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Total Flavonoid, Total Phenolic Content and Antioxidant Activity of *Erechtites valerianifolia* Herb Extracts

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Erechtites valerianifolia **(Link ex Wolf.) Less. ex DC. or "Jonggolan" (Indonesian name), a member of the family Asteraceae, was collected from Meru Betiri Forest, Indonesia. This study investigated the antioxidant activity of *E. valerianifolia* and its total phenolic and total flavonoid content. Methanol, ethyl acetate, dichloromethane, and hexane extracts of *E. valerianifolia* herbs were tested using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals scavenging assay. Antioxidant activity was expressed in IC_{50} values of the n-hexane, dichlormethane, ethyl acetate and methanol extracts (367.8000 ppm, 139.5200 ppm, 911.0540 ppm, and 401.9530 ppm, respectively) with the best antioxidant activity observed in dichloromethane (DCM) extract. Total phenolic content (TPC) of the n-hexane, dichloromethane, ethyl acetate and methanol extracts were 10.6400 ± 0.0338 mg GAE/g; 6.4500 ± 0.0000 mg GAE/g; 6.6900 ± 0.0323 mg GAE/g; and 3.2300 ± 0.0000 mg GAE/g, respectively (with gallic acid as reference). Total flavonoid content (TFC) of the n-hexane, dichlormethane, ethyl acetate and methanol extracts were 1.4000 ± 0.0784 mg QE/g; −2.3900 ± 0.0000 mg QE/g; 0.4400 ± 0.0000 mg QE/g, and 0.1100 ± 0.0000 mg QE/g, respectively (with quercetine as reference). Total flavonoid content of the n-hexane, dichloromethane, ethyl acetate and methanol extracts were 2.3700 ± 0.1180 mg RE/g; −2.7900 ± 0.0000 mg RE/g; 0.9100 ± 0.0000 mg RE/g; and 0.2400 ± 0.0000 mg RE/g, respectively (with rutin as reference). The correlation between total phenolic/total flavonoid content and antioxidant test was less than 50%, indicating no direct/negative correlation between polyphenolic content of the extracts and antioxidant activity.**

Key Words: *Erechtites valerianifolia*, total flavonoid, total phenolic content, antioxidant activity

Abbreviations: DPPH – 1,1-diphenyl-2-picrylhydrazyl, GAE – gallic acid equivalent, QE – quercetine equivalent, SOD – superoxide dismutase, TFC – total flavonoid content, TPC – total phenolic conent

INTRODUCTION

Free radicals are often associated with physiological events such as inflammation, aging, and cancer (Bhaigyabati et al. 2011). Free radicals are atoms or molecules that have unpaired electrons that are formed as intermediates in an organic reaction through the homolysis of covalent bonds. Due to their reactivity, free radical compounds are able to attack the cellular components such as lipid compounds, lipoproteins, proteins, carbohydrates, RNA, and DNA. The next consequence of free radical reactivity is the occurrence of structural damage or cell function. In the human body, free radicals are formed continuously, both in the form of normal cell metabolic processes, inflammation, malnutrition, and due to the response to external influences, such as environmental pollution, ultraviolet (UV), cigarette smoke and others (Ebrahimzadeh et al. 2008; Ko et al. 1998). Natural free radicals produced in the human body that compromise the immune system can be inhibited by antioxidants. However, as a person ages, the body cells degenerate and affect the immune response in the body. As a result, free radicals formed in the body are not balanced by the production of antioxidants. Therefore, the body needs an exogenous antioxidant that can be obtained from fruits and vegetables. Adequate antioxidant consumption is reported to reduce the incidence of degenerative diseases such as liver disease, cancer, atherosclerosis, and osteoporosis, among others. Use of synthetic antioxidants such as butylated

hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butylhydroquinone (TBHQ) has been restricted to food products because these antioxidants are considered to have a carcinogenic effect (Mongkolsilp et al. 2004; Roberfroid and Calderon 1995). The current study was conducted to investigate the antioxidant properties and total phenolic and total flavonoid content of *Erechtites valerianifolia* (Link ex Wolf.) Less. ex DC. extracts using different solvents.

E. valerianifolia (locally named "Jonggolan"), a member of the family Asteraceae, is an herb that grows up to 1 m. It was collected from the Meru Betiri Forest in East Java, Indonesia and is normally consumed as a vegetable in the local communities (Hernandez et al. 2013; Umiyah 2011). This plant is commonly utilized to treat fever, diarrhea, tonsillitis, wounds, and eczema (Umiyah 2011). However, scientific information and research on *E. valerianifolia* is limited and not widely explored.

Antioxidants are molecules that terminate oxidative reaction via a wide range of mechanisms. The mechanisms could be radical scavenging reactions, as in the action of tocopherols; complexation of transition metals, linked in peroxidebond decomposition; and initiation of lipoperoxidative reactions, as in the case of the iron chelator deferoxaminemesylate; and stabilization of peroxide derivatives as in the case of vitamin C (Mongkolsilp et al. 2004; Roberfroid and Calderon 1995).

MATERIALS AND METHODS

Plant Material

Samples of herbs of *Erechtites valerianifolia* (Link ex Wolf.) Less. ex DC. were collected from Meru Betiri Forest, Indonesia from January to June, 2016 and stored at room temperature. The plant was identified by the Indonesian Institute of Sciences (LIPI), Indonesia.

Extraction

E. valerianifolia herbs (30 kg) freshly obtained from Meru Betiri were dried at 50 °C in the oven to produce dry powder. The dry powder was extracted using methanol, ethyl acetate, dichloromethane, and n-hexane by percolation method and evaporated using a rotary evaporator (Riyanto 2003). The crude extracts were tested for total flavonoid content, total phenolic content and antioxidant using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay.

DPPH Free Radical Scavenging Activity Assay

The antioxidant assay was performed on scavenged effect of DPPH, a stable free radical compound with red color. If free radicals have been scavenged, DPPH will change in color intensity to yellow. This test uses a chemical reaction to show sample free radical scavenging activity, which changes 1,1-diphenyl-2-picrylhydrazyl to 1,1-diphenyl-2-picrylhydrazine. Four extracts were diluted with methanol to obtain concentrations of 5, 10, 25, 50, 100, 250, 500, and 1000 μg/mL. The samples were mixed with methanol solution of 300 μM DPPH (Sigma) and incubated at 37°C for 60 min. The absorption was measured at 515 nm. Ascorbic acid was used as positive control (Molyneux 2004; Shimada et al. 1992).

Inhibition percentage (free radical scavenging activity) was measured by % inhibition of *E. valerianifolia* extracts as follows:

$$[1-(B/A)] \times 100\%$$

where A is absorbance in the absence of sample and B is absorbance in the presence of sample.

IC_{50} value was calculated based on the concentration of sample DPPH free radical scavenging activity at 50%.

Total Phenolic Content

Analysis of total phenolic content was tested by Folin-Ciocalteu method at 765 nm absorbance. The standard gallic acid was made at a concentration of 2–100 ppm. Samples (10 mg) were dissolved in 0.5 mL methanol, 2.5 mL of aquadest and 2.5 mL of 50% Folin-Ciocalteau reagent. The mixture was allowed to stand for 5 min and added with 2 mL of 7.5% Na_2CO_3 and mixed. Following incubation for 15 min at 45 °C, absorbance was measured at 765 nm using a UV-VIS spectrophotometer (Pourmorad et al. 2006; Singleton and Rossi 1965). Total phenolic content (TPC) was calculated using the formula:

$$TPC = C.V.fpg$$

where C is phenolic concentration (value x), V is the volume of extract (mL), *fp* is dilution factor, and g is weight of the sample.

Total Flavonoid Content

Total flavonoid content (TFC) was measured by aluminum trichloride methods using quercetin and rutin as reference compounds. Extracts (125 μL) were added in 75 μL of 5 % $NaNO_2$ solution. The mixture was allowed to stand for 6 min and added with 150 μL of aluminum chloride (10%), incubated for 5 min, and adjusted to 750 μL of NaOH (1 M) for a final volume at 2500 μL with distilled water. Following incubation for 15 min, absorbance was measured at 510 nm. The color of the mixture changed to pink. TFC was expressed as the equivalent of quercetin and rutin 100 g^{-1} using the formula $y = mx + c$ (Zhishen et al. 1999).

RESULTS

The n-hexane, dichloromethane, ethyl acetate and methanol extracts were as much as 42.9 g (1:43% w/w), 21.4 g (0.71% w/w), 29 g (0.97% w/w) and 135.8 g (3:53% w/w), respectively. The highest amount is found in the polar methanolic extract. DPPH free radical scavenging assay of the extracts is shown in Table 1.

The total phenolic content of each extract is expressed as the equivalent of gallic acid or gallic acid equivalent (GAE). GAE is a general reference for measuring the amount of phenolic compounds present in a material (Molyneux 2004; Mongkolsilp et al. 2004). The total flavonoid content of each extract is expressed as the equivalent of quercetin/rutin equivalent (QE/RE). QE/RE is a general reference for measuring the amount of flavonoid compounds present in a material. Total phenolic content (gallic acid as reference) and total flavonoid content (quercetin and rutin as reference) of *E. valerianifolia* extracts are shown in Tables 3 and 4.

Relationship between total flavonoid content, total phenolic content, and antioxidant activity (IC_{50}) in this study is shown in Figures 2, 3, and 4.

Table 1. Antioxidant activity (DPPH free radical scavenging test) of *E. valerianifolia* extracts.

No.	Extract	IC_{50} (µg/mL)
1	*n*-hexane	367.8000
2	Dichloromethane	139.5200
3	Ethyl acetate	911.0540
4	Methanol	401.9530

Table 2. Total phenolic content of *E. valerianifolia* extracts (gallic acid as reference).

No.	Extract	% GAE ± SD
1	*n*-hexane	10.640 ± 0.0338
2	Dichloromethane	6.4500 ± 0.0000
3	Ethyl acetate	6.6900 ± 0.0323
4	Methanol	3.2300 ± 0.0000

Table 3. Total flavonoid content of *E. valerianifolia* extracts (quercetin as reference).

No.	Extract	% QE ± SD
1	*n*-hexane	1.4000 ± 0.0784
2	Dichloromethane	−2.3900 ± 0.0000
3	Ethyl acetate	0.4400 ± 0.0000
4	Methanol	0.1100 ± 0.0000

Table 4. Total flavonoid content of *E. valerianifolia* extracts (rutin as reference).

No.	Extract	% RE ± SD
1	*n*-hexane	2.3700 ± 0.1180
2	Dichloromethane	−2.7900 ± 0.0000
3	Ethyl acetate	0.9100 ± 0.0000
4	Methanol	0.2400 ± 0.0000

DISCUSSION

The antioxidants or radical scavengers may be categorized as a) natural and synthetic enzymatic antioxidants or b) natural and synthetic nonenzymatic antioxidants. The former includes superoxide dimutases (SOD), catalases, copper coordination compounds as SOD -like products, seleno derivatives with glutathione-like peroxidase activity, while the latter includes vitamin E and its homologues, ascorbic acid, carotenoids, glutathione and derivatives, flavonoids, lazaroids, and captodative olefins (Roberfroid and Calderon 1995).

Based on the result of the antioxidant acitivity test, the IC_{50} of the dichloromethane extract of *E. valerianifolia* was 139.5200 mg/mL and it has the best significant antioxidant activity against DPPH free radical scavenging among the other extracts, which is classified as middle activity (Table 1). Antioxidant activity is classified as either very strong (<50 ppm), strong (50–100 ppm), medium (101–150 ppm), or weak 151–200 ppm (Blois 1958). The activity of the four extracts of *E. valerianifolia* is lower than that of ascorbic acid (21.2250 µg/mL IC_{50}) that means very good antioxidant activity (Kumawat et al. 2012). The antioxidant of dichlorometane extracts was less active than that of the positive control ascorbic acid which was used in this experiment.

Antioxidants are electron-donating compounds (electron donors) or reductants. These compounds have a low molecular weight, but they are able to decrease oxidation reactions by inhibiting the production of radicals. The DPPH method is easy, effective, and practical for screening the radical activity of several compounds (Molyneux 2004). This activity was measured by counting the color intensity reduction of DPPH equivalent to DPPH concentration reduction. The antioxidant reaction is produced by the reaction of diphenyl-picrylhydrazyl molecule and hydrogen atom which was released to change the diphenyl-picrylhydrazine compound and change the color of DPPH from purple to yellow (Shimada et al. 1992).

The hexane extract had the highest total phenolic content of 10.640 ± 0.0338 mg GAE/g (Table 2). The

Fig. 1. *Erechtites valerianifolia* (Link ex Wolf.) Less. ex DC.

hexane extract also had the highest total flavonoid content of 2.3700 ± 0.1180 mg RE/g (rutin as reference) and 1.4000 ± 0.0784 mg QE/g (quercetin as reference) (Tables 3 and 4).

The statistical result using one-way ANOVA followed by LSD showed a significant difference between total phenolic content (with gallic acid as reference) at α = 0.000, and total flavonoid content (rutin and quercetin as reference) at α = 0.0000, of each extract ($\alpha \leq 0.05$). Further one-way ANOVA followed by LSD test results on total phenol content of each extract showed that the hexane extract gave a significant difference to the dichloromethane, ethyl acetate, and methanol extracts. The decreasing order of the total phenolic content in the extracts was: hexane extract, ethyl acetate extract, dichloromethane extract, and methanol extract, respectively. The order of the total flavonoid content (rutin and quercetin as reference) in the extracts was: hexane extract, ethyl acetate extract, methanol extract, and dichlormethane extract, respectively.

The solubility of the phenolic compound depends on the solvent used. The polyphenol component has a wide spectrum with different solubility properties, making the procedure difficult for plant extraction using suitable solvents. In this study, hexane extracts had the highest total phenolic and flavonoid content among the other extracts (Koffi et al. 2010; Naczk and Shahidi 2004; Nurhanan and Wan Rosli 2012).

There was a very strong/positive correlation between total phenolic content (mg GAE/g sample) and antioxidant activity (IC_{50}) based on several studies (Agbo et al. 2015; Banji et al. 2018; Rahman et al. 2012; Shirazi et al. 2014). Some studies reported that the total polyphenolic content (total phenolic and flavonoid content) of many plants has a very strong correlation with antioxidant activity (having a correlation value of greater 80%), but in this study, there was a contrasting result because the extracts might consist of various components possibly encouraging a mechanism of antagonism compared to single compounds (Hernandez et al. 2013;

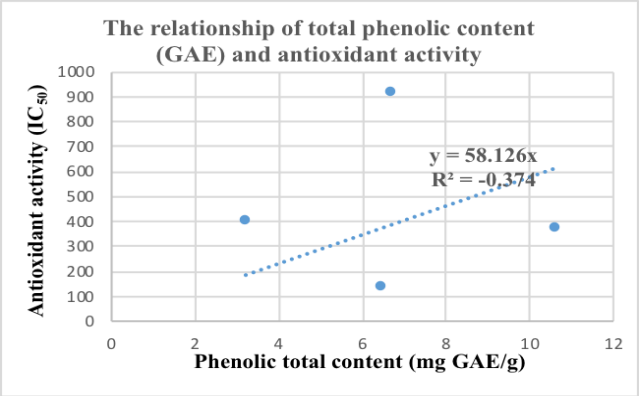

Fig. 2. Total phenolic content (GAE) (gallic acid as reference) and antioxidant activity of *Erechtites valerianifolia* herb extracts.

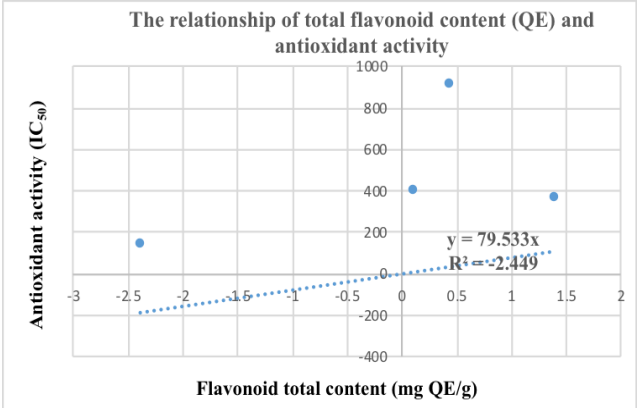

Fig. 3. Total flavonoid content (QE) (quercetin as reference) and antioxidant activity of *Erechtites valerianifolia* herb extracts.

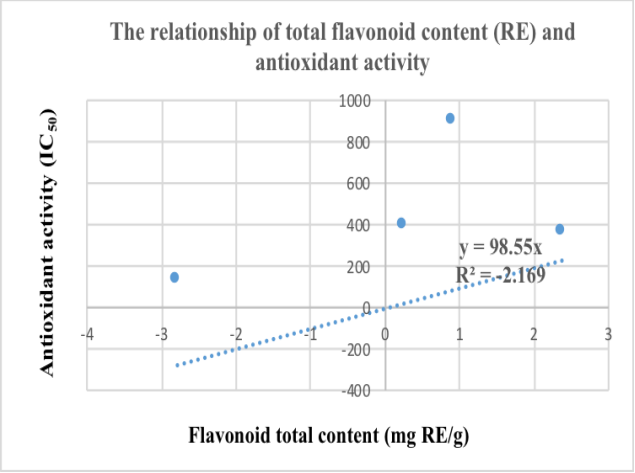

Fig. 4. Total flavonoid content (RE) (rutin as reference) and antioxidant activity of *Erechtites valerianifolia* herb extracts.

Lorenzo et al. 2001; Manske and Holmes 1950). The results showed no correlation or a negative correlation between total phenolic/total flavonoid content and antioxidant activity (Ying et al. 2013). The relationship

between total flavonoid/ total phenolic content and antioxidant activity in this study can be seen in Figures 2, 3, and 4. Correlation between total phenolic/ total flavonoid content and antioxidant test was less than 50% that indicated no direct/negative correlation between polyphenolic content of the extracts and antioxidant activity.

CONCLUSION

The hexane extract has high total phenolic/total flavonoid content but it is not correlated with antioxidant activity. The order of the total phenolic content in the extract is the hexane extract, ethyl acetate extract, dichlormethane extract, and methanol extract, respectively. The order of the total flavonoid content (rutin and quercetin as reference) in the extract is hexane extract, ethyl acetate extract, methanol extract, and dichloromethane extract, respectively. IC_{50} of the dichloromethane extract (139.520 mg/mL) indicates that it has the best significant antioxidant activity among the other extracts, and is classified as middle activity. Correlation between total phenolic/total flavonoid content and antioxidant test was less than 50% that indicated no direct/negative correlation between polyphenolic content of the extracts and antioxidant activity.

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