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In Vitro Bioassay of n-buthanol Isolate of *Acorus calamus* L. on Inhibitory of Activity α-Glucosidase

Ahmad Najib[1]*, Sri Hartati[2], and Berna Elya[3]

[1]Faculty of Pharmachy Moslem Univesity of Indonesia, Makassar 90132, Indonesia
[1]Research Center for Chemistry, Indonesian Institute of Science, Serpong, Indonesia
[3]Departmen of Pharmacy Faculty of Mathematics and Natural Science University of Indonesia; Depok, Indonesia, 16424

Corres.author: ahmad.najib@ymail.com
Phone : +6281524045514

Abstract: Compound existence at one particular plant extract ingredient that has ability to inhibitory α-glucosidase enzyme, give a chance to develop a natural medicine in the effort therapy of diabetic. This aim of study to see effectivity of n-buthanol isolate from plants Acorus calamus L. on inhibitory α -glucosidase enzyme. Extraction done with masceration method use methanol. The gummy methanol extract followed by partition using n-heksan, ethyl acetate and n-butanol. The n-buthanol extract isolate using column chromatography method with stationer phase is resin diaion hp 20 and eluent ethanol: water in various concentration, isolation result is got 5 fraction. In each fraction was assayed for inhibitory activity on α-glucosidase enzyme. The potential inhibition effect was got in 5th fraction and from purified process using column chromatography method with stationer phase is silica gel and eluent dichloromethane : methanol 95:5 was got ACB as active compound with IC_{50} 17.89 µg/ml. This result shows that plants Acorus calamus L. has a potency to develop upon which diabetes therapy.
Key word : enzyme α-glucosidase, n-butanol Isolate, Acorus calamus L.

Introduction

Diabetes mellitus (DM) is in the top 5 of the most significant diseases in the developed world, and is gaining in significance there and elsewhere. Present number of diabetics worldwide is 171 million and this is likely to increase to 340 million or more by the year 2030[1,2] For a long time, diabetics have been treated with several medicinal plants or their extracts based on the folklore medicine[3]. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigation on hypoglycemic agent from medicinal plants has been more important[4].

Type 2 diabetes mellitus (DM) is a disorder characterized by insulin resistance and a progressive decline in pancreatic beta-cell

function associated with increasing hyperglycaemia. Defective betacell function occurs early and can be detected in individuals with impaired fasting and/or post-prandial glucose levels (the so-called 'pre-diabetics')[4]. The α-glucosidase inhibitors inhibit the activity of the glucosidase enzymes which are present in the brush border of enterocytes in the intestinal villi. Disaccharide and oligosaccharide cleavage is prevented with a net decrease in intestinal carbohydrate absorption. Overall, the α-glucosidase inhibitors reduce postprandial insulin concentrations through the attenuated rise in postprandial glucose levels[6].

Less than 2% of the drug is absorbed. It is broken down by intestinal amylases and certain intestinal bacteria. Some degradation products are taken up and subsequently eliminated in the urine. The drug should be taken with the first bite of food during a meal and not more than 15 minutes after the start of the meal. This drug is ideal for initiation of pharmacotherapy in type 2 diabetic patients[7].

Acorus calamus L.(AC), family Araceae, have been used in the Indian and Chinese systems of medicine for hundreds of years for its beneficial role in several kinds of diseases especially the central nervous system abnormalities[8,9]. The hypolipidemic activity of AC in rats has been reported by Parab and Mengi, 2002, but it has demonstrated that the extract of this plant had no effects on the glucose level of hyperglycemic murines induced by either adrenalin or alloxan[10]. In this study we investigated the inhibitory effect of n-buthanol fraction of AC. on α-glucosidase enzyme.

Material And Methods

This study conducted in Laboratory of Natural Product and Pharmaceuticals, Research Center for Chemistry-Indonesian Institute of Science Kawasan Puspitek, Serpong on December 2009-May 2010

Plant Materials

Dried rhizome of AC. collected from farmer on Jogjakarta. Plant samples were identified at Herbarium Bogoriensis -Indonesian Institute of Science.

Extraction and Isolation of Plant Materials

Dried rhizome (20 kg) macerated with 100 liter methanol in five days (this process was done in two times) than evaporated. The viscous extract was dissolve in water 1:1, followed by fractionations with n-hexane, ethyl acetate and n-buthanol. The n-buthanol extract was concentrated using a rotary evaporator at 55^0C and left on the laboratory bench for 5 days to obtain a dark gummy residues (123 g). The column was packed with resin diaion hp 20, 600 g and 10 g of the gummy extract placed on top of the resin and eluted with ethanol: water (0:100), (25:75), (50:50), (75:25) and (100:0), elution in each eluent end if the solution of fraction was clear then replace with the other eluent until the last variation. The each fraction from variation of eluent collected and evaporated with rotary evaporator. After fractionation the active fraction isolate with column chromatography using sephadex as the stationer phase and eluted with methanol : dichloromethane (1:1), each isolate collected and left on laboratory bench until the eluent vaporate. The bioassay was done in each isolate and the active isolate purified with column chromatography method with stationer phase is silica gel and eluent dichloromethane : methanol 95:5

Inhibition Assay for α-glucosidase Enzyme

The reaction mixture consisting 250μL 0f 20 mM p-nitrophenyl α-D-glucopyranoside (Sigma Chemical Co.), 495 μL phosphate buffer (pH 7.0) adding to flask contain 5 μL of sample dissolved in DMSO at various concentration (3.135-25 μg mL^{-1}). The reaction mixture was pre-incubated for 5 min at 37^0C, the reaction was start by adding 250 μL α-glucosidase (0.075 unit) (EC 3.2.1.20 from Wako Pure Chemical Industry) incubation was continued from 30 min. The reaction stopped by adding 1 ml 0f 0.1 M Na_2CO_3. Activity of α-glucosidase was determined by measuring release of p-nitrophenol at 400 nm. Koji extract from *Aspegillus terreus* used positive control of α-glucosidase.

Result And Discussion

Study from the radix *of* AC. is widely used in the therapy of diabetes in traditional folk medicine of America[11], and it prevails in Merak, Banten, Indonesia to improve diabetes. However, the antidiabetic effects of AC. have not been fully studied as yet. The hypolipidemic activity of AC[12].

In this investigation will be showed the potency isolate of n-buthanol extract AC. as inhibitory agent on α-glucosidase enzyme. This study using the fraction from n-buthanol extract AC. with column chromatography method to separated it, table 1 show result of process. We use the resin to separated fraction because it suitable with the crude extract with high polarity (hydrophilic). After the bioassay test done in each fraction the active fraction isolate with column chromatography using sephadex, table 2 show result of process.

The result of inhibition assay for α-glucosidase enzyme from isolate show that the ACB compund purified from 3th Isolat is active with IC_{50} value 17.89 µg ML^{-1} and the other fraction has not activity (Table 3).

The investigation use a koji extract as control from *Apergillus terreus* is an especially prolific producer of secondary metabolites has biological activities such as inhibitory of α-glucosidase and it has a most potential activity therefore examined the effect on postprandial blood glucose level after a meal in mice[13].

Conclusion

ACB as active compound with IC_{50} 17.89 µg/ml. This result shows that plants *Acorus calamus* L. has a potency to develop upon which diabetes therapy.

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Table 1.: Result of Isolation n-Buthanol Extract AC Using Colomn Resin Chomatography Method

Fraction	Eluent	Total Weight (mg)
I	Ethanol : Water (0%:100%)	2171.8
II	Ethanol : Water (25%:75%)	4444.4
III	Ethanol : Water (50%:50%)	7343.5
IV	Ethanol : Water (75%:25%)	5286.9
V	Ethanol : Water (100%:0%)	506.2

Table 2.: Result of Isolation n-buthanol Extract AC Using Colomn Sephadex Chomatography Method

Isolate	Eluent	Total Weight (mg)
I	Diclorometan:Methanol (1:1)	279.5
II	Diclorometan:Methanol (1:1)	285.2
III	Diclorometan:Methanol (1:1)	40.6
IV	Diclorometan:Methanol (1:1)	49.2
V	Diclorometan:Methanol (1:1)	26.2

Table 3.: Inhibitory Activity of Isolate From n-buthanol Extract AC

Isolate	IC_{50} (µg ML^{-1})
I	$4{,}4.10^{9}$
II	83.92
III	28.76
IV	NA
V	NA
ACB	17.89

Note : NA = No Activity

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