

# Antioxidant and Alpha Glucosidase Inhibitor Screening of *Merremia peltata* L. as Potential Traditional Treatment for Diabetes Mellitus

Bannan Muthi'atul Af-idah<sup>1</sup>, Muhammad Hanafi<sup>2</sup>, Berna Elya<sup>1,\*</sup>

Bannan Muthi'atul Af-idah<sup>1</sup>,  
Muhammad Hanafi<sup>2</sup>, Berna Elya<sup>1,\*</sup>

<sup>1</sup>Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok, INDONESIA.

<sup>2</sup>Indonesian Institute of Sciences and Faculty of Pharmacy, University of Pancasila, INDONESIA.

## Correspondence

Berna Elya

Professor and Lecturer at the Faculty of Pharmacy, Universitas Indonesia, INDONESIA.

E-mail: berna.elya@farmasi.ui.ac.id

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## ABSTRACT

**Introduction:** *Merremia peltata* is ethnomedicine plant used as traditional medicine in Sulawesi, Sumatra, Maluku and Papua. *M. peltata* is used for diabetic. Diabetes mellitus therapy with inhibit activity of alpha glucosidase enzyme could delay absorption of monosaccharides after a meal and interrupt glucose transport into the circulation. **Objective:** This research purpose is to investigate in vitro antioxidant activity and alpha glucosidase enzyme inhibitor leaves and stem extract of *M. peltata*. Method: The Stem and leaves of *M. peltata* were extracted sequentially using the UAE method using hexane, ethyl acetate, and methanol as mobile phase/solvent. The *M. peltata* extracts were subjected to the antioxidant activity assay by the DPPH radical scavenging and FRAP method. Antidiabetic activity was determined by an enzymatic alpha glucosidase inhibitor. **Result:** The extract which had best performance in antioxidant activity was stem ME with value of IC<sub>50</sub> in DPPH 47.41 µg/mL and total antioxidant power 340.04 µmol/g. This study showed that leaves and stem extract of *M. peltata* have potential alpha glucosidase inhibitors for diabetic therapy. Stem ME had the best activity with IC<sub>50</sub> value 47.44 µg/mL, almost two times better than acarbose as a positive control (IC<sub>50</sub> = 98.38 µg/mL). Leaves ME, leaves EA, and stem EA also give better activity of alpha glucosidase inhibitors than acarbose with IC<sub>50</sub> value 67.24 µg/mL, 69.38 µg/mL, and 72.85 µg/mL, respectively. **Conclusion:** *M. peltata* has potential antioxidant and alpha glucosidase inhibitor activity for diabetic therapy.

**Key words:** *Merremia peltata*, Antidiabetic, Alpha-glucosidase inhibitor, Antioxidant.

## INTRODUCTION

Diabetes mellitus is a metabolic disorder that is marked by the rise in blood sugar due to a decrease in insulin secretion by pancreatic beta cells and insulin function or disorder<sup>1</sup>. World Health Organization (WHO) estimates 422 million adults over the age of 18 lived with diabetes globally in 2014 and caused 1.5 million deaths in 2012<sup>2</sup>. The whole world diabetes mellitus prevalence increases continuously and predicted in 2030 people with diabetes mellitus will reach 550 million people, this is equivalent to about three new cases every 10 seconds, or nearly 10 million per year<sup>3</sup>.

One of the therapies used in the treatment of diabetes is alpha glucosidase inhibitor (eg: Acarbose, Miglitor, and Voglibose)<sup>1</sup>. Acarbose is Food and Drug Administration (FDA) approved for the treatment of adults with type 2 diabetes mellitus. Acarbose is a complex oligosaccharide that acts as a competitive, reversible inhibitor of pancreatic alpha amylase and membrane-bound intestinal alpha glucosidase enzymes. The enzyme breaks down oligosaccharides, trisaccharides, and disaccharides (sucrose, maltose) to monosaccharides (glucose, fructose) in the brush border of the small intestine, so the absorption of monosaccharides after a meal is delayed and transport through the

mucosal surfaces into the circulation is interrupted<sup>4</sup>. Unfortunately, there are some side effects of acarbose therapy, like flatulence, diarrhea, and abdominal pain causing uncomfortable to patients<sup>5</sup>.

Indonesia has so many biodiversity consisting of more than 40.000 endemic plants, and 7000 among them reported as medical plants<sup>6</sup>. More than 400 plants have reported as hypoglycemic agent with various mechanism. the plant has some bioactive constituent such as components, phenolics, glycosides, alkaloids, terpenoids, flavonoids<sup>7</sup>. Traditional medicines have been used for a long time and play an important role as alternative medicines. Using herbal medicine prove has less side effects and more acceptable in the Indonesian society also in diabetic therapy. Since ancient times, the ancestors of the Indonesian people have used plants to maintain health and treat various diseases, called "Jamu". Jamu is a traditional herb that can be made from plants, animals, minerals, preparations (galenic) or a mixture of these ingredients, which have been used from generation to generation for treatment based on experience<sup>8</sup>.

*Merremia peltata* (L.) Merr.) is perennial herbaceous vine from Convolvulaceae family, which often wrapped around the other plants. *M. peltata* known as "Aka Lambuang" is an ethnobotanical plant used

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as traditional medicine in Sulawesi, Sumatra, Maluku and Papua. Phytochemical screening showed that leaves extract of *M. peltata* contains terpenoid, steroid, saponin, and phenolic<sup>9</sup>. Traditionally, *M. peltata* is used as a potion for inflammation, abdominal pain, wound healing, infectious disease, diabetic therapy<sup>10</sup>. Despite of *M. peltata* is used traditionally as diabetic therapy, antidiabetic activity of *M. peltata* has not been investigated.

Previous in vitro and in vivo studies showed that some plants from merremia genus have antidiabetic activity. Plants in the merremia genus are reported to contain phenolic compounds, flavonoids, sulfates, aliphatic pyrrolidine amides, tropane, and alkaloid<sup>11</sup>. In vitro study showed that leaves ethanol extract of *Merremia hederacea* and hexane fraction of *Merremia mammosa* had inhibitor activity of enzyme alpha amylase and alpha glucosidase<sup>6,11</sup>. In vivo study using streptozotocin induced diabetic rats showed extract ethanol of *Merremia tridentata*, *Merremia mammosa*, *Merremia emarginata*, and *Merremia hederacea* have potential antidiabetic activity<sup>11,12,13,14</sup>. Based on the chemotaxonomy of chemical compounds in the same genus, *M. peltata* has the possibility of having the same pharmacological activity as alpha glucosidase inhibitor for potential antidiabetic agent, but such tests have not been conducted. This research purpose is to investigate in vitro antioxidant activity and alpha glucosidase enzyme inhibitor of leaves and stem extract of *M. peltata*.

## MATERIAL AND METHOD

### Plant material

Samples used in this study were the bark and leaves of *M. peltata* which obtained from Ratatotok district, North Sulawesi and identified by Herbarium Bogoriensis, Biological Research Centre, Indonesian Institute of Science.

### Chemical

Chemicals used in this study were Phosphate buffer pH 7, Alpha-glucosidase (Wako Pure Chemical Industries Ltd., Japan), Acarbose, ethanol, ethyl acetate, methanol, n-hexane, para nitrophenyl alpha-D-glucopyranoside (Wako Pure Chemical Industries Ltd. EC 3.2.1.20, Jepang), dichloromethane, dimethyl sulphoxide (Merck cat. 3.17275, Germany), Sodium carbonate (Merck cat. 1.09940, Germany), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid (Sigma-Aldrich, A5960), Ferrous sulfate heptahydrate (MERCK, Germany).

### Sample preparation

Fresh Stem and leaves of *M. peltata* were washed using fresh water and dried in the oven, then crushed with a grinder to get powder simplisia.

### Extraction

The dried powder of stem and leaves *M. peltata* were extracted sequentially using Ultrasonic Assisted Extraction (UAE). The sample (100 g) extracted using N-Heksan (NH) (500 ml) for 30 minutes three times, then filtered to obtain filtrate and residue. The residue was dried and then re-extracted using Ethyl Acetate (EA) then Methanol (ME) using the same method. The filtrate was evaporated using a rotary evaporator to obtain crude extract.

### Microscopic observation by light and SEM microscope

The microscopic part of dried powder of *M. peltata* leaves and stem were observed using light and Scanning Electron Microscope (SEM) microscope.

### Antioxidant assay by DPPH inhibition

The antioxidant ability of extract was determined using (1,1-diphenyl-2-picrylhydrazyl) DPPH radical scavenging activity method described

by Burda & Oleszek (2001) with slight modification. Ascorbic acid was used for positive control. Five milligram extracts were dissolved in 10 ml methanol solution, and diluted to get sample concentrations 20, 40, 60, 80, and 100 ppm. 500 µL sample solution were added into 1.5 mL DPPH solution, mixed for 2 minutes, and incubated in dark room for 30 minutes. After incubation, the sample absorbance was measured using Spectrophotometer UV-Vis in 517 nm. The color change from purple to yellow means free radical scavenging efficiency<sup>15</sup>. Free radical scavenging activity was calculated as the percentages of color decreasing of DPPH solution using the following equation:

$$\text{Free radical scavenging activity (\%)} = 1 - \frac{\text{sample absorbance}}{\text{control absorbance}} \times 100$$

### Antioxidant assay by (Ferric Reducing Antioxidant Power) FRAP

The total antioxidant potential of stem and leaves *M. peltata* determined using the method described by Wojdylo et al (2007) with slight modification. The FRAP reagent was made with a mixture of TPTZ (2,4,6-tripyridyl-s-triazine) 10 mM in 40 mM HCl, FeCl<sub>3</sub>.6H<sub>2</sub>O (20 mM) and Acetate buffer (300 mM, pH 3.6) in the ratio 1:1:10. Extract sample (100 µg/ml) was mixed with 3 mL FRAP reagent then the absorbance was measured after 10 min. Ascorbate acid was used as positive control. The calibration curve prepared using FeSO<sub>4</sub>.7H<sub>2</sub>O methanol solution in concentration 100 to 2000 mM and measured concentration of Fe<sup>2+</sup> at 593 nm using Spectrophotometer UV-Vis. The amount of Fe<sup>2+</sup> produced from the reduction of Fe<sup>3+</sup> by the extract was calculated from the standart curve prepared from ferrous sulphate solution and results were expressed as mg Fe<sup>2+</sup> / 100 g dry sample. All determinations were performed in triplicate<sup>16</sup>.

### Alpha glucosidase inhibitor activity assay

The alpha glucosidase inhibitor was determined using adapted published protocol from Elya et al (2015). Acarbose used as a positive control. Extract of stem and leaves *M. peltata* were dissolved in maximum 10% DMSO and phosphate buffer solution pH 6.8. Five various concentrations of sample and acarbose were made to determine IC<sub>50</sub> of alpha glucosidase inhibitor. Thirty microliter of sample, 36 µL of phosphate buffer solution, and 17 µL PNPG substrate at concentration of 4 mM were put in 96 well microplate. The mixture was incubated at 37°C for 5 min. After incubation, 17 µL of alpha glucosidase enzyme solution 0.025 U/ml was added into each well. The mixture was incubated at 37°C for 15 min. The reaction was stopped by adding 100 µL of Sodium Carbonate (Na<sub>2</sub>CO<sub>3</sub>) 200 mM. Absorbance was measured at 405 nm using a microplate reader<sup>17</sup>. Each test was repeated three times.

Percent inhibition of the sample was determined using following equation:

$$\text{Inhibition (\%)} = \frac{(A1 - A2)}{A1} \times 100$$

Where:

- A1 : Absorbance of blanko (B\*) - control of blanko (KB\*\*)
- A2 : Absorbance of sample (S) - control of sample absorbance (KS\*\*\*)
- \* : Blanko contains substrate + enzyme, without extract
- \*\* : Control of blanko contains substrate and buffer, without enzyme and extract
- \*\*\* : Control of sample contains substrate + extract with the addition of the enzyme after incubation

Percent inhibition obtained in each sample was processed in the form of a graph, which x as concentration and y as percent inhibition of sample to get linear regression equation Y= ax + b. The Inhibition Concentration (IC<sub>50</sub>) was determined using following equation:

$$IC_{50} = \frac{(50 - a)}{b}$$

## RESULT AND DISCUSSION

### Extraction

Five hundred gram dried powder of stem and leaves of *M. peltata* were extracted using UAE method. The ultrasonic extraction method was faster and more effective to extract secondary metabolites from plants than conventional methods like maceration or soxhletation<sup>18</sup>. The high power ultrasound (20 to 25 kHz) could degrade the cell wall and increase the penetration of solvent through the plant cells, enhancing the solubility of phytochemicals.

Extraction was carried out sequentially with different polarity solvent to maximize solubility of phytochemical from the sample, with increasing polarity solvent starting from N-Hexane (NH), Ethyl Acetate (EA), then Methanol (ME). Solvent polarity would affect the type and amount of chemical compounds to be extracted, the antioxidant capacity, and biological activity of the extract<sup>19</sup>. The yield of UAE sequential extraction was displayed in the following table 1. Table 1 showed that NH extract from stem and leaves had the highest amount indicating that the sample contained more non polar than polar substituents.

### Antioxidant assay by DPPH inhibition

Antioxidant assay using (DPPH) free radical scavenging method was the first approach for evaluating the antioxidant potential of a compound, developed by Blois (1958)<sup>20</sup>. A stable free radical  $\alpha$ ,  $\alpha$ -diphenyl- $\beta$ -picrylhydrazyl (DPPH; C<sub>18</sub>H<sub>12</sub>N<sub>5</sub>O<sub>6</sub>, M=394.33) had odd electron of nitrogen atom. An antioxidant compound donated a hydrogen atom formed hydrogen bond with nitrogen atom in DPPH, marked with decolorization of violet DPPH solution turned to yellow. The absorbance of DPPH at 517 nm was interrupted by light, oxygen, pH, and type of solvent in addition to the antioxidant. Polar solvent may decrease the odd electron density of nitrogen atoms in DPPH and increase the reactivity of DPPH. DPPH in methanol solution had good stability under the light. Incubation under dark room was needed to prevent photochemical decomposition of DPPH that positively correlated with the absorbed light energy<sup>21</sup>

Ascorbic acid (AA) was used as a positive control because of its strong antioxidant properties. AA acts primarily as a donor of single hydrogen atoms, and the radical anion monodehydroascorbate reacts mainly with radicals. Together these properties account for the remarkable antioxidant actions of ascorbic acid<sup>22</sup>.

IC<sub>50</sub> is the concentration of compounds that have 50% inhibition of DPPH. The compound with higher antioxidant activity will have the lower value of IC<sub>50</sub>. According to Phongpaichit et al (2007), a compound stated as free antiradical very strong when the IC<sub>50</sub> value <10  $\mu$ g / mL, strong if the IC<sub>50</sub> value is between 10-50  $\mu$ g / mL, moderate if the IC<sub>50</sub> value ranges from 50-100  $\mu$ g /mL, weak when the IC<sub>50</sub> value is between 100-250  $\mu$ g / mL and is inactive when IC<sub>50</sub> above 250  $\mu$ g / mL. DPPH inhibition of stem and leaves *M. peltata* were showed in picture 2. IC<sub>50</sub> obtained from a regression linear equation with plotting sample concentration in x axis and % inhibition in y axis of graph. Regression equation and IC<sub>50</sub> of the sample were presented in table 3.

AA as a positive control showed very strong antioxidant activity with IC<sub>50</sub> value 10.49  $\mu$ g/mL. The stem ME showed the best antioxidant

**Table 1: The yield of *M. peltata* extraction using UAE.**

	Stem NH	Stem EA	Stem ME	Leaves NH	Leaves EA	Leaves ME
Weight of extract (g)	20.3	14.7	13.4	43.4	32	28.6
Yield (%)	4.1%	2.9%	2.7%	8.7%	6.4%	5.7%

**Table 2: Antioxidant activity of *M. peltata* extract using DPPH assay.**

Sample Cons ( $\mu$ g/mL)	% inhibition DPPH			
	Asam Ascorbat	Stem NH	Stem EA	Stem ME
Regression Eq.	$y = 0.3788x + 46.026$	$y = 0.4824x + 1.9886$	$y = 0.5847x + 4.2386$	$y = 0.7291x + 15.436$
IC <sub>50</sub> ( $\mu$ g/mL)	10.49	99.53	81.94	47.37

Sample Cons ( $\mu$ g/mL)	% inhibition DPPH		
	Leaves NH	Leaves EA	Leaves ME
Regression Eq.	$y = 0.5028x - 4.625$	$y = 0.9045x - 17.114$	$y = 0.6295x + 5.8409$
IC <sub>50</sub> ( $\mu$ g/mL)	108.64	74.20	70.15

activity of all sample extracts with the lowest IC<sub>50</sub> values 47.37  $\mu$ g/mL. The leaves ME, leaves EA, stem EA, and stem NH were categorized as moderate antioxidant activity with IC<sub>50</sub> value respectively 70.15  $\mu$ g/mL, 74.20  $\mu$ g/mL, 81.94  $\mu$ g/mL, and 99.53  $\mu$ g/mL. The Leaves NH were categorized as a weak antioxidant activity with IC<sub>50</sub> value 108.64  $\mu$ g/mL.

### Antioxidant assay by FRAP

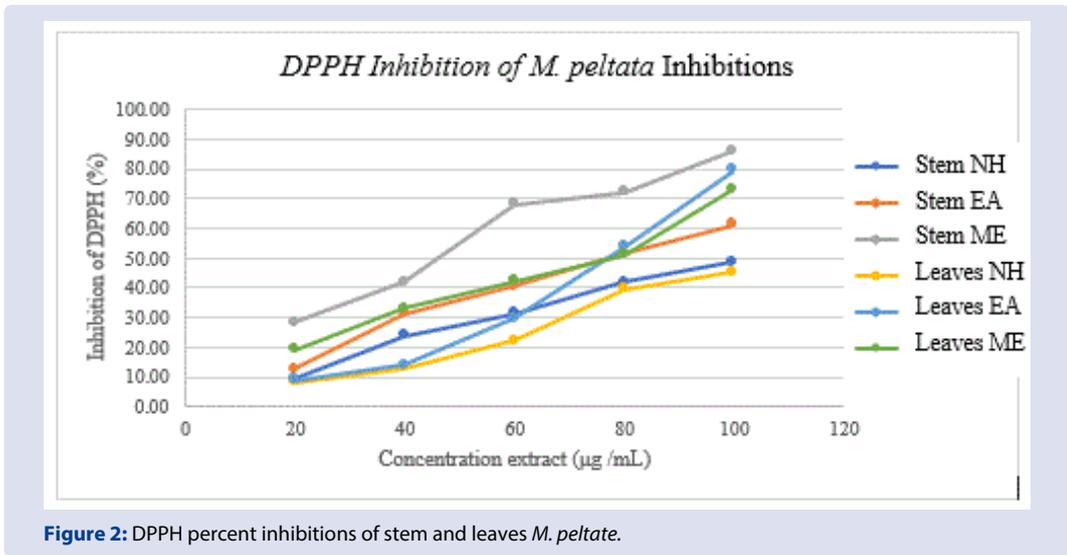
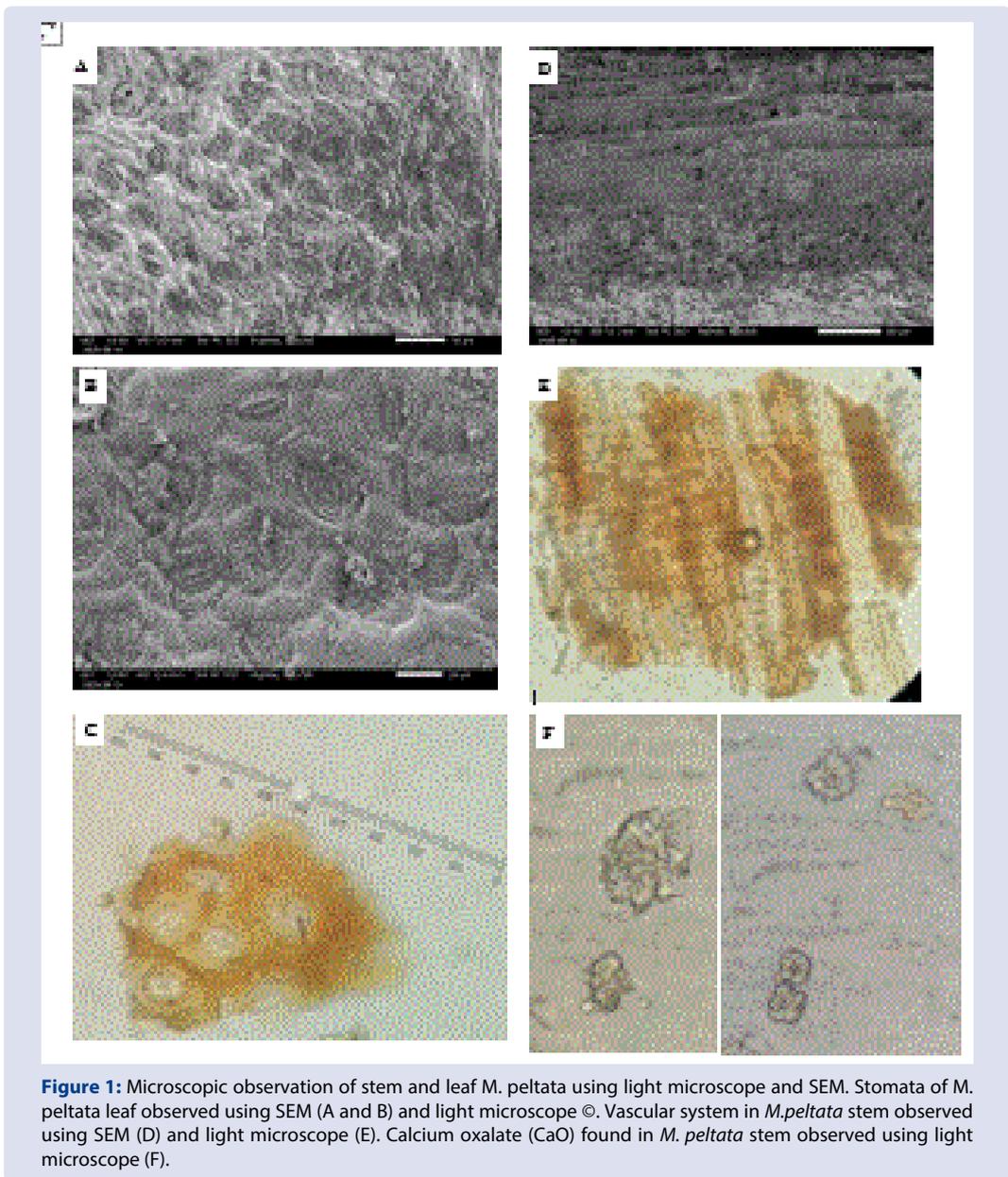
The FRAP assay is a relatively simple, quick, and inexpensive method for measuring total antioxidant activity of plant samples. The assay uses the reduction of ferric ions (Fe<sup>3+</sup>) to ferrous ions (Fe<sup>2+</sup>), indicated by a colour change from pale yellow color to intensive blue, and absorbance at 598 nm<sup>23</sup>. Calibration curve from ferrous sulfate heptahydrate (FSH) was made as a standart and obtained regression equation  $y = 0.00257x + 0.04715$ . The total antioxidant activity of the sample shown in following table 4.

Based on the FRAP assay, stem ME also had the highest total antioxidant power of the plant extract with value 207.08  $\mu$ mol/g after ascorbic acid as a positive control antioxidant with value 340.04  $\mu$ mol/g. The stem EA, leaves ME, leaves EA, leaves NH, and stem NH had total antioxidant value 164.53  $\mu$ mol/g, 137.33  $\mu$ mol/g, 77.37  $\mu$ mol/g, 65.18  $\mu$ mol/g, and 59.75  $\mu$ mol/g, respectively. Methanol fraction showed good capacity of antioxidant than ethyl acetate or n-hexane because polarity of the solvent could dissolve polar compounds like polyphenol and flavonoid, that had good antioxidant activity. Wakeel et al (2019) reported polarity of the solvent affected phenolic content and flavonoid content of the extract sample, directly correlated with reducing power, antioxidant, and free radical scavenging capacity. The amount of phenolic compound, flavonoid compound and antioxidant capacity were significantly increased with increasing polarity and abruptly decreasing at a very high polarity index such as water. It means that the plants have different biochemical compounds with a range of polarity<sup>24</sup>.

### Alpha glucosidase inhibitor activity assay

The principle of this test is that a substance that acts as an inhibitor will bind to the  $\alpha$ -glucosidase enzyme so that the enzyme activity in hydrolyzing the pNPG substrate (p-nitrophenyl- $\alpha$ -D-glucopyranoside) becomes p-nitrophenol which is yellow in color will be inhibited. The absorbance is measured at 405 nm based on the amount of p-pyrophanol formed.

The in vitro alpha glucosidase inhibitor assay of the *M. peltata* stem and leaves extract used five variations of sample concentration to get a graph which x axis as concentration and y axis as percent inhibition. Regression equation from the graph used to determine IC<sub>50</sub> of the extract. The result showed that Stem ME had the best activity with IC<sub>50</sub> value 47.44  $\mu$ g/mL, almost two times better than acarbose as a positive



**Table 3: Antioxidant power of *M. peltata* extract by FRAP assay.**

Sample	FRAP ( $\mu\text{mol Fe}^{2+}/\text{mg}$ )
Acarbose	340.04 $\pm$ 3.0
Stem NH	56.75 $\pm$ 1.7
Stem EA	164.53 $\pm$ 1.3
Stem ME	207.08 $\pm$ 4.8
Leaves NH	65.18 $\pm$ 0.5
Leaves EA	77.37 $\pm$ 0.6
Leaves ME	138.33 $\pm$ 0.5

**Table 4: IC50 alpha glucosidase inhibitor activity of stem and leaves *M. peltata*.**

Sample	Regression Equation	IC50 ( $\mu\text{g}/\text{mL}$ )
Acarbose	$y = 0.2156x + 28.79$	98.38
Stem NH	$y = 0.2775x - 7.6194$	207.64
Stem EA	$y = 0.7474x - 4.4503$	72.85
Stem ME	$y = 0.6613x + 18.631$	47.44
Leaves NH	$y = 0.3793x - 12.923$	165.89
Leaves EA	$y = 0.7489x - 1.9571$	69.38
Leaves ME	$y = 0.8114x - 4.5593$	67.24

control (IC50 = 98.38  $\mu\text{g}/\text{mL}$ ). Leaves ME, leaves EA, and stem EA also give better activity of alpha glucosidase inhibitors than acarbose with IC50 value 67.24  $\mu\text{g}/\text{mL}$ , 69.38  $\mu\text{g}/\text{mL}$ , and 72.85  $\mu\text{g}/\text{mL}$ , respectively. Stem NH and leaves NH had lower inhibitory activity than acarbose with IC50 value 207.64  $\mu\text{g}/\text{mL}$  and 165.89  $\mu\text{g}/\text{mL}$ . The regression equation and IC50 value showed in following table 5.

Previous phytochemical study by Perez et al (2015), ethanol extract of *M. peltata* contained flavonoid, alkaloid, and tannin<sup>9</sup>. Many bioactive compounds from different plants have been reported to have hypoglycemic effect, in that mostly phenolics, resin glycosides, and flavonoids have a positive correlation as antidiabetic agents<sup>11</sup>. The ethanol extract shows greater inhibition activity in both stem or leaves of *M. peltata* compared to other extracts. The presence of flavonoids and phenolic compounds in ethanol extract of *M. peltata* may act against diabetes mellitus either through their capacity to avoid glucose absorption.

## CONCLUSION

*M. peltata* has potential antioxidant and alpha glucosidase inhibitor activity for diabetic therapy. Antioxidant power of *M. peltata* extract had positive correlation in alpha glucosidase inhibitor activity. Stem ME has the best antioxidant and better alpha glucosidase inhibitor activity than acarbose as positive control. Phytochemical content of phenolics, resin glycosides, and flavonoids of the *M. peltata* extract had positive correlation of hypoglycemic activity and had proven as antidiabetic agent in previous study.

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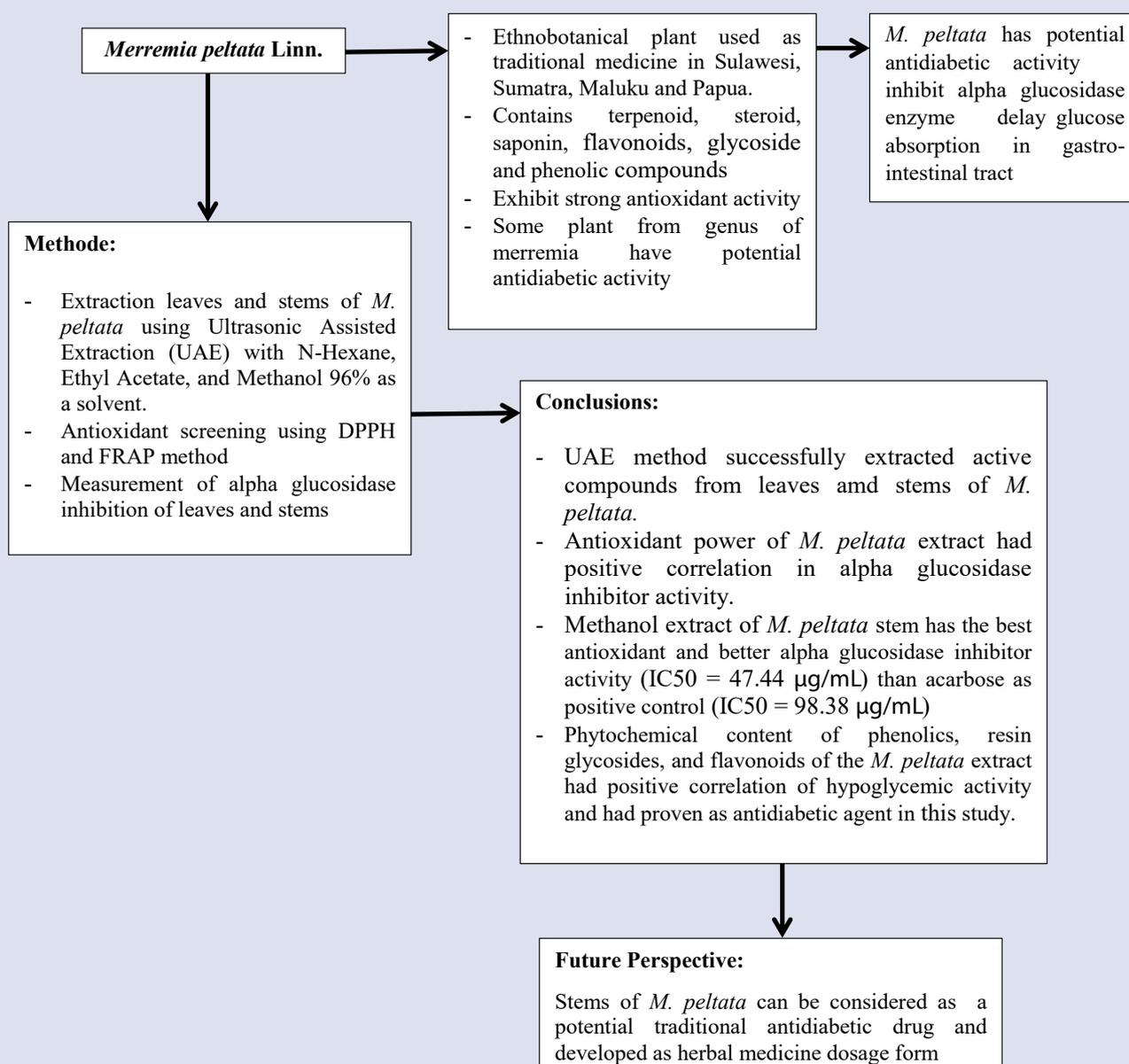
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## GRAPHICAL ABSTRACT



## ABOUT AUTHORS



**Bannan Muthiatul A** is a Pharmacist and Magister Pharmacy Student at the Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok, West Java 16424, Indonesia. She conducted research on pharmacognosy study of natural product and biotechnology of pharmacy.



**Muhammad Hanafi** is a Researcher at the Research Center for Chemistry, Indonesian Institute of Sciences, PUSPITEK area, Serpong, South Tangerang, Banten, Indonesia and Lecturer at the Faculty of Pharmacy, University of Pancasila, Srengseng Sawah, Jakarta, Indonesia. He has research experience in the field of Natural Product.



**Berna Elya** is a Professor and Lecturer at the Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok, West Java 16424, Indonesia. She develops works in the area of Pharmacognosy and Phytochemistry (Natural Product).

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