Saudi Pharmaceutical Journal 31 (2023) 101677

HOSTED BY

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

The α-glucosidase inhibitory activity of avicularin and 4-O-methyl gallic acid isolated from *Syzygium myrtifolium* leaves

Islan Nor [a,b], Komar Ruslan Wirasutisna [a], Rika Hartati [a], Muhamad Insanu [a,*]

[a] *Departement of Pharmaceutical Biology, School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia*
[b] *Faculty of Pharmacy, University of Muhammadiyah Banjarmasin, Banjarmasin, Indonesia*

ARTICLE INFO

Article history:
Received 10 April 2023
Accepted 13 June 2023
Available online 19 June 2023

Keywords:
α-glucosidase
Diabetes
Syzygium
Avicularin
4-O-methyl gallic acid

ABSTRACT

Diabetes Mellitus is the main cause of death on a global scale. In 2019, there were 463 million people with diabetes, and WHO predicts that by 2030, there will be 578 million. As an antidiabetic agent, α-glucosidase inhibitors are one of the methods employed to reduce the prevalence of diabetes. Diabetes is traditionally treated with *Syzygium* as a primary material, medicine, fruit, ornamental plant, and source of carpentry. This investigation aimed to examine the inhibitory effect of seven species of *Syzygium* against α-glucosidase enzyme using an *in vitro* assay and isolate active substances and ascertain their concentrations in each sample. As a solvent, ethanol was used in maceration to extract the substance. Afterward, the extract underwent a series of fractionation techniques, including liquid–liquid extraction, vacuum liquid chromatography, column chromatography, and preparative Thin Layer Chromatography (TLC) for purification and isolation. The compound's structures were elucidated using TLC, UV–Visible spectrophotometry, and nuclear magnetic resonance (NMR) spectroscopy. Based on concentrations of 100 and 200 μg/mL, *Syzygium myrtifolium* exhibited the most significant inhibitory effect, followed by other species of *Syzygium*. The proportion of ethyl acetate had the strongest activity (IC_{50} 0.40 ± 0.02 μg/mL) contrasted to positive control acarbose (IC_{50} 55.39 ± 0.67 g/mL) and quercitrin (IC_{50} 6.47 ± 0.40 μg/mL). Avicularin and 4-O-methyl gallic acid were discovered in the ethyl acetate fraction of *Syzygium myrtifolium* with IC_{50} values of 17.05 ± 0.75 μg/mL and 25.19 ± 0.21 μg/mL, respectively. As α-glucosidase inhibitory, the results of this study indicate *Syzygium myrtifolium* can be used as a dietary supplement to manage hyperglycemia.

1. Introduction

Diabetes is a metabolic condition distinguished by untreated high blood sugar (World Health Organization, 2019). High glucose levels in the body, known as hyperglycemia, can interact non-enzymatically with plasma proteins. This interaction can generate glycated hemoglobin and glycated albumin. The accumulation of progressed glycation final products in various body parts, including the kidneys and retina, can contribute to diabetes-related complications (Priya et al., 2018). World Health Organization (WHO) projects that there will be 578 million individuals with diabetes by 2030, up from 463 million in 2019. Prevalence increases by 51% between 2019 and 2045 (Saeedi et al., 2019).

Inhibiting carbohydrate hydrolysis enzymes (α-glucosidase found in the digestive organs) and lower postprandial glucose levels are critical for managing type 2 diabetes mellitus (Li et al., 2005). These medications include acarbose, miglitol, and voglibose (Kumar et al., 2011). Numerous hypoglycemic agents have undesirable side effects, for instance, bloating, nausea, diarrhea, and flatulence. Therefore, the development of natural α-glucosidase inhibitors is widespread because of fewer adverse effects (P et al., 2011).

Syzygium can be used as fruit trees, ornamental plants, and carpentry sources for nutritional and medicinal purposes (Rabeta et al., 2013). *Syzygium* is a Myrtaceae family member with between 1200 and 1800 species found in Africa, southern Asia, and the Pacific (Rocchetti et al., 2019). *Syzygium* is a plant with fruit numerous chemical constituents and bioactivity. Fruits may enhance the

* Corresponding author.
E-mail address: insanu99@itb.ac.id (M. Insanu).

Peer review under responsibility of King Saud University. Production and hosting by Elsevier.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2023.06.010

human diet's amount of vitamins, minerals, and bioactive compounds (Vasco et al., 2008). Several *Syzygium* species have been investigated for their chemical constituents and bioactivity. They were reported for antioxidant, antiviral, antidiabetic, and hepatoprotective properties (Sobeh et al., 2018b).

Syzygium aromaticum is traditionally used to treat dental infection, toothaches, burn, and wounds (Batiha et al., 2020). *Syzygium cumini* seeds and leaves are used in India and Brazil to manage diabetes (Ayyanar and Subash-babu, 2012). *Syzygium aqueum* leaves have alleviated childbirth pains (Manaharan et al., 2014). The intake of *Syzygium polyanthum* leaves is linked to treating diabetes, hypertension, ulcers, diarrhea, skin disease, and infection (Ismail and Wan Ahmad, 2019). *Syzygium myrtifolium* addresses stomach aches (Memon et al., 2015). In India, the fruit tonic of *Syzygium jambos* is known to enhance the health of the brain and liver, whereas the fruit infusion exhibits diuretic properties (Ochieng et al., 2022). *Syzygium malaccense* has been traditionally used to treat mount ulcers and irregular menstruation (Uddin et al., 2022).

This investigation aims to analyze the potential of *Syzygium* species as α-glucosidase inhibitors, including *Syzygium aqueum*, *Syzygium aromaticum*, *Syzygium cumini*, *Syzygium jambos*, *Syzygium malaccense*, *Syzygium myrtifolium*, and *Syzygium polyanthum* leaf extracts. Most prospective species were determined by NMR analysis following the isolation of active compounds.

2. Material and method

2.1. Chemical and reagents

α-glucosidase (*Saccharomyces cerevisiae*) G5003-100UN Cas number 56180-94-0, and 4-nitrophenyl-α-D-glucopyranoside (pNPG) N1377-1G Cas number 3767-28-0 were acquired from Sigma-Aldrich (St. Louis, MO, USA). Acarbose hydrate was obtained from TCI (Tokyo, Japan). Quercitrin was purchased from Rotichrom. Citroborate and 10% H_2SO_4 in MeOH were specific spray reagents to TLC.

2.2. Sample and ethanolic extract preparation

The leaves of seven *Syzygium* species were collected in West Java, Indonesia. They were harvested and gathered between October and December 2019. The leaves were cleansed with running water. The items were then desiccated in a drying cabinet. The leaves, which were dried, were pulverized into a powder using a grinder. The pulverized leaves were mixed with a 1:10 (w/v) 96% ethanol concentration (redistilled). Macerate was conducted at ambient temperature for 3x24 hours. The collected filtrate was filtered through Whatman filter paper. The filtrate of ethanol was condensed using a rotary evaporator maintained at a temperature of 50 °C using a rotary evaporator.

2.3. Isolation and identification of active compounds from Syzygium myrtifolium leaves

The specimens with specimen B-149/2021 were authenticated at the Indonesian Institute of Sciences' Research Center for Biology in Bogor, West Java, Indonesia. The extraction was conducted at Bandung Institute of Technology's Department of Biology Pharmacy in Bandung, Indonesia. The juvenile red leaves were separated from the other leaves. Two thousand grams of powdered *Syzygium myrtifolium* leaves macerate with ethanol 96% at a proportion of 1:10 (w/v) (3x24 hours). Then, a 500 g portion of the extract underwent fractionation by employing a liquid–liquid extraction technique using solvents of escalating polarity, including *n*-hexane, ethyl acetate, and methanol.

Further purification was performed on the ethyl acetate fraction using vacuum liquid chromatography. A gradient elution system comprising *n*-hexane, ethyl acetate, and methanol was employed for this purpose. It produced 21 fractions. The fraction with identical chromatogram and promising activity were combined and rechromatographed using column chromatography with isocratic elution, chloroform/methanol (8:1.5). The same chromatogram fraction was merged and rechromatographed using TLC preparative with isocratic elution using chloroform/methanol (8:1.5) to yield 37.2 mg of compound IN1 and 17.5 mg of compound IN2. Compound IN1, amorphous yellow powder. Compound IN2, needle-shaped white crystal.

2.4. α-Glucosidase inhibitory activity

The α-Glucosidase inhibition investigation adopted an adapted method (Vonia et al., 2022). In 96 well microplates, 30 μL of the sample, 36 μL of 0.1 M phosphate buffer solution (pH 6.8), and 17 μL of 6 mM p-nitrophenyl-α-D-glucopyranoside substrate were added, and preincubated at 37 °C for 5 min. After a 5 min preincubation at 37 °C, 17 μL (0.2 units/ml) of the α-glucosidase enzyme were combined, followed by 15 min of incubation at 37 °C to complete the reaction. The chemical response was ended by combining 100 μL of 200 mM Na_2CO_3 in every single well. The absorbance of the chemical process was assessed using a microplate reader at 400 nm (Tecan Infinite 200 pro). Each measurement was conducted in triplicate. Positive control was acarbose, and negative control was the absence of any inhibitor. Calculating the percentage of inhibition:

$$\%\,\text{Inhibition} = \frac{B1 - B2}{B1} \times 100\%$$

B1 = Blank absorbance * – control of blank absorbance **.
B2 = Sample absorbance – control of sample absorbance ***.
* Blank comprises PBS + pNPG + enzyme + Na_2CO_3.
** Control of blank comprises PBS + Na_2CO_3, excluding enzyme.
*** Control of sample comprises extract + PBS + Na_2CO_3, excluding enzyme.

The IC_{50} was determined by employing a linear regression equation (y = a + bx), where the x-axis corresponds to the concentration of the samples and the y-axis represents the percentage of inhibition. The IC_{50} value represents the model's strength to inhibit 50% of α-glucosidase activity below the experimental conditions.

2.5. Qualitative analysis of active compound

The analysis employed 2D TLC, TLC with three distinct eluents, and HPLC. 2D TLC using two distinct eluents: chloroform/methanol/formic acid (8:2:0.25) and ethyl acetate/methanol/water (8:2:1.5). TLC with three different eluents using chloroform/methanol/formic acid (8:1.5:0.25), ethyl acetate/methanol/water (8:2:1.5), and methanol/acid formic (10:0.25). HPLC analysis was performed on the Shimadzu LC-20AD with stationary phase using Phenomenex HPLC Column Kromasil 5u 100A C18 with an injection number of 20 μL volume and mobile phase using 0.02% H_3PO_4 in water and methanol. The elution system was gradient and isocratic, 0.01 to 3.00 min using a gradient from 40 to 60% methanol, 3.01 to 7.00 min using isocratic 60% methanol, 7.01 to 12.00 min using isocratic 70% methanol, 12.01 to 15.00 min using isocratic 40% methanol and stop at 15.01 min. HPLC Detector utilized UV 210 and 360 nm.

2.6. NMR analysis

NMR spectrums were obtained using an Agilent 500 MHz with a DD2 console system operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Compound IN1 was extracted with solvent CD_3OD and analyzed for ^{1}H, ^{13}C, COSY, HMBC, and HSQC. Compound IN2 was analyzed for ^{1}H and ^{13}C using solvent DMSO-d_6.

2.7. Statistical analysis

The data on α-glucosidase inhibitory activity belonging to extracts, fractions, and isolated compounds were expressed as the mean value and the standard deviation (SD). Statistical analysis of the data was performed using SPSS version 26.0. The independent-sample *t*-test and one-way analysis of variance (ANOVA) with Tukey's posthoc test were conducted to evaluate the significance. A value of $p < 0.05$ was considered statistically significant.

3. Result

3.1. Phytochemical screening

The standard phytochemical analysis detected flavonoid, phenol, tannin, quinone, saponin, and steroid/triterpenoid in the crude drug and ethanolic extract of *Syzygium myrtifolium* (Harborne, 1984).

3.2. In vitro α-Glucosidase inhibitory activity

Leaves of seven species of *Syzygium* were assessed with *in vitro* α-glucosidase inhibitory using pNPG as substrate (Table 1). α-glucosidase enzyme inhibition percentages were displayed for each sample. The sample extract with a significant inhibitory effect will be forwarded to the IC_{50} values and then isolated for the active compound. *Syzygium myrtifolium* exhibited the most significant inhibitory effect, followed by *Syzygium jambos*, *Syzygium polyanthum*, *Syzygium aromaticum*, *Syzygium malaccence*, *Syzygium aqueum,* and *Syzygium* cumini, respectively based on concentration 100 and 200 μg/mL.

The most inhibitory activity of α-glucosidase was fractionated from *Syzygium myrtifolium* by liquid–liquid extraction from nonpolar to polar solvents (Table 2). The IC_{50} values of 0.40 ± 0.02 g/mL and 0.44 ± 0.03 g/mL for ethyl acetate and water fraction indicate a significant inhibitory effect. The Ethyl acetate fraction was sent to isolate the active compound, yielding IN1 and IN2. IC_{50} values are 17.05 ± 0.75 μg/mL and 25.19 ± 0.21 μg/mL, respectively.

Table 1
The percentage α-glucosidase inhibitory activity of *Syzygium* leaf extract.

Samples (leaf extract)	Inhibition (%)	
	100 μg/mL	200 μg/mL
Syzygium aqueum	26.51 ± 3.90^{a,d}	48.58 ± 5.23
Syzygium aromaticum	51.69 ± 2.92^{b}	81.93 ± 1.29^{a}
Syzygium cumini	23.13 ± 5.83^{a}	34.55 ± 4.15
Syzygium jambos	83.75 ± 1.37^{c}	92.27 ± 1.13^{a,b}
Syzygium malaccense	37.64 ± 4.37^{d}	62.23 ± 3.30
Syzygium myrtifolium	90.88 ± 1.21^{c}	99.70 ± 1.26^{b}
Syzygium polyanthum	59.45 ± 4.49^{b}	91.22 ± 3.83^{a,b}
Acarbose	50.73 ± 1.27	66.03 ± 1.11*

Note: Data are the mean of three replicates (n = 3) ± standard deviation. At $p < 0.05$, the means in the vertical column with a similar alphabet are not significantly different. Values are the final concentration. * (Vonia et al., 2022).

Table 2
The IC_{50} values for α-glucosidase inhibitory activity of extract, fraction, and active compound of *Syzygium myrtifolium.*

Samples	IC_{50} (μg/mL)
Ethanol extract	0.42 ± 0.65^{a}
n-hexane fraction	11.61 ± 0.94
Ethyl Acetate fraction	0.40 ± 0.02^{a}
Water fraction	0.44 ± 0.03^{a}
IN1	17.05 ± 0.75
IN2	25.19 ± 0.21
Quercitrin	6.47 ± 0.40
Acarbose	55.39 ± 0.67

Note: Data are the mean of three replicates (n = 3) ± standard deviation. At $p < 0.05$, the means in the vertical column with a similar alphabet are not significantly different.

3.3. Isolated compound

IN1 was obtained as a yellow amorphous powder. The compound was eluted with Chloroform/Methanol/Formic acid (8:1.5:0.25) on TLC silica gel GF_{254} and reacted with citroborat to produce a green-to-yellow spot, which was then transformed into a yellow dot with 10% H_2SO_4. Analysis of IN1 using 2D TLC and TLC with three distinct eluents revealed one spot. This compound's UV peaks at 298 and 357 nm indicated the existence of flavonoids, mainly the flavonol group (Harborne et al., 1975). The HPLC chromatogram showed one peak at a retention time of 5.7 min with mobile phase Methanol/Water (4:6) using UV 210 and 360 nm detectors. The ^{1}H NMR spectrum of IN1 (Supplementary Fig. 1s) detected two single protons at flavonoid's A ring with *meta*-coupled at positions 6 and 8 at δ 6.19 and 6.38. Chemical shifts at 7.75, 6.86, and 7.57 indicated three single aromatic proton signals at position 2′, 5′, and 6′ and indicated 3′,4′-disubtituted flavonoid's B ring. Sugar is present at δ 3.45–5.15 as arabinofuranose. The ^{13}C NMR spectrum of IN1 (Supplementary Fig. 2s) showed 20 carbon. Several correlations with heteronuclear single quantum coherence (HSQC) (Supplementary Fig. 3s). 2D NMR, heteronuclear multiple bond connectivity (HMBC) (Supplementary Fig. 4s), and correlation spectroscopy (COSY) (Supplementary Fig. 5s) spectra exhibited a correlation between $^{1}H \rightarrow {}^{13}C$ and $^{1}H \leftrightarrow {}^{1}H$. The result of the NMR spectrum has been confirmed as avicularin and compared with previously published data (Ahn et al., 2019; Da Silva Sa et al., 2017).

IN2 was obtained as a white needle crystal. The compound was eluted with Chloroform/Methanol/Formic acid (8:1.5:0.25) on TLC silica gel GF_{254} and did not react when sprayed with citroborat. IN2 did exhibit a dark spot when treated with 10% H_2SO_4. Analysis of IN2 using 2D TLC and TLC with three distinct eluents revealed one spot. This compound showed that UV maximum of 289 nm. The HPLC chromatogram exhibited a single peak at the retention time of 5.1 min with mobile phase Methanol/Water (4:6) and detector UV 210. The proton NMR spectrum (Supplementary Fig. 6s) detected three peaks. The broad single peak at δ 9.21 indicated three substituted OH at positions 2, 5, and 7. The proton signal at δ 6.93 was identified single proton at positions 2 and 6 in aromatic substitution. The peak at δ 3.74 indicates the presence of CH_3 at position 8. Six peaks with different intensities were detected in the ^{13}C NMR for the remaining signals (Supplementary Fig. 7s). The spectrum at δ 108.5 and 145.6 exhibits higher intensity peaks than the others, indicating two C for each peak. These data agree with previous reports on 4-O-methyl gallic acid (Abhishek et al., 2019; Farag et al., 2015).

4. Discussion

Hyperglycemia characterizes a group of metabolic disorders known as diabetes. Long-term specific effects cause additional secondary conditions, including retinopathy, nephropathy, and neuropathy, and increase the risk of developing other diseases (World Health Organization, 2019). Inhibiting α-glucosidase found in the digestive organs delayed glucose absorption. That is essential to type 2 diabetes management (Li et al., 2005).

By inhibiting the work of the α-glucosidase enzyme, it is possible to prevent diabetes mellitus, primarily type 2 diabetes mellitus, thereby reducing diabetes-related mortality. The α-glucosidase enzymes (maltase, isomaltase, glucomaltase, and sucrase) function in the small intestine wall to hydrolyze oligosaccharides and disaccharides. This enzyme can be inhibited to effectively decrease the digestion and absorption of complex carbohydrates. The condition helps minimize the postprandial glucose spike in people with diabetes (Shinde et al., 2008). Many gastrointestinal adverse effects are associated with α-glucosidase inhibitors, such as bloating, nausea, diarrhea, and flatulence. The α-glucosidase inhibitors from naturally derived can be used to treat postprandial hyperglycemia, and they have lesser adverse effects and are less expensive in contrast to synthetic antihyperglycemic drugs (P et al., 2011).

Most species of *Syzygium* were determined to have hypoglycemic effects *in vivo* and *in vitro*. On *Syzygium* species, the following *in vitro* hypoglycemic effects were examined: α-glucosidase inhibitory activity, α-amylase inhibitory activity, DPP-IV inhibitory activity, and antiglycation assay (Priya et al., 2018). A previous *in vitro* study of multiple *Syzygium* revealed their potential as α-glucosidase inhibitors. Other species include *Syzygium aqueum* (Manaharan et al., 2012), *Syzygium aromaticum* (Adefegha and Oboh, 2012; Oboh et al., 2015), *Syzygium cumini* (Ajiboye et al., 2020; Franco et al., 2020; Perera et al., 2018; Liu et al., 2018; Omar et al., 2012; Priya et al., 2018; Trinh et al., 2016), *Syzygium malaccence* (Arumugam et al., 2016, 2014); *Syzygium polyanthum* (Abdulrahman et al., 2019; Dewijanti et al., 2020; Elya et al., 2015; Rahayu et al., 2019).

Although previous studies have investigated the α-glucosidase inhibitory activity of the genus *Syzygium*, neither *Syzygium myrtifolium* nor *Syzygium jambos* have been mentioned. This study compared the inhibitory activity of α-glucosidase enzyme from seven Species of *Syzygium* and found that *Syzygium myrtifolium* had the most significant inhibitory effect (Table 1), followed by *Syzygium jambos*, *Syzygium polyanthum*, *Syzygium aromaticum*, *Syzygium malaccence*, *Syzygium aqueum*, and *Syzygium cumini*, respectively. The isolation phase of *Syzygium myrtifolium* was continued to determine its active components. The ethyl acetate and water components of *Syzygium myrtifolium* demonstrated the most significant inhibitory activity. The ethyl acetate fraction was selected for the next step. Two active compounds, Avicularin (Fig. 2) and 4-O-methyl gallic acid (Fig. 3), were determined by NMR Analysis from the ethyl acetate fraction. Their purity was evaluated using HPLC (Fig. 1). Tables 3 and 4 exhibits the information obtained from NMR analysis.

Avicularin is derived from quercetin, but little is known about its biological activity (Fujimori and Shibano, 2013). Avicularin exerts its effect on lipid accumulation in 3T3-L1 cells by inhibiting C/EBP-activated GLUT4-mediated glucose uptake, leading to reduced lipid accumulation (Fujimori and Shibano, 2013), as reported in previous investigations to have biological activity as antibacterial (Da Silva Sa et al., 2017). The previous study showed avicularin exhibited a significant concentration-dependent reduction in cell proliferation in Hcc. Furthermore, it demonstrated a significant inhibitory effect on the moving and invasive capabilities of Huh7 cells. Avicularin also inhibits G0/G1-phase cell population and reduces the aggregation of S-phase cells in the cell stages while inducing cell apoptosis (Wang et al., 2019). Avicularin demonstrated antioxidant activity at a concentration of 100 μg/L, showing a scavenging rate of 87.54% against OH radicals (Lee et al., 2019). Avicularin successfully decreases hepatic inflammation, endoplasmic reticulum stress (ERS), and glucose metabolism problem caused by Pb (Qiu et al., 2022).

Similarly, 4-O-Methyl gallic acid was identified as a gallic acid derivative via NMR spectroscopy (Dao et al., 2019). Compared to gallic acid, 4-O-methyl gallic acid was less effective against distinct influenza strains (Dao et al., 2019). In addition, 4-O-methyl gallic acid inhibits bacteria and yeast, particularly against *Streptococcus faecalis*, *Cryptococcus neoformans* (Abhishek et al., 2019), and *R. solanacearum* (Farag et al., 2015). Other investigations have also reported the efficacy of 4-O-methyl gallic acid in reducing the expression of AT1R and CD36 mRNA in Ang II-treated MDPs. This indicates a potent restraint of Ang II-triggered pro-atherogenic process involved in foam cell formation (Oliveira et al., 2004). 4-O-methyl gallic acid effectively suppressed the expression and synthesis of genes and signaling molecules related to inflammation, containing NO, PGE2 (iNOS, COX-2, and TNF-α expression), and IL-1β, in both RAW264.7 cells and primary macrophages when activated with lipopolysaccharide (LPS) (Na et al., 2006). 4-O-methyl gallic acid not only restrains VEGF creation below the hypoxic state but also suppresses the creation of ROS in endothelial cells that are activated with VEGF. The compound also effectively blocks the invasion of endothelial cells and the formation of tubes when activated with bFGF (Jeon et al., 2005). Based on previous studies, no existing literature documenting the inhibition of the α-glucosidase enzyme by avicularin and 4-O-methyl gallic acid. Therefore, this study is the initial report on the inhibitory ability of these compounds on the α-glucosidase enzyme.

Avicularin and 4-O-methyl gallic acid have not previously been reported in *Syzygium myrtifolium*. Active compounds that have been reported from *Syzygium myrtifolium* include anthocyanin, betulinic acid, dimethyl cardamonin, ursolic acid, (2S)-7-Hydroxy-5-methoxy-6,8-dimethyl flavanone, (S)-5,7-dihydroxy-6,8-dimethyl flavanone, (E)-2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethyl chalcone, (E)-2′,4′-dihydroxy-6′-methoxy chalcone (Aisha et al., 2013; Anggraini, 2017; Memon et al., 2015). Avicularin was isolated from *Euphorbia humifusa* (Ahn et al., 2019), *Taxillus kaempferi* (Fujimori and Shibano, 2013), *Myrcia tomentosa* (Da Silva Sa et al., 2017), *Vaccinum vitis-idaea* (Riihinen et al., 2013), whereas

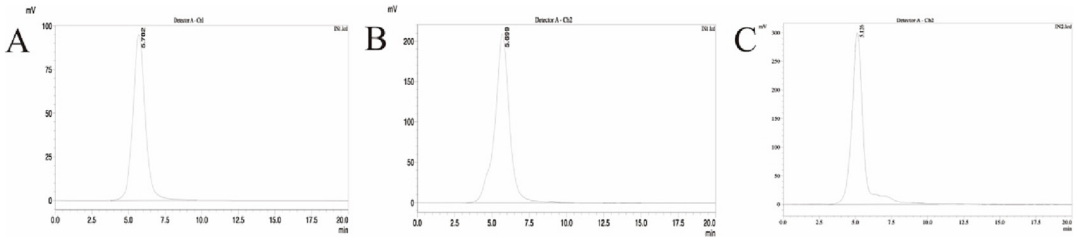

Fig. 1. HPLC Chromatogram of Active Compound. (A) IN1 at 360 nm, (B) IN1 at 210 nm, (C) IN2 at 210 nm.

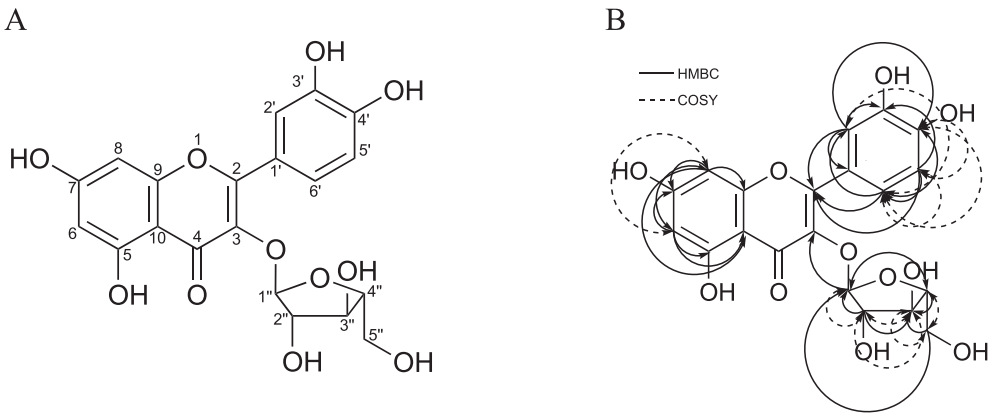

Fig. 2. (A) Structure of IN1 (Avicularin), (B) Correlation of HMBC and COSY of IN1 (Avicularin).

Table 3
1D and 2D NMR data of IN1 (Avicularin).

Position	^{13}C	HSQC	HMBC	COSY
	δ_C	δ_H (J in Hz)	$^{1}H \rightarrow {}^{13}C$	$^{1}H \leftrightarrow {}^{1}H$
1				
2	149.9			
3	135.6			
4	179.4			
5	162.9			
6	99.8	6.19 (1H, d, 2.1)	C-5, C-7, C-8, C-10	H-6, H-8
7	165.9			
8	94.7	6.38 (1H, d, 2.1)	C-6, C-7, C-9, C-10	H-8, H-6
9	158.37			
10	105.6			
1′	123.0			
2′	117.4	7.75 (1H, d, 2.2)	C-2, C-1′, C-3′, C-4′	H-2′, H-6′
3′	145.9			
4′	158.6			
5′	116.1	6.86 (1H, d, 8.45)	C-2, C-3′, C-6′	H-5′, H-6′
6′	122.8	7.57 (1H, dd, 2.2, 2.2)	C-2, C-2′, C-4′	H-6′, H-5′, H-2′
1″	104.6	5.15 (1H, d, 6.6)	C-3, C-4″	H-1″, H-2″
2″	72.8	3.9 (1H, t, 7.15)	C-1″, C-3″	H-2″, H-1″, H-3″, H-5″
3″	74.1	3.65 (1H, dd, 3.25, 3)		H-3″, H-2″, H-5″
4″	66.9	3.45 (1H, dd, 3.15, 3.2)	C-1″, C-3″	H-4″, H-5″
5″	69.1	3.83 (2H, dd, 3.7, 3.75)	C-1″, C-3″	H-5″, H-2″, H-4″, H-3″

d, duplet; ***dd***, duplet of duplet; ***t***, triplet.

4-O-methyl gallic acid was isolated from *Elaocarpus tonkinensis* (Dao et al., 2019), *Phyllanthus polyphyllus* (Abhishek et al., 2019), *Acacia arabica* and *Punica granatum* (Farag et al., 2015). Our study represents the initial documentation of the isolation of Avicularin and 4-O-methylgallic acid from the leaves of *Syzygium myrtifolium*.

Many active compounds have been found in other *Syzygium* species, and some have been reported to possess antidiabetic activity. 4-hydroxybenzaldehyde; myricetin-3-O-rhamnoside; europetin-3-O-rhamnoside; phloretin; myrigalone-G; myrigalone-B were found off *Syzygium aqueum*, which showed inhibitory activity against α-glucosidase and α-amylase enzymes (Manaharan et al., 2012). Clove bud essential oils contained dominant volatile compounds such as α-pinene; β-pinene, and 1,8-cineole also showed α-glucosidase and α-amylase inhibitory activity (Oboh et al., 2015). Active compounds such as jamutannins; *iso*-oenothein C; oenothein C; cornussin B; swertisin; gallic acid; chlorogenic acid; syringic acid; coumaric acid; ellagic acid; myricetin; ferulic acid; cinnamic acid; quercetin; phloridzin; and p-coumaric acid were found in the seeds of *Syzygium cumini*. These compounds also demonstrated anti-hyperglycemic activity (Liu et al., 2018; Omar et al., 2012; Priya et al., 2018). Moreover, the leaves were found to contain quercetin; quercetin glycoside; gallic acid; myricetin glycoside; kaempferol glycoside; luteolin glycoside; rosmarinic acid, epicathechin-3-gallate (Franco et al., 2020). Nonanoic acid, methyl ester; Eicosanoic acid, methyl ester; 9,12-Octadecadienoic acid(Z, Z)-, methyl ester; 9-Octadecenoic acid, methyl ester; Tricosanoic acid; (-)-Caryophyllene oxide; Cyclopropane, 1-(2-methylene-3-butenyl)-1-(1-methylene propyl)-; 1-Tridecene; Tetradecane; 1-Decene; Hexadecane; 2-Pentadecanone were found in *Syzygium polyanthum* and reported to have antidiabetic activity (Widjajakusuma et al., 2019). Myricitrin was identified in *Syzygium malaccense* and exhibited anti-hiperglycemic properties (Arumugam et al., 2014). Various compounds were identified in the leaves of *Syzygium jambos*,

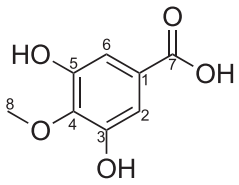

Fig. 3. Structure of IN2 (4-O-methyl gallic acid).

Table 4
^{1}H and ^{13}C data of IN2 (4-O-methyl gallic acid).

Position	^{13}C	Type of C	^{1}H
	δ_C		δ_H (J in Hz)
1	119.3	C—C	
2	108.5	CH	6.93 (2H, s) – H
3	145.6	C—OH	9.21 (3H, brs) – OH
4	138.43	C—O	
5	145.6	C—OH	9.21 (3H, brs) – OH
6	108.5	CH	6,93 (2H, s) – H
7	166.35	COOH	9.21 (3H, brs) – OH
8	51.63	CH_3	3.74 (3H, s) – CH_3

brs, broad singlet; ***s***, singlet.

including anacardic acid analog, myricitrin, myricetin, ursolic acid, gallic acid, squalene, malic acid, citric acid, castalagin, hexahydroxydiphenoyl-hexoside, casuarinin, ellagic acid, and myricetin rhamnoside (Sharma et al., 2013; Sobeh et al., 2018a).

Although the α-glucosidase inhibitory activity of *Syzygium myrtifolium* and its isolates is promising, additional progress is required for comprehensive evaluation. This includes conducting toxicity assays, preclinical studies, and clinical trials. Further development is necessary to assess the potential side effects of *Syzygium myrtifolium* and its isolates, such as avicularin and 4-O-methyl gallic acid. Advance research on avicularin and 4-O-methyl gallic acid is also needed to determine if, in the future, these compounds can be used in the treatment as hypoglycemic agents in addition to acarbose, miglitol, and voglibose.

5. Conclusion

Syzygium myrtifolium and *Syzygium jambos* leaf extracts inhibited the α-glucosidase enzyme more effectively than other Syzygium species in this study. Two bioactive compounds: Avicularin (IN1) and 4-O-methyl gallic acid (IN2), were determined as the active compounds isolated from *Syzygium myrtifolium*. Two bioactive compounds have the potential to inhibit α-glucosidase enzyme, in contrast to the positive control acarbose. Avicularin is more potent than 4-O-Methyl gallic acid, but the extract and fraction of ethyl acetate and water are more promising to be developed. This study's findings suggest that *Syzygium myrtifolium* leaf extract and its bioactive fraction can be used as a dietary supplement to manage hyperglycemia associated with type 2 diabetes mellitus with further development.

Funding

This work is supported by World Class Research program 2019 managed by the Bandung Institute of Technology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

Thank you for the support facilities provided by the World Class Research program 2019.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2023.06.010.

References

Abdulrahman, M.D., Fatihah, H.N.N., Khandaker, M.M., Ali, A.M., Mat, N., 2019. *In vitro* biological investigations on *syzygium polyanthum* cultivars. Int. J. Agric. Biol. 22, 1399–1406. https://doi.org/10.17957/IJAB/15.1214.

Abhishek, R.U., Venkatesh, H.N., Sudharshana, T.N., Mohana, D.C., 2019. Antimicrobial Activity of 4-O-methylgallic Acid Isolated from *Phyllanthus polyphyllus* L. against Foodborne Pathogenic Bacteria and Yeasts. J. Biol. Act. Prod. Nat. 9, 120–134. https://doi.org/10.1080/22311866.2019.1605932.

Adefegha, S.A., Oboh, G., 2012. *In vitro* inhibition activity of polyphenol-rich extracts from *Syzygium aromaticum* (L.) Merr. & Perry (Clove) buds against carbohydrate hydrolyzing enzymes linked to type 2 diabetes and Fe^{2+}-induced lipid peroxidation in rat pancreas. Asian Pac. J. Trop. Biomed. 2, 774–781. https://doi.org/10.1016/S2221-1691(12)60228-7.

Ahn, S., Jung, H., Jung, Y., Lee, J., Shin, S.Y., Lim, Y., Lee, Y.H., 2019. Identification of the active components inhibiting the expression of matrix metallopeptidase-9 by TNFα in ethyl acetate extract of *Euphorbia humifusa* willd. J. Appl. Biol. Chem. 62, 367–374. https://doi.org/10.3839/jabc.2019.051.

Aisha, A.F.A., Ismail, Z., Abu-Salah, K.M., Siddiqui, J.M., Ghafar, G., Abdul Majid, A.M. S., 2013. *Syzygium campanulatum* korth methanolic extract inhibits angiogenesis and tumor growth in nude mice. BMC Complement. Altern. Med. 13. https://doi.org/10.1186/1472-6882-13-168.

Ajiboye, B.O., Ojo, O.A., Oyinloye, B.E., Akuboh, O., Okesola, M.A., Idowu, O., Kappo, A. P., 2020. In vitro antioxidant and inhibitory activities of polyphenolic-rich extracts of Syzygium cumini (Linn) Skeels leaf on two important enzymes relevant to type II diabetes mellitus. Pak. J. Pharm. Sci. 33, 523–529.

Anggraini, T., 2017. Antioxidant activity of *syzygium oleana*. Pakistan J. Nutr. 16, 605–611. https://doi.org/10.3923/pjn.2017.605.611.

Arumugam, B., Manaharan, T., Heng, C.K., Kuppusamy, U.R., Palanisamy, U.D., 2014. Antioxidant and antiglycemic potentials of a standardized extract of *Syzygium malaccense*. LWT - Food Sci. Technol. 59, 707–712. https://doi.org/10.1016/j.lwt.2014.06.041.

Arumugam, B., Palanisamy, U.D., Chua, K.H., Kuppusamy, U.R., 2016. Potential antihyperglycaemic effect of myricetin derivatives from *Syzygium malaccense*. J. Funct. Foods 22, 325–336. https://doi.org/10.1016/j.jff.2016.01.038.

Ayyanar, M., Subash-babu, P., 2012. Syzygium cumini (L.) Skeels: a review of its phytochemical constituents and traditional uses. Asian Pac. J. Trop. Biomed. 2, 240–246. https://doi.org/10.1016/S2221-1691(12)60050-1.

Da Silva Sa, F.A., De Paula, J.A.M., Dos Santos, P.A., Oliveira, L.D.A.R., Oliveira, G.D.A. R., Liao, L.M., De Paula, J.R., Silva, M.D.R.R., 2017. Phytochemical analysis and antimicrobial activity of *Myrcia tomentosa* (Aubl.) DC. leaves. Molecules 22, 1–10. https://doi.org/10.3390/molecules22071100.

Dao, N.T., Jang, Y., Kim, M., Nguyen, H.H., Pham, D.Q., Dang, Q.L., van Nguyen, M., Yun, B.S., Pham, Q.M., Kim, J.C., Vu, D.H., 2019. Chemical constituents and anti-influenza viral activity of the leaves of vietnamese plant *Elaeocarpus tonkinensis*. Rec. Nat. Prod. 13, 71–80. https://doi.org/10.25135/rnp.76.18.04.266.

Dewijanti, I., Mangunwardoyo, W., Dwiranti, A., Hanafi, M., Artanti, N., 2020. Short communication: Effects of the various source areas of Indonesian bay leaves (*Syzygium polyanthum*) on chemical content and antidiabetic activity. Biodiversitas J. Biol. Divers. 21, 1190–1195. https://doi.org/10.13057/biodiv/d210345.

El-Saber Batiha, G., Alkazmi, L.M., Wasef, L.G., Beshbishy, A.M., Nadwa, E.H., Rashwan, E.K., 2020. *Syzygium aromaticum* l. (myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. Biomolecules 10 (2), 202. https://doi.org/10.3390/biom10020202.

Elya, B., Handayani, R., Sauriasari, R., Hasyyati, U.S., Permana, I.T., Permatasari, Y.I., 2015. Antidiabetic activity and phytochemical screening of extracts from indonesian plants by inhibition of alpha amylase, alpha glucosidase and dipeptidyl peptidase IV. Pakistan J. Biol. Sci. 18 (6), 279–284.

Farag, M.A., Al-Mahdy, D.A., Salaheldine, R., Fahmy, S., Yassin, A., Porzel, A., Brandt, W., 2015. Structure-Activity Relationships of Antimicrobial Gallic Acid Derivatives from Pomegranate and Acacia Fruit Extracts against Potato Bacterial Wilt Pathogen. Chem. Biodivers. 12, 955–962. https://doi.org/10.1002/cbdv.201400194.

Franco, R.R., Ribeiro Zabisky, L.F., de Lima, P., Júnior, J., Mota Alves, V.H., Justino, A.B., Saraiva, A.L., Goulart, L.R., Espindola, F.S., 2020. Antidiabetic effects of *Syzygium cumini* leaves: a non-hemolytic plant with potential against process of oxidation, glycation, inflammation and digestive enzymes catalysis. J. Ethnopharmacol. 261. https://doi.org/10.1016/j.jep.2020.113132.

Fujimori, K., Shibano, M., 2013. Avicularin, a plant flavonoid, suppresses lipid accumulation through repression of C/EBPα-activated GLUT4-mediated glucose uptake in 3T3-L1 cells. J. Agric. Food Chem. 61, 5139–5147. https://doi.org/10.1021/jf401154c.

Harborne, J.B., 1984. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*, Springer Netherlands, retrieved from internet: https://books.google.co.id/books?id=6VIVAQAAIAAJ.

Harborne, J.B., Mabry, T.J., Mabry, H., 1975. *The Flavonoids, Springer-Science+Business Media*, Springer Science+Business Media Dordrecht. https://doi.org/10.1007/978-1-4899-2909-9.

Ismail, A., Wan Ahmad, W.A.N., 2019. *Syzygium polyanthum* (Wight) Walp: a potential phytomedicine. Pharmacogn. J. 11, 429–438. https://doi.org/10.5530/pj.2019.11.67.

Jeon, K.S., Na, H.J., Kim, Y.M., Kwon, H.J., 2005. Antiangiogenic activity of 4-O-methylgallic acid from *Canavalia gladiata*, a dietary legume. Biochem. Biophys. Res. Commun. 330, 1268–1274. https://doi.org/10.1016/j.bbrc.2005.03.109.

Kumar, S., Narwal, S., Kumar, V., Prakash, O., 2011. α-glucosidase inhibitors from plants: a natural approach to treat diabetes. Pharmacogn. Rev. 5, 19–29. https://doi.org/10.4103/0973-7847.79096.

Lee, J.S., Lee, A.Y., Quilantang, N.G., Geraldino, P.J.L., Cho, E.J., Lee, S., 2019. Antioxidant activity of avicularin and isovitexin from *Lespedeza cuneata*. J. Appl. Biol. Chem. 62, 143–147. https://doi.org/10.3839/jabc.2019.020.

Li, Y., Wen, S., Kota, B.P., Peng, G., Li, G.Q., Yamahara, J., Roufogalis, B.D., 2005. *Punica granatum* flower extract, a potent α-glucosidase inhibitor, improves postprandial hyperglycemia in Zucker diabetic fatty rats. J. Ethnopharmacol. 99, 239–244. https://doi.org/10.1016/j.jep.2005.02.030.

Liu, F., Ma, H., Wang, G., Liu, W., Seeram, N.P., Mu, Y.u., Xu, Y., Huang, X., Li, L., 2018. Phenolics from *Eugenia jambolana* seeds with advanced glycation endproduct formation and alpha-glucosidase inhibitory activities. R. Soc. Chem. 9 (8), 4246–4254.

Manaharan, T., Appleton, D., Cheng, H.M., Palanisamy, U.D., 2012. Flavonoids isolated from *Syzygium aqueum* leaf extract as potential antihyperglycaemic agents. Food Chem. 132, 1802–1807. https://doi.org/10.1016/j.foodchem.2011.11.147.

Manaharan, T., Chakravarthi, S., Radhakrishnan, A.K., Palanisamy, U.D., 2014. *In vivo* toxicity evaluation of a standardized extract of *Syzygium aqueum* leaf. Toxicol. Rep. 1, 718–725. https://doi.org/10.1016/j.toxrep.2014.09.006.

Memon, A.H., Ismail, Z., Al-Suede, F.S.R., Aisha, A.F.A., Hamil, M.S.R., Saeed, M.A.A., Laghari, M., Majid, A.M.S.A., 2015. Isolation, characterization, crystal structure elucidation of two flavanones and simultaneous RP-HPLC determination of five major compounds from *Syzygium campanulatum* Korth. Molecules 20, 14212–14233. https://doi.org/10.3390/molecules200814212.

Na, H.J., Lee, G., Oh, H.Y., Jeon, K.S., Kwon, H.J., Ha, K.S., Lee, H., Kwon, Y.G., Kim, Y.M., 2006. 4-O-Methylgallic acid suppresses inflammation-associated gene expression by inhibition of redox-based NF-κB activation. Int. Immunopharmacol. 6, 1597–1608. https://doi.org/10.1016/j.intimp.2006.06.004.

Oboh, G., Akinbola, I.A., Ademosun, A.O., Sanni, D.M., Odubanjo, O.V., Olasehinde, T. A., Oyeleye, S.I., 2015. Essential Oil from Clove Bud (*Eugenia aromatica* Kuntze) Inhibit Key Enzymes Relevant to the management of type-2 diabetes and some pro-oxidant induced lipid peroxidation in rats pancreas *in vitro*. J. Oleo Sci. 64, 775–782. https://doi.org/10.5650/jos.ess14274.

Ochieng, M.A., Ben Bakrim, W., Bitchagno, G.T.M., Mahmoud, M.F., Sobeh, M., 2022. *Syzygium jambos* L. Alston: an insight into its phytochemistry, traditional uses, and pharmacological properties. Front. Pharmacol. 13, 1–15. https://doi.org/10.3389/fphar.2022.786712.

Oliveira, M.V.B., Badia, E., Carbonneau, M.A., Grimaldi, P., Fouret, G., Lauret, C., Léger, C.L., 2004. Potential anti-atherogenic cell action of the naturally occurring 4-O-methyl derivative of gallic acid on Ang II-treated macrophages. FEBS Lett. 577, 239–244. https://doi.org/10.1016/j.febslet.2004.10.015.

Omar, R., Li, L., Yuan, T., Seeram, N.P., 2012. α-Glucosidase Inhibitory Hydrolyzable Tannins from Eugenia jambolana Seeds. J. Nat. Prod. 75 (8), 1505–1509.

P, S., Zinjarde, S.S., Bhargava, S.Y., Kumar, A.R., 2011. Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. BMC Complement. Altern. Med. 11 (1). https://doi.org/10.1186/1472-6882-11-5.

Perera, H.K.I., Poongunran, J., Kalpani Premadasa, W.K.V., Jayasinghe, J.A.V.P., 2018. *In vitro* antiglycation and hypoglycaemic effects of *Syzygium cumini* leaf extracts *In vitro* antiglycation and hypoglycaemic effects of *Syzygium cumini* leaf extracts. J. Natl. Sci. Foundation Sri Lanka 46 (3), 281.

Priya, H., Karthika, S., Arun, B., Sinumol, G., Dhanya, R., Suseela, S., Nisha, A.P., 2018. Syzygium cumini seed exhibits antidiabetic potential via multiple pathways involving inhibition of a -glucosidase, DPP-IV, glycation, and ameliorating glucose uptake in L6 cell lines. J. Food Process. Preservation 1–13. https://doi.org/10.1111/jfpp.13464.

Qiu, T., Shi, J.X., Cheng, C., Jiang, H., Ruan, H.N., Li, J., Liu, C.M., 2022. Avicularin Attenuates Lead-Induced Impairment of Hepatic Glucose Metabolism by Inhibiting the ER Stress-Mediated Inflammatory Pathway. Nutrients 14, 1–9. https://doi.org/10.3390/nu14224806.

Rabeta, M.S., Chan, S., Neda, G.D., Lam, K.L., Ong, M.T., 2013. Anticancer effect of underutilized fruits. Int. Food Res. J. 20, 551–556.

Rahayu, I., Heng, P.H., Timotius, K.H., 2019. *In vitro* antioxidant properties and α-glucosidase inhibition of combined leaf infusions from *Psidium guajava* L., *Syzygium polyanthum* L., and *Annona muricata* L. Pharmacogn. J. 11, 1269–1277. https://doi.org/10.5530/pj.2019.11.197.

Riihinen, K.R., Mihaleva, V.V., Gödecke, T., Soininen, P., Laatikainen, R., Vervoort, J. M., Lankin, D.C., Pauli, G.F., 2013. 1H-NMR fingerprinting of *Vaccinium vitis-idaea* flavonol glycosides. Phytochem. Anal. 24, 476–483. https://doi.org/10.1002/pca.2444.

Rocchetti, G., Lucini, L., Ahmed, S.R., Saber, F.R., 2019. *In vitro* cytotoxic activity of six Syzygium leaf extracts as related to their phenolic profiles: An untargeted UHPLC-QTOF-MS approach. Food Res. Int. 126,. https://doi.org/10.1016/j.foodres.2019.108715 108715.

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A.A., Ogurtsova, K., Shaw, J.E., Bright, D., Williams, R., 2019. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res. Clin. Pract. 157, 107843.

Sharma, R., Kishore, N., Hussein, A., Lall, N., 2013. Antibacterial and anti-inflammatory effects of *Syzygium jambos* L. (Alston) and isolated compounds on acne vulgaris. BMC Complement. Altern. Med. 13, 1–10. https://doi.org/10.1186/1472-6882-13-292.

Shinde, J., Taldone, T., Barletta, M., Kunaparaju, N., Hu, B., Kumar, S., Placido, J., Zito, S.W., 2008. a -Glucosidase inhibitory activity of *Syzygium cumini* (Linn.) Skeels seed kernel in vitro and in Goto – Kakizaki (GK) rats. Carbohydr. Res. 343, 1278–1281. https://doi.org/10.1016/j.carres.2008.03.003.

Sobeh, M., Esmat, A., Petruk, G., Abdelfattah, M.A.O., Dmirieh, M., Monti, D.M., Abdel-Naim, A.B., Wink, M., 2018a. Phenolic compounds from *Syzygium jambos* (Myrtaceae) exhibit distinct antioxidant and hepatoprotective activities *in vivo*. J. Funct. Foods 41, 223–231. https://doi.org/10.1016/j.jff.2017.12.055.

Sobeh, M., Mahmoud, M.F., Petruk, G., Rezq, S., Ashour, M.L., Youssef, F.S., El-Shazly, A.M., Monti, D.M., Abdel-Naim, A.B., Wink, M., 2018b. *Syzygium aqueum*: A polyphenol- rich leaf extract exhibits antioxidant, hepatoprotective, pain-killing and anti-inflammatory activities in animal models. Front. Pharmacol. 9, 1–14. https://doi.org/10.3389/fphar.2018.00566.

Trinh, B.T.D., Staerk, D., Jäger, A.K., 2016. Screening for potential α -glucosidase and α -amylase inhibitory constituents from selected Vietnamese plants used to treat type 2 diabetes. J. Ethnopharmacol. 186, 189–195. https://doi.org/10.1016/j.jep.2016.03.060.

Uddin, A.B.M.N., Hossain, F., Reza, A.S.M.A., Nasrin, M.S., Alam, A.H.M.K., 2022. Traditional uses, pharmacological activities, and phytochemical constituents of the genus *Syzygium*: a review. Food Sci. Nutr. 10, 1789–1819. https://doi.org/10.1002/fsn3.2797.

Vasco, C., Ruales, J., Kamal-Eldin, A., 2008. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. Food Chem. 111, 816–823. https://doi.org/10.1016/j.foodchem.2008.04.054.

Vonia, S., Hartati, R., Insanu, M., 2022. *In Vitro* alpha-glucosidase inhibitory activity and the isolation of luteolin from the flower of *Gymnanthemum amygdalinum* (Delile) Sch. Bip ex Walp. Molecules 27, 2132. https://doi.org/10.3390/molecules27072132.

Wang, Z., Li, F., Quan, Y., Shen, J., 2019. Avicularin ameliorates human hepatocellular carcinoma via the regulation of NF-κB/COX-2/PPAR-γ activities. Mol. Med. Rep. 19, 5417–5423. https://doi.org/10.3892/mmr.2019.10198.

Widjajakusuma, E.C., Jonosewojo, A., Hendriati, L., Wijaya, S., Surjadhana, A., Sastrowardoyo, W., Monita, N., Muna, N.M., Fajarwati, R.P., Ervina, M., Esar, S.Y., 2019. Phytochemical screening and preliminary clinical trials of the aqueous extract mixture of *Andrographis paniculata* (Burm. f.) Wall. ex Nees and *Syzygium polyanthum* (Wight.) Walp leaves in metformin treated patients with type 2 diabetes. Phytomedicine 55, 137–147.

World Health Organization, 2019. Classification of diabetes mellitus, World Health Organization. World Health Organization. Doi: 10.5005/jp/books/12855_84.