ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Activity of Gaharu (Aquilaria malaccensis Lam) Leaves Extract and Fraction as Herbal Drug Against α-Glucosidase Inhibition

Aris Suhardiman*[1], Ketut Adnyana I.[2], Ika Kurnia Sukamawati[1], Titin Rostinawati[3]

[1]Faculty of Pharmacy, Universitas Bhakti Kencana, Soekarno Hatta Street No. 754 Bandung, West Java- 40614 Indonesia
[2]Pharmacy School, Bandung Institute Technology, Ganesha Street No. 10. Lb. Siliwangi, Coblong District, Bandung City, West Java 40132, Indonesia
[3]Non akademisi, The Pharmacist of the Pharmacy Fita Farma, Pangeran Santri Street No. 62, South Sumedang District Sumedang Regency West Java, Indonesia

Article History:

Received on: 15 Sep 2021
Revised on: 20 Oct 2021
Accepted on: 03 Nov 2021

Keywords:

Agarwood leaves (Aquilaria malaccensis Lam),
Diabetes mellitus,
IC50 value,
alpha-glucosidase enzyme inhibitors

ABSTRACT

Diabetes mellitus in 2020 in the world was 422 million people, and 1,6 million deaths are directly attributed to diabetes each year. Diabetes mellitus is a disease characterized by increased levels of glucose in the blood due to impaired function of the pancreas gland in producing the hormone insulin. One of the mechanisms of action of the antidiabetic drug class is α-glucosidase enzyme inhibitors. Compounds that play a role in the inhibitory activity of the α-glucosidase enzyme from plant extracts are flavonoids. Agarwood (Aquilaria malaccensis Lam) plants contain secondary metabolites such as alkaloids, flavonoids, saponins, quinones, tannins and steroids/triterpenoids. Gaharu leaves were extracted by maceration method using 96% ethanol solvent, then the extract obtained was fractionated using a liquid-liquid extraction method with n-hexane, ethylacetate and methanol-water as solvents. The extracts and fractions obtained were tested for inhibition of the α-glucosidase enzyme as measured by a microplate reader at a wavelength of 405 nm and acarbose as a comparison. The inhibitory activity of the α-glucosidase enzyme is expressed by the IC^{50} value. The inhibitory activity of α-glucosidase enzyme from extracts and fractions of n-hexane, ethylacetate and methanol-water were 90,87 μg/ml; 908,53 μg/ml; 89,78 μg/ml and 35,89 μg/ml and acarbose 19,08 μg/ml. The methanol-water fraction showed a very strong inhibitory activity against the α-glucosidase enzyme.

*Corresponding Author

Name: Aris Suhardiman
Phone: +6285220247902
Email: aris.suhardiman@bku.ac.id

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v13i1.17

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

According to WHO related to diabetes mellitus in the world, in 2020 about 422 million people worldwide have diabetes, the majority living in low and middle-income countries and 1,6 million deaths are directly attributed to diabetes each year [1]. Diabetes mellitus consists of two types, the first type is diabetes mellitus which is caused by heredity and the second type is caused by lifestyle. In general, the prevalence of type 2 diabetes mellitus is 80%, this is due to an unhealthy lifestyle which is the main trigger for the increasing prevalence of diabetes mellitus.

One class of antidiabetic drugs is an inhibitor of the

glucosidase enzyme in the digestive tract with the mechanism of action of slowing down the absorption of glucose in the small intestine so that it has the effect of lowering blood glucose levels. [2]

α-glucosidase enzyme inhibitors can be obtained naturally from plants. One of the plants that can be developed as traditional medicine in inhibiting the α-glucosidase enzyme is the gaharu plant (*Aquilaria malaccensis* Lam). The use of gaharu plants traditionally has wide use in the world of health as painkillers, toothaches, rheumatism drugs and poison repellents. The purpose of this study was to test the α-glucosidase enzyme inhibitors from the extracts and fractions of gaharu (*Aquilaria malaccensis* Lam) leaves.

The extracts of *A.malaccensis* in different solvent polarities and drying method showed variable α-glucosidase inhibitory activities. The highest α-glucosidase inhibition percentage was exhibited by the Air Dry Methanol and Oven Dry Methanol extracts with 75,84% and 71,61%. [3]

The results of the study will provide an overview of the inhibition of the α-glucosidase enzyme in vitro, which is then measured the absorbance results using UV-Vis Spectrophotometry at a wavelength of 405 nm with a comparison of acarbose.

METHODOLOGY

Materials

The tools used in the research are beaker glass (Pyrex®), measuring cup (Pyrex), mortar and stamper, the crucible, evaporating dish, test tube (Pyrex®), spatel, stirring rod, label paper, filter paper, analytical balance, oven, micropipette (Dragon Lab®), Eppendorf, maceration apparatus, separating funnel, rotary vaporator (Butchi Rotavapor R-100®), UV Lamp (Spectroline®), 96

Wella microplate, (iwaki®), microplate reader (Thermo Scientific®), Sonication (Elamasonil®), pH meter (Mettler Toledo®) and Incubator (memert®).

The materials used in this study were gaharu (*Aquilaria malaccensis* Lam) leaves obtained from PT. Mitra Dulur Sejahtera, Traditional Medicine Factory (Herbal), Palembang, South Sumatra. The solvent used with technical grade is 96% ethanol, n-hexane, ethylacetate solvent. Acarbose (TCI Chemicals®), α-glucosidase enzyme (Sigma-Aldrich®), pnitrophenyl-α-D- glucopyranoside (p-NPG) substrate (Sigma-Aldrich®), Sodium hydroxide (NaOH), Chloroform, $FeCl^3$, Dragendorff reagent (bismuth III nitrate, nitric acid, potassium iodide) Mayer's reagent (Mercury III chloride, potassium iodide) Liebermann-Burchard reagent (Acetic anhydrous, concentrated sulfuric acid), 10% HCl, 10% H_2SO_4, Mg powder, amyl alcohol, 5% $AlCl_3$, micropipette tip, sodium carbonate, phosphate buffer, and aqua dest.

Determination of Agarwood Leaves

Agarwood leaves were obtained from Palembang and were determined by the Indonesian Institute of Science (LIPI Bogor) by certificate number SK:2438 / IPH.1.01 / II.07 / XI / 2018. Sample According to Taxonomic Hierarchy Serial No: 845890, *Aquilaria malaccensis* Lam. [4]

Simplicia Characterization

The Simplicia characterization was carried out on the examination of the determination of the total ash content, the determination of the acid-insoluble ash content, the determination of the water-soluble extract content, the determination of the ethanol-soluble extract content and the determination of drying shrinkage. [5]

Determination of total ash content

Put 2 grams of gaharu leaf simplicia powder into a silicate crucible that has been ignited and grounded. Then incandescent slowly until the charcoal runs out, cooled and weighed. Then the filtrate is put into a crucible, evaporated and incandescent until the weight remains, the ash content was calculated in %w/w of the weight of the agarwood leaf simplicia powder used. [5].

Determination of acid-insoluble ash content

Boiled agarwood leaf simplicia ash was obtained from the determination of the total ash content with 25 ml of dilute H_2SO_4 for 5 minutes. The acid-insoluble part was collected by filtering through a glass crucible or using ash-free filter paper, washed with hot water, ignited to a constant weight and weighed. The acid-insoluble ash content was calculated in % w/w against the weight of the agarwood leaf simplicia powder used. [5].

Determination of ethanol-soluble extract content

Macerated 5 grams of dried gaharu leaf simplicia powder for 24 hours with 100 ml of 96% ethanol using a stoppered flask while shaking many times for the first 6 hours and then allowed to stand for 18 hours. The macerate was filtered quickly by avoiding the evaporation of ethanol, then 20 ml of the filtrate was taken to be evaporated to dryness in a shallow, flat bottomed dish that had been tared. Drying was carried out at a temperature of 105ºC until the weight was constant.

Calculated levels in percent of compound soluble in ethanol 96% of the initial weight of gaharu leaf simplicia. [5].

Determination of water-soluble extract content

Macerated 5 grams of dried gaharu leaf simplicia powder for 24 hours with 100 ml of chloroform saturated water using a corked flask while shaking many times for the first 6 hours and then allowed to stand for 18 hours. The macerate is filtered and then 20 ml of the filtrate is taken to be evaporated to dryness in a shallow, flat-bottomed dish that has been leveled. Drying was carried out at a temperature of 105ºC until the weight was constant. Calculated levels in percent of water-soluble compounds to the initial weight of agarwood leaf simplicia. [5]

Determination of drying shrinkage

Put 2 grams of gaharu leaf simplicia powder into the *Moisture balance* tool using a container lined with aluminum foil that has been tarred and then the drying shrinkage level is measured at a temperature of 105ºC until the tool shows a constant number. [5]

PHYTOCHEMICAL SCREENING

Phytochemical screening is one of the medicinal plants in research to determine the class of compounds contained in gaharu leaves. Phytochemical screening carried out included examination of alkaloids, flavonoids, saponins, tannins, quinonens, steroids and triterpenoids. [6]

Alkaloid Test

Gaharu leaf extract of 10 mg was added with 5 ml of 25% ammonia and then 20 ml of chloroform was added. The mixture is filtered to obtain a water layer and an organic layer. The water layer is added with 2 drops of Dragendorff's reagent or Mayer's reagent. If a white precipitate is formed with the addition of Mayer's reagent, it means that the gaharu leaf extract contains alkaloids. [7]

Flavonoid Test

Gaharu leaf extract was added 0,1 mg magnesium powder and 4 ml amyl alcohol (30% HCl and 90% ethanol mixture with the same volume) and 4 ml alcohol then the mixture was shaken. A positive result is indicated by a red, yellow or orange color on the amyl alcohol layer. [7]

Saponin Test

2 grams of gaharu leaf extract is dissolved in hot water and 1 drop of 2N HCl is added and then shaken vigorously. Saponins will produce a stable foam visible for 5 minutes and will not disappear will showing a positive result of saponins in gaharu leaf extract. [7].

Quinone test

Boil 1 gram of gaharu leaf extract in 100 ml of hot water for 5 minutes and filter 5 ml of the filtrate then add 2 drops of 1N NaOH solution. The formation of red color indicates the presence of quinone [7].

Tannin Test

Gaharu leaf extract 2 grams dissolved in 100 ml of hot water, boiled for 5 minutes and filtered 5 ml of the filtrate then reacted with 10% $FeCl_3$ solution, if dark blue or greenish-black color occurs indicates positive tannin. Then 5 ml of the filtrate was added with a gelatin solution, if A white precipitate was formed, which indicated the presence of tannin. Furthermore, 5 ml of another filtrate is added with Setasny reagent (1 part formaldehyde; 2 part HCl) and heated in a water bath if a pink precipitate forms indicating the presence of catechate tannins. The precipitate was filtered, then the filtrate was saturated with sodium acetate and added $FeCl_3$. If a black-blue color is formed, it indicates the presence of faulty tannins. [7].

Steroid/triterpenoid test

Macerated 1 gram of gaahru leaf extract with 20 ml of ether for 2 hours, then filtered. 5 ml of filtrate was evaporated in an evaporating cup. Three drops of Liebermann-Burchard reagent was added to the residue. If a red-purple color is formed, it indicates the presence of triterpenoids and if a blue-green color is formed, it indicates the presence of steroids. [7].

Extraction

The gaharu leaf simplicia powder was weighed and then soaked in 96% ethanol for 3x24 hours with a ratio of 1:10, every 1x24 hours replaced with a new solvent then filtered. The maserate is collected and concentrated with a rotary vaporator. The concentrated extract obtained was weighed, and the extract yield was calculated. [8].

Fractionation

The ethanol extract obtained and had been concentrated was then fractionated using a liquid-liquid extraction method with n-hexane, ethylacetate and methanol-water as solvents, respectively. The first sequence of fractionation using n-hexane solvent, and each fraction was extracted 3 times. The results of the n-hexane fractionation were carried out with ethylacetate solvent and carried out 3 times. Then the results of the fractionation of h-hexane, ethylacetate and methanol-water were collected. The obtained fraction is concentrated with a rotary vaporator.

Extraction and Fractionation Monitoring

Gaharu leaf extract and fraction were monitored using thin-layer chromatography with silica gel F254 as stationary phase and polar mobil phase namely ethylacetate:methanol: water (8:1:1), semi-polar Chloroform: Methanol (9:1) mobile phase, and non-polar mobil phase n-hexane: ethyl acetate (9:1). [9]

α-Glucosidase Enzyme Inhibition Activity Test

Gaharu leaf extract and fraction were tested for α-glucosidase enzyme inhibitory activity, including optimization of enzyme concentration, sample testing, calculation of the percentage of α-glucosidase enzyme inhibitory activity and calculation of IC^{50}, and using acarbose as a comparison. [10].

The substrate used in the study was p-NPG (p-nitrofenil-α-D-glukopiranosida), which is hydrolyzed by α-glucosidase to release p-nitrophenol, a color agent that can be monitored at 405 nm. The enzyme inhibition activity for α-glucosidase was assessed according to the methods. [11]

Testing the inhibitory activity of the α-glucosidase enzyme by extracts and fractions of gaharu leaves using spectrophotometric methods in vitro and measuring absorbance at a wavelength of 405 nm. The activity test was carried out on several sample concentrations, namely from a dilution of 1000 μg/ml. For the concentration of ethanol extract of gaharu leaves 70-120 μg/ml, methanol fraction:water 30-50 μg/ml, ethylacetate fraction 50-110 μg/ml and n-hexane fraction 400-800 μg/ml and acarbose 1020 μg/ml.

Acarbose are competing for α-glucosidase inhibitors with enzymes from the small intestine that break down complex carbohydrates. These drugs inhibit the absorption of carbohydrates and reduce postprandial blood glucose concentration but there are side effects on gastrointestinal including flatulence (42%-74%), abdominal discomfort (12%-19%) so that user has been restricted. [12]

RESULTS AND DISCUSSION

Simplicia characterization

The purpose of simplicia characterization is to ensure the quality of the simplicia used. The results of simplicia characterization can be seen in Table 1.

Determination of total ash content aims to provide an overview of the internal and external mineral content of agarwood leaf simplicia. In addition to the total ash content, the determination of the acid-insoluble ash content is also carried out, which aims to provide an overview of the external mineral content in the material. A small acid insoluble ash content indicates the least impurities such as sand or silicates in the simplicia [13].

Table 1: Simplicia characterization results and extract density

Characterization Test	Results (% b/b)
Total Ash Content	3,92
Acid Insoluble Ash Content	1,17
Water Soluble Level	8,95
Ethanol Soluble Extract Level	9,27
Drying Loss	9.85

Table 2: Gaharu Leaf Fraction Yield Results

Fraksi	Bobot (g)	Rendemen (%)
n-hexane	40,52	20,26
Ethylacetate	34,92	12,46
Methanol: water	120,53	60,27

Determination of water-soluble and ethanol-soluble extracts was carried out with the aim of knowing the initial description of the amount of content of a compound that is soluble in organic solvents and non-organic solvents. The results of the determination of the sari content showed that the simplicia of gaharu leaves was more soluble in ethanol.

Determination of drying shrinkage is carried out to provide a maximum limit for the amount of compound that is lost or evaporates at a set temperature of 105º C. [14]

Phytochemical Screening

Phytochemical screening was carried out to determine the secondary metabolite compounds contained in simplicia. The results of phytochemical screening showed positive results in all classes of compounds tested so that gaharu leaves contain secondary metabolites consisting of alkaloids, flavonoids, saponins, quinones, tannins and steroids/triterpenoids. This shows that gaharu leaves can have activity against the inhibition of the α-glucosidase enzyme because it has flavonoid compounds.

Extraction

One of the quality parameters of the extract is the yield of the resulting extract. Yield is the ratio between the extract obtained and the initial simplicia. The higher the yield value, the higher the extracted value.

The results of the extraction process carried out

obtained a thick extract of 2693,3 g, while the yield of gaharu leaf extract obtained was 55,11%. (Figure 1, Figure 2 and Figure 3)

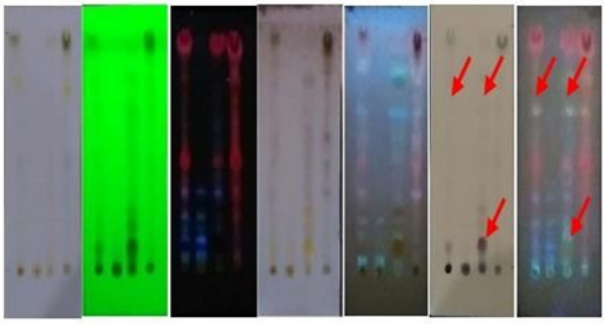

Figure 1: TLC of extracts and fractions by using n-hexane: ethyl acetate (9:1, v/v) as mobile phase

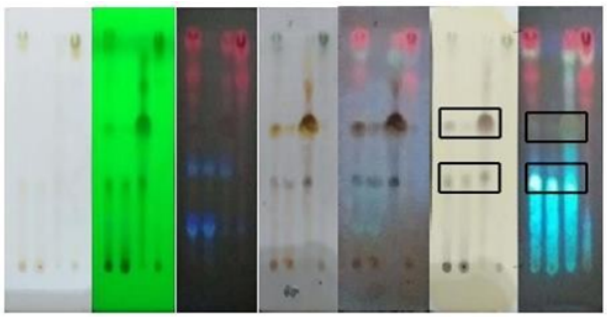

Figure 2: TLC of extracts and fractions by using chloroform: methanol (9:1, v/v) as mobile phase

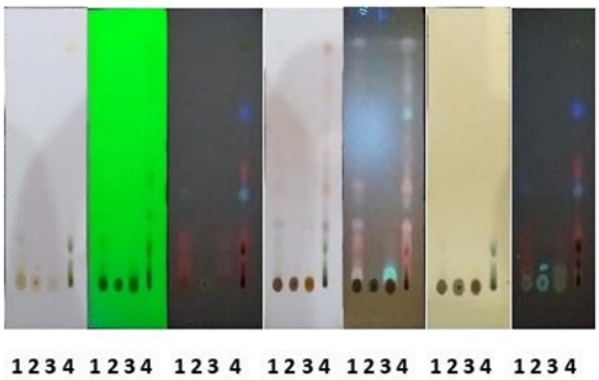

Figure 3: TLC of extracts and fractions by using ethyl acetate: methanol:water (8:1:1, v/v) as mobile phase

Fractionation

Fractionation is the process of withdrawing a compound using two solvents of different polarity. Fractionation was carried out using a liquid-liquid extraction method with n-hexane, ethylacetate and methanol: water as solvents. Gaharu leaf fraction yield results can be seen in Table 2.

The results of the obtained fraction yields, many compounds in gaharu leaves are dissolved in the solvent methanol: water so that many compounds are dissolved, such as flavonoid compounds. Flavonoid compounds have a role in the inhibitory activity of the α-glucosidase enzyme.

Extract and Fraction Monitoring

Monitoring of extracts and fractions was carried out to see the compounds contained in the extracts and fractions qualitatively using the TLC (Thin Layer Chromatography) and mobile phase methods.

Monitoring of the extract using thin-layer chromatography showed that with 10% H_2SO_4 spots, it would produce black spots after heating. Yellow fluorescence after spraying with $AlCl_3$ reagent indicated the presence of flavonoid compounds in the extract and fraction of gaharu leaves. There are also spots that fluoresce in green and blue under 366 nm UV lamp after spraying with $AlCl_3$ spots. With the appearance of 10% $FeCl_3$ spots, black spots appeared on a yellow background which indicated that the extract and fraction had phenol group compounds. From the results of the monitoring of extract and fractions, it shows that gaharu leaves have flavonoid compounds where both of these compounds have a role in the activity of α-glucosidase enzyme inhibition. The results of monitoring extracts and fractions can be seen in the Figure 3.

Testing of α-Glucosidase Enzyme Inhibitory Activity

Inhibition of α-glucosidase enzymes causes inhibition of glucose absorption in patients with diabetes mellitus. Testing the inhibitory activity of α-glucosidase aims to determine the inhibitory activity of α-glucosidase by extracts and fractions of gaharu leaves and acarbose as a comparison. [15]

The bioassay α-glucosidase inhibitor was employed in this investigation to direct the discovery of bioactive compounds. Leaf Extract *A.sinensis*, petroleum ether fraction, ethanol fraction, n- butanol fraction, water-soluble fraction were evaluated for their α-glucosidase inhibitory activity. The ethanol fraction showed very strong inhibitory effects against α-glucosidase. [16]

Prior to testing, optimization of the α-glucosidase enzyme was carried out to obtain the optimal concentration. The results of the enzyme concentration of 0,4 U/ml α-glucosidase were the most optimum, and the substrate concentration used was 15 mM which resulted in absorbance of 0,797. The results

of testing the inhibitory activity of the α-glucosidase enzyme from the extracts and fractions of gaharu leaves and acarbose as a comparison can be seen in Figure 4.

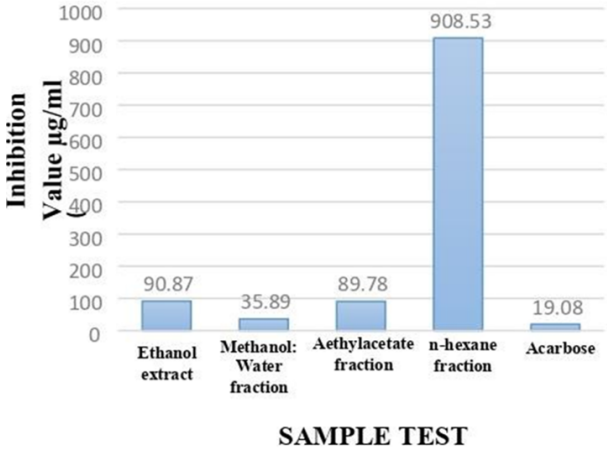

Figure 4: Effect of extracts on inhibition of cancer cell growth (MCF-7)

The results of testing the inhibitory activity of the α-glucosidase enzyme are indicated by the IC^{50} value, the smaller the IC^{50} value means the greater the inhibitory power of the α- glucosidase enzyme sample. The test results from the standard, namely acarbose, showed a high inhibitory ability, and the test sample that gave the best inhibitory power was the methanol: water fraction with an IC^{50} value of 35,89 μg/ml.

Based on the results of phytochemical screening and monitoring of extracts and fractions, it showed the presence of flavonoid compounds in the extracts and fractions. The presence of a class of flavonoid compounds plays a role in the inhibitory activity of the α-glucosidase enzyme. Good inhibition of the methanol: water fraction is thought to contain higher flavonoid compounds because many flavonoids are dissolved compared to the extract, ethylacetate fraction and n-hexane fraction. [17].

CONCLUSION

The results showed that there was an inhibitory activity of the alpha glucoside enzyme by the extract and fraction of gaharu leaves. The best results from the test sample was the methanol: water fraction with an IC^{50} value of 35,89 μg/ml and the compounds that played a role were flavonoid compounds. So that agarwood leaves have the potential for the development of herbal medicines in the treatment of diabetes mellitus.

Thank You Note

This research was assisted by research funds from the Ministry of education, culture, research and technology in the Inter-University Cooperation Research (PKPT) scheme. Thank you also to the research team as follows Prof. I Ketut Adnyana, Ika Kurnia Sukmawati and Titin Rostinawati non academics and Bhakti Kencana University and the Bandung Institute Technology.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding Support

The authors declare that they have no funding support for this study.

REFERENCES

[1] WHO. Diabetes Mellitus, 2020. Health Topics. World Health Organization. Accessed on September 10, 2021.

[2] IDF. Diabetes Atlas 8th Edition, 2017. International Diabetes Federation.

[3] Wan Nadilah et al. Evaluation of DPPH Free Radical Scavenging, alpha-glucosidase inhibitory, and antimicrobial activities of Aquilaria malaccensis leaf extracts. *J.Agrobiotech*, 10(1):36–45, 2019.

[4] Gerald Guala and Markus Döring. Integrated taxonomic information system (ITIS) Taxonomic Hierarchy, 2019. Aquilaria malaccensis. Accessed on 18 July 2019.

[5] A J Harborne. Phytochemical Methods a Guide to Modern Techniques of Plant Analysis, springer science and business media. page 302, 1988. ISBN: 978-0-412-57270-8.

[6] N Adelina et al. Aquilaria malaccensis Lam", Seed Leaflet. Forest and Landscape Denmark (103). *The University of Copenhagen*, pages 1–3, 2004.

[7] N R Farnsworth. Biological and phytochemical screening of plants. *Journal of pharmaceutical sciences*, 55(3):225–276, 1966.

[8] Indonesian Health Technology Assessment Committee. Ministry of Health of the Republic of Indonesia. Indonesian Herbal Pharmacopoeia edition II. *Health Technology Assessment (HTA)*, 2017.

[9] A Suhardiman et al. Cytotoxic Test of Fraction of Gaharu Leaves (Aquilaria malaccensis) on Cervic Cancer Cells (Hela Cells) Using MTT Assay Method. *Int. J. Res. Pharm. Sci*, 12(2):1624–1631, 2021.

[10] S Kumar et al. 'α-glucosidase inhibitors from

plants: A natural approach to treat diabetes. *Pharmacognosy Reviews*, 5(9):19–29, 2011.

[11] M Deutschlander et al. Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes. *Journal of Ethnopharmacology*, 124(3):619–624, 2009.

[12] M A C Burns, T L Schwinghammer, B G Wells, P M Malone, J M Kolesar, and J T Dipiro. Pharmacotherapy: Principles and Practise, 4th Edition. New York, 2016. Mc Graw Hill Education. ISBN 978-0-07-183502-2.

[13] Khan Saikia. Aquilaria malaccensis Lam., a Red-listed and highly exploited tree species in the Assamese home garden. *Current Science*, 102(4):546–547, 2012.

[14] L.P.A Oyen and Dung Nguyen Xuan. Plants Resources of South-East Asia. No. 19 Essential Oils Plants. In *Plants Resources of South-East Asia. No. 19 Essential Oils Plants*, pages 64–67, 1999. Backhuys Publishers, Leiden the Netherlands. ISBN : 90-5782-010-2.

[15] J T Dipiro, G Barbara Well, L Terry Schwinghammer, and Cecily V Dipiro. Pharmacotherapy Handbook, 9th Edition. New York, 2015. McGraw-Hill Education. ISBN: 978-0-07-182129-2.

[16] J Feng, X W Yang, and R F Wang. Bioassay-guided isolation and identification of α- glucosidase inhibitors from the leaves of Aquilaria sinensis. *Phytochemistry*, 72(2-3):242–247, 2011.

[17] Goutam Brahmachari. Bio-Flavonoids with Promising Antidiabetic Potentials: A Critical Survey. *Research Signpost Opportunity, Challenge and Scope Of Natural Products in Medicinal Chemistry*, pages 187–212, 2011. ISBN:978-81-308-0448-4.