In-vitro α-Glucosidase Inhibitory Activity of *Litsea petiolata* Hk. f

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Abstract. Alpha-glucosidase is an enzyme that plays a role in the process of hydrolysis of carbohydrates into glucose so that the enzyme inhibitor can inhibit the process of glucose absorption. One synthetic drug that has a mechanism of action to inhibit the activity of the alpha-glucosidase enzyme is acarbose, however in the long term has side effects, such as digestive system disorders. Therefore, the use of natural medicines as alternative therapies can be considered for use. This study aimed to determine the inhibitory activity of the enzyme alpha-glucosidase from extracts and fractions of *Litsea petiolata* Hk. f bark. The extraction method used maceration, and fractionation using column chromatography. The results showed that the IC_{50} values for extract, fraction B, fraction D, and acarbose were 444.17 µg/ml, 682.85 µg/ml, 1683.45 µg/ml, and 110.60 µg/ml, respectively. In conclusion, the activity extract as the alpha-glucosidase was higher than the fractions but lower than acarbose.

Keywords: *Litsea petiolata* Hk. F, extract, fraction, alpha-glucosidase inhibitory

1 Introduction

Litsa petiolata Hk. f belongs to Family Lauraceae, this plant commonly found in Hutan Simpan Rimba Teloi, Sik, Kedah, Malaysia [1]. *L. petiolata* is native to southern Thailand wahich is also called “Thummong” and commonly used to give flavor to food dishes, because containing 7 aldehydes, 5 ketones, and 3 esters [2]. Some natural compounds that have been successfully isolated on the stem of this plant such as arbine, norharman reticuline, isboldine, and thalifoline [1]. In the leaves, there is leaf oil which has been widely studied as an antibacterial [3].

The enzyme of α-glucosidase catalyzes the final step in the process of digestion of carbohydrates [4]. Inhibition of action of α-glucosidase can inhibit the liberation of d-glucose from complex carbohydrates so that absorption is delayed [4]. This will cause a decrease in

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plasma glucose levels [4]. In this study, an in vitro α-glucosidase inhibitory activity test was carried out from *Litsea petiolata* Hk. f bark.

The ability to reduce glucose levels in blood plasma by extract of plant can add information on the precense of herbal medicines for type-2 diabetes [5]. Diabetes is a disease that continues to grow and is a heavy economic burden for both patients and countries. This chronic metabolic disease can also cause complications that endanger the patient [5].

Diabetes mellitus is a systemic metabolic disease characterized by hypoinsulinemia, hyperlipidemia, hyperglycemia, and hyperaminoacidemia which causes the secretion and the act of insulin decrese [6]. Diabetes mellitus therapy is available in the form of insulin and oral antidiabetic, such as biguanide, sulfonylureas, α-glucosidase enzyme inhibitors, and glinides [6]. This study aimed to examine the extract and fraction of *L. petiolata* as an inhibitor of the α-glucosidase enzyme which is one of the methods of diabetes mellitus therapy. Acarbose is a diabetic drug with a mechanism to inhibit the α-glucosidase enzyme. So in this study, acarbose was used as a control [7].

2 Method

Plant Material: The stem of *L. petiolata* was obtained from Sirnpan Rimba Teloi forest, Sik, Kedah, Malaysia in February 2017 [1]. The firstly, a fat was removed from dried powder sample by soaking it using n-hexane for 3 days with moistened by NH_4OH. The sample was then extracted using a soxhletation method with dichloromethane for 18 hours. Fractionation of the extract was carried out using column chromatography withincreasing polarity solvent (dichlormethane and methanol) [1].

α-Glucosidase inhibitory assay: The assay was performed using microplate reader (Versamax ELISA, USA) according to previously study by Mahayasih et al [8]. To obtain 50% inhibition concentration (IC_{50}), 30 µl samples with various concentrations were put in 96 microplate wells, then was added by 36 µl of phosphate buffer pH 6.8 and, as the substrate, 17 µl of 4 mM p-NPG (p-nitrofenil-α-D-glucopiranoside) (Sigma-Aldrich, Switzerland) [8]. The microplate was inserted into a microplate reader, shaked and incubated at 37^{o}C for 5 minutes, then was added 17 µl of enzyme α-glucosidase (Saccharomyces cerevisiae, Sigma-Aldrich-Germany) at concentration 0.8 unit/L [8]. Subsequently re-incubated at 30 ^{o}C for 15 minutes, the reaction was then terminated by adding 100 µl of 267 mM Sodium carbonate solution [8]. The absorbance of the solution was measured with a microplate reader at 400 nm. Each test was repeated three times. A solution system contains substrate and enzyme, without extract was used as a blank and a solution system without enzyme was used as a control. Acarbose was used as a positive control. The percent inhibition of the enzyme by samples was calculated by following formula [8]:

$$\text{Inhibition (\%)} = [(\text{blank absorption} - \text{sample absorption}) / \text{blank absorption}] \times 100. \quad (1)$$

The IC_{50} value showed the concentration of extract or fraction required for inhibiting 50% of α-glucosidase enzyme activity.

3 Result

The result of α-glucosidase inhibitory activity assay of extract and fraction of *L. petiolata* and also acarbose (positive control) can be seen in Figure 1-4,

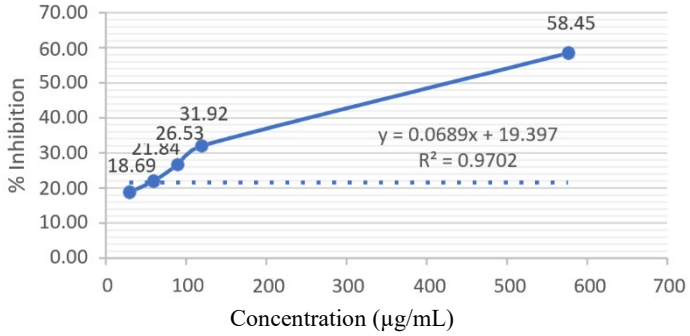

Fig. 1. Average results of the enzyme α-glucosidase inhibition percentage of *L. petiolata* stem extract

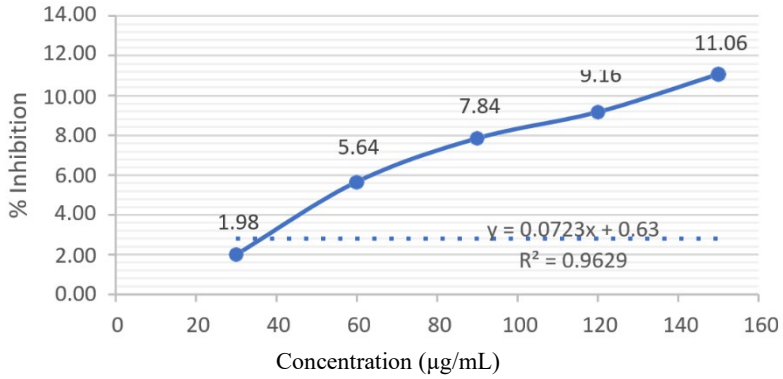

Fig. 2. Average results of the enzyme α-glucosidase inhibition percentage of *L. petiolata* stem fraction B

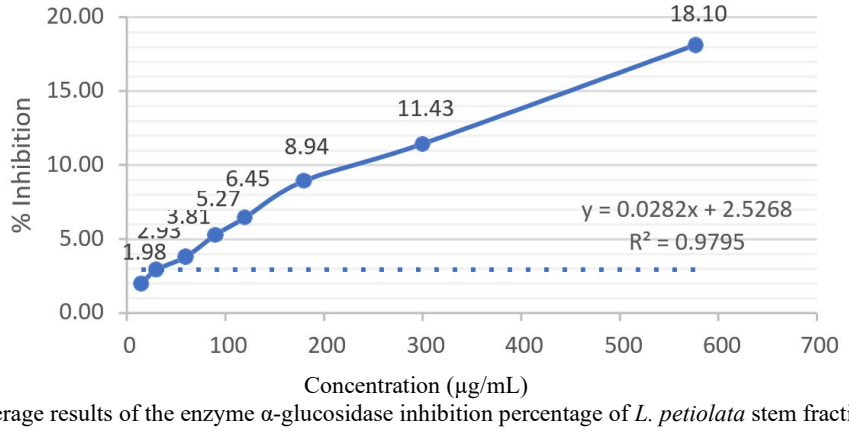

Fig. 3. Average results of the enzyme α-glucosidase inhibition percentage of *L. petiolata* stem fraction D

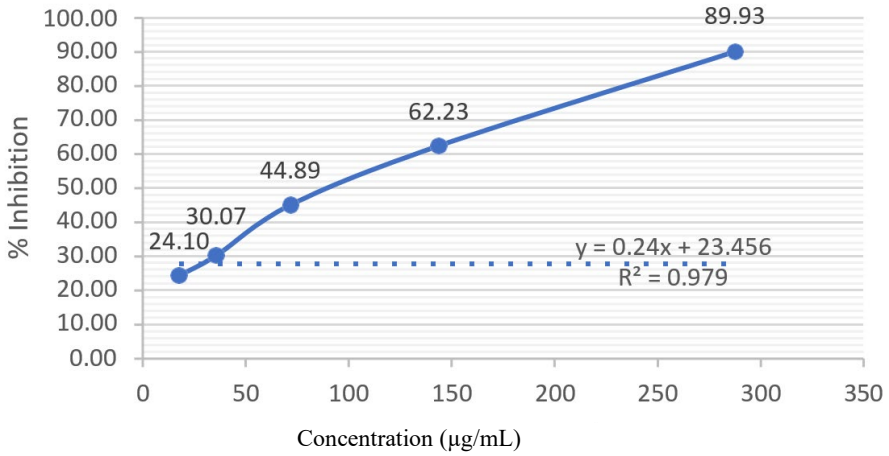

Fig. 4. Graph of the average results of the measurement of the enzyme α-glucosidase inhibition percentage of acarbose (positive control) with five concentrations

According to the results of the α-glucosidase inhibitory activity the dichloromethane extract has an IC_{50} value of almost four times that of IC_{50} of acarbose and its fractions are even greater. IC_{50} calculation results can be seen in Table 1.

Table 1. The result of IC_{50} calculation of α-glucosidase inhibitory activity

Samples	IC_{50} (µg/mL)
The *Litsea petiolata* Hk. f bark dichloromethane extract	444.17
The fraction B of the extract	682.85
The fraction D of the extract	1683.45
Acarbose	110.6

4 Discussion

An α-glucosidase is one of the enzymes in addition to the amylase enzyme that plays an important role in the process of carbohydrate hydrolysis so that the activity of the α-glucosidase can inhibit the digestion of carbohydrates to reduce the speed of glucose uptake into the blood [9]. The α-glucosidase enzyme inhibiting agent can be used as a therapeutic approach to control hyperglycemia [9]. High blood glucose levels are a sign of chronic metabolic disorders, namely diabetes mellitus [4]. Treatment of diabetes mellitus can be done by controlling high blood glucose levels [10]. The action of α-glucosidase inhibitors can block the activity of glucosidase thereby slowing the digestion process and absorption of carbohydrates. This causes the level of glucose in the blood to decrease [10].

Some α-glucosidase inhibitors, such as acarbose and voglibose, which are obtained from natural sources, can clinically treat diabetes mellitus [10]. Acarbose is one of the drugs commonly used to inhibit glucose absorption, only it has side effects such as liver toxicity and undesirable effects on gastrointestinal [9]. Therefore, it is necessary to find alternative treatments with natural ingredients that can inhibit the activity of the α-glucosidase enzyme without causing side effects [10]. This study tested the inhibitory activity of the enzyme α-

glucosidase from the *L. petiolata* bark to determine the effectiveness of the extract and its fractions.

The activity of α-Glucosidase inhibitors was demonstrated by Genus Litsea (Lauraceae) that is oleum of *L. coreana*, where it was collected from Ningguo, Mingshan, dan Wuxi, Cina with IC_{50} value 1.71, 2.64, and 3.88 mg/mL, respectively [11]. The IC_{50} of dichloromethane extract of *L petiolata* stem from Kedah Malaysia is still lower than *L. coreana* leaf oil from China. *L. coreana* var. Lanuginosa leaf, which is commonly consumed as a tonic in China, has been distilled using water to obtain essential oils and 50 compounds have been identified by Gas Chromatography-Mass Spectrometer which are citral, caryophyllene, dodecanal, α humulene, and decanal [12]. While, some alkaloids have been isolated from *L. petiolata* bark diclorometha ne extracts, namely arbine, norharman, reticuline, isoboldine, dan thalifoline [1].

In addition, *L. lancifolia* originating from Uttarakhand, India has also been studied, reported to have potential as an antidiabetic drug with IC50 values inhibiting the α-glucosidase 229.61 µg / mL, with IC_{50} acarbose values (positive control) 50.26 ± 9.57 µg/mL [13]. Epigallocatechin gallate from L. coreana has also been investigated for its inhibition of the α-glucosidase enzyme with an IC_{50} value of 3.8 mg / mL [14].

5 Conclusion

Antidiabetic activity of dichloromethane extracts of *L. petiolata* bark and both fractions have been investigated through the α-glucosidase inhibition assay using a microplate reader spectrophotometric method and acarbose as a positive control. The results showed the potential of *L. petiolata* bark dichloromethane extract to be further developed as an antidiabetic, with better results than its fractions.

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