BIODIVERSITAS
Volume 26, Number 6, June 2025
Pages: 3047-3053

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260647

Quantification of 1-deoxynojirimycin content, α-amylase inhibition activity, and secondary metabolites in four different mulberry accession leaves from Indonesia

YASINTA RATNA ESTI WULANDARI[1,♥], WILLIAM FRANS[1], YANTI[2]

[1]Department of Biotechnology, Faculty of Bioscience, Technology, and Innovation, Universitas Katolik Indonesia Atma Jaya. Jl. Raya Cisauk-Lapan No. 10, Cisauk 15345, Tangerang, Indonesia. Tel.: +62-21-80827200, Fax.: +62-21-80827207, ♥email: yasinta.ratna@atmajaya.ac.id
[2]Graduate Program of Biotechnology, Faculty of Bioscience, Technology, and Innovation, Universitas Katolik Indonesia Atma Jaya. Jl. Raya Cisauk-Lapan No. 10, Cisauk 15345, Tangerang, Indonesia

Manuscript received: 9 March 2025. Revision accepted: 12 June 2025.

Abstract. *Wulandari YRE, Frans W, Yanti. 2025. Quantification of 1-deoxynojirimycin content, α-amylase inhibition activity, and secondary metabolites in four different mulberry accession leaves from Indonesia. Biodiversitas 26: 3047-3053.* Mulberry (*Morus* spp.) is known to be one of the plants with potential as an antidiabetic. However, there is still limited information on the potential of mulberry leaves against diabetes, particularly mulberry from Indonesia. One of the compounds found in mulberry, namely 1-deoxynojirimycin (1-DNJ), has shown significant potential for diabetes. Therefore, this study aimed to analyze the content of 1-DNJ, the inhibitory activity of α-amylase enzyme, and secondary metabolites found in 4 types of mulberry accessions from various regions in Indonesia. Mulberry plant samples were obtained from Bogor, Pati, as well as Situbondo, and their leaves were made into extracts. The samples were analyzed for 1-DNJ content using High Performance Liquid Chromatography (HPLC), α-amylase inhibitory activity through in-vitro tests, and secondary metabolite was determined with LCMS-QTOF. The results showed that accession 3 from Bogor had the highest 1-DNJ content with 0.357±0.003 mg 1-DNJ/g leaves. In terms of α-amylase inhibitory activity, accession 3 also performed the best with an IC_{50} value of 5.1075±1.734 mg/mL compared to the acarbose with 2.062±0.424 mg/mL. Further analysis of extract found flavonoid groups such as luteolin, quercetin, and cyanidin 3,5-glucosidase, which could potentially contribute to the inhibition activity of α-amylase. Compared to others, accession 3 from Bogor showed the most potential result due to its high 1-DNJ content and low IC_{50} value. Moreover, future studies were recommended to conduct in-vivo tests and examine the ability of mulberry leaves accession 3 in handling antidiabetic disease healing.

Keywords: 1-deoxynojirimycin, α-amylase inhibition, diabetes, flavonoid, mulberry

INTRODUCTION

The mass development of global urbanization and modernization is significantly affecting various lifestyle aspects such as highly processed food, lack of exercise, and environmental factors. The combination of these factors causes the growth of metabolic syndrome diseases such as Type 2 Diabetes Mellitus (T2DM) worldwide (Chandrasekaran and Weiskirchen 2024). In 2021, approximately 19.49 million of the population in Indonesia suffered from diabetes (Ministry of Health, Republic Indonesia 2024). Most of these patients have chronic complications including heart failure, kidney disease, and coronary artery disease (Soeatmadji et al. 2023).

T2DM patients are treated with drugs and improvements in lifestyle, although chemical drugs often cause side effects when consumed in the long term (Soeatmadji et al. 2023). One of the generic drugs used to treat T2DM is acarbose, semi-synthetic compound that inhibits carbohydrate-digesting enzymes. Despite the promising effects, the use of this drug has associated side effects including flatulence, abdominal discomfort and diarrhea. (Mabate et al. 2021) The side effects have led to the search for alternative medicine, such as herbal medicine. In this context, botanical derivatives can be useful for treating or preventing metabolic syndrome disease due to their biologically active metabolites with multiple target effects compared to a single chemical substance (Shomi 2022).

One of the botanical derivatives is mulberry (*Morus* spp.), a deciduous woody tree species of the Moraceae family with origin in India and China. Mulberry plant needs specific requirements, such as high precipitation densities and elevated altitudes, typically ranging from 400 to 800 meters above sea level (Qiao et al. 2021). These specific requirements to grow mulberry plant perfectly fits the Indonesian climate, which made several regions in Indonesia begin to cultivate this plant. Usually, the mulberry species planted in Indonesia are *Morus cathayana* Hemsl., *M. alba* L., *M. indica* L., and *M. australis* Poir. The focal points of mulberry cultivation in Indonesia are situated within the regencies of Soppeng and Wajo in the South Sulawesi province (Andadari et al. 2022), Tabanan, Jembrana, and Karangasem, Bali province (Andhita et al. 2020).

Mulberry has been long used as a traditional herbal medicine, and extensive research conducted in China, Korea, and India has highlighted its potential as a treatment for T2DM. This is due to the high concentration of alkaloid iminosugar compounds, one of which is 1-Deoxynojirimycin

(1-DNJ). 1-DNJ can inhibit α-glucosidase activity through the binding of disaccharides and preventing the breakdown of glucose (Ma et al. 2019). Previous studies suggest that *Morus* spp. exhibits variability among different species, which may influence their medicinal properties. Additionally, 1-DNJ content is influenced by environmental factors such as temperature and precipitation (Hao et al. 2018). Therefore, strategies to enhance the productivity and quality of mulberry leaves, such as variety selection and hybridization, have been explored and shown to improve yields (Andadari et al. 2022). Indubitably *Morus* spp. also contains variety of other secondary metabolites, some of which may offer promising potential remedy for metabolic disease. This highlights the value of further research to explore and optimize the therapeutic applications of mulberry.

Some research also shows that mulberry can inhibit the α-amylase (EC 3.2.1.1) activity by binding to the enzyme competitively due to its flavonoid contents for instance luteolin (Martinez-Gonzalez et al. 2019), cyanidin (Yamane et al. 2019), and quercetin (Rahmani et al. 2023). Furthermore, the inhibition of this enzyme relies on the structural features of flavonoids to bind effectively to the enzyme's active site (Cui et al. 2019). There is still little information about the effect of mulberry plantation region in Indonesia on 1-DNJ content in their leaves and its effect on the inhibition of α-amylase activity. The purpose of this study is to quantify 1-DNJ content, α-amylase potential inhibition, and secondary metabolites analysis of the mulberry leaves of accessions 2 and 5 (*M. australis*), accession 3 (*M. indica*), and accession 4 (*M. alba*) from various regions in Indonesia.

MATERIALS AND METHODS

Study region

Samples were obtained from 3 different regions in Indonesia (Table 1). Stem cuttings obtained were planted in the experimental garden of Universitas Katolik Indonesia Atma Jaya, for 10 months. Subsequently, leaves extraction and inhibition analysis of α-amylase enzyme were carried out in the Biochemical and Enzyme Technology Laboratory, and Plant Cell and Culture Laboratory at Universitas Katolik Indonesia Atma Jaya. Analysis of alkaloids, flavonoids, and terpenoids using Liquid Chromatography-Mass Spectrofotometry-Quadrapole Time of Flight (LCMS-QTOF) was performed in PT. Saraswanti Indo Genetech, Bogor, Indonesia.

Sample preparation

Mature leaves were taken in the 5th position from the shoot, frozen in a freezer overnight, and freeze-dried at -10°C for 7 days. The samples obtained were ground and passed through an 80-mesh sieve.

Sample extraction

According to Jeszka-Skowron et al. (2014), mulberry powder was extracted with a 75% ethanol solution in a ratio of 1:10 (mulberry powder: ethanol 75%) for 24 hours, with the first 6 hours shaken at 125 rpm. Filtrate was then filtered, collected, and total extract was concentrated by evaporation at 50°C using a rotary evaporator. This maceration process was repeated three times to ensure maximum yield.

Preparation of 1-DNJ stock solution

The preparation of stock solution was adhered to Ji et al. (2016), where 1-DNJ standard stock solution of 75 mg/L was prepared by weighing and dissolving the compound in water for Liquid Chromatography (LC). Subsequently, the standard obtained was stored in a refrigerator at 4°C.

Derivatization of 1-DNJ

Derivatization of 1-DNJ procedures adhered to the protocols outlined by Kim et al. (2003). Initially, extract or 1-DNJ standard solution of 10 µL was mixed with 10 µL of 0.4 M potassium borate buffer (pH 8.5). This was followed by adding 20 µL of 5 mM FMOC-Cl in acetonitrile, the solution was vortexed for 10 seconds and allowed to stand at 25°C for 20 minutes in the water bath. Finally, to stop the reaction 10 µL of 0.1 M glycine was added. The solution was diluted with 950 µL of 0.1% acetic acid to stabilize FMOC-1-DNJ, then filtered using a 0.2 µm nylon syringe filter. Furthermore, 10 µL of the filtrate was injected into HPLC to measure 1-DNJ concentration, where every sample was repeated three times.

Preparation of standard solution

1-DNJ stock solution was chemically modified following the process outlined in 1-DNJ derivatization method. Initially, the standard solution was made using a derivatized stock solution diluted with distilled water for LC at a concentration of 2.28, 4.56, 9.13, 18.25, 37.5, and 75 ppm. HPLC was used to analyze the standard solutions and establish a standard curve based on the relationship between the peak area in the chromatogram and 1-DNJ concentration (Hu et al. 2013).

HPLC analysis

The derivatized extracts of all 4 accessions and standard solutions passed through HPLC analysis to quantify their 1-DNJ content. HPLC analysis used a mobile phase of acetonitrile-acetic acid 0.1% (v/v) at a 50:50 % (v/v), flowing at 1.0 mL/minute for 14 minutes. A silica C18 column (Poroshell 120, 4.6 × 150 nm) was used as the stationary phase at ambient temperature, coupled with a VWD detector set at 254 nm. Post-analysis, the column passed through a 10-minute equilibration with the mobile phase (Kim et al. 2003). All chemicals utilized in the HPLC analysis were of HPLC grade, and double-distilled water was employed.

The concentration of 1-DNJ in leaf samples was quantified using a standard curve to determine the amount of 1-DNJ in milligrams per gram of leaves. Each sample underwent HPLC analysis in triplicate, and the data presented were the mean±Standard Error (SE).

Table 1. Mulberry variety accession and region altitude

Accession	Region	Region altitude (m asl.)	Mulberry species
2	Sukamantri, Bogor, West Java	532	*Morus* australis Poir
3	Sukamantri, Bogor, West Java	532	*Morus indica* L.
4	Muktiharjo, Pati, Middle Java	88	*Morus alba* L.
5	Asem Bagus, Situbondo, East Java	5.5	*Morus australis* Poir

Determination of α-amylase inhibitory activity

Referring to the method from Kifle et al. (2021) methods with a slight modification. The extracts were diluted to different concentrations of 1.25, 2.5, 5, and 10 mg/mL in phosphate buffer pH 6.94. Firstly, 250 µL of α-amylase solution and 250 µL of crude extract were mixed inside the Eppendorf (EP) tube. Then, the sample was incubated at 37°C for 10 minutes using a water bath. After that, 250 µL of starch 1% was added to the corresponding tubes, then incubated at 37°C for 3 min. The incubated tube was then added to 250 µL of 3,5-Dinitrosalicylic acid (DNS) solution. The solutions were incubated at 95°C for 15 minutes, then cooled using running water. Finally, the OD value of each well was measured using a microplate reader at 540 nm. In the positive control group, the samples were replaced with acarbose 1.25, 2.5, 5, and 10 mg/mL in phosphate buffer. All assays were performed in triplicate in three independent experiments. The calculation of α-amylase inhibition activity was measured by IC_{50} value, which represented the concentration of a compound inhibiting 50% of α-amylase activity. Furthermore, IC_{50} value was calculated from the dose-response curve from the linear regression analysis. The enzyme used in this study was Liquozyme supra 2.2x from Sagu, which comes from *Bacillus licheniformis,* this enzyme has characteristic unit of 333.90/g Density 1.253 g/mL, total viable count less than 100/g, and coliform bacteria <4/g as described from its label.

Secondary metabolites analysis

Leaves extract with the highest 1-DNJ content was selected and analyzed using LCMS- QTOF instrument. The analysis was carried out to determine the content of alkaloids, flavonoids, and terpenoids as representative of the other varieties. LCMS was used the parameter for the mass error ≤5 ppm, isotope match MZ RMS PPM and percentage ≤6 ppm and isotope match MZ RMS % ≤10%, intensity/response ≥300, and fragment match ≥1 mass fragment. Furthermore, the stationary phase was capillary column (HSS T3) 2.1 mm × 100 mm, alongside TOF-MSE with Ionization ESI (-) and ESI (+), and flow rate at 0.6 mL/min. Meanwhile, the mobile phase was formic acid 0.1% in acetonitrile and formic acid 0.1% in ddH_2O. All chemicals in LCMS-QTOF were HPLC grade, double-distilled water was used, and repeated three times.

Data analysis

The data of HPLC, water content, and IC_{50} value was analyzed by Analysis of Variance (ANOVA) using SPSS 27.0 (SPSS Inc., Chicago, Il, USA). The standard for significance was at $p<0.05$ to determine the normality was assessed by using Saphiro-Wilk. Additionally, post-hoc analysis was based on the Duncan test to determine the differences among means.

RESULTS AND DISCUSSION

Extraction sample

The extraction of mulberry leaves used ethanol 75% because it is the most optimal concentration to extract the most compounds from the previous paper by Guo et al. (2023).

HPLC analysis

The chromatogram of the standard solutions showed that 1-DNJ was detected at a retention time of 3.5 min (Figure 1). As presented in Figure 2, the standard curve was used to determine 1-DNJ concentration of the chromatogram result.

From the four samples analyzed, the results showed fluctuations in 1-DNJ content, where the highest concentrations were found in accession 3, with 0.357±0.003 mg 1-DNJ/g leaves showing a significant difference to others (Figure 3). Furthermore, accession 4 showed the second highest 1-DNJ content with 0.246±0.054 mg 1-DNJ/g leaves, without significant difference from others. Accession 5 showed a 1-DNJ concentration of 0.199±0.050 mg 1-DNJ/g leaves making accession 2 the lowest with 0.190±0.014 mg 1-DNJ/g leaves, without significant difference.

α-amylase inhibition assay

The result for α-amylase inhibition was presented using IC_{50} value determined by using standard curve (data not shown). The result showed that acarbose had an IC_{50} value of 2.062 mg/mL, while the lowest value of 5.107 mg/mL was observed in accession 3, without a significant difference from acarbose. Furthermore, accessions 2, 4, and 5 showed no significant difference with IC_{50} value of 8.708 mg/mL, 10 mg/mL, and 10.592 mg/mL, respectively. As shown in Table 2, IC_{50} is the concentration of a compound that inhibits 50% of α-amylase activity in degrading starch.

Table 2. IC_{50} value of α-amylase inhibition (mg/mL) mulberry leaves extract

Accession code	IC_{50} value (mg/mL)±SE
Acarbose	$2.062±0.424^{a}$
2	$8.708±0.603^{b}$
3	$5.107±1.734^{a}$
4	$10±1.547^{b}$
5	$10.592±0.434^{b}$

Note: Different superscript letters indicate significant differences ($p<0.05$) among different IC_{50} value for the same assay

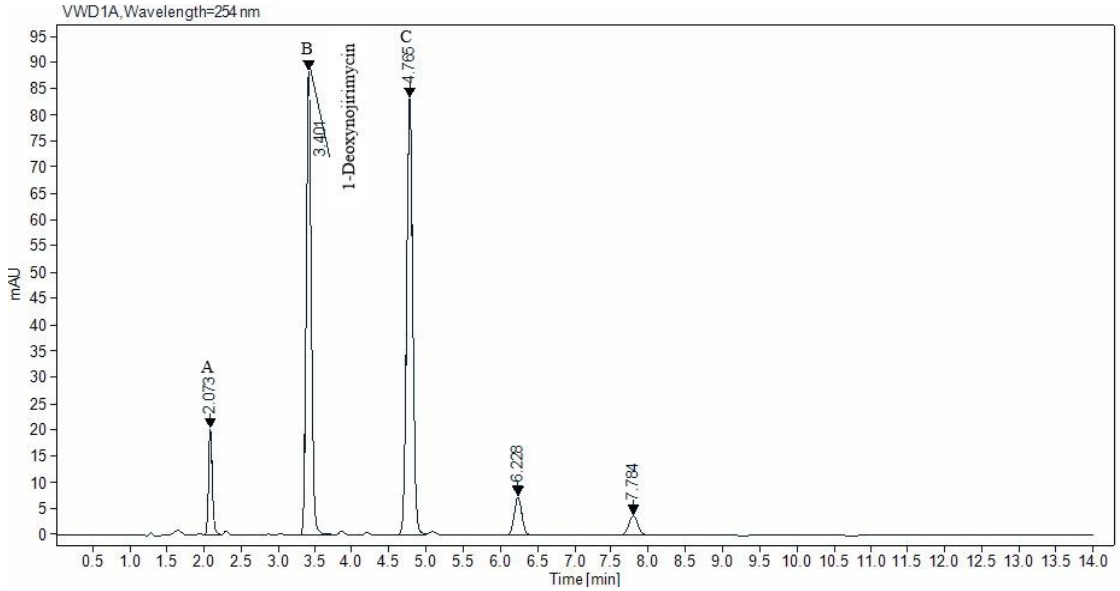

Figure 1. Chromatogram standard solution at concentration 75 ppm. Each peak represents a different compound with: A. FMOC-OH, B. FMOC-1-DNJ, and C. FMOC-Gly

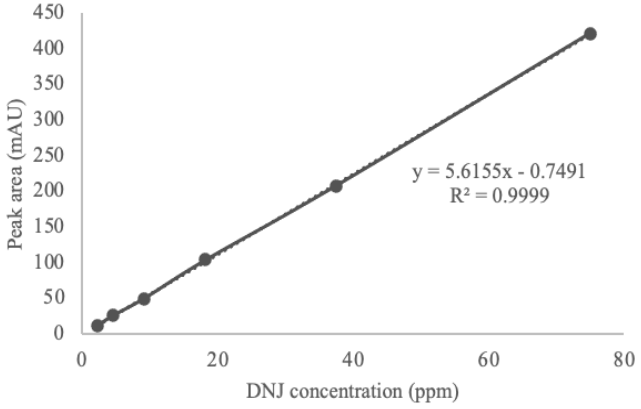

Figure 2. 1-DNJ standard curve, created using 1-DNJ standard solution in several concentration

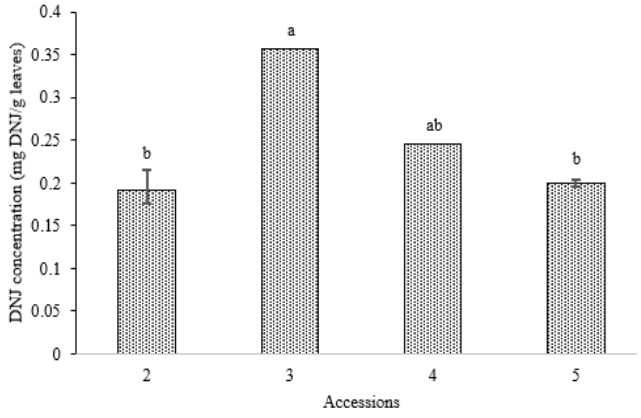

Figure 3. Comparison of 1-DNJ concentration from different accessions. The graph displays 1-DNJ content (mg 1-DNJ/g leaves) for various accessions: 2. *Morus australis*, 3. *Morus indica*, 4. *Morus alba*, and 5. *Morus australis*

Secondary metabolites analysis

The result of LCMS-QTOF detected 48 phytochemicals, comprising 2 alkaloids, 34 flavonoids, and 12 terpenoids. Specifically, 1-DNJ was detected inside the extract and categorized as alkaloid class (bold letter), with details of the compound name shown in Table 3. The analysis showed 16 glycoside compounds including 1-DNJ, kaempferol, quercetin, and genistein.

Discussion

The result of HPLC showed a difference of 1-DNJ concentration between each accession due to differences in mulberry varieties and regional plantations (Wang et al. 2021). In previous studies, the determination of 1-DNJ inside *M. alba* and *M. australis* was performed in China mulberry plant, with a 1-DNJ concentration ranging from 0.20-3.88 mg 1-DNJ/g leaves and 0.0163-1.019 mg 1-DNJ/g leaves (Ji et al. 2016; Wang et al. 2021). However, there is no previous information about 1-DNJ content in *M. indica*. The difference in results can be attributed to internal factors, particularly the efficiency of MnGUTB1 gene between varieties. This gene is responsible for converting 2-Amino-2-Deoxy-D-Mannitol (ADM) into nojirimycin, making the content different between species (Straube 2023). Additionally, the timing of harvesting mulberry leaves becomes crucial because the activation of MnGUTB1 gene requires sunlight. The optimal period to harvest 1-DNJ-rich leaves is June-August, with peak content in August showing the factors that cause lower 1-DNJ concentration observed in this study (Sugiyama et al. 2017). The data also showed no significant difference in 1-DNJ content of accessions 2 and 5. The slight difference is possibly due to variations in altitude and temperature where mulberry originally grows, which causes changes in its chemical composition (Bai et al. 2023). Previous studies from Hao et al. (2018) also show that higher precipitation and temperature result in higher 1-DNJ content.

Table 3. LCMS-QTOF result analysis of alkaloid, flavonoid, and terpenoid compounds from mulberry leaves extract

Class	Compound name
Alkaloids	Dictysine
	2-O-α-D-Glycosides of galactose-1-deoxynojirimycin
Flavonoids	2′,7-Dihydroxy-4′,5′-dimethoxyisoflavone
	7-Hydroxy-1-methoxy-2-methoxyxanthone
	Isoophiopogonone A
	Kaempferol-3,7-di-O-β-D-glucopyranoside
	Kaempferol-3-glucuronide
	Kaempferol-3-O-(2G-α-L-rhamnosyl)-rutinoside
	Quercetin-7-O-[β-D-glucopyranosyl(1→6)-β-D-glucopyranoside]
	(2S)-5,7-Dihydroxy-6-methoxy-flavanone-7-O-β-D glucopyranoside
	2′-Hydroxy-4,4′,6′-trimethoxydihydrochalcone
	2"-O-Acetylrutin
	6-Hydroxykaempferol-3-O-glucoside
	6-Methoxy-2-[2-3'-methoxy-4'-hydroxy-phenyl)ethyl]chromone
	7-Hydroxy-5,3',4'-trimethoxy flavone
	7-Methyltectorigenin
	7-α-L-Rhamnosyl kaempferol 3-O-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside
	8,3′-Dihydroxy-7,4′-dimethoxy-isoflavone
	Cassiaside
	Cyanidin 3,5-diglucoside
	Delphin
	Delphinidin-3- glucoside
	Eriodictyol-7-O-β-D-methyl-glucuronopyranoside
	Genistein-7,4'-di-O-β-D-glucoside
	Kaempferol-3-glucuronide
	Luteolin 7-O-β-D-(6''-acetyl)-glucopyranoside
	Luteolin-7-O-[β-D-`1`apiofuranosyl(1→6)]β-D-glucopyranoside
	Nelumboroside A
	Noririsflorentin
	Quercetin-3-gentiobioside
	Quercetin-3-O-(6''-O-acetyl)-β-D-glucopyranoside
	Quercetin-3-O-(6-O-feruloyl-β-D-glucopyr-anosyl)-(1→2)-β-D-ga-lactopyranosyl-(1→2)-β-D-glucopyranoside
	Quercetin-7-O-[β-D-glucopyranosyl(1→6)-β-D-glucopyranoside]
	Safflor yellow B
	Sativanone
	Undulatoside A
Terpenoid	(S)-Shihulimonin A
	6,17-Epoxylathyrol-5,15-diacetate-3-phenylacetate
	Yadanzioside P
	(E)-Aldosecologanin
	Cimicifugic acid B
	Isodihydrofutoquinol B
	Lactinolide
	Ligustroside
	Oxyphyllenone A
	Rindoside
	Sonchuside A
	Yadanzioside C

Note: Bold letter: 1-DNJ, Italic letter: Glycoside compounds

The research for a new α-amylase inhibitor derived from medicinal plants is a better method for the treatment of postprandial hyperglycemia. Inhibition of α-amylase, through the active site binding, delayed carbohydrate digestion to monosaccharides resulting in lowering post-prandial hyperglycemia. In this study, the extract of *Morus* spp. leaves showed α-amylase inhibitory activity. In this study, the IC_{50} value of the acarbose and mulberry extract was quite high compared to the previous research using pancreatic α-amylase for acarbose at 0.02 mg/mL (Ji et al. 2021), and mulberry leaves at 0.3 mg/mL (Kim et al. 2011). The high differences in the IC_{50} value are possibly related to differences in enzyme purity, as enzyme purity highly affecting the enzyme activity. Unpurified enzyme contain impurities that hinders the enzyme reaction with the substance (Valles et al. 2022).

In the preceding study, secondary metabolites from flavonoids are the main phytochemical that bears α-amylase inhibitory activity. Furthermore, it can be explained because of the high flavonoid content inside the accession 3 extract compared to the other extracts. This was confirmed by the phytochemicals detected using LCMS-QTOF of mulberry extract, which have α-amylase inhibitory properties such as luteolin (Martinez-Gonzalez et al. 2019), quercetin (Su and Tang 2019), and cyanidin-3-glucoside which can bind to the α-amylase active site (Ji et al. 2021).

There are several mechanisms that promote T2DM; one of them is oxidative stress via molecular mechanisms. Fortunately, antioxidant activity in plant sources comes from phenolic compounds like flavonoids. LCMS-QTOF results detected several compounds that have the potency of antidiabetic activity. The content of secondary metabolite compounds in mulberry leaves was also analyzed using the LCMS-QTOF instrument. 48 phytochemical compounds were detected consisting of 2 alkaloids, 34 flavonoids, and 12 terpenoids. These phytochemical compounds act as antioxidants that play a role in inhibiting the α-amylase enzyme. It was recorded that 16 compounds were in glycosides form, including 1-DNJ, kaempferol, quercetin, and genistein. These compounds are known to have antioxidant properties and can be trusted as antidiabetics because of their ability to fight free radicals. From the alkaloid group, 1-DNJ detected that other than inhibiting the α-glucosidase could also regulate insulin secretion inside the body (Ren et al. 2022). From the flavonoids group, the biochanin A (2′,7-Dihydroxy-4′,5′-dimethoxyisoflavone) (Amri et al. 2022), kaempferol (Wang et al. 2020), genistein (Dkhar et al. 2018), and quercetin (Rahmani et al. 2023) can regulate the insulin production inside the rats Streptozotocin (STZ)-induced in vivo testing. Other than insulin, there is methoxyxanthone through mimicking the effects of AMP, which activates AMPK when ATP levels drop, and activate the 5'-Adenosine Monophosphate-activated Protein Kinase (AMPK), which is responsible for the ATP production inside the body. Activated AMPK will start the translocation of GLUT4 glucose transporters to cell membrane and increase the uptake of glucose from the bloodstream (Chen et al. 2019). Compound cyanidin 3,5-diglucosidase could inhibit DPP IV, which is responsible for glucagon production and insulin inhibitor (Yamane et al. 2019). All these compounds have been proven to decrease the glucose blood level and detected inside the mulberry leaf extract by using LCMS-QTOF.

In conclusion, accession 3 of mulberry from Bogor has the highest 1-DNJ content and % inhibition α-amylase activity at 0.350±0.009 mg DNJ/g leaves and IC_{50} of 5.1075±1.734 mg/mL, suggesting it may have the greatest potential for antidiabetic effects among the accessions studied. The potential flavonoids detected from LCMS-QTOF are luteolin, quercetin, and cyanidin can inhibit the α-amylase enzyme. In addition to 1-DNJ, various flavonoids are identified in the mulberry extract, such as biochanin A, kaempferol, genistein, quercetin, and methoxyxanthone, which have antidiabetic properties. These findings highlight the importance of both alkaloid and flavonoid compounds in the antidiabetic potential of mulberry extracts.

ACKNOWLEDGEMENTS

The authors express gratitude for the support provided by the *Penelitian Penugasan* 2024 research grant No. 0203/III/LPPM-PM.10.01/03/2024 from Universitas Katolik Indonesia Atma Jaya.

REFERENCES

Amri J, Alaee M, Babaei R, Salemi Z, Meshkani R, Ghazavi A, Akbari A, Salehi M. 2022. Biochanin-A has antidiabetic, antihyperlipidemic, antioxidant, and protective effects on diabetic nephropathy via suppression of TGF-β1 and PAR-2 genes expression in kidney tissues of STZ-induced diabetic rats. Biotechnol Appl Biochem 69 (5): 2112-2121. DOI: 10.1002/bab.2272.

Andadari L, Yuniati D, Supriyanto B, Murniati, Suharti S, Widarti A, Steven E, Sadapotto A, Winarno B, Minarningsih, Agustarini R, Muin N, Isnan W, Heryati Y, Adalina Y, Yeny I, Dewi R, Nurlia A, Riendriasari SD, Maharani KE, Nugraha LM, Narendra BH. 2022. Lens on tropical sericulture development in Indonesia: Recent status and future directions for industry and social forestry. Insects 13 (10): 913. DOI: 10.3390/insects13100913.

Andhita LTM, Suamba IK, Artini NWP. 2020. Pengaruh kepuasan petani ulat sutra terhadap loyalitas sebagai plasma pada pola kemitraan agrowisata Sutera Sari Segara. Jurnal Agribisnis dan Agrowisata 9 (1): 79-88. [Indonesian]

Bai H, Jiang S, Liu J, Tian Y, Zheng X, Wang S, Xie Y, Li Y, Jia P. 2023. Planting conditions can enhance the bioactivity of mulberry by affecting its composition. Front Plant Sci 14: 1133062. DOI: 10.3389/fpls.2023.1133062.

Chandrasekaran P, Weiskirchen R. 2024. The role of obesity in type 2 diabetes mellitus-An overview. Intl J Mol Sci 25 (3): 1882. DOI: 10.3390/ijms25031882.

Chen T-H, Tsai M-J, Fu Y-S, Weng C-F. 2019. The exploration of natural compounds for anti-diabetes from distinctive species *Garcinia linii* with comprehensive review of the *Garcinia* family. Biomolecules 9 (11): 641. DOI: 10.3390/biom9110641.

Cui H, Lu T, Wang M, Zou X, Zhang Y, Yang X, Dong Y, Zhou H. 2019. Flavonoids from *Morus alba* L. leaves: Optimization of extraction by response surface methodology and comprehensive evaluation of their antioxidant, antimicrobial, and inhibition of α-amylase activities through analytical hierarchy process. Molecules 24 (13): 2398. DOI: 10.3390/molecules24132398.

Dkhar B, Khongsti K, Thabah D, Syiem D, Satyamoorthy K, Das B. 2018. Genistein represses PEPCK-C expression in an insulin-independent manner in HepG2 cells and in alloxan-induced diabetic mice. J Cell Biochem 119 (2): 1953-1970. DOI: 10.1002/jcb.26356.

Guo Z, Lai J, Wu Y, Fang S, Liang X. 2023. Investigation on antioxidant activity and different metabolites of mulberry (*Morus* spp.) leaves depending on the harvest months by UPLC-Q-TOF-MS with multivariate tools. Molecules 28 (4): 1947. DOI: 10.3390/molecules28041947.

Hao J-Y, Wan Y, Yao X-H, Zhao W-G, Hu R-Z, Chen C, Li L, Zhang D-Y, Wu G-H. 2018. Effect of different planting areas on the chemical compositions and hypoglycemic and antioxidant activities of mulberry leaf extracts in Southern China. PLoS One 13 (6): e0198072. DOI: 10.1371/journal.pone.0198072.

Ministry of Health, Republic Indonesia. 2024. Saatnya mengatur si manis. sehatnegeriku.kemkes.go.id. [27 July 2024]. https://sehatnegeriku.kemkes.go.id/baca/blog/20240110/5344736/saatnya-mengatur-si-manis/. [Indonesian]

Hu X-Q, Jiang L, Zhang J-G, Deng W, Wang H-L, Wei Z-J. 2013. Quantitative determination of 1-deoxynojirimycin in mulberry leaves from 132 varieties. Ind Crops Prod 49: 782-784. DOI: 10.1016/j.indcrop.2013.06.030.

Jeszka-Skowron M, Flaczyk E, Jeszka J, Krejpcio Z, Król E, Buchowski MS. 2014. Mulberry leaf extract intake reduces hyperglycemia in Streptozocin (STZ)-induced diabetic rats fed high fat diet. J Funct Foods 8: 9-17. DOI: 10.1016/j.jff.2014.02.018.

Ji T, Li J, Su S-L, Zhu Z-H, Guo S, Qian D-W, Duan J-A. 2016. Identification and determination of the polyhydroxylated alkaloids compounds with α-glucosidase inhibitor activity in mulberry leaves of different origins. Molecules 21: 206. DOI: 10.3390/molecules21020206.

Ji Y, Liu D, Jin Y, Zhao Juan, Zhao Jiang, Li H, Li L, Zhang H, Wang H. 2021. In vitro and in vivo inhibitory effect of anthocyanin-rich mulberry extract on α-glucosidase and α-amylase. LWT 145: 111484. DOI: 10.1016/j.lwt.2021.111484.

Kifle ZD, Debeb SG, Belayneh YM. 2021. In vitro α-amylase and α-glucosidase inhibitory and antioxidant activities of the crude extract and solvent fractions of *Hagenia abyssinica* leaves. Biomed Res Intl 2021: 6652777. DOI: 10.1155/2021/6652777.

Kim G-N, Kwon Y-I, Jang H-D. 2011. Mulberry leaf extract reduces postprandial hyperglycemia with few side effects by inhibiting α-glucosidase in normal rats. J Med Food 14 (7-8): 712-717. DOI: 10.1089/jmf.2010.1368.

Kim J-W, Kim S-U, Lee HS, Kim I, Ahn MY, Ryu KS. 2003. Determination of 1-deoxynojirimycin in *Morus alba* L. leaves by derivatization with 9-fluorenylmethyl chloroformate followed by reversed-phase high-performance liquid chromatography. J Chromatogr A 1002 (1-2): 93-99. DOI: 10.1016/S0021-9673(03)00728-3.

Ma Y, Lv W, Gu Y, Yu S. 2019. 1-deoxynojirimycin in mulberry (*Morus indica* L.) leaves ameliorates stable angina pectoris in patients with coronary heart disease by improving antioxidant and anti-inflammatory capacities. Front Pharmacol 10: 569. DOI: 10.3389/fphar.2019.00569.

Mabate B, Daub CD, Malgas S, Edkins AL, Pletschke BI. 2021. A combination approach in inhibiting type 2 diabetes-related enzymes using *Ecklonia radiata* fucoidan and acarbose. Pharmaceutics 13 (11): 1979. DOI: 10.3390/pharmaceutics13111979.

Martinez-Gonzalez AI, Díaz-Sánchez ÁG, de la Rosa LA, Bustos-Jaimes I, Alvarez-Parrilla E. 2019. Inhibition of α-amylase by flavonoids: Structure Activity Relationship (SAR). Spectrochim Acta A Mol Biomol Spectrosc 206: 437-447. DOI: 10.1016/j.saa.2018.08.057.

Qiao Y, Ito M, Kimura T, Ikeuchi T, Takita T, Yasukawa K. 2021. Inhibitory effect of *Morus australis* leaf extract and its component iminosugars on intestinal carbohydrate-digesting enzymes. J Biosci Bioeng 132 (3): 226-233. DOI: 10.1016/j.jbiosc.2021.05.005.

Rahmani AH, Alsahli MA, Khan AA, Almatroodi SA. 2023. Quercetin, a plant flavonol attenuates diabetic complications, renal tissue damage, renal oxidative stress and inflammation in streptozotocin-induced diabetic rats. Metabolites 13 (1): 130. DOI: 10.3390/metabo13010130.

Ren XX, Xing Y, He L, Xiu Z, Yang L, Han A, Jia Q, Dong Y. 2022. Effect of 1-deoxynojirimycin on insulin resistance in prediabetic mice based on next-generation sequencing and intestinal microbiota study. J Ethnopharmacol 289: 115029. DOI: 10.1016/j.jep.2022.115029.

Shomi N. 2022. Lifestyle-Related Diseases and Metabolic Syndrome. IntechOpen, London.

Soeatmadji DW, Rosandi R, Saraswati MR, Sibarani RP, Tarigan WO. 2023. Clinicodemographic profile and outcomes of type 2 diabetes mellitus in the Indonesian cohort of discover: A 3-year prospective cohort study. J ASEAN Fed Endocr Soc 38 (1): 68-74. DOI: 10.15605/jafes.038.01.10.

Straube H. 2023. Am-aza-ing antidiabetic: Mulberry dehydrogenase $MnGUTB_1$ contributes to the biosynthesis of 1-deoxynojirimycin. Plant Physiol 192 (2): 700-702. DOI: 10.1093/plphys/kiad140.

Su J, Tang Z. 2019. Effects of (-)-epigallocatechin gallate and quercetin on the activity and structure of α-amylase. Trop J Pharm Res 18 (3): 585-590. DOI: 10.4314/tjpr.v18i3.20.

Sugiyama M, Katsube T, Koyama A, Itamura H. 2017. Seasonal changes in functional component contents in mulberry (*Morus alba* L.) leaves. Hortic J 86 (4): 534-542. DOI: 10.2503/hortj.okd-053.

Valles M, Pujals S, Albertazzi L, Sánchez S. 2022. Enzyme purification improves the enzyme loading, self-propulsion, and endurance performance of micromotors. ACS Nano 16 (4): 5615-5626. DOI: 10.1021/acsnano.1c10520.

Wang T, Wu Q, Zhao T. 2020. Preventive effects of kaempferol on high-fat diet-induced obesity complications in C57BL/6 mice. Biomed Res Intl 2020: 4532482. DOI: 10.1155/2020/4532482.

Wang Z, Dai F, Tang C, Xiao G, Li Z, Luo G. 2021. Quantitative determination of 1-deoxynojirimycin in 146 varieties of mulberry fruit. Intl J Food Prop 24: 1214-1221. DOI: 10.1080/10942912.2021.1955923.

Yamane T, Imai M, Handa S, Yamada K, Sakamoto T, Ishida T, Inui H, Yamamoto Y, Nakagaki T, Nakano Y. 2019. Reduction of blood glucose and HbA1c levels by cyanidin 3, 5-diglucoside in KKAy mice. J Funct Foods 58: 21-26. DOI: 10.1016/j.jff.2019.04.038.