

Total phenolic content and antioxidant activities of binahong (Anredera cordifolia.)

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ABSTRACT

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Background: Binahong plants are reportedly containing polyphenols, flavonoids, and steroid compounds. The content of polyphenols and flavonoids plays an important role as antioxidants. Research using the method of FRAP (Ferric Reducing Ability of Power) indicates that the antioxidant content of the binahongleaves extract is 3.68 mmol/100 g in dried leaves. This study offers new proof of antioxidant activity of all the aerial part of binahong.

Objective: This research aims to determine the total phenolic content from binahong extract and its ability as free radical scavenger using DPPH method.

Methods: The plant used in this research is every part of plants on the ground. The binahong extract is obtained by a maceration method with various solvents (hexane, chloroform, and methanol). Total phenolic content in the extract is spectrophotometrically determined with the Folin Ciocalteu reagent. Antioxidant activity assay is performed in vitro by the method of DPPH, with Gallic acid as a comparator. ES_{50} parameter is determined from the linear regression equation between the concentration of extracts and % scavenging.

Results: The results showed that the total phenolic content of hexane, chloroform and methanol extract of Binahong was 8.54 ± 0.49 GAE mg/g, 17.30 ± 0.47 GAE mg/g and 32.5 ± 1.11 GAE mg/g. Hexane extract, chloroform extract and methanol extract of Binahong have a free radical scavenger activity. The value of ES_{50} extracts of hexane, chloroform, methanol, and successive acid errors is 58.601 ± 2.533 μ g/ml, 446.219 ± 2.268 μ g/ml, 237.683 ± 13.373 μ g/ml and 2.058 ± 0.002 μ g/ml.

Conclusion: The capability of the three of binahong extract as a free radicals DPPH scavenger were weaker than gallic acid.

Latar Belakang: Tanaman binahong dilaporkan mengandung senyawa polifenol, flavonoid, dan steroid. Kandungan polifenol dan flavonoid memiliki peranan penting sebagai antioksidan. Penelitian dengan menggunakan metode FRAP (Ferric Reducing Ability of Power) menunjukkan bahwa aktifitas antioksidan dari daun binahong .3,68 mmol/100 g pada simplisia daun kering. Penelitian ini akan mencari sebuah bukti baru aktivitas antioksidan semua bagian binahong yang berada di atas permukaan tanah.

Tujuan: Penelitian ini bertujuan untuk mengetahui seberapa besar kandungan fenolik total dari ekstrak binahong serta mengetahui kemampuan ekstrak binahong sebagai penangkap radikal bebas dengan menggunakan metode DPPH.

Metode: Bagian tanaman binahong yang digunakan pada penelitian ini adalah seluruh bagian tanaman yang ada diatas tanah. Ekstrak binahong diperoleh dengan cara maserasi bertingkat dengan menggunakan pelarutheksan, kloroform dan metanol. Kandungan fenolik total dalam ekstrak ditentukan secara

spektrofotometri dengan pereaksi Folin Ciocalteu. Uji penangkapan radikal bebas dilakukan secara *in vitro* dengan metode DPPH, dengan asam galat sebagai pembanding. Harga ES_{50} ditentukan dari persamaan regresi linear antara konsentrasi ekstrak dan % penangkapan.

Hasil: Hasil penelitian menunjukkan bahwa Kandungan fenolik total ekstrak heksan, kloroform dan metanol berturut-turut adalah $8,54 \pm 0,49$ GAE mg/g, $17,30 \pm 0,47$ GAE mg/g dan $32,5 \pm 1,11$ GAE mg/g. Ekstrak heksan, ekstrak kloroform ekstrak metanol binahong mempunyai kemampuan sebagai penangkap radikal bebas. Nilai ES_{50} ekstrak heksan, kloroform, metanol dan asam galat berturut-turut adalah $583,601 \pm 2,533$ μ g/ml, $446,219 \pm 2,268$ μ g/ml, $237,683 \pm 13,373$ μ g/ml dan $2,058 \pm 0,002$ μ g/ml.

Kesimpulan: Potensi ketiga ekstrak binahong sebagai penangkap radikal bebas DPPH lebih lemah dibanding asam galat.

INTRODUCTION

Free radicals may play a role in various degenerative diseases such as cancer, atherosclerosis, ageing, inflammation, diabetes, hair loss, and Parkinson's.^{1,2} The effects of free radicals can be mitigated if the body has enough antioxidants and by regulating diet, such as by consuming foods and drinks that contain a high level of antioxidants. Therefore, people are starting to change their lifestyles by using nature-sourced materials which are believed to be healthier and safer for the body.³

The human body is capable of producing limited amounts of endogenous antioxidants. However, the limited amount of antioxidants is not able to fight the increasing free radicals inside the body. Therefore, exogenous antioxidants are needed to fight the increase in free radicals.⁴

Antioxidants can eliminate, clean (scavenging), resist formation, or negate the effects of free radicals.^{4,5} There are 2 types of antioxidants; synthetic and natural antioxidants. Natural antioxidants have higher effectiveness but lower toxic properties compared to synthetic antioxidants.⁶ Therefore, the food and medicine industry has shifted to develop natural antioxidants.

Binahong (*Anredera cordifolia*) or Tenore Steen is found in South America and one of the

interesting plants to study. Growing evidence suggests that the binahong plant has clinical potentials. Binahong leaves and rhizoma are known to be useful as a healing agent for scars, typhus, inflammation of the intestine, lowering uric acid, dysentery, and haemorrhoid. Binahong leaves contain triterpenoid saponins, flavonoid compounds, and ursolic acid.^{7,8}

Binahong plants need to be explored to obtain its active compounds of the antioxidants property. It is necessary to screen the antioxidant potential with some extraction methods and compound with various levels of polarity. Previous studies have shown that the ethanol extract of binahong leaves has antioxidant activity.^{9,10} Accordingly, this study using all parts of plants above ground with hexane (non-polar), chloroform (semipolar), and methanol (polar) extracts to obtain extracts tested for free radical scavenging activity. The results of this study suggest that the use of binahong can be optimized, especially in the health sector.

METHODS

Binahong plants were obtained from the Bantul Pleret area of Yogyakarta. Determination of plants was carried out in the Laboratory of Pharmaceutical Biology, Gadjah Mada University.

Extraction procedures

The process of extraction in this study was done by using a gradual maceration method. 20 g of dried powder from all parts of the Binahong plant above ground level was extracted with gradually organic solvents from non polar to polar organic solvents (hexane, chloroform and methanol). After maceration periods, the soaked powder-solvent mixtures were filtered by using Buchner funnel. The residue left in the funnel was re-extracted twice follow the same procedure and filtered. Each extract was concentrated and dried by by using rotary evaporator to obtain a thick extract.

Antioxidant activity

The free radical scavenging activity of hexane, chloroform, and methanol extracts of Binahong

was measured using DPPH assay. The extracts were added into tubes containing 0,15 mM methanolic DPPH solution. The reduction of absorbance at 516 nm was measured twice, before and 30 minutes after extracts were added into tubes.

Antioxidant activity is calculated by the following equation¹¹:

$$\% \text{ Capture} = [(A_{\text{control}} - A_{\text{test}} / A_{\text{control}}) \times 100\%]$$

Note: A_{control} = Control absorbance

A_{test} = Control absorbance test sample

The concentration of extract needed to reduce absorbance by 50% from the initial state (ES_{50}) was determined by the regression equation between the percentage of capture and concentration. The smaller the value of ES_{50} means the greater the antioxidant power of the compound. As a comparison for this method used gallic acid, and all tests were replicated 3 times.

Determination of total phenolic binahong extract

Determination of Total Phenolic Content (TPC) was performed by visible spectrophotometry with Folin Ciocalteu reagent. This method is based on the formation of a blue complex that is read at a wavelength of 746 nm. Gallic acid was used as a comparison. The third TPC value of the extract was expressed in Gallic Acid Equivalent (GAE) mg / g extract. A total of 10.00 mg of each

type of extract was dissolved in methanol to amount to 10.0 ml. Three hundred microliters of the extract solution were added to 1.5 ml of the Folin-Ciocalteu reagent (which had been diluted 10 times). After settling for 3 minutes, the solution is added with 1.2 ml of 7.5% Na_2CO_3 . The absorbance will be read at 765 nm after 1 hour. As a comparison used Galat acid (12) All tests were replicated 3 times. ES_{50} data and TPC content were statistically analyzed, namely, Anova continued with LSD with a 95% confidence level using the SPSS program.

RESULTS

The extract yield obtained from each solvent was 1.2% Hexane; chloroform 4.2%; and 6.3% methanol respectively. The ability of compounds as free radical scavengers is shown by a decrease in absorbance of DPPH at λ 516.4 nm. The results of antioxidant activity tests by DPPH method as shown in Table 1 and Figure 1 below:

Table 1. The results of antioxidant activity tests by DPPH

Sample	$ES_{50} \pm SD$ ($\mu\text{g/ml}$)
Gallic Acid	2.058 \pm 0.002*
Methanol extract	237.68 \pm 13.373*
Chloroform extract	446.219 \pm 2.268*
Hexane extract	583.601 \pm 2.533*

Note: n=3 *p<0.05

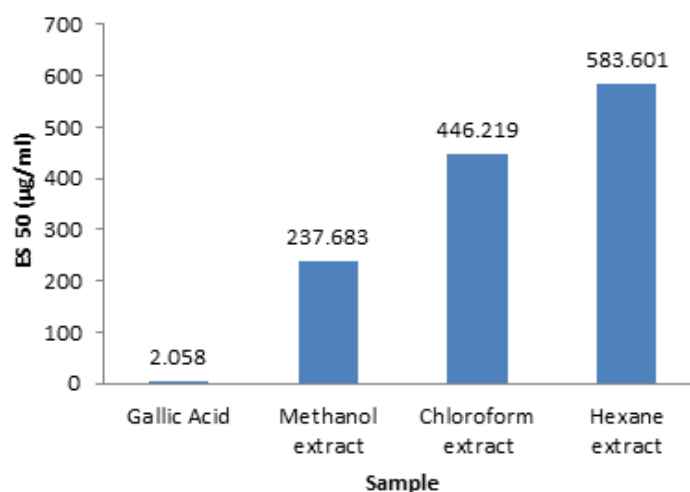


Figure 1. Comparison of ES_{50} between Gallic acid and binahong extract

The results of determining the extract TPC content are presented in Figure 2.

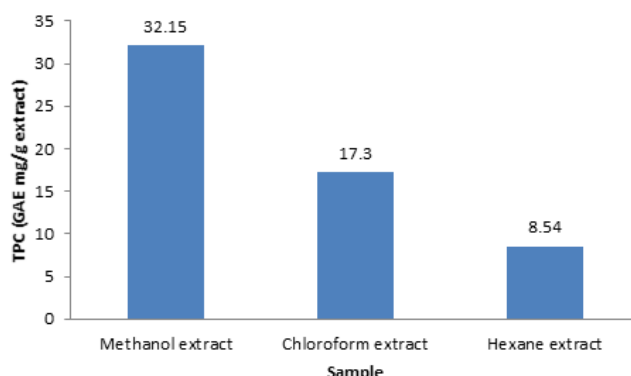


Figure 2. Profile of phenolic total Binahong extract

DISCUSSION

Figure 1 shows that methanol extract has the most powerful ability as the free radical scavenger compared to chloroform extract and hexane extract. Based on the ES_{50} value of methanol extract, the smaller the ES_{50} value of a substance, the greater its ability as a free radical scavenger. The results of this study are in line with another research which states that the methanol extract of binahong leaves has higher free radical scavenging activity than hexane extract.¹³ However, the ability of binahong extract as a free radical scavenger is still weaker than gallic acid. According to Blois criteria, those three extracts are in the inactive category because they have an ES_{50} value of more than $200 \mu\text{g} / \text{ml}$.¹⁴ Different from the previous research, this study uses not only the leaves but also all parts of the plant above ground level (both stems, young leaves, and old leaves).¹⁵ Moreover, we studied the content of flavonoids and the total antioxidant capacity of the ethanol extract of binahong leaves. Antioxidant test methods used were also different, in which this study uses the DPPH method while Selawa et al. using the FRAP method.

The results of TPC determination showed that methanol extract has the highest TPC content followed by chloroform extract and hexane extract. This shows that more polar solvents tend to find polyphenol compounds.

Based on these data, it is seen that there is a correlation between TPC content and its ability as a free radical scavenger with a correlation coefficient (R) value of 0.994. Accordingly, recent research states that the TPC content, as well as its antioxidant potential of lampes leaf extract, is greater than the seed extract.¹⁶ This is supported by Büyüktuncel et al which suggests that antioxidant activity is strongly correlated with total phenolic content in red wine.¹⁷

CONCLUSION

The antioxidant activity of methanol extract was higher compared to chloroform extract and hexane extract. The total phenolic level of total methanol extracts was higher compared to chloroform extract and hexane extract.

CONFLICT OF INTEREST

None declare.

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