JURNAL KARTIKA KIMIA

J. Kartika Kimia, May 2025, 8, (1), 85-91
Journal homepage: http://jkk.unjani.ac.id/index.php/jkk
P-ISSN: 2655-1322 E-ISSN: 2655-0938

Exploring the Alpha-Amylase Inhibitory Potential of Roselle Calyx Extracts (Hibiscus sabdariffa L) in n-Hexane and Ethyl Acetate Fractions

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DOI: https://doi.org/10.26874/jkk.v8i1.899

Received: 24 March 2025, *Revised*: 9 June 2025, *Accepted*: 11 June 2025, O*nline*: 12 June 2025

Abstract

Diabetes mellitus is a metabolic disorder characterized by elevated blood sugar levels, which result from impaired insulin excretion or sensitivity. Among the various medicinal plants utilized by the Indonesian population, roselle (Hibiscus sabdariffa L) stands out as a potential remedy for several diseases including diabetes mellitus. This study aims to assess the inhibitory potential of roselle on α-amylase enzyme activity. The roselle calyx extraction method was performed through multilevel maceration using n-hexane and ethyl acetate solvents. Testing the activity of inhibiting the α-amylase enzyme was carried out in vitro using acarbose as a comparison. The findings revealed that the n-hexane extract of roselle calyx exhibited potent α-amylase enzyme inhibition with an IC_{50} value of 20.43 µg/mL. Similarly, roselle calyx ethyl acetate extract demonstrated strongl α-amylase enzyme inhibitory activity, characterized by an IC_{50} value of 10.13 µg/mL. Meanwhile, acarbose exhibited robust α-amylase inhibitory activity, possessing an IC_{50} value of 4.04 µg/mL. It is worth noting that all these substances displayed α-amylase enzyme inhibitory activity in the very active category, although their efficacy was not equivalent to acarbose.

Keywords: Acarbose, Antidiabetic, α-amylase, Hibiscus sabdariffa L., Roselle

1 Introduction

According to the from 2018 Indonesian Ministry of Health's National Health Survey, the prevalence of diabetes among children under the age of 15 was estimated to be 2%. This prevalence varied across different regions in Indonesia, with the rates observed in DKI Jakarta, Yogyakarta, Kalimantan Timur, Sulawesi Utara, and Jawa Timur. Genetic and epigenetic factors were identified as the contributing factors to the higher prevalence in these regions [1]. However, concerning projections, the International Diabetes Federation in 2021 predicted a significant increase in the number of individuals with diabetes in Indonesia. The IDF estimated that Indonesia would have 19.47 million people living with diabetes. This represents a notable increase of 1.6% from the initial prevalence rate of 6.9% to a projected rate of 8.5% [2].

One of the mechanisms employed by commercially available anti-diabetic pharmaceuticals involves the inhibition of the α-glucosidase enzyme, which comprises the α -amylase enzyme. The α-amylase enzyme is responsible for catalysing the breakdown of α-1,4 glycosidic bonds, converting polysaccharides into a mixture of smaller oligosaccharides. In other words, the α -amylase enzyme plays a crucial role in starch hydrolysis. This process contributes to the accelerated carbohydrate degradation, potentially leading to increased postprandial hyperglycaemia [3]. The mechanism of the α-amylase enzyme inhibitor has the same mechanism as the α-glucosidase (acarbose) mechanism. However, it is important to point out that long-term use of the drug acarbose has been associated with a range of side effects, such as gastrointestinal disorders including flatulence, malabsorption, stomach ache, diarrhoea and bloody diarrhoea, severe constipation to cause impaired function and can cause the patient to bruise or bleed easily [4]. Given the limitations and side effects associated with synthetic α-amylase and α-glucosidase inhibitors like acarbose, there is growing interest in exploring natural alternatives derived from medicinal plants.

Indonesia is blessed with an abundant array of medicinal flora, among of which is *Hibiscus*

sabdariffa commonly known as roselle, stands out due to its notable anti-diabetic pharmacological effects. Roselle's potential as an antidiabetic was demonstrated by testing its effects on diabetic animal models using dried, aqueous, or ethanolic extracts. Overall, the administration of dried roselle, aqueous, or ethanolic extracts at doses ranging from 30 to 500 mg/kg bw has the potential to lower blood glucose levels in test animals that have been stimulated by alloxan or streptozotocin.[5] Interestingly, extracts and water fractions derived from red roselle flower calyx have demonstrated α-glucosidase enzyme inhibitory activity, while white roselle flower calyx exhibits α-amylase enzyme inhibitory activity [6]. However, there appears to be a dearth of available information in the existing literature concerning the specific activity of α-amylase enzyme inhibitors derived from red roselle calyx extracts using n-hexane and ethyl acetate. Extraction using non-polar (n-hexane) and semi-polar (ethyl acetate) solvents aims to extract metabolite compounds [7] that have the potential to inhibit the α-amylase enzyme, for example terpenoids borapetoside C and borapentol B in non-polar solvents [8] and flavonoid compounds luteolin in semi-polar solvents [9].

Considering the possible effects of non-polar and semi-polar extracts of roselle calyx, it is very important to conduct research on the effects of these extracts, which can later add information on their potential for the treatment of diabetes mellitus. Therefore, this study was conducted as an initial screening of the effects of n-hexane and ethyl acetate extracts of roselle calyx as α-amylase inhibitors.

2 Method

2.1 Chemical materials

Acarbose BPFI, α-glucosidase enzyme from *Saccharomyces cerevisiae* (Sigma Aldrich®), p-nitrophenyl-α-D-glucopyranoside (Sigma Aldrich®), DMSO (Merck®), Dragendorff's reagent, Mayer's reagent, Liebermann-Bhouchard's reagent, 10% vanillin-sulfuric acid reagent, iron (III) chloride, potassium hydroxide, concentrated sulfuric acid, 2N hydrochloric acid, chloroform, ether, dilute ammonia, amyl alcohol, magnesium powder, 1% gelatine solution, potassium dihydrogen phosphate, sodium hydroxide, phosphoric acid, ethyl acetate, n-butanol, formic acid.

2.2 Roselle flower calyx collection, characterisation and extraction

Roselle flowers used in this study were obtained from the Gombong area, West Java. A comprehensive characterization and phytochemical screening examination was carried out on red roselle calyx simplicia [10]. The extraction of red roselle calyx (*Hibiscus sabdariffa* L) was carried out using maceration with increased solvent polarity.

The first level of extraction was carried out by soaking 100 g of roselle calyx simplicia in 1 L of non-polar solvent (n-hexane) for the first 6 hours, with regular stirring every hour, followed by an 18 hour period of maceration. After this, the macerate was collected. The second level of extraction was carried out by soaking the residue from the first level of extraction in 1 L of semi-polar solvent (ethyl acetate). Similar to the first extraction, the roselle calyx was soaked in ethyl acetate for the first 6 hours, stirred hourly, and left to stand for up to 18 hours before collecting the macerate. Each process was repeated three times. Subsequently, each macerate obtained using the same solvent was collected and concentrated using a rotary evaporator, employing at a temperature of 50ºC and a pressure of 20 psi. After that, the concentrated extract was evaporated in a water bath at 70ºC to obtain a thick extract.

2.3 α-Amylase Enzyme Inhibition Test

The concentrations of n-hexane extracts used were prepared at 10, 40, 60, 80 and 100 µg/mL, while the concentrations of ethyl acetate extract used were set at 4, 6, 10, 20 and 40 µg/mL. Acarbose was used as a reference, with concentrations of 2.5, 5, 10, 40, and 60 µg/mL.

The α-amylase enzyme inhibition test was carried out in twos replicates, with the following procedure being followed: (1) a total of 200 µL of control solution/each concentration variation of the test solution (acarbose, n-Hexane extract or ethyl acetate extract of roselle calyx) was dispensed into separate containers; (2) to each of these containers, 200 µL of α-amylase enzyme solution was added. The mixture was then incubated for 10 minutes at 37ºC; (3) subsequently, 200 µL of substrate was added to each test solution, and the mixture was incubated once more again at room temperature for 3 minutes; (4) after that, 200 µL of 3,5-dinitrosalicylic acid (DNSA) reagent was added to stop the reaction, then heated for 10 minutes at 95ºC; (5) after that, the absorbance of the mixture was measured using a UV-Visible

spectrophotometer at a wavelength of 540 nm; and (6) the percentage of α-amylase inhibition was calculated using **Eq. 1**.

$$\alpha - \text{amylase inhibition } (\%) = \frac{K-U}{K}\ x\ 100\% \qquad \text{(Equation 1)}$$

K = Absorbance of enzyme activity without sample; U = Absorbance of enzyme activity with the addition of the tested sample.

Inhibition Concentration 50 (IC_{50}) value represents the concentration of a substance that can inhibit the activity of the α-amylase enzyme by up to 50%. To calculate the $IC_{50,}$ a linear regression equation derived from the calibration curve between the percentage of inhibitory activity and the concentration of the sample is utilized to obtain the concentration of the extract with inhibitory activity against the α-amylase enzyme. The linear regression equation is y = ax + b, where x is the concentration of the test sample and y is the percent inhibition. So IC_{50} can be calculated using **Eq. 2**.

$$IC_{50} = \frac{50-a}{b} \qquad \text{(Equation 2)}$$

a = The intercept of the x and y axis plots, b = Slope plot of the x and y axes

3 Result and Discussion

At the beginning of the study, the simplicia characteristics were examined to ensure its quality [11]. The results of this characterization process are presented in **Table 1**.

Table 1 Results of Examination of the Characterization of Roselle Flower Calyx Simplicia (*Hibiscus sabdariffa* L.)

Parameter	Result
Water content (%v/w)	7.70±0.14
Water Soluble Content (%w/w)	14.93±0.61
Ethanol Soluble Content (%w/w)	16.76±0.35
Total Ash (%w/w)	5.51±0.20
Water Soluble Ash (%w/w)	4.53±0.29
Acid Insoluble Ash (%w/w)	0.22±0.03

Data presented in mean±SD

In this extraction procedure, a sequential extraction method using two solvents with distinct polarities was applied, beginning with a non-polar solvent and then proceeding with a semi-polar solvent. The rationale behind this sequential approach lies in the notion that the two solvents, with their differing polarities, can yield extracts containing diverse secondary metabolite compounds. The polarity of these secondary metabolites can greatly impact their efficacy as α-amylase enzyme inhibitors [12]. Thus, this method facilitates the identification of which secondary metabolite compounds play a role, or exert a more pronounced effect, in inhibiting the α-amylase enzyme. The results of secondary metabolite screening for each extract are tabulated in **Table 2**.

Table 2 Results of Phytochemical Screening of Simplicia, n-hexane Extract and Ethyl Acetate Extract of Roselle Flower Calyx (*Hibiscus sabdariffa* L)

Parameter	Roselle Flower Calyx	n-hexane Extract	Ethyl Acetate Extract
Flavonoids	+	-	+
Alkaloids	-	-	-
Tannins	+	-	+
Saponins	+	-	+
Polyphenols	+	-	+
Steroids	+	+	+
Triterpenoids	-	-	-
Monoterpenoids-Sesquiterpenoids	+	+	+
Quinone	+	+	+

(+) Secondary metabolites were detected
(-) No secondary metabolites were detected

A review conducted by Kashtoh and Baek (2023) showed the potential of various types of polar, semi-polar and non-polar extracts that are effective in inhibiting α-amylase. The group of compounds that inhibit α-amylase include flavonoids (luteolin, isoquercitrin, epicatechin gallate, tricetin, rutin), triterpenoids (glochidon, ligularoside A), phenolic compounds (ellagic acid, chlorogenic acid), tannins (chingiitannin A) [13]. Phytochemical screening (**Table 2**) showed that the n-hexane extract contained steroids, terpenoids and quinones, while the ethyl acetate extract contained flavonoids, tannins, saponins, polyphenols, steroids, terpenoids and quinones. This shows the potential of the compounds contained in roselle extract as α-amylase inhibitors.

Sequential extraction was selected due to its ability to yield high quantity extracts. The extract obtained from 200 grams of red roselle flower calyx simplicia with n-hexane solvent yielded 2.67 g of thick extract (yield 1.34% w/w), whereas ethyl acetate solvent produced 8.98 g of thick extract (yield 4.48% w/w).

In vitro testing was carried out to determine the functioning of the α-amylase enzyme in degrading starch as simpler sugars, such as into maltose and glucose. In testing α-amylase enzyme inhibitors, we adopted the DNSA method, a well-established approach in enzymology. In this method, DNSA functions as an oxidizer agent, which undergoes reduction when interacting with test samples, ultimately forming 3-amino-5-nitrosalicylic acid. Simultaneously, the aldehyde group in glucose undergoes oxidation by 3,5-dinitrosalicylic acid leading to the formation of carboxyl groups occurring in an alkaline environment. The resultant colour changes observed in the reaction solutions are primarily attributed to the formation of 3-amino-5-nitrosalicylic acid. This compound exhibits the remarkable property of absorbing strong electromagnetic wave radiation within a specific wavelength, typically falling between 540 nm to 550 nm [14]. In our experiment, cassava starch served as the substrate for the α-amylase enzyme. Phosphate buffer pH 6.9 was used to sustain the enzyme stability and ensure its proper functioning during the testing process, because the optimum pH for the α-amylase enzyme is in the range of 5.6-7.2. The incubation process involving heat can alter the structure of the α-amylase enzyme and halt its activity; consequently, the hydrolysis reaction of the α-amylase enzyme will cease due to a change in the enzyme's configuration. The 3,5-dinitrosalicylic acid reagent will change colour from yellow to orange after the reaction is complete, based on the concentration and percent inhibition of the being tested extract [15].

Several variations in the concentration of each test substance were used to determine the percent inhibition, which was then used to calculate the IC_{50} value when testing the activity of α-amylase. The IC_{50} value was calculated to ascertain the concentration at which 50% of enzyme activity is inhibited. The graph of the results of the α-amylase inhibition measurements at each concentration can be seen in **Fig. 1**. Based on the resulting linear regression equation, the IC_{50} value can be calculated, the results can be seen in **Table 3**.

The test results revealed that the IC_{50} values for the active substances—namely acarbose, n-hexane extract and ethyl acetate extract—stood at 4.04, 20.42 and 10.13 µg/mL, respectively. Despite having a lesser yield, ethyl acetate extract is more effective at inhibiting the α-amylase enzyme, as seen by its lower IC_{50} value (10.13 µg/mL) as compared to n-hexane (20.42 µg/mL). The variation in solvent polarity has a significant impact on the type of drug extracted: more polar ethyl acetate extracts polar compounds with potentially stronger inhibitory effect, whereas n-hexane extracts non-polar compounds. Although the higher yield of ethyl acetate (4.48%) indicates a bigger amount of substance extracted overall, the potential activity is dependent on both the amount and the potency of the active component present.

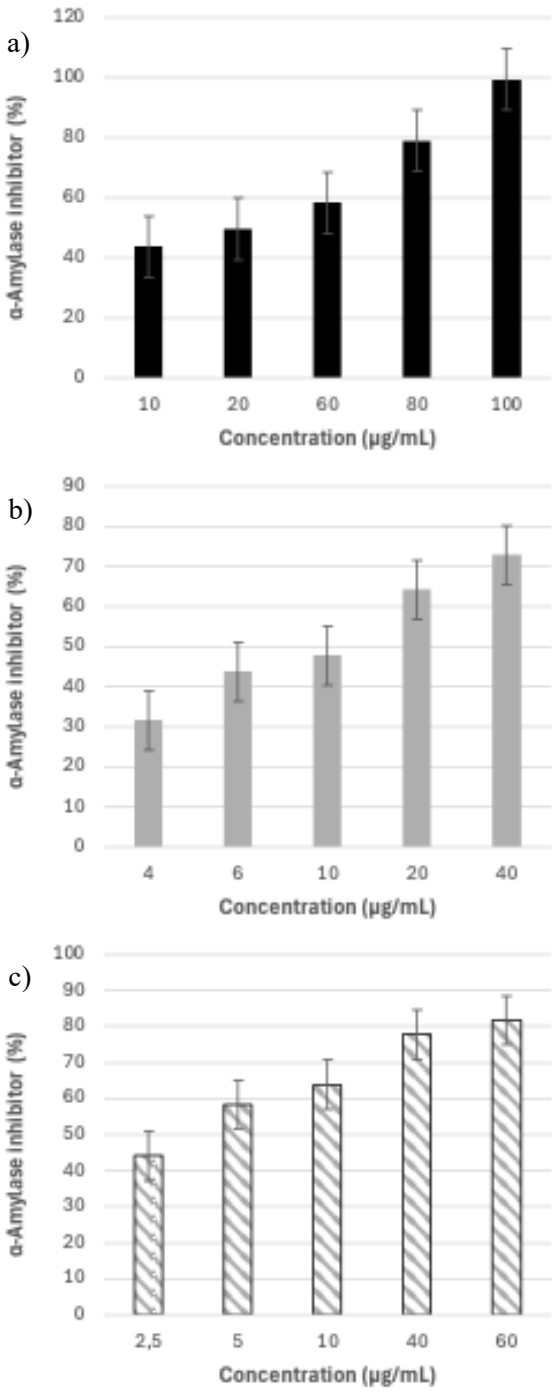

Figure 1 Graph of α-amylase inhibition effect of extracts, a) n-hexane extract, b) ethyl acetate extract, c) acarbose.

Table 3 Results of α-amylase inhibitor test

Compound	Equation	IC_{50} (µg/mL)
Acarbose	y=0.6021x+47.566	4.04
n-hexane	y=0.5499x+38.768	20.42
Ethyl Acetate	y=0.9095x+40.786	10.13

All test materials showed IC_{50} values below 50 µg/mL indicating a very strong α-amylase inhibitory ability [16]. Acarbose and compounds in the extract are thought to be able to compete with the enzyme's substrate, binding to the enzyme's active center and thus preventing the conversion of the substrate into glucose.

As mentioned earlier, the n-hexane extract of red roselle flower calyx contains steroids, monoterpenoids-sesquiterpenoids, and quinones, whereas the ethyl acetate extract contains flavonoids, tannins, saponins, polyphenols, steroids, monoterpenoids-sesquiterpenoids, and quinones. *Salacia oblonga* contains phenols, flavonoids, alkaloids, terpenoids, tannins, and saponins that have α-amylase inhibitory properties [17]. The competitive relationship between polyphenol and α-1,4-glucosidic bonds in terms of enzyme binding was responsible for α-amylase enzyme inhibition. Thus, the variation in substrate concentration altered the quantity of α-1,4-glucosidic bonds that served as effective binding sites for α-amylase, which in turn altered the likelihood of enzyme-polyphenol binding [18]. Even though flavonoids are effective α-amylase inhibitors, the results were weaker when compared to α-glucosidase activity. The diverse inhibitory effects of flavanol's on human -glucosidases depend on their structure, the source of the enzyme, and the substrates used. C6-OH A ring hydroxylation and reduced B ring hydroxylation are two crucial structural components of flavonoids for α-glucosidase inhibition enhancement in humans [19].

Based on data taken from the KNApSAcK database (https://www.knapsackfamily.com/), compounds contained in the rocelle plant include eugenol, myricetin, trigoneline, 3,4-dihydroxybenzoic acid, ergosterol, spinasterol, quercetine, gossypetin, hibiscetin, hibiscetin 3-glucoside, delphinidin 3-sambubioside, citric acid, apha-terpineol, daphniphylline, and malic acid. The limitation of this study is that the identification of compounds contained in n-hexane and ethyl acetate extracts was not carried out. Therefore, one of the next steps is to determine the compounds in each extract using LC-MS/NMR.

It is necessary to conduct *in vivo* research on the anti-diabetic effects of n-hexane and ethyl acetate extracts, as *in vitro* assessment of their anti-diabetic properties is a limitation of this study. *In vivo* testing can be performed on alloxan or streptozotocin induced animal models. It is believed that secondary metabolite compounds present in n-hexane and ethyl acetate extracts of red roselle flowers can also reduce blood glucose levels *in vivo*. In addition, its mechanism of action as a cellular and molecular antidiabetic requires extensive investigation. The reference to *Tinospora crispa*'s alkaloid, flavonoid, and steroid/triterpenoid components highlight the multifaceted nature of plant-derived metabolites in releasing insulin from the pancreas and lowering blood glucose levels [20]. Referring to research on *Gongronema latifolium* Benth., it is known that several steroidal pregnane compounds (e.g.: marsectohexol, 3-O-[6-deoxy-3-O-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranos-yl]-11,12-di-O-tigloyl-17β-marsdenin, and their derivatives) are able to inhibit the α-amylase enzyme *in vitro* and *in vivo* [21]. The potentials of alkaloids, phenols, flavonoids, saponins, tannins, terpenes, and steroid to influence cellular and molecular pathways involved in glucose metabolism is a promising opportunity for further exploration. Their capacity to protect pancreatic beta cells, correct insulin signalling abnormalities, reduce oxidative stress and inflammation, activate AMP-activated protein kinase (AMPK), and modulate carbohydrate digestion and absorption suggest a multi-functional approach in addressing diabetes at multiple levels [22]. In addition, computational chemistry approaches (e.g., basic docking or interaction prediction targeting AMPK or GLUT4 pathways.) or bioautographic analysis can be carried out to enrich information on the working mechanisms of compounds contained in roselle extract.

4 Conclusion

The n-hexane and ethyl acetate extracts inhibited α-amylase activity with IC_{50} values of 20.42 and 10.13 µg/mL, respectively. These results indicate a significant anti- α-amylase effect for both extracts, although they were less potent compared to acarbose (IC_{50} 4.04 µg/mL). However, further *in silico, in vitro* or *in vivo* studies are needed to support this hypothesis and gain a deeper understanding of the specific compounds responsible for the observed effects.

Acknowledgement

We extend our sincere gratitude to the UNJANI Research and Community Service Institute for their funding, which covered all the expenses associated with the research activities. Their support played a pivotal role in enabling the successful completion of this study, contributing valuable insights to the field of science.

References

[1] Badan Penelitian dan Pengembangan Kesehatan. Laporan Nasional Riset Kesehatan Dasar 2018, Jakarta: Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan; 2019, 152–62 p.

[2] IDF. International Diabetes Federation. 10th ed. Boyko EJ, Magliano DJ, Karuranga S, Piemonte L, Riley P, Saeedi P, et al., editors. IDF Diabetes Atlas. International Diabetes Federation; 2012. 37 p.

[3] Tiwari SP., Srivastava R., Singh CS., Shukla K., Singh RK., Singh P., ... Sharma R., 2015, Amylases: an overview with special reference to alpha amylase, *J Global Biosci*, *4*(1), 1886-1901.

[4] DiNicolantonio JJ., Bhutani J., O'Keefe JH., 2015, Acarbose: safe and effective for lowering postprandial hyperglycaemia and improving cardiovascular outcomes, *Open heart*, *2*(1).

[5] Jamrozik D., Borymska W., Kaczmarczyk-Żebrowska, I., 2022, Hibiscus sabdariffa in diabetes prevention and treatment—Does it work? An evidence-based review, *Foods*, *11*(14), 2134. https://doi.org/10.3390/foods11142134

[6] Ademiluyi AO., Oboh G., 2013, Aqueous extracts of Roselle (Hibiscus sabdariffa Linn.) varieties inhibit α-amylase and α-glucosidase activities in vitro, *Journal of medicinal food*, *16*(1), 88-93. https://doi.org/10.1089/jmf.2012.0004

[7] Abubakar AR., Haque M., 2020, Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes, *Journal of Pharmacy and Bioallied Sciences*, *12*(1), 1-10. https://doi.org/10.4103/jpbs.JPBS_175_19

[8] Pujiyanto S., Wijanarka W., Raharjo B., Anggraeni V., 2019, Aktivitas inhibitor α-amilase ekstrak etanol tanaman brotowali (Tinospora crispa L.), *Bioma: Berkala Ilmiah Biologi*, *21*(2), 91-99. https://doi.org/10.14710/bioma.21.2.91-99

[9] Martinez-Gonzalez AI., Díaz-Sánchez ÁG., De La Rosa LA., Bustos-Jaimes I., Alvarez-Parrilla EJSAPAM., 2019, Inhibition of α-amylase by flavonoids: Structure activity relationship (SAR), *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *206*, 437-447. https://doi.org/10.1016/j.saa.2018.08.057

[10] Direktorat Pengawasan Obat Tradisional. Parameter standar mutu ekstrak tumbuhan obat. Jakarta: Departemen Kesehatan RI; 2020, 13–33 p.

[11] Indrayanto G., 2018, Recent development of quality control methods for herbal derived drug preparations, *Natural Product Communications*, *13*(12),1934578X1801301208. https://doi.org/10.1177/1934578X1801301208

[12] Zhang QW., Lin LG., Ye WC., 2018, Techniques for extraction and isolation of natural products: A comprehensive review, *Chinese medicine*, *13*, 1-26.

[13] Kashtoh H., Baek KH., 2023, New insights into the latest advancement in α-amylase inhibitors of plant origin with anti-diabetic effects, *Plants*, *12*(16), 2944. https://doi.org/10.3390/plants12162944

[14] Guzmán GG., Garza BAA., Ríos RC., Minsky NW., Aranda RS., 2022, Assessment of α-amylase inhibition activity by an optimized and validated in vitro microscale method, *Química Nova*, *45*(9), 1146-1152.15. https://doi.org/10.21577/01004042.20170919

[15] Keharom S., Mahachai R., Chanthai S., 2016, The optimization study of α-amylase activity based on central composite design-response surface methodology by dinitrosalicylic acid method, *International Food Research Journal*, *23*(1).

[16] Melinda NA., Kusumo DW., Sari DIK., 2023, Aktivitas antidiabetes beberapa fraksi daun mimba (Azadirachta indica) secara in vitro berdasarkan penghambatan enzim α-Amilase, *Majalah Farmasi dan Farmakologi*, *27*(3), 82-87. https://doi.org/10.20956/mff.v27i3.28301

[17] Chelladurai GRM., Chinnachamy C., 2018, Alpha amylase and Alpha glucosidase inhibitory effects of aqueous stem extract of Salacia oblonga and its GC-MS analysis, *Brazilian Journal of Pharmaceutical Sciences*, *54*(01), e17151. https://doi.org/10.1590/s217597902018000117151

[18] Zhang J., Li C., Wang G., Cao J., Yang X., Liu, X., & Sun L., 2022, α-Amylase inhibition of a certain dietary polyphenol is predominantly affected by the concentration of α-1, 4-

glucosidic bonds in starchy and artificial substrates, *Food Research International*, *157*, 111210. https://doi.org/10.1016/j.foodres.2022.111210

[19] Barber E., Houghton MJ., & Williamson G., 2021, Flavonoids as human intestinal α-glucosidase inhibitors, *Foods*, *10*(8), 1939. https://doi.org/10.3390/foods10081939

[20] Elfahmi E., Santoso W., & Anggardiredja K., 2019, Uji Aktivitas Antidiabetes Produk Obat Herbal yang Mengandung Ekstrak Bratawali (Tinospora crispa (L.) Miers ex Hoff. f & Thoms.), *Jurnal sains farmasi & klinis*, *6*(3), 213-219. https://doi.org/10.25077/jsfk.6.3.213-219.2019

[21] Ogunyemi OM., Gyebi GA., Saheed A., Paul J., … Olaiya CO., 2022, Inhibition mechanism of alpha-amylase, a diabetes target, by a steroidal pregnane and pregnane glycosides derived from Gongronema latifolium Benth, *Frontiers in molecular biosciences*, *9*, 866719. https://doi.org/10.3389/fmolb.2022.866719

[22] Shehadeh MB., Suaifan GA., & Abu-Odeh AM., 2021, Plants secondary metabolites as blood glucose-lowering molecules, *Molecules*, *26*(14), 4333, 1-46. https://doi.org/10.3390/molecules26144333