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Antidiabetic Effect of Standardized Extract of Indonesian Kanunang Leaves (*Cordia myxa* L.)

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Abstract : Diabetes mellitus (DM) is a metabolic disease that is affected by high blood sugar (glucose). This is occured because of either insulin secretion or insulin resistance. Prevalence of DM in Indonesia is very high case, and it is predicted about 21.3 millions people in 2030 will be suffer from its disease. DM can be treated by modern drugs and herbal medicine. One of several plants to treat of DM is Kanunang (*Cordia myxa* L. Boraginaceae Family). The extract of Kanunang was strandardised by Pharmacognostic methods to determine of water, total ash, and acid-insoluble ash contens that are 9.04; 19.53; and 0.843 respectively. Microbiological assay is showed that Kanunang extract is relatifely safe from pathogen microorganisme. In vitro study have been done with inhibiting of α-glucosidase enzyme. This research was conducted that *C. myxa* leaves extract could be inhibiting activity of α-glucosidase enzyme with IC_{50} value is 35.89 µg/mL (compared with positive standard is 117.20 µg/mL). The conclusion of this research that the *C. myxa* extract is potent to develop as an antidiabetic medicine.
Keywords: Diabetes mellitus, α-glucosidase, Kanunang, *Cordia myxa* L.

Introduction

Diabetes melitus (DM) is a metabolic disease of carbohydrate, fat, and protein. It is occurred because the body have a high blood sugar contents (hyperglychemia) caused by disruption of insulin secretion, insulin resistance or both of them[1,2]. Because of this disruption, the body can not supply of insulin to metabolize of sugar[2].

Insulin, an essential hormone, is produced by pancreatic beta-cells in the body, and producing insulin is affected by some respons such as glucose[3]. The mayor function of insulin is to counter of hyperglycemia. It breaks down of carbohydrate to monomers of sugar, so that the intake of sugar is easy to penetrate into the muscle. However, if the insulin function is disturbed, concentration of blood sugar will increase.

DM is disease with high prevalence in the world especially in Indonesia. Refers to Wild *et al.* (2004) that in 2000, Indonesia will be occupying to the fourth after India, China, and USA. They predicted that the prevalence will increase from 8.4 to 21.3 in 2030. This is the serious problem in the healthy sistem in Indonesia[4].

Controling of DM disease can be managed by diet, physical exercise, and medicine either synthetic or herbal medicine[5,6]. Herbal medicine is an alternative to treat of DM an having low risk with some mechanism such as α-glukosydase inhibition enzyme.

One of herbal medicine is *Cordia myxa* L. (in Enrekang people called Kanunang). This plant grows around in Asia especially in Indonesia. Base on emphirical data by Enrekang people that *C. myxa* is a folk medicine to treat DM. Currently research by Hussain and Kakoti in 2013 showing that the infusum at 500 mg/kgBW could be decreasing of hyperglicemic animal model induced of aloxan. In addition research by Al-Musayeib *et al*. 2011, reporting that the plant contains protocatechuic acid, caffeic acid, and flavonoid glycosides[7,8,9,10].

Base on the previously described, *C. myxa* needs to be depeloped as a DM herbal medicine candidate and focused investigation on α-glucosidase inhibitor. It is a potential plant to upgrade for the herbal value as a standardized herbal medicine or Phytopharmaca.

Experimental

Plant : Kanunang leaves (*Cordia myxa* L. Fam. Boraginaceae**)**

Chemicals : α-glucosidase, acarbose, sodium carbonate, phophate buffer pH 6,8, PNPG Substrate 5 Mm, bovine serum albumin, methanol, aquadest, chloroform, $AlCl_3$, $FeCl_3$, citroborate, TLC aluminium plate F_{254}.

Standardization of Extract

Kanunang (*C. myxa*) leaves were collected from Enrekang district, South Sulawesi. It was determined at centre for research of Biologi, LIPI Cibinong, Jawa Barat. *C. myxa* was sorted to get leaves, washed to clean from the impurity, dried, cutted and powdered for the easily extraction procceses. **The extract of Kanunang (*Cordia myxa* L.) was standardized according to guideline of** Depkes RI[11]**.**

Determination of Water Contain

Preparing the crucible porcelain with heating at a temperature of 105^{o}C for 30 minutes. Kaunang leaves powder of 1,0 gram put on porcelain heated at a temperature of 100^{o}C to 105^{o}C for 5 hours, weighted and repeated of heating up untill the constant weigh reached[11].

Determination of Total Ash Content

As much as 2 grams of simplisia put in a porcelain crucible that has been heated and calibrated, then heated until the charcoal forming, cooled and weighted. If in this way the charcoal can not be eliminated, added hot water, stirred, filtered through ash-free filter paper. Filter paper along filtering rest was heated in the same crucible. The filtrate was put into the crucible, evaporated and heated until the constant weight. Total ash content was calculated to weight of the extract (%w/w)[12].

Determination of Acid-insoluble Ash

The ash produced on the determination of total ash level was heated with 25 mL of concentrated hydrochloride acid, washed using hot water, heated untill the mass constant. The ash insoluble in acid was calculated to the weight of simplisia (w/w)[12].

TLC (Thin Layer Chromatography) Profile

The chromatography profile of methanolic extract of kanunang leaves was made using TLC aluminium plate F_{254} as a stationary phase and chloroform : methanol (1:1) as mobile phase. TLC profile shows in UV_{254} and UV_{366} [11].

Enzyme Preparation

Enzyme solution was made about 1,0 mg α-glucosidase/100 mL phosphat buffer pH 7 with 200 mg bovine serum albumin. Before used about 1 mL of enzyme solution, it was diluted to 25 times with phosphate buffer pH 7.

α-glucosidase Inhibitory Assay

The α-glucosidase inhibitory assay has been used refers to Saijyo *et. al.* (2008) with some modified. In a 96-well plate reader, a reaction mixture containing 30 µL of extract solution with varying consentrations (100, 200, 300, 400, 500 and 600 µg/mL) and 36 µL of phosphate buffer, 17 µl of PNPG as a substrate and pre-incubated for 5 min at 37°C, and then 17 µL of α-glucosidase was added. After further incubation at 37°C for

15 min, the reaction was stopped by adding 100 µl of sodium carbonate (200 mM). All the enzyme, inhibitor and substrate solutions were made using the same buffer. Acarbose was used as a positive control and water as negative control. The yellow colour produced (due to paranitrophenol formation) was measured by colorimetric analysis, and absorbance was read at 405 nm. Each experiment was performed in duplo, along with appropriate blanks[13].

Table 1. Procedure design of α-glukosidase assay

Reagent	**Volume (µL)**					
	S	**SC**	**B**	**BC**	**BS**	**PC**
Phophate buffer pH 6,8	36	36	66	66	200	36
Extract (100, 200, 300, 400, 500, and 600 µg/mL)	30	30	-	-	-	-
Acarbose	-	-	-	-	-	30
PNPG Substrate 5 Mm	17	17	17	17	-	17
Incubated for 5 minute at 37 ^{o}C						
α-Glucosidase 0,15 U/mL	17	-	17	-	-	17
Incubated for 15 minute at 37 ^{o}C						
$NaCO_3$ 200 Mm	100	100	100	100	-	100
α-Glucosidase 0,15 U/Ml	-	17	-	17	-	-
Total Volume	200	200	200	200	200	200
Measured of absorbance at λ = 405 nm						

Table 2. Design of *microplate reader*

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	SC	SC	SC	SC	SC	SC	SC	SC	SC	SC	SC	SC
B	S	S	S	S	S	S	S	S	S	S	S	S
C	PC	PC	PC	PC	PC	PC	PC	PC	PC	PC	PC	PC
D	S	S	S	S	S	S	S	S	S	S	S	S
E	PC	PC	PC	PC	PC	PC	PC	PC	PC	PC	PC	PC
F	S	S	S	S	S	S	S	S	S	S	S	S
G									BC	BC	BC	SC
H									B	B	B	BC

Abbr.:

S = Sample
SC = Sample control
B = Blank
BC = Blank control
BS = Baseline
PC = Positive control

The % inhibition has been obtained using the formula:

$$\% \text{ inhibition} = \frac{\{\text{Absorb. (control)} - \text{Absorb. (sample)}\}}{\text{Absorb (control)}}$$

IC_{50} value is defined as the concentration of extract inhibiting 50% of alpha-glucosidase activity under the stated assay conditions. In case of significant inhibition, IC_{50} values were determined by linear regression by fitting to a sigmoidal dose-response equation with variable slope. All values are represented as Mean ± Standard Deviation. The IC_{50} was calculated using the equation:

$$IC_{50} = \frac{50 - a}{b}$$

Results and Discussion

Preparation of Plant

The air-dried powdered leaves of about 2900.0 gram were extracted by maceration method with ethanol 70% for 72 hours. Ethanol 70% was used because there is a universal solvent for extracting either polar or non polar of chemical compounds from plants[14,15].

Table 3. Extraction

No	Name	Weight (g)	Rendemen (%)
1	Fresh material	2900,0	-
2	Dry material	1380,0	-
3	Extracted material	350,0	-
4	Dry extract	25,0	7,14
5	Solvent	13,7 (L)	-

Standardization process

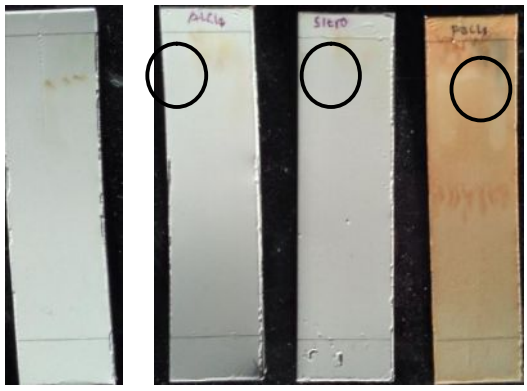
Standardization process was the important step in developing of herbal medicine. This process depends on certain parameters to get the same quality such as water, total ash, and acid insoluble ash contains.

Table 4. Standardized of minerals content in extract

No	Name	Contents (%)
1	Water content	9,04
2	Total ash content	19,53
3	Acid insoluble ash content	0,843

Content of high water in the extract will be affecting to decrease of material quality. Refers to Farmakope Herbal Indonesia (2000), generally the content of water is less than 10%. Whereas total ash and acid insoluble content are non specific parameter[16].

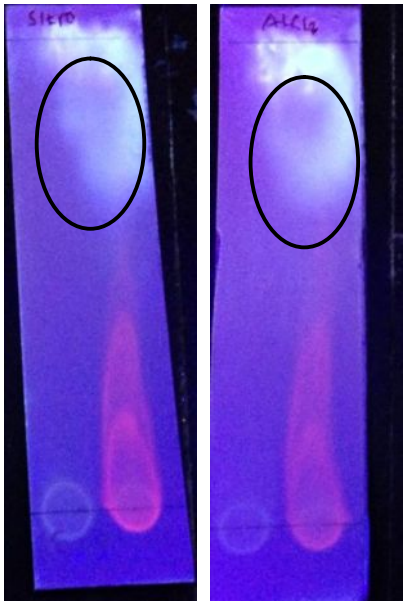
a. TLC profile



(a) (b) (c) (d)

Figure 1. TLC Profile

Stationary phase : TLC plate Silica gel 60 F254 (Merck)
Mobile phase : n-Buthanol: acetic acid : water; 4:1:5
a : without reagent
b : $AlCl_3$ sprayer reagent
c : Citroborate sprayer reagent
d : $FeCl_3$ sprayer reagent



(a) (b)

Figure 2. TLC Profile on UV_{366}

Stationary phase : TLC plate Silica gel 60 F254 (Merck)
Mobile phase : n-Buthanol: acetic acid : water; 4:1:5
a : Citroborate/UV_{366}
b : $AlCl_3/UV_{366}$

TLC profile showed that the Kanunang extract contains a phenol and flavonoid compouns because the spot of extract was changed to positive colour that is blue [15]. $FeCl_3$ is a reagent to identified of phenols and polyphenols. Meanwhile, Citroborat and $AlCl_3$ are specifically identified of flavonoid. The positive colour is fluorescent on UV_{366} light.

b. Microbiological and metal contamination assays

Microbiological assay was used to detect of phatogenic bacteria in the extract at Microbiology Laboratory, Faculty of Pharmacy UMI. The result showed that the the number of bacteria colony was not more than standard maximum value.

Table 5. Number of bacteria colony

Kanunang extract	**Number of bacteria in colony**			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}
NA (Nutrient Agar)	88	120	110	20
PDA (Potato Dextrose Agar)	26	320	128	26

Heavy metal contamination assay was method to measure of metal contaminan in the *C. myxa* leaves extract that has at Laboratory of assay and calibration of BBIHP Makassar, Indonesia. The result showed that metal contaminan at *C. myxa* extract is lower than the standard value (based on Indonesia National Standardization).

Table 6. Heavy metal contents

Metals	**Unit**	**result**	**Methods**
Mercury (Hg)	mg/kg	< 0,1	SNI 01-2896-1998 point 6
Timbal (Pb)	mg/kg	<0,1	SNI 01-2896-1998 point 5
Cadmium (Cd)	mg/kg	<0,05	SNI 01-2896-1998 point 5

c. Preclinical assay

Antidiabetic preclinical assay is using the enzyme as an in vitro test such as inhibition of α-glucosidase enzyme activity. The principle of this method is based on extracts of *C. myxa* ability to inhibit α-glucosidase enzyme that is characterized by decreased glucose levels after treatment were measured on a UV-Vis spectrophotometer instrument.

This research was using the same material such as sample (extract), sample control, blank, and blank control. Extract solutions were several consentration that is 15, 30, 45, 60, 75, and 90 µg/mL. The mixture solution was measured in microplate reader at 405 nm.

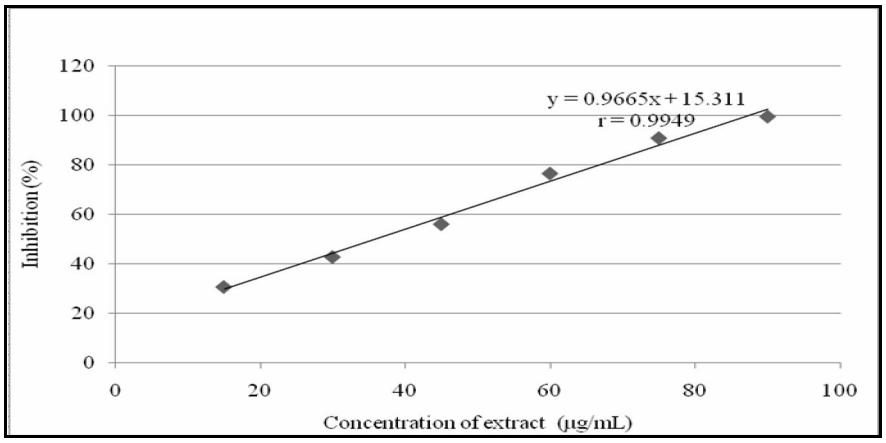


Figure 3. Correlation coefficient of concentration of extract to inhibit α-Glucosidase enzyme

Table 7. IC_{50} of *C. myxa* extract

C	A	SC	A-SC	Average (A2)	Blank (A1)	% inhibition	A	b	R	IC_{50}
15	0,448	0,042	0,406	0,405	0,585	30,77	15,31	0,967	0,995	35,89
	0,445	0,041	0,404							
30	0,395	0,064	0,331	0,335		42,82				
	0,396	0,058	0,338							
45	0,352	0,083	0,269	0,257		56,07				
	0,328	0,083	0,245							
60	0,239	0,100	0,139	0,138		76,50				
	0,231	0,095	0,136							
75	0,179	0,126	0,053	0,054		90,77				
	0,176	0,121	0,055							
90	0,168	0,165	0,003	0,004		99,40				
	0,166	0,162	0,004							

C = Concentration
A = Absorbance
SC = Sample Control
IC_{50} PC = 117,20 µg/mL

The activity of chemical compounds can be seen at IC_{50} value. There are inhibiting of enzyme activity as a converted the carbohydrate to its monomers.

The result of inhibition assay have displayed the kanunang extract inhibit of α-glucosidase enzyme with IC_{50} value 35.89 µg/mL. In this point showed that the extract is stronger than positive control with IC_{50} value 117.20 µg/mL. On the other hand, increasing the consentrations indicated decreasing absorbance, so the lower glucose has produced and the higher of inhibitory effect the enzyme of α-glucosidase.

Alfa-Glucosidase is the enzyme to metabolism of polysaccharide or olygosaccharide to convert to monosaccharide and increase of glucose in the blood. α-Glucosidase is a catalyst of non prediction glucose at α-1,4 on substrat with α-D-glukose as a result[17]. It is an important in the ending procces for break of carbohydrate. If the activity of enzyme is inhibited, concentration of postprandial glucose in the blood will be decreaced[18,19].

Conclusions

C. myxa leaves exstract can be inhibiting of α-glucosidase activity. IC_{50} value of *C. myxa* exstract is 35,89 µg/mL, and positive control is 117,20 µg/mL. The *C. myxa* leaves exstract is potential as an antidiabetic herbal medicine.

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