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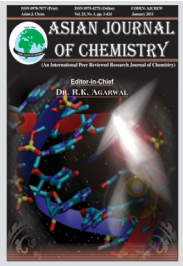

NOTE

Aldose Reductase and α-Glycosidase Inhibition Activities of Active Fraction of *Leucaena leucocephala* (lmk) De Wit Seeds

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The fraction of methanolic extract of *Leucaena leucocephala* (lmk) De Wit seeds was found to showed inhibitory activities on α-glucosidase and aldose reductase, respectively. The assays of aldose reductase and α-glycosidase inhibition activities were conducted in *in vitro* way on the subfractions isolated from the methanol extract of *Leucaena leucocephala* (lmk) De Wit. This work shows that one of the active subfractions (I-5) can inhibit aldose reductase and α-glycosidase enzyms, with the IC_{50} of 12.56 ± 1.23 µg/mL dan 7.67 ± 1.32 µg/mL. These IC_{50} values of I-5 are on par with acarbose.

Key Words: Aldose reductase, α-Glycosidase, *Leucaena leucocephala* (lmk) De Wit seeds, Diabetes mellitus.

Diabetes mellitus with its devastating consequences has assumed epidemic proportion in many countries of the world. There are an estimated 143 million people worldwide with diabetes, which is almost five times more than the estimation of 10 year ago. This number will probably double by 2030. Inhibitors of α-glucosidase and aldose reductase have potential use in the treatment of diabetes mellitus[1].

α-Glucosidase is an important enzyme on dietary carbohydrate digestion and post-translational processing of glycoproteins[2]. Small intestinal α-glucosidase hydrolyzes carbohydrate to glucose, which is absorbed through the gut wall to become the blood glucose[3] and to cause postprandial hyperglycemia. It has been recognized that α-glucosidase inhibitors can prevent against some diseases *e.g.* diabetes, obesity, hyperlipoproteinaemia and hyperlipidaemia[4-6].

Aldose reductase is a rate limiting enzyme in the polyol pathway associated with the conversion of glucose to sorbitol. The enzyme is located in the eye (cornea, retina, lens), kidney, myelin sheath and also in other tissues less involved in diabetic complications. Experiments in diabetic animals have implicated sorbitol accumulation in the lens to the development of cataracts. The use of inhibitors of aldose reductase in animal studies has demonstrated that diabetic complications such as cataracts, nephropathy and slowing of nerve conduction can be ameliorated[7].

The purpose of this work is to identify the aldose reductase and α-glycosidase inhibition activities of several methanol extract fractions, obtained from previous work, which are known to have antidiabetic activities.

Extraction and fractionation: *L. leucocephala* (lmk) DeWit seeds were grounded to powder and refluxed three times in a gradient way using solvents like *n*-hexane, ethyl acetate, methanol and water as well as direct extraction with methanol. Each extract is let to evaporate and held until fractions are yielded. Methanol extracts were fractionated with column chromatography using chloroform-methanol eluents in a gradient way, ranging from (5:1), (4:1), (3:1), (2:1) and (1:1) to categorize the compounds contained in the methanol extracts based on their polarity.

Aldose reductase inhibitory activity: Aldose reductase activity was assayed by the method described previously[8]. The lenses of wistar rats were homogenized in 135 mM Na, K-phosphate buffer (pH 7.0) containing 10 mM 2-mercaptoethanol and centrifuged for 0.5 h. The supernatant fluid was used as the enzyme fraction. The incubation mixture contained 135 mM Na, K-phosphate buffer (pH 7.0), 100 mM Li_2SO_4, 0.03 mM NADPH, 1 mM DL-glyceraldehyde as a substrate and 100 mL of the enzyme fraction, with or without 25 mL of sample solution in a total volume of 0.5 mL. Test samples were dissolved in DMSO. The reaction was initiated by the addition of NADPH at 30 °C. After 0.5 h of incubation, the reaction was stopped by the addition of 150 mL of 0.5 M HCl. Then 0.5 mL of 6 M NaOH containing 10 mM imidazole was added and the solution was heated at 60 °C for 10 min to convert

NADP to a fluorescent product. The fluorescence was measured at room temperature using a spectrofluorometer (type 650-10, Hitachi, Japan) with an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

α-Glucosidase inhibition assay: The enzyme inhibition assay is based on the breakdown of substrate to produce a coloured product, followed by measuring the absorbance over a period of time[9,10] In brief, α-glucosidase (Sigma, type III, from yeast) was dissolved in buffer A (0.1 mol/L potassium phosphate, 3.2 mmol/L-$MgCl_2$, pH 6.8) (0.1 units/mL), *p*-nitrophenyl-α-D-gluco pyranoside dissolved in buffer A at 6 mmol/L was used as substrates. 102 µL buffer B (0.5mol/L potassium phosphate, 16 mmol/L-$MgCl_2$, pH 6.8), 120 µL sample solution (0.6 mg/mL in dimethyl sulfoxide), 282 µL water and 200 µL substrate were mixed. This mixture was in-cubated in water-bath at 37 ºC for 5 min and then 200 µL enzyme solution was added and mixed. The enzyme reaction was carried out at 37 ºC for 0.5 h and then 1.2 mL 0.4 mol/L glycine buffer (pH 10.4) was added to terminate the reaction. Enzymatic activity was quantitied by measuring the absorbance 410 nm.

Fractionation with column chromatograms aimed at isolating compounds within the methanol fractions with the expectation that a pure compound could be obtained. Methanol extract at preliminary test was subsequently fractionated with column chromatography by using appropriate eluents. Fractionation was done with chloroform-methanol eluents in a gradient way with respective solvent proportions of 5:1, 4:1, 3:1, 2:1, 1:1. Eluate from each extract was retained well. Every fraction was also treated with TLC. Fractions with similar pattern of isolation with chromatograms were combined; yielding simpler fractions.

The results of combining TLC examination was given in Table-1.

TABLE-1
RESULT OF COMBINING TLC
EXAMINATION OF ALL FRACTIONS

Sub-fraction	Combi-nation	Spot colour	No. of spots	R_f
I-1	1-10	Brown	1	0,88
I-2	11-17	Tosca green	1	0,48
I-3	13-27	Brown, tosca green, brown	3	0.29, 0.19, 0.097
I-4	37-60	Bright green, dark green, brown	3	0.25, 0.19, 0.097
I-5	61-90	Dark green	1	0,19
I-6	91-137	Dark green	1	0,16

All the 6 subfractions were tested for their aldose reductase and α-glycosidase inhibition activities. The results are shown in Table-2. Table 2 shows that the subfraction I-5 is more active to inhibit aldose reductase and a-glycosidase enzyms, with the with the IC_{50} of 12.56 ± 1.23 µg/mL dan 7.67 ± 1.32µg/mL. These IC_{50} values of I-5 are on par with acarbose.

Acarbose inhibits enzymes (glycoside hydrolases) needed to digest carbohydrates: specifically α-glucosidase enzymes in the brush border of the small intestines and pancreatic

TABLE-2
α-GLUCOSIDASE AND ALDOSE REDUCTASE
INHIBITORY ACTIVITY OF SUBFRACTION

Subfraction	IC_{50} (µg/mL) α-glucosidase	IC_{50} (µg/mL) Aldose reductase
I-1	231.23 ± 2.13	>100
I-2	121.23 ± 2.33	>100
I-3	234.23 ± 4.21	>100
I-4	123.24 ± 3.24	>100
I-5	7.67 ± 1.32	12.56 ± 1.23
I-6	78.12 ± 2.13	98.25 ± 1.56
Acarbose	6.78 ± 1.26	23.43 ± 2.31

α-amylase. Pancreatic α-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine, whereas the membrane-bound intestinal α-glucosidases hydrolyze oligosaccharides, trisaccharides and disaccharides to glucose and other monosaccharides in the small intestine[10].

α-Glucosidase inhibitors work to control diabetes by inhibition the enzymes that break down starches and carbohydrates in the body, lowering the necessity for insulin. This is accomplished through competitive, reversible inhibition of pancreatic α-amylase and membrane bound intestinal α-glucoside hydrolase enzymes[9]. Aldose reductase is an enzyme that is normally present in the eye and in many other parts of the body. It helps change glucose (sugar) into a sugar alcohol called sorbitol. Too much sorbitol trapped in eye and nerve cells can damage these cells, leading to retinopathy and neuropathy. Drugs that prevent or slow (inhibit) the action of aldose reductase are being studied as a way to prevent or delay these complications of diabetes[8].

Conclusion

One of the active subfractions isolated from *L. leucocephala* (lmk) De Wit seeds can inhibit the activity of aldose reductase and α-glycosidase enzyms so that this subfraction can be developed as an antidiabetic drug.

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