Vol. 13(3): 849-864, September 2025 DOI: https://doi.org/10.23960/jsl.v13i3.1197

Jurnal Sylva Lestari

P-ISSN: 2339-0913
E-ISSN: 2549-5747

Journal homepage: https://sylvalestari.fp.unila.ac.id

Full Length Research Article

Antidiabetic Activities of Agarwood (*Aquilaria malaccensis*) Leaf Extracts via Enhanced Insulin Secretion in BRIN-BD11 Pancreatic Beta-Cells

Yanico Hadi Prayogo[1, 2], Rita Kartika Sari[1, 2, *], Zikri Hamidi[1], Wasrin Syafii[1], Eva Harlina[3], Bayu Febram Prasetyo[3]

[1] Department of Forest Products, Faculty of Forestry and Environment, IPB University, Bogor, Indonesia
[2] Tropical Biopharmaca Research Center, IPB University, Bogor, Indonesia
[3] Department of Clinical, Reproductive, and Pathology, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia
[*] Corresponding Author. E-mail address: rita_kartikasari@apps.ipb.ac.id

ARTICLE HISTORY:

Received: 30 May 2025
Peer review completed: 29 July 2025
Received in revised form: 26 August 2025
Accepted: 22 September 2025

KEYWORDS:

Agarwood
Alpha-glucosidase
Insulinotropic
Molecular docking

ABSTRACT

Agarwood leaves (*Aquilaria malaccensis*), a non-timber forest products, shows significant potential as a source of antidiabetic compounds. This study aims to evaluate the antidiabetic activity of agarwood leaves and their cytotoxicity on pancreatic beta-cells, as well as predict their compound bioactivity through an in silico approach. The leaves were extracted using ethanol, water, and a mixture of ethanol–water with the assistance of ultrasound irradiation. The extracts were then tested in vitro for their antidiabetic potential by assessing their ability to inhibit the α-glucosidase enzyme and their effect on insulin secretion, as well as their cytotoxicity on BRIN-BD11 pancreatic beta-cells. The phytocompounds in the extract were identified using liquid chromatography-mass spectrometry, and their binding behavior was studied by in silico molecular docking. Among the three, the ethanol–water extract showed the highest extraction yield. Cytotoxicity assays revealed that the ethanol–water extract was cytotoxic at high concentrations (1000 µg/mL), but safe at lower concentrations. The alpha-glucosidase inhibition was relatively weak. Nevertheless, the extracts significantly stimulated insulin secretion in BRIN-BD11 cells up to fivefold compared to untreated cells. In silico studies indicated that xanthone glycoside, flavonoid glycoside, and coumarin compounds exhibit strong binding affinities to multiple insulin-secretion-related proteins. These findings suggest that agarwood leaf extract, particularly ethanol–water extract, possesses promising antidiabetic activity through an insulinotropic mechanism.

1. Introduction

Diabetes mellitus is a chronic degenerative disease that poses a major health problem in Indonesia and is a priority in the management of non-communicable diseases. Indonesia ranks fifth in the world in terms of the number of diabetes mellitus cases, with 20.4 million cases in 2024, and this figure is projected to increase to 28.6 million by 2050 for the population aged 20–79 years (IDF 2025). Diabetes mellitus is characterized by hyperglycemia (blood glucose levels exceeding normal levels) due to insulin deficiency caused by β-pancreatic cell damage (type 1) or insulin resistance and impaired insulin secretion due to β-pancreatic cell dysfunction (type 2).

Diabetes mellitus (DM) can lead to tissue and organ damage, as well as complications affecting the eyes, kidneys, nervous system, and blood vessels due to metabolic dysfunction at the cellular level. Long-term diabetes mellitus complications include macrovascular complications (coronary heart disease, peripheral vascular disease, and stroke), microvascular complications (nephropathy, retinopathy, and neuropathy), and a combination of both (diabetic foot) (Hardianto et al. 2020). The complex and costly management of diabetes mellitus has resulted in an increasing economic burden on the Indonesian government, with healthcare costs rising by 4.9% (Adiarso et al. 2020).

Currently, type 1 DM is treated with insulin injections. In contrast, type 2 DM is managed with oral medications containing synthetic active ingredients from several drug classes, including sulfonylureas (glibenclamide), meglitinides (repaglinide), biguanides (metformin), and thiazolidinediones (rosiglitazone), which work by increasing insulin secretion and sensitivity. Additionally, α-glucosidase inhibitors (acarbose and miglitol) are used to slow down starch digestion in the small intestine, delaying carbohydrate absorption into the bloodstream and reducing blood glucose spikes. However, these drugs have side effects such as hypoglycemia and the risk of kidney and liver damage (Hardianto et al. 2020). Furthermore, approximately 95% of active pharmaceutical ingredients in Indonesia, including DM drugs, are imported (Adiarso et al. 2020). Therefore, the utilization of natural antidiabetic compounds from Indonesia's abundant plant resources has great potential to reduce dependency on imported drugs and minimize the side effects of synthetic medications (Prajapati et al. 2022).

One of the natural resources with potential as a natural source of antidiabetics is the agarwood tree. The agarwood tree (*Aquilaria malaccensis*) is a highly valuable plant, with the highest-quality wood priced at IDR 5–10 million/kg and the lowest-quality wood at around IDR 500,000/kg (Hidayat et al. 2020). The increasing demand for agarwood has led to its cultivation, including the utilization of agarwood leaves for tea production. In vitro studies have shown that the ethanol extract from agarwood leaves has a half-maximal inhibitory concentration (IC_{50}) value of 90.87 µg/ml against the α-glucosidase enzyme, while the methanol–water fraction exhibits stronger antidiabetic activity with an IC_{50} of 35.89 µg/ml (Suhardiman et al. 2022). In vivo studies have demonstrated that administering 500 mg/kg body weight (BW) of methanol extract from agarwood leaves to rats reduced blood glucose levels by 57.1% without causing toxicity (Zulkifle 2018). Additionally, kombucha derived from agarwood leaves at a dosage of 1 mL/20 g BW in mice showed antidiabetic effects comparable to those of the commercial drug glibenclamide (Khoerunnisa et al. 2023). The aqueous fraction of ethanol extract from agarwood leaves at a dose of 10 mg/kg BW in rats also enhanced glucose uptake by increasing glucose transporter type 4 expression in muscle tissue by 24.5% (Said et al. 2016).

Although studies have shown the potential of agarwood leaves in lowering blood glucose levels, the active compounds in methanol extracts of agarwood leaves and their underlying mechanisms remain largely unexplored. While the ethanol extract exhibits lower activity, it is considered more promising due to the use of a safer solvent. Therefore, this study aims to investigate the effects of extraction methods and solvent variations on the antidiabetic activity and cytotoxicity of BRIN-BD11 pancreatic β-cells. The BRIN-BD11 pancreatic β-cell line is a hybrid cell line generated through the electrofusion of a primary culture of NEDH rat pancreatic islets with RINm5F cells, which are derived from an NEDH rat insulinoma (McClenaghan et al. 1996). This cell line is suitable for investigating the antidiabetic activities related to the effects of various treatments on pancreatic insulin secretion, as pancreatic β-cells are responsible for secreting insulin.

Additionally, the mechanisms by which phytochemical compounds exert antidiabetic activity are also predicted through an in silico approach.

2. Materials and Methods

2.1. Extraction

Agarwood leaves were dried and pulverized to a particle size of 40–60 mesh. The resulting powder was first immersed in *n*-hexane to remove lipophilic extractives. After immersion in *n*-hexane, the powder was subjected to separate extractions with ethanol, 50% ethanol, and water. The extraction process employed the ultrasound-assisted extraction (UAE) method, with an extraction duration of 45 minutes (Familasari et al. 2023). The powder-to-solvent ratio was maintained at 1:10. Immersion was repeated three times for each solvent type. The resulting filtrates from the separate extractions with ethanol, 50% ethanol (ethanol:water), and water were concentrated, and the extracts were weighed to determine the extraction yield.

2.2. α-glucosidase Inhibition Assay

The evaluation of α-glucosidase inhibition activity was conducted through an in vitro enzymatic reaction, following established research protocols. Acarbose was used as the positive control. A concentration series of the extract was used, including 6.25, 12.5, 25, 50, and 100 µg/mL. For the assay, 50 µL of 0.1 M phosphate buffer (pH 6.8), 25 µL of 10 mM p-NPG substrate solution, and 10 µL of the sample solution were added to a 96-well microplate. The mixture was incubated at 37°C for 5 minutes. Next, 25 µL of α-glucosidase enzyme solution (0.4 U/mL in 0.1 M phosphate buffer, pH 7) was added to the wells, followed by a 30-minute incubation at 37°C. To terminate the reaction, 100 µL of 0.2 M Na_2CO_3 was added, and the absorbance was measured at 410 nm. The assay was performed on both fractionated and crude extracts, with each test conducted in triplicate.

2.3. Cytotoxicity Assay

The cytotoxicity of the leaf extracts was assessed spectrophotometrically using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The cell line used in this study was BRIN-BD11, a pancreatic β-cell line. Cell cultures were seeded into a 96-well microplate with a final volume of 100 µL and incubated for 24 hours. The extract samples, dissolved in the medium at concentrations of 10, 100, and 1000 µg/mL, were then added separately to the wells containing the cultured cells and incubated for 48 hours. After incubation, 10 µL of MTT solution (2000 mg/L) was added, followed by another 4-hour incubation at 37°C. Subsequently, 100 µL of 70% ethanol was added to each well, and the absorbance was measured at 565 nm using a microplate reader. The use of three different test concentrations was intended to illustrate the effects of the extract treatment at low (10 µg/mL), medium (100 µg/mL), and high (1000 µg/mL) concentrations.

2.4. BRIN-BD11 Cell Secreted Insulin Quantification

The insulin-stimulating effect of agarwood leaf extracts was tested on BRIN-BD11 cells. About 150×10^5 cells were cultured in bottles and incubated at 37°C with 5% CO_2 for 24 hours. Then, the cells were transferred to 96-well plates, with 5000 cells per well in RPMI 1640 medium. After 24 hours, the cells were expected to attach to the bottom of the wells. The wells were rinsed

three times with 1 mL of modified Krebs-Ringer Buffer (KRB-1), which contains various salts, glucose (1.11 mM), and was gassed with 5% CO_2 and 95% O_2 for 15 minutes. Before treatment, the cells were pre-incubated for 40 minutes with 1 mL of KRB-1 at 37°C and 5% CO_2. To test the extract's effect, cells were incubated for 60 minutes under the same conditions using 1 mL of agarwood leaf extract at concentrations of 100 µg/mL, dissolved in KRB-3 (a version of the buffer with 16.7 mM glucose) and KRB-1. After treatment, the media was collected, centrifuged at 158 g for 5 minutes, and insulin levels were measured using a Finetest ELISA kit (INS Rat cat#ER113). Absorbance was read at 450 nm using a BioRad Microplate Reader (model 3550). The insulin standard curve was prepared using the same method.

2.5. Phytochemical Analysis using Liquid Chromatography Mass Spectrometry (LC-MS)

Metabolite profiling using LC-MS was conducted according to the protocol by Prayogo et al. (2023a), with certain modifications. The analysis was performed using a Vanquish LC system coupled with a Q Exactive Plus Orbitrap HRMS (Thermo Scientific, USA). Three *A. malaccensis* extracts were prepared by dissolving them in LC-MS grade methanol (Merck, Germany) and filtering through a 0.2 µm polytetrafluoroethylene membrane. The resulting filtrates were then analyzed via liquid chromatography. Separation was carried out using an Accucore C18 column (100 × 2.1 mm, 1.5 µm; ThermoScientific) maintained at 30°C. A gradient elution program was employed as follows: 0–1 min (5% B), 1–25 min (5–95% B), 25–28 min (95% B), and 28–30 min (5% B). The mobile phases consisted of water with 0.1% formic acid (eluent A) and acetonitrile with 0.1% formic acid (eluent B), delivered at a flow rate of 0.2 mL/min. Mass spectrometric detection was performed using electrospray ionization in positive (M+H), with a capillary voltage of 3.80 kV and temperature of 320°C. Data were acquired at a resolution of 70,000 across an m/z range of 150–1500, with a mass accuracy tolerance of 10 ppm. Compound identification and annotation were performed using Compound Discoverer 3.1 (Thermo Scientific, USA).

2.6. In-silico Molecular Docking Study

Four target proteins were chosen due to their relevance to insulin secretion activity in pancreatic β-cells (**Table 1**). Before conducting docking simulations with the metabolites, the receptor structures were obtained from the Protein Data Bank (PDB, https://www.rcsb.org/), with selection based on the quality of their resolution. To ensure reliability, validation was performed by redocking the original co-crystallized ligands into their respective receptors (Kontoyianni et al. 2004). Protein preparation was performed using AutoDockTools 1.5.7. The docking process was deemed valid when the root mean square deviation (RMSD) between the docked and original ligand was below 2 Å (Prayogo et al. 2024; Wati et al. 2020).

The validated receptor structures were subsequently employed to evaluate the binding interactions of phytochemicals present in the extract. Molecular structures of the compounds identified via LC-MS were retrieved from databases such as PubChem and ChemSpider. These structures were further refined through geometry optimization using the Newton-Raphson algorithm based on molecular mechanics, executed via the Open Babel/Obabel software (O'Boyle et al. 2011). Molecular docking was performed using the AutoDock Vina algorithm (Eberhardt et al. 2021), with each compound docked in 10 replicates. The resulting docking scores were ranked, and the top-performing compounds were further examined using PyMOL version 3.1 and LigPlot version 2.28.

Table 1. The protein target (receptor) for molecular docking analysis

Proteins: PDB code	Binding coordinate (x, y, z)	RMSD(Å)	Physiological role
Glucagon-like peptide-1 receptor (GLP-1R): 6X19	130.657, 111.088, 86.069	0.2804	It modulates insulin secretion in response to glucose levels, suppresses pancreatic exocrine activity, and helps protect pancreatic β-cells from apoptosis (Kusunoki et al. 2024; Zhang et al. 2020)
Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3Kα): 7TZ7	27.475, 9.505, 4.166	0.4273	It is involved in transmitting insulin signals, promoting glucose absorption, and supporting cell survival and growth (Fairhurst et al. 2022).
Protein kinase RNA-like endoplasmic reticulum kinase (PERK):4X7L	3.925, -9.793, 6.889	0.2028	It manages the endoplasmic reticulum stress response by phosphorylating eIF2α, thereby regulating protein synthesis and promoting cell viability (Le et al. 2025; Smith et al. 2015).
Insulin receptor kinase (IRK): 4IBM	-2.453, 9.724, -19.914	0.3582	It enhances the insulin signaling cascade, improving glucose uptake, energy metabolism, and maintaining glucose balance in the body (Anastassiadis et al. 2013).

3. Results and Discussion

3.1. Extraction Yield and Cytotoxicity on BRIN-BD11 β-pancreatic Cell

Extraction of agarwood leaves produced varying yields. The D-E50 extract resulted in the highest yield and was significantly different from ethanol and water extract (**Fig. 1a**). Meanwhile, the water extract had the lowest yield, indicating that the extraction yield trend for agarwood leaves was leaf ethanol–water extract (LEW) > leaf ethanol extract (LE) > leaf water extract (LW). This difference in yield is attributed to the use of different extraction solvents, which influence the ability to extract various types or classes of compounds present in agarwood leaves. The 1:1 ethanol and water mixture acts as a solvent with the capability to extract compounds with a broader range of polarities. Water is highly polar, while ethanol has lower polarity compared to water. Based on the yield trend, the higher extraction yield of LE compared to LW suggests that relatively low-polarity compounds likely dominate the compounds in agarwood leaves. Methanol extracts of *Aquilaria malaccensis* leaves contain flavonoids, phenolics, triterpenoids, and steroids, while the methanol–water fraction contains flavonoids, triterpenoids, and phenolics (Hidayah et al. 2023). Consistent with the findings of this study, extracts obtained using polar solvents tend to contain compounds of similar polarity. For example, the methanol extract of *A. auriculiformis* wood was dominated by flavonoid compounds (Prayogo et al. 2023b). However, the types of compounds present in the extract are also influenced by the specific plant part or plant species itself (Andianto et al. 2024).

Concentration-dependent inhibition was observed in the cytotoxicity of the extracts against BRIN-BD11 cells. The inhibitory effect became more pronounced as the concentration increased from 10 to 1000 µg/mL (**Fig. 1b**). In general, the extracts exhibited less than 50% inhibition of cell proliferation, except for the LEW extract at a concentration of 1000 µg/mL. Based on linear regression analysis, the IC_{50} value of LEW was determined to be 663.98 µg/mL, whereas the IC_{50} values of LE and LW were greater than 1000 µg/mL. These findings indicate that LEW possesses higher cytotoxic potential compared to LE and LW. At 10 µg/mL, the LW extract even showed a negative inhibition value (-7.85%), which may suggest stimulation of cell proliferation or experimental variability. Its effect became significant only at 1000 µg/mL (28.78%). The trend at 1000 µg/mL was: LEW > LW > LE, suggesting that ethanol–water extracts contain more cytotoxic or antiproliferative compounds compared to ethanol-only or water-only extracts. This quantitative

data was also supported by cell morphology, as observed in the micrographs. Visual reduction in cell density was seen, indicated by the decreased presence of purple-stained cells (**Fig. 1c**).

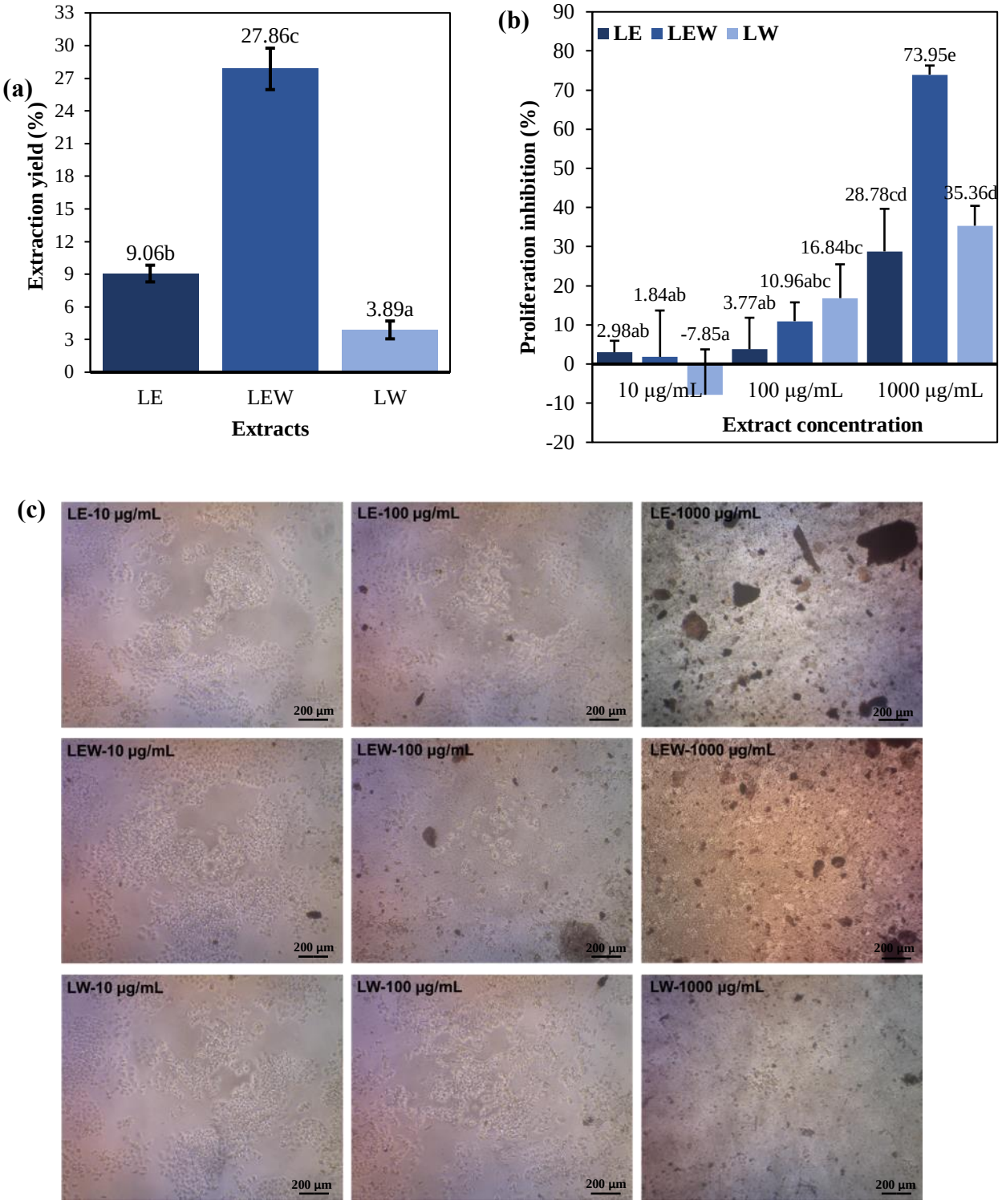

Fig. 1. Extraction yield (a), cytotoxicity against BRIN-BD11 cells (b), and micrographs showing BRIN-BD11 cell morphology (c) treated with agarwood leaf extract at concentrations of 10, 100, and 1000 µg/mL. Values followed by different letters indicate significant differences based on Duncan's multiple range test ($p < 0.05$).

3.2. Agarwood Leaf Extract Treatment Effect on α-glucosidase Enzyme and BRIN-BD11 Cell Insulin Secretion

The antidiabetic activity, attributed to α-glucosidase enzyme inhibition, demonstrated a dose-dependent inhibitory effect, with a positive correlation between increasing concentration and increased inhibitory activity (**Fig. 2**). The LE extract exhibited the highest inhibitory activity compared to the LEW and LW extracts. At most concentrations (particularly from 25 to 100 µg/mL), the LE extract consistently showed the strongest inhibition, reaching up to 15.579% at 100 µg/mL. Meanwhile, LEW and LW showed similar inhibition levels, with LEW demonstrating higher inhibition than LW at concentrations of 12.5, 25, and 50 µg/mL, but showing lower inhibition than LW at 100 µg/mL. The trend of LE > LEW > LW suggests that ethanol-based solvents (especially pure ethanol) are more effective in extracting bioactive compounds responsible for α-glucosidase inhibition compared to water or ethanol–water mixtures.

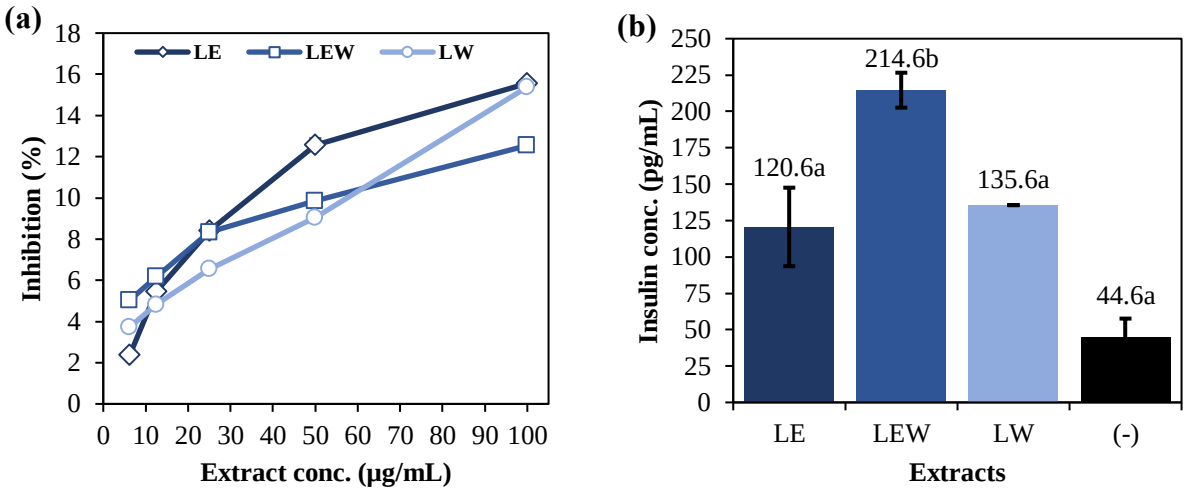

Fig. 2. α-Glucosidase enzyme inhibition (a) and insulin secretion levels (b) in BRIN-BD11 cells treated with extracts at a concentration of 100 µg/mL and negative control (-). Values followed by different letters indicate significant differences based on Duncan's multiple range test ($p < 0.05$).

The antidiabetic activity of the extracts, based on α-glucosidase enzyme inhibition, showed relatively low potential. All extracts exhibited inhibition values of less than 50% at the highest test concentration used. This indicates that the IC_{50} values for all extracts are greater than 100 µg/mL. Ethanol and ethanol–water extracts are often known to contain bioactive compounds, such as phenolic acids, flavonoids, tannins, and polyphenols, which are associated with their antidiabetic and antioxidant properties (Akyüz 2022; Karagecili et al. 2023). However, in this study, even though polar solvents like ethanol and water were used for extraction, the IC_{50} values exceeding 100 µg/mL suggest that the active compound content may not be sufficiently potent or may require higher concentrations to achieve significant inhibition. Optimizing the extraction method in ultrasound-assisted extraction by varying the solvent composition, amplitude, and extraction time is effective in enhancing antidiabetic activity (Maser et al. 2024). Additionally, synthesized thiazolidinediones with various substitutions at C3 and C5 show promising activity in inhibiting α-glucosidase and α-amylase, as well as reducing glucose uptake by yeast cells (Gharge et al. 2024). This confirms that structural modification of bioactive compounds may help improve their α-glucosidase inhibitory activity. Furthermore, agarwood leaf extract is likely to contain antidiabetic bioactive constituents that warrant isolation and evaluation in their purified form. A similar

phenomenon has been reported for mangiferin, which, when isolated from both unripe and ripe mango pulp, exhibited markedly stronger inhibitory activity against α-glucosidase and α-amylase than the crude extract (Sekar et al. 2019). In line with this, Mistry et al. (2023) demonstrated that administration of mangiferin as a single compound elicited more pronounced antidiabetic effects compared to the corresponding extract in alloxan-induced diabetic rats.

The α-glucosidase enzyme inhibition values indicate that the antidiabetic activity of *A. malaccensis* extract does not occur through the mechanism of α-glucosidase enzyme inhibition, but rather through another mechanism, namely the stimulation of insulin secretion. BRIN-BD11 cells treated with agarwood leaf extract showed an increase in insulin secretion compared to the negative control (**Fig. 2b**). The extract treatment concentration used was 100 µg/mL, with low cytotoxicity levels based on **Fig. 1**. An increase in insulin secretion was observed following treatment with all types of extracts (**Fig. 2b**). The order of insulin secretion concentration from highest to lowest was: LEW > LW ≈ LE > Control. Treatment with the LW and LE extracts resulted in insulin concentration increases of 304% and 270%, respectively, compared to the control. Meanwhile, LEW showed remarkable potential, with an increase in insulin concentration of up to 481% compared to the control. To date, there have been no previous studies reporting the potential of agarwood leaf extract to increase secreted insulin concentration in BRIN-BD11 pancreatic β-cells.

3.3. Phytochemical Profile of Agarwood Leaf Extracts

The choice of extraction solvents results in distinct phytochemical profiles, as evidenced by the chromatograms of the three agarwood leaf extracts (LE, LEW, and LW), which share overall pattern similarities but exhibit differences in peak heights at specific retention times (**Fig. 3**). In the early retention time (0–2.5 minutes), peak heights followed the trend LE > LEW > LW, suggesting LE's superior efficiency for extracting polar or low-molecular-weight compounds. Mid-retention times (5–10 and 15–20 minutes) showed variations in intermediate-polarity compounds, reflecting solvent selectivity, while the late retention times (27.5–30 minutes) revealed differences in less polar compounds, likely influenced by solvent properties such as polarity and viscosity.

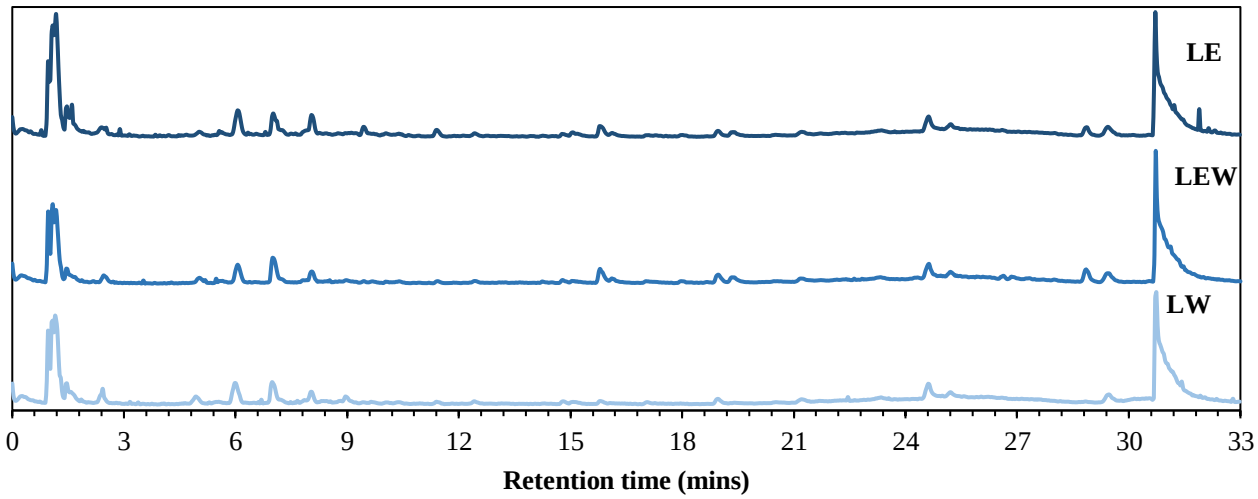

Fig. 3. Total ion chromatogram of agarwood leaf extracts.

Glycosides and carboxylic acids dominate agarwood leaf extracts. The compound 1,5-anhydro-1-(1,3,6,7-tetrahydroxy-9-oxo-9H-xanthen-2-yl)hexitol was identified as the most abundant

compound in the LEW extract (**Table 2**). Meanwhile, D-(–)-quinic acid was the predominant compound in both LE and LW extracts. Other identified glycosides include khelloside, with a relative abundance range of 7.5–8.7%, and glycitein, which ranges from 1.8% to 9.6%. Several other compounds detected belong to the short- and long-chain carboxylic acid (fatty acid) groups. Rudiana et al. (2021) reported different compounds compared to this study, identifying several phytocompounds in fractions of *A. malaccensis* bark extract using LC-MS, namely acetaminophen, aminosamptothesin, isophorone, diethyl phthalate, 3-hydroxy-6H-benzochromen-6-one, isolongifolenic acid, 2-(2-phenethyl)chromone, 4′,7-dihydroxy-2′,5-dimethoxyisoflavone, 4-hydroxy-3-(1-phenylpropyl)-2H-chromone-2-one, 6-methylchromone-2-carboxylic acid, 3,4-dimethyl-2-phenylmorpholine, and β-calacoren. Moreover, to date, there have been no other reports on the phytocompounds of leaf extracts.

Table 2. Phytocompounds of agarwood leaf extracts

RT (min)	Identified compound	Class	Mass error (ppm)	Relative abundance (%)		
				LE	LEW	LW
7.05	1,5-anhydro-1-(1,3,6,7-tetrahydroxy-9-oxo-9H-xanthen-2-yl)hexitol (ATX)	Xanthone glycoside	-2.46	8.4	14.1	9.9
1.16	D-(–)-Quinic acid	Carboxylic acid	-6.00	11.8	10.2	17.3
15.83	Glycitein	Flavonoid glycoside	-3.07	6.4	9.6	1.8
6.08	Khelloside	Coumarin	-2.73	8.7	7.7	7.5
1.25	Citric acid	Carboxylic acid	-5.61	1.7	4.7	9.8
1.22	Glucoheptonic Acid	Carboxylic acid	-3.99	1.4	1.2	2.3
1.22	DL-Malic acid	Carboxylic acid	-9.08	3.4	4.3	1.1
1.13	1-O-acetyl-α-maltose	Carbohydrate	-2.59	2.3	1.8	2.5
12.45	(10E,15Z)-9,12,13-Trihydroxy-10,15-octadecadienoic acid	Fatty acid	-2.38	1.5	1.1	1.2

Note: RT= retention time.

3.4. Bioactivity Mechanism Prediction through In Silico-Molecular Docking

Molecular docking analysis revealed that ATX, glycitein, and khelloside exhibited good binding affinities. Among them, ATX and khelloside demonstrated the strongest binding affinities toward insulin receptor kinase (IRK) and glucagon-like peptide-1 receptor (GLP-1R), suggesting their potential as promising candidates for targeting insulin signaling pathways (**Table 3**). Glycitein exhibited the highest binding affinity toward phosphoinositide 3-kinase alpha (PI3Kα) and protein kinase R-like endoplasmic reticulum kinase (PERK), suggesting its potential as a significant inhibitor in these pathways. Additionally, both ATX and glycitein displayed strong interactions with PI3Kα and PERK. When compared with the binding affinity of commercial drugs, the phytocompounds exhibited stronger interactions with the PI3Kα and PERK proteins. Glycitein showed a higher binding affinity than glimepiride and glibenclamide against PI3Kα and PERK. Meanwhile, ATX, glycitein, and khelloside demonstrated higher binding affinities than glimepiride and glibenclamide against PERK. Overall, khelloside, ATX, and glycitein demonstrated consistently strong binding across GLP-1R, PI3Kα, PERK, and IRK, suggesting their potential for broad-spectrum antidiabetic activity.

Table 3. Binding affinity of agarwood leaf extract phytocompounds

Compound name	Binding affinity (kcal/mol)			
	GLP-1R	**PI3Kα**	**PERK**	**IRK**
ATX	**-7.696**	**-7.655**	-8.364	**-8.580**
D-(−)-Quinic acid	-5.271	-5.215	-5.571	-5.292
Glycitein	-7.628	**-8.755**	**-8.820**	-7.785
Khelloside	**-7.915**	-7.612	**-8.711**	**-8.101**
Citric acid	-4.967	-5.145	-5.334	-5.521
Glucoheptonic Acid	-4.768	-5.135	-5.311	-4.851
DL-Malic acid	-4.160	-4.716	-4.482	-5.135
1-O-acetyl-α-maltose	-6.284	-5.940	-6.968	-6.640
(10E,15Z)-9,12,13-Trihydroxy-10,15-octadecadienoic acid	-7.055	-6.017	-7.114	-5.914
Glimepiride*	-9.860	-8.195	-6.716	-8.861
Glibenclamide*	-9.708	-7.775	-7.367	-8.644

Notes: Bolded numbers represent the top two highest binding affinities; commercial antidiabetic drug (*).

Compounds such as citric acid, DL-malic acid, and D-(−)-quinic acid, which have relatively high abundance in several extracts, also show fairly good binding affinity values of −4.967, −4.16, and −5.271 kcal/mol, respectively. The combination of high abundance and the ability to interact with molecular targets supports their potential as antidiabetic candidates. Additionally, the measured ligand efficiency reveals how effectively these compounds interact at the molecular level, despite the torsion values indicating varying degrees of molecular flexibility. This factor is important in the design of structure-based drug candidates. Among the three best-docked compounds, glycitein exhibited the highest ligand efficiency (**Table 4**). The same phenomenon was also observed when compared with glibenclamide and glimepiride. This indicates that the atoms within the glycitein ligand contribute more effectively to the binding energy.

Table 4. Ligand efficiency of agarwood leaf extract phytocompounds

Compound name	Ligand efficiency (kcal/mol)			
	GLP-1R	**PI3Kα**	**PERK**	**IRK**
ATX	-0.2566	-0.2539	-0.2566	-0.2860
D-(−)-Quinic acid	-0.4048	-0.4008	-0.4048	-0.4072
Glycitein	-0.3637	-0.4170	-0.3637	-0.3710
Khelloside	-0.2734	-0.2594	-0.2734	-0.2794
Citric acid	-0.3824	-0.3961	-0.3824	-0.4302
Glucoheptonic Acid	-0.3967	-0.4282	-0.3967	-0.4046
DL-Malic acid	-0.4609	-0.5248	-0.4609	-0.5708
1-O-acetyl-α-maltose	-0.2432	-0.2394	-0.2432	-0.2444
(10E,15Z)-9,12,13-Trihydroxy-10,15-octadecadienoic acid	-0.3054	-0.2594	-0.3054	-0.2486
Glimepiride	-0.2900	-0.2410	-0.1975	-0.2606
Glibenclamide	-0.2942	-0.2356	-0.2232	-0.2620

Previous studies support these findings; D-(–)-Quinic acid has been reported to inhibit the α-glucosidase enzyme and impede nonenzymatic glycosylation and glucose absorption in Caco-2 cells, which plays a role in blood sugar regulation (Han et al. 2024). Moreover, citric acid has been shown to treat glucose metabolism disorders induced by hyperlipidemia by improving insulin sensitivity (Yadikar et al. 2022). The combination of relative abundance and molecular affinity data suggests that agarwood leaf extract has potential as a source of active compounds for developing natural compound-based therapies for diabetes.

Strong binding affinity is related to the types of interactions formed between the ligand (phytocompound) and the receptor. Khelloside interacts with the GLP-1R receptor (**Fig. 4a1**) without forming hydrogen bonds, whereas the ATX–GLP-1R interaction involves hydrogen bonding (**Fig. 4a2**). However, the khelloside–GLP-1R complex exhibits stronger binding affinity, which is likely due to the greater number of van der Waals interactions formed. A similar phenomenon is observed in the ligand–IRK receptor interaction (**Fig. 4d1** and **4d2**). Meanwhile, the influence of π-interaction quantity on binding affinity is seen in ligand interactions with the PI3Kα and PERK receptors (**Fig. 4b1**, **4b2**, **4c1**, and **4c2**).

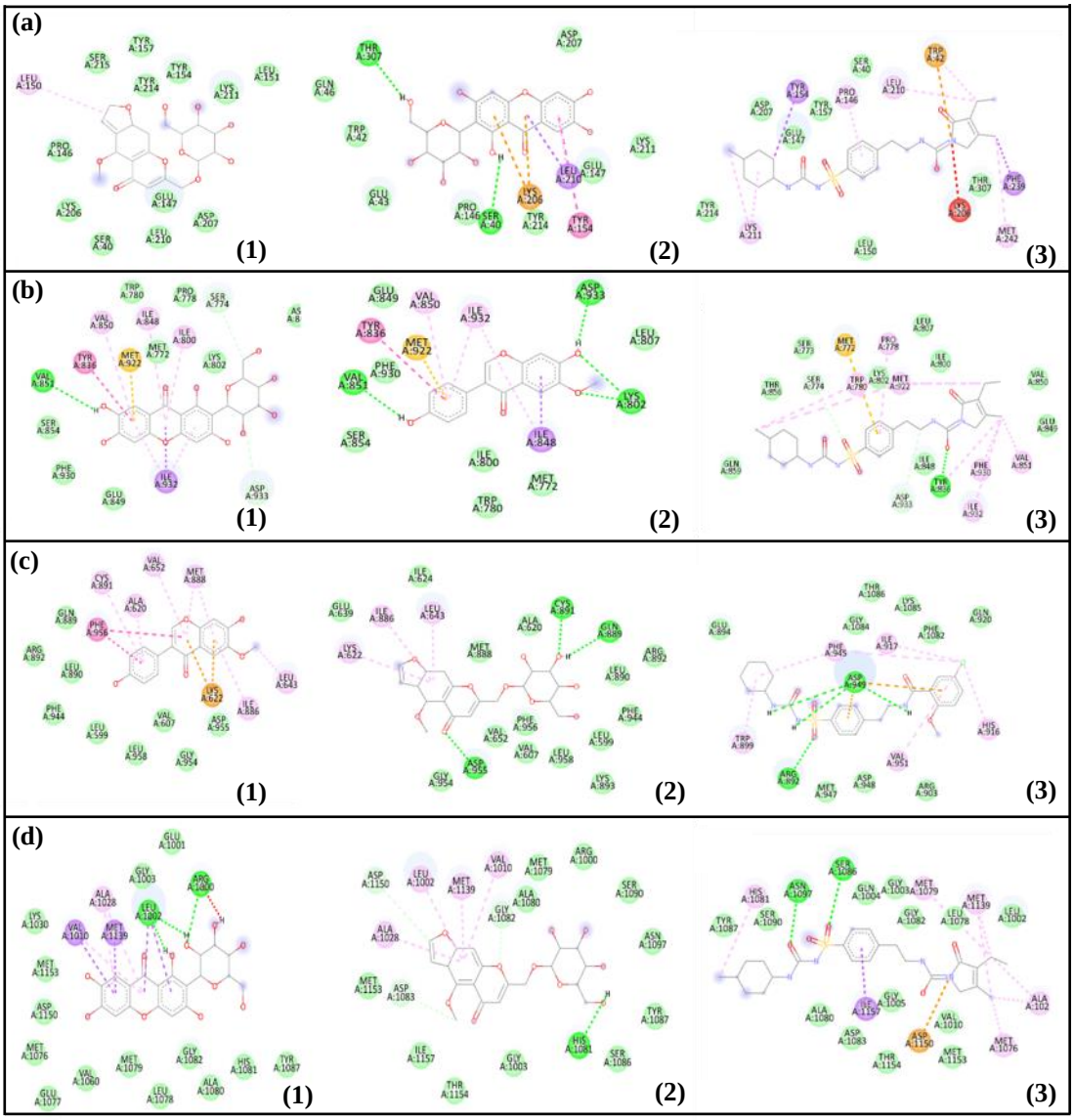

Fig. 4. Ligand-receptor complex of agarwood leaf phytocompounds interacts with GLP-1R (a), PI3Kα (b), PERK (c), and IRK (d). The top 1 (1) and top 2 (2) docked phytocompound-receptor interaction; the best docked positive control ligand-receptor interaction (3); van der Waals (light green), conventional hydrogen bond (bright green), carbon hydrogen bond (pale pink), Pi-Sigma (violet), Pi-Sulfur/Pi-Cation (orange), Pi–Pi T-shaped (pink), carbon hydrogen bond (light grayish green), and Pi-Alkyl (light purple).

Compounds with the highest binding affinity to PI3Kα and PERK also exhibit a greater number of π-interactions. In general, hydrophobic interactions such as van der Waals and π-

interactions are more commonly formed in ligand–receptor interactions than hydrogen bonds. A systematic review has also reported this phenomenon through bond-type analysis in the Protein Data Bank (Ferreira et al. 2017). Additionally, ATX and khelloside are glycoside compounds that demonstrate better binding capability compared to other types of compounds. Previous studies have demonstrated that glycosylated flavonoids exhibit stronger binding activity than their aglycone forms, as indicated by in silico studies (Prayogo et al. 2024).

The molecular docking results show that ligand-receptor interaction with the GLP-1R protein does not exhibit a significant effect. Although compounds such as ATX, glycitein, and khelloside exhibit relatively high binding affinities, these values are not sufficient to produce strong insulinotropic activity through the interaction with GLP-1R. This suggests that the antidiabetic activity of compounds in agarwood leaf extract is not primarily mediated through GLP-1R inhibition. The correlation between insulinotropic activity, phytochemical profile, and molecular docking analysis predicts the mechanism of action of agarwood leaf extract. The LEW extract, with the best bioactivity in enhancing insulin secretion in BRIN-BD11 cells, contains the highest levels of ATX and glycitein compared to other extracts. ATX exhibits a strong binding affinity to the IRK protein and a relatively strong affinity to other proteins. Meanwhile, glycitein exhibits strong binding affinity to PI3Kα and PERK proteins. This suggests that the insulinotropic activity of the LEW extract is highly influenced by the high abundance of ATX and glycitein, working through multiple mechanisms, with the dominant mechanism being ATX binding to IRK.

The increase in insulin secretion upon treatment with LE and LW extracts is also linked to the phytochemical components and their binding activity with target proteins. LE and LW both show comparable insulinotropic activity, with no significant difference. The LE extract contains higher levels of glycitein and khelloside compared to LW, while LW contains more ATX than LE. This is thought to be a factor contributing to the similar insulinotropic activity between the two extracts. Both extracts also contain quinic acid in high abundance, but this compound does not show strong potential based on molecular docking studies. The LE extract is believed to operate through a mechanism dominated by khelloside–receptor interactions (main mechanism), supported by ATX–receptor interactions. Conversely, the LW extract appears to work mainly via ATX–receptor interactions (the primary mechanism), supported by khelloside–receptor and glycitein–receptor interactions.

4. Conclusions

Agarwood leaf extracts, prepared using ethanol, water, and their mixtures, have shown potential as antidiabetic agents through a mechanism that enhances insulin secretion. Treatment with agarwood leaf extract increased insulin secretion from BRIN-BD11 pancreatic β-cells by approximately fivefold compared to untreated cells. The strong insulinotropic activity of the agarwood leaf extract is attributed to the presence of polar compounds, including glycosylated xanthones, coumarins, and flavonoids. The dominant compounds in the agarwood leaf extract exhibited multitarget mechanisms, with high binding affinity values to four key proteins: the glucagon-like peptide-1 receptor, phosphatidylinositol 4,5-bisphosphate 3-kinase, protein kinase RNA-like endoplasmic reticulum kinase, and the insulin receptor kinase.

Acknowledgments

The authors acknowledge the support from the Directorate General of Higher Education (DIKTI), Ministry of

Education, Culture, Research, and Technology of the Republic of Indonesia, through the Regular Fundamental Research Grant (Penelitian Fundamental Reguler/PFR), contract number 027/E5/PG.02.00.PL/2024, awarded to IPB University. This article constitutes an additional output of the PFR project.

Author Contributions

Y.H.P.: Conceptualization, Project Administration, Resources, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing – Original Draft Preparation, Writing – Review and Editing, Visualization; R.K.S.: Conceptualization, Project Administration, Resources, Methodology, Validation, Formal Analysis, Investigation, Data Curation; W.S.: Conceptualization, Project Administration; E.H.: Conceptualization, Project Administration; B.F.P.: Conceptualization, Project Administration; Z.H.: Validation, Formal Analysis, Data Curation, Visualization.

Conflict of Interest

The authors declare no conflict of interest.

Declaration of Generative AI and AI-Assisted Technologies in the Manuscript Preparation

Not applicable.

References

Adiarso, Marwanto, B., Ismariny, Z., Zubair, M., Widyastuti, N., and Prihawantoro, S. 2020. *Outlook Teknologi Kesehatan 2020: Inisiatif Penguatan Rantai Pasok Bahan Baku Obat*. BPPT, Jakarta.

Akyüz, M. 2022. The Determination of Antidiabetic, Anticholinesterase and Antioxidant Properties of Ethanol and Water Extracts of Blackberry (*Rubus fruticosus* L.) Fruits at Different Maturity Stages. *South African Journal of Botany* 151(18): 1035–1048. DOI: 10.1016/j.sajb.2022.11.012

Anastassiadis, T., Duong-Ly, K. C., Deacon, S. W., Lafontant, A., Ma, H., Devarajan, K., Dunbrack, R. L., Jr, Wu, J., and Peterson, J. R. 2013. A Highly Selective Dual Insulin Receptor (IR)/Insulin-Like Growth Factor 1 Receptor (IGF-1R) Inhibitor Derived from an Extracellular Signal-Regulated kinase (ERK) Inhibitor. *The Journal of Biological Chemistry* 288(39): 28068–28077. DOI: 10.1074/jbc.m113.505032

Andianto, Wahyudi, I., Sari, R. K., Pari, G. and Prayogo, Y. H. 2024. Phytochemical Profile of *Rhizophora apiculata*, *Bruguiera gymnorhiza*, and *Bruguiera cylindrica* for Wood Identification. *Jurnal Sylva Lestari* 12(2): 435–444. DOI: 10.23960/jsl.v12i2.884

Eberhardt, J., Santos-Martins, D., Tillack, A. F., and Forli, S. 2021. AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. *Journal of Chemical Information and Modeling* 61(8): 3891–3898. DOI: 10.1021/acs.jcim.1c00203

Fairhurst, R. A., Furet, P., Imbach-Weese, P., Stauffer, F., Rueeger, H., McCarthy, C., Ripoche, S., Oswald, S., Arnaud, B., Jary, A., Maira, M., Schnell, C., Guthy, D. A., Wartmann, M., Kiffe, M., Desrayaud, S., Blasco, F., Widmer, T., Seiler, F., Gutmann, S., Knapp, M., and Caravatti, G. 2022. Identification of NVP-CLR457 as an Orally Bioavailable Non-CNS-Penetrant pan-Class IA Phosphoinositol-3-Kinase Inhibitor. *Journal of Medicinal Chemistry* 65(12): 8345–8379. DOI: 10.1021/acs.jmedchem.2c00267

Familasari, S., Sari, R. K., Syafii, W., Carolina, A., Cahyaningsih, U. and Sa'diah, S. 2023. Ultrasound-Assisted Extraction of Antimalarial Compounds from the Bidara Laut (*Strychnos ligustrina*) Heartwood. *Jurnal Sylva Lestari* 11(3): 360–369. DOI: 10.23960/jsl.v11i3.682

Ferreira, R., and Schapira, M. 2017. A systematic Analysis of Atomic Protein-Ligand Interactions

in the PDB. *MedChemComm* 8(10): 1970–1981. DOI: 10.1039/c7md00381a

Gharge, S., Alegaon, S. G., Jadhav, S., Ranade, S. D. and Kavalapure, R. S. 2024. Design, Synthesis, Characterization and Antidiabetic Evaluation of 3,5-Substituted Thiazolidinediones: Evidenced by Network Pharmacology, Molecular Docking, Dynamic Simulation, in Vitro and in Vivo Assessment. *European Journal of Medicinal Chemistry Reports* 12: 100213. DOI: 10.1016/j.ejmcr.2024.100213

Han, Z., Wang, L., Sun, P., Huang, M., Yu, F., Liu, J., Wu, Y., He, P., Tu, Y., and Li, B. 2024. Quinic Acid as an Inhibitor of α-glucosidase Activity, Nonenzymatic Glycosylation, and Glucose Transport in Caco-2 Cells. *Food Frontiers* 5(6): 2545–2555. DOI: 10.1002/fft2.486

Hardianto, D. 2020. Telaah komprehensif Diabetes Melitus: Klasifikasi, Gejala, Diagnosis, Pencegahan, dan Pengobatan. *Jurnal Bioteknologi dan Biosains Indonesia* 7(2): 305–317. DOI: 10.29122/jbbi.v7i2.4209

Hidayah, N., Ruga, R., and Saleh, C. 2023. Anti-Inflammatory Effect of Extract and Fractions of Gaharu (*Aquilaria malaccensis* Lamk.) Leaves. *Jurnal Kimia Mulawarman* 21(1): 62–66. DOI: 10.30872/jkm.v21i1.1022

Hidayat, H., Siburian, R., and Yuliana, C. I. 2020. Gaharu Alam, Jaringan Perdagangan, dan Gaharu Budidaya: Studi Kasus Kalimantan Timur. *Jurnal Biologi Indonesia* 16(1): 99–110. DOI: 10.47349/jbi/16012020/99

IDF. 2025. *IDF Diabetes Atlas. 10th Edition*. International Diabetes Federation. Brussels, Belgium.

Karagecili, H., İzol, E., Kireçci, E. and Gülçin, İ. 2023. Antioxidant, Antidiabetic, Antiglaucoma, and Anticholinergic Effects of Tayfi Grape (*Vitis vinifera*): A Phytochemical Screening by LC-MS/MS Analysis. *Open Chemistry* 21(1): 20230120. DOI: 10.1515/chem-2023-0120

Khoerunnisa, D. A., Choesrina, R., and Suwendar. 2023. Uji Aktivitas Antidiabetes Kombucha Daun Gaharu (*Aquilaria malaccensis* Lam.) terhadap Mencit Jantan (*Mus musculus*) Swiss Webster yang diinduksi Aloksan. *Pharmacy* 3(2): 591–599.

Kontoyianni, M., McClellan, L. M., and Sokol, G. S. 2004. Evaluation of Docking Performance: Comparative Data on Docking Algorithms. *Journal of Medicinal Chemistry* 47(3): 558–565. DOI: 10.1021/jm0302997

Kusunoki, M., Hirota, K., Shoji, T., Uba, T., Matsuo, Y., and Hayashi, M. 2025. Dexmedetomidine Suppresses Glucose-Stimulated Insulin Secretion in Pancreatic β-cells. *FEBS Open Bio* 15(4): 634–646. DOI: 10.1002/2211-5463.13960

Le, H. T., Yu, J., Ahn, H. S., Kim, M. J., Chae, I. G., and Cho, H. N. 2025. eIF2α Phosphorylation-ATF4 Axis-Mediated Transcriptional Reprogramming Mitigates Mitochondrial Impairment during ER Stress. *Molecules and Cells* 48(2): 100176. DOI: 10.1016/j.mocell.2024.100176

Maser, W. H., Maiyah, N., Karnjanapratum, S., Nukthamna, P., Thompson, A. K., Huda, N., and Bavisetty, S. C. B. 2024. Antidiabetic Property Optimization from Green Leafy Vegetables Using Ultrasound-Assisted Extraction to Improve Cracker Production. *Preventive Nutrition and Food Science* 29(1): 47–62. DOI: 10.3746/pnf.2024.29.1.47

McClenaghan, N. H., Barnett, C. R., Ah-Sing, E., Abdel-Wahab, Y. H., O'Harte, F. P., Yoon, T. W., Swanston-Flatt, S. K., and Flatt, P. R. 1996. Characterization of a Novel Glucose-Responsive Insulin-Secreting Cell Line, BRIN-BD11, Produced by Electrofusion. *Diabetes* 45(8): 1132–1140. DOI: 10.2337/diab.45.8.1132

Mistry, J., Biswas, M., Sarkar, S., and Ghosh, S. 2023. Antidiabetic Activity of Mango Peel Extract and Mangiferin in Alloxan-Induced Diabetic Rats. *Future Journal of Pharmaceutical Sciences* 9(1): 22. DOI: 10.1186/s43094-023-00472-6

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., and Hutchison, G. R. 2011. Open Babel: An Open Chemical Toolbox. *Journal of Cheminformatics* 3(1): 33. DOI: 10.1186/1758-2946-3-33

Prajapati, A. K., Sagar, S., and Kumar, R. 2022. Past and Current Prospectives of Herbal Product. *Journal of Research in Applied Sciences and Biotechnology* 1(5): 145–160. DOI: 10.55544/jrasb.1.5.16

Prayogo, Y. H., Batubara, I., Wahyudi, S. T., Sari, R. K., and Syafii, W. 2023a. Metabolite Profile, Antiproliferative, and Antioxidant Activity of *Acacia auriculiformis* and *Acacia crassicarpa* Extracts. *Journal of Saudi Chemical Society* 27(6): 101742. DOI: 10.1016/j.jscs.2023.101742

Prayogo, Y. H., Batubara, I., Sari, R. K., Egra, S., Yamauchi, K., Mitsunaga, T. and Syafii, W. 2023b. Chemical Constituent of *Acacia auriculiformis* Wood Extractives and Their Antioxidant Activity. *Jurnal Sylva Lestari* 11(3): 370–381. DOI: 10.23960/jsl.v11i3.753

Prayogo, Y. H., Wahyudi, S. T., Batubara, I., Sari, R. K., and Syafii, W. 2024. Multitarget Ensemble Docking of Potent Anticancer and Antioxidant Active Compounds from the *Acacia auriculiformis* and *Acacia crassicarpa*. *Research Journal of Pharmacy and Technology* 17(2): 707–716. DOI: 10.52711/0974-360x.2024.00110

Rudiana, T., Merru, E. S. Y., Hendrawati, H., and Sukandar, D. 2021. Characterization and Anticancer Activity from Gaharu (*Aquilaria malaccensis*) Stem Bark Extract. *EduChemia: Jurnal Kimia dan Pendidikan* 6(2): 197–207. DOI: 10.30870/educhemia.v6i2.10983

Said, F., Kamaluddin, M. T., and Theodorus. 2016. Efficacy of the *Aquilaria malaccensis* Leaves Active Fraction in Glucose Uptake in Skeletal Muscle on Diabetic Wistar Rats. *International Journal of Health Sciences and Research* 6(7): 162–167.

Sekar, V., Chakraborty, S., Mani, S., Sali, V. K., and Vasanthi, H. R. 2019. Mangiferin from *Mangifera indica* Fruits Reduces Post-Prandial Glucose Level by Inhibiting α-glucosidase and α-amylase Activity. *South African journal of botany* 120: 129–134. DOI: 10.1016/j.sajb.2018.02.001

Smith, A. L., Andrews, K. L., Beckmann, H., Bellon, S. F., Beltran, P. J., Booker, S., Chen, H., Chung, Y. A., D'Angelo, N. D., Dao, J., Dellamaggiore, K. R., Jaeckel, P., Kendall, R., Labitzke, K., Long, A. M., Materna-Reichelt, S., Mitchell, P., Norman, M. H., Powers, D., Rose, M., Shaffer, P. L., Wu, M. M., and Lipford, J. R. 2015. Discovery of 1H-Pyrazol-3(2H)-Ones as Potent and Selective Inhibitors of Protein Kinase R-like Endoplasmic Reticulum Kinase (PERK). *Journal of Medicinal Chemistry* 58(3): 1426–1441. DOI: 10.1021/jm5017494

Suhardiman, A., Adnyana, I. K., Sukamawati, I. K., and Rostinawati, T. 2022. Activity of Gaharu (*Aquilaria malaccensis* Lam) Leaves Extract and Fraction as Herbal Drug Against α-Glucosidase Inhibition. *International Journal of Research in Pharmaceutical Sciences* 13(1): 35–41. DOI: 10.26452/ijrps.v13i1.17

Wati, W., Widodo, G. P., and Herowati, R. 2020. Prediction of Pharmacokinetics Parameter and Molecular Docking Study of Antidiabetic Compounds from *Syzygium polyanthum* and *Syzygium cumini*. *Jurnal Kimia Sains dan Aplikasi* 23(6): 189–195. DOI: 10.14710/jksa.23.6.189-195

Yadikar, N., Ahmet, A., Zhu, J., Bao, X., Yang, X., Han, H. and Rozi, P. 2022. Exploring the Mechanism of Citric Acid for Treating Glucose Metabolism Disorder Induced by Hyperlipidemia. *Journal of Food Biochemistry* 46(12): e14404. DOI: 10.1111/jfbc.14404

Zulkifle, N. L. 2018. Antidiabetic Activity of *Aquilaria malaccensis* (Agarwood) Leaves Extracts.

[Thesis]. Universiti Malaysia Pahang. Pahang, Malaysia.

Zhang, X., Belousoff, M. J., Zhao, P., Kooistra, A. J., Truong, T. T., Ang, S. Y., Underwood, C. R., Egebjerg, T., Šenel, P., Stewart, G. D., Liang, Y. L., Glukhova, A., Venugopal, H., Christopoulos, A., Furness, S. G. B., Miller, L. J., Reedtz-Runge, S., Langmead, C. J., Gloriam, D. E., Danev, R., *et al*. 2020. Differential GLP-1R Binding and Activation by Peptide and Non-peptide Agonists. *Molecular Cell* 80(3): 485–500. DOI: 10.1016/j.molcel.2020.09.020