

**ISOLASI DAN UJI PENGHAMBATAN ALPA AMILASE  
SENYAWA KUERSETIN DARI BUAH OKRA (*Abelmoschus  
esculentus* L.)**

**ISOLATION AND INHIBITION TEST OF QUERCETIN COMPOUND  
FROM OKRA FRUIT (*Abelmoschus esculentus* L.)**

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**Submitted:** 21-10-2021

**Revised:** 15-11-2021

**Accepted:** 30-12-2021

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**ABSTRAK**

Okra (*Abelmoschus esculentus* L.) adalah tanaman dari suku Malvaceae. Senyawa metabolit sekunder yang cukup besar pada buah Okra adalah golongan flavonoid yang diduga mampu memberikan aktifitas farmakologi untuk menurunkan gula darah. Penelitian ini bertujuan melakukan isolasi, identifikasi terhadap isolat kuersetin yang telah mampu dipisahkan dan mengetahui hasil uji penghambatannya terhadap enzim alpa amilase. Ekstraksi dilakukan menggunakan metode remaserasi dengan pelarut etanol 80% dilanjutkan fraksinasi dengan n-heksana, eter, etil asetat dan air. Senyawa penanda diisolasi menggunakan metode KLT Preparatif dengan fase diam silika gel dan fase gerak n-butanol : asam asetat : air (4:1:5). Isolat berupa serbuk kuning dengan rendemen 0,18% dan memiliki titik lebur 307-309,5°C. Hasil identifikasi dengan KLT Densitometri diperoleh hasil harga Rf dan bentuk spektra dari baku kuersetin dan isolat hampir sama atau dikatakan identik. Pengujian terhadap daya inhibisi senyawa kuersetin terhadap enzim alpa amilase diperoleh hasil senyawa kuersetin mempunyai daya penghambat sebesar 49,74% dibanding dengan acarbose 58,90%. Nilai IC<sub>50</sub> isolat kuersetin adalah 8,358 mg/ml dan acarbose 7,598 mg/ml dan secara statistik, kedua kelompok tersebut tidak menunjukkan perbedaan yang signifikan.

**Kata Kunci:** Okra, *Abelmoschus esculentus*, enzim alpha amilase, kuersetin

**ABSTRACT**

Okra (*Abelmoschus esculentus* L.) is one of the plants from the Malvaceae. The secondary metabolite compounds with quite large composition flavonoid compound that is able to provide pharmacological activity to lower blood sugar. This study aimed to isolate, identify find out the results of the inhibition test on the alpha amylase enzyme one of enzyme that affects blood sugar levels. Herein, the extraction was initially carried out using the remaceration method of okra fruit powder using 80% ethanol followed by fractionation process using n-hexane, ether, and ethyl acetate. The marker was isolated using the preparative TLC method with a silica gel and n-butanol:acetic acid : water (4:1:5) as stationary and mobile phases. The isolates were a yellow powder with a yield of 0.18% and a melting point of 307-309.5°C. TLC Densitometry was used to determine the Rf value and the spectral form of the standard quercetin and isolates wick are found to be identical. The isolate showed that quercetin compounds had the inhibitory power of the amylase enzyme by 49.74% compared to acarbose 58.90%. IC values, by quercetin isolates were 8,358/ml and acarbose 7,598mg/ml and statistically, the t-group showed no significant differences.

**Keywords:** Okra, *Abelmoschus esculentus*, alpha amylase enzyme, quercetin

**1. INTRODUCTION**

The increase of blood sugar above normal amount is a degenerative disease that suffered by many Indonesian people and 1.5 million deaths 2012 the largest percentage of these deaths

occurred before the age of 70 for developing countries such as Indonesia due to this disease (Suyono, 2006). What is very concerning is that many diabetic patients in Indonesia are not diagnosed and do not take medication because in the early stages the signs or complaints of a lot of urination, excessive thirst, increased appetite and drastic weight loss are often not realized in time long enough to allow the emergence of various other comorbidities (Ramadhan & Marissa, 2015). Diabetes mellitus consists of several types some are given insulin or by taking diabetes drugs orally to regulate blood sugar levels in normal conditions. But certainly regulating the blood sugar need the huge amount of funds, besides that it is not uncommon for these diabetes drugs to cause side effects, making sufferers look for other alternatives. Improper management for treat diabetics can result uncontrolled increase in blood sugar with the risk of causing complications such as diabetic nephropathy, stroke, blindness and others (Kocurek, 2009). Traditionally many plants can be used empirically for antidiabetic. However, it is often used only based on experience and there is no scientific data to support it. One of the plants used for diabetes therapy is okra (*Abelmoschus esculentus*) is a type of vegetable (Xiao et al., 2011).

The main flavonoid content in okra is isoquercetin and quercetin compounds that is able to provide antidiabetic activity in vitro through the action of increasing glucose uptake in tissues and increasing insulin resistance (Jeong et al., 2012). Quercetin is a bioactive compound functioning as an antioxidant that has the ability to scavenge free radicals (Roy et al., 2014), prevent and protect from oxidative stress. Oxidative stress conditions will be able to cause uncontrolled diabetes mellitus and causes the complications. The efforts that can be made for diabetics, one of which is to control post-prandial or postprandial glucose levels, is an important strategy in preventing type 2 diabetes from developing further in the body. This condition is highly dependent on the absorption of monosaccharides whose breakdown process is catalyzed by alpha amylase enzymes. By inhibiting these enzymes, there will be a delay in glucose absorption due to inhibition of the breakdown of carbohydrates into monosaccharides that occur in the digestive system that plays a role in controlling blood sugar levels (Sabitha et al., 2011). Nowadays natural ingredients are developed to control blood sugar due to affordable price and lower side effect. In this study, quercetin was isolated from okra fruit and then the isolates were analyzed by melting point and identification by TLC densitometry. Furthermore, the isolate was tested for its activity in inhibiting the amylase enzyme. The inhibition test was carried out with a UV-Vis spectrophotometer to measure the absorption at the maximum wavelength. In measuring the inhibition of the alpha amylase enzyme, the comparison of acarbose was used as one of the oral antidiabetic agents whose mechanism is to inhibit the activity of alpha amylase.

## **2. METHODS**

### **Research Tools**

Blenders, glassware, maceration vessels, rotary evaporator Lab Tech, water baths, vials, test tubes, drip plates, analytical balances, flannel, porcelain crucible, UV lamp, melting point apparatus, volume pipette, incubator, vortex, TLC Densitometer CAMAG.

### **Research Material**

Green okra (*Abelmoschus esculentus* L.) solvents used in the extraction process were ethanol 80%, n-hexane, ether, ethyl acetate, quercetin raw, filter paper, silica gel plate GF 254, n-butanol, glacial acetic acid, aquadest, chloroform, methanol, ammonia vapor, metal Mg,

concentrated sulfuric acid, alpha amylase enzyme, acarbose, iodine reagent, quercetin standard p.a, starch p.a, DMSO p.a, NaOH p.a, Sodium phosphate p.a.

## **Method**

### ***Sample extraction***

The sifted green okra fruit simplicia was extracted using the remaceration method with 80% ethanol as solvent (Dmitrienko et al., 2012). Then green okra fruit powder many as 800 grams or more then put in a closed jar, then added 80% ethanol solvent as much as 4000mL. The extraction process was carried out for 5 days, the filtrate obtained was separated and the process was repeated at second time with the same amount of 80% ethanol as solvent. The resulting filtrate was combined and then filtered. The filtrate was collected and concentrated in a water bath at a temperature of 78<sup>0</sup>C until obtaining thick extract.

### ***Flavonoid phytochemical screening***

- a. Wilstarter reagent (Gulo et al., 2021): sample was added with concentrated HCl and Mg powder, positive flavonoids gave a yellow color.
- b. 10% NaOH reagent (Harborne, 1998): sample plus 10% NaOH positive of flavonoids gave orange/orange on the drip plate.
- c. Bate Smite-Metcalf reagent: the sample was added with concentrated H<sub>2</sub>SO<sub>4</sub> placed in a water bath, positive for flavonoids giving a red color.

### ***Identification of Flavonoids by TLC***

Confirmation test using TLC was carried out using the system as the stationary phase: silica gel GF 254 and eluted with n-butanol:acetic acid:water (4:1:5). After elution was completed, the plates were detected under a 254 nm UV light lamp, and evaporated with ammonia vapor. Positive contains flavonoids will be formed a brownish yellow stain is formed (K. R. Markham, 1982).

### ***Okra Fruit Fractionation***

As much as 25 grams of thick extract dissolved in 100 mL of distilled water. The solution was partitioned liquid by adding 50 mL of n-hexane three times then shake in a separating funnel. Let stand until two layers formed and then separated. The water fraction was partitioned with 50 mL ether three times. The remaining water fraction from the ether fractionation process was added with 50 mL ethyl acetate three times. The n-hexane fraction, ether fraction, and ethyl acetate fraction obtained were concentrated in a water bath with a temperature of 50<sup>0</sup>C (Sangavi et al., 2014).

### ***Separation of Flavonoid Compounds from Okra by Preparative TLC***

The viscous ethylacetate fraction was dissolved with 1 mL of methanol then spotted on the stationary phase GF60 254 forming a band and eluted on the best mobile phase from the results of mobile phase orientation by TLC, namely BAA (4:1:5) eluent. The results of the separation can be seen under UV light 254 nm and 366 nm. The same band stain quercetin standard was scraped off and dissolved in ethyl acetate and centrifuged for 10 minutes at 3500 rpm.

### ***Identification of Quercetin Compounds***

The compounds obtained from the preparative TLC results were determined by melting point and yield of the isolates and identification by TLC densitometry.

### ***Alpha Amylase Enzyme Inhibition Test***

In the early stages of testing isolate activity Quercetin performed by operating time (OT) and maximum wavelength. To find the maximum wavelength, it can be done by making a mixture of 1 ml of starch, 1 ml of isolate with the concentration of 5 mg/ml, 1 ml of alpha amylase enzyme, 2 ml of phosphate buffer and wait for 30 minutes and then to stop the reaction, 1 ml of 1 M HCl and 0.5 ml were added and the iodine solution as a coloring reagent. After this process the wavelength absorption readings were carried out using a UV-Vis spectrophotometer at a wavelength of 400-800nm. To determine the operating time was done by mixing 1 mL of starch, 1 mL isolate 5mg/mL, 1ml alpha amylase enzyme, 2 mL phosphate buffer. It was done for 10 measurements with 5-10 minute intervals of 5-60 minutes. Then the reaction was stopped by adding 1mL of 1M HCl and 0.5mL of iodine-iodide solution as a color reagent. Maximum wavelength absorption was read on a UV-Vis spectrophotometer. Tests for the inhibition of the alpha amylase enzyme were carried out in the following way: quercetin isolates as samples were made in 4 concentrations, namely the concentration of 10 mg/ml; 5 mg/ml; 2.5 mg/ml; 1.25mg/ml was dissolved using 1% DMSO. The isolate test solution was made by mixing 1 ml of starch solution, 1 ml of sample solution, 2 ml of phosphate buffer solution, 1 ml of enzyme solution in a reaction tube with an OT time of 10 minutes. After the OT time was reached, 1mL of 1 M HCl and 0.5mL of iodine-iodide solution were added and the absorbance was read at a wavelength of 662.5 nm. For the measurement, a blank solution was used: a mixture of 1 ml of starch; 1 ml DMSO and 3 ml phosphate buffer. The solution mixture was waited for 10 minutes and then 1 ml of 1 M HCl was added and 0 of iodine-iodide was added. 5 ml absorbance was read at a wavelength of 662.5 nm. Control solution: 1 ml starch mixture; 1 ml solution of each isolate concentration and 3 ml phosphate buffer solution. Wait until 10 minutes for the entire solution, then add 1 ml of 1 M HCl and 0.5 ml of iodine-iodide, the absorbance was read at a wavelength of 662.5 nm. Positive control: 50 mg of acarbose tablets with 5 concentrations of 10 mg/ml; 5 mg/ml; 2.5 mg/ml; 1.25mg/ml dissolved in 1% DMSO. The positive control solution was made by the mixture of 1 ml of starch solution, 1 ml of each concentration of acarbose solution, 1 ml of enzyme solution and 2 ml of phosphate buffer solution. Acarbose control solution was prepared from a mixture of 1 ml of starch, 1 ml of each concentration of acarbose solution, and 3 ml of phosphate buffer. The acarbose solution and the acarbose control were waited for 10 minutes (Salam & Dewanti., 2020); (Yuana et al., 2014). The percent inhibitor was calculated using the formula:

$$\% \text{ inhibitor} = 1 - \frac{A_1}{A_0} - A_2 \times 100\%$$

Where

A1 = sample absorbance

A2 = control absorbance

A0 = blank absorbance

### **3. RESULTS AND DISCUSSION**

The results of the extraction of okra (*Abelmoschus esculentus* L.) macerated with 80% ethanol in this research showed the highest yield percentage. The thick extract of okra (*Abelmoschus esculentus* L.) obtained was 251.34 grams and the yield was 31.42%. On research (Prakoso et al., 2016) okra fruit (*Abelmoschus esculentus* L.) macerated with 96% ethanol

solvent for 5 days obtained 11.80%. Differences in results due to differences in the concentration of ethanol solvent affect the level of polarity. 80% ethanol was more polar than 96% ethanol, the higher the polarity of the solvent, the higher the yield obtained, the more polar the solvent, the better the extraction power (Indah et al., 2016). The viscous extract obtained was then fractionated to separate the compounds according to their level of polarity in solvent levels with different polarities starting from n-hexane solvent, followed by ether and ethyl acetate solvents. The result of the screening showed that the fraction containing flavonoid was ether and ethyl acetate fraction, but base on previous research in this study, the isolation of flavonoid would only be carried out on the ethyl acetate fraction. The results of fractionation with ethyl acetate solvent obtained a yield of 5.36%. Phytochemical screening for flavonoid of ethyl acetate fraction can be done by chemical test using NaOH reagent, concentrated sulfuric acid called Bate Smite–Metcalf, magnesium metal and hydrochloric acid called Wilstater reaction. The test results showed that the ethyl acetate fraction of okra contains flavonoids, 10% NaOH reagent was used because there will be a decomposition reaction of flavonoid compounds into yellow to brown acetophenone. Because of the reduction of the benzopyron core, Wilstater reagent reaction product was yellow to orange or orange. For the Bate Smite–Metcalf reagent which is an acid catalyst, electrophilic substitution occurs which is marked by a color change to red. The results of chemical identification on the ethylacetate fraction were obtained as shown in Table 1:

**Table 1.** Result of Color Reaction Flavonoid Okra (*Abelmoschus esculentus* L.)

No	Reactor	Reaction Results
1	NaOH	yellow
2	Wilstater	orange
3	Bate Smite-Metcalf	red

After identifying the phytochemicals, it was continued with the confirmation of flavonoid compounds using the Thin Layer Chromatography (TLC) method. Flavonoid compounds were identified using the mobile phase n-butanol: acetic acid: water (4:1:5) and the stationary phase silica gel GF 254. TLC test on flavonoids confirmed the ethyl acetate fraction of okra positive contain flavonoids with the appearance of a brownish yellow color after being given ammonia vapor (K. Markham, 1982). Flavonoid compounds in the ethylacetate fraction were detected at Rf 0.65 and standard quercetin at Rf 0.50 with the chromatogram in Figure 1.

Furthermore, preparative TLC was to obtain flavonoid compounds from the ethyl acetate fraction using a chromatographic system as the mobile phase was n-butanol: acetic acid: water (4:1:5) and the stationary phase was silica gel GF 254. On the preparative TLC plate, 3 spots were obtained with an Rf value of 0.34; 0.66 and 0.92. The stain at Rf 0.66 which indicated positive flavonoid were then scraped and identified. The purity tes was carried out with 3 eluent compositions with different polarity levels. The result of TLC with 3 eluent compositions of the mobile phase obtained one spot indicated that the isolated obtained was pure. Analysis using two-dimensional TLC with developers ethanol:ethyl acetate : n-hexane ( 2:3:2) after that the TLC plate was rotated 90° and then eluted again using ethanol eluent : chloroform : n-hexane (2:2:1) also showed the presence of one stain, so it can be ascertained that the isolates that were

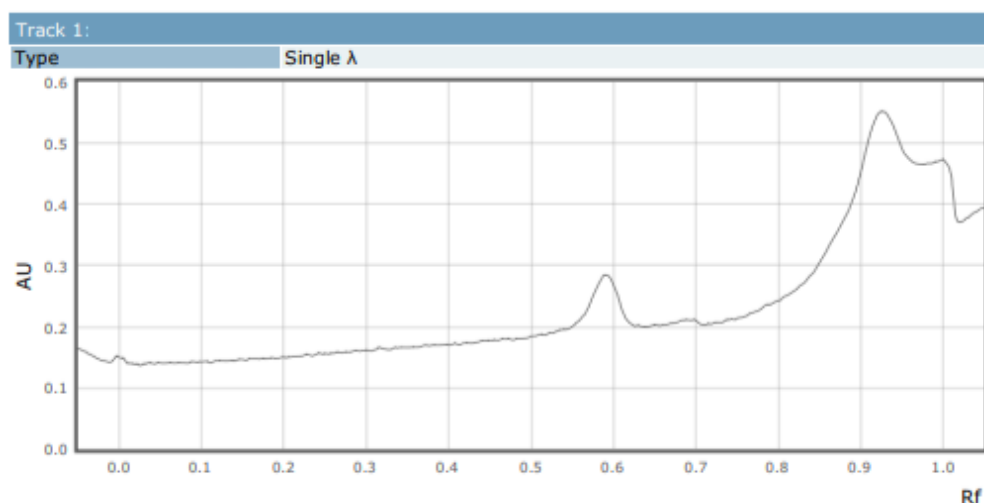
successfully separated were flavonoid isolates. The isolate was a yellow powder with a yield of 0.18% and a melting point of 307-309.5°C.



1 2 3

**Figure 1.** Identification of Flavonoid by TLC identification, namely the mobile phase was n-butanol: acetic acid: water (4:1:5), the stationary phase was silica gel GF 254 and with ammonia vapor spotting (1 = rutin standard;  $R_f=0$  no spot appear; 2 = ethyl acetate fraction  $R_f = 0,65$ ; 3 = kuersetin standard  $R_f = 0,50$ )

Identification of Pure Okra Fruit Isolates (*Abelmoschus esculentus* L.) followed by TLC Densitometry. Based on the  $R_f$  value and the form of the isolate spectra obtained compared to the standard quercetin, it has the identical values as seen in **Figure 2** and **Figure 3**:



**Figure 2.** Results Identification of Standard Quercetin by TLC densitometry. The mobile phase is ethanol: ethyl acetate: n-heksan (2:2:6) dan the stationary phase silica gel GF 254 with value  $R_f = 0,57-0,515$

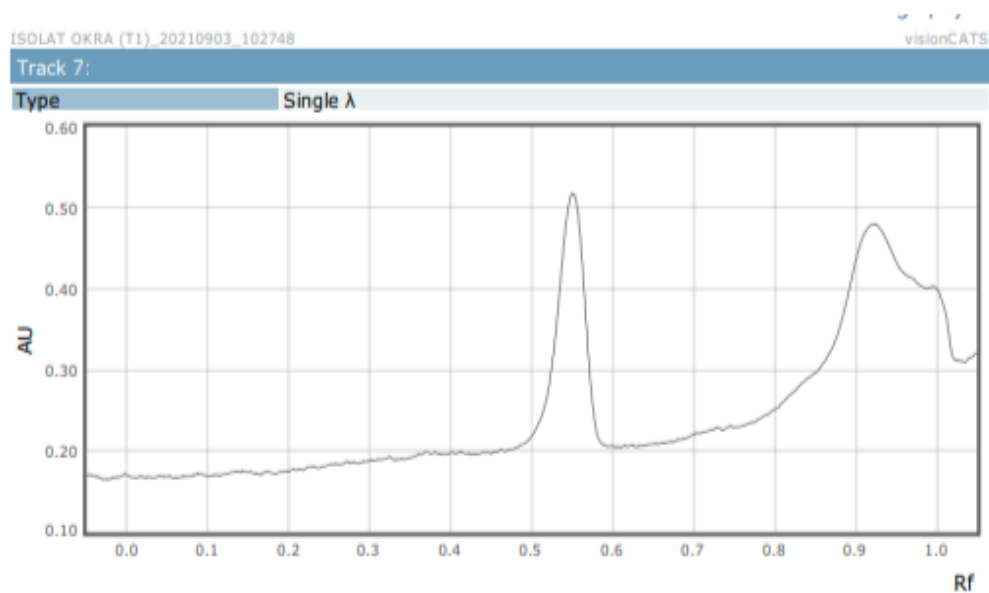


Figure 3. Results of isolate Quercetin by TLC densitometry. The mobile phase is ethanol: ethyl acetate: n-hexsan (2:2:6) dan the stationary phase silica gel GF 254 with value Rf = 0,58-0,550

Testing the activity of alpha amylase enzymes carried out *in vitro* is based on the principle of breaking down the capable substrate in producing a color with absorbance can be measured at a certain wavelength. In this study, the test was chosen for the inhibition of the alpha amylase enzyme because it is one of the therapeutic approaches for diabetes mellitus that can be done. Amylase enzyme is a digestive enzyme to speed up the reaction rate in the initial step of glucose hydrolysis, but it does not change chemically at the end of the reaction. Inhibition of the intestinal alpha amylase enzyme will later be able to delay hydrolysis into monosaccharides and simultaneously glucose absorption, resulting in decreased postprandial glucose levels (Chakrabarti et al., 2002). The test started with determining the maximum wavelength and operating time, the results were 635 nm wavelength with an absorbance value of 0.749 and an operating time of 10 minutes with an absorbance value of 0.721. The alpha amylase activity inhibition test was carried out using starch iodine testing method, by measuring the absorption of the remaining starch as a substrate formed after the amylase enzyme treatment and measured by a spectrophotometer with a maximum wavelength of 635nm. In this study, using acarbose as a positive control in testing the enzyme inhibitory activity with various concentration variants resulted the IC50 average value of 7,598 mg/ml. For the test results of quercetin isolates with various concentration variants, an average IC50 value of 8,358 mg/ml was obtained. The IC50 value is the value of the sample concentration to inhibit enzyme activity by 50%. The IC50 value was obtained from the linear regression equation which is the relationship between concentration and percent inhibition.

To find out the difference in each concentration in the group or the difference between groups, statistical testing was carried out with an unpaired T test. The results of the T-test showed  $p > 0.05$  indicating that the t-group showed no significant differences.

Table 2 IC<sub>50</sub> Value Acarbose standard and isolate Quercetin OkraFruit

Sampel	Persamaan Regresi Linear	r	IC <sub>50</sub> (mg/mL)
Acarbose Replication 1	$Y = 3,15x + 27,23$	0,996	7,23
Acarbose Replication 2	$Y = 3,87x + 21,89$	0,991	7,26
Acarbose Replication 3	$Y = 2,66 x + 30,42$	0,993	7,35
Acarbose Replication 4	$Y = 3,11 x + 24,73$	0,999	8,10
Acarbose Replication 5	$Y = 2,61 x + 28,98$	0,990	8,05
IC <sub>50</sub> Average			7,598
Isolat Replication 1	$Y = 3,61x + 20,86$	0,993	8,07
Isolat Replication 2	$Y = 2,56x + 28,23$	0,992	8,30
Isolat Replication 3	$Y = 3,82x + 21,98$	0,990	8,76
Isolat Replication 4	$Y = 2,71x + 27,16$	0,996	8,42
Isolat Replication i 5	$Y = 3,11x + 15,46$	0,997	8,24
IC <sub>50</sub> Average			8,358

#### 4. CONCLUSION

The results of flavonoid compounds isolation from okra fruit (*Abelmoschus esculentus* L.) was a quercetin compound with inhibitory effect on the alpha amylase enzyme in vitro with an average IC<sub>50</sub> value of 8,358 mg/ml and IC<sub>50</sub> for acarbose positive control of 7,598 mg/ml and statistically, the t-group showed no significant differences.

#### 5. ACKNOWLEDGMENT

Author would like to thank to STIFAR Pharmasi Foundation Semarang for providing facilities for this research. Author would like to thank to Mrs. Novy Sulistyowati and Achiro Akib who helped and supported this research.

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