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Isolation and Characterization of Phenolic Contents, Tannin, Vitamin C and E from Water Lettuce *(Pistia stratiotes)*

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ABSTRACT

Water lettuce (*Pistia stratiotes*) is macrophyte plant that grows in nearly all tropical fresh waterways and floats on the water surface. The purpose of this study was to quantify the phytochemical constituents and vitamins which contribute to antioxidant activity of this plant. *N*-hexane, ethyl acetate, and methanol extracts of water lettuce leaf were screened for the presence of phenols and tannins following Folin-Ciocalteu method. The vitamin C and E content of fresh leaves were determined using HPLC. The results show that the phenolic content of methanol extract was the highest with the value of 109.05 ppm while the ethyl acetate extract exhibits the highest tannins (138.86 ppm). Total contents of 0.52 mg and 3.36 mg were respectively obtained for vitamin C and vitamin E in 100 g of the sample. These results indicate the natural presence of antioxidants in the extract.

Keywords: Antioxidant, bioactive, *Pistia stratiotes*, water lettuce.

INTRODUCTION

Water lettuce (*Pistia stratiotes*) is a plant that lives as macrophyte freshwater and floats on the surface spread of tropical to subtropical regions. This plant is known in many countries as a weed. The leaf and stems of water lettuce are composed of 92.9% moisture, 1.4% protein, 0.3% crude fat, 2.6% carbohydrate, 0.9% crude fibre, and 1.9% minerals (mainly phosphorus and potassium). Vitamins, stigmasterol, and palmitate acids are also found in the leaf of this plant. Water lettuce efficacious as a drug of influenza, fever, cough throw, allergic itch, dysentery and measles. This plant can also be used as an absorber of pollutants for example metals industry waste. The presence of bioactive compounds thought to play a role in the efficacy and use of water lettuce.

Bioactive compounds are naturally produced in animals and plants to use for various diseases. Some bioactive compounds are normally occur in plants, namely alkaloids, flavonoids, phenols, tannins, steroids and triterpenoids.

Antioxidants are compounds able to inhibit or prevent oxidation of the substrate is easily oxidized in the form of simple or complex shapes,

such as the fat, fatty acids, proteins, and DNA. Antioxidants based on their source can be grouped into two, namely a natural antioxidant and synthetic antioxidants. Natural antioxidants can be obtained from natural materials. There are fears the side effects of synthetic antioxidants, the natural antioxidant is a source of antioxidant potential to be developed. Natural antioxidants can be obtained from a number of bioactive compounds, for example phenols and tannins and also from vitamin. As for some of the vitamins that can act as antioxidants are vitamin A, vitamin C, and vitamin E.

Phenolic compounds were higher in the methanol extract of the *Lantana camara* generate IC_{50} most effective with DPPH radical scavenging assay[1]. Tannins fraction in *Rubus occidentalis* extract also has a very effective IC_{50} value as a source of antioxidants[2]. Vitamin C also has a very strong antioxidant activity and is generally used as a positive control of the test the antioxidant activity[3]. Meanwhile, vitamin E acts as an antioxidant in the cell membrane, or more precisely in cell membrane lipids, circulating low density lipoprotein (LDL) cholesterol, lungs, liver, and adrenal tissue. Based on this information, the study of bioactive compounds and vitamin content of plants is important to do. The purpose of this study was to determine the levels of phenols, tannins, vitamin C, and vitamin E in water lettuce (*Pistia stratiotes*) extracted by n-hexane, ethyl acetate, and methanol.

MATERIALS AND METHODS

Water lettuce (*Pistia stratiotes*) collected from Banyuasin, South Sumatra, Indonesia. Other materials used are solvent for extraction (*n*-hexane, ethyl acetate, methanol). The tools are used, the rotary vacuum evaporator, vortex and high-performance liquid chromatography (HPLC Hitachi 7100).

This study began from sampling, preparation, and measure of vitamin C and vitamin E. The next stage is the extraction and testing of the content of total phenols and tannins. The leaf of the water lettuce was used and then reduced-size. Most of the samples assayed in fresh form for vitamin C and vitamin E using HPLC and a part of samples were dried by oven-dried and then extracted for measure of phenols and tannins. Extraction aims to separate certain components of a sample to obtain bioactive compounds. Before extracted samples first dried in oven at a temperature of 45°C until the moisture content below 10%. A total of 100 g sample was macerated in stages using 400 mL *n*-hexane (1:4, w/v). Extraction is done at room temperature for 48 h, assisted by stirring using a magnetic stirrer. Stirring is done to increase the chances of interaction between the sample and the solvent so as to maximize the yield of the extract. The sample is then filtered using Whatman No.01 filter paper and the filtrate is evaporated using a rotary vacuum evaporator (40°C) to obtain a crude extract. The extract is then stored in dark coloured bottles and wrapped in aluminium foil and kept at chilling temperatures before it is used for testing the content of total phenols and tannins. The process also repeated for ethyl acetate and methanol.

Total phenolic content

The content of total phenols was determined by Folin-Ciocalteu method[4]. It was based on the ability of phenol reduces the phosphomolybdate in Folin-Ciocalteu to blue molybdenum compounds[5]. A total of 0.01 g of extract dissolved in 25 mL of 96% ethanol and 2 mL of the solution was taken into a test tube and then added 5 mL distilled water and 0.5 mL of 50% Folin-Ciocalteu and homogenized used vortex. The mixture was incubated at room temperature for 5 min. and then added 1 mL of 5% Na_2CO_3 and stirred, then incubated at room temperature for 60 minute. The absorbance was recorded using spectrophotometry at 725 nm.

Tannin content

A sample (0.2 g) was inserted into Erlenmeyer flask containing 10 ml of methanol and homogenized and incubated at room temperature for 60 minute. A total of 1.0 ml of the supernatant was mixed distilled water in tube was then added 0.3 mL of 0.1 M $FeCl_3$ and homogenized. The mixture was then added 0.3 mL $K_3Fe(CN)_6$ 0,008 M homogenized and incubated at room temperature for 10 min. The absorbance was measured at a 725 nm.

RESULTS AND DISCUSSION

Vitamin C

Vitamin C in fresh water lettuce leaf was 0.52 mg/100 g. The vitamin C indicated that the leaf of this plant can be used as a source of natural antioxidants. Yellow orange (*Citrus sinensis*) had higher levels of vitamin C reached 984 mg/100 g reported by Dumbrava *et al*.,[6] whereas once extracted with water only reached 44.03 mg/100 g and alcohol extract was 26.42 mg/100 g. Vitamin C is water soluble vitamin. The results of another study conducted by Yang *et al*.,[7] (2014) reported that the vitamin C in a water extract in water spinach (*Ipomoea aquatica*) was 42 mg/100 g of dried-powder. The extract also has antioxidant activity as tested using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) with activity of 238 mg/100 g *eq*.vitamin C. Dumbrava *et al*.,[6] also reported the presence of the antioxidant activity of vitamin C in the *Citrus sinensis* fruit through DPPH radical scavenging assay. Vitamin C (*l*-ascorbic acid) has been widely used as a positive control for comparison activity antioxidant with antioxidant testing samples, for example in large-leafed mangrove (*Bruguiera gymnorrhiza*) fruit by Sudirman *et al*.,[3]. Differences in vitamin C and antioxidant activity due to the differences in types of crops, crop conditions, type of solvent used for extraction and methods of antioxidants assay.

Vitamin E

Vitamin E in fresh water lettuce leaf was 3.36 mg/100 g. The content of vitamin E in apples 0.46 mg/100 g, banana 0.33 mg/100 g, and strawberries 0.19 mg/100 g. This proves that the water lettuce leaf is a potential source of vitamin E. The existence of vitamin E on fresh water lettuce leaf can be used as a source of natural antioxidants. Rukmiasih *et al*.,[8] reported that the duck meat was given vitamin E during storage 10 weeks have a higher fatty acid composition compared with those given vitamin C. It is proved that vitamin E provides good protection from fatty acids and have antioxidant activity. Vitamin E is a fat-soluble vitamin that is more effective in providing protection against fatty acids than vitamin C which is water soluble vitamin. Vitamin E or tocopherol acts as an antioxidant because it is easily oxidized. Therefore, it can protect other compounds from oxidation. vitamin E is the main defence against oxygen radical, lipid peroxides and free radicals and stop the chain reaction of free radicals.

Total phenol

Total phenol of water lettuce leaf has high in the methanol extract was 109.05 ppm (Table 1). Rumiyati *et al*.,[9] reported that more phenolic compounds of methanol (polar) extract of *Cayratia trifolia* was 57.7 ppm than the fraction of the semi-polar and non-polar. Based on Kahkonen *et al*.,[10] the phenol content in the leaf greatly influenced by the age of leaf, soil, fertilizer and physical environment, biological, and chemical. According Andayani *et al*.,[11] phenolic compounds have antioxidant activity which has a hydroxy group substituted in the *ortho* and *para* positions. Total phenol content of these positively correlated to the antioxidant activity. Meenakshi *et al*.,[12] reported an association between total phenol and antioxidant activity. A material has a high content of phenolic compounds, the antioxidant activity in the material is also high.

Tannin

Tannin content of water lettuce has high in the ethyl acetate extract was 138.86ppm (Table.1). Rahman *et al*.,[13] reported that the tannin content contained in the petroleum ether extract (76.27mg/100 g) of *Jangomas flacourtia* plants larger than the methanol extract (35.14 mg/100 g) and chloroform (25.54 mg/100 g). The high total polyphenols in ethyl acetate solvent suspected class of polyphenols that have the same molecular weight solvent ethyl acetate for example tannins and flavanols. Tannins are polyphenolic compounds that can form insoluble complex compounds with proteins. These compounds are found in various types of plants used both for food and animal feed.

Table.1: Total phenol and tannin of water lettuce extract (*Pistia stratiotes*)

Bioactive compounds	Value (ppm)		
	N-Hexane	Ethyl Acetate	Methanol
Total phenol	63.33	99.23	109.05
Tannin	72.74	138.86	95.41

CONCLUSION

Water lettuce (Pistia stratiotes) is macrophyte plant that grows in nearly all tropicalfresh waterways and floats on the water surface. The fresh leaf of this plant contain vitamin C was0.52 mg/100 g and vitamin E was3.36 mg/ 100 g. Total phenol of water lettuce leaf has highin the methanol extract was 109.05 ppm. Tannin content of water lettuce has highin the ethyl acetate extract was 138.86 ppm.

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