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Inhibition Activity of Leaves, Flower and Root Extracts of Ruellia tuberosa L on α-Glucosidase Enzymes

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Abstract

Previous study stated that the herba of *Ruellia tuberosa* L has inhibition activity on α-glucosidase enzymes. The leaves, flowers, and roots of *Ruellia tuberosa* L contain flavonoids but have different activities. This study aims to determine the inhibitory activity by the leaves, flowers and roots of *Ruellia tuberosa* on the α-glucosidase enzyme. Extraction was performed by maceration method using ethanol 96% as solvent. Inhibition activity was measured based on the formation of a yellow solution of p-nitrophenol compound as a result of the reaction between p-nitrophenyl-α-D-glucopyranoside (as substrate) and α-glucosidase enzyme at a wavelength of 405 nm. The system reaction of inhibition study is divided into two reactions, namely S0 (contains substrates without enzyme) and S1 (contain substrate and enzyme). The results showed that inhibition activities of leaves, flower and root extract were 9.97 ± 4.97 % at 7.8125 µg/mL concentration of extract; 13.98 ± 5.18 % at 7.8125 µg/mL concentration of extract; 99.08 ± 0.12 % at 31.25 µg/mL concentration of extract, respectively. Based on data, the extract of leaves, flowers and roots of *Ruellia tuberosa* L. has the potential as an inhibitor of the enzyme α-glucosidase.

Keywords: α-glucosidase; leaves; root; flower; extract Ruellia tuberosa L

INTRODUCTION

Diabetes mellitus is a chronic disease characterized by a decrease in the production of insulin produced by the pancreas so that insulin which functions as a hormone that regulates the balance of blood sugar levels in the body does not work effectively and causes an increase in the concentration of glucose in the blood (hyperglycemia). According to the latest IDF estimate, there were 382 million people living with diabetes in 2013. A total of 6.9% of the 176689336 population aged over 15 years in Indonesia suffer from diabetes mellitus and it increases with age. The number of people with diabetes mellitus 90% is type 2 diabetes mellitus (Kemenkes RI, 2014).

One of the most important efforts in the management of type 2 diabetes mellitus is controlling blood sugar levels after eating, by slowing down the breakdown of

carbohydrates into glucose so as not to cause an increase in blood glucose concentrations in a short time (postprandial hyperglycemic) (Li et al., 2005). The enzyme -glucosidase is an enzyme that mediates the process of breaking down carbohydrates into glucose. To slow down the glucose breakdown process in a short time, an -glucosidase enzyme inhibitor agent is needed (Loranza, 2012).

Amylase and α-glucosidase inhibitors compounds which are already on the market and used for the treatment of type 2 diabetes mellitus area acarbose and miglitol, marketed as Glucobay® and Glyset®. However, these drugs have various side effects (Feng et al., 2011), such as bloating, nausea, diarrhea, and increased diabetes complications (Nampoothiri et al., 2011). Therefore, it is necessary to find alternative treatments that are effective, efficient and have minor side effects.

Ruellia tuberosa L. reported contains flavonoids and has the potential to be developed as an antihyperglycemic agent, especially on a inhibition of α-glucosidase enzyme (Rajan et al., 2012). The extract of the ethyl acetate fraction of the *Ruellia tuberosa* L. was reported to have antidiabetic activity in rabbits models induced by alloxan (Shahwara et al., 2011). It has also been reported that 70% ethanol extract could inhibit the α-glucosidase enzyme with an IC_{50} value of 83.23 µg/mL (Amri, 2014). Likewise, the ethanolic extract of *R. tuberosa* L leaves has been reported to have inhibitory activity of the α-glucosidase enzyme with an IC_{50} value of 98.5 µg/mL (Mun'im et al., 2013). Flower parts containing flavonoids have antioxidant activity using DPPH assay with %inhibitor of 28 ± 0.82% (Vankar and Srivastava, 2010). In addition, the roots contain higher content of flavonoids and phenolics which have been reported as antidiabetic activity in rats induced by streptozotocin. Moreover, it could lower MDA (Malondialdehyde) levels from 3.5 ± 0.3 g/mL to 1.7 ± 0.4 g/mL. It has been proved to help in recovery of liver impairments (Laily Kurniawati et al., 2018). Although the leaves, flowers, and roots of *R. tuberosa* L contain flavonoids (Chothani and Mishra, 2012; Chothani et al., 2010), they have different activities. This research was to determine activity of leaves, flowers and roots of *R tuberosa* L on inhibition of α-glucosidase enzyme.

METHODS

The materials used in this study consisted of a 96-well microplate (Iwaki), ELISA reader (ELX 800 Bio Tech), and glassware, *Ruellia tuberosa* L herbs taken in the district of Sukoharjo, α-glucosidase enzyme (Sigma Aldrich), phosphate buffer (pH 6.8), p-nitrophenyl glucopyranoside (Sigma Aldrich), Na_2CO_3(Merck).

1. Extraction

Leaves, flowers, and roots of *R tuberosa* were soaked in 96% ethanol as much as 7.5 times the weight of the simplicia powder. The liquid extract obtained then evaporated to reduce the amount of solvent. Liquid extract then stands in a water bath until a thick extract is obtained.

2. Flavonoid Identification Test

A solution of 2 mL of the extract was added to the Mg plate and then 5 drops of concentrated HCl were added. The flavonoid compounds contained in the extract will produce a red to orange color (Wilstater Method).

3. Sample Solution Preparation

Each of the ethanol extracts of leaves, flowers, and roots of purple gold leaf was weighed as much as 100 mg which was then dissolved with DMSO up to 5 mL so that the concentration of the mother liquor sample was 2%.

4. Enzyme Inhibition Test

The inhibitory activity of the α-glucosidase enzyme performed followed table 1. Mix 49 L of phosphate buffer pH 6.8 and 25 L of the enzyme α-glucosidase 0.005 U/ml then add 1 L of sample and incubate at 37°C for 15 minutes, then add 25 L of 1 mM pNPG and incubate at 37°C for 30 minutes. The reaction was stopped by adding 100 µL of 0.1 M Na_2CO_3 (Ankita Bachhawat et al., 2011). Yellow color formed then measured absorbance by ELISA reader at 405 nm. Replication was carried out for 3 times.

Table 1. Reaction systems of inhibition activity of samples on α-glucosidase enzyme

Materials	Blank (µL)	Control (µL)	S0 (µL)	S1 (µL)
Extracts	-	-	1	1
DMSO	1	1	-	-
Buffer	49	49	49	49
Enzyme	-	25	-	25
Incubated on waterbath at 37°C for 15 minutes				
Substrate	25	25	25	25
Buffer	25	-	25	-
Incubated on waterbath at 37°C for 30 minutes				
Na_2CO_3	100	100	100	100

Note:
S1 : absorbance of sample with enzyme
S0 : absorbance of sample without enzyme

5. Preparation of Acarbose Solution

Acarbose tablet powder equivalent to 250 mg was dissolved in phosphate buffer pH 6.8 and 2N HCl (1:1) in 5 ml then centrifuged and the supernatant was taken so that the concentration of the mother liquor sample was obtained at 5%.

6. Enzyme Inhibition Test

Absorbance data was calculated using equation (1) to determine inhibition activity.

$$\text{Inhibition activity (\%)} = \frac{(Cp-NP\ control)-(Cp-NP\ Samples)}{Cp-NP\ control} x100 \qquad (1)$$

Note: Cp-NP control= concentration of p—NP without sample; Cp-NP samples = S1-S0

Furthermore, from the data above, as the x-axis is the concentration of p-nitrophenol and % inhibition as the y-axis, so that the linear regression results will be obtained y = a + bx which is used to calculate IC_{50}. using equation (2):

$$IC_{50} = \frac{(50-b)}{a} x\ 100\% \qquad (2)$$

RESULTS AND DISCUSSION

Maceration is a cold extraction method which is carried out by immersing simplicia in an appropriate solvent with stirring or shaking at room temperature (MOH RI, 2000). The extraction results obtained 39.43 grams of thick leaf extract, 6.68 grams of thick flower extract and 1.30 grams of thick root extract.

Table 2. Extraction Yields of Samples of *R. tuberosa L*

No	Sample	Yield (%)
1	**Leaves**	49.29
2	**Flower**	8.68
3	**Root**	3.90

The extraction process in this study used 96% ethanol as a solvent because it was able to attract semi-polar compounds such as flavonoids and generally produced high yields (Saifudin, 2014). Table 2 shows that the highest extract yield was leaves with a yield of 42.29% and the lowest yield was roots with a yield of 3.90%.

Flavonoid Identification Test

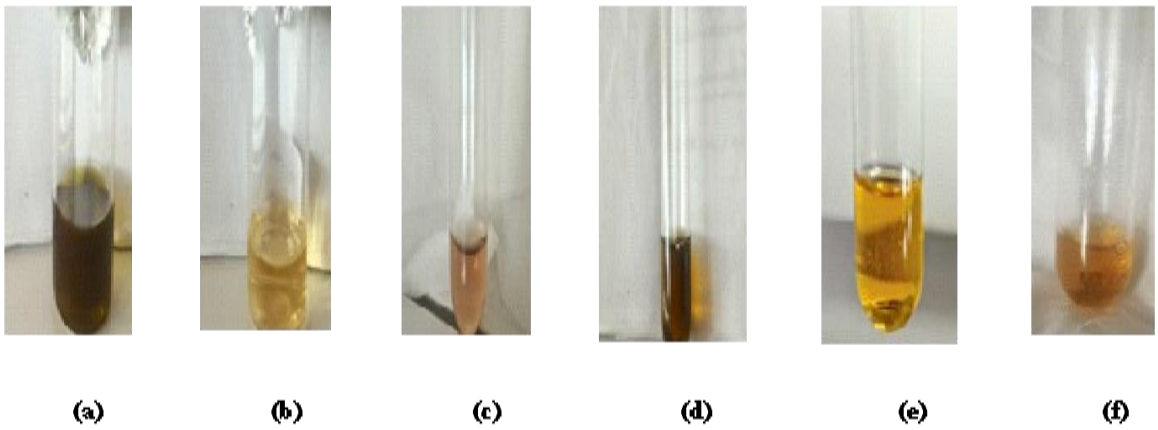

Figure 2. Flavonoid test of samples, a-c. before reaction [a. Leaves; b. flower; c. root extract], d-f. after reaction [red-orange color formed as positive reaction]

In this study, only flavonoid identification tests were carried out due to flavonoid compounds are the most common compounds found in natural materials, especially plants and in other studies stated that flavonoids are compounds that can inhibit α-glucosidase and α-amylase enzymes (Tadera et al., 2006).

α -Glucosidase Enzyme Activity Inhibition

The inhibitory activity of the α -glucosidase enzyme performed in vitro through an enzymatic reaction using p-nitrophenyl-α-D-glucopyranose (p-NPG) as a substrate. The α-glucosidase enzyme will hydrolyze p-NPG into yellow -D-glucose and p-nitrophenol (Fig. 3).

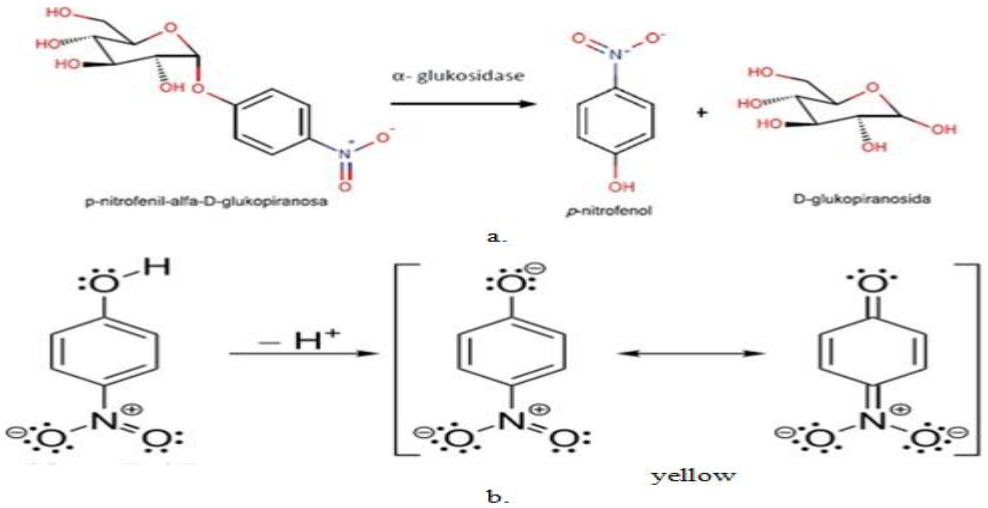

Figure 3. Enzymatic reaction of α-glucosidase and p-nitrophenyl- α-D-glucopyranoside (a), Resonance of chromophore of p-NP which formed yellow color

The yellow color change that occurs above is due to a bathochromic shift caused by the -glucosidase enzyme that hydrolyzes p-NPG resulting in the π→ π* electron transition, the intensity of which is higher so that the chromophore groups (phenol and -NO_2) formed due to hydrolysis are p-nitrophenol which in alkaline conditions becomes anion yellow and sensitive to light intensity. The higher the inhibitory power of an inhibitor, the less p-nitrophenol formed, it can be seen through the color produced by p-nitrophenol which is fading with the presence of obstacles in the formation of p-nitrophenol (Najib et al., 2011). The standard p-nitrophenol curve (Fig. 4) was used as an equation to calculate the p-NP formed from the enzymatic reaction.

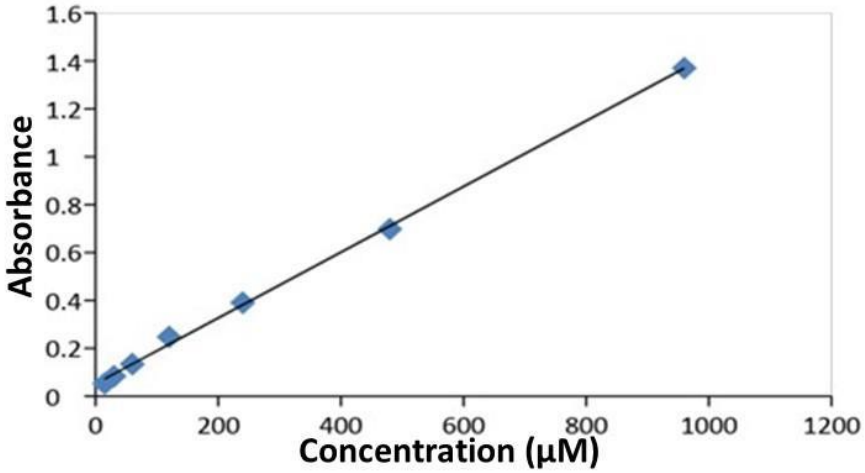

Figure 4. Graphic relation of Concentration of p-NP (x-axis) vs absorbance (y-axis)

Linear regression equation obtained was y = 0.0014x + 0.0516 with an r^2 value of 0.9987. This shows that there is a strong relationship between the concentration of p-NPG vs absorbance, because the value of r^2 is close to 1. Furthermore, based on b (slope) value, it can be concluded that this method is less sensitive, as well as the selectivity of this method is not appropriate due to intercept value being relatively high compared to slopes.

The inhibitory study reaction system was divided into two reactions, namely S0 and S1 (Table 1). S0 is a reaction using a substrate without an enzyme as well as a correction of the absorbance of the extract and S1 is a reaction using a substrate and an enzyme. The percentage of inhibitory power obtained in this study fluctuated so that the IC_{50} value was not calculated. Theoretically, the higher the concentration of the extract, the greater the inhibitory power of an inhibitor so that less p-nitrophenol is formed. Therefore, a point that has a consistent pattern is chosen as indicated by a small standard deviation value (Table 3).

Table 3. Inhibition Activity (%) of Extracts on α-glucosidase enzyme (n=3)

Samples	Inhibition activity (%) (x ± SD)	Concentration (ppm)
Leaves extract	9.97 ± 4.97	7.81
Flower extract	13.98 ± 5.18	7.81
Root Extract	99.08 ± 0.12	31.25
Acarbose	78.02 ± 0.56	3.125

Amri (2014) result showed that the ethanolic extract of *R. tuberosa* L have IC_{50} value of 83.23 µg/mL, in line with Mun'im et al (2013) stated that ethanolic extract of *R. tuberosa L* leaves has inhibitory activity on α-glucosidase enzyme with an IC_{50} value of 98.5 µg/mL. Based on the data above and previous studies, although the extracts of leaves, flowers, and roots of *R. tuberosa* L IC_{50} value was not obtained, but it was showed potential as inhibitors of the α-glucosidase enzyme.

CONCLUSION

Extracts of leaves, flowers, and roots of *Ruellia tuberosa L*. have potential as α-glucosidase enzyme inhibitors.

REFERENCES

Amri A.D.F., 2014, Uji aktivitas antidiabetes dari ekstrak etanol 70% tumbuhan pecah beling hutan(Ruellia tuberosa L.) menggunakan metode penghambatan enzim α-glukosidase secara In Vitro, Skripsi. Fakultas Kedokteran dan Ilmu Kesehatan. Program Studi Farmasi. UIN Syarief Hidayatullah, Jakarta.

Ankita Bachhawat J., Shihabudeen M.S. and Thirumurugan K., 2011, Screening of Fifteen Indian ayurvedic plants for alpha-glucosidase inhibitory activity and enzyme kinetics, International Journal of Pharmacy and Pharmaceutical Sciences, 3 (SUPPL. 4), 267–274.

Chothani D.L. and Mishra S.H., 2012, In vitro antioxidant activity of Ruellia tuberosa root extracts, Free Radicals and Antioxidants, 2 (4), 38–44.

Chothani D.L., Patel M.B., Mishira S.H. and Vaghasiya H.U., 2010, Review on Ruellia tuberosa (cracker plant), Pharmacognosy Journal, 2 (12), 506–512.

DEPKES RI, 2000, Parameter standar umum ekstrak tumbuhan obat, 1-68

Feng J., Yang X.W. and Wang R.F., 2011, Bio-assay guided isolation and identification of alpha-glucosidase inhibitors from the leaves of Aquilaria sinensis, Phytochemistry, 72 (2–3), 242–247.

Hanani E., Mun A. and Sekarini R., 2005, Identifikasi senyawa antioksidan dari kepulauan seribu, Majalah Ilmu Kefarmasian, II (3), 127–133.

Kemenkes RI, 2014, Situasi dan Analisis Diabetes, Pusat Data dan Informasi Kementerian Kesehatan RI, 2.

Laily Kurniawati A.N., Aulanni'am, Srihardyastutie A. and Safitri A., 2018, The Effects of Root Extract Ruellia tuberosa L on Histopathology and Malondialdehyde Levels on the Liver of Diabetic Rats, IOP Conference Series: Materials Science and Engineering, 299, 12022.

Li Y., Wen S., Kota B.P., Peng G., Li G.Q., Yamahara J. and Roufogalis B.D., 2005, Punica granatum flower extract, a potent α-glucosidase inhibitor, improves postprandial hyperglycemia in Zucker diabetic fatty rats, Journal of Ethnopharmacology, 99 (2), 239–244.

Loranza B., 2012, Uji Penghambatan Aktivitas Enzim Alfa-glukosidase dan Identifikasi Golongan Senyawa Kimia dari Fraksi Teraktif Daun Buni (Antidesma bunius L.), Skripsi. Fakultas Matematika dan Ilmu Pengetahuan. Program Studi Farmasi. Universitas Indonesia, Depok.

Mun'im A., Katrin, Azizahwati, Andriani A., Mahmudah K.F. and Mashita M., 2013, Screening of α- Glucosidase Inhibitory Activity of Some Indonesian Medicinal Plants, International J. Med. Arom. Plants, 3 (2), 144–150.

Najib A., Hartati S. and Elya B., 2011, In vitro bioassay of n-buthanol isolate of Acorus calamus L. on inhibitory of activity a-glucosidase, International Journal of PharmTech Research, 3 (4), 2085–2088.

Nampoothiri S. V., Prathapan A., Cherian O.L., Raghu K.G., Venugopalan V. V. and Sundaresan A., 2011, In vitro antioxidant and inhibitory potential of Terminalia bellerica and Emblica officinalis fruits against LDL oxidation and key enzymes linked to type 2 diabetes, Food and Chemical Toxicology, 49 (1), 125–131.

Rajan M., Kumar V.K., Kumar P.S., Swathi K.R. and Haritha S., 2012, Research Article Antidiabetic, antihyperlipidaemic and hepatoprotective activity of methanolic extract of Ruellia tuberosa Linn leaves in normal and alloxan induced diabetic rats, Journal of Chemical and Pharmaceutical Research, 4 (6), 2860–2868.

Saifudin A., 2014, Senyawa Alam Metabolit Sekunder Teori, Konsep, dan Teknik Pemurnian, 1st ed., deepublish, Yogyakarta.

Shahwara D., Ullah S., Ahmad M., Ullah S., Ahmad N. and Khan M.A., 2011, Hypoglycemic activity of Ruellia tuberosa linn (Acanthaceae) in normal and alloxan-induced diabetic rabbits, Iranian Journal of Pharmaceutical Sciences, 7 (2), 107–115.

Sugiwati S., Setiasih S. and Afifah E., 2009, Antihyperglycemic Activity of The Mahkota dewa [Phaleria macrocarpa (Scheff.) Boerl.] Leaf Extract As An Alpha-Glucosidase Inhibitor, J Logika, 13 (2), 74–78.

Tadera K., Inami Y.M., Akamatsu K.T. and Atsuoka T.M., 2006, Inhibition of Alpha-Glucosidase and Alpha-Amylase by Flavonoids, J Nutr Sci Vitaminol, 52, 149–153.

Vankar P.S. and Srivastava J., 2010, Evaluation of Anthocyanin Content in Red and Blue Flowers, International Journal of Food Engineering, 6(4), 1-13.