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**Study of The Pharmacological Activity and
Heavy Metal Content of *Eichhornia crassipes*
Extract**

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Abstract: *Eichhornia crassipes* is well-known as water hyacinth. Water hyacinth grows rapidly in the nutrient-rich water and high light intensity places. The uncontrollable growth of water hyacinth has caused many negative impacts to the environment. For instance, interrupted water transport and decreased population of aquatic lives. The capacity of utilising water hyacinth is slower than water hyacinth growth and water hyacinth is still considered as a threat to the ecosystem. This work was focused on the study of the pharmacological activity and heavy metal content of water hyacinth in Lake Cipondoh, Tangerang. Fresh water hyacinth was pre-treated through oven-drying and milling process. After that, each part of the plant was macerated by using multiple extraction method with 96% ethanol/water and three variations of sample-to-solvent ratios (1:30, 1:50, and 1:75 w/v). The result of the experiment showed that water hyacinth leaves produced an extract with lowest IC₅₀ (55.76 ± 6.73 ppm) compared to other parts. The most optimum solvent used to achieve this result was 96% ethanol/water (1:1 v/v). In order to obtain the lowest antioxidant activity, the sample to solvent ratio used was 1:50 and the heavy metal in the extract was very low. With this result, it was concluded that there is a promising opportunity to apply the water hyacinth growing in Lake Cipondoh, Tangerang as herbal medicine ingredient. Through this utilization, the overall number of water hyacinth in Indonesia can be reduced or at the least be controlled, so that the environmental problem caused by this plant can be minimized.

Keywords: Water hyacinth, phenolic content, heavy metal content, phytochemicals, antioxidant activity

1. Introduction

1.1. *Eichhornia crassipes*

Eichhornia crassipes is well-known as water hyacinth (WH) or Eceng G²⁰lok in Indonesia. Water hyacinth is a tropical and sub-tropical South America aquatic macrophyte plant which belongs to the family *Eichhorniaceae*. The attractive six petals of blue-violet flowers, it has spread worldwide through botanical gardens (Silva, de Melo, Silvestre, & Silva, 2015). This plant is known as one of the world's worst aquatic weed due to its rapid growth in lakes and rivers in which there are adequate amount of nutrients in the water and sunlight (Gichuki, et al., 2011). Through sexual reproduction, *E. crassipes* produces seeds which could remain up to thirty years (Adeyemi & Osubor, 2016) and caused environmental degradation such as blockage of waterways, obstruct navigation and loss of biodiversity (Ndimele & Jimoh, 2011).



Figure 1. Water Hyacinth from Lake Situ Cipondoh, Indonesia.

Most of the 500 lakes in Indonesia have been covered with water hyacinth for years. For instance, Lake Rawa Pening in Semarang with an area of 2.670 Ha is one out of 500 lakes where water hyacinth has grown out of control and has caused many negative impacts to the environment and the society near the lake. Water hyacinth has grown out of control and has been interrupting water transport, power and tourism activity. Even more, it has decreased the population of aquatic lives in the lake. In 2002, 20% - 30% of the lake surface were covered by water hyacinth. In 2016, 60% of the surface area of the lake has been covered and contributed to at least 150.000 m³ of sedimentation (Utomo, 2017).

In order to deal with water hyacinth growth, various approaches have been carried out. Scientific studies indicate water hyacinth has the ability to absorb heavy metals from water body, animal feedstock and alternative fuel source (Ndimele & Jimoh, 2011). The absorbed heavy metals are accumulated in roots, stems and leaves. The extracts of distinct parts of *E. crassipes* show it contains phenolic compounds and exhibits good antioxidant activity (Surendraraj, Farvin, & Anandan, 2013). Each place where water hyacinth grows has different distribution of heavy metals, different activity of antioxidant. So far, no studies of *E. crassipes* in Lake Situ Cipondoh, Tangerang, Indonesia have been done.

1.2. Objective

The aim of this research is to find out whether water hyacinth can be used as one of many pharmaceutical or food ingredients. The main objective of this research were to: (a) develop an optimum extraction method which maximises the pharmacological activity and minimises the heavy metal content of water hyacinth; (b) study the pharmacological activity such as antioxidant activity water hyacinth extract; and (c) study the heavy metal content of water hyacinth powder and extract which are obtained from each water hyacinth parts.

2. Materials and Methods

2.1. Chemicals

For the extraction of antioxidant activity, the chemicals needed were ethanol (Mallinckrodt, United Kingdom), 2,2-Diphenyl-1-Picrylhydrazyl hydrate (DPPH) (Sigma-Aldrich, USA).

2.2. Raw Materials

Water hyacinth was collected from Lake Situ Cipondoh, Tangerang, Indonesia in February 2018. The collected water hyacinth was washed and separated into roots, stems and leaves. All the separated parts were oven-dried at 45°C for 24 – 48 hours until the moisture content below 10% was recorded. Then, the dried water hyacinth was milled by using food processing miller. Water hyacinth which was passed through 60 mesh sieves with an opening of 250 μm was collected and stored at room temperature until extraction.

2.3. Maceration Extraction

Maceration extraction was performed for each part of *E. crassipes*. The extractions were carried out in duplicate and the powder from each parts of water hyacinth were extracted with the mixture of 96% ethanol/water (1:1, v:v) and 3 different sample-to-solvent ratios (1:30, 1:50, 1:75).

2.4. Multiple Stage Solid – Liquid Extraction

Following the work done by Surendraraj, Farvin, & Anandan (2013), multi-stage extraction was used to extract all water hyacinth plant parts by using ethanol and water. The mixture of 96% ethanol/water and two different concentrations of the solvent were added to the sample preparation.

For the preparation of the 96% ethanol/water (1:1, v:v of 120 mL : 50 mL) extract with sample-to-solvent ratio of 1:30, 5 grams of powdered water hyacinth was extracted with 50 mL of 96% ethanol/water at room temperature for a day. It was then centrifuged at 11,000 rpm for 10 minutes. The supernatant was filtered. Under the same condition, the residue was re-extracted two times. All extracts were later combined.

The extraction steps were repeated for different sample-to-solvent ratio, which were 1:50 for 3 grams in 50 mL 96% ethanol/water and 1:75 for 2 grams in 50 mL 96% ethanol/water. These extracts from different sample to solvent ratios were analysed of its total antioxidant activity by using UV-VIS Spectrophotometer.

2.5. Antioxidant Activity of The Extracts of *E. crassipes*

Antioxidant activity in the extracts was assessed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH), following Surendraraj, Farvin, & Anandan (2013) method with modification. DPPH solution (500 µL, 0.25 mM in ethanol) was mixed with 500 µL of extract (at four different concentrations). The mixture was shaken and kept at room temperature for 30 min. The absorbance was measured at 517 nm using an UV-Vis spectrophotometer and compared with a control without extract and the assays were carried out in duplicate. For the blank, 500µL water was mixed with 500 µL 96% ethanol. While for sample control, 500 µL of 96% ethanol was mixed with 500 µL of DPPH. The A_{500} of the samples is compared with ascorbic acid which acts as a standard. The antioxidant activity (AA) or scavenging activity was expressed as percentage inhibition of DPPH radical.

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100\% \quad (1)$$

where, $\text{abs}_{\text{sample}}$ is absorbance of sample and $\text{abs}_{\text{control}}$ is absorbance of sample control.

2.6. Heavy Metal Analysis of *E. crassipes* Powder and Extracts

2.6.1. Arsenic (As) and Mercury (Hg) Concentration

Arsenic and mercury stock solution (1000 ppm) was diluted into 100 ppb arsenic and mercury standard solution. A standard series for arsenic and mercury solution 1, 3, 5, 7 and 10 ppb were made by mixing with HNO₃ 0.5N. 0.3 – 0.5 mg of the sample was weighed and added with 9 mL of HNO₃ p.a. and 1 mL of H₂O₂ p.a. were added and put into Teflon vessel. This solution was allowed to stand for 10 minutes and then, it was heated in microwave digestion for ±30 minutes. After the microwave digestion was cooled down, the sample solution was moved into 100 mL volumetric flask and filtered using filter paper no.41. 25 mL of the filtered solution was pipetted and mixed with 2 M HCl 8M and 0.1 mL KI 20%.

By using Atomic Absorption Spectrophotometer GBC HG 3000, for arsenic analysis, the standard and the sample were measured at 193.7 nm with condition: the fuel is 1.2 – 1.3 L/min, smallest flame, NaBH₄ as reductor and HCl 3M as acid. The sample concentration (mcg/L) was calculated based on arsenic standard solution's linear regression $y = mx + c$. So, Arsenic concentration can be calculated with the following equation:

$$\text{As concentration (mcg/kg)} = \frac{C \times V \times D_F}{W} \quad (2)$$

where, C is sample concentration reading (mcg/L), V is volumetric Flask Volume (L), D_F is dilution factor, and W is sample weight (g).

Meanwhile, for mercury analysis, By using Atomic Absorption Spectrophotometer GBC HG 3000, the standard and the sample were measured at 253.7 nm with condition: no flame (using cold vapour technique), NaBH₄ as reductor and HCl 3M as acid. The sample concentration (mcg/L) was calculated based on mercury standard solution's linear regression $y = mx + c$. So, Arsenic concentration can be calculated with Equation (2).

2.6.2. Lead (Pb) and Cadmium (Cd) Concentration

Lead and cadmium stock solution (1000 ppm) was diluted into 10 ppb lead and cadmium standard solution. A standard series for lead solution 0, 0.5, 1, 2, 3 and 5 ppb, and cadmium solution 0, 1, 3, 5, 7 and 10 ppb were made by mixing with HNO₃ 0.5N. 0.3 – 0.5 mg of the sample was weighed and added with 9 mL of HNO₃ p.a. and 1 mL of H₂O₂ p.a. were added and put into Teflon vessel. This solution was allowed to stand for 10 minutes and then, it was heated in microwave digestion for ±30 minutes. After the microwave digestion was cooled down, the solution was moved into 100 mL volumetric flask. 5 µL of matrix modifier Mg(NO₃)₆. 6H₂O 0.7% were added to the sample solution.

By using Atomic Absorption Spectrophotometer GF, the standard and the sample were measured at 228.8 nm for cadmium and 217.0 nm for lead. The sample concentration (mcg/L) was calculated based on lead and cadmium standard solution's linear regression $y = mx + c$. So, lead and cadmium concentration can be calculated with the following equation:

$$\text{Pb or Cd concentration (mg/kg)} = \frac{C \times V \times D_F}{W} \tag{3}$$

where, c is sample concentration reading (mg/L), v is volumetric flask volume (L), D_F is dilution factor, and W is sample weight (g).

3. Results and Discussions

3.1. Antioxidant Activity

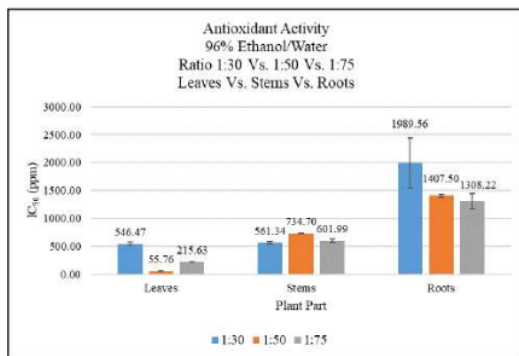


Figure 4. IC₅₀ of WH Part in Different Ratios

While 96% ethanol/water had been chosen as the solvent to analyse antioxidant activity. A lower half-maximal Inhibition Concentration (IC₅₀) means higher antioxidant activity. From Figure 4, the extraction with ratio 1:30, the lowest IC₅₀ was found in WH leaves and followed by WH stems and roots. As same as ratio 1:30, the lowest IC₅₀ in ratio 1:50 and 1:75 was found in WH leaves and followed by WH stems and roots as well. Overall, the lowest IC₅₀ was obtained in WH leaves of ratio 1:50. Because not all phenolic compounds are phenolic antioxidants. Hence, different phenolic antioxidant may vary.

3.2. Heavy Metal Content

Based on the results obtained by using Atomic Absorption Spectrophotometer, the heavy metal content (Pb, Cd, As, Hg) in WH leaves, stems and roots powder were not detected or there was only a small amount of heavy metal content. The same case happened to the 96% ethanol/water extract of WH leaves, stems and roots. Heavy metal content was not detected. The limit of detection of lead (0.012 mg/kg), cadmium (0.004 mg/kg), arsenic (0.028 mg/kg) and mercury (0.013) by the Atomic Absorption Spectrophotometer were all below the maximum standard of heavy metal content established by WHO (2007) and BPOM-RI (2014). Hence, the extracts can be used as pharmaceutical ingredients such as herbal medicine.

The low concentration of heavy metals could be due to the good environment in Lake Situ Cipondoh. Besides, heavy metals in plants usually chelated by metal chelator inside the plant. This is called as metal-chelate complexes.

4 Conclusion

In this research, it is shown that water hyacinth from Lake Situ Cipondoh, Tangerang has high antioxidant activity by drying at 45°C. Water hyacinth leaves of 96% ethanol/water extract with mass to solvent ratio of 1:50 has the lowest IC₅₀ (55.76 ± 6.73 ppm). The heavy metals in all WH powder and 96% ethanol/water extracts were very low.

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