

LC-MS Based Metabolite Profiling of Ethanol Extract From the Sungkai (*Peronema canescens* Jack) and *In Silico* Prediction of Antidiabetic Activity With α -Glucosidase

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ABSTRACT. Sungkai (*Peronema canescens* Jack) is a medicinal plant in the Verbenaceae family that is known to have antidiabetic activity. The secondary metabolite content found in sungkai leaves is in the form of alkaloids, flavonoids, triterpenoids, steroids, phenolics, and saponins. This research aimed to identify the biological activity of compounds in sungkai plants as antidiabetic agents using α -glucosidase inhibitors *in silico*. Sungkai leaf extract with ethanol solvent was identified using a Liquid Chromatography Mass Spectrometer (LC-MS/MS). There are 15 compounds resulting from LC-MS/MS analysis which will then predict antidiabetic activity using molecular docking. Molecular docking was carried out using AutoDock Tools software and visualized with Discovery Studio. The highest scoring result obtained from the molecular docking test was the compound $C_{13}H_{23}N_6S$ with a free bond energy result of -7.31 and an inhibition constant value of 4.41 μ M, which binds three hydrogen bonds in GLU 78, ALA 75, and GLU 198.

Keywords: *In silico*, metabolite profiling, *Peronema canescens*

INTRODUCTION

Indonesia is a country with a tropical climate that has biodiversity. There are around 940 plant species out of 40,000 types of plants in the world that are used as medicine. Indonesia has 30,000 kinds of plants, which is an advantage as a country with a tropical climate (Brata & Wasih, 2021). One of the plants that can be used as alternative medicine in Indonesia is the sungkai plant (*Peronema canescens* Jack). The secondary metabolite content in sungkai leaves can help the body's homeostatic function. Sungkai leaves also contain furano diterpenoid compounds of the type clerodane diterpenoid, alkaloids, flavonoids, triterpenoids, steroids, phenolics and saponins (Latief *et al.*, 2023). Flavonoids are a class of phenolic compounds that have various biological activities. Flavonoids can reduce blood glucose levels with their ability as antioxidants. Natural flavonoids play a role in preventing diabetes and its complications and are protective against β -cell damage, as well as increasing insulin sensitivity (Primal and Ahriyashna, 2022).

Phenolic compounds are widely recognized for their antioxidant and antidiabetic properties. These compounds, which include flavonoids, phenolic acids, tannins, and lignans, have been studied for their ability to neutralize free radicals, improve insulin

sensitivity, and regulate blood glucose levels (Deka *et al.*, 2022)

The crude extract of sungkai leaves from ethanol solvent was tested using Liquid Chromatography Mass Spectrometry (LC/MS-MS). LC/MS-MS analysis is an analytical technique that combines the physical separation capabilities of liquid chromatography with the detection specificity of mass spectrometry. Liquid chromatography separates the sample components, and then the charged ions are detected by a mass spectrometer (Mangurana *et al.*, 2019). From the results of the LC/MS-MS analysis, it was found that there were compounds contained in sungkai leaf extract. This compound will be tested *in silico* to determine its antidiabetic activity using an α -Glucosidase inhibitor. Diabetes is a metabolic disorder that occurs due to the pancreas not being able to produce enough insulin, which is characterized by hyperglycemia. One pharmacological therapy that can be used to reduce blood glucose levels is by inhibiting the action of the α -Glucosidase enzyme, which can delay glucose absorption in the digestive tract (Alvionitasari *et al.*, 2022).

Computer-aided drug discovery (CADD) is an approach used for the development of drug compounds that has advantages in terms of cost and time. CADD has a vital role in innovation in low-cost

drug discovery, as well as reducing the use of animals in pharmacological tests and helping to design new drug candidates. One of the methods used is an *in silico* test, which can identify drug targets by computationally predicting target proteins (Brogi et al., 2020). The approach used in this test is molecular docking simulation. This simulation makes it possible to discover new compounds that can be used as drugs and can also predict how the target molecule will interact with the ligand and assess the structural activity of the compound (Rahardian et al., 2022).

Materials and Methods

The materials used in this research were sungkai leaves obtained from Muaro Jambi Regency, Jambi Province, Ethanol, α -glucosidase, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu, and 3D test compounds, which were saved in pdb format, as well as the structure of the receptor (target protein) α -Glucosidase (3A4A), which is stored in pdb form on the web server of each database. The tools used in this research are hardware, namely Lenovo PC IdeaCentre AIO 5i 24IAH7 F0GR006RID Storm Gray (Intel Core i7 12700H, Win11 Home, 16GB DDR4, Intel ARC A370M 4GB GDDR6) and software PyRx, ChemDraw Ultra version 22.0, Chem 3D version 22.0, AutoDockTools, Discovery Studio Visualizer 2021 and UCSF Chimera for the webserver used rscb (Research Collaboratory for Structural Bioinformatics).

Extraction Process

Extraction was carried out using the maceration method with a ratio of 1:50. 100 grams of sungkai leaves powder was weighed, then macerated with 500 ml of ethanol solvent and left for 1x24 hours. The macerate obtained was concentrated using a rotary evaporator. The concentrated extract obtained was then weighed.

Determination of Total Phenolic Content and Antioxidant Activity

Phenolic compound intake may play a fundamental role in antioxidant activity and diabetes management, as it can reduce blood glucose levels, oxidative stress, protein glycation, and other mechanisms (Farias et al, 2012). The phenolic content was assayed using the Folin-Ciocalteu reagent, following Taamalli et al, 2014. An aliquot (0.125 mL) of a suitable diluted ethanol extract was added to 0.5 mL of deionized water and 0.125 of the Folin-Ciocalteu reagent. The total phenolic content was measured at 760 nm and expressed as milligrams of gallic acid per gram of dry matter (mg GAE/g DM). The test was repeated three times.

The DPPH solution was made by dissolving 5 mg of DPPH powder in 50 mL of ethanol. Make a blank solution with 3 mL of DPPH solution and take 1 mL of ethanol. Then, a sample solution was made by dissolved 50 mg of extract in 50 mL of ethanol (p.a.). Next, the mixed solution was made by mixing the sample solution in various concentrations (20, 40, 60, 80, 100) ppm, which was added with 3 mL of DPPH

solution, then homogenized and left for 30 minutes. Furthermore, it was tested using a UV-Vis Spectrophotometer at a wavelength of 518 nm (Delviani et al., 2024).

LC-MS/MS Test

The crude extract of sungkai leaves from ethanol solvent was analyzed using LC/MS-MS. The results of the LC/MS-MS data analysis obtained a chromatogram in the form of a peak height plot, and the molecular weight of the compounds contained in the extract can be obtained so that you can know the number of compounds contained in each sample.

Ligand Preparation

Ligand preparation is carried out by drawing the 2D structure of the test compound (ligand) using ChemDraw Ultra version 22.0 and then converting it into 3D form using Chem 3D version 22.0 and saving it in pdb format. The test ligands were optimized by minimizing energy using PyRx software with the open Babel feature and then saved in pdbqt format.

Macromolecule Preparation

Macromolecular preparation is carried out by downloading the receptor in the <https://www.rcsb.org> database with the receptor code 3A4A (α -Glucosidase). Macromolecules are separated from solvents and native ligands or nonstandard residues using the UCSF Chimera application. Native ligands and unnecessary residues are removed by clicking the select feature, then clicking residues and selecting all nonstandard, then selecting the actions feature, clicking atoms/bonds, and then clicking delete. Macromolecular (receptor) files are saved in pdb format. Next, the macromolecules were optimized using AutoDockTools by adding hydrogen ions and Kollman charges and saved in pdbqt file format.

Molecular Validation and Docking

Validation of the molecular docking method was carried out using AutoDockTools software. This is done by re-docking the natural ligands of each macromolecule (receptor). The parameter used is Root Mean Square Deviation (RMSD). The results obtained in this process are the grid box parameters and RMSD values. The docking method is valid if it has an RMSD value $< 2 \text{ \AA}$. The molecular docking process is carried out using AutoDock Tools software. The macromolecule (receptor) and ligand structures that have been optimized separately are stored in one folder. The molecular docking process uses a grid box and energy minimization parameters according to validation results. Grid box parameter settings are carried out using grid box coordinates, which are determined based on the ligand coordinates of the receptor used in the docking validation process. Next, mooring is carried out using AutoDock Tools software. The docking data displayed is in the form of binding affinity values and amino acid residue interactions. Docking results are saved in pdbqt format.

Visualization and Analysis of Docking Results

The visualization process is carried out to see the interactions that occur in the docking results between the receptor and the ligand. Visualization of docking results was carried out using Discovery Studio Visualizer 2021 software.

RESULTS AND DISCUSSION

The extraction process of sungkai leaves uses the maceration method, where the dried simplicia obtained weighing 100 grams is added with 500 ml of ethanol. The extract obtained was then concentrated using a rotary evaporator. The ethanol extract of sungkai leaves obtained was tested for antioxidant activity using the DPPH (2,2-diphenyl-1-picryl hydrazyl) method. DPPH is a synthetic radical that dissolves in polar solvents such as methanol and ethanol. DPPH is a stable radical whose intensity can be measured at a wavelength of 518 nm. The presence of antioxidant compounds can change the colour of the DPPH solution from purple to yellow; this is because free radicals that do not have an electron pair will be purple and will change to yellow when their electrons are paired (Rizkayanti et al., 2017). Changes in absorbance resulting from this reaction have been widely used to test the ability of several molecules to scavenge free radicals. From the results of the antioxidant activity test using the DPPH method, a linear regression equation was obtained and the IC_{50} value was determined to be 27.92 ppm (**Table 1**).

Next, an LC-MS/MS test was carried out on the crude extract of sungkai leaves from ethanol solvent. Liquid Chromatography Mass Spectrometry (LC/MS-MS) is an analytical technique that combines the physical separation capabilities of liquid chromatography with the detection specificity of mass spectrometry. Liquid chromatography separates the components of the sample, and then the charged ions are detected by a mass spectrometer. LC-MS data can be used to provide information about the molecular weight, structure, identity, and quantity of specific sample components. Compounds are separated on the basis of relative interactions with the chemical layer of the particles (stationary phase) and solvent elution through the column (mobile phase). The results of LC/MS-MS data analysis will produce a chromatogram in the form of a peak height plot, and the molecular weight of the compounds contained in the extract will be obtained so that you can know the number of compounds contained in each sample (Mangurana et al., 2019).

From **Table 2**, 15 active compounds are found in

sungkai leaves extract. The 15 compounds obtained will then be screened using the SWISSADME webserver (<http://www.swissadme.ch/>). The initial screening in carrying out molecular docking, namely in the ligand selection process, must be in accordance with Lipinski's rules. A test compound (ligand) is considered to have the potential to enter cell membranes and be absorbed by the body if it meets Lipinski's constraints with the following criteria: (1) molecular weight < 500 grams/mol, (2) number of hydrogen proton donor groups < 5, (3) number of hydrogen bond proton acceptor groups < 10, (4) value of the logarithm of the partition coefficient in water and 1-octanol < 5 (Bela et al., 2024). By analyzing the physicochemical characteristics of the ligand according to Lipinski's rules, it is possible to determine the hydrophobic/hydrophilic character of a substance to diffuse passively through the cell membrane. The log P value represents the solubility coefficient in fat/water, which has a range of -0.4 – 5. Molecular weights of more than 500 Da cannot diffuse across the cell membrane. The greater the log P value, the more hydrophobic the molecule is. Molecules that are too hydrophobic tend to have a high level of toxicity because they are retained longer in the lipid bilayer and are distributed more widely in the body, thereby reducing the selectivity of binding to the target enzyme. Apart from that, a log P value that is too negative is also not good because the molecules cannot penetrate the lipid bilayer membrane. The number of hydrogen bond donors and acceptors shows that the energy required for the absorption process to continue increases as the hydrogen bond capacity increases. In general, Lipinski's rule describes the solubility of certain compounds in penetrating cell membranes by passive diffusion (Syahputra et al., 2014). From the screening results, seven compounds were obtained that had biological potential.

The visualization process is carried out by taking data from docking results or scoring values. Molecules with the lowest scoring value show affinity with good stability and can be visualized with software. The smaller the result of the docking process, the more stable the protein-ligand complex is, so that the compound is more patent (Akbar et al., 2022). Visualization of docking results can be done with the help of Discovery Studio Visualizer 2021 software. Visualization aims to see the interaction between the ligand and the amino acid residues on the receptor. The visualization process is carried out to see the interactions that occur as a result of docking between the receptor and the ligand.

Table 1. Phenolic compound, antioxidant, and α -glucosidase inhibition

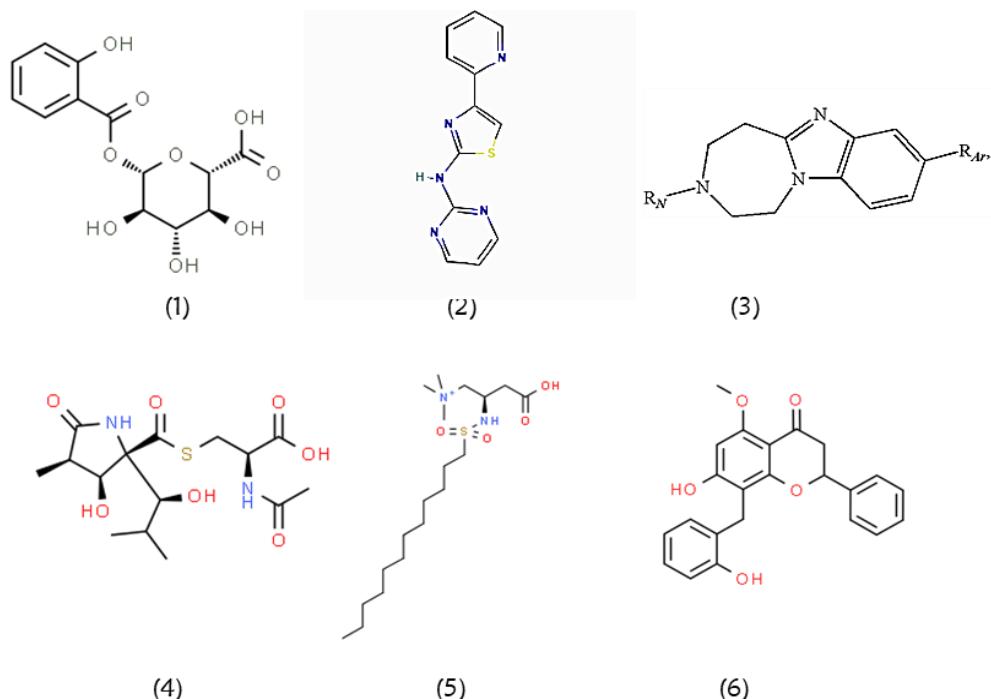
Samples	Phenolic compound	Antioxidant (IC_{50})	α -glucosidase
Ascorbic acid	-	10.13 ppm	0.144 ppm
EtOH Extract	7.2038 mg EAG/g	27.92 ppm	38.18 ppm

Table 2. LC-MS/MS metabolites profile

Peak	RT (min)	Measured <i>m/z</i>	Formula	Proposed metabolite	Potential
P2	13.318	375.1253	C ₂₃ H ₂₀ O ₅	5- <i>O</i> -Methylchamanetin	Anticancer and alzheimer (Quimque <i>et al.</i> , 2021)
P1	15.473	313.0555	C ₁₃ H ₁₄ O ₉	Salicyl Acyl Glucuronide	Acylate proteins directly and undergo intramolecular rearrangement produce reactive aldehydes that cause protein glycation (Smith <i>et al.</i> , 2018)
P2	14.036	693.1954	C ₃₄ H ₃₄ N ₂ O ₁₄	Dexylosyl Pradimicin C	Antibiotic and Antifungal (Tomita <i>et al.</i> , 1989)
P7	13.45	399.1213	C ₁₅ H ₂₄ N ₂ O ₇ S	Lactacystin	As a proteasome inhibitor (Tomoda & Omura, 2000)
P3	13.809	383.1269	C ₄₉ H ₇₀ N ₁₄ O ₁₁	Asn Asn Asn	Anticancer, Antimicrobial, and Antioxidant (Hamadon <i>et al.</i> , 2022)
P3	14.694	535.2431	C ₂₅ H ₄₀ N ₂ O ₇ S	Lipoxin D4	Treatment of coronary heart disease (Liang <i>et al.</i> , 2022)
P7	16.491	699.609	C ₄₉ H ₇₈ O ₂	Cholesteryl Ester	Antidiabetic (LaBarre, 2020)
P1	20.994	607.272	C ₃₁ H ₄₂ O ₁₂	Clerodendrin A	Anti-inflammatory, antioxidant, and antitumor (Ku'zma & Gomulski, 2022)
P2	9.857	437.2138	C ₁₉ H ₂₈ N ₆ O ₆	Arg Asp Phe	Antioxidants and amino acids (Wang <i>et al.</i> , 2021)
P1	3.654	317.0385	C ₁₄ H ₁₇ Cl ₃ N ₂	Tetrahydro-azepinoquinolines	Analgesic activity (Hinschberger <i>et al.</i> , 2003)
P1	17.928	295.1703	C ₁₃ H ₂₃ N ₆ S	(dimethyl-[2-[(4-pyrimidin-2-yl)piperazine-1-carbothioyl]amino]ethyl)azanium	Anticancer (Tadesse <i>et al.</i> , 2017)
P2	17.568	293.1905	C ₂₁ H ₂₅ O	2-methylbuta-1,3-diene:styrene;hydroxide	ND
P1	18.839	353.2475	C ₁₆ H ₃₇ N ₂ O ₄ S	<i>N,N</i> -dimethyllethanamine;dodecylazanide;sulfate	ND
P1	21.354	621.2868	C ₂₇ H ₄₅ N ₂ O ₁₄	(hydrogen peroxide;4-(4-oxocyclohexyl)cyclohexan-1-one;5-(7-oxooxepan-4-yl)oxepan-2-one) urea	ND
P2	19.724	393.2782	C ₁₉ H ₄₁ N ₂ O ₄ S	(2 <i>R</i>)-3-Carboxy-2-[(dodecylsulfonyl)amino]- <i>N,N,N</i> -trimethyl-1-propanaminium	Drug carrier (Wang & Wang, 2022)

Tabel 3. The properties of compounds are based on Lipinski's rules

Bioactive Compounds	Coefficient Log P	Molecular Weight (BM)	HBA	HBD
C ₁₃ H ₁₄ O ₉ (1)	-1.5588	314.245	9	5
C ₁₃ H ₂₃ N ₆ S (2)	0.6427	295.427	6	2
C ₁₄ H ₁₇ C ₁₃ N ₂ (3)	3.2136	246.7353	2	1
C ₁₅ H ₂₄ N ₂ O ₇ S (4)	-0.5623	376.4253	9	5
C ₁₉ H ₄₁ N ₂ O ₄ S (5)	4.6931	395.62072	6	4
C ₂₃ H ₂₀ O ₅ (6)	4.4037	376.401	5	2
Native ligand	-3.2214	180.1559	6	5

**Figure 1.** Five bioactive compounds

The results obtained from the docking process are in the form of bond energy and type of interaction (hydrogen bonds). The calculation (scoring function) of the ligand conformation formed in a macromolecule under equilibrium conditions is known as the bond energy. The binding free energy will reach balance if it is negative. The binding free energy will also be directly proportional to the inhibition constant. The greater the negative value of a compound's ΔG , the more spontaneous its ability to interact with the target receptor. The inhibition constant can be declared strong if it has a value of $\leq 100 \mu\text{M}$ and conversely weak if it is $\geq 100 \mu\text{M}$ (Putri et al., 2023). A low binding value indicates that the ligand-protein complex formed is stable. Based on this, the conformation of the test compound, which has the lowest binding energy and interacts with the amino acid residues at the binding site, is selected (Susanti et al., 2019). From the results obtained from the molecular docking test in **Table 4**, the best scoring results were obtained for the compound C₁₃H₂₃N₆S with a free bond energy of -7.31 and an inhibition constant value of 4.41 μM , which binds three hydrogen bonds in GLU 78, ALA 75

and GLU 198.

Hydrogen bonds are used as a parameter that characterizes the existence of pharmacological interactions in the mechanism between the drug and the receptor. Hydrogen bonds are the central bonds that can maintain protein stability, so the hydrogen bonds formed in molecular dynamics simulations indicate the affinity between the compound and the target receptor. Meanwhile, hydrophobic interactions can increase protein stability by changing the nature of amino acids that have hydrophilic properties in a hydrophobic environment and can also determine amino acid residues that have a significant contribution to the aim of maintaining protein stability (Akbar et al., 2022). The results of molecular docking using α -Glucosidase inhibitors can have antidiabetic potential. α -Glucosidase is an enzyme that plays a role in metabolism in the process of carbohydrate metabolism found at the edge of the surface of small intestinal cells and the process of forming glycoproteins and glycolipids. A compound that has the potential to be antidiabetic because it can reduce blood sugar levels (Sari et al., 2020).

Tabel 4. Results of the molecular docking test on the α -glucosidase receptor

Bioactive Compound	Binding free energy (kcal-mol ⁻¹)	Inhibition Constant (μ M)	Hydrogen Bond	Hydrogen bond distance (Å)	Amino acid residues
C ₁₃ H ₁₄ O ₉	-2.69	10.59	LYS 523	2.23555	LYS 523, GLU 322, PHE 321, LEU 323, PHE 360, PHE 543, TRP 581, LYS 524, SER 544
C ₁₃ H ₂₃ N ₆ S	-7.31	4.41	GLU 78 ALA 75 GLU 198	2.25692 3.52453 3.38709	GLU 198, ALA 195, SER 199, TRP 119, HIS 117, ASN 76, GLU 118, ALA 75, TYR 203, GLU 78,
C ₁₄ H ₁₇ C ₁₃ N ₂	-6.81	10.27	ASP 242 ARG 315	1.48278 2.78926	GLU 411, HIS 280, ASP 242, SER 240, PRO 312, LEU 313, LYS 156, PHE 314, ARG 315, TYR 316, TYR 158, ASN 415
C ₁₉ H ₂₈ N ₆ O ₆	-1.01	182.57	LYS 406 ASP 473 TRP 402	2.08181 1.95569 2.23316	ARG 476, ASP 473, TRP 402, LYS 400, LYS 406, ASN 401
C ₁₅ H ₂₄ N ₂ O ₇ S	-2.56	13.31	PHE 321	2.11616	LYS 523, LEU 323, GLU 322, PRO 320, VAL 319, PHE 21, GLY 361, SER 544, PHE 360, ASP 362, TRP 581, PHE 543, ASRG 359
C ₁₉ H ₄₁ N ₂ O ₄ S	-3.85	1.51	LYS 523 PHE 321	1.62075 3.24106	TRP 581, PHE 543, PRO 320, LYS 523, PHE 321, GLU 322, GLY 361, LEU 323, ARG 359, TRP 326, PHE 360
C ₂₃ H ₂₀ O ₅	-6.19	29.23	HIS 280 ASP 242 ASP 307 PRO 312	2.72721 1.97236 2.5701 3.47687	SER 311, ARG 315, PHE 314, ASP 242, LEU 246, HIS 280, ASP 307, SER 304, VAL 308, GLY 309, THR 310, PRO 312
Native ligand	-3.37	3.41	HIS 112 ASP 215 ASP 69 HIS 315 ASP 352 GLN 182	2.12037 2.12487 1.92463 2.19047 1.99107 3.49291	VAL 109, GLN 182, PHE 159, ARG 442, VAL 216, PHE 178, GLU 277, ASP 352, ARG 213, HIS 315, ASP 69, TYR 72, ASP 215, HIS 112

Tabel 5. Visualization of molecular docking results

Bioactive compound	2D	3D
C ₁₃ H ₂₃ N ₆ S		
Native ligand		

CONCLUSIONS

From the results of the LC-MS/MS test analysis on sungkai leaves, 15 compounds were obtained. The compounds obtained were subjected to a molecular docking test against α -Glucosidase to determine its biological activity as an antidiabetic. From the results obtained, the $C_{13}H_{23}N_6S$ compound has the best scoring value with a free bond energy value of -7.31 and has an inhibition constant value of 4.41 μM , which binds three hydrogen bonds in GLU 78, ALA 75, and GLU 198.

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REFERENCES

Alvionitasari, D. N., Sulastri, L., Desmiaty, Y., Syamsudin, S., & Simanjuntak, P. (2022, February). Isolasi dan identifikasi senyawa kimia penghambat enzim α -glukosidase dari fraksi air daun salam (*Syzygium polyanthum* (Wight) Walp.). *In Prosiding Seminar Nasional Kimia* (16-22).

Bela, A., Dewi, T.T., Prabawa, S., Khatib, A., Yusnaidar, Latief, M., Tarigan, I.L. (2024). In silico study of bioactive compounds of putat leaf extract (*Planchonia valida*) as anti-cancer against the VEGFR2 receptor. *Journal of the Indonesian Society of Integrated Chemistry*. 16(1). <https://doi.org/10.22437/jisc.v16i1.32049>

Brata, A., & Wasih, E. A. (2021). Uji efek antipiretik infusa daun sungkai (*Peronema canescens*) pada mencit putih jantan (*Mus Musculus*) (Test the antipyretic effect of sungkai leaf infusion (*Peronema canescens*) on male white mice (*Mus Musculus*). *Riset Informasi Kesehatan*, 10(2), 164. <https://doi.org/10.30644/rik.v10i2.554>

Deka H, Choudhury A, Dey BK. (2022). An overview on plant derived phenolic compounds and their role in treatment and management of diabetes. *Journal of Pharmacopuncture*. 30;25(3):199-208. <https://doi.org/10.3831/KPI.2022.25.3.199>

Delviani, Maharani, V.G.R., Shadrina, P.N., Ali, N., Tarigan, I.L. (2024). Synthesis of Carboxymethyl Cellulose from Mangrove Nipah (*Nypa fruticans*) as Vitamin C Coating for Drug Delivery System. *Chempublish*. 8(1). <https://doi.org/10.22437/chp.v8i1.31722>

De Paulo, F D., de Araújo, F F., Neri-Numa, I.A., Pastore, G.M. (2021). Antidiabetic potential of dietary polyphenols: A mechanistic review, *Food Research International*. 145. <https://doi.org/10.1016/j.foodres.2021.110383>.

Dewi, N., & Ginarsih, N. (2021). Molecular Docking Ellagic Acid Sebagai Agen Anti-photoaging Secara *In Silico*. *Acta Holistica Pharmaciana*. 3(1), 22-30.

Hamadon, W.S., N. Bouali, R. Badraouni, R.H. Lajimi, A. Hamdi, M. Alreshidi, M. Patel, M. Adnan, A.J. Siddiqui, E.Noumi, V.R. Pasupuleti and M. Snoussi. 2022. Chemical Composition and the Anticancer, Antimicrobial, and Antioxidant Properties of *Acacia* Honey from the Hail Region: The *In Vitro* and *In Silico* Investigation. *Evidence-Based Complementary and Alternative Medicine*. 7(1): 1-16.

Hinschberger, A., Butt, S., Lelong, V., Boulouard, M., Dumuis, A., Dauphin, F., Bureau, R., Pfeiffer, B., Renard, P., Rault, S. (2003). New Benzo[h][1,6]naphthyridine and Azepino[3,2-c]quinoline Derivatives as Selective Antagonists of 5-HT4 Receptors: Binding Profile and Pharmacological Characterization. *Journal of Medicinal Chemistry*. 46(1): 138-147.

Ku'zma, L and J. Gomulski. 2022. Biologically Active Diterpenoids in the *Clerodendrum* Genus-A Review. *International Journal of Molecular Sciences*. 7(23): 1-42.

LaBarre, J. L. 2020. *Metabolomics: A Promising Tool to Improve the Understanding of the Developmental Origins of Obesity and Metabolic Disease*. Amerika Serikat: University of Michigan.

Latief M, Rahmani, Fahrezi A, Sutrisno, Tarigan IL. Comparison of the Anti-Inflammatory Activity of Flavonoid Bioactive Compounds Acetone Fraction and Steroid Fraction Ethyl Acetate Sungkai Leaves *In Vivo* and *In Silico* Studies. *Pharmacogn Journal*. 15(6): 1068-1076. <https://doi.org/10.5530/pj.2023.15.196>

Liang, Z., Q. Zeng., X. Ou., J. Cai., T. Lan and W. Lu. 2022. Nontargeted Metabolomic Profiling of Huo-Tan-Chu-Shi Decoction in the Treatment of Coronary Heart Disease with Phlegm-damp Syndrome. *Cardiology Research and Practice*. 7(1): 1-11.

Mangurana, W. O. I., Yusnaini, Y., & Sahidin, S. (2019). Analisis LC-MS/MS (liquid chromatograph mass spectrometry) dan metabolit sekunder serta potensi antibakteri ekstrak n-heksana spons *Callyspongia aerizusa* yang diambil pada kondisi tutupan terumbu karang yang berbeda di perairan teluk staring (LC-MS/MS (liquid chromatograph mass spectrometry) analysis and secondary metabolites and antibacterial potential of n-hexane extract of the sponge *Callyspongia aerizusa* taken under different coral reef cover conditions in the waters of Staring Bay). *Jurnal Biologi Tropis*, 19(2), 131-141. <https://doi.org/10.29303/jbt.v19i2.1126>

Akbar, N. A., Amin, S., W. T. W. (2022). *Studi In Silico Senyawa Yang Terkandung Dalam Tanaman Daun Sirih Merah (Piper crocatum RUITZ & PAV) Sebagai Kandidat Anti Sars CoV-2.* 2, 378–391.

Primal, D., & Ahriyasna, R. (2022). Efek ingesti seduhan daun sungkai (*Peronema canescens*) terhadap perubahan glukosa darah dan kerusakan ginjal tikus diabetes mellitus (Effect of ingestion of sungkai leaf infusion (*Peronema canescens*) on changes in blood glucose and kidney damage in diabetic rats). *Jurnal Kesehatan Perintis (Perintis's Health Journal)*, 9(2), 110–124. <https://doi.org/10.33653/jkp.v9i2.879>

Putri, R. G., Safithri, M., Husnawati, H., & Kurniasih, R. (2023). Penambatan molekuler senyawa aktif sirih merah (*Piper crocatum*) pada butirilkolinesterase sebagai kandidat antialzheimer (Molecular docking of the active compound of red betel (*Piper crocatum*) on butyrylcholinesterase as an anti-alzheimer's candidate). *ALCHEMY Jurnal Penelitian Kimia*, 19(1), 68. <https://doi.org/10.20961/alchemy.19.1.59676.68-85>

Quimque, M.T., K.I. Notarte, A. Letada, R.A. Fernandez, D.Y. Pilapil, IV, K.R. Pueblos, J.C. Agbay, H.M. Dahse, A.W. Storjohann, D. Tasdemir, A. Khan, D.Q. Wei and A.P.G. Macabeo. 2021. Potential cancer and alzheimer's disease-targeting phosphodiesterase inhibitors from *Uvaria alba*: Insights from *In Vitro* and consensus virtual screening. *ACS Omega*. 7(6): 8403-8417.

Sari, I. W., Junaidin, J., & Pratiwi, D. (2020). Studi molecular docking senyawa flavonoid herba kumis kucing (*Orthosiphon stamineus* B.) pada reseptor α -glukosidase sebagai antidiabetes tipe 2. *Jurnal Farmagazine*, 7(2), 54. <https://doi.org/10.47653/farm.v7i2.194>

Smith, D.A., T. Hammond and T.A. Baillie. 2018. Safety assessment of acyl glucuronides- a simplified paradigm. *Drug Metabolism and Disposition*. 1(46): 908-912.

Syahputra, G., Ambarsari, L., & Sumaryada, T. (2014). Simulasi docking kurkumin enol, bisdemetoksikurkumin dan analognya sebagai inhibitor enzim12-lipokksigenase (Simulation of docking of curcumin enol, bisdemethoxy curcumin and their analogues as enzyme12-lipoxygenase inhibitors). *Jurnal Biofisika*, 10(1), 55–67.

Tadesse, S., M. Yu, L.B. Mekonnen, F. Lam, S. Islam, K. Tomusange, M.H. Rahaman, B. Noll, S.K.C. Basnet, T. Teo, H. Albrecht, R. Milne and S. Wang. 2017. Highly potent, selective, and orally bioavailable 4-thiazol-n-(pyridin-2-yl) pyrimidin-2-amine cyclin-dependent kinases 4 and 6 inhibitors as anticancer drug candidates: Design, synthesis, and evaluation. *Journal of Medicinal Chemistry*. 1(60): 1892-1915.

Tomita, K., Nishio, M., Saitoh, K., Yamamoto, H., Hoshino, Y., Ohkuma, H., Konishi, M., Miyaki, T., Oki, T. (1989). Pradimicins A, B, and C: New antifungal antibiotics. *XLIII(7). The Journal of Antibiotics*.

Tomoda, H and S. Omura. 2000. Lactacystin, a proteasome inhibitor: Discovery and its application in cell biology. *Yakugaku Zasshi*. 120(10): 935-949.

Wang, J., G. Wang, N. Chen, F. An, R. Zhang, Y. Zhang, M.U. Rahman and Y. Zhang. 2021. Characterization of structural, functional and antioxidant properties and amino acid composition of pepsin-derived glutelin-1 hydrolysate from walnut processing by-products. *Royal Society of Chemistry*. 1(11): 19158-19168.

Wang, Y., Wang., C. (2022). Novel eye drop delivery systems: Advance on formulation design strategies targeting anterior and posterior segments of the eye. *e. Pharmaceutics* 2022, 14, 1150. <https://doi.org/10.3390/pharmaceutics14061150>