

Standardization and α -Glucosidase Inhibitory of Extract from *Anredera Cordifolia* Leaves

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Abstract

Medical plants traditionally used by the people of Indonesia to overcome various diseases. *Anredera cordifolia* leaves empirically used as an anti-diabetic. This research aims to investigate standardizations of extract from *Anredera cordifolia* and to conduct in vitro assay of α -glucosidase enzyme inhibitory effects of the extracts. Extraction of leaf powder was conducted with maceration by using 70% ethanol as solvent, followed by extract quality determination that involved extract specific parameters, such as organoleptic, ethanol soluble content, water soluble content and extract nonspecific parameters, such as loss on drying, water content, total ash content, acid not soluble ash, ethanol residual content, heavy metals contaminant, and microbial contaminant. The research used in vitro assay for α -glucosidase enzyme inhibitory effects. Organoleptic results of the extract showed the extract has a thick consistency, blackish green colour, taste rather chelates and hasn't a specific aromatic odor. Phytochemical screening result indicate the presence of the simplicia and ethanol extract from *Anredera cordifolia* leaves contain flavonoid, saponin, steroid/triterpenoid, and coumarins. The water soluble content 72.1414%, ethanol soluble content 69.6382%, loss on drying 12.8366%, water content 8.4567%, total ash content 1.2154%, acid not soluble ash 0.0407, ethanol residual content 0.2540%, Pb and Cd metals contaminant are 0.2326 mg/kg and 0.0024 mg/kg, microbial contamination of total plate number showed 0.4550×10^3 colony/g, molds and yeasts number 0.1067×10^3 colony/g and had α -glucosidase enzyme inhibitory effects, with IC_{50} values of 47.8855 μ g/mL. Standardized extract of *Anredera cordifolia* have α -glucosidase enzyme inhibitory activity.

Keywords: *Anredera cordifolia*, binahong leaves, antidiabetic, α -glucosidase

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Introduction

Diabetes Mellitus (DM) is a major health problem in the world, including Indonesia. Besides using anti diabetic drugs, traditional medicine is still an alternative for the treatment of diabetes mellitus. Traditional medicine is the ingredient or ingredients in the form of plant material, animal ingredients, mineral materials, galenic, or mixtures of these materials that have historically been used for treatment, and may be applied according with the norms in the society.

Some medicinal plants have been scientifically studied the mechanisms of the anti-diabetic activity, including activity as α -glucosidase inhibitor, induces insulin secretion and improve insulin function. One of the plants commonly used in the traditional medicine in Indonesia as anti-diabetic is binahong (*A. cordifolia*). Elin Yulinah Sukandar conduct research about effect of

methanol extract *A. Cordifolia* leaves on blood sugar in diabetes mellitus model mice. The research showed that the methanol extract of *A. cordifolia* leaves at a dose of 50, 100, 200 mg/kg bw could lower blood glucose level significantly different compared to control group ($p < 0.05$) either on day 7 or on day 14 and increased the number and repaired the damage of β -pancreas cell.

The study began with extraction process using 70% ethanol solvent, followed by phytochemical screening test for the extracts, by referring to extract standardization by using some quality parameters. Standardization is the process of delivering product with a specified minimum level of one or more phyto constituent, where we can make sure about the quality of the product; broadly, it covers the qualitative and quantitative part of analysis. Standardization of extraction for herbal plants in Indonesia is an

important phase in the development of native medicine in Indonesia. Standardization is necessary to obtain various raw materials and eventually to ensure optimum pharmacological effects of the plant. In the development of traditional medicine, particularly from herbal plants, which have been traditionally used as anti-diabetic, extraction of *binahong* leaves was conducted in reference to extract standardization, followed by the test for α -glucosidase enzyme inhibitory activity.

Methodology

Materials and Reagents

Simplicia powder from *binahong* (*Anredera cordifolia*) leaves, ethanol 70%, aquadest, chloroform, silica gel, Karl Fischer reagents, ammonia 30%, hydrochloric acid 1:10, Dragendorff reagent, Mayer reagent, magnesium powder, hydrochloric acid (c), amyl alcohol, ferri chloride 1%, Stiasny reagent, sodium hydroxide 1 N, ether, anhydrous acetic acid, sulphuric acid (c), ammonia 10%, alcohol, nitric acid 10%, Potato Dextrose Agar (PDA), Nutrient agar (NA), buffer phosphate pH 7.2, buffer phosphate pH 7.0, p-nitrophenyl- α -D-glucopyranoside, enzyme α -glucosidase, bovine serum albumin, dimethylsulfoxide (DMSO), sodium carbonate 0.2 M, acarbose.

Method

This research was conducted by collecting and providing materials. Furthermore determined and phytochemical screening simplicia powder, then extraction of simplicia powder with kinetic maceration using ethanol 70%. Maserat obtained was concentrated by rotary evaporator to obtain thick extract *binahong* leaves. Furthermore, the phytochemical screening of the extract, determination of specific and non-specific parameters of the extract, and optimization α -glucosidase enzyme inhibition in vitro from *binahong* leaves (*Anredera cordifolia*).

Steps of Research

Sample collection

Samples of *A. cordifolia* leaves were collected from the Balitro, Bogor, West Java. The samples were identified in Research Centre of Biology-Bogoriense Herbarium at Cibinong Bogor, West Java. Hot air-dried samples were processed for further analysis.

Preparation extracts

A. cordifolia leaves (500 g of simplicia powder) were extracted separately five, with ethanol 70% (10 L) under maceration. The filtrate was concentrated with a rotary evaporator until obtained a thick extract *binahong* leaves then stored at 4 °C for analysis.

Phytochemical screening

The phytochemical analysis of *A. cordifolia* leaf extract has been performed to find the presence of major secondary metabolites like flavonoids, tannins, saponins, steroid, glycosides, coumarins, anthraquinones and alkaloids.

Determination the quality parameters of extract

1. Specific parameters determination

Organoleptic, water soluble content, ethanol soluble content

2. Nonspecific parameters determination

Loss on drying, water content, total ash content, acid not soluble ash, ethanol residual content, heavy metals contaminant, microbial contaminant

In vitro inhibition test on α -glucosidase enzyme

Inhibition test extracts to α -glucosidase with thick extract *binahong* leaves were obtained, inhibition test which caused p-nitrophenol produced will be reduced by the enzyme α -glucosidase and then absorbance was measured by Absorbance Microplate Reader ELx800 at λ 405 nm to obtain IC₅₀ values. IC₅₀ values were obtained from the extract compared to the IC₅₀ value of a carbose as a positive control.

α -glucosidase inhibitory activity assay was calculated using the equation:

$$\% \text{ inhibition} = \frac{C-S}{C} \times 100\%$$

C = absorbance of the enzyme activity without inhibitors

S = absorbance of the enzyme activity with the addition of the samples tested (S₁-S₀)

S₁ = absorbance of p-nitrophenol as a result of the addition of enzyme first

S₀ = absorbance of p-nitrophenol as a result of the addition of sodium carbonate first

Results and Discussion

Yield of extracts

Extraction was conducted by using 70% ethanol solvent, it is a polar solvent. Therefore, it is expected that most of the chemical components are soluble in it. Table 1 present yield of extract. DER-native is ratio between simplicia powder (gram) and amount of extract (gram). Rendement is ratio between amount of extract (gram) and simplicia powder (gram).

Phytochemical screening

Phytochemical screening in this research aims to determine the group of chemical compounds of the powder and extract *A. cordifolia* leaves. Table 2

present results of phytochemical screening of simplicia powder and extract *binahong* leaves.

Table 1. Yield of extract *binahong* leaves

Simplicia powder (gram)	Amount of extract (gram)	DER-native	Rendement (%)
500	91.602	5.4584	18.3204

Table 2. Phytochemical screening of simplicia powder and extract *binahong* leaves

No.	Group of chemical compound	Simplicia powder of <i>binahong</i> leaves	Extract <i>binahong</i> leaves
1.	Alkaloids	-	-
2.	Flavonoids	+	+
3.	Saponins	+	+
4.	Quinone	-	-
5.	Tannin	-	-
6.	Steroid/triterpenoid	+/+	+/+
7.	Essential oils	-	-
8.	Coumarins	+	+

Results of phytochemical screening showed that the powder and extract *A. cordifolia* leaves secondary metabolites containing flavonoids, saponins, steroids/triterpenoids, and coumarins.

Specific parameters determination

Quality determination involved extract specific parameters, such as organoleptic, ethanol soluble content, water soluble content. Table 3 present results of specific parameters determination.

Organoleptic results of the extract showed the extract has a thick consistency, blackish green colour, taste rather chelates and hasn't a specific aromatic odor. Assay of water soluble content showed the amount of inorganic compounds in the extract. While the assay of ethanol soluble content showed the amount of organic compounds in the extract. The results showed that water soluble content (72.1414%) greater compared to ethanol soluble content (69.6382), suggesting that extract of *A. cordifolia* leaves contains more polar compounds.

Table 3. Specific parameters determination

Specific parameters	Result
Organoleptic	
✓ Form	Thick extract
✓ Colour	Blackish green
✓ Smell	Hasn't a specific aromatic odor
✓ Taste	Rather chelates
Measurement of soluble compound content	
✓ Ethanol soluble compound content (%)	69.6382
✓ Water soluble compound content (%)	72.1414

Non-specific test for the extract parameter

Quality determination that involved extract nonspecific parameters, such as loss on drying, water content, total ash content, acid not soluble ash, ethanol residual content, heavy metals contaminant, and microbial contaminant. Table 4 present results of nonspecific parameters determination.

Table 4. Nonspecific parameters determination

Nonspecific parameters	Result
Loss on drying (%)	12.8366
Water content (%)	8.4567
Total ash content (%)	1.2154
Acid insoluble ash content (%)	0.0407
Ethanol residual content (%)	0.2540
Heavy metal contamination	
✓ Pb metal contamination (mg/kg)	0.2326
✓ Cd metal contamination (mg/kg)	0.0024
Microbial contamination	
✓ Total plate number (colony/g)	0.4550x10 ³
✓ Molds and yeasts number (colony/g)	0.1067x10 ³

Loss on drying was determined to find out the water content and evaporating compound in the extract after a gravimetric drying process in an oven under a temperature of 105 °C. The result showed that loss on drying 12.8366%. Water content measurement was conducted to find out the water contained in the extract. The lower water content is more stable the extract will be for a longer term. The result showed that water content 8.4567%.

Measurement of total ash content and acid insoluble ash content in the extract aimed to finding out mineral

elements in the extract, this is known as inorganic substance or ash. In the heating process in an oven under a temperature of 450 °C, it was found that organic matters in the extract could be burnt, while the inorganic matters, like ash, could not. Measurement of total ash content aimed to finding out mineral compounds, both physiological compound like K, Mg, and non-physiological compounds, like pollutant, ash, and soil in the extract.

The results of the determination of residual solvent ethanol with gas liquid chromatography in extracts obtained 0.2540% ethanol content. The result of the determination still fulfil the requirements of maximum residual solvent in the extract is less than 1%. The result showed that the extract obtained can be used as raw material preparation because it contained low levels of ethanol.

The content of Pb and Cd in the extracts can be derived from the environment the plants grow and the production process. The content of heavy metals such as Pb and Cd that into the body should be limited in number because it is dangerous for health. The content of Pb excess in the body can cause nerve damage, urogenetal, reproduction and hemopoitic. While the content of Cd excess in the body can cause poisoning and organ damage one for example is kidney damage. In the results showed levels of Pb in the extracts of 0.2326 mg/kg whereas Cd levels of 0.0024 mg/kg.

The result showed the Total Plate Number and Mold and Yeast Number in extracts is 0.4550 x 10³ colonies/g and 0.1067 x 10³ colonies/g. The presence of microbes in the extract may result from processing the samples into extracts, the air and the storage of extract. The low of microbial growth in the extract caused the compound in the extract has efficacy as anti-bacterial. Testing Total Plate Number to determine the growth of aerobic bacteria mesophyll, while the testing Mold and Yeasts number to determine the presence of the fungus.

α-glucosidase inhibitory activity

α-glucosidase enzyme hydrolysed nitrophenyl-α-D-glucopyranoside substrate into yellow p-nitrophenyl and glucose. The extract showed α-glucosidase inhibitory effects, which were determined from p-nitrophenyl absorption that had been formed and measured using Microplate Reader with a wavelength of 405 nm. The IC₅₀ value showed the extract concentration that could inhibit 50% of the α-glucosidase enzyme. Table 5 present that *A. cordifolia* extract had α-glucosidase enzyme inhibitory effects of 47.8855 µg/mL. These are comparable with the positive controls, named 45.0704 µg/mL for acarbose.

Table 5. IC₅₀ value of the extract in comparison with acarbose

Chemical/extract compound	IC50 (µg/ml)
Acarbose	45.0704
<i>A. cordifolia</i>	47.8855

The anti hyperglycemic action of acarbose was result from a competitive, reversible inhibition of pancreatic α-amylase and membrane-bound intestinal α-glucosidase hydrolase enzyme. Pancreatic α-amylase hydrolyses complex starches to oligosaccharides in the lumen of the small intestine, while the membrane-bound intestinal α-glucosidase hydrolyse oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharides in the brush border of the small intestine. In diabetic patients, this enzyme inhibition results in a delayed glucose absorption and a lowering of postprandial hyperglycemia.

Conclusion

1. Phytochemical screening result indicate the presence of the simplicia and ethanol extract from *Anredera cordifolia* leaves contain flavonoid, saponin, steroid/triterpenoid, and coumarins.
2. Organoleptic results of the extract showed the extract has a thick consistency, blackish green colour, taste rather chelates and hasn't a specific aromatic odor. The water soluble content 72.1414%, ethanol soluble content 69.6382%, loss on drying 12.8366, water content 8.4567%, total ash content 1.2154%, acid not soluble ash 0.0407, ethanol residual content 0.2540%, Pb and Cd metals contaminant are 0.2326 mg/kg and 0.0024 mg/kg, microbial contamination of total plate number showed 0.4550 x 10³colony/g, molds and yeasts number 0.1067 x 10³colony/g.
3. Extract *binahong* leaves had α-glucosidase enzyme inhibitory effects, with IC₅₀ values of 47.8855 µg/mL.

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