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In Vitro And *in Vivo* Antidiabetic Activity of Ethanol Extract and Fractions of *Hibiscus surattensis* L Leaves

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Abstract

Hisbiscus surattensis L the local name "tamoenju" is utilized by ethnic Sumari (Central Sulawesi) as an antidiabetic herbal, but definitive studies on efficacy and safety are lacking. Therefore, this study was aimed to investigate the antidiabetic activity of ethanolic extract of the leaves and fractions using in vitro and in vivo models. Ethanol extract (EE) of the leaves of *H. surattensi*s L was prepared by maceration. The ethanol extract was separated into three fractions by liquid/liquid extractions to yield n-hexane (HF), ethyl acetate (EAF) and water fraction (WF). Firstly, the extract and fractions were tested for its inhibitory activity on α-glucosidase in vitro. The results showed IC_{50} EE, HF and WF more than 5000 ppm while the IC_{50} fraction of EAF 3888.34 ppm. Then, oral glucose tolerance test (OGTT) of the extract/fractions were carried out in normal mice. On OGTT, the ethanol extract, EAF, and WF at doses of 150 and 300 mg/kg BW were able to inhibit increasing the level of glucose in the blood. The results showed that the extract and fraction of *H. surattensis* L leaves in vitro had no effect as α-glucosidase inhibitor but in vivo had the hypoglycemic effect especially on the fraction of ethyl acetate and the water fraction.

Keywords: α-glucosidase, antidiabetic, *Hibiscus suratttensis*, glucose tolerance.

Aktivitas Antidiabetes Ekstrak Etanol dan Fraksi *Hibiscus surattensis* L Leaves secara *in vitro* dan *in vivo*

Abstrak

Tanaman *Hisbiscus surattensis* L yang dikenal dengan nama "tamoenju" di desa Sumari (Sulawesi Tengah) telah diperkenalkan sebagai obat antidiabetes secara turun-temurun oleh masyarakat. Namun demikian belum ada penelitian ilmiah yang membuktikan tentang adanya efek dan keamanan tanaman tersebut. Oleh karena itu penelitian ini bertujuan untuk mengetahui efek antidiabetes ekstrak dan fraksi daun *H. surattensis* L secara in vitro dan in vivo. Ekstraksi dilakukan dengan metode maserasi menggunakan etanol 96% kemudian difraksinasi secara ekstraksi cair-cair (ECC) sehingga diperoleh fraksi n heksan (HF), etil asetat (EAF) dan air (WF). Uji antidiabetes secara in vitro ekstrak dan masing-masing fraksi dengan parameter penghambatan enzim α glukosidase sedangkan uji in vivo dilakukan dengan metode uji toleransi glukosa. Hasil menunjukkan IC_{50} ekstrak etanol, fraksi n heksan dan fraksi air lebih dari 5000 ppm sedangkan IC_{50} fraksi etil asetat 3888,34 ppm. Pada ada uji toleransi glukosa oral menunjukkan kelompok yang diberikan ekstrak etanol, fraksi etil asetat dan fraksi air dengan dosis 150 mg/kg BB dan 300 mg/kg BB memiliki kemampuan untuk menghambat kenaikan kadar glukosa darah akibat pemberian glukosa yang lebih baik dibandingkan fraksi n heksan. Hasil penelitian menunjukkan ekstrak dan fraksi daun *H. surattensis* L secara in vitro tidak mempunyai efek sebagai inhibitor α glukosidase namun secara in vivo memiliki efek hipoglikemik terutama pada fraksi etil asetat dan fraksi air.

Kata kunci: α-glukosidase, antidiabetes, *Hibiscus surattensis*, toleransi glukosa.

1. Introduction

Diabetes mellitus (DM) refers to a complex and diverse group of disorders that affect the metabolism of carbohydrates, lipids, and proteins. It is characterized by high blood glucose levels that result from an absolute or relative insulin deficiency or reduced sensitivity of the tissues to insulin[1].

Diabetes mellitus type 2 is one of the major's world health problems and has reached epidemic proportions[2]. The aim of oral therapy in type 2 diabetes is to reach normoglycemia to prevent later complications (retinopathy, nephropathy, neuropathy, and microangiopathy)[3].

α-Glucosidase inhibitors (AGIs) are among the available glucose-lowering medications. The α-glucosidase enzyme is located in the brush border of the small intestine and is required for the breakdown of carbohydrates to absorbable monosaccharides. The AGIs delay, but do not prevent, the absorption of ingested carbohydrates, reducing the postprandial glucose and insulin peaks[3].

The Oral Glucose Tolerance test (OGTT) measures the body's ability to use a type of sugar, called glucose that is the body's main source of energy. OGTT, a test of immense value and sentiment, in favor of using fasting plasma glucose concentration alone was seen as a practical attempt to simplify and facilitate the diagnosis of diabetes[4].

Because of the complex mechanism involved in DM, many of the current antidiabetic chemical agents have some limitations and even some severe adverse effects[5]. Therefore, these situations have encouraged the searches for alternative therapeutic agents from not only synthetic chemicals but also natural plants.

As a plant belonging to *Hibiscus* genus, *Hibiscus surattensis* L (Malvaceae), locally named as "Tamoenju", has long been used in the folk medicine to treat diabetes in Central Sulawesi, especially, in the area of Sumari ethnic. Previous studies have shown that the ethanolic leaves extract of *H. surattensis* L was also known to possessed potent antidiabetic.6 The constituents reported in ethanol extract of *H. surattensis* L leaves were alkaloids, flavonoids, tannins, steroids and triterpenoids, saponins and polyphenols[6]. Therefore, the present study was aimed to investigate the antidiabetic activity of ethanolic extract of the leaves and fractions using in vitro and in vivo models to ascertain their ethnobotanical uses.

2. Materials and Methods

2.1. Plant Material

Leaves of *H. surattensis* L were collected in January, 2017 from Alindau, Sindue Tobata, Central Sulawesi, Indonesia. The plants were identified by an herbarium staff from Departement of Biology, School of Life Science and Technology, Bandung Institute of Technology, Bandung, Indonesia.

2.2. Chemicals material

α-glucosidase from *S. cerevisiae* (Sigma-Aldrich, Singapore), substrate p-nitrophenyl-α-D-glucopyranoside (Sigma-Aldrich, Singapore), Sodium hydroxide, Potassium dihydrogen phosphate (Merck), Sodium carbonate (Merck), Bovine serum albumin (Sigma-Aldrich, Singapore), Acarbose (Glucobay® PT. Bayer Indonesia). All other chemicals used in the experiment of technical grade.

2.3. Equipment

Laboratory equipment which we used was Biotek Epoch Microplate Spectrophotometer. Blood glucose level was measured using One Touch Ultra Easy (LifeScan Inc.).

2.4. Animals

Male Swiss Webster mice (weight 25-30 g, age 2-3 month) were obtained from School of Pharmacy, Institute of Technology Bandung. Then, as a negative control of samples, the mixture of reagent above was used without enzyme and for positive control, the mixture of reagent without sample was used. The animals were maintained in an animal laboratory with a controlled environment, while all of animals had free access to food and water. On the day of experimentation, mice were fasted for 5 h (beginning at 7 AM),

and an OGTT was performed using a 3 g/kg glucose oral.

2.5. Methods

2.5.1. Plant extraction and fractionation

The dried leaves of *H. surattensis* L (1.3 Kg) were powdered and extracted with 96% ethanol by maceration method (5x24 hours) and evaporated by rotary evaporator. The yield percentage of *H. surattensis* L was 11.08%. 50 grams extract of *H. surattensis* L crude extract was fractionated using the liquid-liquid partition. The partition was done using n-hexane and ethyl acetate.

2.5.2. Phytochemical screening

Phytochemical screening of the crude extract and fractions was carried out employing standard procedures[7], to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, and quinones.

2.5.3. *In vitro* α-glucosidase inhibition activity

The inhibitory activity of α-glucosidase was performed according to the modified method[8]: 0.25 mg of α-glucosidase enzyme was diluted in phosphate buffer (pH 7.0) contains 20 mg of Bovine serum albumin. The enzyme solution was diluted 10x before assay. Reagen mixtures are as follow: 250 µl of 20 mM p-nitrophenyl α –D- glucopyranoside and 495 µl of phosphate buffer was mixture with 5 µl of samples in various concentration (150; 350; 625; 1250; 2500; and 5000 µg/mL). The reaction was pre incubated in water bath at 37ºC for 5 minutes, the reaction began after addition of 250 ul of enzyme solution and the incubation was continued for 15 minutes. The reaction was stopped by addition of 1 mL 200 mM Na_2CO_3 and the amount of p-nitrophenol resulted was measured using microplate reader at λ=400 nm. Acarbose at various concentrations (0.1; 0.5; 1.0; 5.0; and 10 µg/mL) was included as a standard. Inhibition activity was calculated using the following formula: [C-S]/C x 100%. Where C is absorbance of positive control and S is absorbance of the sample after reduced by absorbance of negative control of the sample. The IC_{50} value which defined as the concentration of the sample needed to inhibit 50% of α-glucosidase activity in assay conditions.

2.5.4. Oral Glucose Tolerance Test (OGTT)

The animals were randomly divided into eleven groups of 5 mice in each group and treated as follows: group 1 (normal control) and 2 (positive control) were administered with sodium CMC 0.5% solution (vehicle) per oral (p.o.); group 3 received acarbose (13 mg/kg BW in the same vehicle; group 4-11 were administered orally with 150 mg/kg BW dan 300 mg/kg BW of extract and fractions suspended in sodium CMC 0.5%. After 1 hour of drug administration, the mice were orally treated with 3g/kg BW of glucose. The blood samples were collected after 30, 60, 90 and 120 min of drug administration from the tail vein in accordance with the procedures outlined in the institutional animal care and use committee guideline. Plasma glucose concentration was measured using a glucometer with its corresponding glucose-test strips. Areas under the curves (AUC) for OGTT were calculated to evaluate glucose tolerance[9].

2.5.5. Statistical analysis

Plasma glucose levels are expressed as the mean ± standard deviation. Plasma glucose levels of the different group and times were statistically analyzed by one-way ANOVA followed by LSD test. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Phytochemical screening

It is known that plants are rich in a variety of secondary metabolites such as tannins, steroid/triterpenoids, alkaloids, flavonoids, and saponins. It is necessary to identify the phytochemical components employed by herbalists in the treatment of diseases[10]. The presence or absence of certain phytochemicals could be used to explain some of the biological activity of certain plant extracts.

Alkaloids, triterpenoids, flavonoids, tannins, saponins, and quinones were identified in the preliminary phytochemical investigation of *H. surattensis*. After fractionation of the ethanol extract, alkaloids, flavonoids and triterpenoids were found in n-hexane fraction; ethyl acetate fraction and water fraction was shown positive results for alkaloids, flavonoids, tannins, triterpenoids and quinones.

3.2. Alpha-glucosidase inhibitory activity

In the present study, ethanol extract and three fractions of *H. surattensis* L were evaluated for their inhibitory effect on α-glucosidase enzymes by the in-vitro method. Alpha-glucosidase inhibitory activity for the ethanolic extract and fractions of *H. surattensis* L presented in Table 1.

3.3. Effect of ethanolic extract/fractions on oral glucose tolerance test

The ethanol extract, ethyl acetate, and water fraction showed a significant reduction in blood glucose levels from 30 min onwards when compared to control group of animals.

All value is expressed as mean Standard Deviation (n=5). *P<0.05, as compared to positive control value. Values with different superscripts in same column are significantly different from control at each specific hour after the administration of standard and different doses of the extract and fraction. One-way ANOVA followed by LSD tests was performed to analyze the comparison. NC: Normal control; PC: positive control; S: standard acarbose (13 mg/kg BW); EE: ethanol extract; HF: n-hexane fraction; EAF: etyl acetate fraction; WF: water fraction.

The effects of extract/fractions of *H. surattensis* L on glucose tolerance test are shown in Figure 1. Before administration of glucose, blood plasma glucose level were similar among groups. At 30 minutes after glucose administration acarbose could attenuate the increase of plasma glucose concentration significantly compared to positive control. A similar result was observed as well in the group of ethanol extract, ethyl acetate fraction and the water fraction in two doses (150 mg/kg BW and 300 mg/kg BW).

4. Discussions

α-Glucosidase plays a central role in modulating postprandial hyperglycemia, which breaks down α-1,4-glucosidic linkages of disaccharides, resulting in simpler sugars. A previous study reported the established α-glucosidase inhibitors and their effects on delaying the expeditious generation of blood glucose after food uptake[11].

In the present study, the ethanol extract of *H. surattensis* L was divided into three fractions by polarity and the α-glucosidase inhibitory activities of these extract/ fractions were detected using pNPG as the reaction substrate. As shown in Table 1, no α-glucosidase inhibition was observed in the ethanol extract, n-hexane, ethyl acetate and water fractions. So this plant might have another mechanism for reduction of blood glucose.

For Oral Glucose Tolerance Test, the blood samples were analyzed for glucose

Table 1. The α-glucosidase inhibitory activity extract, n hexane fraction, ethyl acetate fraction, water fraction, and acarbose.

No	Sample	IC_{50} (ppm)
1	Ethanol extract	>5000
2	n-hexane extract	>5000
3	Ethyl acetate fraction	3888.34
4	Water fraction	>5000
5	Acarbose	0.17

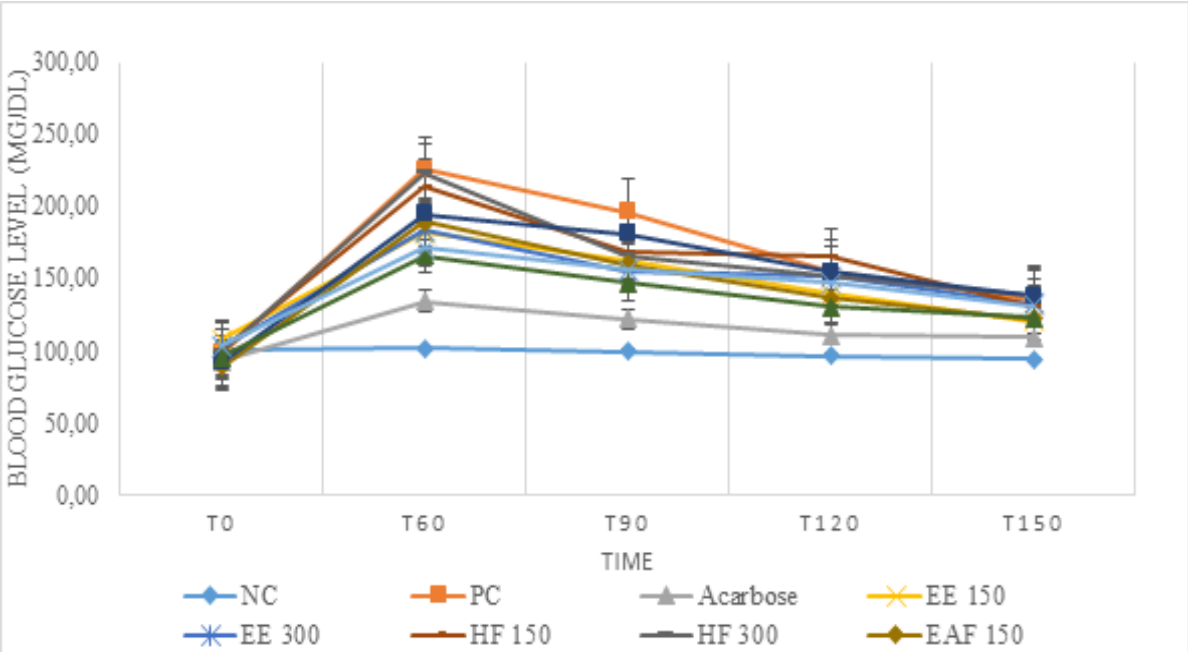

Figure 1. Effect of ethanol extract of *H. surattensis* L on oral glucose tolerance

content at 0, 60, 90, 120 and 150 minutes, respectively. The blood sugar levels of ethanol extract, n-hexane, ethyl acetate and water fraction (150 and 300 mg/kg BW) treated groups were found compared to be a positive control.

In this study, the ethanol extract, ethyl acetate, and water fraction treated mice suppress its rise in blood glucose level with 150 mg/kg and 300 mg/kg BW as compared with positive control. Groups EAF and WF glucose lowering efficiency between 120-150 minutes and were comparable to the diabetic standard shown in Fig 1. Thus the EAF and WF enhanced glucose utilization and improves tolerance in glucose loaded mice.

The experiment showed that Glucose Tolerance Test (GTT) measures the body ability to use glucose, the body's main source of energy. This test can be used to diagnose prediabetes and diabetes[12]. Glucose lowering effects were found after oral administration of EE, EAF, and WF in mice. This may be due to the presence of hypoglycemic flavonoids, alkaloids, or triterpenoids that also requires further investigation.

Impaired glucose tolerance is reflected in a larger incremental AUC of the plasma glucose disappearance curve. Results of OGTT revealed that the AUC (0- 150 min) significantly increased in positive control, ethanol extract, and n-hexane fraction in comparison to normal control. In other treatment groups (EAF and WF), AUC was significantly lowered as compared to positive control. The acarbose treatment was associated with the strongest hypoglycemic effects among all groups. The hypoglycemic activity of the EAF and WF could be due to the enhancement of beta-cell sensitivity to the insulin-releasing effect of glucose, the improvement of insulin action and the extra pancreatic effects of glucose-6-phosphate dehydrogenase activation or suppression of hepatic glucose production but does not affect to inhibition α-glucosidase[13].

5. Conclusion

The ethanol extract, EAF, and WF of *H. surattensis* L leave possesses antidiabetic effects, possibly mediated by the stimulatory effect on insulin secretion and/or by sensitization of target organs to insulin action but not through the inhibition of α-glucosidase. These findings suggest that the plant may be a potential source for the development of new oral hypoglycemic agent.

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