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Antioxidant Activity and Flavonoid Contents of Daun Dewa (*Gynura pseudochina)* in Various Substrates with Humic Acid Treatment

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Antioxidant Activity and Flavonoid Contents of Daun Dewa (*Gynura pseudochina*) in Various Substrates with Humic Acid Treatment

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Abstract. Daun dewa (*Gynura pseudochina*) is a potential producer of a medicinally active compound. Several active compounds contained were steroid, saponin, flavonoid, tannin, and essential oils. The benefit of *G. pseudochina* as medicine includes anticoagulant (prevent blood clot) and blood liquefaction. The use of zeolite and sand as substrates in this study shall give information about the active compound in the plant. Thus, the objectives of this study were to know the effect of substrate towards the active compound and flavonoid contents, also to determine the effective substrate that leads to the higher activity of antioxidant and flavonoid contents in *G. pseudochina*. This study used an experimental method with a two-factor factorial, completely randomized design. The first factor was substrates, consist of zeolite and sand substrates. The second factor was the treatment of humic acid concentrations, 0 g/kg; 4g/kg; 8 g/kg; 12g/kg. There were a total of 24 units of experiment, which consist of 8 combinations of treatments with three replications each. The result showed that both zeolite and sand substrates affected the growth and antioxidant activity of *G. pseudochina*. Zeolite substrate enhanced the antioxidant activity, yet it did not increase the growth. Meanwhile, the sand substrate increased growth. The treatment of 8 g/kg humic acid in a zeolite substrate enhanced the antioxidant activity. The treatment of humic acid in both zeolite and sand substrates could be used to improve the growth and antioxidant activity in *G. pseudochina*.

1. Introduction

Daun dewa (*G. pseudochina*) is known as a medicinal plant and rich in antioxidants. An antioxidant is a substance that can inhibit and prevent the oxidation of other molecules. It may reduce the negative impact of free radicals inside the body. The interesting issue in this study is how the modification of the substrate may improve the biomass production of medicinal plants. The use of humic acid in zeolite and sand substrates is expected to be the reference that informs the utilization of the substrate in improving medicinal plants with various active compounds. Zeolite has a high negative charge so that it can absorb nutrients and release it gradually. Zeolite has a high cation exchange capacity (120 – 180 me/100g) that is used as a cation adsorber, binder, and trader [1].

Moreover, the zeolite may enhance the nutrients absorption by plant roots [2]. Humic acid is amorphous dispersed colloid substances, ranging in color from brown to black, having high molecular weight, and used to improve soil texture, aeration, water permeability, and affinity [3]. Humic acid has a high exchange capacity, thus helping the nutrients movements from soil to plant [4]. This study is expected to provide information to get high-quality medicinal plants with substrate modification

technology. This study will contribute to rural resource development. Antioxidant stabilizes the excess of free radicals in the body. Vegetable plants or medicinal plants produce antioxidant molecules. The natural antioxidant resource is vegetables and fruits.

An antioxidant is a substance that inhibits oxidation reaction by free radicals, which can cause denaturation of unsaturated fatty acid, cell membranes, blood vessels, DNA base, and lipid tissues that lead to illnesses [5]. Plants may have antioxidant activity if they contain a compound that can inhibit free radicals, like phenol and flavonoid. Several studies have been done to observe the relation between phenol, flavonoid, and antioxidant activities. The previous study stated that the spore of sterile sea fern has high flavonoid content and categorized as a strong antioxidant [6]. Some plants that have flavonoid contents and antioxidant activity are noni/mengkudu (*Morinda citrifolia* L.) [7] and bark of Devil's tree/pulai (*Alstonia scholaris* R.br) [8]. Flavonoid content and antioxidant activity have been studied in several plants, such as bay cedar/jati belanda (*Guazuma ulmifolia*), tree bean/kedaung (*Parkia roxburghii*), kumis kucing (*Orthosiphon stamineus*), green cera/sambiloto (*Andrographis paniculata*), arrowleaf sida/sidaguri (*Sida rhombifolia*) and field sowthistle/tempuyung (*Sonchus arvensis*) [9].

An antioxidant is a molecule that inhibits or prevents the oxidation of other molecules. It can be differentiated into endogenous and exogenous antioxidants. Endogenous antioxidant or primary antioxidant is known as an enzymatic antioxidant. Exogenous antioxidants or secondary antioxidants capture free radicals and break the lipid peroxidation chain reaction, thus also known as chain reaction breaker antioxidants. There are complex metabolism reactions in each of the body cells, and among those occur reactions, there is oxygen involved that can be very reactive. The involvement of oxygen in metabolism generates "Reactive Oxygen Species" or ROS, such as hydrogen peroxide (H_2O_2), hydroxyl radical (OH-), and superoxide (O2-). Oxidation produces free radicals that stimulate chain reaction and harm the cells. The free radical compounds inside the cell are very reactive and always try to find an electron pair in order to be stable. The cell damage may cause the cell to be unstable, so that potentially lead to aging and cancer. For instance, flavonoids, terpenoids, and alkaloids are secondary metabolites. Polyphenolic compounds, such as flavonoid and gallic acid, are able to inhibit oxidation reactions via radical scavenging mechanism by donating an electron to the unpaired electron in free radicals so that the radicals decrease [10].

The objectives of this study were to know the effect of substrate to the antioxidant activity and flavonoid contents and to determine the effective substrate to improve antioxidant activity and flavonoid contents in daun dewa (*G. pseudochina*).

2. Methods

This study used an experimental method with a two-factor factorial completely randomized design. The first factor was substrate, consist of two types of substrates, zeolite, and sand. The second factor were humic acid, that consist of four concentrations, 0 g/kg; 4g/kg; 8 g/kg; 12g/kg. There was a total of 24 units of experiments, consisting of 8 combinations of treatments and with three replications each. The observation was conducted after the plants were 12 weeks old.

2.1 Biomass analysis and sample preparation

Biomass is the dry weight of cleaned plants. Dried sample of daun dewa was grinded with mortar and pestle until become powder, and then dried on open air.

2.2 Antioxidant activity test with DPPH method

A total of 100 µL extract (various concentrations) was added with 1.0 mL DPPH 0.4 mM and ethanol until 5.0 mL. The mixture then was shaken well with vortex and being left for 30 minutes. The absorbance of the mixture was measured at 517 nm using a spectrophotometer. Blank absorbance was also measured. The result of antioxidant determination was compared with vitamin E.

The antioxidant activity was calculated based on the formula below:

$$Antioxidant\ activity = \frac{Blank\ abs - Sampel\ abs}{Blank\ abs}\ x\ 100\%$$

2.3 Total flavonoid quantitative analysis

Total flavonoid determination was done by aluminum chloride colorimetric assay [11]. A total of 5 g of dried daun dewa was put into a beaker, added with methanol solvent until it was submerged and left for 48 hours. The mixture then strained to get the extract. The macerations were done again repeatedly with methanol solvent. The methanol extract was collected and concentrated using a rotary evaporator at 60^0C.

A total of 0.5 mL of dissolved extract (1:10 g/ml ethanol) was added with 1.5 mL ethanol; 0.1 mL AlCl3 10%; 0.1 mL natrium acetate 1 M; and 2.8 mL distilled water. The mixture was being left for 30 minutes; then, absorbance was measured at 417 nm. Quercetin was used to make a calibration curve. Total flavonoid content in ethanol extract was expressed as mg quercetin/g dry powder.

3. Results

The observation result of daun dewa biomass, antioxidant activity and flavonoid content showed that substrate had significant effect; the addition of 4 g/kg humic acid on sand substrate showed the highest biomass (Figure 1).

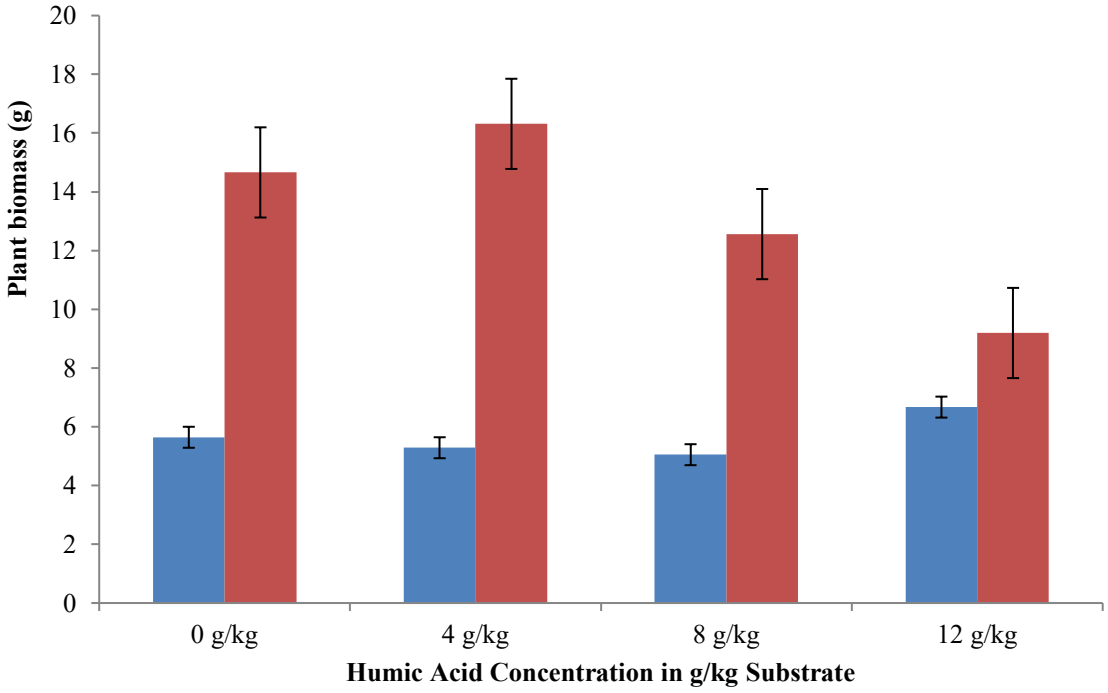

Figure 1. Plant biomass on zeolite and sand substrate with humic acid treatments (blue: zeolit, red: sand)

DPPH Anti-Free Radicals Assay by Spectrophotometry observation result that zeolite substrate caused the increasing of antioxidant activity (Figure 2).

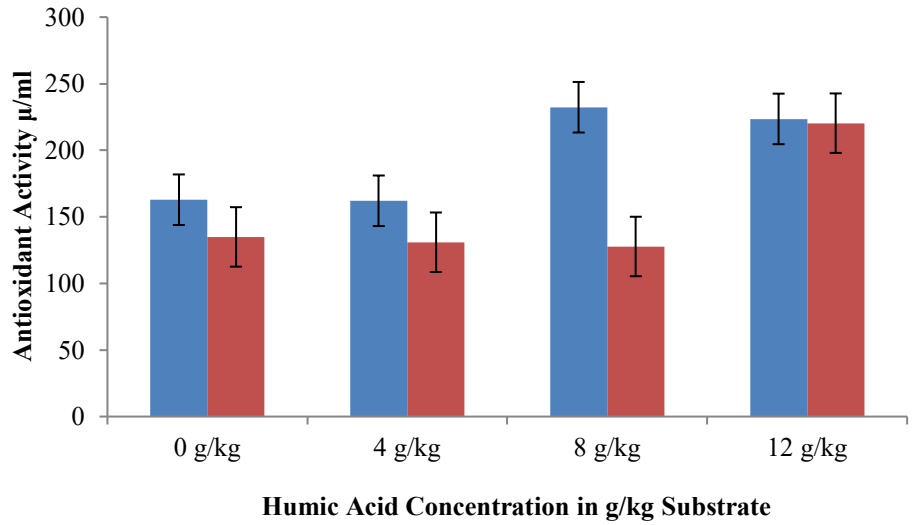

Figure 2. Antioxidant activity of daun dewa in zeolite and sand substrate with humic acid treatments (blue: zeolit, red: sand)

The result of flavonoid analysis showed that zeolite substrate was able to improve flavonoid content; zeolite substrate treated with 8 g/kg humic acid.

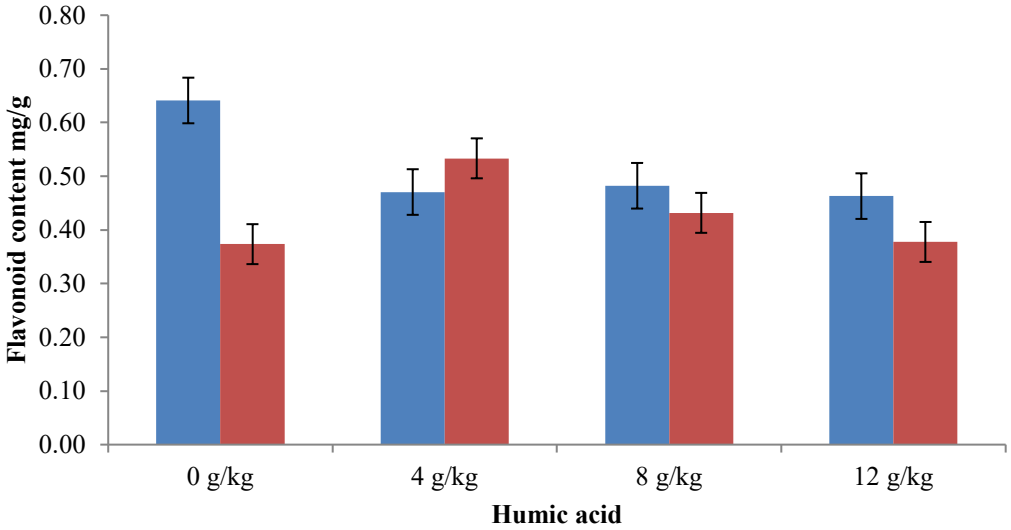

Figure 3. Flavonoid content of daun dewa in zeolite and sand substrate with humic acid treatments (blue: zeolit, red: sand)

4. Discussion

Based on this result, it can be said that daun dewa grew better in sand substrate than the zeolite substrate. The nutrients in the substrate highly affect growth. The availability of nutrients in the sand

substrate was proven better so that the root had optimum nutrient absorption, and the translocation process within the plant occur perfectly.

Growth is the result of the primary metabolism process, while flavonoid is the result of the secondary metabolism process. The flavonoid, as color pigments like anthocyanin and proanthocyanin. Colorless flavonoid compound (ex. flavanol) is useful as an antioxidant. Based on this study result, it can be explained that sand substrate increased growth, while zeolite enhanced secondary metabolites, such as antioxidant activity and flavonoid content. The plants were allegedly under stress so that the metabolism produced secondary metabolite and reactive oxygen species as a by-product. Reactive oxygen species may be reduced and controlled by a compound that has antioxidant activity and antioxidative enzymes.

5. Conclusion

Zeolite and sand were able to affect the growth and antioxidant activity of daun dewa. Zeolite could enhance antioxidant activity but did not affect growth. Meanwhile, the sand substrate could increase growth. Humic acid could be used to improve the antioxidant activity of daun dewa if it was added in the zeolite substrate.

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