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Comparative study of antioxidant activity and phenolic content in Indonesian *Amaranthus viridis* L and *Amaranthus Hybridus* L as a cardioprotective candidate

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Abstract

Non communicable diseases are the biggest cause of global, regional and national mortality, and one of which is heart disease. Recently, various diseases have been stated to be the result of free radical compounds which cause oxidative stress, therefore preventing free radicals with phytochemical can be alternative to reduce the risk of heart disease because of Indonesia is country with the second mega biodiversity in the world. Medicinal plant such as *Amaranthus viridis* L and *Amaranthus hybridus L* rich in antioxidants and are known have cardioprotective effect, however less information comparative study of antioxidant activity and phenolic content of Indonesian *Amaranthus viridis* L and *Amaranthus hybridus L* especially in decoction extraction method. This research was descriptive quantitative and aim at determining the content of phenolic compound and antioxidant activity of *Amaranthus viridis* L and *Amaranthus hybridus* L in decoction extraction method. Phenolic compound test was conducted by utilizing Folin-Ciocalteu method; while antioxidant activity test was performed by DPPH method. Both tests used Spectrophotometer UV-Vis. Based on t-test independent sample test *Amaranthus viridis* L have higher total phenolic (5.12± 0.6 mg GAE/g) than of *Amaranthus hybridus L*(2.14 ± 0.6 mg GAE/g). *Amaranthus viridis* L have antioxidant activity higher (87.96 ±0.2%) than of *Amaranthus Hybridus* L (75.1 1.3 %). Conclusion of this research is total phenolic content and antioxidant activity of *Amaranthus viridis* L higher than *Amaranthus hybridus* however both have antioxidant > 50 %.

Keywords: Antioxidant activity; Extraction; Amaranthus; Phenolic content

1. Introduction

An unhealthy diet and sedentary life style have a high chance of increasing diseases such as cardiovascular, which until now is still the number one cause of death in Indonesia (SKI) [1]. Indonesia has GERMAS (a healthy living community movement program) consists of several steps including exercise, eating fruit and vegetables, not smoking, and regular health checks [2], therefore research of the content of compounds in vegetables that are beneficial for health is important. Spinach is one of vegetables that is easy to grow in Indonesia.

Two type of spinach that are commonly consumed are *Amaranthus viridis L* and *Amaranthus hybridus*. *Amaranthus viridis* L is generally cultivated and Amaranthus Hybridus beside being cultivated also grows wild. *Amaranthus viridis* L. and *Amaranthus hybridus* L rich in nutrient and contain phytochemical that are beneficial for health one of which is cardioprotective [3,4, 5]. All part of plant can actually be use for health, however in Indonesia it is common to use the leaves to make juice or cook them.[6] State that extracts from all parts of the plant can be used for health or can act as a nutraceutical and clinical potential or as functional food. *Amaranthus . viridis* L have the highest total phenolic content

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than *Amaranthus tricolor* L and *Amaranthus spinosus* L. Base on HPLC and LCMS its known derivate phenolic of *Amaranthus viridis* L are as follows Quercetin, hyperoside, Rutin, Kaemferol and rutin is generally found in the genus Amaranth [7]

Recently there has been an increase in interest to use compound that decrease the effect of oxidative stress that plays an important role in the development of chronic and degenerative diseases [8]. Antioxidant can be categorized base on their activity such as enzymatic and non-enzymatic antioxidant or in the solubility in water or in lipid antioxidants. Enzymatic antioxidants such as CAT, GSH, SOD, non-enzymatic such as natural antioxidants and synthetic antioxidants. Vitamin C is antioxidant that solubility in water and vitamin E is lipid solubility antioxidant. Bioflavonoid as a natural antioxidants or as non-enzymatic antioxidants [9]. Base on [10] Antioxidants mechanism of flavonoid compound are as follows:

- Exhibit activity base on hydrogen transfer (HAT)
- Single electron transfer via proton transfer (SE-PT)
- Transition metal chelation (TMC)

Mechanism flavonoid through hydrogen transfer (HAT) as shown in figure 1 and single electron transfer via proton transfer (SET-PT) as shown in figure 1

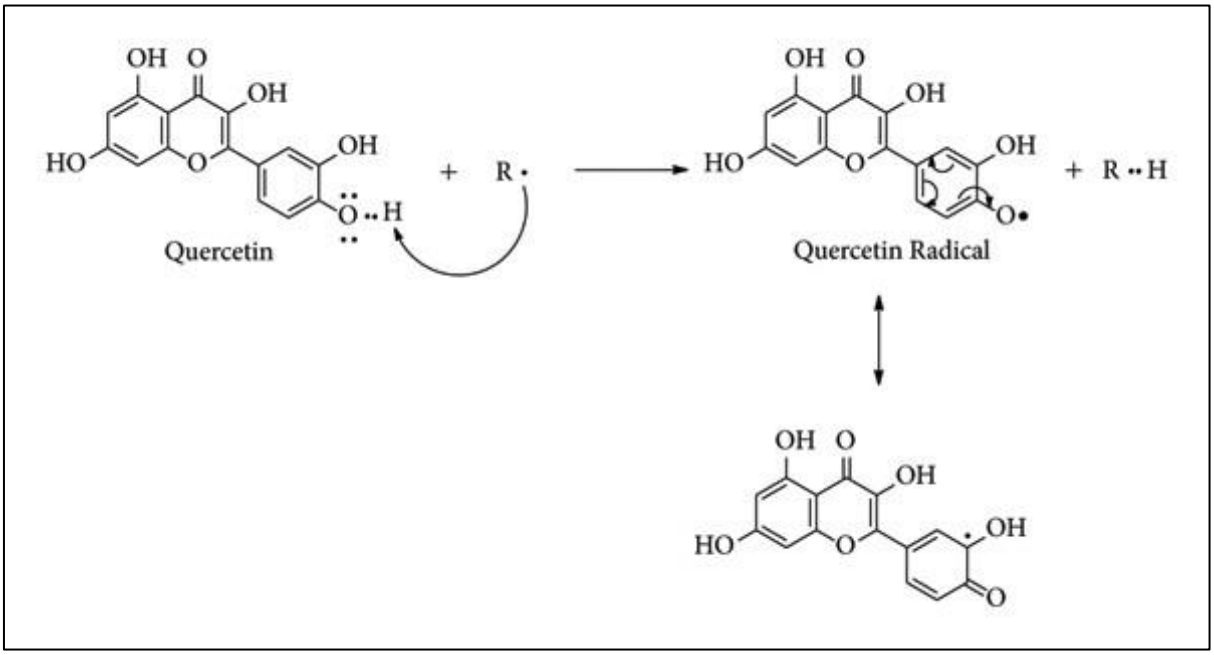

Figure 1 Reaction Quersetin with Radical Species [9]

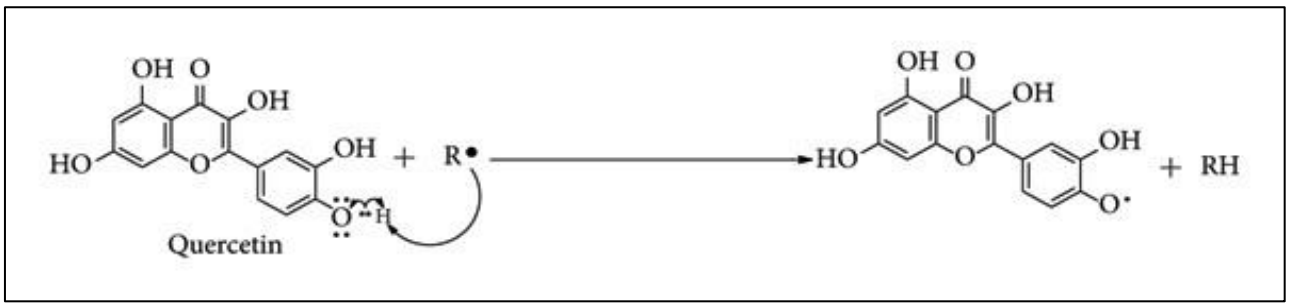

Figure 2 Single Electron Transfer (SET-PT) of Quersetin. [10]

Active compounds in plant can be obtained through extraction method. Extraction is the first step to separate which are expected bioactive component from row material and solvent is the most widely used method [11, 12}. Several steps are required to obtain bioactive compound such as first solvent enter the matrix of the sample and finally the dissolve substance pass in to the solvent [12,13]. Conventional extraction such as maceration, percolation, infusion, decoction often used to extract plants, and maceration which are method that are very often used because of it is very simple and can be used for thermolabile compound but maceration has disadvantage of long extraction time [14]. Previous research with method maceration extraction method in Indonesian *Amaranthus hybridus* L showed that presence of strong antioxidant with IC 50 54.99 µg/mL [15], Base on review in [16] maceration method is used to extract of *Amaranthus viridis* L and the result of the research as follows IC 50 by DPPH 14.25- 83.43µg/ml. Indonesia spinach widely used for vegetables by cooking as a local wisdom so that the decoction extraction method often carried out in everyday habits as a nutraceutical and a herbal medicine. Decoction method is mostly used in Chinese Medicine Clinics but it not recommended for chemicals that degrade quickly in the presence of heat [11] Base on previous research maceration extraction method has been used for study of antioxidant activity of Indonesian Amaranthus hybridus L or *Amaranthus viridis* L but less information about comparative study of *Amaranthus hybridus* L *and Amaranthus viridis* L on antioxidant activity and phenolic content especially in decoction extraction method. Decoction extraction method was chosen because people generally consume spinach by boiling or steaming it, therefore this study aims to determine total phenolic content and antioxidant activity in Based on previous research, this study aims to determine total phenolic content and antioxidant activity in *Amaranthus hybridus* L and *Amaranthus viridis* L in decoction extraction method

2. Material and methods

2.1. Plant Materials

Leave of *Amaranthus hybridus* L and *Amaranthus viridis* L from local market. The obtained Leave of *Amaranthus hybridus* L and *Amaranthus viridis* L were then washed and dray and then put in the blender for grinder. 20 g of *Amaranthus hybridus* L and *Amaranthus viridis* L powder then were added by 400 mL water, then put in boiling water for ± 10 minutes, cooling and filtered for further analysis [17,18,19]

2.2. Reagent and Chemical

Folin-Ciocalteu phenol reagent, Na2CO3, gallic acid from Sigma Aldrich, DPPH (1.1 diphenyl, 2-picrylhydrazyl) from Sigma Aldrich were all prepared in analytical grades.

2.3. Determination of Total Phenolic Content

Determination of base of total phenolic content base [18,20] the steps were: 1 ml sample (1mg/ml) was added into 1 ml Folin-Ciocalteu phenol reagent, and then incubated for 5 minutes. It was then added with 10 ml of Na2CO3 7 %, then with 13 ml of distilled water shaken and incubated in dark environment with the temperature of 23 degree Celsius. The samples were measured by Spectrophotometer UV Vis in the absorbance of 750 nm. The total of phenolic content was then determined by comparing with a standard curve of gallic acid where the total of phenolic acid was indicated by the value of mg GAE/g of dry sample

2.4. Antioxidant Activity Determination

Antioxidant Activity Determination was also conducted based on a modified method [18,20] as follows: Liquid solution of 24 mg DPPH in 100 mL of methanol and stored at a temperature of 20 °C. DPPH can be used by dissolving DPPH in methanol to obtain 0.98 ± 0.02 DPPH absorbance at 517 nm with spectrophotometer. 3 mL DPPH was then added to 100 µL of sample (concentration might vary from 10-500 µg/mL). Mixed until homogeny, incubated in dark room for more or less 15 minutes in room temperature; it could be read by absorbance in spectrophotometer.

3. Result

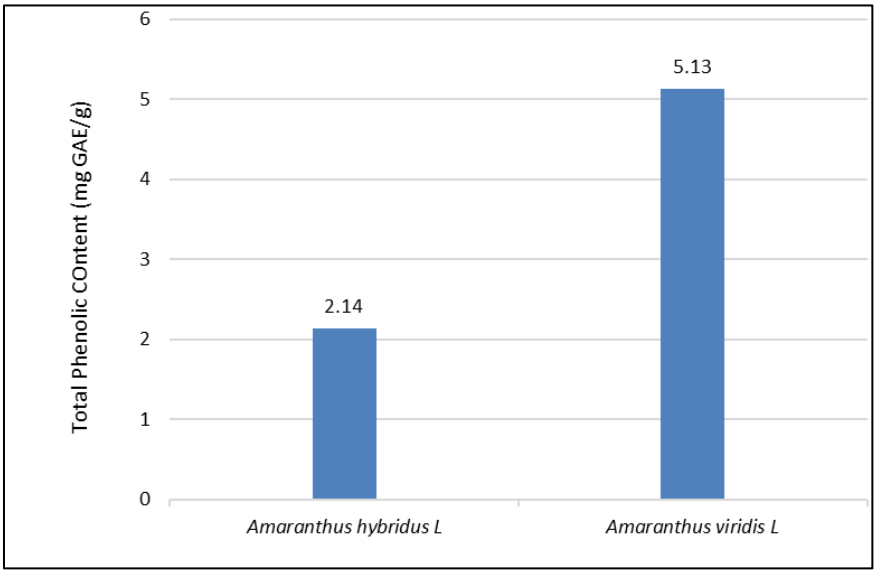

Figure 3 Total phenolic compounds in decoction extracts of *Amaranthus hybridus L* and *Amaranthus viridis L* (All analysis are the means of triplicate measurement ± standard deviation a: expressed as mg Gallic acid (GAE)/ g of dry plant material)

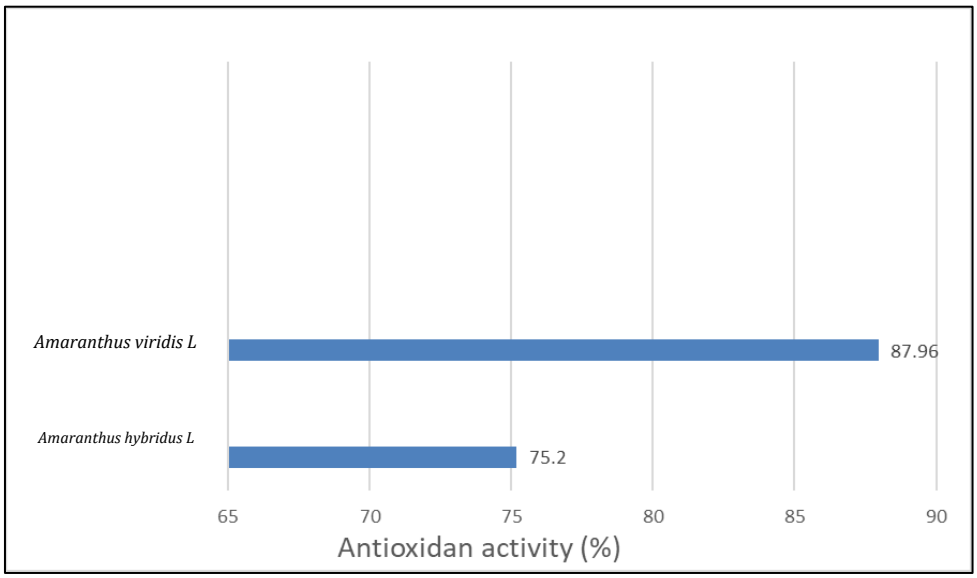

Figure 4 Number of antioxidant activity in decoction extracts of *Amaranthus hybridus L* and *Amaranthus viridis L* (All analysis are the means of triplicate measurement ± standard deviation a: expressed as mg percent of free radical inhibitions)

Total phenolic compound and antioxidant activity in decoction extraction in *Amaranthus hybridus* L and *Amaranthus viridis* L are presented in figure 3 and Figure 4 respectively.

4. Discussion

Plant generally contain phytochemical which is the result of secondary metabolism and phenolic compounds are the most abundant in plant. They contain benzene rings with one or more hydroxyl substituents and ring from simple phenolic molecules to highly polymerized compound [21]. Phenolic compound quantification depends on different parameters such as the chemical nature of compounds, extraction method used, particle size, interfering substances and impurities [22] Decoction the extraction method chosen in this study base on Indonesian local wisdom which generally utilizes spinach by boiling it (decoction). Base on t-test independent sample test *Amaranthus viridis* L have higher total phenolic (5.12± 0.6 mg GAE/g) than of *Amaranthus hybridus* L (2.14 ± 0.6 mg GAE/g). *Amaranthus viridis* L have antioxidant activity higher (87.96 ±0.2%) than of *Amaranthus Hybridus* L (75.1 1.3 %). Base [16] a review in *Amaranthus viridis* L show 1-03 -3.64 GAE g/100g of phenolic contents. The result is not different with in this research even though the extraction method used generally with maceration. Base on the research result decoction method can be alternative for quantification phenolic compound from *Amaranthus viridis L.* Modern extraction method with sonicated in *Amaranthus viridis* L *and Amaranthus hybridis* L obtained total phenol higher than conventional extraction that is 121 mg GAE/g and 124.9 respectively, however the result of this study and in previous research are known Amaranthus both have strong antioxidant.

5. Conclusion

Conclusion of this research is total phenolic content and antioxidant activity of *Amaranthus viridis* L higher than *Amaranthus hybridus* L however both have strong antioxidant.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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