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Original Article

ELISA Test on *Cordia myxa* L. Leaf Extract for α-Glucosidase Inhibitor

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ABSTRACT

Aimed: Determine the potential of *Cordia myxa* L. leaf on inhibited **α**-glucosidase. **Material:** ELISA Kit, Ethanol 96%, Colomn Chromatography, n-hexane, ethyl acetate, Glocobay®. **Method:** Sample from *Cordia myxa* L. leaf extracted by ethanol 96% then evaporated to get the sticky extract. The sticky extract of Cordia myxa L. leaf fractionated by column chromatography with n-hexane, n-hexane: ethyl acetate (90:10; 80:20; 75:25; 70:30; 65:35; 60:40; 55:45; 50:50) **Assay:** The fractions assayed by ELISA (Enzyme-Linked Immunosorbent Assay) with acarbose (Glucobay ®) as the comparator. **Result:** The results showed that the n-hexane fraction is the highest potency on inhibited **α**-glucosidase with the noncompetitive mechanism. The IC_{50} of n-hexane fraction is 0.53 ppm been while the acarbose is 6.85 ppm. **Conclusion:** The n-hexane fraction of *Cordia myxa* L. leaf has the highest potency to use for possible decrease blood glucose level.

Key words: *Cordia myxa* L., ELISA, α-Glucosidase, IC_{50}, Acarbose.

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INTRODUCTION

Cordia myxa L. belongs to family Boraginaceae, this plant family around 2740 species distributed in 148 genera. The genus Cordia is one of the most presenting of this family. Cordia is a genus of trees or shrubs the borage family.[1] About 300 species have been identified worldwide that use as folk medicine in Indonesia.[2] The phytochemical qualitative analysis showed that *Cordia myxa* L. contain the presence of oil, glycosides, flavonoids, sterols, saponins, terpenoids, alkaloids, phenolic acids, coumarins, tannins, resins, gums and mucilage.[3] The methanolic extracts from this plants species have reported as the antioxidant and α-amylase and α-glucosidase enzyme inhibitory activity.[4]

Nondependent insulin Diabetes Mellitus (NIDM) or type 2 diabetes mellitus (DM) is correlated with the α-glucosidase inhibitors, this enzyme which is present in the brush border of enterocytes in the intestinal villi. Overall, the α-glucosidase inhibitors reduce postprandial insulin concentrations through the attenuated rise in postprandial glucose levels.[5]

The mechanism of α-glucosidase inhibitors base on inhibited the enzyme subtract react with an enzyme on room temperature.[6] Data resources from the absorbance measurement by the spectrophotometer on specific maximum wave length.[5] The resulting base on the IC_{50} value of sample compares with the IC_{50} of positive control.[7] The on this case IC_{50} value will show the best activity if equal or less than IC_{50} value of positive control.[8] On this method the researcher will need relatively more sample to investigate[5] so to reduce the needs of the sample it will use by another method such as ELISA.[9] This research will use it to investigate the inhibitory of α-glucosidase by *Cordia myxa* L. leaf.

EXPERIMENTAL METHOD

Sample Preparation

Cordia myxa L. leaf from Enrekang regency South Celebes-Indonesia. The taxonomic identification by the Botanical division of Pharmacognosy-Phytochemistry Laboratory, Faculty of Pharmacy, Universitas Muslim Indonesia. The 2.15 Kg dried sample by room temperature was grind then extracted by 5 liters' ethanol 95%, this process was done four times. The liquid extract then evaporated to give the gummy extract.[10]

Sample Fractionation

The gummy extract fractioned by column chromatography with silica gel G. 60 (Merck®) with n-hexane, n-hexane: ethyl acetate (90:10; 80:20; 75:25; 70:30; 65:35; 60:40; 55:45; 50:50). Results of fractions identify by Thin Layer Chromatography (TLC)[11] to investigate the chromatogram profile.[12]

ELISA Test on Fractions

Enzyme Linked Immuno Sorbent Assay (ELISA)[13] on fraction base on scale down from previous research[5] using the reaction mixture consisting 25 µL of 2 mM p-nitrophenyl α-D-glucopyranoside (Sigma Chemical Co.), 49.5 µL phosphate buffer (pH 7.0) adding to flask contains 0.5 µL of sample dissolved

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in DMSO at various concentration (0.31-2.5 µg mL-1). The reaction mixture was pre-incubated for 5 min at 37°C, the reaction was started by adding 25 µL α-glucosidase (Sigma) incubation was continued from 30 min. The reaction stopped by adding 1 ml of 0.01 M Na_2CO_3. The activity of α-glucosidase was determined by measuring the release of p-nitro phenol at 400 nm. Acarbose (Glucobay®) positive control of α-glucosidase.[5]

Kinetics of Inhibition Against α-glucosidase

Inhibition of sample with α-glucosidase activities was measured by increasing concentration of p-nitrophenyl α-D-glucopyranoside as a substrate in the absence or presence of samples at different concentrations. Inhibition type was determined by Lineweaver-Burk plot analysis of the data, which were calculated from the result according to Michaelis-Menten kinetics.[14]

RESULT AND DISCUSSION

After four times maceration with ethanol 96%, the result of extraction shown Table 1.

This research used ethanol because it can extract more active compound than the others.[15]Water and various concentrations (50%, 75% and 100%) This solvent has the lower toxicity and record experiments results were investigated to find that ethanol concentration, solvent/solid ratio and time to increase the extraction yiled[16]The effect of different solvents (methanol, ethanol, acetone and distilled water) and the extraction yield increases with increasing polarity of the solvent used in extraction.[15]Water and various concentrations (50%, 75% and 100%)

After evaporated and fractioned with n-hexane and n-hexane: ethyl acetate on various concentration then obtained to investigate the chromatogram[17] as shown in Figure 1.

Table 1: The results of the calculations percent yield of ethanol extract of *Cordia myxa* L. leaf.

Solvent	The weight of the sample (Kg)	The weight of extract (g)	Yield (%)
Ethanol 96 %	2.15	100	4.65

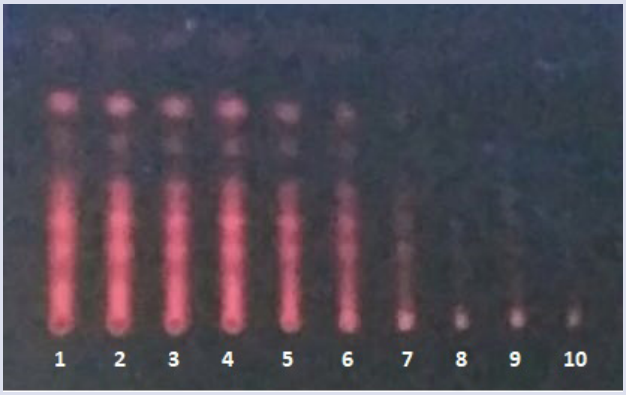

Figure 1: Chromatogram on a fraction.
Description: Stationary phase = Silica gel F_{254}
Mobile phase = n-hexane: ethyl acetate (7:3)
Apparatus: Camag Nanomat 4
Detector: UV light 366 nm

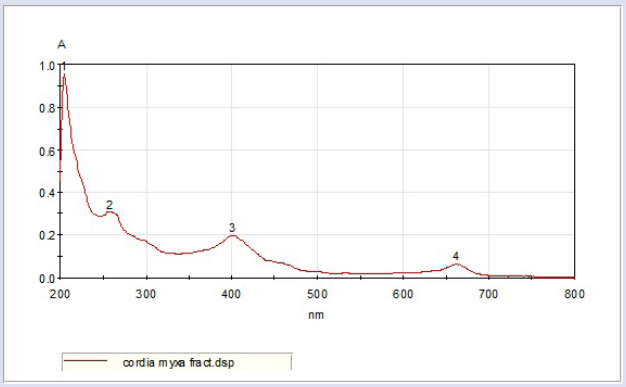

Figure 2: UV-Vis Spectrum on Sample.

Table 2: The results of the ELISA assay on a fraction of *Cordia myxa* L. leaf.

Sample	Comparison of solven (n-hex : EtoAc)	Activity from IC_{50} value (ppm)
Ethanol extract	-	4.44
Acarbose (Glucobay®)	-	6.85
1st Fraction	n-hex	0.53
2nd Fraction	90:10	5.81
3rd Fraction	80:20	0.89
4th Fraction	75:25	2.43
5th Fraction	70:30	0.91
6th Fraction	65:35	7.55
7th Fraction	60:40	9.28
8th Fraction	55:45	12.60
9th Fraction	50:50	16.52

Chromatogram showed that there are 10 fraction obtained some spot representing of many compound.[18] To clarify the amount of compound on the sample we use UV-Vis spectrophotometer[19] as shown in Figure 2.

Analysis of UV-Vis spectrum on sample identified 4 peaks. It important to know that sensitivity of this equipment can detect the compound which is cannot detect by TLC.[20] The data above will assist to investigated for furthermore for the compounds from the sample by elucidation method to determinate the potential compound that can inhibit the α-glucosidase.[21] ELISA assay on the sample as showed on Table 2.

The results on Table 2 regarding the potential for inhibitory of α-glycosidase have been proofed on sample[22] even the IC_{50} there are more potent than acarbose. Since the 6th fraction showed that the activity has been decreasing; it mean the active compound are reducted from the fractions before.

The mechanism of kinetic reaction base on Lineweaver-Burk plot analysis from the sample has been calculated and plotted on Figure 3, below

Regarding on graphic above the inhibitory base on noncompetitive mechanism because of intercept on each line not on Y axis.[23] This mechanism makes a chance to investigate on *in silico* method on our next research as we have been determining on another sample.[24]

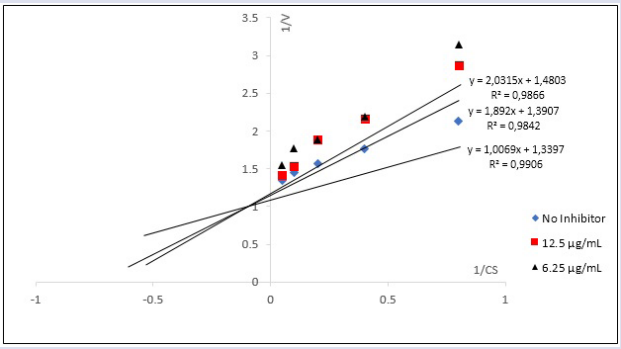

Figure 3: Lineweaver-Burke plot of the reaction α-glucosidase in the presence of sample.

CONCLUSION

The n-hexane fraction of *Cordia myxa* L. leaf have a potential activity to inhibit α-glucosidase related by the highest potency to use for decrease blood glucose level blood with IC_{50} value 0.53 ppm with kinetic mechanism of inhibitory from sample base on noncompetitive.

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CONFLICT OF INTEREST

The authors declare that their is no conflict of interest.

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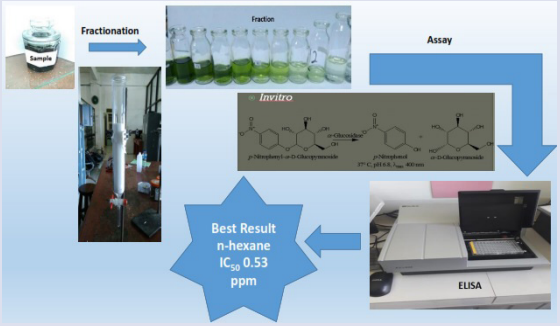
GRAPHICAL ABSTRACT



SUMMARY

- Since *Cordia myxa* L. use to cure disease related to diabetes mellitus as a folk medicine this research finding the data to support the empirical base. This research focused on the mechanism of α-glucosidase inhibition one of three mechanisms that can cure the diabetic patient. By Enzyme-Linked Immunosorbent Assay (ELISA) this research has conducted. There are many fractions have been assayed thus resulted that n-hexane fraction has the best inhibition with IC_{50} 0.53 ppm.

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