Antioxidant Stability Testing On Liquid And Powder *Eichhornia Crassipes* Extract

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Antioxidant Stability Testing On Liquid And Powder *Eichhornia Crassipes* Extract

Nandhita Octavia Nugriani[1], Dr. –Ing. Diah I. W., S.T, M.Sc[1], Silvya Yusri, S.Si, M.T [1]

[1)] Department of Chemical Engineering, Faculty of Life Sciences and Technology, Swiss German University, Tangerang Selatan, Banten, Indonesia.

E-mail: nandhitanugriani@gmail.com

Abstract. Water hyacinth or *Eicchornia crassipes* is a free-floating aquatic plant and is easily found in swamps or rivers. The rapid growth of water hyacinth can disrupt the aquatic environment, thus water hyacinth is categorized as one of the world's worst weed. The challenge is not on how to eliminate the number of this weed, but on how to take advantage by the presence of this water plant. Water hyacinth is known to contain high phenolic content and antioxidant activity. Water hyacinth was extracted using 96% ethanol/water with mass to solvent ratio of 1:30 at 3 different temperatures (30°C, 40°C, 50°C). Spray drying was conducted to produce extract powder with the addition of maltodexrin and Arabic gum as the encapsulating agents. The stability of liquid and powder extract was compared based on the Total Phenolic Content (TPC) and antioxidant activity. The highest TPC was obtained from extraction at 30°C whereas the highest antioxidant activity was obtained from extraction at 50°C. In powder extract, the highest TPC and antioxidant activity was obtained from powder with ratio 2:1 of TSS to encapsulating agents. The stability was performed under different storage conditions. The degradation of TPC was faster in room temperature.

1. Introduction

Eichhornia crassipes or water hyacinth (WH) is a floating warm water aquatic plant. It belongs to the world's worst weeds and accordingly is considered as a plant with a very high growth rate that can harm other aquatic life due to its natural behavior in consuming huge quantities of nutrients in water. In Indonesia, there are 15 critical lakes, which are threatened by the growth of this water hyacinth. As in Rawa Pening, East Java, the growth of water hyacinth has covered around 800 hectares of the total surface water in the area [1]. Billions of dollars have been spent on removing water hyacinth from waterways, but in most cases, this removal is almost impossible to be done. Thus this condition presents its own challenge, not on how to eliminate the number of these weeds but on how to take advantage by the presence of this water plant.

Based on the present studies, *Eichhornia crassipes* is found to have antioxidant properties, as antioxidizing agents are detected in the leaves and its ethanol extract showed good DPPH radical scavenging activities [2]. It could be used as a potential source of antioxidant for food ingredients as well as pharmaceutical products. Antioxidants are compounds that inhibit the oxidation reaction rates, in which the oxidation reaction itself is a chemical reaction that will produce free radicals. Free radicals are molecules, ions or atoms containing unpaired electron or more. Due to the free radical's presence, these radicals are unstable and tend to have a degree of reactivity to chemical reaction of

other molecules [3]. They act by either accepting electron from or donating electron to other molecules. For this reason, they behave as both reductants and oxidants. Free radicals have the capability to attack the macromolecule in the body, causing damage in cells.

efore being further applied, requirements should be fulfilled includes the stability studies of *Eichhornia crassipes* extract. There has been a research regarding the stability of *Eichhornia crassipes* liquid extract conducted by Givianty [4], but It still need to be improved and developed because not only liquid extract available in markets, there are still many choices of dosage forms and the demand for powder form is still increasing; therefore the stability testing should be conducted for powder extract as well to determine which forms have the higher stability. Thus, this problem leads to this research that aims to determine and compare the stability of *Eichhornia crassipes* liquid and powder extract under different temperature conditions.

2. Research Methods

2.1. Chemicals and Equipment

2.1.1. Chemicals. Eichhornia crassipes or water hyacinth was used as the main material in this research, which is known to contain high antioxidant activity and was analyzed for its stability in different kind of forms during storage. Eichhornia crassipes was taken from Situ Cipondoh Lake, Tangerang. Another materials that used for analysis during the research process were listed below. For extraction, the required chemicals were 96% ethanol (v/v), and distilled water that was purchased from Harum Kimia, a chemical supplier in Jakarta. Folin Ciocalteu reagent (Merck, Germany), Gallic acid (Merck, Germany), and Sodium Carbonate (Na2CO3) (Sigma-Aldrich, UK) were used for the determination of total phenolic content of the extract. For antioxidant activity analysis, the extract of Eichhornia crassipes was being assessed based on the radical scavenging effect against DPPH, as the stable free radical. Thus, 2,2-Diphenyl-1-Picrylhydrazyl hydrate (DPPH) (Sigma-Aldrich, USA) was required. Other materials needed were maltodextrin and arabic gum, as the encapsulating agents during spray-drying process that were purchased from Harum Kimia, a chemical supplier in Jakarta.

2.1.2. Equipments. The equipments listed are equipment that were needed for all processes in this research, including the extraction process, determination of total phenolic content in the extract, analysis of antioxidant activity, encapsulation process and shelf life test. The equipment includes were automatic experimental drying oven (EXDRY-51, Indonesia), digital balance (Mettler, Switzerland), weighing scales (O'haus, USA), miller (Fomac, Indonesia), sieve shaker (CISA, Spain), vacuum pump (GAST, USA), cuvette (Brand GmbH, Germany), micropipette (Eppendorf, Germany), vortex (Heidolph, Germany), centrifuge (Pro Research Centrifuges, UK) and UV-VIS spectrophotometer (PG Instruments, UK), that were available in Laboratory of Swiss German University, whereas other equipment needed for spray-drying method was spray dryer (Buchi 190 Mini Spray Dryer, Germany), that available in Bogor Agricultural University, Bogor.

2.2. Experimental Procedure

2.2.1. Preliminary Treatment of Eichhornia crassipes. Preliminary treatment for Eichhornia crassipes was conducted before extraction, where the collected Eichhornia crassipes taken from Situ Cipondoh Lake was washed with water several times to remove the dirt and other possible contaminants which might interfere with the analysis process. It was then dried in shade and the stems, roots and leaves of Eichhornia crassipes were separated, for the reason that only the leaves were used in the extraction process. The leaves of Eichhornia crassipes was then weighed and dried in oven with a temperature of 55°C until reaching moisture content of below 10%. The dried samples was milled using food-processing miller in order to produce smaller size of particle. It was sieved in mechanical

sieve shaker; powder that passed the 60 mesh sizes was collected and stored in refrigerator until extraction.

2.2.2. Stage I: Extraction, Total Phenolic Content and Antioxidant Activity Analysis. For the extraction process, 10 gr of dried Eichhornia crassipes leaves was mixed into 300 mL solution of 96% (v/v) ethanol and distilled water (1:1) as the solvent. The sample was prepared for duplicate analysis. It was extracted under three different extraction temperatures (30°C, 40°C, 50°C) to determine which extract have the highest total phenolic content and antioxidant activities. Extraction process was done under the optimum condition of 3 hours. Filtration was then done using vacuum filter and filter paper to separate the floating materials and to get only the supernatant. Centrifuge was also used before vacuum filter, to firstly separate the floating materials from the solution so that the filtration process was done easier.

For total phenolic content analysis, Folin-Ciocalteu method with gallic acid as a standard was applied. The extract samples was mixed with Folin Ciocalteu reagent and Sodium Carbonate (Na2CO3). The standard curve using the standard solution of gallic acid was prepared. For the ethanolic/water extract of *Eichhornia crassipes,* 200 L will be mixed with the same reagents. The absorbance was then measured after 30 minutes incubation to obtain the total phenolic content in the extract. The absorbance was measured at 765 nm wavelengths.

Antioxidant activity of the extract was assessed using 2,2-diphenyl-1-picryhydrazyl or DPPH solution, as a free radical. DPPH solution was mixed with the extract at minimum four different concentrations. The mixture was allowed to stand for 30 minutes under room temperature. The absorbance was measured at 517 nm, and was compared with a control solution without extract in UV-Vis spectrophotometer. The assays were conducted in duplicate.

2.2.3. Stage II : Pulverization of Extract. Spray - drying method was applied in producing powder extract of Eichhornia crassipes, because it is known as the best drying process in the industry. This facilitates quick drying compared to any other drying method. In this process, the liquid extract was sprayed into a stream of hot vapor through a nozzle and being vaporized. The inlet temperature was set at 170°C, according to the common method of spray drying. The pulverization of Eichhornia crassipes extract was conducted using spray-drying method with the addition of encapsulating agents. The encapsulating agents used were maltodextrin and arabic gum, with the ratio 60:40 respectively. The encapsulating agent was added with ratio 1:1, 1:2 and 2:1 of the total soluble solids of extract to the encapsulating agents, in order to see the effect of encapsulating agents' quantity to the product stability.

2.2.4. Stage III : Stability Studies. After extraction, pulverization, analysis of total phenolic content and antioxidant activity, the last step was the shelf life analysis that involving two different storage conditions; shelf life testing at 30°C in room temperature and shelf life testing in refrigerator temperature at 4°C. By interacting the result with kinetic order reaction principles, the product shelf life can be observed in each storage temperatures.

2.3. Analytical Procedure

2.3.1. Total Phenolic Content Analysis

2.3.1.1. Preparation of Folin-Ciocalteu reagent, Sodium carbonate and Gallic Acid Standard Curve. Total Phenolic Content analysis was conducted using Folin - Ciocalteu method with gallic acid as the standard. The folin reagent and sodium carbonate was prepared. The Folin reagent was prepared by diluting the concentrated Folin- Ciocalteu reagent with distilled water with a ratio of 1:10. The sodium carbonate was prepared by diluting 7.5 grams of solid sodium carbonate into 100 mL distilled water to

get 75% (w/v). For standard curve preparation, 1000 mg/L stock solution was prepared by dissolving 0.1 grams of solid gallic acid into 100 mL distilled water. The stock solution then was diluted to 5, 40, 80, 120, 160 and 200 ppm or mg/L gallic acid solution. From that diluted solution, 200 µL was taken into test tube, mixed with 1 mL Folin reagent and 800µL of sodium carbonate solution (Na2CO3). All mixtures were incubated for 30 minutes at room temperature were then poured into cuvettes and the absorbance was measured using UV-Vis Spectrophotometer at 765 nm. The Gallic Acid standard curve was obtained by plotting a linear regression based on the measured absorbance.

2.3.1.2. Total Phenolic Content of Eichhornia crassipes Extract. For obtaining total phenolic content of the extract, 200 µL of extract was taken into test tube, mixed with 1 mL Folin reagent and 800 µL of sodium carbonate solution (Na2CO3). It was incubated for 30 minutes at room temperature, and was then poured into cuvettes and the absorbance was measured using UV-Vis Spectrophotometer at 765 nm. The total phenolic content was calculated using Gallic Acid Equivalent (GAE) according to the equation below.

$$Total\ phenolic\ content\ \left(\frac{mg\ GAE}{L}\right) = \frac{(Abs - c)x\ DF}{m} \quad (1)$$

where;

Abs : Absorbance (A)

M : Gradient of the Gallic Acid standard curve (A/mg L)

c : Intercept of the Gallic Acid standard curve (A/mg L)

DF : Dilution Factor

2.3.1.3. Total Phenolic Content of Eichhornia crassipes Powder Extract. For obtaining total phenolic content of the powder extract, 0.02 grams of extract powder was dissolved in 10 mL solvent (distilled water and ethanol) in order to obtain 2000 mg/L solution. Then, 200 µL of extract was taken into test tube, mixed with 1 mL Folin reagent and 800 µL of sodium carbonate solution (Na2CO3). It was allowed to stand for 30 minutes for incubation at room temperature, and was then poured into cuvettes and the absorbance was measured using UV-Vis Spectrophotometer at 765 nm. The total phenolic content was calculated using Gallic Acid Equivalent (GAE) according to the mentioned equation.

2.3.2. Antioxidant Activity Analysis by DPPH Assay

2.3.2.1. Preparation of 0.25 mM DPPH Stock Solution. The antioxidant activity of Eichhornia crassipes extract was tested using DPPH Assay method. In order to prepare the 0.25 mM DPPH stock solution, 10 mg of powder DPPH was diluted into 100 mL 96% (v/v) pro-analysis ethanol solution. The stock solution was stored in refrigerator and stored in amber colored bottle that wrapped with aluminium foil.

2.3.2.2. Antioxidant Activity Analysis of Eichhornia crassipes Extract Solution. Antioxidant activity was assessed using 2,2-diphenyl-1-picryhydrazyl or DPPH solution. For control preparation, 0.5 mL of ethanol pro-analysis was taken in a test tube and added with 0.5 mL of 0.25 mM DPPH solution. For obtaining the sample absorbance, 0.5 mL of ethanol was replaced with extract solution at minimum of four different concentrations. The mixture was shaken well, and allowed to stand for 30 minutes in a dark room at room temperature. The absorbance was then measured using UV-Vis Spectrophotometer at 517 nm. From the collected absorbance data, the DPPH inhibition percentage was calculated using the following equation. IC_{50} value is expressed as the concentration of the sample to be able to inhibit 50% of free radical (DPPH).

$$Radical\ scavenging\ activity\ (\%) = \frac{Ac - As}{Ac}\ x\ 100\% \qquad (2)$$

Where,

Ac : Absorbance of control (A)

As : Absorbance of sample (A)

3. Results and Discussions

3.1. Preliminary Treatment and Extraction of Eichhornia crassipes

Preliminary treatment was done in order to separate the raw plant into its stems, roots and leaves as only the leaves were taken for analysis. The leaves were washed with tap water several times to remove dirt and any possible contaminants that might interfere the analysis process, and was dried in shade before cut. The leaves were cut into smaller pieces to facilitate further drying process in oven. The leaves that used for analysis were the leaves with the size ranging from 13cm to 17cm diameter, the other were excluded during the separation of this plants part. The cut leaves were dried in oven at 55℃ for 24-48 hours until the weight was constant and moisture content to below 10% were obtained. After drying, the dried sample was milled using food-processing miller for 15 minutes to reduce the size and sieved using sieve shaker to obtain powder, powder that passed the 250 micrometer pores or 60 mesh size was collected in plastic zipper bag and stored in refrigerator until extraction in order to inhibit or slow down the growth of microorganism so that the product has a longer shelf life [5].

The next step after pre-treatment of *Eichhornia crassipes* was solid-liquid extraction. The extraction process was done and partially adopted from the previous extraction process done by Jimmy [6] and Givianty [4], where the optimum condition for extracting *Eichhornia crassipes* within 3 hours was already selected. In this research, extraction of *Eichhornia crassipes* was carried out in 3 different

temperatures within 3 hours, means that the dried sample of *Eichhornia crassipes* was extracted at 30℃, 40℃ and 50℃ with the combination of distilled water and 96% (v/v) ethanol as the solvent. It was extracted with 1:30 sample to solvent ratio. Vacuum filtration and centrifuge was used in order to filtrate the extract solution and to remove the insoluble impurities to get a clear solution. The filtrate was then centrifuged with speed of 6000 rpm for 15 minutes to precipitate solids followed, so that the supernatant is obtained. Because in general, one of the requirements for using UV-Vis spectrometry is the sample must be in the form of a clear solution to avoid the misleading in data interpretation [7].

3.2. Pulverization of Eichhornia crassipes Extract

In order to produce powder extract, the liquid extract from extraction condition 1 (30℃), 2 (40℃) and 3 (50℃) was spray-dried. It was firstly added with encapsulating agents (maltodextrin and arabic gum) with ratio 1:1 of the total soluble solids to total encapsulating agents and other variation ratios (1:2 and 2:1) was then conducted to analyze the effect of encapsulating agents to the TPC and IC_{50} value of the extract. The inlet temperature of spray drying was 170 ℃ and the outlet was 70℃, based on the default setting parameter in mini spray drier in IPB, Bogor.

Encapsulation is a technique of core material entrapment in certain coatings. The advantage of encapsulation techniques is to protect and control the release of active ingredients [8]. The coating material used must be food grade, biodegradable, and stable during the processing process. Coatings commonly used in encapsulation include carbohydrate-based ingredients, proteins and fats. Maltodextrin is composed of glucose units, and is not effective for stabilizing oil or flavor in a viscosity solution. For this reason, maltodextrin is usually combined with ingredients such as arabic gum or other modified starches for the purposes of material stability. As has been stated by Supriyadi and A. S. Rujita [9], maltodextrin has a good ability to inhibit oxidation reactions. While Arabic gum is soluble in water. In addition to its high solubility, the main characteristics of arabic gum are texture-forming, film forming, binding and also good emulsifier in the presence of protein components in arabic gum. Gum arabic can retain the flavor of food dried by spray drying method because these gum can form layers that can protect from the process of destructive change. Hence, the combination of Arabic gum and maltodextrin was chosen in this research.

3.3. Stability based on Total Phenolic Content

Stability testing for liquid and powder extract was conducted based on Total Phenolic Content. The changes in TPC result from day to day were analyzed.

TPC Stability of Liquid Extract – at Room Temperature Storage

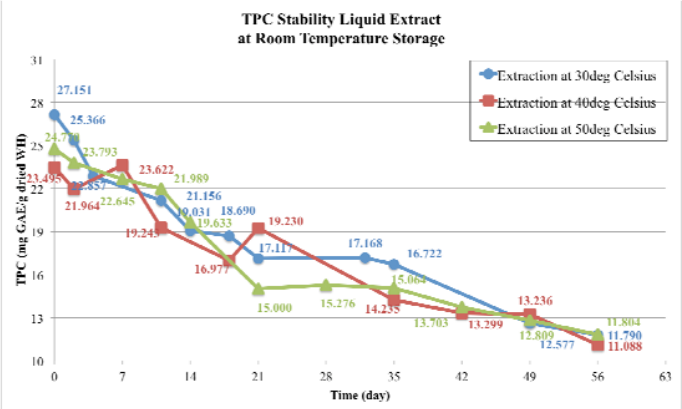

Figure 1. The TPC Stability of Liquid Extract – Room Temperature Storage