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Original Article

ALPHA-GLUCOSIDASE INHIBITOR ACTIVITIES AND PHYTOCHEMICALS SCREENING OF THE PEPEROMIA GENUS CULTIVATED IN INDONESIA

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ABSTRACT

Objective: Peperomia is a genus belong to Piperaceae family, which is valuable as ornamental and has several medical uses but not widely explored in their pharmacological activities. Some peperomia plant has been investigated and reported to have various activities, recently as diabetes mellitus. This research was conducted to screening phytochemical profile and to determine alpha-glucosidase inhibitor activities of five species in genus Peperomia that are easy to grow and has been cultivated in Indonesia.

Methods: Dried leaves were macerated with 70% ethanol and vaporized by rotary evaporator. Phytochemical screening was conducted using qualitative chemical analysis and inhibition of alpha-glucosidase was conducted using p-nitrophenyl-α-D-glucopyranoside as substrate, and absorbance was measured with a spectrophotometer UV-Vis.

Results: The phytochemical screening of the leaves extracts demonstrated the presence of various secondary metabolites, such as flavonoids, phenol, tannins, quinone, alkaloids, saponins, steroids, and triterpenoids. The inhibition of alpha-glucosidase showed that the IC_{50} value of ethanol extract of *P. obtusifolia*, *P. clusiifolia*, *P. caperata* (green), *P. caperata* (red), and *P. argyreia* leaves were 2.90; 18.05; 21.46; 23.81; and 48.70 µg/ml respectively.

Conclusion: The highest inhibition of alpha-glucosidase activity was showed by *P. obtusifolia* with an IC_{50} value of 2.90 µg/ml. Further research is needed to explore its potential as an antidiabetic.

Keywords: Alpha-glucosidase inhibitor, Phytochemical screening, Peperomia

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INTRODUCTION

Peperomia is the second largest genus group after Piper in the Piperaceae family. Peperomia has about 1500 species distributed in the tropics and subtropics spread across the Americas, East Florida, and Asia [1, 2]. Peperomia contribute for 15% of the chemical content information in the Piperaceae family; nevertheless, more than 200 compounds of the Peperomia species have been described and can be classified as chalcones, phenylpropanoids, lignans, terpenoids, monoterpenoids, chromanes, flavonoids, polyketides, and amides [3].

Some Peperomia plants have been applied in traditional medicine. Traditional Chinese medicine mentions that *P. dindygulensis* is used to cure cough, respiratory disorders, and stomach, lung, and kidney cancers [4]. *P. sui* found in Taiwan has activity as an antinfluenza by increasing the viability of cells infected with the H6N1 virus [5]. *P. obtusifolia* has been investigated as antifungal, antibacterial and anti-inflammatory [6–8]. In Indonesia, *P. pellucida* is known as sasaladaan or herba suruhan is used traditionally to lower blood sugar levels, treat fever, bruises, and skin diseases. Previous studies have been conducted on *P. pellucida* as an antidiabetic herbal remedy [9, 10]. *P. pellucida* has inhibitory activity against alpha-amylase with an IC_{50} value of 6.950 µg/ml and alpha-glucosidase inhibitory activities of *P. pellucida* ethyl acetate extract at 500 ppm were 28.19% [11, 12]. Herbaceous ethyl acetate extract of *P. pellucida* can reduce blood glucose levels by 56.32%. Then in further research found peperochromen A and 8,9-dimethoxy ellagic acid as active antidiabetic compounds [13, 14]. Natural resources provide a variety of chemical compounds where potential therapeutic agents can be traced by targeted bioactive screening [15].

Chemical compounds from the genus Peperomia allow this plant to contribute research for antidiabetic chemical compounds [3]. Therefore, more information is needed regarding the use of plants of the genus Peperomia in the pharmaceutical field in the future. Based on chemotaxonomy (the theory of kinship through a systematic approach to plants), plants with the same family generally have similar chemical compounds so they may just have the same potential for the treatment of disease [16]. Some plants of the genus Peperomia such as *P. pellucida*, *P. obtusifolia*, *P. clusiifolia*, *P. argyreia,* and *P. caperata* were found in Indonesian to be investigated for phytochemical screening qualitative chemical analysis and determine their alpha-glucosidase inhibitor activity using p-nitrophenyl-α-D-glucopyranoside as a substrate and absorbance was measured with a spectrophotometer UV-Vis.

MATERIALS AND METHODS

Plant material

P. obtusifolia, P. clusiifolia, *P. argyreia*, *P. caperata* red, and *P. caperata* green leaves taken from Kampung Pasir Kunci, Bandung, West Java. The plant identification was determined in the Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia.

Chemical materials

The materials used were 70% ethanol, alpha-glucosidase enzyme (*Saccharomyces cerevisiae*-Sigma Aldrich), p-nitrophenyl-α-D-glucopyranoside (Sigma Aldrich), Na_2HPO_4, NaH_2PO_4, Acarbose and reagents for phytochemical screening.

Extraction

Dried leaves *P. obtusifolia, P. clusiifolia*, *P. argyreia*, *P. caperata* red and *P. caperata* green were extracted by maceration method using 70% ethanol solvent at room temperature and protected from light. This extraction was repeated three times until most of the chemical components were extracted. All extracts were evaporated at 50 °C and 60 rpm with a rotary evaporator and obtained concentrated ethanol extract.

Phytochemical analysis

Phytochemical screening was conducted by qualitative chemical analysis. The different phytochemical compounds present in the

leaves extracts were carried out using chemical methods [17–19] described as the following:

Flavonoid test

Each sample (2 ml) was mixed with 500 mg of Magnesium powder and 1 ml of concentrated hydrochloride acid. The development of pink, red or yellow color indicated the presence of flavonoid.

Phenolic test

Each sample (0.2 g) was mixed with 5 ml of distilled water and a few drops of 1% ferric chloride solution. The presence of phenolic compounds was suggested by the development of a dark blue or green color.

Tannins test

Each sample (0.2 g) was mixed with 5 ml of distilled water and mixed with 2 ml 1% gelatin in 10% sodium chloride. Presence of a precipitate indicated the occurrence of tannins.

Quinone test

Each sample (0.2 g) was mixed with 5 ml of distilled water and a few drops of concentrated sodium chloride. The intense red color will disappear when a concentrated hydrochloride acid is added indicating a positive result of the quinone.

Alkaloids test

Each sample (1 g) was mixed with 2 ml 10% ammonia and added 4 ml chloroform. The chloroform solution is acidified with 2 ml hydrochloric acid. The acid layer is separated and then added with a few drops of Dragendorff reagent. The presence of a reddish-brown color indicates positive alkaloids.

Saponins test

Each sample (0.2 g) was mixed with 5 ml distilled water and shaken vigorously for 10 s. the development of persistent foam indicated the presence of saponins

Triterpenoid test

Each sample (0.5 g) was dissolved in 5 ml chloroform and then filtered. The filtrate was mixed with a few drops of Liebermann-Burchard reagent. A positive result of triterpenoids is indicated by a dark red color.

Steroid tests

Each sample (0.5 g) was added with 10 ml of chloroform and then filtered. The filtrate was mixed with 2 ml of concentrated anhydrous acetate and a few drops of concentrated sulfuric acid. The formation of a green ring on the test tube indicates the presence of steroids.

Alpha-glucosidase inhibition activity

Alpha-glucosidase inhibition activity of five Peperomia leaves ethanol extract was performed using the method described by Amiri *et al.* (2015) with some modifications. A total of 10 µl samples with concentration variations were premixed with 690 µl of phosphate buffer (pH 6.8) and 200 µl alpha-glucosidase (0.2 units/µl) and then incubated at 39 °C for 5 min. After incubation, 100 µl of the p-nitrophenyl-α-D-glucopyranoside solution was added and then incubated at 39 °C for 30 min. The alpha-glucosidase activity was determined at a wavelength of 400 nm on a UV-Vis spectrophotometer (Shimadzu 1800, Japan) by measuring the quantity of p-nitrophenol released from p-NPG. Acarbose was used as the positive control. The variation concentration of the leaves ethanol extracts required inhibiting 50% of alpha-glucosidase activity under the assay conditions. The inhibition percentages of alpha-glucosidase were assessed by the following:

$$\% \text{ Inhibition} = [(\text{AbsBlank} - \text{AbsSamples})/\text{AbsBlank}] \times 100$$

The inhibitory percent of alpha-glucosidase was plotted against sample concentration and a linear regression curve was obtained in order to calculate the IC_{50} value which is the concentration of sample (µl/ml) necessary to decrease the absorbance of alpha-glucosidase was defined as the IC_{50} value.

RESULTS

Five different of plants were taxonomically determined at Plants Taxonomy Laboratorium, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran collection-number 110-114/HB/01/2020. Five plants identified as *Peperomia clusiifolia*, *Peperomia caperata* (green), *Peperomia caperata* (red), *Peperomia obtusifolia*, and *Peperomia argyreia* from Piperaceae family.

Dried leaves were macerated with 70% ethanol and the yields were *P. obtusifolia* (31.10% or 70.89 g); *P. clusiifolia* (29.53% or 7.74 g); *P. argyreia* (30.03% or 20.32 g); *P. caperata* red (29.73% or 2.70 g) and *P. caperata* green (23.39% or 2.77 g). The results of phytochemical screening were identified by the presence of flavonoids, phenol, tannins, quinone, alkaloids, saponins, steroids and triterpenoids (table 1).

The alpha-glucosidase inhibitory activity of five Peperomia sp leaves extracts against alpha-glucosidase were determined using p-nitrophenyl-α-D-glucopyranoside as a substrate and these were compared to Acarbose as control positive. The inhibition (%) of alpha-glucosidases for each concentration (sample and positive control) then made a graph between % inhibition and concentration and obtain the linear regression equation. Linear regression equation was then used to obtain the IC_{50} value of each sample (fig. 1-6). The results of the IC_{50} (table 2) revealed that Peperomia sp leaves ethanol extracts have potential to inhibit alpha-glucosidase. *P. obtusifolia* leaves ethanol extract showed the highest inhibitory activity against alpha-glucosidase enzyme with an IC_{50} value was of 2.90 µg/ml.

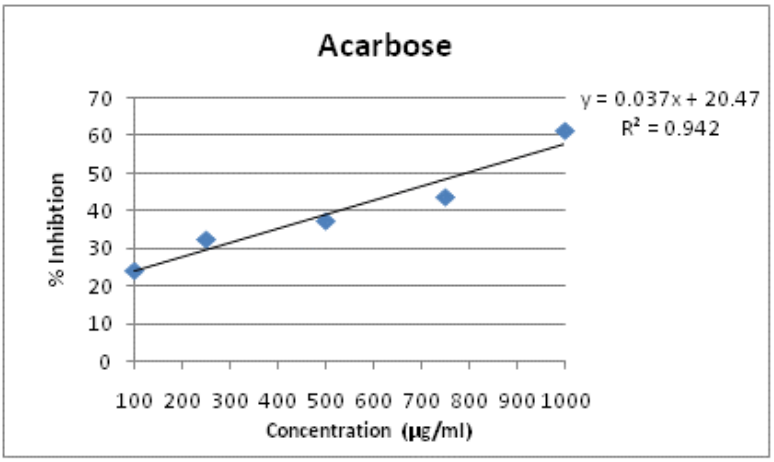

Fig. 1: Inhibition (%) of Acarbose as positive control

Table 1: Phytochemical screening of species in genus Peperomia cultivated in Indonesia

Phytochemical compound	*P. obtusifolia*	*P. clusiifolia*	*P. argyreia*	*P. caperata* (red)	*P. caperata* (green)
Flavonoid	+	+	+	+	+
Phenol	+	+	+	+	+
Tannin	+	+	-	-	-
Quinone	+	+	+	+	+
Alkaloid	-	-	+	-	+
Saponin	+	+	+	+	+
Triterpenoid	-	+	+	-	-
Steroid	+	+	-	-	-

(+) detected; (-) not detected

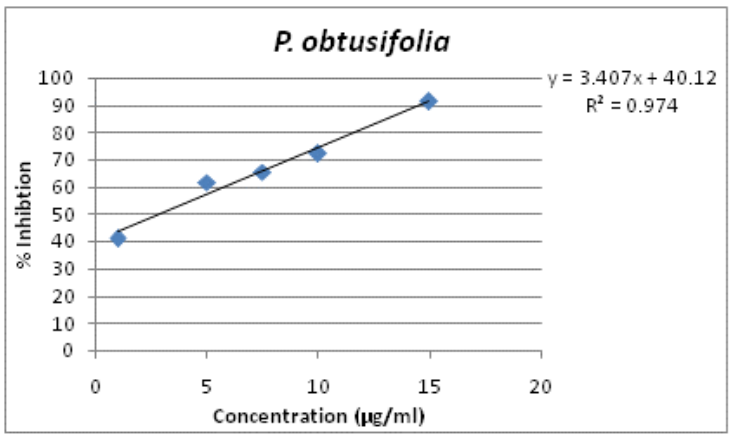

Fig. 2: Inhibition (%) of *P. obtusifolia* leaves ethanol extract

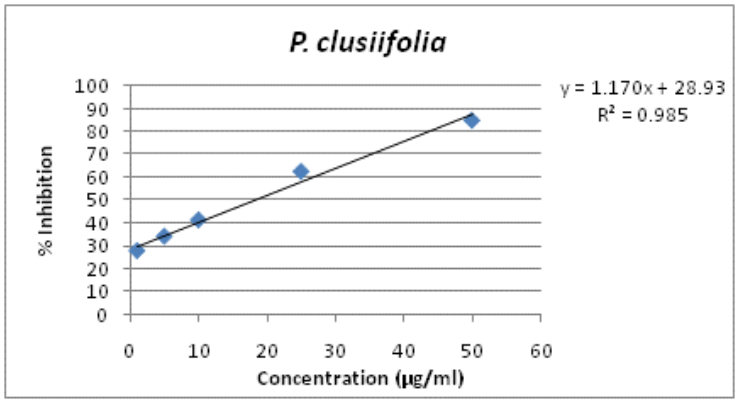

Fig. 3: Inhibition (%) of *P. clusiifolia* leaves ethanol extract

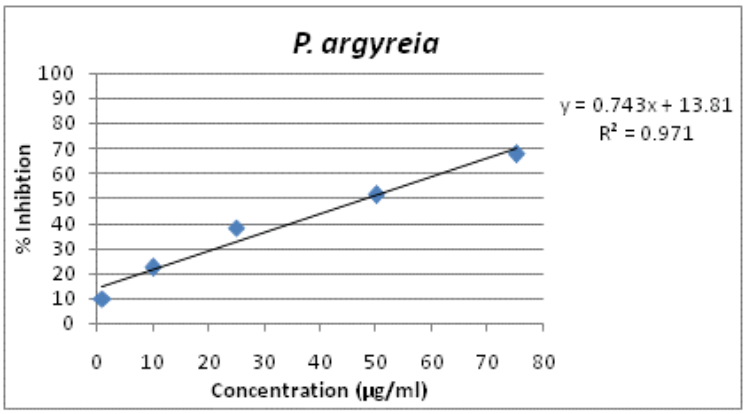

Fig. 4: Inhibition (%) of *P. argyreia* leaves ethanol extract

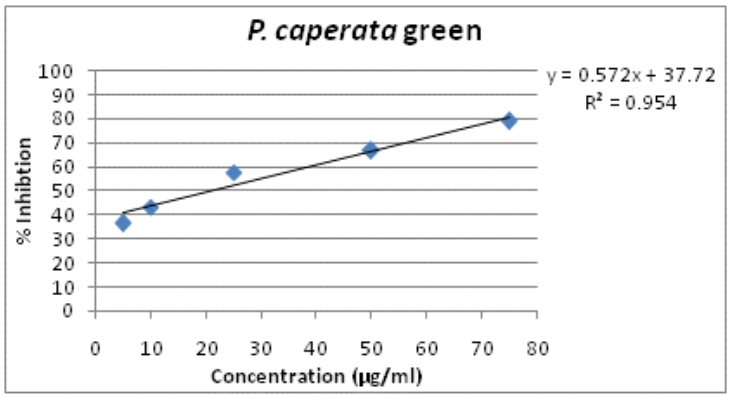

Fig. 5: Inhibition (%) of green *P. caperata* leaves ethanol extract

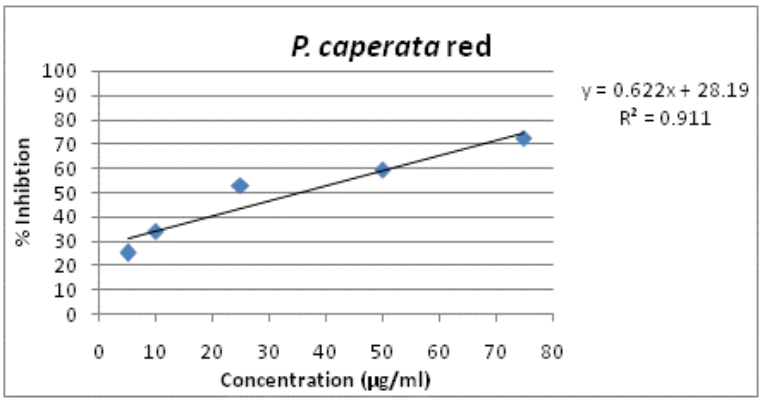

Fig. 6: Inhibition (%) of red *P. caperata* leaves ethanol extract

Table 2: IC_{50} of Peperomia sp extracts against alpha-glucosidase enzyme

Sample	IC_{50} (µg/ml)
Acarbose	798.10
P. obtusifolia	2.90
P. clusiifolia	18.05
P. argyreia	48.70
P. caperata (green)	21.46
P. caperata (red)	23.81

DISCUSSION

Determination of the plant was needed to obtain taxonomy information from Peperomia species. The result of the determination showed that plant species used in this study was *P. pellucida*, *P. obtusifolia*, *P. clusiifolia*, *P. argyreia*, and *P. caperata* from Peperomia genus, and belong to Piperaceae family. Although it is said that the plant species of the genus Peperomia are the second largest after the genus Piper, and it is known that there are around 1500 species, they are still wild plants. In Indonesia it is known that there are 5 species that are commonly found because they have been cultivated as ornamental plants [3, 33].

The leaves of the plant were subjected to extraction with maceration. Maceration is chosen because of suitable for all secondary metabolites and high yield. Maceration conducted with an ethanol solvent to extract all secondary metabolites. Maceration works by molecular diffusion until equilibrium has been reached [20]. After all extract was concentrated, phytochemical screening was conducted to identify secondary metabolites in the plant using many chemical reagents. Phytochemical compound from Peperomia species have been described and can be classified as chalcones, phenyl propanoids, lignans, terpenoids, meroterpenoids, chromanes, flavonoids, polyketides, and amides [3]. In the study five ethanol extract presence of flavonoids, phenols, quinone, and saponins (table 1). Recently new phenolic compound from *P. obtusifolia* has been reported; they are peperomic ester and peperoside [21]. Tannin and steroid were present in *P. obtusifolia* and *P. clusiifolia* leaves ethanol extract. The presence of alkaloid was showed in *P. argyreia* and *P. caperata* (green). Triterpenoid was the presence in *P. clusiifolia* and *P. argyreia*. Theres not many information was obtained regarding of secondary metabolites from *P. argyreia*, *P. clusiifolia*, and *P. caperata*. In 2016, Gutierrez *et al.* described there were 34 species of the genus Peperomia that have been studied; this number is very small, looking at the number of Peperomia species which is around 1600 species. Likely due to the small size of these plants, this could preclude the large-scale phytochemical studies [3].

Inhibitors of alpha-glucosidase are drugs that are useful for lowering glucose levels in diabetes mellitus patients by inhibiting the hydrolysis of complex carbohydrates after meals. Acarbose is an alpha-glucosidase inhibitor class drug that is often used in diabetes mellitus therapy. Acarbose binds to various amino acids located in the catalic region of the enzyme and inhibits catalysis activity in disaccharides and oligosaccharides [22]. The alpha-glucosidase inhibitory activity of five Peperomia leaves ethanol extracts has been determined using colorimetric assay by Spectrophotometer UV-Vis. The principle of this method is the hydrolysis of the *p*-nitrophenyl-α-D-glucopyranoside substrate by the enzyme alpha-glucosidase to form α-D-and p-nitrophenol (yellow color), which the absorbance of p-nitrophenol was measured using a UV-Vis spectrophotometer at a wavelength of 400 nm [16, 23, 24].

The IC_{50} value for five Peperomia leaves ethanol extracts is in the range of 2.90–48.70 µg/ml. *P. obtusifolia* leaves ethanol extract with a concentration of 2.90 µg/ml showed a strong inhibition of the

enzyme alpha-glucosidase, while *P. clusiifolia*, *P. caperata* green, *P. caperata* red and *P. argyreia* showed moderate inhibition. The IC_{50} values of Acarbose showed a concentration of 798.108 µg/ml indicating weak inhibition of the enzyme alpha–glucosidase [25, 26]. Based on IC_{50} values, a concentration of<17 µg/ml is classified as a strong inhibitor [27].

P. obtusifolia has the highest IC_{50} value to inhibit activity of alpha-glucosidase. The phytochemical compound of *P. obtusifolia* has been carried out and is classified as flavonoid, lignan, chromene, khalcon, sesquiterpene and monoterpenes [8, 28–30]. In this research, *P. obtusifolia* presence of flavonoid, phenolic, tannin, quinone, saponin, and steroid compounds. Flavonoid group compounds have a fairly strong activity in inhibiting alpha-glucosidase. The high inhibitory activity of flavonoid compounds is due to the presence of a dihydroxy group in C-3' and C-4' in the flavonoid group. The presence of hydroxy groups in flavonoid compounds can effectively bind to the active sites of alpha-glucosidase. The existence of a dihydroxy group on ring B contributes to conducting electron clouds so that they can contribute hydrogen atoms to form hydrogen bonds with active sites of alpha-glucosidase [31]. Inhibitor of alpha-glucosidase activity of Acarbose was lower than ethanol extracts. This result is related to chemical compounds in the extract that can inhibit alpha-glucosidase synergistically [24]. In the other side, the homologous structure of the enzyme alpha-glucosidase of *Saccharomyces cerevisiae* is known to be different from the alpha-glucosidase enzyme found in mammals. The alpha-glucosidase inhibitors such as acarbose and voglibose inhibit the alpha-glucosidase enzyme obtained from rats, rabbits, and pig intestines but have a weak effect on inhibition of the enzyme alpha-glucosidase obtained from *Saccharomyces cerevisiae*. The use of isoforms alpha-glucosidase derived from *Saccharomyces cerevisiae* allows for easier *in vivo* testing and is a comparison for human variants [32].

CONCLUSION

Phytochemical screening obtained secondary metabolites profiles of five species from genus Peperomia such as flavonoids, phenolics, tannins, quinones, alkaloids, saponins, steroids and triterpenoids. *P. obtusifolia, P. clusiifolia, P. caperata* (green), *P. caperata* (red), and *P. argyreia* can inhibit alpha-glucosidase with IC_{50} value of 2.90, 18.05, 21.46, 23.81, and 48.70 µg/ml, respectively. *P. obtusifolia* extract shows the highest inhibition with concentration 2.90 µg/ml. Further, bioassay-guided discovery of alpha-glucosidase inhibitors and *in vivo* research are required to confirm the present observations. Findings on the active substances contained in the extract and *in vivo* studies are necessary to recognize a potential *P. obtusifolia* in the therapy of diabetes and other related disorders.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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