Inhibition activity of α-glucosidase enzyme, phenolic content and toxicity of Mara (Macaranga tanarius (L.) Mull. Arg) Leaf

Megawati1, Hariyanti2, Resta Pebriani2 and Sofa Fajriah1

1Research Center for Chemistry, Indonesian Institute of Sciences, Kawasan PUSPIPTEK Serpong, Tangerang Selatan, Banten 15314, Indonesia.
2Faculty of Pharmacy and Science, Universitas Muhammadiyah Prof. DR. HAMKA, Jakarta, Indonesia

*Corresponding author: megarafandi@gmail.com

Abstract. Previous studies have shown that mara leaves (Macaranga tanarius (L) Mull. Arg) can be used as medicinal plants because they have antibacterial and anti-inflammatory activity. Compounds containing flavonoids, tannins, saponins. But there is still little research on mara leaves as inhibition α-glucosidase. The purpose of this study was to determine the antidiabetic activity by the α-glucosidase method and its toxicity by the BSLT method and the results of fractionation. This test can be done by looking at the IC50 values obtained from linear regression analysis. Whereas the acute toxicity test uses BSLT (method of Shrimp Lethality Test). Using concentrations of 1000 ppm, 500 ppm, 100 ppm and 10 ppm. At each concentration using 10 larvae Artemia salina Leach and analyzed mortality. LC50 values were obtained from linear regression of mortality percentages and log doses. The results of this study prove the ethyl acetate fraction of Macaranga tanarius (L) Mull. Arg leaves has a high value inhibition activity that is 0.74 μg / mL and is non-toxic so it can be considered as inhibition α-glucosidase.

1. Introduction

The genus Macaranga is a useful species of 250 species in tropical forests where its distribution is in tropical forests of Asia and the Pacific. Investigation of the potential of Macaranga plants as medicinal plants and cosmetic ingredients is still not optimal. However, several natural chemical chemistry researchers from the Faculty of Forestry and FMIPA of Mulawarman University have conducted searches in the form of phytochemical screening and isolation of active compounds from Macaranga in East Kalimantan. Phytochemical and pharmacological studies of Macaranga plants that have been carried out generally show that there are variations in the content of secondary metabolites such as terpenoids, terpenylated flavonoids and stylbenoid, and some phenolic derivatives [1].

More than 26 species of Macaranga have been studied phytochemically with more than 190 secondary metabolite compounds have already been identified, with phenolic compound as a major compound group [2]. Our previous study was successfully identified some phenolic compounds such as macarangin, apigenin, apigenin glycoside, and scopoletin [3-5] from M. Gigantifolia, 5,7,3',4'-tetrahydroxy-3,6-diprenylflavone and kaempferol 7-O-β-glucose from M. gigantifolia and M. hispida (Blume) [3,6] and scopolotin compound from M. hispida (Blume) [7]. Previous studies reported that flavonoids and terpenoids are the main components of the genus Macaranga [8]. Apigenin and active compounds were isolated from Portulaca oleracea L. which has potential antibacterial activity and potentially used as an antibacterial for the treatment of diseases associated with pathogenic bacteria.
Phenolic or polyphenols are one of the most widely distributed groups of natural products in the plant kingdom. More than 8,000 phenolic structures are currently known, and more than 4,000 flavonoids have been supported [10].

The α-glucosidase inhibitors are one of the therapeutic approaches in decreasing postprandial hyperglycemia by delaying the digestion of poly- and oligosaccharides to absorbable monosaccharides [11]. Furthermore, α-glucosidase inhibitors have been a huge concern to researchers working in the field of medicinal chemistry since their antidiabetic and anti-obesity activity, is also associated with their activity against human immunodeficiency virus (HIV) and hepatitis [12-14].

One of the most common diseases throughout the world is diabetes mellitus, a chronic metabolic disorder characterized by hyperglycemia accompanied by various chronic vascular complications. Therefore, control of postprandial blood glucose spikes is crucial for the treatment of diabetes. A number of treatments has been used by patients with type II diabetes are synthetic amylase inhibitors and glucosidases, such as, acarbose, however, they report side effects. As a result, safer natural amylase inhibitors and glucosidases and many compounds have been reported from plant sources [15-19].

Therefore, a further study of the phytochemical content of the Macaranga genus needs to be done especially from M. tanarius (L.) Mull. Arg. In this study, we evaluated the total phenol content from methanol extract, n-hexane, ethyl acetate and butanol fractions, antidiabetic activities from M. tanarius.

2. Research Methods

2.1. General
Sample extraction performed using methanol (Merck), evaporated with Buchi R214-Switzerland rotary evaporator, biological activity reagent for antidiabetic enzyme using α-glucosidase enzyme, total phenolic content and toxicity test using Artemia salina Leach larvae.

2.2. Plant material
Macaranga tanarius leaves was collected in March 2012 from Mekongga forest, Southeast Sulawesi, Indonesia. The plant was identified by the staff of Herbarium Bogoriense, Research Center for Biology, LIPI and a voucher specimen had been deposited at the herbarium.

2.3. Extraction and isolation
The dried leaves (2 kg) of M. tanarius was macerated with methanol for 24 hours (3 times). The methanol extract (220 g) was partitioned with n-hexane, ethyl acetate and n-butanol, respectively, and all fractions were evaluated for their total phenolic content using Follin-Ciocalteu methods.

2.4. Inhibition assay for α-glucosidase activity
α-glucosidase inhibitory activity evaluation of the extracts was performed using an established procedure [13,20,21]. The α-glucosidase enzyme solution was dissolved in a phosphate-buffer solution (pH 7) containing 200 mg albumin serum. Prior to its application, enzyme solution was diluted 25 times in buffer solution. The mixture contained of 20 mM p-nitrophenyl α-D-glucopyranose as the substrate and 100 mM phosphate buffer (pH 7). Ten µL of the extract dissolved in DMSO was prepared. The reaction mixture was then water-bath incubated at 37°C for 5 min. The enzyme solution (250 µL) was added and the solution was incubated for 15 min. The enzyme reaction was stopped by the addition of 200 mM sodium carbonate solution. The resulted p-nitrophenol from the reaction was measured at λ = 400 nm. Quercetin solution was measured. The commercial α-glucosidase anti-diabetic drug, glucobay, was available in the laboratory only in sustain release tablets. Sample concentrations for activity evaluation were 6.25, 12.5, 25, 50, 100 µg/mL. Inhibition was calculated using formula:

\[
\text{Inhibition (\%): } \left( \frac{C - S}{C} \right) \times 100
\]

(Eq. 1)

In which: S = Sample absorbance; C = blank absorbance
2.5. Total Phenolic Content in Extract
The total phenolic content of the extract was determined by the Folin–Ciocalteu method [22]. Various concentration of gallic acid solution were pipetted and placed into test tubes. Each tube was added with 1 mL of the sample, and distilled water to 7.5 mL volume. Added with 0.5 mL of Folin–Ciocalteu reagent, and mixed thoroughly for 8 min, followed by the addition of 1.5 mL of 20% (w/v) of sodium carbonate. The mixture was allowed to stand for further 60 min in the dark, and absorbance was measured at 765 nm. The total phenolic content was calculated based on the calibration curve, and the results were expressed as mg of gallic acid equivalent per g of dry weight.

2.6. Toxicity test
Toxicity test conducted by brine shrimp lethality test method developed by Meyer [23]. Artemia salina Leach. The eggs incubated in sea water for 48 hours until hatch into larvae. About 10 A. salina larvae put in microplate and added with sample in various concentration of samples (10, 100, 500, and 1000 ppm), incubated for 24 hours at room temperature. Toxicity calculated based on the number of dead larvae after incubated. LC$_{50}$ value indicated of sample concentration required to make 50% of the A. salina larvae dead.

3. Result and Discussion
In this study the simplicia used was leaves which had been pollinated. The mara leaves were then extracted using 96% Methanol. The extraction method used is maceration. Maceration has the advantage of simple procedures and equipment; the extraction method is not heated so that the natural ingredients do not decompose. Cold extraction allows many extracted compounds, although some compounds have limited solubility in extraction solvents at room temperature. The maceration process is carried out by soaking the simplicia with methanol 96% for 24 hours in a tightly closed container and protected from light. After 24 hours filtering is done and the pulp is macerated again with a new solvent. Maceration is done three times by soaking 2 kg of simplicia powder in a maceration bottle. After all the macerates were collected, solvent separation was carried out using vacuum rotary evaporator at a temperature of 48-50 ºC until thick extract was obtained. Through a rotary vacuum evaporator, the solvent liquid can be separated without any overheating of the samples by lowering the pressure above the liquid and thus lowers the boiling point [24].

| Table 1. Methanol extract of M. tanarius (L) Mull. Arg. |
|----------------------------------|-----------------|---|
| Extract                          | Weight          | % Yield |
| Simplisia M. Tanarius            | 2 kg            | -       |
| Methanol Extract                 | 220 g           | 10.23   |

After obtaining methanol extract of Mara leaves as much as 220 g with percent yield of 10.23 percent then partitioned (fractionation) using n-hexane-water (1: 1) obtained n-hexane fraction and water fraction, then the results were concentrated by using vacuum rotary evaporator and the water fraction was subsequently partitioned with ethyl acetate - water as much as (1:1), then the results of the ethyl-acetate fraction were concentrated using vacuum rotary evaporator and The water fraction was then partitioned again with butanol-water as much as (1:1), then the results of the butanol fraction were concentrated using vacuum rotary evaporator. The last result was the fraction of the methanol extract of Mara leaves as follows: hexane fraction (1.52 gram), ethyl acetate fraction (9.005 gram), butanol fraction (5.881 grams) and water fraction (3.219 gram). Further, each extract and fraction were tested for inhibition alpha glucosidase [7].
Table 2. Yield of Extract *M. tanarius* (L) Mull. Arg.

| Samples                | Extract Weight (gram) | % Yield |
|------------------------|-----------------------|---------|
| *n*-Hexane Fraction    | 1.522                 | 7.60    |
| Ethyl Acetate Fraction | 9.005                 | 45.22   |
| Butanol Fraction       | 5.881                 | 29.40   |
| Water Fraction         | 3.219                 | 16.09   |

Methanol extract and all fractions (hexane, ethyl acetate, butanol) were evaluated for their antidiabetic using \( \alpha \)-glucoside, total phenolic content using Folin-Ciocalteu method.

Table 3. Total phenolics content of extract and fraction from *M. tanarius* (L) Mull. Arg.

| Samples                | % Total Phenolic Content (GAE (w/w)) |
|------------------------|-------------------------------------|
| Methanol extract       | 27.117 ± 1.06                       |
| Hexane Fraction        | 10.435 ± 0.45                       |
| Ethyl Acetate Fraction | 37.518 ± 1.37                       |
| Butanol Fraction       | 34.646 ± 1.05                       |
| Water Fraction         | 6.775 ± 0.346                       |

GAE = Gallic Acid Equivalent

In this study, total phenolic content of methanol extract was 27.117 ± 1.06 (w/w). It means that each 100 g dry weight of methanol extract contained total phenolics that was equivalent to gallic acid of (Table 2). Partition step of methanol extract with *n*-hexane, ethyl acetate and *n*-butanol yielded highest total phenolic content in the ethyl acetate fraction with the total phenolic content was 37.518 ± 1.37 (Table 2). Ethyl acetate fraction was further purified using various chromatography techniques.

![Figure 1. Comparative solution calibration curve (gallic acid), total phenolic content.](image)

Supported by the data analysis of all biological activities test resulted, showed that ethyl acetate extract of *M. tanarius* leaves has strong activity as antidiabetic with \( IC_{50} \) 0.74 ppm compared with quercetin as positive control (\( IC_{50} \) 5.601 ppm) (Table 4). \( IC_{50} \) value indicated the concentration of sample that can inhibit enzyme activity 50%.

Toxicity tests are used to determine the toxic effects of a compound that will occur in a short time exposure period or the administration of a single concentration of test compounds in test animals. The BSLT (*Brine Shrimp Lethality Test*) method is used for initial screening of active compounds contained in plant extracts since it is easy, inexpensive, fast, does not require special labs and the results are reliable [25].
Table 4. Inhibition activity of α-glucosidase from extract and fractionasi from M. tanarius leaves.

| Samples          | Concentration | % inhibition IC<sub>50</sub> |
|------------------|---------------|-----------------------------|
| Quarcetin        | 10            | 60.207                      | 5.601                  |
|                  | 5             | 48.932                      |                        |
|                  | 2.5           | 36.276                      |                        |
|                  | 1.25          | 15.141                      |                        |
|                  | 0.625         | 10.468                      |                        |
| Methanol extract | 10            | 58.672                      | 8.628                  |
|                  | 5             | 30.272                      |                        |
|                  | 2.5           | 29.508                      |                        |
|                  | 1.25          | 28.464                      |                        |
|                  | 0.625         | 22.150                      |                        |
| Hexane Fraction  | 10            | 40.312                      | 12.46                  |
|                  | 5             | 12.531                      |                        |
|                  | 2.5           | 10.157                      |                        |
|                  | 1.25          | 10.014                      |                        |
|                  | 0.625         | 9.020                       |                        |
| Ethyl Acetate    | 10            | 96.445                      | 0.74                   |
| Fraction         | 5             | 94.939                      |                        |
|                  | 2.5           | 93.972                      |                        |
|                  | 1.25          | 39.550                      |                        |
|                  | 0.625         | 37.555                      |                        |
| Butanol Fraction | 10            | 97.709                      | 1.619                  |
|                  | 5             | 90.785                      |                        |
|                  | 2.5           | 90.800                      |                        |
|                  | 1.25          | 37.437                      |                        |
|                  | 0.625         | 36.935                      |                        |

Table 5 shows that only the fraction n-hexane which is a toxic compound that has an IC value of 50 (301.99 μg / mL) compared to other extracts is not classified as a toxic compound because it has an LC<sub>50</sub> of more than 1000 μg / mL, which according to the theory that an extract is declared toxic according to the BSLT method if it has an LC<sub>50</sub> of less than 1000 μg / mL [26].

Table 5. Toxicity test results with BSLT method extract and fraction.

| No  | Macaranga tanarius (L) Mull.Arg. | LC<sub>50</sub>(μg/mL) | Conclusion     |
|-----|----------------------------------|-------------------------|----------------|
| 1   | Extract of Methanol              | >1000                   | Non-toxic      |
| 2   | Fraction n-Hexane                | 301.99                  | Toxic          |
| 3   | Ethyl Acetate                    | >1000                   | Non-toxic      |
| 4   | Butanol Fraction                 | >1000                   | Non-toxic      |
| 5   | Water Fraction                   | >1000                   | Non-toxic      |

From the results obtained it turns out that the ethyl acetate fraction has a resistance value of α-glucosidase of 0.74 μg / mL more active and approaching the standard, quarcetin (5.601 μg / mL) when compared to other extracts, in the BSLT test the ethyl acetate extract did not show any salina artemia larvae death after adding this extract. Meyer [23] in Farihan [27] reported that an extract showed toxic activity in BSLT if the extract could cause the death of 50% of test animals at concentrations of less than 1000 ppm. The statement above shows that ethyl acetate extract is not toxic to Artemia because it has an LC50 value> 1000 ppm. The ethyl acetate extract in the phenolic content test has a higher content. α-glucosidase and safe to consume without showing mortality in this extract when tested for toxicity by the BSLT method.
Differences in antidiabetic activity were obtained from total phenol levels and total flavonoids. Phenol and flavonoid compounds have a linear increase in anti-diabetic activity, so the higher the level, the better the anti-diabetic [28]. Phenolics and flavonoids have an antidiabetic or antihyperglycemic effect which is possible in several ways by inhibiting absorption, stimulating insulin secretion or acting like insulin and can help the work of enzymes that play a role in carbohydrate metabolism. One of the flavonoid compounds is quercetin which is known as a strong anti-diabetes. Quercetin as part of a flavonoid which is considered as a glucose transport inhibitor in the small intestine which is responsible for the absorption of glucose in the small intestine so that it can reduce blood sugar levels [29,30].

4. Conclusion
This study displayed that ethyl acetate fraction of Macaranga tanarius (L) Mull leaf extract. Arg. consists of phenolic compounds that potentially inhibit α-glucosidase enzyme. There is forceful correlation between the ability to inhibit α-glucosidase and the safety of the compounds contained in the ethyl acetate extract, which does not show its toxicity. Taken together, ethyl acetate extract from M. tanarius leaves potentially developed as a candidate for the prevention and treatment of diabetes. Further pre-clinical study using animal model is necessary to figure out potent anti-diabetic properties of M. tanarius leaves extract as anti-diabetic.

References
[1] Ramadhan R and Kusuma I W 2017 Samarinda: Mulawarman University Press pp 47-63
[2] Magaduila J J 2014 J. Med. Plant Res. 8(12) 489-503
[3] Darmawan, A, Suwarso, W P, Kosela S and Kardono L B S 2015 Ind. J. of Pharm. 26 52–6
[4] Primahana, G and Darmawan A 2017 J. Pure App. Chem. Res. 6(1) 22-6
[5] Fajriah S, Megawati and Darmawan A 2016 J. Life Sci. 6(1) 7-9
[6] Megawati, Saepudin E, Hanafi M, Darmawan A and Lotulung P D N 2015 Makara J. Sci. 19 96–100
[7] Megawati, Hanafi M, Saepudin E and Fajriah S 2016 J. Ilmu Kefarmasian Indonesia 14(1) 38-42
[8] Cai J, Zhao X L, Liu AW, Nian H and Zhang S H 2011 Phytomedicine 18 366-73
[9] Nayaka H B, Ramesh L. L, Madire K U and Asha T 2014 Int. J. Bacteriol. 2014 175851
[10] Cheynier V 2005 Am. J. Clin. Nutr. 81 223–9
[11] Takahashi T and Miyazawa M 2012 Phytother. Res. 26 722-6
[12] Mehta A, Zitzmann N, Rudd P M, Block T M and Dwek R A 1998 FEBS Lett. 430 17–22
[13] Du Z Y, et al 2006 Eur. J. Med. Chem. 41(2) 213-8
[14] Zhu Y, Yin L, Cheng Y and Yamaki K 2008 Food Chem. 109 737–42
[15] Yang Z, Wang Y C, Wang Y and Zhang Y 2012 Food Chem. 13 617-25
[16] Zhang L, Bai B, Liu X, Wang Y, Li M. and Zhao D 2011 Food Chem. 126 203-6
[17] Ye X P, Song C Q, Yuan P and Mao R G 2001 Chin. J. Nat. Med. 8(5) 0349-52.
[18] Ieyama T, Puteri M D P T G and Kawabata J 2011 Food Chem. 128 308-11
[19] Lee S S, Lin H C and Chen C K 2008 Phytochem. 69 2347-53
[20] Lee D S and Lee S H 2001 FEBS Lett. 501 84-6
[21] Fajriah S, Darmawan A, Megawati and Hanafi M 2018 Res. J. Chem. Environ. 22 (Special Issue II) 114-9
[22] Kaur C and Kapoor H C 2002 J. Food Sci. Technol. 37 153-61
[23] Meyer B N, Ferrigni N R, Putnam J E, Jacobsen L B, Nichols D E and McLaughlin 1982 J. Plant Med. 45 31-4
[24] Miller K A and Liu D 2017 Rotary Evaporator Standard Operating Procedure Department of Material Science eng Engineering. Becman Institute p 1
[25] I Made A G and Rasmaya N 2006 General toxicology. F. Pharmacy Pharmacy of Udayana University: Edition II. Bali.
[26] Jazilah N, Fasya N and Abtokhi 2014 Alchemy 3(2) 118-24
[27] Farihah 2006 *Uji Toksisitas Ekstrak Daun Ficus benjamina L terhadap Artemia salina Leach dan Profil Kromatografi Lapis Tipis*. Skripsi Diterbitkan. Surakarta: Fakultas Farmasi Universitas Muhammadiyah Surakarta.

[28] Ghasemzadeh A dan Ghasemzadeh N 2011 *Med. Plants Res.* 5(31) 6697-703

[29] Ratya A 2014 *J. Agromed. Unila*. 1(1) 61-6

[30] Sasmita F W, Eko S, Husamah dan Yuni P 2017 *Biosfera*. 34 22-31