# ISOLATION AND IDENTIFICATION OF BIOACTIVE FLAVONOID GLYCOSIDES FROM *SYZYGIUM JAMBOS* AND THEIR A-GLUCOSIDASE INHIBITORY ACTIVITY

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**ABSTRACT**

Diabetes has grown into a demanding global public health problem, with generally 537 million individuals afflicted worldwide. Without appropriate intervention, the risk of developing chronic complications increases. One of the practical actions for controlling type 2 diabetes mellitus is suppressing carbohydrate-hydrolyzing enzymes such as α-glucosidase. *Syzygium jambos* has a long history as a traditional medicine. It's commonly used to treat diabetes, rheumatism, inflammatory pain, and as a diuretic. This study aimed to investigate the α-glucosidase inhibitory properties of *S. jambos* leaves and their isolated compounds. Ethanol was used as the solvent for maceration, followed by fractionation using liquid-liquid extraction, vacuum liquid chromatography, column chromatography, and radial chromatography. The isolated compounds were identified using thin-layer chromatography (TLC), TLC densitometry, shifting reagents, infrared (IR) spectroscopy, mass spectrometry, and nuclear magnetic resonance (NMR) spectrometry. The ethyl acetate fraction presented the most potent activity of α-glucosidase inhibitory, with an $IC_{50}$ value of 0.31 ± 0.01 μg/mL, exceeding the acarbose as positive control ($IC_{50}$ 62.86 ± 1.24 μg/mL). Two flavonoid glycosides, quercetin 3-O-xylosyl-(1→2)-rhamnoside and myricetin 3-O-xylosyl-(1→2)-rhamnoside, were identified inside this fraction, with $IC_{50}$ values of 65.63 ± 1.36 μg/mL and 188.96 ± 2.85 μg/mL, respectively. These findings suggest that *S. jambos* has potential as a natural source for developing dietary supplements to control hyperglycemia affiliated with type 2 diabetes mellitus.

**Keywords**: α-glucosidase; Diabetic; *Syzygium jambos*; Quercetin 3-O-xylosyl-(1→2)-rhamnoside; myricetin 3-O-xylosyl-(1→2)-rhamnoside

# ISOLASI DAN IDENTIFIKASI GLIKOSIDA FLAVONOID BIOAKTIF DARI *SYZYGIUM JAMBOS* DAN AKTIVITAS PENGHAMBAT ENZIM A-GLUKOSIDASE

**ABSTRAK**

Diabetes telah berkembang menjadi masalah kesehatan global yang serius, dengan sekitar 537 juta orang di seluruh dunia menderita penyakit ini. Tanpa intervensi yang tepat, risiko berkembangnya komplikasi kronis akan meningkat. Salah satu tindakan yang efektif untuk mengendalikan diabetes melitus tipe 2 adalah dengan menekan enzim penghidrolisis karbohidrat seperti α-glukosidase. *Syzygium jambos* memiliki sejarah panjang dalam penggunaannya pada pengobatan tradisional, umumnya digunakan untuk mengobati kondisi seperti kencing manis, rematik, nyeri inflamasi, dan sebagai diuretik. Penelitian ini bertujuan untuk menyelidiki sifat penghambat α-glukosidase dari daun *S. jambos* dan senyawa yang diisolasi. Etanol digunakan sebagai pelarut untuk maserasi, diikuti oleh fraksinasi menggunakan ekstraksi cair-cair, kromatografi cair vakum, kromatografi kolom, dan kromatografi radial. Senyawa yang diisolasi diidentifikasi menggunakan kromatografi lapis tipis (KLT), densitometri KLT, analisis pergeseran yang diinduksi reagen, spektroskopi inframerah (IR), spektrometri massa, dan spektrometri resonansi magnetik nuklir (NMR). Fraksi etil asetat menunjukkan aktivitas penghambatan α-glukosidase terkuat, dengan nilai $IC_{50}$ sebesar 0,31 ± 0,01 μg/mL, melebihi akarbose sebagai kontrol positif ($IC_{50}$ 62,86 ± 1,24 μg/mL). Dua glikosida flavonoid, quercetin 3-O-xylosyl-(1→2)-rhamnoside dan myricetin 3-O-xylosyl-(1→2)-rhamnoside, diidentifikasi di dalam fraksi ini, dengan nilai $IC_{50}$ masing-masing sebesar 65,63 ± 1,36 μg/mL dan 188,96 ± 2,85 μg/mL. Temuan ini menunjukkan bahwa *S. jambos* berpotensi sebagai sumber alami untuk mengembangkan suplemen makanan guna mengendalikan hiperglikemia yang terkait dengan diabetes melitus tipe 2.

**Kata kunci**: α-glucosidase; Diabetes; *Syzygium jambos*; Quercetin 3-O-xylosyl-(1→2)-rhamnoside; myricetin 3-O-xylosyl-(1→2)-rhamnoside

## INTRODUCTION

Diabetes has appeared as a dominant global epidemiology concern, exerting a potent strain on healthcare systems worldwide, with its prevalence regularly increasing over recent decades (Lin *et al*. 2020). In agreement with the International Diabetes Federation (IDF), over 537 million people globally are presently affected by diabetes, with projections suggesting this statistic will increase to 643 million by 2030 and 783 million by 2045 (Hossain *et al*. 2024). In Indonesia presently, there are 18.69 million recorded cases of diabetes, with projections suggesting an increase to 22.99 million by 2025, 27.13 million by 2030, and 40.70 million by 2045 (Wahidin *et al*. 2024).

Type 2 diabetes mellitus (T2DM) constitutes more than 90% of diabetes cases worldwide, establishing it as a highly prevalent metabolic disorder (Vonia *et al*. 2022). Without timely intervention, the risk of developing chronic complications increases, including macroangiopathy, retinopathy, nephropathy, neuropathy, diabetic foot, increased vulnerability to infections, myopathy, osteoporosis, arthropathies, and liver damage (Farmaki *et al*. 2020).

Inflated postprandial blood glucose is a significant risk factor responsible for the onset and progression of type 2 diabetes (Feng *et al*. 2022). Controlling type 2 diabetes mellitus is affiliated with suppressing carbohydrate hydrolyzing enzymes and α-glucosidase in the digestive system to decrease postprandial glucose levels (Nor *et al*. 2023). Acarbose, miglitol, and voglibose are known as α-glucosidase inhibitors, but their use usually results in side effects like abdominal bloating, flatulence, diarrhea, and pneumatosis cystoides intestinalis. This has prompted the search for new plant-based alternatives for α-glucosidase inhibition (Sheliya *et al*. 2015).

*Syzygium jambos* (L.) Alston, commonly called the rose apple, belongs to the Myrtaceae family and is extensively found across regions like South Africa, Central America, and Asia (Jiang *et al*. 2024). *S. jambos* has a rich history in traditional medicine. Its leaf decoctions are used as diuretics to treat rheumatism, inflammatory pain, and diabetes. Crushed leaf juice is used to manage fever, sore throat, and eye discomfort, while leaf powder is used to cool smallpox patients. Additionally, fruit, flowers, seeds, and bark are traditionally used to manage diarrhea, dysentery, asthma, bronchitis, and dysphonia (Wong *et al*. 2024). Previous studies have shown that *S. jambos* leaves exhibit a range of pharmacological activities, in addition to antibacterial, anti-acne, antioxidant, anti-inflammatory, anti-arthritic, and anti-quorum sensing activities (Mohanty and Cock 2010; Sharma *et al*. 2013; Ghareeb *et al*. 2017; Sobeh *et al*. 2018; Apaza Ticona *et al*. 2021). Besides, the leaves are evident to manage hepatoprotective (Sobeh *et al*. 2018), antifungal (Noé *et al*. 2019), anticancer (Rocchetti *et al*. 2019), antihyperglycemic (Nor *et al*. 2023), and anti-acetylcholinesterase (Amir Rawa *et al*. 2022).

Previous studies have shown that *S. jambos* reveals strong potential as an α-glucosidase inhibitor, similar to *S. myrtifolium*, and comparable to other species like *S. aqueum*, *S. aromaticum*, *S. cumini*, *S. malaccense*, and *S. polyanthum* (Nor *et al*. 2023). Building on these findings, the present study aims to investigate further the α-glucosidase inhibitory activity of *S. jambos* leaf extract and isolate compounds. This research provides valuable insights into *S. jambos* as a promising alternative treatment for diabetes mellitus.

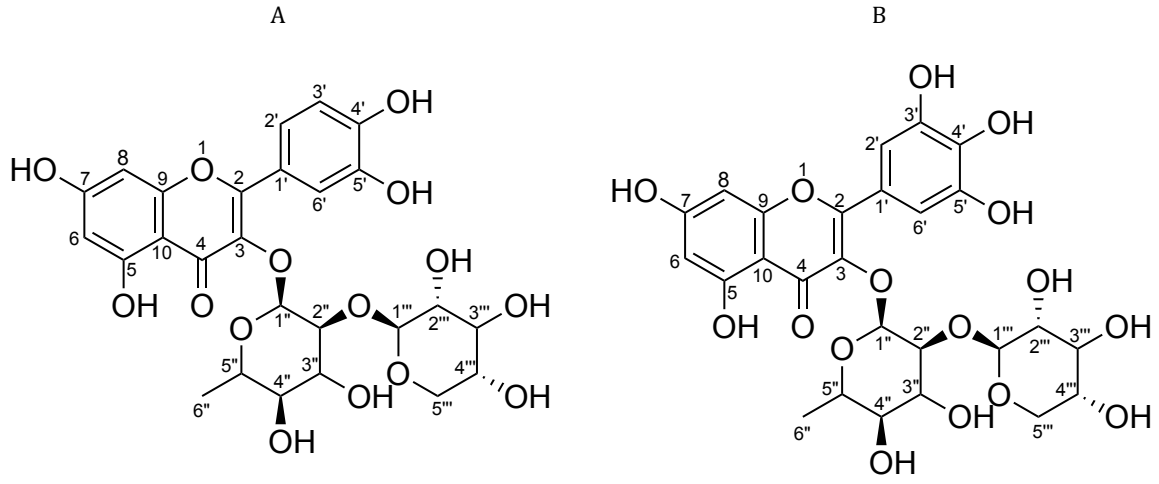


**Figure 1.**(A) Quercetin 3-O-xylosyl-(1→2)-rhamnoside, (B) Myricetin 3-O-xylosyl-(1→2)-rhamnoside

## MATERIAL AND METHOD

### Chemical and reagents

α-Glucosidase from *Saccharomyces cerevisiae* (G5003-100UN) and 4-nitrophenyl-α-D-glucopyranoside (pNPG, N1377-1G) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acarbose hydrate was purchased from TCI Chemicals (Tokyo, Japan). Citroborate was used as a specific spray reagent for thin-layer chromatography (TLC). Sodium hydroxide (NaOH), aluminum chloride ($AlCl_3$), hydrochloric acid (HCl), sodium acetate (NaOAc), and boric acid ($H_3BO_3$) were utilized for chemical shift analysis. Methanol (LiChrosolv hypergrade for LC-MS, Merck Supelco) was used for mass spectrometry analysis.

### Sample and extract preparation

The leaves of *S. jambos* were collected in Dago, Kabupaten Bandung, West Java, Indonesia, on December 30, 2019. The plant material was authenticated under voucher specimen B-151/2021 at the Research Center for Biology, Indonesian Institute of Sciences, Bogor, West Java, Indonesia. The leaves were washed with running water and dried in a drying cabinet at 40-50°C. Once thoroughly dried, the leaves were ground into a fine powder using a mechanical grinder. The powdered leaves were macerated in 96% ethanol at a 1:10 (w/v) ratio for 3x24 hours at room temperature. The resulting extract was filtered through Whatman filter paper and concentrated using a rotary evaporator at 50°C. All extraction and concentration procedures were performed at the Department of Biological Pharmacy, Bandung Institute of Technology, Bandung, Indonesia.

### Isolation and identification of active compounds

Compound isolation was guided by α-glucosidase inhibition assay. Fractionation was performed using liquid-liquid extraction with solvents of increasing polarity: n-hexane, ethyl acetate, and methanol. A total of 30 g of the ethyl acetate fraction was further separated by vacuum liquid chromatography (VLC) using a gradient elution system consisting of n-hexane, ethyl acetate, and methanol. This process yielded 21 fractions. Fractions exhibiting similar chromatographic profiles and promising bioactivity were pooled and subjected to further purification via column chromatography, using an isocratic elution system of chloroform/methanol (8:2). The fractions with a significant activity was then purified using radial chromatography (chromatotron) with an isocratic elution system of chloroform/methanol/water (7:2.75:0.25), leading to the isolation of compounds A and B.

### Identification isolate

The identification of isolated compounds was carried out using several analytical techniques.

Two-dimensional thin-layer chromatography (2D-TLC) was employed, followed by TLC with three different solvent systems. The spots were visualized using a citroborate spray reagent. TLC densitometry was performed to determine wavelength absorption and assess the purity of the isolates. Reagent-induced shift analysis was conducted using NaOH, $AlCl_3$, $AlCl_3$/HCl, NaOAc, and NaOAc/$H_3BO_3$. Further characterization involved infrared (IR), mass spectrometry, and nuclear magnetic resonance (NMR) spectroscopy.

2D-TLC was conducted with two solvent systems: ethyl acetate/methanol/water/acetic acid (8.5:1:0.5:0.25) and chloroform/methanol/water/acetic acid (7:2.75:0.5:0.25). Additionally, TLC analysis with three distinct eluents was performed using ethyl acetate/methanol/water/acetic acid (8.5:1:0.5:0.25), chloroform/methanol/water/acetic acid (7:2.75:0.5:0.25), and toluene/acetone/water/acetic acid (1:8.1:0.25).

TLC densitometry was performed using the chloroform/methanol/water/acetic acid (7:2.75:0.5:0.25) solvent system for the eluents of the isolates, with the densitometric analysis used to assess purity using Camag® TLC scanner 3.

The IR spectra were documented using a Jasco FTIR-4200 instrument. Mass spectra were collected using Waters Xevo TQD, running in negative mode with $[M-H]^-$ ionization. The conditions were as follows: capillary voltage of 2.5 kV, source temperature of 150°C, desolvation temperature of 450°C, nitrogen gas flow rate of 900 L/h, cone gas flow rate of 1 L/h, cone voltage of 50 V, and collision energy of 30 V. NMR spectra were recorded on an Agilent 500 MHz system with a DD2 console, operating at 500 MHz for proton ($^1$H) and 125 MHz for carbon ($^{13}$C) analysis. Isolates A and B were dissolved in $CD_3OD$ and analyzed for $^1$H and $^{13}$C NMR.

**α-Glucosidase inhibitory activity**

The α-glucosidase inhibition assay was tested using a modified version of the method reported before (Nor *et al*. 2023). In a 96-well microplate, 30 µL of the sample, 36 µL of 0.1 M phosphate buffer (pH 6.8), and 17 µL of 6 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate were added. The mixture was preincubated at 37°C for 5 minutes. Afterward, 17 µL of α-glucosidase enzyme (0.2 U/mL) was added to each well, followed by an additional incubation for 15 minutes at 37°C to complete the reaction. The reaction was terminated by adding 100 µL of 200 mM sodium carbonate ($Na_2CO_3$) to each well. The absorbance of the reaction mixture was measured at 400 nm using a microplate reader (Tecan Infinite 200 Pro). All experiments were performed in triplicate. Acarbose was used as the positive control, while the negative control consisted of wells without any inhibitor.

The percentage of inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \left(\frac{B1 - B2}{B1}\right) x\ 100\%$$

Where:

- B1 is the absorbance of the blank (*PBS + pNPG + enzyme + $Na_2CO_3$*),
- B2 is the absorbance of the sample (*sample extract + PBS + pNPG + enzyme + $Na_2CO_3$*).

Controls included:

- Blank control: *PBS + $Na_2CO_3$*, excluding the enzyme,
- Sample control: *sample extract + PBS + $Na_2CO_3$*, excluding the enzyme.

The $IC_{50}$ value, representing the concentration required to inhibit 50% of α-glucosidase activity, was determined using a linear regression model based on the relationship between sample concentration (x-axis) and percentage inhibition (y-axis).

**Statistical analysis**

The α-glucosidase inhibitory activity of the plant extracts, fractions, and isolated compounds was expressed as the mean ± standard deviation (SD). The data were analyzed using one-way analysis of variance (ANOVA), followed by the Games-Howell post hoc test to determine significant differences between groups. Statistical analysis was performed using SPSS software, version 26.0, and a *p*-value of less than 0.05 was considered statistically significant.

## RESULT AND DISCUSSION

This study investigates the pharmacological activity of extracts, fractions, and active compounds from *S. jambos* leaves as inhibitors of the α-glucosidase enzyme for managing type 2 diabetes mellitus. The research extends previous work on the potential of *S. jambos* as an α-glucosidase inhibitor. The α-glucosidase inhibitory activity of the extract, fractions, and isolated compounds was assessed using an *in vitro* assay (Table 1). Among the tested samples, the ethyl acetate fraction, obtained through liquid-liquid extraction, exhibited the greatest inhibitory activity, with an $IC_{50}$ value of 0.31 ± 0.01 µg/mL. In contrast, Isolate B showed the weakest inhibition, with an $IC_{50}$ value of 188.96 ± 2.85 µg/mL. Acarbose, used as the positive control, had an $IC_{50}$ value of 62.86 ± 1.24 µg/mL. At concentrations of 100 µg/mL and 200 µg/mL, the *S. jambos* leaf extract demonstrated inhibition rates of 83.75 ± 1.37% and 92.27 ± 1.13%, respectively, comparable to *S. myrtifolium* and outperforming *S. cumini*, *S. aqueum*, *S. malaccense*, *S. aromaticum*, and *S. polyanthum* (Nor *et al*. 2023). The ethanol extract of *S. jambos* leaves exhibited an $IC_{50}$ value of 1.22 ± 0.03 µg/mL (Table 1), consistent with earlier findings that reported an $IC_{50}$ value of 0.90 ± 0.07 µg/mL. Moreover, the most potent inhibition was observed in the 70% ethanol extract with an $IC_{50}$ value of 0.52 ± 0.01 µg/mL (Wong *et al*. 2024).

The ethyl acetate fraction demonstrated the strongest α-glucosidase inhibition activity compared to the water and n-hexane fractions, making it the focus for further isolation of active compounds. Using vacuum column chromatography, column chromatography, and radial chromatography, two isolates were obtained.

Isolate A was analyzed using two-dimensional thin-layer chromatography (2D-TLC) and TLC with three different solvent systems, revealing a single spot. This spot reacted with citroborate, producing a green coloration. TLC densitometry indicated absorption maxima at 350 and 255 nm, with a purity of 100%. The presence of two absorption bands suggests the existence of a flavonoid (Harborne JB and TJ Mabry 1975). Isolate A was further characterized by reagent-induced shifts using NaOH, $AlCl_3$, $AlCl_3$/HCl, NaOAc, and NaOAc/$H_3BO_3$. The addition of 2 M NaOH produced a bathochromic shift of 45 nm in band 1 without a reduction in intensity, indicating the presence of a 4'-OH group. $AlCl_3$ caused an 18 nm bathochromic shift in band 1, suggesting the presence of a 5-OH group with oxygenation at position 6. $AlCl_3$/HCl resulted in a 40 nm bathochromic shift in band 1, indicating an O-dihydroxy substitution on ring B. NaOAc produced a 15 nm bathochromic shift at 0 minutes with an absorbance of 1.584, suggesting a 7-OH group. After 5 minutes, the absorbance slightly decreased to 1.575, indicating the presence of 6,7 or 7,8 or 3',4'-dihydroxy groups (ortho-dihydroxy). Adding NaOAc/$H_3BO_3$ resulted in an 18 nm bathochromic shift in band 1, confirming a dihydroxy substitution on ring B (Markham 1982).

The infrared (IR) spectrum of Isolate A showed absorption bands at 3401.82 $cm^{-1}$ (O–H), 2927.41 $cm^{-1}$ ($sp^3$ C–H), 1650.77 $cm^{-1}$ (C=C aromatic), 1874.47 $cm^{-1}$ (benzene ring), 1095.37 $cm^{-1}$ (C–O), 1419.35 $cm^{-1}$ ($CH_3$ bending), and 1735.62 $cm^{-1}$ (C=O). Mass spectrometry (MS) analysis showed a molecular ion peak at m/z 579.10 $[M-H]^-$. The $^1H$ NMR spectrum of Isolate A in $CD_3OD$ showed two singlet protons at δ 6.15 and 6.30, appropriate to meta-coupled protons at positions 6 and 8 on the flavonoid's A-ring. Chemical shifts at δ 7.35, 6.92, and 7.29 indicated three aromatic proton signals at positions 2', 5', and 6', consistent with a 3',4'-disubstituted B-ring. The sugar moiety was identified as a disaccharide based on the two anomeric proton signals at δ 5.35 and 4.23, corresponding to rhamnose and xylose, respectively. The $^{13}C$ NMR spectrum detected 26 carbon signals. Chemical shifts at δ 116.71 and 116.49 corresponded to carbons at positions 2' and 5', while signals at δ 146.6, 150.12, and 122.63 were assigned to carbons at positions 3', 4', and 6', respectively, confirming that the aglycone is quercetin. Based on the NMR data (Table 2), Isolate A was identified as quercetin 3-O-xylosyl-(1→2)-rhamnoside (Fig. 1), consistent with previously reported data (Soicke *et al*. 1990; Slowing *et al*. 1994).

Isolate B was analyzed using two-dimensional thin-layer chromatography (2D-TLC) and TLC with three different solvent systems, revealing a single

spot. This spot reacted with citroborate, producing a green coloration. TLC densitometry indicated absorption maxima at approximately 353 nm and 258 nm, with a purity of 91.71%. Similar to Isolate A, two absorption bands indicated the existence of a flavonoid, specifically a flavonol with three free or substituted hydroxyl (OH) groups (Harborne JB and TJ Mabry 1975). Isolate B was further analyzed through reagent-induced shifts using NaOH, $AlCl_3$, $AlCl_3$/HCl, NaOAc, and NaOAc/$H_3BO_3$. The addition of 2 M NaOH decreased intensity, indicating the presence of 3',4'-OH groups, O-dihydroxy groups on ring A or B, or adjacent 3-OH groups. $AlCl_3$ produced a bathochromic shift of 40 nm in band 1, suggesting the presence of a 5-OH group. NaOAc induced a 13 nm bathochromic shift in band 2, with an absorbance of 1.494, indicating a 7-OH group. After 5 minutes, the absorbance decreased to 1.452, supporting the presence of 3',4'-dihydroxy groups (ortho-dihydroxy). Following the addition of NaOAc/$H_3BO_3$, band 1 exhibited a 19 nm bathochromic shift, confirming the presence of ortho-dihydroxy groups on ring B (Markham 1982).

The infrared (IR) spectrum of Isolate B showed absorption bands at 3401.82 $cm^{-1}$ (O–H), 2923.56 $cm^{-1}$ ($sp^3$ C–H), 1600.63 and 1650.77 $cm^{-1}$ (C=C aromatic), 1801 $cm^{-1}$ (benzene ring), 1083.08 $cm^{-1}$ (C–O), 1376.93 $cm^{-1}$ ($CH_3$ bending), and 1716.34 $cm^{-1}$ (C=O). Mass spectrometry (MS) analysis displayed a molecular ion peak at m/z 595.10 $[M-H]^-$. The $^1H$ NMR spectrum of Isolate B in $CD_3OD$ revealed two singlet protons on the A-ring of the flavonoid at δ 6.15 and 6.30, corresponding to meta-coupled protons at positions 6 and 8. A chemical shift at δ 6.95 indicated two protons at positions 2' and 6' with identical chemical environments. As with Isolate A, the sugar moiety in Isolate B was identified as a disaccharide, based on the two anomeric proton signals at δ 5.28 and 4.46, corresponding to rhamnose and xylose, respectively. The $^{13}C$ NMR spectrum detected 26 carbon signals. A chemical shift at δ 109.21 was assigned to carbons at positions 2' and 6', while a signal at δ 147.02 corresponded to carbons at positions 3' and 5'. A signal at δ 138.15 indicated the carbon at position 4'. This chemical shift data confirmed that the aglycone was myricetin. Based on the NMR spectrum (Table 2), Isolate B was identified as myricetin 3-O-xylosyl-(1→2)-rhamnoside (Fig. 1), consistent with previously reported data (Soicke *et al*. 1990; Slowing *et al*. 1994).

Quercetin 3-O-xylosyl-(1→2)-rhamnoside and myricetin 3-O-xylosyl-(1→2)-rhamnoside are flavonol diglycosides previously reported in *S. jambos* leaves (Slowing *et al*. 1994; Nawwar *et al*. 2016; Sobeh *et al*. 2018). In addition to being present in *S. jambos*, these two compounds have also been identified in *Moghania faginea* (Soicke *et al*. 1990). There are few reports on these compounds. However, previous studies have noted that quercetin 3-O-xylosyl-(1→2)-rhamnoside is also found in *Erythrospermum monoticolum* (Harborne and Williams 2000) and *Schizophragma hydrangeoides* (Oh *et al*. 2022). In this study, both compounds were assessed for their α-glucosidase inhibitory properties. There are no prior reports on the α-glucosidase inhibition evaluation of these two compounds. The $IC_{50}$ values for α-glucosidase inhibition were 65.63 ± 1.36 μg/mL for quercetin 3-O-xylosyl-(1→2)-rhamnoside and 188.96 ± 2.85 μg/mL for myricetin 3-O-xylosyl-(1→2)-rhamnoside (Table 1). Previous research has reported that quercetin 3-O-xylosyl-(1→2)-rhamnoside is active against acute inflammation (Harborne and Williams 2000) and exhibits protective effects against UV-induced skin aging (Oh *et al*. 2022).

Many reports have documented various compounds isolated from *S. jambos* leaves, aside from quercetin 3-O-xylosyl-(1→2)-rhamnoside and myricetin 3-O-xylosyl-(1→2)-rhamnoside. These include anacardic acid analogue; myricitrin; myricetin; ursolic acid; gallic acid; squalene (Sharma *et al*. 2013); 7-phloroglucinol (Jambone A-G); oleanic acid; betulinic acid; 3β-O-trans-p-coumaroylalphitolic acid; 3β-O-cis-p-coumaroylalphitolic acid; 5,7-dihydroxy-6,8-dimethyl-4'-methoxyflavone; 6-desmethyl-sideroxylin (Li *et al*. 2015); quercetin 3-O-xylosyl-(1→2)-xyloside; quercetin 3-O-glucuronide; myricetin 3-O-glucoside; myricetin 7-methylether 3-O-xylosyl-(1→2)-rhamnoside; myricetin 3',5'-dimethyl ether 3-O-xylosyl-(1→2)-rhamnoside; myricetin 3',5'-dimethyl ether 3-O-rhamnoside; 1,2,3-tri-O-galloyl-β-glucose; ellagic acid (Nawwar

*et al*. 2016); malic acid; citric acid; hexahydroxydiphenoyl-hexoside; castalagin/vescalagin isomer; galloyl-HHDP-DHHDP-hexoside; bis-HHDP-hexoside; galloyl-bis-HHDP-hexoside (casuarinin); ellagic acid pentoside; ellagic acid rhamnoside; myricetin rhamnoside; rosmarinic acid rhamnoside (Sobeh *et al*. 2018); quercetin-3-O-rutinoside; prenylbenzoic acid 4-β-D-glucoside; morolic acid 3-O-caffeate; 5,4'-dihydroxy, 7-methoxy, 6-methyl-flavone; 3,4,5-trihydroxybenzoic acid; quercetin; isoetin-7-O-β-D-glucopyranoside; 4'-hydroxy-3'-methoxyphenol-β-D-[6-O-(4"-hydroxy-3",5"-dimethoxybenzoate)] glucopyranoside (Ghareeb *et al*. 2017); 5-hydroxymethyl-2-furfural; betulin (Rajkumari *et al*. 2018); stigmasterol; lupeol; syringic acid; beta-amyrin; bergenin derivatives; myricetin derivatives; kaempferol derivatives; friedelin; stigmasterol glucoside; myrigalone G; galloyl castalagin; tellimagrandin II; coriariin A; coriariin B; praecoxin A; praecoxin B; vescalagin; vescalin; trigalloyl glucose; di-HHDP glucose; pomolic acid (Wong *et al*. 2024).

The α-glucosidase inhibition of *S. jambos* leaf extracts and fractions exceeds that of the isolated compounds (Table 1). Plant extracts often contain various bioactive compounds, and the therapeutic efficacy of medicinal plants can be attributed to the synergistic interactions between these compounds (Vaou *et al*. 2022). Compared to isolated compounds, the enhanced enzyme inhibition observed in extracts and fractions suggests a synergistic or potentiating effect where the combined activity exceeds that of individual components (Rasoanaivo *et al.* 2011). This finding supports further exploration of *S. jambos* to isolate additional compounds and evaluate their α-glucosidase inhibitory effects, individually and in combination. The potent inhibition shown by the extracts and fractions demonstrates significant potential for further development. Future research on *S. jambos* should focus on detailed evaluations, including toxicity assessments, preclinical studies, and clinical trials, to advance its potential as an alternative α-glucosidase inhibitor alongside current options like acarbose, miglitol, and voglibose.

**Table 1.** The $IC_{50}$ Value of Extract, Fraction, and Isolate Compound of *S. jambos* against α-Glucosidase Enzyme

| Samples | $IC_{50}$ (µg/mL) |
|---|---|
| Ethanol extract | 1.22 ± 0.03[a] |
| n-hexane fraction | 41.53 ± 0.56 |
| Ethyl Acetate fraction | 0.31 ± 0.01 |
| Water fraction | 1.21 ± 0.08[a] |
| Isolate A | 65.63 ± 1.36[b] |
| Isolate B | 188.96 ± 2.85 |
| Acarbose | 62.86 ± 1.24[b] |

Note: Data are expressed as the mean ± standard deviation (n = 3). Means within the same column followed by identical letters are not statistically different at $p < 0.05$.

**Table 2.** NMR Data of Isolate A and Isolate B

| Position | Isolate A (Quercetin 3-O-xylosyl-(1→2)-rhamnoside) | | Isolate B (Myricetin 3-O-xylosyl-(1→2)-rhamnoside) | |
|---|---|---|---|---|
| | $^{13}C$ | 1H | $^{13}C$ | 1H |
| | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) |
| 1 | | | | |
| 2 | 158.73 | | 158.69 | |
| 3 | 136.53 | | 136.67 | |
| 4 | 179.43 | | 179.51 | |
| 5 | 159.84 | | 158.84 | |
| 6 | 100.95 | 6.15 (1H, s) | 100.78 | 6.15 (1H, d, 2.05) |
| 7 | 166.16 | | 168.5 | |
| 8 | 95.51 | 6.30 (1H, s) | 94.98 | 6.30 (1H, d, 2) |
| 9 | 163.04 | | 163.05 | |
| 10 | 104.93 | | 104.96 | |
| 1' | 122.8 | | 121.73 | |

| Position | Isolate A (Quercetin 3-O-xylosyl-(1→2)-rhamnoside) | | Isolate B (Myricetin 3-O-xylosyl-(1→2)-rhamnoside) | |
|---|---|---|---|---|
| | $^{13}C$ | 1H | $^{13}C$ | 1H |
| | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) |
| 2' | 116.71 | 7.35 (1H, d, 2.1) | 109.21 | 6.95 (2H, s) |
| 3' | 146.6 | | 147.03 | |
| 4' | 150.12 | | 138.15 | |
| 5' | 116.49 | 6.92 (1H, d, 8.25) | 147.03 | |
| 6' | 122.63 | 7.29 (1H, dd, 2.05, 8.2) | 109.21 | 6.95 (2H, s) |
| 1" | 103.17 | 5.35 (1H, s) | 103.23 | 5.28 (1H, d, 1.55) |
| 2" | 82.73 | 3.05 - 3.89 | 82.82 | 3.05 - 3.84 |
| 3" | 70.96 | 3.05 - 3.89 | 70.97 | 3.05 - 3.84 |
| 4" | 73.7 | 3.05 - 3.89 | 73.80 | 3.05 - 3.84 |
| 5" | 71.9 | 3.05 - 3.89 | 71.91 | 3.05 - 3.84 |
| 6" | 17.67 | 1.02 (3H, d, 6.2) | 17.7 | 1.02 (3H, d, 6.2) |
| 1"' | 107.72 | 4.23 (1H, d, 7.65) | 107.81 | 4.46 (1H, d, 7.65) |
| 2"' | 75.25 | 3.05 - 3.89 | 75.28 | 3.05 - 3.84 |
| 3"' | 77.75 | 3.05 - 3.89 | 77.76 | 3.05 - 3.84 |
| 4"' | 71.77 | 3.05 - 3.89 | 71.74 | 3.05 - 3.84 |
| 5"' | 67.02 | 3.05 - 3.89 | 66.97 | 3.05 - 3.84 |

***d***, duplet; ***dd***, duplet of duplet; ***s***, singlet

## CONCLUSION

*S. jambos* exhibits significant α-glucosidase inhibitory activity. The ethyl acetate fraction demonstrated the highest inhibitory effect among the extracts and fractions tested. Quercetin 3-O-xylosyl-(1→2)-rhamnoside and myricetin 3-O-xylosyl-(1→2)-rhamnoside, both flavonoid glycosides isolated from *S. jambos*, showed α-glucosidase inhibition, though less potent compared to the extract and its fractions. Quercetin 3-O-xylosyl-(1→2)-rhamnoside exhibited inhibitory activity comparable to acarbose, with no statistically significant difference. These findings highlight the potential of *S. jambos* extracts and fractions as effective α-glucosidase inhibitors, offering promise for further development into dietary supplements to manage hyperglycemia associated with type 2 diabetes mellitus.

## FUNDING

The author(s) received no financial support for the research, authorship, and/or publication of this article.

## ACKNOWLEDGEMENT

The researchers would like to thank the NMR Laboratory, Faculty of Natural Science and Mathematics, Institut Teknologi Bandung.

## DECLARATION OF COMPETING INTEREST

The authors declare that there are no conflicts of interest, financial or personal, that could have influenced the work presented in this paper.

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