Inhibitory Activity of Enzyme α-Glucosidase Ethanol Extract Combination of Mareme Plant (*Glochidion arborescens* (Müll. Arg.) Boerl.) and Leaves of the Sala Plant (*Cynometra ramiflora* Linn)

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Abstract

Diabetes Mellitus (DM) is still one of the world's major health problems, contributing to many complications leading to increased morbidity and mortality. Different approaches have been performed to enhance the patient's quality of life. Over the past decade, due to a trend in herbal medicines, numerous plants, including Mareme and Sala plants, have been claimed to be advantageous in alleviating DM features in pre-clinical research. Mareme and Sala leaves contain flavonoid compounds, which are potential as antidiabetic agents. This study aimed to determine the in vitro inhibitory activity of the ethanolic extracts of both plants on the α-glucosidase enzyme that plays a pivotal role in diabetic pathophysiology. The inhibition potential of the combination of Mareme and Sala leaf ethanol extracts and the positive control, namely acarbose, were measured using an ELISA reader at 450 nm based on the formation of p-nitrophenol. The ELISA reader measurements showed that the combination of Mareme leaves and Sala leaf ethanol extract could not inhibit α-glucosidase activity *in vitro*, indicated by the IC50 calculation of 374.47 ppm. Nevertheless, our research revealed the presence of diverse secondary metabolites, including flavonoids, in both extracts, which could potentially be responsible for further pharmacological activities.

Keywords: *Glochidion arborescens* (Müll. Arg.) Boerl., *Cynometra ramiflora Linn*, α- glucosidase, Diabetes Mellitus

Significance | Mareme and Sala leaves mix may be a strong blood sugar blocker, but it contains useful chemicals for potential medical research.

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Introduction

Diabetes Mellitus (DM) is a metabolic illness that is defined by insufficient insulin production and/or impaired insulin use within the body. Diagnosis of DM involves the observation of a notable elevation in blood glucose levels (Azis et al., 2020). The global prevalence of diabetes mellitus (DM) has exhibited a consistent upward trend across diverse nations, encompassing both high-income, low-income, and middle-income countries. According to data collected by the World Health Organization (WHO) since 1980, there has been a fourfold increase in the global population of people diagnosed with diabetes mellitus (DM), reaching a staggering 422 million individuals. According to the World Health Organization (2016), diabetes was responsible for around 1.5 million deaths out of a total of 89 million mortalities in the year 2012.

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According to the findings of the Sample Registration Survey conducted in 2014, it is evident that diabetes mellitus (DM) ranks as the third leading cause of mortality in Indonesia, accounting for 6.7% of deaths. This places DM behind stroke, which accounts for 21.1% of deaths, and coronary heart disease, responsible for 12.9% of deaths. According to Riska (2016), the International Diabetes Federation reported an estimated population of 10 million individuals diagnosed with diabetes mellitus in Indonesia in 2015. If this pattern persists, it may have adverse consequences for the human population, such as reduced productivity, disability, and early mortality.

Diabetes mellitus (DM) has the potential to give rise to numerous problems, hence elevating the likelihood of macrovascular and microvascular abnormalities in multiple organs, including the heart, kidneys, eyes, and brain. Disorders of the metabolism of carbohydrates may give rise to DM. α-glucosidase is the principal enzyme involved in the metabolism of carbohydrates. At the periphery of small intestinal cells, this enzyme facilitates the hydrolysis of complex oligosaccharides into monosaccharides or glucose, which are subsequently absorbed into the bloodstream (Chatsumpun, et al, 2017). Despite ongoing advancements in the scientific understanding of the biology of diabetes, current therapeutic interventions mostly focus on antihyperglycemic effects and do not effectively mitigate the development of diabetes-related comorbidities. A therapeutic strategy for managing diabetes mellitus involves the use of measures to impede the uptake of glucose into the bloodstream, namely by the inhibition of α-glucosidase enzyme activity (Garber, et al, 2013). Inhibition of this enzyme will have an impact on delaying glucose absorption (Khatri and Juvekar, 2014). One of the therapies to prevent diabetes is to inhibit the activity of the enzyme α-glucosidase, in this process there is an inhibition of glucose absorption into the flow. Therefore, it is imperative to investigate potential innovative antidiabetic compounds in order to improve the overall well-being of individuals with diabetes in the future. Numerous individuals suffering from chronic illnesses, including diabetes patients, hold the belief that herbal medications might serve as a complementary form of therapy to enhance their overall well-being. This underscores the need for additional investigation into the potential benefits of this therapeutic approach for such patients (Ghorbani, 2017).

In a study conducted by Indra in 2019, it was found that the ethanol extract derived from the leaves of the *Glochidion arborescens* (Müll. Arg.) Boerl. the plant showed promising qualities as a natural antioxidant. The extract demonstrated a total polyphenol content of 33.32 mg QE/g and a flavonoid content of 3.02 mg QE/g. The free radical reduction test results, specifically the DPPH assay, indicated an IC_{50} value of 5.62 µg/mL. This value was higher than that of vitamin C, which exhibited an IC50 value of 3.34 µg/mL. Furthermore, it has been demonstrated that the ethanol extract derived from the leaves of the Mareme plant exhibits significant antioxidant properties. Mareme fraction leaves also have activity that could inhibit an active α-amylase enzyme inhibitory activity with an IC_{50} value of 28.262 ppm (Ambarsari and Haryoto, 2022, Khan et al., 2006), and antioxidants (Bunyapraphatsara et al., 2003)

According to Rostiani (2019), the concentration of 1.60 ppm aligns with the findings of antidiabetic experiments conducted on male mice. These experiments demonstrated a significant reduction in blood glucose levels by 72.2% following a seven-day period of supplementation. According to Li et al. (2019), the potential antidiabetic benefits of flavonoid compounds can be attributed to their ability to enhance insulin production, modulate enzymes involved in glucose metabolism, improve insulin sensitivity, and facilitate glucose absorption by skeletal muscle and adipose tissue.

The plant often referred to as Sala, scientifically known as *Cynometra ramiflora* Linn, is frequently employed as a medicinal remedy for many ailments such as gout, diabetes, hypertension, and other medical conditions inside the Kraton Kasunanan Surakarta. The Sala plant is considered to be a plant species that is relatively uncommon. Therefore, there is a limited body of research on the pharmacological actions and chemical ingredients of this substance. The water extract (godogan) derived from the leaves and stems of the Sala plant is purported to possess curative properties for various ailments, based on empirical evidence.

The medical conditions encompassed in this list consist of hypertension, diabetes, gout, and hypercholesterolemia. Prior research has indicated that Cynometra ramiflora Linn plants originating from several nations had promising qualities as antidiabetic agents, antimicrobial agents (Khan et al., 2006), and antioxidants (Bunyapraphatsara et al., 2003). Consequently, it is imperative to examine the chemical composition, pharmacological properties, potential toxicity, and appropriate formulations of this plant to establish standardized herbal medications, thereby establishing a robust scientific basis (Haryoto et al., 2013). This study aimed to investigate the potential antidiabetic properties of the ethanol extract derived from the leaves of the Mareme plant (*Glochidion arborescens* (Müll. Arg.) Boerl.). The inhibitory activity of the leaves of the *Cynometra ramiflora* Linn plant against the α-glucosidase enzyme was assessed in vitro. Additionally, chemical ingredients were identified by the utilization of phytochemical screening methods.

Materials And Methods

Materials

The foliage of the Mareme plant (*Glochidion arborescens* (Müll. Arg.) Boerl.) was procured from the region of Ciamis. In contrast,

the leaves of the Sala plant (*Cynometra ramiflora* Linn.) were gathered in the vicinity of Kasunanan, the Palace Surakarta, located in Central Java, employs many chemical substances in its research and experiments. These substances include ethanol with a concentration of 96%, aquadest, pyrogen-free aquadest, dimethyl sulfoxide (DMSO), phosphate buffer with a pH value of 6.8, the enzyme α-glucosidase, p-nitrophenyl-D-glucopyranoxide (pNPG), acarbose, sodium carbonate (Na_2CO_3), concentrated sulfuric acid, and ferric chloride ($FeCl_3$).

Extraction

A total of 200 grams of powdered leaves from both plants were individually subjected to extraction using 96% ethanol in a round bottom flask. The solvent was replaced at four-hour intervals, while the extraction process was repeated three times.

The filtrate underwent concentration through the utilization of a rotary evaporator and subsequent vaporization in a water bath maintained at a temperature of 60°C, as described by Indra et al. (2019).

Phytochemical tests

The identification and recognition of steroids

The sample was solubilized in 0.5 mL of chloroform and combined with 0.5 mL of acetic acid anhydride. Subsequently, a volume of 2 mL of concentrated sulfuric acid was carefully introduced into the mixture by means of the tube's walls. The bluish-green color indicates the presence of sterols, whereas the presence of triterpenoids is suggested by the brown ring or violet coloration (Jones & Kinghorn, 2006; Evans, 2009).

The identification of tannins

A quantity of 1 gram of extract was introduced into a test tube. Subsequently, 10 mL of heated water was introduced into the tube. The solution was heated to its boiling point for 5 minutes, followed by adding approximately 3 to 4 drops of $FeCl_3$. The presence of tannin catechol is indicated by the production of a green-blue (green-black) color, whereas the presence of tannin pyrogallol and triterpenoids is suggested when the color turns blue-black (Jones & Kinghorn, 2006; Evans, 2009).

The identification and quantification of polyphenols

A quantity of 0.5 grams of powdered samples was dissolved in 10 milliliters of distilled water, heated, and subsequently filtered while still hot. $FeCl_3$ was added dropwise to the filtrate. According to Kachkoul et al. (2018), the filtrate will exhibit a blackish-blue or dark-green coloration due to the presence of polyphenols.

The identification and quantification of flavonoids

A test tube contained a quantity of leaf oil, namely 3-7 drops. Subsequently, a small quantity of concentrated sulfuric acid solution (H_2SO_4) was introduced into the test tube. The study documented variations in coloration, with the emergence of dark red or yellow pigmentation serving as an indicator of the existence of flavonoid chemicals (Harbone, 1987).

The identification of saponins

3-7 drops of leaf oil were introduced into a test tube, followed by adding 5 mL of water (H^2O). The combination was subjected to agitation for a duration of 30 seconds, during which the observation of foam development served as an indicator for the presence of saponins. The solution was thereafter allowed to equilibrate at ambient temperature briefly. The confirmation of saponin compounds was established if the foam lasted within the range of 1-10 cm, as stated by Harbone (1987).

The assay for inhibiting the α-glucosidase enzyme.

Preparation of the Sample Solution. A stock solution with a concentration of 1000 ppm was prepared by measuring 5 mg of an ethanol extract containing 96% ethanol from the leaves of *Glochidion arborescens* (Müll. Arg.) Boerl. Additionally, 5 mg of leaves from the *Cynometra ramiflora* Linn. plant was dissolved in DMSO to a final volume of 10 mL. The concentration series comprises five different concentrations, namely 50, 100, 150, 200, and 250 ppm, which are obtained from the stock solution in a sequential manner. The activity of the α-glucosidase enzyme to liberate pNPG, as the substrate, takes place at a temperature of 37°C under conditions of pH 6.8 (Nursalinda, et al., 2021). The activity of the α-glucosidase enzyme to liberate pNPG, as the substrate, takes place at a temperature of 37°C under conditions of pH 6.8 (Nursalinda, et al., 2021). The α-glucosidase enzyme inhibition experiment was conducted in accordance with the instructions provided by the kit's manufacturer, adhering to the specific reaction protocol outlined in Table 1. The measurement of absorbance for the solution was thereafter conducted at a wavelength of 450 nm using an ELISA reader.

The procedure for preparing an acarbose solution

Ten mg of acarbose powder was mixed into 10 mL DMSO. A 1000 ppm stock solution of acarbose was made. A viable resolution was achieved with serial dilution, wherein concentrations of 50, 100, 150, 200, and 250 ppm were utilized.

Data analysis

The data analysis was conducted by determining the percentage of α-glucosidase inhibition based on absorbance measurements. This was achieved by the utilization of the following equation:

$$\%Inhibition = \frac{Blank\ Absorbance - Sample\ Absorbance}{Blank\ \ Absorbance}\ x\ 100\ \%$$

The IC50 value was determined by employing a linear regression model, which involved plotting the sample concentration on the x-axis and the corresponding percentage of inhibition on the y-axis. The IC50 value was then calculated using the formula IC50 = (50 - a)/b according to Sugiwati et al. (2009).

Results and Discussion

The initial step in this project involved conducting phytochemical screening. Phytochemical screening serves as a preliminary step in identifying compound groups present in certain plant species

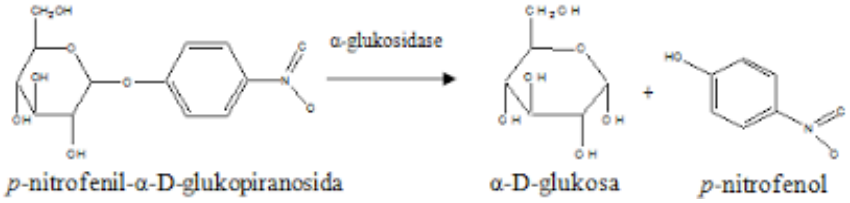

Figure 1. Enzyme α-glucosidase reactions and substrates

Table 1. α-glucosidase inhibition reaction system

	Blank (without extracts &; enzymes)	C (without extracts)	S_0 (without enzim with extract)	S_1 (enzim with extract)
Extract (µL)	-	-	5	5
DMSO (µL)	5	5	-	-
Buffer (µL)	5	5	5	5
Substrat	10	10	10	10
Incubation at 37°C for 5 minutes				
Buffer (µL)	5	-	5	-
Enzim (µL)	-	5	-	5
Incubation at 37°C for 5 minutes				
Na_2CO_3	175	175	175	175

Blanks = Reaction system without extracts and enzymes, C = Mixture without extract (Blank Control), S0 = Mixture without enzyme with extract (Sample), S1 = Mixture of enzymes and extracts (Sample Control)

Table 2. Phytochemical Screening of Mareme and Sala Plant Leaves

Extract	Targeted compound group	Reagent	Result	Interpretation
Mareme leaves	Steroids	Chloroform acetic acid anhydride, Concentrated sulfuric acid	Bluish green	+
	Tannins	$FeCl_3$	Black Green	+
	Polyphenols	Ethanol, $FeCl_3$	Green	+
	Flavonoids	Concentrated sulfuric acid	Reddish orange	++
	Saponins	Vigorous shaking	Froth Formed	+
Sala leaves	Steroids	Chloroform acetic acid anhydride, Concentrated sulfuric acid	Bluish green	+
	Tannins	$FeCl_3$	Black Green	+
	Polyphenols	Ethanol, $FeCl_3$	Green	+
	Flavonoids	Concentrated sulfuric acid	Redness	++
	Saponins	Vigorous shaking	Froth Formed	+

Table 3. The results of alpha-glucosidase enzyme inhibition assay

ACARBOSE	
Concentration (ppm)	IC_{50} (ppm)
50	14,17
100	
150	
200	
250	

ETHANOL EXTRACT COMBINATION OF MAREME AND SALA PLANT LEAVES				
Concentration (ppm)	**Ave. inhibition (%)**	**SD**	**Ave. IC_{50} (ppm)**	**SD**
50	58,00	38,11	374,47	181,10
100	52,67	16,50		
150	38,00	21,70		
200	45,33	19,73		
250	41,33	17,47		

*Ave= average, **Average of % inhibition and IC_{50} values were calculated from two replicates for acarbose and three replicates for combination extracts

(Dewatisari et al., 2018). Table 2 displays the outcomes of the phytochemical screening conducted on the leaves of Mareme and Sala.

The tube test method validated the presence of steroids, tannins, polyphenols, flavonoids, and saponins group compounds, utilizing different reagents as outlined in Table 2. Notably, the test for the flavonoids group exhibited the most pronounced alteration in color, suggesting a substantial concentration of flavonoid compounds in both plant specimens. The pharmacological activities attributed to flavonoid families encompass a range of effects, notably antioxidant, anti-inflammatory, and antidiabetic properties (Ullah et al., 2020).

The assay for inhibiting the α-glucosidase enzyme was conducted using combinations of Mareme and Sala leaf extracts at final concentrations of 50, 100, 150, 200, and 250 ppm. The positive control in this study was acarbose, a commonly prescribed antihyperglycemic medicine that functions as an α-glucosidase inhibitor. The positive control was assessed by conducting experiments on two reaction systems, one of which involved the reaction without the presence of a specific variable.

The extraction process involves obtaining a substance (S0) and subsequently subjecting it to a reaction using an extract (S1). The IC_{50} value was established in order to quantify the effectiveness of inhibition. This was achieved by employing a linear regression equation that relates the concentration of the extract (in parts per million) or acarbose (in parts per million) to the percentage of inhibition. The IC50 value represents the level at which 50% of enzyme inhibitory activity is observed (Meila & Purwandarie, 2017). Mohan et al. (2015) observed a significant suppression of the α-glucosidase enzyme, as indicated by the reduced IC_{50} value.

Table 3 shows that acarbose has an IC50 value of 14.17 ppm, showing a significant level of effectiveness in inhibiting α-glucosidase activity. The findings of the enzyme inhibition experiment are presented in Table 3. In the present study, the IC50 value of acarbose was determined to be 14.17 ppm, indicating a significant level of effectiveness in inhibiting α-glucosidase activity. In contrast, the co-administration of Mareme and Sala leaf extracts did not exhibit significant inhibitory effects compared to acarbose, as seen by the IC_{50} value of 374.47 ppm. According to the categorization of IC_{50} values, acarbose demonstrates activity as an antidiabetic agent, but the combination extract of Mareme and Sala leaves exhibits inactivity as an antidiabetic agent. According to Ariani et al. (2017), the results indicate that acarbose exhibits inhibitory potential against the enzyme α-glucosidase, as evidenced by its IC_{50} value. On the other hand, the combination of 96% ethanol extract of Mareme and Sala leaves has a relatively modest inhibitory potential against the same enzyme.

In this investigation, acarbose was employed to test its efficacy against conventional medication in managing diabetes mellitus. The mechanism by which acarbose exerts its effects involves the inhibition of the enzyme α-glucosidase, so preventing the hydrolysis of polysaccharides into glucose. This inhibition ultimately leads to a reduction in blood sugar levels.

According to the study conducted by Darmawi et al. (2015), The ethanol extract derived from the leaves of Mareme and Sala plants has been found to contain flavonoid chemicals, which have been previously identified as inhibitors of the alpha-glucosidase enzyme (Proenca et al., 2017). The inhibition of α-glucosidase enzymes leads to a subsequent delay in the absorption of carbohydrates, hence contributing to the reduction of blood glucose levels (Madduluri, 2013). According to Rostiani (2019), prior studies have indicated that Mareme leaves possess flavonoid compounds with a concentration of 3.02 mg QE/g. Similarly, Afrin et al. (2016) found that Sala leaves contain a higher concentration of flavonoid compounds, specifically 86.2 mg QE/g. Nevertheless, irrespective of the flavonoid composition, our research demonstrates that a combination of Mareme and Sala leaves extracted using 96% ethanol did not exhibit any inhibitory effects on α-glucosidase enzymes. It is noteworthy that previous research by Patel et al. (2012) has identified the leaves of Mareme and Sala as effective antidiabetic drugs, operating through an alternative mechanism of action by promoting the secretion of insulin. According to Nadilah et al. (2019), the lack of inhibition of the enzyme α-glucosidase suggests that the antidiabetic properties of the combination of Mareme Sala plant leaves extract may not be mediated through the enzymatic pathway.

In addition, various additional parameters can contribute to variations in IC_{50} values and α-glucosidase enzyme activity. These factors include temperature, enzyme reaction rate, substrate concentration, and pH levels (Champe et al., 2010).

The experiment for evaluating the inhibitory activity of the α-glucosidase enzyme involved the use of a positive control, acarbose, as well as 96% ethanol extract samples derived from a combination of Mareme and Sala leaves. This assay was designed to measure the hydrolysis reaction of the substrate, namely p-nitrophenyl-α-D-glucopyranoside (PNPG). The effectiveness of enzyme inhibition was assessed by measuring the absorbance value of p-nitrophenol, which served as a substrate. The intensity of the resulting yellow color was quantified using an ELISA reader set at a wavelength of 450 nm (Maryam et al., 2020). The enzyme α-glucosidase catalyzes the hydrolysis of p-nitrophenyl-α-D-glucopyranoside, forming p-nitrophenol and glucose, as depicted in Figure 1. The magnitude of the yellow hue generated is proportional to the quantity of glucose synthesized. According to Ariani et al. (2017), the presence of a vibrant yellow hue during the reaction signifies a substantial production of glucose, which suggests a diminished inhibition of the α-glucosidase enzyme.

Conclusion

The study's results indicate that the ethanolic extracts of Mareme and Sala leaves, when combined, yielded an IC_{50} value of 374.47 ppm. This value classifies the combination as an inactive inhibitor of α-glucosidase. However, the results of the phytochemical screening indicated that the ethanolic extracts of Mareme and Sala leaves exhibited the presence of steroids, tannins, polyphenols, flavonoids, and saponins. These findings have potential significance for future investigations into additional pharmacological properties.

Author Contributions

H. conceptualized, prepared the method, managed project and wrote. H., A. V. A. investigated, collected data, and prepared graphs. P. I., C. H. M. wrote, drafted, edited, prepared graphs and analysed data. K. H. Y. wrote, reviewed and edited the paper.

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Competing financial interests

The authors have no conflict of interest.

References

Ariani N.; Kartika I. Ratna and ; Kurniadewi F., 2017, Uji aktivitas inhibisi enzim α-glukosidase secara In vitro dari ekstrak metanol daun Cryptocarya densiflora Blume dan Fraksi-Fraksinya, Jurnal Riset Sains dan Kimia Terapan, 7 (1), 14-20. https://doi.org/10.21009/JRSKT.011.03

Azis W.A., Muriman L.Y. and Burhan S.R., 2020, Hubungan Tingkat Pengetahuan dengan Gaya Hidup Penderita Diabetes Mellitus, Jurnal Penelitian Perawat Profesional, 2 (1), 105-114. https://doi.org/10.37287/jppp.v2i1.52

Ambarsari, N, and Haryoto, 2022, The Inhibitory Effect of α-amylase Enzyme of Fraction and Isolate of Mareme Leaves (Glochidion arborescens Blume), Journal of Smart Science and Technology, 2(2), 32-39, https://doi.org/10.24191/jsst.v2i2.31

Awin T, Mediani A, Faudzi SMM, Maulidiani, Leong SW, Shaari K, et al. Identification of α-glucosidase inhibitory compounds from Curcuma mangga

fractions. International Journal of Food Properties. 2020;23(1):154–66. doi:

10.1080/10942912.2020.1716792. Bunyapraphatsara N., Jutiviboonsuk A., Sornlek P., Therathanathorn W., Aksornkaew S., Fong H.H.S., Pezzuto J.M. and Kosmeder J., 2003, Pharmacological studies of plants in the mangrove forest, Thai Journal of Phytopharmacy, 10 (2), 1-12.

Champe P., Harvey R. and Ferrier D., 2010, Lippincott's Illustrated Reviews: Biochemistry, Wolker Kluwer, London.

Chatsumpun N, Sritularak B, Likhitwitayawuid K. , 2017, New biflavonoids with α-glucosidase and pancreatic lipase inhibitory activities from Boesenbergia rotunda. Molecules. 2017 Okt 30;22(11):1862. doi: 10.3390/molecules22111862.

Darmawi A.R., Saleh C. and Kartika R., 2015, Aktivitas antihiperglikemik dari ekstrak etanol dan Ary Rizki Darmawi, Chairul Saleh, Rudi Kartika, Jurnal Kimia Mulawarman, 12 (2), 59-63.

Dewatisari W.F., Rumiyanti L. and Rakhmawati I., 2018, Rendemen dan Skrining Fitokimia pada Ekstrak Daun Sanseviera sp., Jurnal Penelitian Pertanian Terapan, 17 (3), 197. https://doi.org/10.25181/jppt.v17i3.336

Evans, C.W., 2009, Pharmacognosy Trease and Evans, 16th Ed. London: Saunders Elvesier.

Garber AJ, Abrahamson MJ, Barzilay JI, Blonde L, Bloomgarden ZT, Bush MA, et al., 2013, AACE comprehensive diabetes management algorithm 2013. Endocrine Practice. 2013;19(2):327-36. doi: 10.4158/endp.19.2.a38267720403k242.

Harbone, J. B., 1987, Metode Fitokimia: Penentuan Cara Modern Menganalisa Tumbuhan, Terbitan Kedua, Terjemahan: Kosasih Padmawinata dan Iwang Soediro, ITB: Bandung

Haryoto, Muhtadi, Indrayudha P., Azizah T., Suhendi A. and Haryoto, Muhtadi, Peni Indrayudha, Tanti Azizah A.S., 2013, Aktivitas Sitotoksik Ekstrak Etanol Tumbuhan Sala (Cynometra ramiflora Linn) Terhadap Sel HeLa, T47D dan WiDR, Journal Penelitian Saintek, 18, 21-28.

Indra ; Nurmalasari, N.; Kusmiati, M; (2019) 'Fenolik Total , Kandungan Flavonoid , dan Aktivitas Antioksidan Ekstrak Etanol Daun tumbuhan Mareme (Glochidion arborescens Blume .)', Jurnal Sains Farmasi & Klinis, (22), pp. 206-212. https://doi.org/10.25077/jsfk.6.3.206-212.2019

Jones, W.P. dan Kinghorn, A.D., 2006, Extraction of plant secondary metabolites, In: Sarker, S.D., Latif, Z. dan Gray, A.I., eds. Natural Products Isolation. 2nd Ed. New Jersey: Humana Press. https://doi.org/10.1385/1-59259-955-9:323

Kachkoul R., Sqalli Houssaini T., El Habbani R., Miyah Y., Mohim M. and Lahrichi A., 2018, Phytochemical screening and inhibitory activity of oxalocalcic crystallization of Arbutus unedo L. leaves, Heliyon, 4 (12), e01011. https://doi.org/10.1016/j.heliyon.2018.e01011.

Khatri DK, Juvekar AR. 2014, α-glucosidase and α-amylase inhibitory activity of Indigofera cordi-folia seeds and leaves extract. Int J Pharm Pharm Sci. 2014;6(11):152-5

Li Jian, Bai L., Li X., He L., Zheng Y., Lu H., Li Jinqi, Zhong L., Tong R., Jiang Z. and Shi J., 2019, Antidiabetic potential of flavonoids from traditional Chinese medicine: A review, American Journal of Chinese Medicine, 47 (5), 933-957. https://doi.org/10.1142/S0192415X19500496

Maryam S.M., Suhaenah A. and Amrullah N.F., 2020, Uji aktivitas penghambatan enzim α-glukosidase ekstrak etanol biji buah alpukat sangrai (Persea americana Mill.) secara in vitro, Jurnal Ilmiah As-Syifaa, 12 (1), 51-56. https://doi.org/10.33096/ja.v12i1.619

Meila O. and Purwandarie D., 2017, Uji Aktivitas Antidiabetes Dari Ekstrak Etanol 70% Buah Kiwi (Actinidia deliciosa), Jurnal Farmagazine, IV (1), 19-27.

Meutia, Z. Z., & Dewi, B. E. (2017). Effects of C. ramiflora Linn. leaf extract against dengue virus replication in vitro on Huh7it-1 cell. Advanced Science Letters, 23(7), 6834-6837. https://doi.org/10.1166/asl.2017.9411

Nadilah W., Wan Adibah, Ali A.M., Nur W., Wan Amalina, Hasima N., Campus B., Iman T.D., Campus B. and Iman T.D., 2019, Aquilaria malaccensis, 10 (1), 36-45.

Nursalinda, Haryoto, Peni Indrayudha, 2021, Inhibition of Alpha-Glucosidase Enzyme by Neem Leaf (Azadirachta indica) and Mango Ginger (Curcuma mangga), Jurnal Kefarmasian Indonesia, 2021:11(1):56-64, https://doi.org/10.22435/jki.v11i1.3950

Patel D.K., Prasad S.K., Kumar R. and Hemalatha S., 2012, An overview on antidiabetic medicinal plants having insulin mimetic property, Asian Pacific Journal of Tropical Biomedicine, 2 (4), 320-330. http://dx.doi.org/10.1016/S2221-1691(12)60032-X.

Sabiha, S., Serrano, R., Hasan, K., da Silva, IBM., Rocha, J., Islam, N., Silva, O., 2022, The Genus Cynometra: A Review of Ethnomedicine, Chemical, and Biological Data, Plants, 11 (24), 3504. https://doi.org/10.3390/plants11243504

Sugiwati S., Setiasih S. and Efy Afifah dan, 2009, Antihyperglycemic Activity of The Mahkota Dewa [Phaleria macrocarpa (Scheff.) Boerl.] leaf extracts as an alpha-glucosidase inhibitor, Makara Kesehatan, 13 (2), 74-78. https://doi.org/10.7454/msk.v13i2.364

Tjay TH, Rahardja K.,2007, Obat-obat penting khasiat, penggunaan, dan efek-efek sampingnya. Edisi 6. Jakarta: PT Elex Media Komputindo Gramedia; 2007.

Yusuf, A .,Dewi, B. and Sjatha, F. 2018, Antiviral Activity of Cynometra ramiflora Linn Leaves Extract Against Replication of Dengue Virus Serotype 2 on Huh7.5Cell In Vitro, Proceedings of BROMO Conference (BROMO 2018), pages198-201, DOI:10.5220/0008359901980201