Antioxidant activity of Indonesian water spinach and land spinach (*Ipomoea aquatica*): A comparative study

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Antioxidant activity of Indonesian water spinach and land spinach (*Ipomoea aquatica*): A comparative study

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Abstract. *Ipomoea aquatica* is Convolvulaceae plant that is widely consumed in Southeast Asia as a vegetable. The plant contains various bioactive components, e.g. phenols and flavonoids. Water spinach and land spinach are two varieties of *Ipomoea aquatica* in Indonesia. Both extracts have been analyzed for their free radical scavenging activity using DPPH (2,2-diphenyl-1-picrylhidrazyl). Total phenol was measured using Folin Ciocalteu and the total flavonoid was measured using $AlCl_3$. Both extracts also have been analyzed by TLC (Thin layer chromatography) using DPPH and $AlCl_3$ spray reagents. Water spinach has higher antioxidant activity than land spinach and there was a correlation between antioxidant activity and total phenol/flavonoid content. Based on TLC results, it was supposed that the compound responsible for antioxidant activities in water spinach and land spinach was flavonoid.

1. Introduction

Antioxidants are beneficial for human health because they can eliminate free radicals that contribute to most chronic diseases. One source of antioxidants is green vegetables that contain various nutrients and antioxidant compounds. Vegetable consumption is considered advantageous to reduce the risk of cancer and other degenerative diseases [1].

Ipomoea aquatica Forsk, commonly known as Kangkung in Indonesia, is a perennial herb that belongs to the family of Convolvulaceae. This plant can be found in India, Ceylon, Tropical Asia, Africa, and Australia, growing wild in India and the USA and is believed to come from China. In Southeast Asia, such as in Malaysia, China, Hong Kong, Singapore and Indonesia, these plants are widely cultured and consumed as daily vegetables [2].

Ipomoea aquatica contains carbohydrates and nutrients, especially such minerals as K, Fe, Mg and Mn. It also contains bioactive compounds such as flavonoids and phenols. Moreover, there are so many activities possessed by water spinach such as antioxidants, anticancer, antidiabetic, anti-inflammatory, anti-ulcer, anxiolytic, and antiepileptic. The antioxidant activity of water spinach has been observed by many researchers with various methods including the DPPH method [1,3-6].

In Indonesia, there are two kinds of *I. aquatica*, namely water spinach and land spinach. Therefore, the aim of this study was to determine the antioxidant activity of the two types of water spinach by using the DPPH method. In addition, each type of *I. aquatica* was determined for their respective content of total phenols and flavonoids. Both extracts also were analyzed by thin layer chromatography (TLC) using DPPH and $AlCl_3$ spray reagents.

2. Methods

2.1. Materials

The primary materials for this study were DPPH (2,2-diphenyl-1-picrylhidrazyl), gallic acid, Ciocalteu reagent, vitamin C and quercetin from Sigma and some other reagents and solvents.

2.2. Sample preparations

Leaves from the two varieties *of Ipomoea aquatica*, water spinach and land spinach, were collected from Situgede, Wanaraja, Garut, Indonesia. The two plants were determined in the Herbarium Bandungense School of Life Science and Technology, Bandung Institute of Technology. Those plants were cleansed, dried and pounded into powder.

2.3. Extraction

Two hundred grams of the dried powder was macerated with ethanol for 24 hours. The maceration process was carried out 3 times. The ethanol extract was dried using a rotary evaporator.

2.4. Antioxidant activity with DPPH

Antioxidant activity was determined by DPPH based on the method of Rohman et al [7] with some modifications. Each extract and vitamin C were prepared in various concentrations. Each sample concentration was piped as much as 1 ml and put into a vial, then 1 ml of DPPH solution 100 µg / ml and 2 ml ethanol pa. were added. The mixture was homogenized and left for 30 minutes in a dark place. Absorbance was measured using a UV-VIS spectrophotometer at the maximum wavelength 516 nm with blank ethanol. The control absorbance measurements were also carried out (1 ml of DPPH 100 µg / ml solution added with 3 ml ethanol pa and left 30 minutes in a dark place). The tests were carried in triplicate. After the observed absorbance value was measured for inhibition % of the solution using the following equation:

$$\text{Inhibition \%} = [(CA-SA)/(CA)] \times 100\% \quad (1)$$

Note:
CA : Control Absorbance
SA : Sample Absorbance

From the inhibition %, the IC50 value (the concentration that can inhibit 50% DPPH) was determined. The IC50 value was calculated from the linear regression curve between inhibition % and various sample concentrations.

2.5. Determination of total phenolic content

Total phenolic content was determined by the Folin-Ciocalteu method done by Rohman et al [7] with some modifications. One ml of sample was put into a 10 ml volumetric flask, then 500 µl of Folin Ciocalteu reagent was added and they were mixed until homogeneous for 1 minute.

Before the eighth minute, 4 ml of Na2CO3 7.5% b/v was added, and they were shaken for 1 minute and aquadest was also added then they were shaken until homogeneous. The measurements were carried out with a spectrophotometer at the maximum wavelength; 741 nm. Standard gallic acid solutions (10, 20, 30, 40, 50, 60 µg / ml) were used to make a calibration curve. The tests were carried in triplicate. The total phenolic content was calculated as gallic acid equivalent / g extract (mg / g GAE).

2.6. Total flavonoid content

The total flavonoid content was determined by the method carried out by Pormorad et al [8] with some modifications. A total of 0.5 ml of sample (100 µg / ml in ethanol) was added with 1.5 ethanol, 0.1 ml of AlCl3 10%, 0.1 mL of 1 M sodium acetate and 2.8 mL of aquadest. After that, it was incubated at room temperature for 30 minutes, then the absorbance was measured with a UV spectrophotometer at the maximum wavelength of 442 nm. The quercetin solution (10,20,30, 40, 50 and 60 µg / mL) was

used to make a calibration curve. The tests were carried in triplicate. The total flavonoid content was calculated as quercetin equivalent / g extract (mg / g QE).

2.7. Thin layer chromatography

Both extracts were analyzed by TLC using silica gel 60 F254 plates and n-hexane: ethyl acetate (2:8) which is the mobile phase which provides the best separation. The results of the chromatography were sprayed with the appearance of DPPH 0.2% and AlCl3 reagents.

3. Results and discussion

There are differences in leaf size in both types of *Ipomoea aquatica*. Water spinach has a smaller leaf than land spinach (Figure 1).

(a) (b)

Figure 1. *Ipomoe aquatica* leaves, (a) water spinach, (b) land spinach.

The antioxidant activity test using DPPH method with visible spectrophotometer was based on the color changes that occur in DPPH free radicals. DPPH solution in ethanol will give purple and strong absorption at a wavelength of 517 nm. The color can change because the reaction between DPPH and one hydrogen atom is released by the compounds contained in the sample to form a yellow non-radical DPPH compound. The measured absorbance was the absorbance of the remaining DPPH solution which did not react to the sample antioxidant compounds [9]. The absorbance was measured after a 30-minute incubation time to allow the DPPH to react with the sample antioxidant compounds [10]. The results of measurement of antioxidant activity in the sample (Figure 2) showed that IC50 water spinach extract was lower than land spinach, which means that the antioxidant activity of water spinach extract was greater than land spinach. The antioxidant activity of water spinach extract (15.828 µg / mL) was also lower than vitamin C.

The determination of the total phenol content of water spinach and land spinach ethanol extract was measured using the Folin Ciocalteu principle. The principle of measuring total phenol content with Folin Ciocalteu reagents is the formation of complex blue compounds. The formed blue color will become thicker, consistent with the concentration of the phenolic ion formed. The determination of total phenol content was carried out by making standard gallic acid curves with several series of concentrations. The gallic acid standard curve was obtained by calculating the linear regression between the concentration of gallic acid as X and the absorbance of gallic acid with Folin Ciocalteu reagent as Y. The resulting standard acid linear regression equation was $y = 0.006 x + 0.181$. The results showed (Figure 3) that the total phenolic content of the water spinach extract (76.96 ± 2.245 mg / g GAE) was higher than land spinach extract (31.37 ± 0.849 mg / g GAE).

The examination of total flavonoid content was carried out by the addition of $AlCl_3$ which will form complex bonds with hydroxyl groups from flavonoids. The determination of total flavonoid content was done by making a quercetin calibration curve with several series of concentrations. The standard curve was made with the same function as the standard gallic acid curve in determining the total phenol content, which is to determine the total flavonoid content. The flavonoid content was calculated using a linear regression equation of the standard quercetin, $y = 0.005 x + 0.19$. The results of the examination

of the total flavonoid content of water spinach ethanol extract was 81.28 ± 5.362 mg / g QE, while the total flavonoid content in the water spinach ethanol extract was 24.56 ± 3.043 mg / g QE (Figure 4)

The group of phenol compounds has antioxidant activity in several ways or mechanisms, including the presence of hydroxyl groups which are hydrogen contributors that can reduce free radicals [11]. One of which is flavonoids wherein almost all groups of flavonoids have antioxidant activity. The high reactivity of the hydroxyl group causes free radicals to become inactive [12]. In I. aquatica extract, water spinach antioxidant activity was higher than land spinach and the total phenol content and flavonoid in water spinach was higher than land spinach so that it can be said that the greater the total phenolic content and total flavonoid extract is the greater the antioxidant activity will become.

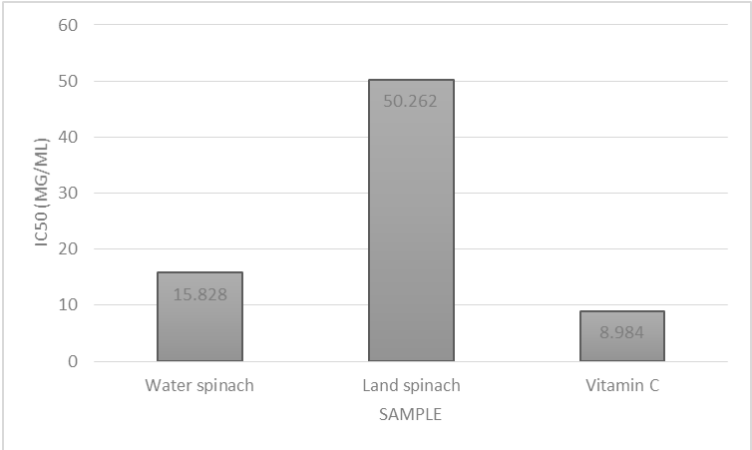


Figure 2. Antioxidant activities of samples.

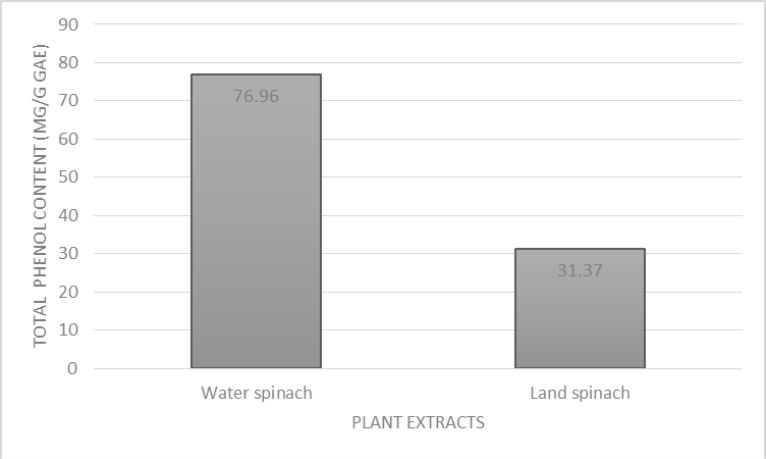


Figure 3. Total phenol content extracts.

The appearance of a 0.2% DPPH reagent was used to identify the presence of antioxidant compounds (yellow spot against a purple background) [13]. While the appearance of $AlCl_3$ reagent was used to detect flavonoid compounds, including the appearance of blue spots under 366 nm UV light [14]. The results of both TLC extract (Figure 5) showed the similarity of the TLC profile of the two extracts. The results of the second TLC extract were yellow spots with Rf 0.73 when sprayed with 0.2% DPPH. Then on the other plates using the same system sprayed with $AlCl_3$, it caused blue spots on uv 366 nm which showed the presence of flavonoids with Rf 0.73. From the results of TLC which is believed to play a role in providing antioxidant activity in water spinach extract and land water spinach are flavonoids because it is seen from the Rf on the sprayed plate that the $AlCl_3$ spray plate has the same Rf value with

a 0.2% DPPH spray plate. This is in accordance with the research conducted by Prasad et al, which has succeeded in isolating flavonoid compound from Ipomoea aquatica, 7-O-β-D-glucopyranosyl-dihydroquercetin-3-O-α-D-glucopyranoside, which has antioxidant activity [15].

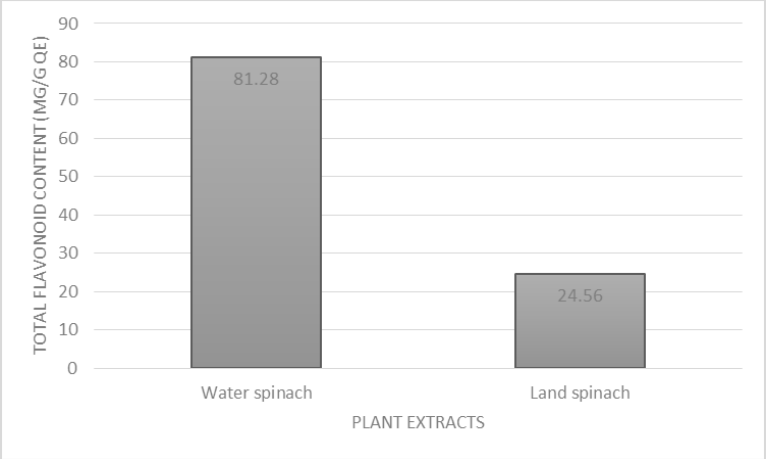
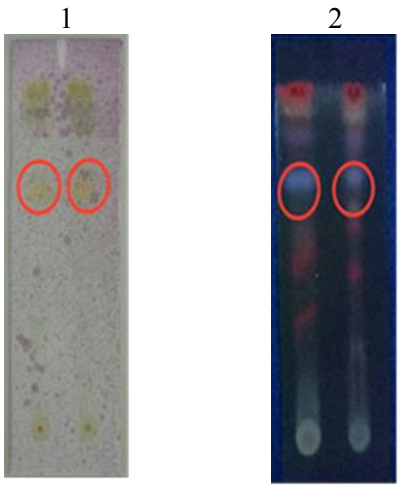


Figure 4. Total flavonoid content extracts.



Notes : (a) = Water spinach extract
(b) = Land spinach extract
1 = On visible light after DPPH 0.2% spray
2 = On UV 366 nm light after $AlCl_3$ spray

Figure 5. TLC results.

4. Conclusion

Water spinach has higher antioxidant activity than land spinach and there was a correlation between antioxidant activity and total phenol / total flavonoid contents. It was supposed that the compound responsible for antioxidant activities in water spinach and land spinach was flavonoid.

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