Artocarpus Altilis Leaves Activity in Inhibiting α-Amylase Enzyme as Oral Antidiabetic Drug Candidate

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Abstract: The search for effective antidiabetic drugs remains to be a focus of research in the world. *Artocarpus* have been used as traditional medicine in South-East Asia. One of the species, *Artocarpus altilis* has been known in Indonesia as traditional treatment for diabetic. This research aimed to discover α-amylase inhibitor activity of *Artocarpus altilis* leaves. Leaves were extracted by maceration method, followed by phytochemical screening and quercetin identification using LC-MS method. Inhibition of α-amylase was measured using UV-Vis spectrophotometer at 524 nm wavelength, while antioxidant activity was determined using DPPH. *Artocarpus altilis* leaves ethanol extract exhibited activity against α-amylase with 155.96 ppm IC50 (0.27 times relative potential compared to acarbose) and the extract showed antioxidant activity with the IC50 of 999.96 ppm. *Artocarpus altilis* showed activity as the α-amylase inhibitor.

1 INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder caused by insulin deficiency resulting in increased levels of sugar in the blood. According to the International Diabetes Federation (2013) predicts an increase in the number of DM patients in Indonesia from 9.1 million in 2014 to 14.1 million by 2035.

One way that can be used to maintain postprandial blood sugar levels in people with diabetes mellitus is to inhibit α-amylase. this enzyme works to inhibit starch by breaking the α-1,4 glycosidic bond (Bender 2004). The α-amylase inhibitor may interfere with or slow down the breakdown of carbohydrates, thereby reducing the availability of blood glucose. Therefore, the development of the α-amylase enzyme inhibitor has become an attractive target for DM treatment therapy.

Some plants have been investigated to have α-amylase inhibitory activity such as *Colocassia esculenta* and *Xanthosoma mafafa* with high flavonoid contain (Eleazu et al. 2018). The inhibitory effect on α-amylase also found on plants that have potent antioxidant activity, like ethanolic extract of *Elaeocarpus Ganitrus* (Talukdar and Nagar 2017). One of the plants that have been used by the people of Indonesia to overcome diabetes is *Artocarpus altilis* leaves, or well known as Breadfruit, or in Indonesia called Sukun.

Ethanol extract of breadfruit (*Artocarpus Communis* Forst) leaves has proven to have antihyperglycaemic activity at a dose of 100 mg/kg BW with 66.77% blood glucose reduction (Ari et al. 2015). Other study showed that plants with the same family of *Moraceae, Arthocarpus heterophyllus*, had been investigated to have activity as α-amylase inhibitor (Kotowaroo et al. 2006). In this study, we investigated the activity of 70% ethanol extract of *Artocarpus altilis* leaves against α-amylase. The test was performed using a DNS (dinitrosalisilate) reagent (Thalapaneni et al. 2008).

2 MATERIAL AND METHODS

2.1 Material

Artocarpus altilis leaves were obtained from Balai Penelitian Tanaman Rempah dan Obat (BALITRO) Bogor. Determination was done at Herbarium Bogoriense, LIPI, Bogor. α-amylase enzyme from *Bacillus licheniformis* (Sigma-Aldrich).

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2.2 Extract Preparation

A total of 500 mg *Artocarpus altilis* leaves powder was macerated with 8 L of 70% ethanol. The powder was soaked (each time using one-third of the total solvent) for 6 hours with occasional stirring then stand for 24 hours. Macerate was then separated by filter paper. The process was repeated 3 times with the same amount of fresh solvent. All macerate was concentrated using a vacuum rotary evaporator.

2.3 Phytochemical Screening

2.3.1 Alkaloid

50 mg of *Artocarpus altilis* leaves extract (ALE) was added with 2N HCl (1 mL) and 9 mL of distilled water on a water bath for 2 mins. These filtrates were continue to screened for Dragendorff's and Mayer's test (Talukdar and Nagar 2017; Prashant Tiwari, Kumar, and Gurpreet Kaur 2011)

2.2.2 Saponin

50 mg of ALE was diluted with warm distilled water to 10 ml and shaken vigorously for 10 seconds. The formation of 1-10 cm frothing which persists for 10 min, then added with 1 drop 2 N HCl. If the frothing persists, it suggests the presence of saponins (Talukdar and Nagar 2017; Prashant Tiwari, Kumar, and Gurpreet Kaur 2011).

2.2.3 Flavonoid

50 mg of ALE was dissolved in 10 mL ethanol, heated over the water bath at 100°C, then filtered. The filtrate then added with concentrated HCl and Mg. The formation of red-orange shows the sample contains flavonoids (Talukdar and Nagar 2017; Prashant Tiwari, Kumar, and Gurpreet Kaur 2011).

2.2.4 Tannin

50 mg of ALE was added with 10 ml of distilled water, boiled over the water bath for 5 minutes, then cooled and filtrated. The filtrate was then added with 1-2 drops 1% FeCl3, if dark blue or dark green colour was formed, indicates the presence of tannin (Talukdar and Nagar 2017; Prashant Tiwari, Kumar, and Gurpreet Kaur 2011).

2.2.4 Terpenoid and Steroid

50 mg of ALE was added with 2 drops of anhydrite acetic acid, followed by concentrated H2SO4 along the side of the test tube and observed for the presence of a reddish purple colour indicating the presence of terpenoids while blue-green indicating steroids (Talukdar and Nagar 2017; Prashant Tiwari, Kumar, and Gurpreet Kaur 2011).

2.4 LC-MS Analysis

Identification with LC-MS was done to determine the content of quercetin compound in plant extract test. LC-MS analysis was carried out with the instrument of Agilent Technologies 7890 with Auto Sampler and 5975 Mass Selective Detector and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: HP Ultra 2. Capillary column; length (m) 30x0.25 (mm) I.D x 0.25 (µm) Thickness film, operating in electron impact mode at 70eV; Helium gas was used as carrier gas at a constant flow of 1.2 µl / min and injection volume of 5 µl was employed (split ratio 100: 1) injection port temperature 250ºC; ion source temperature 230ºC. Initial oven temperature was at 80ºC (for 0 minute), increasing at 3ºC/min to 150ºC (hold for 1 minute) and increasing 20ºC/min to 280ºC (hold for 26 minutes).

2.5 Antioxidant Property

5 mg DPPH was dissolved in 250 mL MeOH to obtain 50 µM solution. 3.8 mL of DPPH solution then added with 0.2 MeOH, left for 30 minutes in dark, then scanned between 500-700 nm with UV-Vis spectrophotometer to get maximum absorption wavelength.

ALE was diluted with distillate water to make the concentration of 100, 150, 200, 250 and 300 ppm. Each dilution was pipetted 0.2 mL and mixed with 3.8 mL 50 µM DPPH solution. The absorbance of mixing solutions was measured 30 min after the reaction (in dark) using the maximum wavelength (517 nm). Data obtained were processed using below formula, with absorbance of 50 µM DPPH solution as contro (Hanani, Mun, and Sekarini 2005)l:

$$\% \text{ Inhibition} = \frac{control\ absorbance}{sample\ absorbance}\ x\ 100\%$$

IC50 was calculated using regression equation.

2.6 α-Amylase Inhibition Test

Inhibitory activity against α-Amylase was conducted following Thalapaneni et al. (2008), with modification. A total of 500 µl of the test sample (ALE- 90, 150, 260, 450, and 780 ppm and acarbose- 16, 25, 39, 61, 95 and 150 ppm) were

added to 500 µl of α-amylase solution (0.5 ppm) in 0.02 M phosphate buffer (PH 6.9 with 0.006 M NaCl) then incubated at 25°C for 10 minutes. Each tube then added with 500 µl of 1% (b/v) of starch solution in 0.02 M phosphate buffer at the time interval, then incubated at 25°C for 10 minutes. After the second incubation, 1000 µl of dinitro salicylic acid colour (DNS) reagents was added to stop the reaction. The tubes were then incubated bath for 5 minutes in boiling water, then left to room temperature. Finally, 10.000 µl of distilled water was added, then was measured at 524 nm.

Table 1. Solution Composition of α-Amylase Inhibition Assay

Group	Sample (µl)	Buffer (µl)	Enzym (µl)	Starch (µl)	DNS (µl)	Distilled water (µl)
Blank	-	1000	-	500	1000	10.000
Blank control	-	500	500	500	1000	10.000
ALE control	500	500	-	500	1000	10.000
ALE	500	-	500	500	1000	10.000
Acarbose	500	-	500	500	1000	10.000

The inhibitory Percentage was calculated using following formula (Trinoviani, Kholisoh, and Ar-rifa 2016).

$$\text{Inhibition (\%)} = \frac{C - X}{C} \times 100\%$$

C = Abs. of blank control – Abs. of blank

X = Abs. of sample – Abs, of sample control

Relative potential (RP) was calculated using the following formula (P et al. 2011):

$$RP = \frac{IC_{50} \text{ of acarbose}}{IC_{50} \text{ of ALE}} \times 100\%$$

3 RESULT AND DISCUSSION

3.1 Phytochemical Screening

Flavonoid such as quercetin has been studied to have potent α amylase inhibitor activity (Sales et al. 2012). This research showed that Artocarpus altilis leaves extract (ALE) contains saponin, flavonoid, tannin, and terpenoid **(Table 2.).** Therefore, the search was continued to the determination of quercetin.

Table 2. *Artocarpus altilis* Leaves Extract Phytochemical Screening Results

Secondary metabolites	Results
Alkaloid	-
Saponin	+
Flavonoid	+
Tannin	+
Terpenoid	+
Steroid	-

3.2 Result of LC-MS Analysis

Artocarpus altilis leaves extract (ALE) was further investigated with LC-MS instrument to determine one of the bioactive compound which known has been studied provides a antidiabetic activity that is quercetin (Abdelmoaty et al. 2010; Sunarwidhi, Sudarsono, and Nugroho 2014; Tadera et al. 2006). The result displayed that quercetin presented maximum peak at 19.16 retention time. On the other hand, ALE chromatogram showed no peak at the same retention time (**Figure 1**). Therefore, it showed that ALE did not contain quercetin.

Even though phytochemical screening of ALE was positive for flavonoids, from the results of LC-MS test showed that quercetin is not one of the flavonoid compounds that may play a role as α - amylase Inhibitor in this plant. Other research shows potent flavanol compounds that might contribute as active inhibitor are luteolin and myricetin (Tadera et al. 2006). Further study is needed to investigate these bioactive compounds in Artocarpus altilis leaves.

3.3 α - Amylase Inhibitory Activity of *Artocarpus altilis* leaves Extract

The α-amylase enzyme play role in the hydrolysis of starch by breaking the α-1,4 glycoside bond (Bender 2004). Inhibition of this enzyme has been proven to be useful to prevent increasing postprandial glucose level in patients with diabetes mellitus (Eleazu et al. 2018). It works by affecting the metabolism in the gastrointestinal tract, interfere with the breakdown of carbohydrates so that will reduce the blood glucose (Shinde et al. 2008).

The α-amylase enzyme and the starch-soluble substrate were used in the α-amylase inhibitor activity test. The DNS reacted with reducing sugars to form 3-amino-5-nitrosalicylic acid. These compounds are

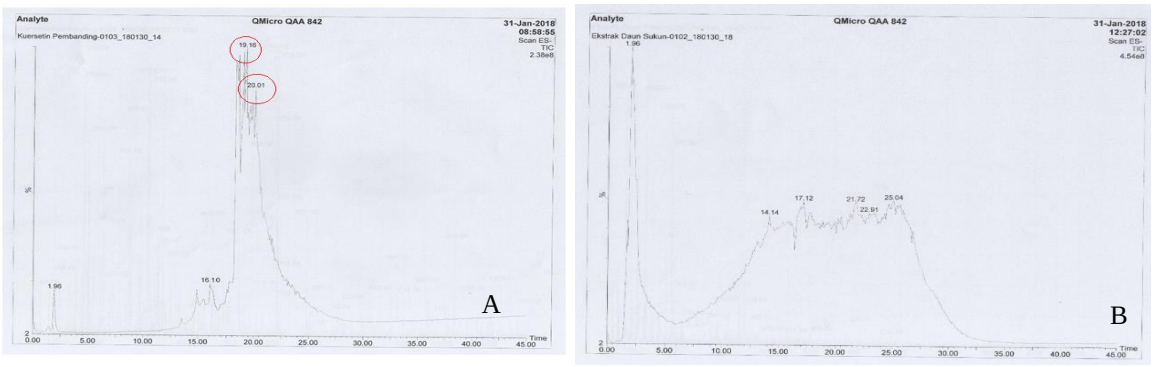

Figure 1. Chromatogram of Quercetin (A) and ALE (B)

Table 3. Absorbance on α-Amylase Inhibitory Assay

ALE Concentration (ppm)	Abs
90	0.168
150	0,155
260	0,119
450	0,086
780	0,045

Table 4. Percentage of α-amylase Inhibition

Sample	Cons (ppm)	Inh. (%)	IC_{50} (ppm)
ALE	90	40,7	155.96
	150	46,0	
	260	59.7	
	450	72,6	
	780	88,2	
Acarbose	16	21,3	42.7
	25	29,7	
	39	46,4	
	61	57,8	
	95	77,2	

The results showed that ALE had inhibitory activity against α-amylase with IC50 of 155.96 ppm. While the value of IC_{50} acarbose was 42.7 ppm

(**Table 4**.). These data indicating that ALE was less potent compared to acarbose with relative potential was 0.27.

Chemical compounds suspected to act as a-amylase inhibitors in this plant are flavonoids. Flavonoids could form complex compounds with starch, which causes the inhibition of hydrolysis of polysaccharides into monosaccharides. The flavonoid structure responsible for inhibiting the α-amylase enzyme is a double bond on the position B of the 2 'and 3', 5-OH rings, the bond at the B ring in position 3 'and the OH group in ring B. The alteration in the structure is thought to be reduced inhibition ability (Tadera et al. 2006). However, it is possible that other contents may also act as inhibitors α-amylase.

Artocarpus altilis leaves ethanol extract has been studied on α-glucosidase with IC50 8.89 ppm (Gustina 2012). While in this study the IC50 was 155.95 ppm for α-amylase. This shows that this plant more potent in inhibiting α-glucosidase than a-amylase.

3.4 Antioxidant Activity of *Artocarpus altilis* leaves Extract

Research shows that the effects of α-amylase inhibitors are found in plants that have antioxidant activity. In this study, we also determined the antioxidant activity using DPPH method. Oxidative stress is an imbalance of reactive oxygen radicals. Antioxidants can prevent cell damage by preventing or slowing down the oxidation process (Fındık, Ceylan, and Elmastaş 2011). Study showed that plants with has potent antioxidant properties could also inhibit α-amylase at low concentrations (Apostolidis and Lee 2010; Ashok et al. 2011; Conforti et al. 2005). The result showed that ALE possesses a potent antioxidant property towards DPPH with IC_{50} of 996.66 ppm (**Table 5**.).

Table 5. Percentage of Inhibition by DPPH method

Sample	Concentration (ppm)	Abs.	IC (%)	IC_{50} (ppm)
ALE	100	0.650	12.57	996.66
	150	0.643	13.51	
	200	0.615	17.34	
	250	0.599	19.42	
	300	0.594	20.10	

4 CONCLUSIONS

In conclusion, 70% ethanol extract of breadfruit (Artocarpus altilis) leaves exhibited antioxidant activity and potential as an α-amylase inhibitor.

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