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Isolation of α-glucosidase enzyme inhibitor from titanus (*Leea aequata* L.)

Roy Indrianto Bangar[a], Kurnia Nastira Ningsih[b], Rahmana Emran Kartasasmita[c], Muhammad Insanu[a]

[a]Department of Pharmaceutical Biology, Bandung Institute of Technology, Bandung, West Java, Indonesia
[b]University Center of Excellence for Nutraceuticals, Biosciences and Biotechnology Research Center, Bandung Institute of Technology, Bandung, West Java, Indonesia
[c]Department of Pharmaceutical Chemistry, Bandung Institute of Technology, Bandung, West Java, Indonesia

ABSTRACT

Diabetes mellitus is a metabolic disease characterized by high blood glucose levels. Indonesia was ranked fourth globally, with over 12 million people suffering from diabetes. Natural-based drugs can be an alternative for treating diabetes with fewer side effects and are expected to be more economical than conventional drugs. One of the plant families used to reduce blood glucose levels is the *Vitaceae* family. One of the *Vitaceae* species currently used for traditional medicine is *Leea aequata*. This study aimed to isolate active compounds with α-glucosidase inhibitor activity from titanus plant. This study began with collecting leaf, fruit, and stem samples from titanus plants that grow in North Sumatra. The extraction method used was maceration using ethanol 96%. The isolation stage started with fractionation, followed by purification with column chromatography, in which TLC monitored for each process. α-glucosidase in testing was carried out on extracts, fractions, and isolates using a reagent Multiscan microplate reader at 400 nm. Compound identification was done using spectrodensitometry and ^{1}H-NMR spectroscopy. The IC_{50} value of the ethyl acetate fraction (2.4±0.02 µg/mL) was higher than the ethanol extract (8.96 ± 0.44 µg/mL), n-hexane fraction (21±0.61 µg/mL), and water fraction (16.96 ± 0.38 µg/mL), so it was continued to the isolation stage. The isolated compound was characterized as 3,3′,4′,5,7-Pentahydroxyflavone 3-rhamnoside (quercitrin) with the IC_{50} was 12.25 ± 0.2 µg/mL.

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*Corresponding authors:
insanu99@itb.ac.id

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1. Introduction

Diabetes mellitus is a metabolic disease characterized by high blood glucose levels. The World Health Organization (WHO) states in 2003 that the number of people with diabetes aged from 20−79 years out of 3.8 billion worldwide was 194 million and is expected to increase to 333 million by 2025. Meanwhile, in Indonesia, the number of people with diabetes ranks fourth in the world after the United States, India, and China, with more than 12 million people (PERKENI, 2021). Controlling postprandial glucose levels is an essential strategy in the prevention of type 2 DM so that a therapeutic approach can be taken by delaying sugar absorption by inhibiting carbohydrate hydrolysis enzymes such as α-glucosidase in digestion (Soelistijo, 2021). Long-term use of synthetic antidiabetic drugs such as acarbose can have several side effects, such as gastrointestinal disorders such as nausea, vomiting, abdominal pain, and bloating (Zhao et al., 2020). α-glucosidase enzymes are a class of exo α-glucosidase enzymes that work to hydrolyze 1,4-glycosidic bonds and release D-glucose from the final substrate (Li et al., 2011). Polysaccharides, one of the sources of nutrition, will be broken down into oligosaccharides by α-amylase in saliva and pancreas. The product of α-amylase hydrolysis will be passed on to the small intestine (Shibano et al., 2008). Disaccharides such as maltose with α-1,4-glycoside bond, isomaltose with α-1,6-glycoside and sucrose with α-1,2-glycoside will be broken down into monosaccharides by α-glucosidase in the brush border membrane of the small intestine (Chen et al., 2021). Three types of oral antidiabetic drugs are inhibitors of α-glucosidase enzyme activity, namely acarbose, magnitol, and voglibose. Among the three drugs, acarbose is commonly used in Indonesia (Chen et al., 2021).

Using natural-based drugs can be an alternative for treating diabetes with fewer side effects and is expected to be more economical than conventional drugs. One of the plant families used to reduce blood glucose levels is the *Vitaceae* family. For example, the ethanol extract of *Leea indica* leaves showed a hypoglycaemic effect by lowering the glucose levels (Kumoro, 2015). Similarly with the ethanol extract of *Leea macrophylia* exhibited a restoration of damaged pancreatic cells in albino rats induced by streptozotocin (Rahman et al., 2018). Based on these studies it can be concluded that *Vitaceae* species can be a new source of antidiabetic agents. One of the *Vitaceae* species currently used for traditional medicine is *Leea aequata*. This plant is known as titanus in North Sumatra, and local people often use it as traditional medicine. Therefore, this study aimed to isolate active compounds with α-glucosidase inhibitor activity. Titanus plant parts that are leaf, stem, and fruit.

2. Materials and methods

2.1. Materials

The instruments used in this study were a rotary evaporator (Buchi Rotavapor), vacuum liquid chromatography, classical column chromatography tools, chromatography chamber, UV lamp with λ 254 nm and 366 nm, hotplate, sonicator, microplate reader (Versamax Elisa Microplate Readertecan infinite 200 pro, USA), X-Rite's 504 Spectrodensitometer and NMR Spectrometer (Agilent 500 MHz).

The main materials used in this study were leaf, stem, and fruit crude drugs of titanus (*Leea aequata* L.) obtained from Karo Regency, North Sumatra. The materials used in this study are ethanol 96%, ethyl acetate, *n*-hexane, acetone, chloroform, distilled water, toluene, hydrochloric acid, sulfuric acid, ammonia, magnesium powder, amyl alcohol, iron (III) chloride solution, anhydrous sodium sulfate, sodium acetate, gelatin, NaOH, potassium iodide, aluminum chloride, glacial acetic acid, citric acid, boric acid, TLC silica gel 60 F_{254} Merck, α-glucosidase from *Saccharomyces cerevisiae* and 4-nitrophenyl α-D-glucopyranoside (p-NPG) were purchased from Sigma Chemical Co. (St. Louis, MO), Bovine Serum Albumin, acarbose hydrate 98% purity from Tokyo Chemical Industry CO., LTD, CertipurÂ® buffer solution pH 7 from Merck, dimethyl sulfoxide, phosphate diluent pH 6.8 and sodium carbonate (Na_2CO_3) solution.

2.2. Methods

2.2.1. Material collection and preparation

To produce a high-quality of crude drugs, the process must begin with harvesting the fresh raw materials. The plant parts such as leaves, stems, and fruits were collected from Berastagi, Karo Regency, North Sumatra. After collection, the samples were identified and sorted. Fresh samples were then sun-dried, ground into powder, packaged and labeled in dry condition, and sealed in containers.

2.2.2. Macroscopic examination

Macroscopic examination is carried out on titanus leaves (*Leea aequata* L.) organoleptically, which includes color, shape, and smell (Kemenkes RI, 2017).

2.2.3. Crude drug characterization

Crude drug characterization (for the selected extract) included determination of lost on drying, total ash content, acid insoluble ash content, water content, and the sum of the ethanol and water-soluble content.

2.2.4. Determination of lost on drying

A total of 2 g of crude drug was weighed in a closed crucible that had been previously weighed. A crucible cup is put into the oven to dry at 105°C until the weight remains (Kemenkes RI, 2017).

2.2.5. Determination of water content

Determination of water content is based on the principle of azeotrope distillation separation. Toluene is saturated first with water in the ratio (200 mL toluene:5 mL water). The volume of water in the tube was recorded as the initial volume. 5 g of plant material was put into the distillation flask. Distillation was carried out until the volume of water did not increase anymore. Moisture content is calculated in % v/w (Kemenkes RI, 2017).

2.2.6. Determination of total ash content

The extract was weighed as much as 2 g and put into a silicate crucible, slowly incinerated until the charcoal ran out at 500-600°C, cooled in a desiccator, and weighed. If the charcoal could not be removed, the silicate crucible was cooled, 2 mL of water was added, and then it was dried on a water bath and heated until the weight remained constant at 500-600°C. The total ash content is calculated against the weight of the extract (WHO, 2011).

2.2.7. Determination of acid insoluble ash content

The ash obtained to determine total ash content was boiled with 25 mL of dilute hydrochloric acid for 5 minutes. The acid-insoluble part is filtered through ash-free filter paper and washed with hot water until the filtrate is neutral, then the filter paper is incinerated in the same crucible until a fixed weight is obtained at 500-600°C. Acid insoluble ash content is calculated against the weight of the extract (WHO, 2011).

2.2.8. Determination of water-soluble content

5 g of plant material was put in a clogged flask, and 100 mL of chloroform water was shaken repeatedly for the first 6 hours and left for 18 hours. Then, 20 mL of filtrate was filtered and evaporated to dry at 105°C until fixed weight. After that, the percent of water-soluble content was calculated (Kemenkes RI, 2017).

2.2.9. Determination of ethanol soluble content

5 g of plant material was put in a clogged flask, and 100 mL of ethanol was shaken repeatedly for the first 6 hours and left for 18 hours. Then, a quick filtration was done to avoid ethanol evaporation, and 20 mL of filtrate was evaporated to dryness at 105ºC until the weight remained. After that, the percent of ethanol-soluble content was calculated (Kemenkes RI, 2017).

2.2.10. Phytochemical screening

Phytochemical screening or phytochemical screening is carried out to determine the presence of alkaloid compounds, flavonoids, polyphenols, quinones, tannins, saponins, and triterpenoids/steroids.

2.2.11. Extraction

200 g of each crude drugs was extracted using 1.5 L ethanol 96% by maceration process during 24 hours. The process was carried out for 3 times and each time was macerated using a new solvent. The macerate obtained was then concentrated with a rotary evaporator and dried using a freeze-dryer. The yield is calculated based on the percentage of weight (%w/w) between the yield and the weight of the crude drugs powder used by weighing (Kemenkes RI, 2017).

2.2.12. Fractionation

The fractionation method used is liquid-liquid extraction. The choux paris method is used: thick ethanol extract dissolved in hot water, filtered, and then extracted successively using n-hexane and ethyl acetate. After that, each filtrate was evaporated to obtain the concentrated fraction of n-hexane, ethyl acetate and water.

2.2.13. Isolation

Fraction of the extract that actively inhibited the α-glucosidase enzyme was fractionated by classical column chromatography (CCC). 4 g of ethyl acetate fraction was fractionated using gradient polarity of the mobile phase from non-polar to polar with specifically ratio of *n*-hexane to ethyl acetate and ethyl acetate to methanol, with 2 mL collected into vials each time. This process yielded 121 subfractions, which were then monitored using thin layer chromatography (TLC) with a stationary phase of silica gel F_{254} and a mobile phase of chloroform: methanol (8:2). Monitoring was done using UV light at 254 nm, 366 nm, and citroborate reagent under UV light at 366 nm. Subfractions with similar spot patterns were combined, resulting in 7 combined subfractions. The most active subfraction (SF5) with an inhibition value of 92.29% was

then taken to the purification stage. Subfraction 5, weighing 900 mg, was further purified using classical column chromatography (CCC) with an isocratic mobile phase of chloroform: methanol (8:2), resulting in 45 sub-subfractions. Final purification was done using preparative thin layer chromatography with a stationary phase of silica gel F_{254} and a mobile phase of chloroform: methanol (8:2), yielding an isolate weighing 15 mg.

Table 1. Test of α-glucosidase enzyme inhibitory activity (Nor et al., 2023)

Reagents	Volume (µL)					
	Negative control	Blank	Sample	Sample control	Acarbose	Acarbose control
Sample	-	-	30	30	30	30
Phosphate Diluent (pH 6,8)	66	83	36	53	36	53
p-NPG 6mM Substrate	17	17	17	17	17	17
Incubation at 37°C for 5 minutes						
Enzyme α-glucosidase 0,2 U/mL	17	-	17	-	17	-
Incubation at 37°C for 15 minutes						
Sodium carbonate	100	100	100	100	100	100
Final volume	200	200	200	200	200	200

2.2.14. Purity test and characterization of isolated compound

Purity testing of the isolate was conducted using single-development TLC with three different mobile phases of varying compositions and polarities, as well as 2-dimensional TLC with the same stationary phase of silica gel F_{254}. The three mobile phases used were: (1) chloroform: acetone: formic acid (5:1:1), (2) ethyl acetate: methanol: formic acid (5:1:1), and (3) chloroform: acetone (2:8). For 2-dimensional TLC, the mobile phase used were: (1) chloroform: acetone: formic acid (5:1:1) and (2) ethyl acetate: methanol: formic acid (5:1:1). Monitoring was conducted using UV light at 254 nm, 366 nm, and a 10% H_2SO_4 in methanol spot detection reagent under visible light. The results of the purity test using both methods showed that the isolate exhibited a single spot. The instrument used to characterize the isolated compound are Spectrodensitometer and NMR Spectrometer.

2.2.15. Preparation of test solution

Weighed the extract and then put it into Erlenmeyer. Ethanol was added and stirred using a magnetic stirrer and then filtered. A series of concentrations of extract was made that are 10; 25; 50; 100; 500; 1000 µg/mL (Kemenkes RI, 2017). A series of concentrations of acarbose and selected extract for IC_{50} calculation are 1; 5; 10; 25; 50; 100 µg/mL. The tested solutions were Na_2CO_3, acarbose, and the series of concentrations of samples solution (Pant et al., 2017).

2.2.16. Stages of enzyme testing

Determination of α-glucosidase enzyme inhibition activity was carried out using the spectrophotometric method using a microplate reader. The test was conducted in a 96-well microplate. The reagents required for the test were p-NPG 6 mM, phosphate-buffered saline pH 6.8, α-glucosidase enzyme 0.2 U/mL, sodium carbonate 200 mM, positive control acarbose, and sample. The assay used a 96-well plate in which the components were negative control, blank, sample, sample control, and acarbose as the positive control. The total volume of each well is 200 µL. Testing was done in triplicate. The test of α-glucosidase enzyme inhibitory activity can be seen in Table 1.

3. Results and discussion

This study aimed to determine the potential activity of α-glucosidase enzyme inhibition from ethanol extracts of titanus plant parts, including leaves, stems, and fruits. Plant parts with the strongest activity will continue to the isolation stage, identifying compounds with activity and conducting inhibition activity of isolated compounds.

The obtained macerate was concentrated using a rotary evaporator until a thick extract was obtained. The thick extract was stored in a clean and tightly closed container. The extract yield obtained from each crude drugs can be seen in Table 2.

Table 2. Extract yield

Sample	Yield (% w/w)
Leave	40.5%
Stem	3.58%
Fruit	16.47%

3.1. Screening the inhibitory activity of α-glucosidase enzyme from titanus plant parts

Each samples extract is first screened for inhibitory activity. This is the initial screening test for selecting which extracts that will proceed to the isolation stage. The screening test was conducted using two extract concentrations, 100 µg/mL and 200 µg/mL, to determine whether doubling the extract concentration would increase the percentage of inhibition and by how many times. The extract that has the strongest activity will continue to the subfraction stage until isolated compound is obtained. The results of the activity screening of the sample extracts can be seen in Table 3. This assay method for screening α-glucosidase inhibitor activity was characterized by its speed and simplicity with using Acarbose as a drug of positive control. Percentage of inhibition on the α-glucosidase activity was calculated by the equation:

$$\left[1-\left(\frac{B}{A}\right)\right]\times 100\% \quad (1)$$

whereas (A) is absorbance in the absence of sample/blank and (B) is absorbance in the presence of sample (Srianta et al., 2013). From the stock solution (1000 µg/mL), then prepared the series of dilutions to create different concentrations of the inhibitor that are 10; 25; 50; 100; 500 µg/mL depending on the expected potency of the inhibitor. The results showed that at a concentration of 100 µg/mL, the activity was already high and there was a significant difference between the samples.

Table 3 shows that the leaf extract had the strongest inhibitory activity compared to stem and fruit extracts, so the leaf extract is continued to the isolation stage, and the IC_{50} value is calculated, which will be compared with acarbose used as a control. The IC_{50} value of acarbose and leaf extract can be seen in Table 4. The IC_{50} value is denotes the concentration of the sample required to inhibit 50% of α-glucosidase activity. To calculate the IC_{50} value, plot the inhibitor concentration (on a logarithmic scale) in the X-axis

against the percentage of inhibition in the Y-axis to generate an inhibition curve and then obtained the linear regression equation as y = 12.280 ln(x) + 40.591 with coefficient relation (R^2) = 0.9725 for acarbose and the linear regression equation as y = 7.952 ln(x) + 14.762 with coefficient relation (R^2) = 0.9861 for the selected extract. The IC_{50} value identified by the concentration that causes close to 50% inhibition and then interpolating between concentrations.

Table 3. Percent inhibition of α-glucosidase enzyme from extracts of plant parts

Sample	Percent of Inhibition	
	Concentration 100 µg/mL	Concentration 200 µg/mL
EEL	95.58% ± 1.86	98.23% ± 0.66
EES	54.61% ± 0.03	69.82% ± 0.28
EEF	74.38% ± 1.58	95.33% ± 0.89

Note: n=3; EEL (Leaf Ethanol Extract), EES (Stem Ethanol Extract), EEF (Fruit Ethanol Extract)

Table 4. IC_{50} value of α-glucosidase enzyme inhibition

Sample	IC_{50} Value (µg/mL)
Acarbose*	74.83 ± 2.55
EEL	8.96 ± 0.44

Table 4 showed that the IC_{50} value of leaf ethanol extract showed more active than acarbose. The difference in the IC_{50} value of acarbose is too far compared to the leaf ethanol extract, where acarbose is a positive control. This is due to acarbose being an α-glucosidase inhibitor which has a high inhibition effect on mammals but does not have an inhibition effect on *Saccharomyces cerevisae* and *Bacillus stearomophillus* (Kim et al., 2008).

3.2. Characterization of selected extract and crude drug

The selected extracts from the α-glucosidase inhibition activity test were characterized. Characterization of crude drug includes macroscopic and microscopic examination and also determination of their characteristics (Febrinda et al., 2013). Phytochemical screening of crude drug and extract was also carried out to examine the presence of flavonoid, tannin, saponin, steroid/triterpenoid, quinone, coumarin, and alkaloid compounds (Zürich et al., 2005). Macroscopic results showed that titanus leaves are single leaves, alternate, tapered tip pointed base, serrated edges, pinnate repetition, protruding below, smooth surface, and color.

3.3. Determination of selected crude drug characteristics

Determination of selected sample (leaves) characteristics in this study includes determination of lost on drying, moisture content, total ash content, acid insoluble ash content, and water-ethanol soluble content carried out to ensure the uniformity of crude drug quality meets predetermined requirements so that the safety of the crude drug used can be guaranteed. The results of the characterization crude drug can be seen in Table 5.

Table 5. Characterization of selected crude drug

Parameters	Sample (Leaves)
	Crude Drug
Lost on drying (w/w)	6.46% ± 0.19
Moisture content (v/w)	7.66% ± 0.03
Water soluble content (w/w)	1.1% ± 0.15
Ethanol soluble content (w/w)	9.87% ± 0.63
Total ash content (w/w)	8.03% ± 0.42
Acid insoluble ash content (w/w)	0.90% ± 0.19

3.4. Phytochemical screening

Phytochemical screening is carried out to determine the content of secondary metabolite compounds in the tested symbiotes and extracts such as alkaloids, flavonoids, saponins, phenols, tannins, triterpenoids, and steroids. The results of phytochemical screening for the crude drug and extract can be seen in Table 6.

Table 6. Phytochemical screening of selected crude drug and extract

Group	Sample (Leaves)	
	Crude drug	Extract
Flavonoid	+	+
Phenol	+	+
Tannin	+	+
Quinone	+	+
Saponins	+	+
Alkaloid	+	+
Steroid/Triterpenoid	+	+

Notes: + (detected); - (not detected)

3.5. Fractionation

Based on the results of preliminary tests, the ethanol extract of titanus leaves is the selected extract to proceed to the fractionation stage because it has the best inhibition activity. Fractionation was carried out by liquid-liquid extraction method with *n*-hexane, ethyl acetate, and water solvents. The thick extract of 10 g was dissolved in 100 mL of hot water and then filtered to remove chlorophyll. The filtrate was then liquid-liquid extracted with ethyl acetate and *n*-hexane solvents. The water, ethyl acetate, and *n*-hexane fractions were then concentrated using a rotary evaporator. The yield of *n*-hexane, ethyl acetate, and water fractions can be seen in Table 7.

3.6. Fractionation activity test on α-glucosidase enzyme inhibition

Activity testing was carried out on 3 fractions of liquid-liquid extraction results, including water fraction, ethyl acetate fraction, and n-hexane fraction. The IC_{50} results of the test can be seen in Table 8.

The most active fraction was the ethyl acetate fraction, with an IC_{50} value of 2.4 µg/mL more active than ethanol extract. This can occur because the ethyl acetate fraction compounds contain many flavonoids which plays an important role in the process of α-glucosidase enzyme inhibition (Proença et al., 2017). The *n*-hexane and water fractions were less active than the ethyl acetate extract. The most active fraction (ethyl acetate) in inhibiting the α-glucosidase enzyme was continued to the isolation stage.

Table 7. Fraction yield weight

Sample (Leaves)	Yield (% w/w)
Water fraction	45 %
n-Hexane fraction	15 %
Ethyl acetate fraction	40 %

Table 8. The IC_{50} value (µg/mL) of each fractions

Sample (Leaves)	IC_{50} value (µg/mL)
Water fraction	16.96 ± 0.38
n-Hexane fraction	21 ± 0.61
Ethyl acetate fraction	2.4 ± 0.02

3.7. Characterization of isolated compound

Characterization of isolated compound were carried out by Spectrodensitometer and NMR Spectrometer.

Based on the spectrodensitogram on Fig. 1 showing there are 2 spectral peaks where band I is around 330-360/385 nm and band II is around 250-280 nm, it can be concluded that the compound has chromophore groups on its structure. This spectrodensitogram showed two characteristics adsorption for a flavonoid with a

flavonol-based structure containing free or substituted 3-OH (Markham, 1988).

^{1}H-NMR (CD_3OD, 500 MHz) of isolated compound showed signals at proton chemical shifts (δ_H): 7.34 (*d, J*=2,1 Hz, 1H, H-2'), 7.32 (*m*, 1H, H-6'), 6.92 (*d, J*=8.3 Hz, 1H, H-5'), 6.38 (*d, J*=2.1 Hz, 1H, H-8), 6.21 (*d, J*=2.1 Hz, 1H, H-6), 5.36 (*d, J*=1.7 Hz, 1H, H-1"), 4.23 (*dd, J*=3.4 & 1.7 Hz, 1H, H-2"), 3.76 (*dd, J*=9.4 & 3.4 Hz, 1H, H-3"), 3.42 (*m*, 1H, H-5"), 3.35 (*d, J*=9.5 Hz, 1H, H-4") and 0.95 (*d, J*=6.2 Hz, 3H, H-6").

This ^{1}H-NMR spectrum on Fig. 2 showed there are 11 proton peaks which distributed on the aromatic (δ_H 6-8 ppm) and aliphatic (δ_H 1-4 ppm) regions. There are five aromatic protons with the multiplicity pattern indicating these protons originate from two different benzene rings: two protons from the A ring of flavonoid at δ_H 6,21 (*d*) (H-6) and 6,38 (*d*) (H-8) which have *meta* position of each other, and the other three protons from the B ring of flavonoid with δ_H 6,92 (H-5'), 7,32 (H-6') and 7,34 (H-2') which have *ortho* position bertween H-5'−H-6' and *meta* position between H-6'−H-2'. These aromatic protons indicated to flavonoid compounds: Quercetin. The isolated compound also has five methine-oxy protons and one methyl proton from glycone part which indicated to rhamnose (hexose sugar), where one methyl proton at δ_H 0,95 (H-6"), followed by four methine-oxy protons at δ_H 3,35 (H-4"), 3,42 (H-5"), 3,76 (H-3") and 4,23 (H-2"), and one the most deshielded methine-oxy proton at δ_H 5,36 (H-1") which is a proton bound to two oxygen atoms. The ^{1}H-NMR data of isolated compound indicate that the isolate is a glycosylated flavonoid compound, with the aglycone part being the flavonoid Quercetin and the glycone/sugar part being Rhamnose which is Quercitrin and the compared data with previous study by Tsague et al. (2020) shown in Table 9.

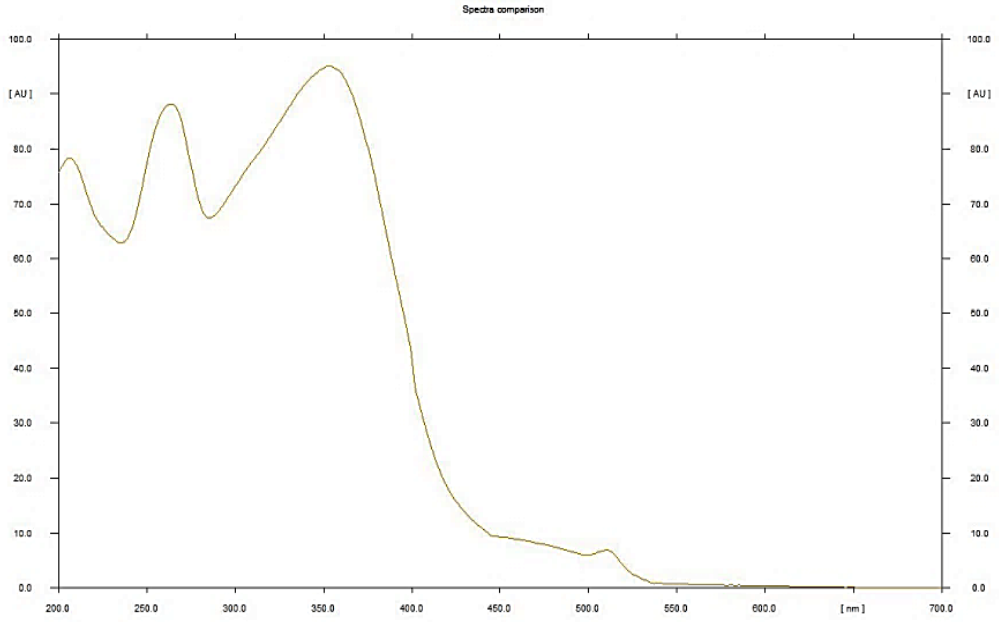

Fig. 1. Spectrodensitogram of isolated compound

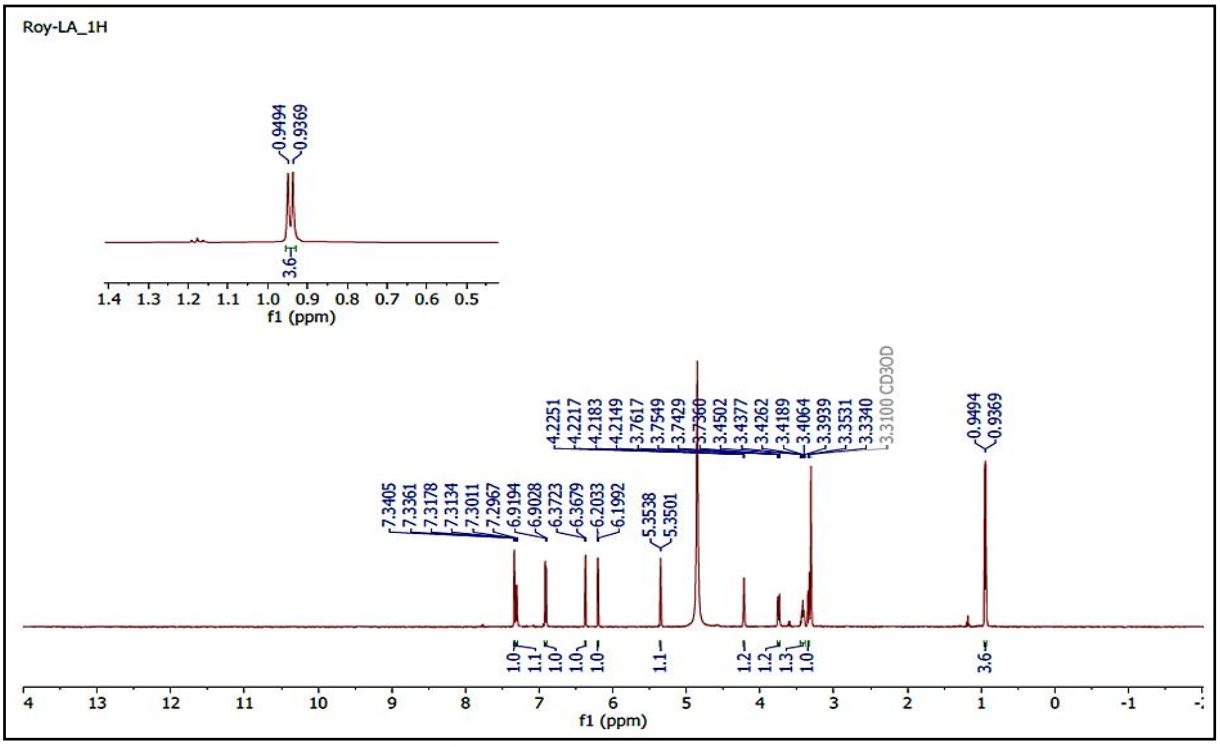

Fig. 2. ^{1}H-NMR spectrum of isolated compound

Table 9. ^{1}H-NMR of isolated compound compared with reference

Position	Isolated compound	Reference Quercitrin (Tsague et al., 2020)
	δ_H in ppm (m, J)	δ_H in ppm (m, J)
H-6	6.21 (d, J=2.1 Hz)	6.21 (d, J=2.1 Hz)
H-8	6.38 (d, J=2.1 Hz)	6.38 (d, J=2.1 Hz)
H-2'	7.34 (d, J=2.1 Hz)	7.35 (d, J=2.1 Hz)
H-5'	6.92 (d, J=8.3 Hz)	6.92 (d, J=8.3 Hz)
H-6'	7.32 (m)	7.32 (m)
Rhamnose		
1"	5.36 (d, J=1.7 Hz)	5.37 (d, J=1.3 Hz)
2"	4.23 (dd, J = 3.4 & 1.7 Hz)	4,24 (dd, J=3.2 & 1.7 Hz)
3"	3.76 (dd, J = 9.4 & 3.4 Hz)	3,77 (dd, J=9.7 & 3.3 Hz)
4"	3.35 (d, J = 9.5 Hz)	3.36 (d, J=9.5 Hz)
5"	3.42 (m)	3.44 (m)
6"	0.95 (d, J = 6.2 Hz)	0.95 (d, J=6.2 Hz)

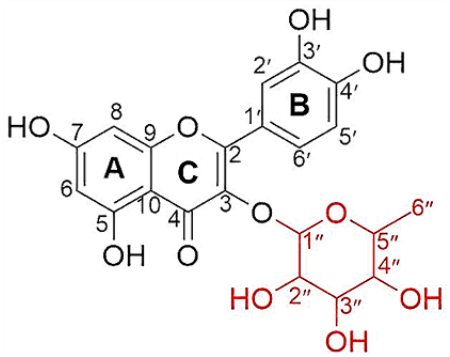

Fig. 3. Structure of isolated compound (quercitrin)

From the results of the ^{1}H-NMR spectrum, it can be concluded that the chemical structure obtained is quercetin linked by the rhamnose glycoside at the C-3 position via the C-O-C bond. With this, it can be concluded that the isolated compounds obtained are 3,3′,4′,5,7-Pentahydroxyflavone 3-rhamnoside; Quercetin 3-rhamnoside; Quercitrin. The structure of the isolated compound can be seen in Fig. 3.

3.8. Isolated compound activity test

Isolated compound was tested for their inhibitory activity against the α-glucosidase enzyme quantitatively using a microplate reader. The results of the isolated compound test obtained an IC_{50} value is 12.25 ± 0.2 µg/ml which higher than the EEL/ Leaf Ethanol Extract (8.96 ± 0.44 µg/ml) and ethyl acetate fraction (2.4 ± 0.02 µg/ml) that means the isolated compound is less active. From these results showed that α-glucosidase inhibitory activity is not only caused by single compound but also probably by the result of synergic effect from any compounds in extract or fraction.

4. Conclusion

It can be concluded that the active compound on α-glucosidase inhibitory activity is 3,3′,4′,5,7-Pentahydroxyflavone 3-rhamnoside (Quercitrin) with an IC_{50} value is 12.25 ± 0.2 µg/mL.

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Conflict of interest

The authors declare there is no conflict of interest in this research.

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