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Postprandial Bioassay and Radical Scavenging on *n*-Hexane Fraction of *Cordia myxa* L. Leaf

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Abstract
Cordia myxa L. *is empirically used as an antidiabetic medication. The aim of this study is to determine the in-vivo effect n-hexane fraction of Cordia myxa* L. *on reducing blood sugar levels and their relationship with the anti-free-radical activity assay using DPPH (1,1-diphenyl-2-picrylhydrazyl). The n-hexane fraction of Cordia myxa* L. *was used as a sample in the post-prandial assay which was analyzed using the ANOVA statistical test followed by post-hoc test. The results showed that there were significant in the extract using a 500 mg dose. In the anti-free-radical test using DPPH (1,1-diphenyl-2-Picrylhydrazyl) at maximum wavelength of 516 nm, 16.49 µg /mL IC_{50} value was obtained. For comparison, Quercetin with IC_{50} 0.13 µg/mL was used. This shows that the sample has strong anti-free radical inhibitory potential ranged from 10 µg/mL to 50 µg/mL. The data from the calculation showed that there is a correlation between its effect of lowering blood sugar levels with anti-free radical activity from the n-hexane fraction of Cordia myxa* L.

I. Introduction

Diabetes is a degenerative and chronic disease (World Health Organization, 2022). As a result of this disease can be related to one mechanism for the emergence of a new symptom caused by oxidation of free radical cause the severity of the disease to increase (Endrini et al., 2007) for example, the emergence of a tumor (Halim & Halim, 2019). This can be prevented by using natural ingredients in the form of nutritious plants as antidiabetic (Chinsembu, 2019). The use of plants as antidiabetic drugs has long been done. This is evidenced by the many studies of plants that have potential as an alternative in the treatment of diabetes(Mohammed et al., 2017).

One of the plants that have been studied and has efficacy as an antidiabetic with a mechanism to inhibited α-glucosidase is a *Cordia myxa* L (Yousef Nasab et al., 2017). The latest research showed that the results of ELISA on *n*-hexane fraction from this plant can inhibit the enzyme α-glucosidase (Najib, Ahmad, et al., 2019). Invitro assay from purified extract on the same plant using it leaves can decrease blood glucose level in mice (Malik & Ahmad, 2016). The inhibitory of α-glucosidase that it was present in the bristle barrier of intestinal villi on enterocytes. This enzyme has a function to cleavage disaccharide and oligosaccharide and affects to increase absorption of carbohydrate. This mechanism will be inhibited with the compounds that have a potency to use decrease the postprandial glucose levels (Najib et al., 2011).

Invivo assay to determine the potential of a plant for treatment in diabetes is to use the postprandial assay is a test of decreasing blood sugar levels after consumption of carbohydrates. Measured at the time after eating is also commonly termed as blood glucose. When this measurement uses experimental animals commonly used are mice classified in several groups, namely the positive control group and negative controls while the samples to be assayed are administered at different concentrations. The assay results can be seen in the decrease in blood sugar at a normal level with taking blood at a certain time (Chiu et al., 2018).

Antioxidant assay commonly used in plants is to add free radicals in the form of compound 1,1-diphenyl-2-picryl hydrazyl (DPPH). It is unstable because in one element there is no electron pair (Zamani et al., 2018). The plant compounds that have potential as anti-free radicals when reacted with this compound will donate a proton so DPPH will be stable This is indicated by the color change in the testing system from purple to yellow (Kandi & Charles, 2019). When this color change occurs then it can be determined that the compounds added

to the system have the potential as radical scavenging (Hara et al., 2018).

This study is to determine the in-vivo effect *n*-hexane fraction of *Cordia myxa* L. on reducing blood sugar levels and their relationship with the anti-free-radical activity assay using DPPH (1,1-diphenyl-2-picrylhydrazyl).

II. Research Method

II.1 Apparatus and Chemicals

The apparatus and chemical used in this research is Nesco Multicheck kit with glucose test strips, Spectrophotometer UV-Vis (Thermo Scientific™ GENESYS 10S), metformin, Na-CMC, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Quercetin (Sigma Aldrich, USA).

II.2 Plant Material

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.

II.3 Preparation of Extract and Fraction

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) (Najib, Ahmad, et al., 2019).

II.4 Postprandial Assay

This experiment used 2 months-old male albino mice divided into 4 groups. Each group there are 5 mice.

Group I administered by Metformin, group II Na-CMC, group III sample 250 mg and group IV sample 500 mg. An animal blood sample is taken after 15, 30, 60 and 120 minutes. Plasma glucose level measured by Nesco Multicheck kit (Malik & Ahmad, 2016).

II.5 Radical Scavenging Assay

The radical scavenging activity of plant extracts against stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) was determined by the slightly modified method of Brand-Williams. DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH. The change in color (from deep violet to light yellow) was measured at a maximum wavelength of 517 nm on a UV visible light spectrophotometer. The solution of DPPH in methanol 6×10^{-5} M was prepared fresh daily before UV measurements. Three mL of this solution was mixed with 100 microgram/mL concentration of individual plant extracts as well as an herbal preparation. The samples were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following Formula 1 (Waris et al., 2016).

$$\% \text{ Inhibition} = [(AB-AA)/AB] \times 100 \quad (1)$$

Where AB = absorption of blank sample (t= 0 min); AA = absorption of test extract solution (t=15 mins)

III. Results and Discussion

Previous research on this plant was using the in vivo method, ELISA method which showed that the *n*-hexane fraction *Cordia myxa* L. leaf had the effect of inhibiting α-glucosidase (Najib, Ahmad, et al., 2019). This research aimed to determine the basic concept of subsequent research was to look into the method related to the radical scavenging effect. The *n*-hexane can inhibit the increase in blood sugar in mice. Table 1 showed that the sample can decrease blood glucose to the normal level on the animal test. Related with the same genus *Cordia obliqua* on streptozotocin-induced diabetic rats (Ramakrishnan et al., 2017). There are twenty-four compounds on *n*-hexane fraction have been determination (Najib, Handayani, et al., 2019). The interaction of This compound can inhibit α-glucosidase with the uncompetitive kinetic mechanism (Najib, Ahmad, et al., 2019).

Table 1: Post Hoc statistical data analysis on standard and sample

Group	Group	Mean different (J)	Std. Error	Sig	95% Confidence Interval	
					Lower bound	Upper bound
Metformin	Na CMC	88.60	54.65	0.124	-204.44	27.24
	Extract 250 mg	-12.80	54.65	0.818	-128.64	103.04
	Extract 500 mg	39.40	54.65	0.481	-76.44	155.24
Na-CMC	Metformin	88.60	54.65	0.124	-27.24	204.44
	Extract 250 mg	75.80	54.65	0.184	-40.04	191.64
	Extract 500mg	128.00*	54.65	0.032	-12.15	243.84
Extract 250 mg	Metformin	12.80	54.65	0.818	-103.04	128.64
	Na CMC	-75.80	54.65	0.184	-191.64	40.042
	Extract 500mg	52.200	54.65	0.354	-63.64	168.04
Extract 500mg	Metformin	-39.40	54.65	0.481	-155.24	76.44
	Na CMC	128.00*	54.65	0.032	-243.84	12.15

Extract 250 mg	-52.200	54.65	0.354	-168.04	63.64

*The mean difference is significant at the 0.05 level

The radical scavenging on Table 2 showed that the sample has strong potency (IC_{50} lower than 50 µg/ml). It means the antioxidant compound in sample can cure the stress oxidative related to diabetes mellitus. Because oxidative stress plays an important role in complications of diabetes mellitus, both microvascular and cardiovascular (Burgos-Morón et al., 2019). DPPH as a model-free radical can be inhibited adequately by the sample. Figure 1 showed the highest potency from quercetin as the standard radical scavenging on plant compound (Hidalgo et al., 2010). Even though the IC_{50} on sample lower than comparator but it still affected to scavenge the DPPH with the color change of system from violet to yellow (Waris et al., 2016). Figure 2 showed the standard curve on the sample with a correlation value R^2 = 0.9978. The correlation can explain that the measurements are valid (Abdi, 2010).

In conclusion *n*-hexane fraction of *Cordia myxa* L. leaf has a potency to develop to cure diabetes mellitus relationship with antioxidant compound activity.

Table 2: Results of radical scavenging assay

Sample	Concentration (ppm)	Blank absorbance	Sample absorbance	Inhibition %	IC_{50} (µg/ml)
Quercetin	0.1	0.854	0.431	49.53	
	0.2	0.854	0.417	51.17	
	0.3	0.854	0.402	52.92	0.1261
	0.4	0.854	0.388	54.56	
	0.5	0.854	0.376	55.97	
n-hexane fraction	10	0.854	0.431	49.53	
	20	0.854	0.425	50.23	16.49
	30	0.854	0.418	51.05	
	40	0.854	0.410	51.99	
	50	0.854	0.403	52.81	

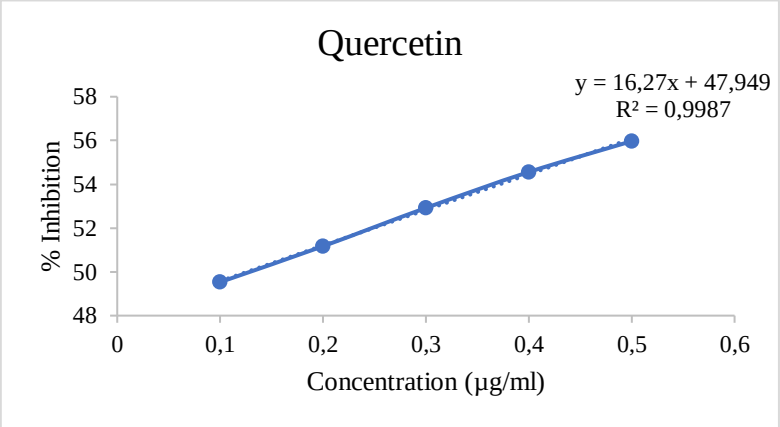

Figure 1. Standard curve on quercetin

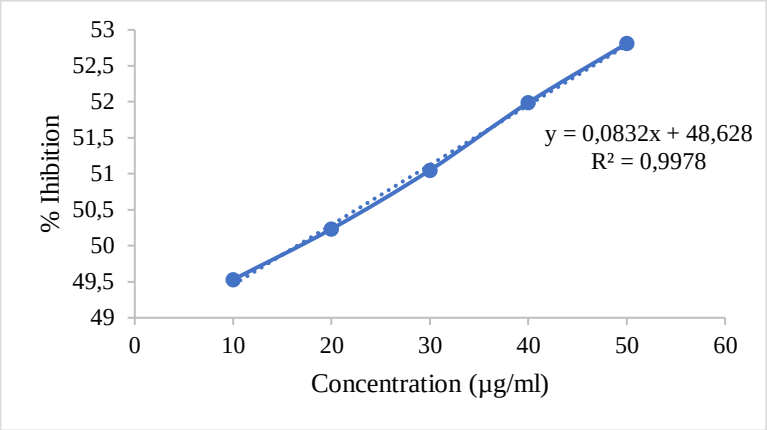

Figure 2. Standard curve on sample

IV. Conclusions

There is a correlation between its effect of lowering blood sugar levels with anti-free radical activity from the *n*-hexane fraction of *Cordia myxa* L.

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