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Research Article

In Silico and *In Vitro* Inhibitory Activity of Indonesian Herbal Compound Extracts against SARS-COV-2 Recombinant Papain-Like Protease

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ABSTRACT

The SARS-CoV-2 papain-like protease (PLpro) is essential for viral replication and a promising target for drug discovery. This study explored the inhibitory potential of compounds from Indonesia herbals Butterfly pea flower (*Clitoria ternatea* L), Star fruit leaves (*Averrhoa carambola* L.), and Java plum leaves (*Syzygium cumini* (L.) Skeels) against PL pro through molecular docking and *in vitro* assays. The molecular docking method utilized the target protein PLpro (PDB ID: 7CMD), with the native ligand obtained from compounds identified in these plant extracts. The compounds were identified using the KNApSAcK database and analyzed for drug-likeness based on Lipinski's Rule of Five. The physicochemical characteristics affecting absorption, distribution, metabolism, excretion, and toxicity (ADMET) were determined using the pkCSM descriptor algorithm protocol. Validation was performed using the redocking method, achieving an RMSD score of 0.728 Å, which indicated validity (RMSD <2.0 Å). The results identified four ligands with the lowest binding affinities from these extracts: (-)-Epicatechin 3-O-gallate, folic acid, petunidin 3-glucoside, and ellagic acid, with binding scores of -8.6, -8.3, -7.1, and -7.1 kcal/mol, respectively. Prior to conducting the PLpro *in vitro* inhibition assay, a fluorescence-based inhibition assay was performed using Z-RLRGG-AMC as the substrate and GRL0617as the control inhibitor. All extracts were subjected to 70% ethanol maceration. The IC_{50} value of GRL0617 was 3.38 µM, while fluorescence tests showed that Java plum leaf extract exhibited the highest inhibition percentage at 66.10±3.22%. These findings indicate that all three plant extracts contain compounds capable of inhibiting PLpro activity.

1. Introduction

COVID-19, an infectious respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged as a pandemic in 2020. On May 5th 2023, the WHO declared the end of COVID-19 as a Public Health Emergency of International Concern (PHEIC) and transition to long-term management of the pandemic. Historically, until June 2023, there have been more than 760 million cases of COVID-19 and over 6.9 million deaths worldwide (https://www.who.int/news-room/fact-sheets/detail/coronavirus-disease-(covid-19), with Indonesia recording more than 6.8 million cases and over 160 thousand deaths by June 2023 (https://www.worldometers.info/coronavirus/country/indonesia/).

Although COVID-19 is currently in its endemic phase, there are still fatal outcomes, particularly among vulnerable populations such as the elderly and those with underlying health conditions. Furthermore, the advent of SARS-CoV-2 variations and drug-resistant mutations necessitates the development of further oral antivirals

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(Tan *et al.* 2024). Hence, research into anti-COVID drugs remains crucial. Currently, therapeutic agents strongly recommended by the WHO for COVID-19 therapy, includes nirmatrelvir, ritonavir, corticosteroids, interleukin-6 receptor blockers, and baricitinib (Reis *et al.* 2023; Mitev 2023). Most of these drugs work by modulating the immune system to suppress infection, except for nirmatrelvir and ritonavir. Nirmatrelvir inhibits 3-chymotrypsin-like protease (3CLpro) in SARS-CoV-2, a crucial enzyme for virus replication. While Ritonavir, an HIV protease inhibitor, is co-administered with nirmatrelvir to enhance its pharmacokinetics. Notably, only nirmatrelvir and ritonavir directly target the structure of SARS-CoV-2, indicating opportunities for novel drugs directly targeting the virus (Napitupulu *et al.* 2023).

The SARS-CoV-2 genomic RNA encodes structural and non-structural proteins. Structural proteins include nucleocapsid (N), membrane (M), envelope (E), and spike (S), while non-structural proteins are comprised of 3-chymotrypsin-like protease (3CLpro), papain-like protease (PL Pro), and RNA-dependent RNA polymerase (RdRp). Both PL Pro and 3CLpro play a critical role in the virus replication process, making them attractive target for therapeutic agents (Ulfah *et al.* 2022). However, compared to 3CL Pro there are very few research for active compounds against PL pro. The SARS-CoV-2 papain-like protease (PLpro) represents a promising but difficult pharmacological target (Tan *et al.* 2024). Whereas, natural ingredients, abundant in Indonesia, hold promise for drug development against COVID-19. Notable examples include turmeric (*Curcuma longa* L.), Java ginger (*Curcuma xanthorrhiza* Roxb.), ginger (*Zingiber officinale* Roscoe), guava (*Psidium guajava* L.), Lagoon Spurge or Meniran (*Phyllanthus niruri*), and Sambiloto (*Andrographis paniculata*) (BPOM 2020).

Molecular docking, an *in silico* method, predicts the inhibitory activity of natural compounds against both PL pro and 3CLpro. This approach aids in selecting potential inhibitors or activators for a target protein, offering insights into the mechanism of action at the molecular level (Rizma *et al.* 2021). Several herbal ingredients have demonstrated inhibitory activity against PL pro in silico, including Seco-4-hydroxylintetralin in Meniran, Brazilin, and Brazilein in secang wood, and Curcumin in turmeric (Laksmiani *et al.* 2020; Firdayani *et al.* 2022).

Additionally, some plant ingredients exhibit inhibitory activity against another SARS-CoV-2 protease, 3CLpro, enhancing their potential for drug development. For instance, Cubebin in Meniran is a potential candidate as a 3CLpro inhibitor (Laksmiani *et al.* 2020). However, several plants, including Butterfly pea flowers, Sweet star fruit leaves, and Java plum leaves, have not yet been tested for their PL pro inhibitory activity (Indayani 2021; Aini *et al.* 2022; Fazadini and Yzzuddin 2022), making them intriguing candidates for investigation.

This research aims to conduct molecular docking of compounds from Butterfly pea flower (*Clitoria ternatea* L. (Ct)), Star fruit leaves (*Averrhoa carambola* L. (Ac)), and Java plum leaves (*Syzygium cumini* (L.) Skeels (Sc)) against PL pro as the target protein. Evaluation parameters include binding affinity and interaction profile. *In vitro* experiments will complement molecular docking results, providing further insights into the potential of these natural compounds as anti-COVID agents for treating COVID-19. For in vitro experiment, previously described purified PL Pro was applied (Napitupulu *et al.* 2024).

2. Materials and Methods

2.1. *In Silico* Study

The hardware utilized for this study was a Dell™ laptop model Inspiron 14 5000 2-in-1, equipped with an AMD® RyzenTM 5 3500U processor, 8 GB RAM, AMD Radeon™ Vega 8 graphics, and running on a Windows 10 Pro 64-bit operating system. The software employed included AutoDock Tools 4.2.6 (Morris *et al.* 2009), AutoDock Vina (Trott and Olson 2009; Eberhardt *et al.* 2021), BIOVIA Discovery Studio 2021 (BIOVIA 2021), Microsoft Excel (Microsoft Corporation 2019), and PyRx (Dallakyan and Olson 2015).

For *in silico* analyses, the target protein utilized was obtained from the Protein Data Bank (PDB) server, namely PL Pro (PDB ID: 7CMD) (Firdayani *et al.* 2022). The native ligand serving as a positive control was obtained from the dissociation of the ligand forming a complex with the target protein. Ligands from compounds found in Butterfly pea flowers, Sweet star fruit leaves, and Java plum leaves, which have been previously published, were employed. Ligand-protein interactions were then visualized using BIOVIA Discovery Studio 2021 (BIOVIA 2021). Amino acid residues involved in the positive control (GRL0617), were compared and analyzed with amino acid residues involved in ligand-protein interactions of compounds from herbal plants (Firdayani *et al.* 2022).

The KNApSAcK database was accessed to identify the compounds present in herbal plants. KNApSAcK

provides information regarding plant species and the metabolites contained in them, accessible via http://www.knapsackfamily.com/knapsack_core/top.php (Afendi *et al.* 2012).

2.2. Drug-Likeness Analysis

The physicochemical properties of bioactive compounds from these herbal plants were analyzed to assess their drug-likeness. This analysis focused on parameters based on Lipinski's Rule of Five, which indicates that a compound with two or more of these parameters is likely to have good drug-likeness (Chaudhary and Mishra 2016). The parameters include fewer than five hydrogen bond donors, fewer than ten hydrogen bond acceptors, a molecular mass less than 500 Da, high lipophilicity (expressed as LogP not exceeding 5), and a molar refractivity range of 40 to 130.

2.3. ADMET Analysis

The physicochemical characteristics affecting absorption, distribution, metabolism, excretion, and toxicity (ADMET) of the tested compounds were determined using the pkCSM ADMET descriptor algorithm. Drug absorption was evaluated based on factors such as membrane permeability, intestinal absorption, skin permeability, and glycoprotein P substrate or inhibition properties. Drug distribution was assessed based on parameters like blood-brain barrier permeability, central nervous system permeability, and distribution volume. Metabolism was evaluated using the CYP model for substrates or inhibitors, while excretion was assessed based on the total clearance model and renal substrates. Drug toxicity considerations included toxicity, hERG inhibition, hepatotoxicity, and skin sensitization. These parameters were evaluated against standard ranges (Firdayani *et al.* 2022).

Data for the ADMET analysis were obtained from several websites, namely pkCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction), SwissADME (http://www.swissadme.ch/), and Protox-II (https://tox-new.charite.de/).

2.4. Extraction of Herbal Plants

Butterfly pea flowers and sweet star fruit leaves were sourced from Bogor Regency, West Java, while Java plum leaves were obtained from Sragen Regency, Central Java. All plants collected were then authenticated by “Eka Karya” Botanical Garden Characterization Laboratories, National Research and Innovation Agency (BRIN). Simplicia from the three herbal plants were extracted at the Laboratory of the Research Institute for Spices and Medicinal Plants, Bogor, Indonesia, using the maceration method with a 70% ethanol solvent. The weight ratios of simplicia to solvent used were 1:8 for Butterfly pea flowers, 1:10 for Sweet star fruit leaves, and 1:5 for Java plum leaves. Each type of plant simplicia weighed 500 grams. The resulting extract yields were 174.5 grams for Butterfly pea flowers, 138.9 grams for Sweet star fruit leaves, and 180.9 grams for Java plum leaves, corresponding to extraction yields of 34.9%, 27.78%, and 36.18%, respectively (Yunarto and Sulistyaningrum 2017; Rachmawati *et al.* 2021; Yumni *et al.* 2022).

2.5. *In Vitro* Test of Plant Extracts against PL Pro

The inhibition of PL Pro activity was evaluated using a fluorescence-based inhibition assay. The reaction buffer, containing 20 mM Tris-Buffer (pH 8.0) and 4 mM DTT (Dithiothreitol), was added to the wells of a 384-well non-binding black plate. Subsequently, the purified PL Pro enzyme (Chen *et al.* 2021; Napitupulu *et al.* 2024) at a concentration of 50 nM was dissolved in the reaction buffer. Herbal extracts acting as inhibitors were added to the reaction mixture at a final concentration of 250 ppm. The reaction solution was incubated for 30 min before adding the Z-RLRGG-AMC peptide conjugate substrate at a final concentration of 30 µM. The total reaction volume was 40 µL. Fluorescence intensity was measured at wavelengths of 345 nm (excitation) and 445 nm (emission) for 1 hour with a data collection interval of every 1 minute using a microplate reader at a reaction temperature of 30°C (Chen *et al.* 2021; Chang *et al.* 2023; Napitupulu *et al.* 2024). Before applying the herbal extract, the positive control was used to check the validity of in vitro condition mixture. When the obtained IC_{50} value closed to previously reported, then the condition of assay could be used for *in vitro* test. The assay results were processed to obtain the percent inhibition value using the formula: %Inhibition = ((Control slope - test slope) / Control slope) × 100%.

3. Results

3.1. Compliance with Lipinski's Rules

Before conducting molecular docking, compounds were screened based on Lipinski's Rule of Five to ensure their pharmacokinetic properties. A total of 33 compounds from Butterfly pea flowers, 32 from Sweet

star fruit, and 20 from Java plum were identified. Among these compounds, 8 from Butterfly pea flowers, 30 from Sweet star fruit, and 18 from Java plum fulfilled Lipinski's rules (Table 1).

3.2. Molecular Docking Analysis

Molecular docking was performed using PyRx, assessing binding affinity (ΔG) to determine potential ligand-protein interactions. While none of the compounds surpassed the positive control GRL0617's binding affinity (-9.3 kcal/mol), (-)-Epicatechin 3-O-gallate exhibited the most negative binding affinity (-8.6 kcal/mol) (Table 2).

3.3. Visualization of Ligand-Protein Interactions

Another aspect that was reviewed to analyze potential inhibitor compounds was the interaction profile. GRL0617 was used as the comparator. Amino acid residues involved in ligand-protein interaction on GRL0617 are Asp164, Gln269, Tyr268, Pro247 and Pro248. (-)-Epicatechin 3-O-gallate showed close similarities in amino acid residues involved which are Gln269, Tyr268, Ans267 and Pro248) (Table 3, Figure 1). Other compounds exhibited similarities in amino acid residues with GRL0617, however none were exactly the same.

3.4. ADMET Analysis of Best Ligands

To evaluate pharmacokinetic and safety profiles, ADMET analysis was conducted. The top ten ligands were classified under toxicity class 5, indicating low toxicity potential. Further, these compounds demonstrated favorable pharmacokinetic properties (Table 4).

3.5. *In Vitro* Assay

To validate the condition for in vitro assay, firstly, the IC_{50} value of the control inhibitor GRL0617 was measured and successfully determined at 3.38 µM. Subsequently, inhibition tests using herbal extracts were performed. Results revealed that Java plum leaves extract exhibited the highest inhibition, while butterfly pea flower extract showed the lowest inhibition (Table 5). The potential of Java plum in inhibiting PL Pro, which is involved in the virus replication, means that it could be used as a potential anti-covid medicine. The extract could also be used as herbal supplement for further prevention of Covid. From *in silico* analyses result, 5 potential active compounds were available in Java plum: Folic acid, Ellagic acid, Quercetin, Myricetin, and Cyanidin. This factor probably explains why Java plum extract has the best inhibition rate compared to others.

4. Discussion

While COVID-19 is currently in the endemic phase, fatalities still occur, particularly among vulnerable populations such as the elderly and those with pre-existing health conditions. Furthermore, the emergence of SARS-CoV-2 variants and drug-resistant mutations necessitates the development of additional oral antivirals (Tan *et al.* 2024). Therefore, research into anti-COVID medicines remains important.

Significant efforts have been made to uncover the antiviral mechanisms of medicinal plants and their natural compounds on various SARS-CoV-2 virus lifecycle stages, including virus entry (S protein, ACE2), replication (3CLpro, PLpro, helicase, N protein, and RdRp), assembly (E and M proteins), viral release, and host-specific interactions via in silico analyses (Abou Baker *et al.* 2023). Like 3CLpro, PLpro is critical in the virus replication process, making it an attractive target for therapeutic agents (Jade *et al.* 2021; Ulfah *et al.* 2022). However, despite decades of optimization in medicinal chemistry and extensive high-throughput screening, the development of PLpro inhibitors has progressed more slowly and significantly lags behind that of 3CLpro inhibitors (Tan *et al.* 2022). The SARS-CoV-2 PLpro represents a promising but challenging pharmacological target (Tan *et al.* 2024). The lack of PLpro binding pocket subsites has made the creation of highly potent PLpro inhibitors difficult (Ma *et al.* 2021). Therefore, studies seeking inhibitors of SARS-CoV-2 PLpro remain of interest.

Medicinal plants are potential sources of SARS-CoV-2 PLpro inhibitors (Jabeen *et al.* 2024). Indonesia holds significant potential for anti-COVID medicinal plants, as at least 80% of the medicinal plant species in Southeast Asia are found there, either as native or introduced species (Cahyaningsih *et al.* 2021). Several potential plants distributed widely in Indonesia are suitable for further research and development as anti-SARS-CoV-2 agents

Table 1. List of compounds that satisfy Lipinski's rule

PubChem CID	Compound name	Chemical formula	Lipinski parameter				Conclusion
			MW (g/mol)	#Donor H	#Acceptor H	LogP	
5280961	Genistein	$C_{15}H_{10}O_5$	270.24	3	5	2.5768	v (4 fulfilled)
5282102	Astragalin	$C_{21}H_{20}O_{11}$	448.4	7	11	-0.2445	v (2 fulfilled)
5280804	Hirsutrin/Isoquercitrin	$C_{21}H_{20}O_{12}$	464.4	8	12	-0.5389	v (2 fulfilled)
5318606	Isomyricitrin	$C_{21}H_{20}O_{13}$	480.4	9	13	-0.8333	v (2 fulfilled)
443651	Petunidin 3-glucoside	$C_{22}H_{23}O_{12}^{+}$	479.4	8	11	0.3906	v (2 fulfilled)
443652	Oenin	$C_{23}H_{25}O_{12}^{+}$	493.4	7	11	0.6936	v (2 fulfilled)
68245	Delphinidin chloride	$C_{15}H_{11}ClO_7$	338.69	6	7	-0.3815	v (4 fulfilled)
159287	Malvidin	$C_{17}H_{15}O_7^{+}$	331.30	4	6	3.2205	v (4 fulfilled)
5280450	Linoleic acid	$C_{18}H_{32}O_2$	280.4	1	2	5.8845	v (3 fulfilled)
22311	Limonene	$C_{10}H_{16}$	136.23	0	0	3.3089	v (4 fulfilled)
31253	beta-Myrcene	$C_{10}H_{16}$	136.23	0	0	3.475	v (4 fulfilled)
6857557	15-cis-Phytofluene	$C_{40}H_{62}$	542.9	0	0	13.61	v (2 fulfilled)
9064	(+)-Catechin/cianidanol	$C_{15}H_{14}O_6$	290.27	5	6	1.5461	v (4 fulfilled)
72276	(-)-Epicatechin	$C_{15}H_{14}O_6$	290.27	5	6	1.5461	v (4 fulfilled)
182232	(+)-Epicatechin	$C_{15}H_{14}O_6$	290.27	5	6	1.5461	v (4 fulfilled)
11005	Myristic acid	$C_{14}H_{28}O_2$	228.37	1	2	4.7721	v (4 fulfilled)
5459879	Sedoheptulose	$C_7H_{14}O_7$	210.18	6	7	-4.0163	v (3 fulfilled)
10712	Cellobiose	$C_{12}H_{22}O_{11}$	342.30	8	11	-5.3972	v (2 fulfilled)
7427	Trehalose	$C_{12}H_{22}O_{11}$	342.30	8	11	-5.3972	v (2 fulfilled)
11727586	Galactinol	$C_{12}H_{22}O_{11}$	342.30	9	11	-6.0104	v (2 fulfilled)
892	Inositol	$C_6H_{12}O_6$	180.16	6	6	-3.8346	v (3 fulfilled)
4276	Myristicin	$C_{11}H_{12}O_3$	192.21	0	3	2.1524	v (4 fulfilled)
171489	5-O-Methylembelin	$C_{18}H_{28}O_4$	308.4	1	4	4.4015	v (4 fulfilled)
8294	Linalyl acetate	$C_{12}H_{20}O_2$	196.29	0	2	3.2406	v (4 fulfilled)
173183	Campesterol	$C_{28}H_{48}O$	400.7	1	1	7.6347	v (3 fulfilled)
222284	(-)-beta-Sitosterol	$C_{29}H_{50}O$	414.7	1	1	8.0248	v (3 fulfilled)
5281691	Rhamnetin	$C_{16}H_{12}O_7$	316.26	4	7	2.291	v (4 fulfilled)
5280934	alpha-Linolenic acid	$C_{18}H_{30}O_2$	278.4	1	2	5.6605	v (3 fulfilled)
72277	(-)-Epigallocatechin	$C_{15}H_{14}O_7$	306.27	6	7	1.2517	v (3 fulfilled)
107905	(-)-Epicatechin 3-O-gallate	$C_{22}H_{18}O_{10}$	442.4	7	10	2.5276	v (3 fulfilled)
445724	Fucitol	$C_6H_{14}O_5$	166.17	5	5	5	v (4 fulfilled)
439174	N-Acetyl-D-glucosamine	$C_8H_{15}NO_6$	221.21	5	6	-3.0776	v (4 fulfilled)
5283387	Oleamide	$C_{18}H_{35}NO$	281.5	1	1	5.5092	v (3 fulfilled)
5376350	Cryptoflavin	$C_{40}H_{56}O_2$	568.9	1	2	10.7878	v (2 fulfilled)
46189015	3-Methoxy-5-undecylphenol	$C_{18}H_{30}O_0$	278.4	1	2	5.4741	v (3 fulfilled)
89782462	2,5-Dimethoxy-3-undecylphenol	$C_{19}H_{32}O_3$	308.5	1	3	5.4827	v (3 fulfilled)
1130	Thiamine	$C_{12}H_{17}N_4OS^{+}$	265.36	2	5	0.60774	v (4 fulfilled)
161557	Ampelopsin/Dihydromyricetin	$C_{15}H_{12}O_8$	320.25	6	8	0.8919	v (3 fulfilled)
5281672	Myricetin	$C_{15}H_{10}O_8$	318.23	6	8	1.6936	v (3 fulfilled)
135398658	Folic acid	$C_{19}H_{19}N_7O_6$	441.4	6	10	-0.0448	v (3 fulfilled)
493570	Riboflavin	$C_{17}H20N_4O_6$	376.4	5	7	-1.72356	v (4 fulfilled)
370	Gallic acid	$C_7H_6O_5$	170.12	4	5	0.5016	v (4 fulfilled)
64971	Betulinic acid	$C_{30}H_{48}O_3$	456.7	2	3	7.0895	v (3 fulfilled)
91472	3-Friedelanone/Friedelin	$C_{30}H_{50}O$	426.7	0	1	8.457	v (3 fulfilled)
5280863	Kaempferol	$C_{15}H_{10}O_6$	286.24	4	6	2.2824	v (4 fulfilled)
5280343	Quercetin	$C_{15}H_{10}O_7$	302.23	5	7	1.988	v (4 fulfilled)
128861	Cyanidin	$C_{15}H_{11}O_6^{+}$	287.24	5	5	2.9089	v (4 fulfilled)
311	Citric acid	$C_6H_8O_7$	192.12	4	7	-1.2485	v (4 fulfilled)
5281855	Ellagic acid	$C_{14}H_6O_8$	302.19	4	8	8	v (3 fulfilled)
10494	Oleanolic acid	$C_{30}H_{48}O_3$	456.7	2	3	7.2336	v (3 fulfilled)
159287	Malvidin	$C_{17}H_{15}O_7^{+}$	331.30	4	6	3.2205	v (4 fulfilled)
10189	Eugenin	$C_{11}H_{10}O_4$	206.19	1	4	1.81562	v (4 fulfilled)
445354	Retinol	$C_{20}H_{30}O$	286.5	1	1	5.5103	v (3 fulfilled)
54670067	Ascorbic acid	$C_6H_8O_6$	176.12	4	6	-1.4074	v (4 fulfilled)

Table 2. Ten best ligands against SarsCov2 PL Pro

Compound name	ΔG (kcal/mol)	Plant origin
Postive control GRL0617	-9.3	-
(-)-Epicatechin 3-O-gallate	-8.6	*Averrhoa carambola* (Sweet star fruit)
Folic acid	-8.3	*Syzygium cumini* (Java plum)
Ellagic acid	-7.1	*Syzygium cumini* (Java plum)
Petunidin 3-glucoside	-7.1	*Clitoria ternatea* (Butterfly pea)
Oenin	-7.0	*Clitoria ternatea* (Butterfly pea)
Quercetin	-7.0	*Syzygium cumini* (Java plum)
Myricetin	-7.0	*Syzygium cumini* (Java plum)
Cyanidin	-7.0	*Syzygium cumini* (Java plum)
Delphinidin chloride	-6.9	*Clitoria ternatea* (Butterfly pea)
Genistein	-6.9	*Clitoria ternatea* (Butterfly pea)

Table 3. Interaction profile for the best ligands for inhibition

Ligand	Hydrogen Bond (H bond distance, Å)	Electrostatic/ Hydrophobic Interaction
(-)-Epicatechin 3-O-gallate	Gln269 (3.08 Å) Tyr268 (2.71 Å) Asn267 (2.53 Å)	Pro248
Folic acid	Tyr268 (3.04 Å) Gln269 (2.12 Å) Asp164 (2.45 Å) Pro248 (2.61 Å)	Glu161
Ellagic acid	Gln269 (2.65 Å) Leu162 (2.08 Å) Pro248 (2.97 Å)	Tyr264 Tyr268 Asp164 Gly163
Petunidin 3-glucoside	Asp164 (3.27 Å) Tyr268 (2.91 Å) Thr301 (3.02 Å)	Tyr264 Pro248
Oenin	Arg166 (2.77 Å) Asp164 (3.27 Å) Gln269 (2.60 Å; 3.42 Å; 3.68 Å)	Glu167
Quercetin	Asp164 (2.49 Å) Tyr273 (1.79 Å)	Pro247 Pro248 Tyr264 Tyr268
Myricetin	Gln269 (2.20 Å) Thr301 (2.75 Å) Gly266 (2.70 Å) Tyr268 (3.02 Å)	Pro247 Pro248 Tyr264
Cyanidin	Asp164 (2.40 Å) Tyr273 (2.00 Å) Gly266 (2.87 Å) Tyr268 (3.00)	Pro247 Pro248
Delphinidin chloride	Gln269 (2.12 Å) Thr301 (2.90 Å)	Pro247 Pro248 Tyr264 Tyr268
Genistein	-	Pro247 Pro248 Tyr264

targeting critical proteins like ACE-2 receptors, spike protein, 3CLpro, PLpro, RdRp, and helicase (Illian *et al.* 2021; Purwitasari *et al.* 2023). While some studies have explored Indonesian herbal plants for PLpro inhibition using in silico approaches, plants such as Butterfly pea flowers (*Clitoria ternatea* L.), Sweet star fruit leaves (*Averrhoa carambola* L.), and Java plum leaves (*Syzygium cumini* (L.) Skeels) have not been included in these reports. Additionally, some plant compounds exhibit inhibitory activity against 3CLpro, enhancing their potential for drug development. For example, cubebin from Meniran has shown potential as a 3CLpro inhibitor (Laksmiani *et al.* 2020). However, the extracts of Butterfly pea flowers, Sweet star fruit leaves, and Java plum leaves have not been tested for PLpro inhibitory activity (Indayani 2021; Aini *et al.* 2022; Fazadini and Yzzudin 2022), making them intriguing candidates for investigation. Hence, a study focusing on the inhibitory effects of these three Indonesian herbs would provide new insights.

Clitoria ternatea, known as Butterfly pea, has long been used in traditional medicine to treat various ailments, including constipation, indigestion, arthritis, skin conditions, liver issues, and digestive troubles, as well as possessing anti-diabetic and potential anti-cancer properties (Jeyaraj *et al.* 2021; Purnamayanti *et al.* 2022). Similarly, Averrhoa carambola has been used for thousands of years to treat conditions such as arthralgia, chronic paroxysmal headache, vomiting, lithangiuria, coughing, diabetes, diabetic nephropathy, inflammation, hepatoprotection, cardioprotection, hypertension, and neuroprotection (Astuti 2017; Luan *et al.* 2021). Java plums (*Syzygium cumini*), native to Indonesia and India, are also known for their therapeutic properties. The plant is rich in alkaloids, flavonoids, phenylpropanoids, terpenes, tannins, and lipids (Afendi *et al.* 2012). The seeds contain bioactive compounds that exhibit important pharmacological properties, such as anti-diabetic effects. Phytoconstituents found in *Syzygium cumini* include tannic acid, 4-hydroxydiphenoyl glucose, 3-hydroxy diphenoyl glucose, 1-galloylglucose, resorcinol, p-coumaric acid, corilagin, ellagic acid, quercetin, β-sitosterol, ferulic acid, folic acid, guaiacol, and other compounds (Afendi *et al.* 2012; Das *et al.* 2023). This plant has also been used in Ayurvedic medicine (Calleja *et al.* 2022; Das *et al.* 2023). Previously, extracts of *Clitoria ternatea* flower, *Averrhoa carambola* leaves, and *Syzygium cumini* leaves demonstrated significant inhibition against 3CLpro, another SARS-CoV-2 protease (Oktavianti *et al.* 2023).

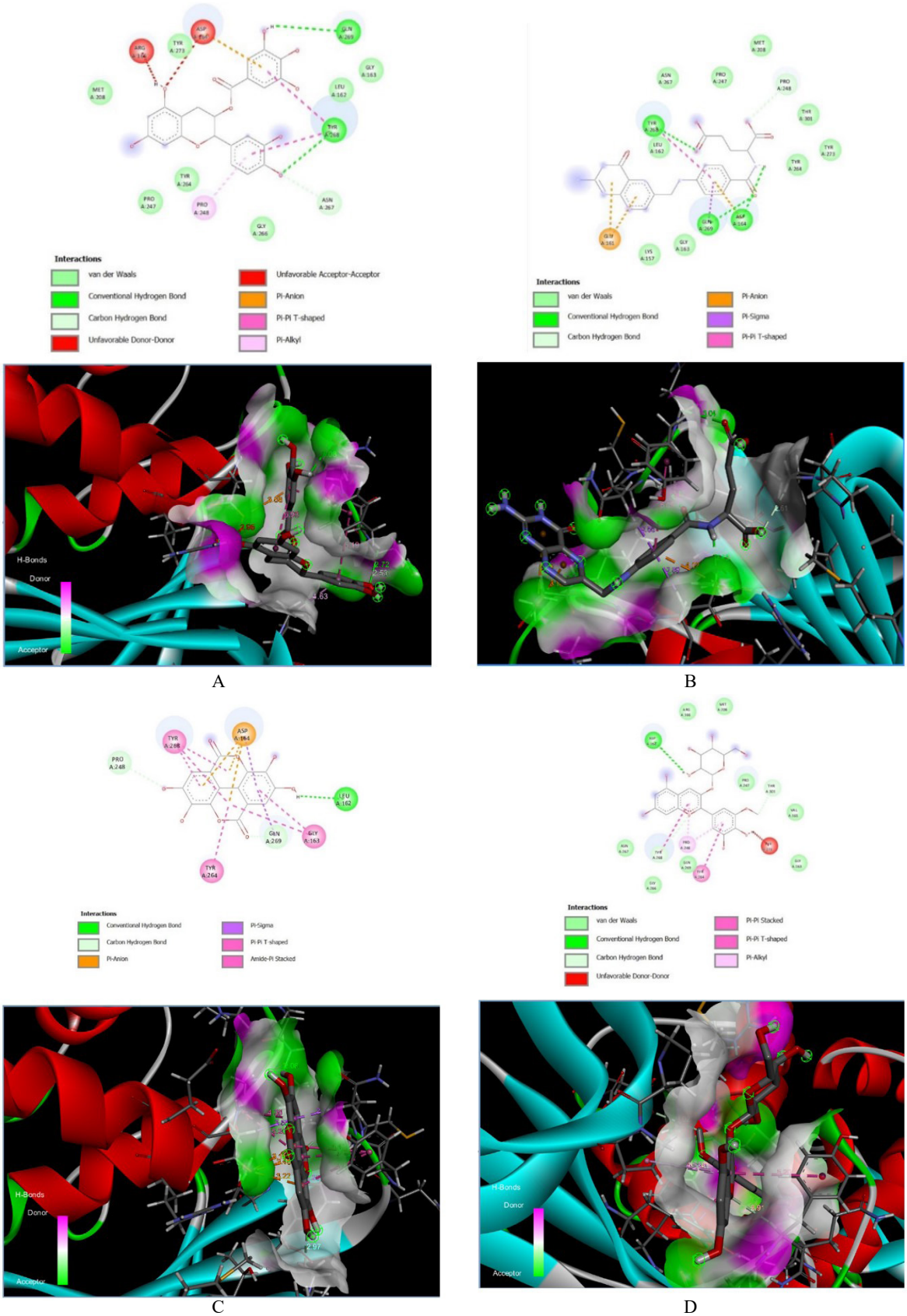

Figure 1. The 2D and 3D visualization of interaction profile of top 4 herbal compounds [(A) (-)-Epicatechin 3-O-gallate; (B) Folic aicd; (C) Ellagic acid; (D) Petunidin 3-glucoside]

Table 4. ADMET profile for the best ligand for inhibition

Compound name	Absorption		Distribution		Metabolism inhibitor CYP					Excretion	Toxicity		
	WS (log mol/L)	HIA	VDss (human)	BBB	1A2	2C19	2C9	2D6	3A4	Total clearance (log ml/min/kg)	AMES toxicity	LD_{50} (mg/kg)	Predicted Toxicity Class
(-)-Epicatechin 3-O-gallate	-2.911	62.096	0.664	-1.847	No	No	No	No	No	-0.169	No	1000	4
Folic acid	-2.88	1.108	0.046	-1.615	No	No	No	No	No	0.527	No	135	3
Ellagic acid	-3.181	86.684	0.375	-1.272	Yes	No	No	No	No	0.537	No	2991	4
Petunidin 3-glucoside	-2.879	42.394	1.112	-1.947	No	No	No	No	No	0.635	No	5000	5
Oenin	-2.835	46.812	0.937	-1.887	No	No	No	No	No	0.676	No	5000	5
Quercetin	-2.925	77.207	1.559	-1.098	Yes	No	No	No	No	0.407	No	159	3
Myricetin	-2.915	65.93	1.317	-1.493	Yes	No	No	No	No	0.422	No	159	3
Cyanidin	-2.935	87.303	0.952	-1.234	Yes	No	No	No	No	0.532	No	5000	5
Delphinidin chloride	-2.916	61.919	0.965	-1.281	No	No	No	No	No	0.572	No	5000	5
Genistein	-3.595	93.387	0.094	-0.71	Yes	Yes	No	No	No	0.151	No	2500	5

WS: Water Solubility. HIA: Human Intestinal Absorption. BBB: Blood Brain Barrier

Table 5. Inhibition percentage value for each herbal extract and its combination

Herbal extract	Percent inhibition
Butterfly pea flower	15.20±1.02
Sweet star fruit	46.14±3.12
Java plum	66.10±3.22
Mix of butterfly pea flower and sweet star fruit	39.45±2.17
Mix of butterfly pea flower and Java plum	36.37±1.81
Mix of sweet star fruit and Java plum	47.10±2.28

Thus, further investigation of these herbs through *in silico* and *in vitro* methods would yield valuable insights.

The *in silico* molecular docking results validated the method with a positive control (GRL0617) binding affinity value of -9.3 kcal/mol, consistent with previous reports (Osorio *et al.* 2022). The top ten compounds identified were (-)-Epicatechin 3-O-gallate, folic acid, ellagic acid, petunidin 3-glucoside, oenin, quercetin, myricetin, cyanidin, delphinidin chloride, and genistein. The inhibitory activity of (–)-epicatechin-3-O-gallate (ECG) against the SARS-CoV-2 spike protein, 3CLpro, PLpro, and NSPs has been reported in other studies (Zu and Xie 2020; Rajak and Ganguly 2023), supporting our findings. Ellagic acid is known for its antiviral properties (Park *et al.* 2014; Acquadro *et al.* 2020; Umar *et al.* 2022), whereas the efficacy of folic acid in combating SARS-CoV-2 remains controversial (Topless *et al.* 2022; Karakousis *et al.* 2023). Molecular docking of compounds from these three herbs revealed their potential as PLpro inhibitors. Of the ten ligands with the most negative binding affinities, five were derived from Java plum, four from Butterfly pea flowers, and one from Sweet star fruit.

The IC_{50} value of the control inhibitor GRL0617 was determined at 3.38 µM, close to the reported range of 1.39–2.4 µM for GRL0617 on Z-RLRGG-AMC (Freitas *et al.* 2020). These findings supported further *in vitro* assays of the three Indonesian herbs. Fluorescence tests confirmed the inhibition of PLpro activity by all the herbal extracts, shown by a decrease in fluorescence intensity and inhibition percentage values. Java plum leaf extract showed the highest inhibition, at 66.10±3.22%, aligning with previous reports of its significant inhibition against 3CLpro (Oktavianti *et al.* 2023). This indicates that Java plum leaf extract can inhibit both PLpro and 3CLpro activity, making it a strong candidate for anti-COVID compounds. However, while the single extract of Java plum leaves had significant inhibitory effects, the combined extracts of the three herbs showed reduced inhibition. While herbal mixtures can sometimes enhance effectiveness, their multiple components often lead to unpredictable and complex interactions (Che *et al.* 2013).

In conclusion, *in silico* and *in vitro* inhibitory assays of compounds from three Indonesian herbs-Butterfly pea flowers, Sweet star fruit leaves, and Java plum leaves-against recombinant SARS-CoV-2 PLpro were conducted. Four ligands with the lowest binding affinities were identified: (-)-Epicatechin 3-O-gallate (Ac), folic acid (Sc), petunidin 3-glucoside (Sc), and ellagic acid (Sc and Ct), with binding scores of -8.6, -8.3, -7.1, and -7.1 kcal/mol, respectively. Java plum leaf extract exhibited significant potential as a PLpro inhibitor, showing the highest inhibition rate. These findings underscore the potential of these herbal extracts as PLpro inhibitors and anti-COVID agents. Further research is needed to isolate

and test individual compounds from these extracts and to conduct animal studies before advancing to clinical trials.

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References

Abou Baker, D.H., Hassan, E.M., El Gengaihi, S., 2023. An overview on medicinal plants used for combating coronavirus: current potentials and challenges. *J Agric Food Res*. 13, 100632. https://doi.org/10.1016/j.jafr.2023.100632

Acquadro, S., Civra, A., Cagliero, C., Marengo, A., Rittà, M., Francese, R., Sanna, C., Bertea, C., Sgorbini, B., Lembo, D., Donalisio, M., Rubiolo, P., 2020. Punica granatum leaf ethanolic extract and ellagic acid as inhibitors of Zika virus infection. *Planta Medica*. 86, 1363–1374. https://doi.org/10.1055/a-1232-5705

Afendi, F. M., Okada, T., Yamazaki, M., Hirai-Morita, A., Nakamura, Y., Nakamura, K., Ikeda, S., Takahashi, H., Altaf-Ul-Amin, Md., Darusman, L. K., Saito, K., Kanaya, S., 2012. KNApSAcK family databases: integrated metabolite–plant species databases for multifaceted plant research. *Plant and Cell Physiology.* 53, e1–e1. https://doi.org/10.1093/pcp/pcr165

Aini, N.S., Kharisma, V.D., Widyananda, M.H., Murtadlo, A.A.A., Probojati, R.T., Turista, D.D.R., Tamam, M.B., Jakhmola, V., Sari, D.P., Albari, M.T., Pernamasari, D., Ghifari, M.A., Ghifari, M.R., Mandeli, R.S., Muhardi, Oktavia, B., Sari, T.K., Sriwahyuni, T., Azhari, P., … Zainul, R., 2022. *In silico* screening of bioactive compounds from *Syzygium cumini* L. and *Moringa oleifera* L. against SARS-CoV-2 via tetra inhibitors. *Pharmacognosy Journal*. 14, 267–272. https://doi.org/10.5530/pj.2022.14.95

Astuti, I.P., 2017. *Averrhoa bilimbi* L., *Averrhoa carambola* L. forma acidis dan *Averrhoa carambola* L. forma dulcis: belimbing tua koleksi bersejarah di Kebun Raya [*Averrhoa bilimbi* L., *Averrhoa carambola* L. forma acidis dan *Averrhoa carambola* L. forma dulcis: old starfruit from the historical collection at the Botanical Gardens]. *Warta Kebun Raya.* 15, 19–24. https://doi.org/10.1201/9780203025901.ch15

BIOVIA. 2021. BIOVIA Discovery Studio

[BPOM] Badan Pengawas Obat dan Makanan 2020. Pedoman Penggunaan Herbal dan Suplemen Kesehatan dalam Menghadapi COVID-19 di Indonesia [Guidelines for the Use of Herbs and Health Supplements in Facing COVID-19 in Indonesia] Available at: https://www.ptonline.com/articles/how-to-get-better-mfi-results. [Date accessed: 23 July 2023]

Cahyaningsih, R., Magos Brehm, J., Maxted, N., 2021. Setting the priority medicinal plants for conservation in Indonesia. *Genet Resour Crop Evol*. 68, 2019–2050. https://doi.org/10.1007/s10722-021-01115-6

Calleja, D.J., Lessene, G., Komander, D., 2022. Inhibitors of SARS-CoV-2 PLpro. *Front Chem*. 10, 1-20. https://doi.org/10.3389/fchem.2022.876212

Chang, J.O., Kim, J., Hong, S., Jeong, K., Kim, S., Lee, W., 2023. Protocol for *in vitro* fluorescence assay of papain-like protease and cell-based immunofluorescence assay of coronavirus infection. *STAR Protocols*. 4, 102295. https://doi.org/10.1016/j.xpro.2023.102295

Chaudhary, K.K., Mishra, N., 2016. A review on molecular docking: novel tool for drug discovery. *JSM Chem*. 4, 1029.

Che, C.T., Wang, Z.J., Chow, M.S.S., Lam, C.W.K., 2013. Herb-herb combination for therapeutic enhancement and advancement: Theory, practice and future perspectives. *Molecules.* 18, 5125-5141. https://doi.org/10.3390/molecules18055125

Chen, Z., Cui, Q., Cooper, L., Zhang, P., Lee, H., Chen, Z., Wang, Y., Liu, X., Rong, L., Du, R., 2021. Ginkgolic acid and anacardic acid are specific covalent inhibitors of SARS-CoV-2 cysteine proteases. *Cell Biosci.* 11, 45. https://doi.org/10.1186/s13578-021-00564-x

Dallakyan, S., Olson, A.J., 2015. Small-molecule library screening by docking with PyRx. In: Hempel J, Williams C, Hong C. (Eds.). *Chemical Biology. Methods in Molecular Biology*, *Vol 1263*. New York: Humana Press. pp. 243–250. https://doi.org/10.1007/978-1-4939-2269-7_19

Das, G.,Nath, R.,Das Talukdar, A., Ağagündüz, D., Yilmaz, B., Capasso, R., Shin, H.S., Patra, J.K., 2023. Major bioactive compounds from Java plum seeds: an investigation of its extraction procedures and clinical effects. *Plants.* 12, 1214. https://doi.org/10.3390/plants12061214

Eberhardt, J., Santos-Martins, D., Tillack, A. F., Forli, S., 2021. Autodock vina 1.2.0: new docking methods, expanded force field, and python bindings. *J Chem Inf Model*. 61, 3891–3898. https://doi.org/10.1021/acs.jcim.1c00203

Fazadini, S.Y., Yzzuddin, A., 2022. *In silico* study: the blue butterfly pea flower (*Clitoria ternatea* L.) compound has potential for herbal medicine for Covid-19. *World J Pharm Res*. 11, 970–985. https://doi.org/10.20959/wjpr20227-24361

Firdayani, Riswoko, A., Helianti, I., 2022. Inhibition of SARS-Cov-2 proteases by medicinal plant bioactive constituents: molecular docking simulation. *IOP Conf Ser Earth Environ Sci.* 976, 1-7. https://doi.org/10.1088/1755-1315/976/1/012054

Freitas, B.T., Durie, I.A., Murray, J., Longo, J.E., Miller, H.C., Crich, D., Hogan, R.J., Tripp, R.A., Pegan, S.D., 2020. Characterization and noncovalent inhibition of the deubiquitinase and deISGylase activity of SARS-CoV-2 Papain-Like protease. *ACS Infect Dis*. 6, 2099-2109. https://doi.org/10.1021/acsinfecdis.0c00168

Illian, D.N., Siregar, E.S., Sumaiyah, S., Utomo, A.R., Nuryawan, A., Basyuni, M., 2021. Potential compounds from several Indonesian plants to prevent SARS-CoV-2 infection: a mini-review of SARS-CoV-2 therapeutic targets. *Heliyon*.

7, e06001. https://doi.org/10.1016/j.heliyon.2021.e06001

Indayani, F.D., 2021. Uji hambatan ekstrak metanol daun belimbing (*Averrhoa carambola* L.) terhadap 3CLpro SARS-CoV-2 [Theses]. Yogyakarta, Indonesia: Universitas Sanata Dharma.

Jabeen, J., Ahmed, N., Shahzad, Z., Shahid, M., Ahmad, T., 2024. Insights for future pharmacology: exploring phytochemicals as potential inhibitors targeting SARS-CoV-2 papain-like protease. *Future Pharmacol*. 4, 510-540. https://doi.org/10.3390/futurepharmacol4030029

Jade, D., Ayyamperumal, S., Tallapaneni, V., Joghee Nanjan, C.M., Barge, S., Mohan, S., Nanjan, M.J., 2021. Virtual high throughput screening: Potential inhibitors for SARS-CoV-2 PLPRO and 3CLPRO proteases. *Eur J Pharmacol.* 901, 174082. https://doi.org/10.1016/j.ejphar.2021.174082

Jeyaraj, E.J., Lim, Y.Y., Choo, W.S., 2021. Extraction methods of butterfly pea (*Clitoria ternatea*) flower and biological activities of its phytochemicals. *J Food Sci Technol.* 58, 2054-2067. https://doi.org/10.1007/s13197-020-04745-3

Karakousis, N.D., Gourgoulianis, K.I., Kotsiou, O.S., 2023. The role of folic acid in SARS-CoV-2 infection: an intriguing linkage under investigation. *J Pers Med.* 13, 561. https://doi.org/10.3390/jpm13030561

Laksmiani, N.P.L, Larasanty, L.P.F, Santika, A.A.G.J, Prayoga, P.A.A, Dewi, A.A.I.K, Dewi, N.P.A.K., 2020. Active compounds activity from the medicinal plants against SARS-CoV-2 using *in silico* assay. *Biomed Pharmacol J.* 13, 873–881. https://doi.org/10.13005/BPJ/1953

Luan, F., Peng, L., Lei, Z., Jia, X., Zou, J., Yang, Y., He, X., Zeng, N., 2021. Traditional uses, phytochemical constituents and pharmacological properties of *Averrhoa carambola* L.: a review. *Front Pharmacol.* 12, 699899. https://doi.org/10.3389/fphar.2021.699899

Ma, C., Sacco, M.D., Xia, Z., Lambrinidis, G., Townsend, J.A., Hu, Y., Meng, X., Szeto, T., Ba, M., Zhang, X., Gongora, M., Zhang, F., Marty, M. T., Xiang, Y., Kolocouris, A., Chen, Y., Wang, J., 2021. Discovery of SARS-CoV-2 papain-like protease inhibitors through a combination of high-throughput screening and a flipGFP-based reporter assay. *ACS Cent. Sci.* 7, 1245–1260. https://doi.org/10.1021/acscentsci.1c00519

Microsoft Corporation. 2019. Microsoft Excel

Mitev, V., 2023. Comparison of treatment of COVID-19 with inhaled bromhexine, higher doses of colchicine and hymecromone with WHO-recommended paxlovid, molnupiravir, remdesivir, anti-IL-6 receptor antibodies and baricitinib. *Pharmacia* 70, 1177-1193. https://doi.org/10.3897/pharmacia.70.e112550

Morris, G.M., Ruth, H., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D.S., Olson, A.J., 2009. Software news and updates AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Comput Chem.* 30, 2785–2791. https://doi.org/10.1002/jcc.21256

Napitupulu, R., Helianti, I., Maimunah, Fatiningtyas, F.A., Malik A., 2023. The development of papain-like protease from SARS-CoV-2, a potential drug target for antiviral screening: A review. *IJBiotech* 28, 158-172. https://doi.org/10.22146/ijbiotech.83376

Napitupulu, R., Malik, A., Ulfah, M., Helianti, I., 2024. Improving soluble PL Pro production in *Escherichia coli* through chaperonin and MBP protein tag: Purification and kinetic characterization. [Manuscript submitted for publication]. Faculty of Pharmacy, Universitas Indonesia

Oktavianti, D., Elya, B., Putra, I.G.E.P., Helianti, I., 2023. Java plum (*Syzygium cumini*) leaves as a potential inhibitor of SARS-CoV-2 3-Chymotrypsine-Like Protease (3CLpro). *Trop J Nat Prod. Res*. 7, 3626-3632. http://www.doi.org/10.26538/tjnpr/v7i8.8

Osorio, M.I., Yáñez, O., Gallardo, M., Zuñiga-Bustos, M., Mulia-Rodríguez, J., López-Rendón, R., García-Beltrán, O., González-Nilo, F., Pérez-Donoso, J.M., 2022. Search for novel potent inhibitors of the SARS-CoV-2 papain-like enzyme: a computational biochemistry approach. *Pharmaceuticals*. 15, 986. https://doi.org/10.3390/ph15080986

Park, S.W., Kwon, M.J., Yoo, J.Y., Choi, H.J., Ahn, Y.J., 2014. Antiviral activity and possible mode of action of ellagic acid identified in *Lagerstroemia speciosa* leaves toward human rhinoviruses. *BMC Complement Altern Med*. 14, 171. https://doi.org/10.1186/1472-6882-14-171

Purnamayanti, A., Budipramana, K.,Gondokesumo, M.E., 2022. The potential application of *Clitoria ternatea* for Cancer treatment. *Pharm Sci Res*. 9, 109-124. https://doi.org/10.7454/psr.v9i3.1253

Purwitasari, N., Siswodihardjo, S., Abdelfatah Alhoot, M., Agil, M., 2023. Pharmacological potential of some Indonesian medicinal plants as promising options for COVID-19 during the pandemic era: a literature review. *J Med Chem Sci*. 6, 2735-2749. https://doi.org/10.26655/JMCHEMSCI.2023.11.18

Rachmawati, N., Maulidiyah, G., Aminah., 2021. Uji daya hambat dan toksisitas ekstrak daun Jamblang [*Syzygium cumini* (L.) skeels] terhadap pertumbuhan bakteri *Staphylococcus epidermidis* [Test of the inhibitory and toxicity of Jamblang [*Syzygium cumini* (L.) skeels] leaf extract on the growth of *Staphylococcus epidermidis*]. *Jurnal Biologi Indonesia*. 17, 39–46. https://doi.org/10.47349/jbi/17012021/39

Rajak, P., Ganguly, A., 2023. *In silico* study unfolds inhibitory potential of epicatechin gallate against SARS-CoV-2 entry and replication within the host cell. *Mechanobiol Med.* 1, 100015.

Reis, S., Metzendorf, M.I., Kuehn, R., Popp, M., Gagyor, I., Kranke, P., Meybohm, P., Skoetz, N., Weibel, S., 2023. Nirmatrelvir combined with ritonavir for preventing and treating COVID-19. *Cochrane Database Syst Rev*. 11, CD015395. DOI:10.1002/14651858.CD015395

Rizma, B.R.P., Ananto, A.D., Sunarwidhi, A.L., 2021. The study of potential antiviral compounds from Indonesian medicinal plants as Anti-COVID-19 with molecular docking approach. *J Mol Docking.* 1, 32–39. https://doi.org/10.33084/jmd.v1i1.2307

Tan, H., Hu Y., Jadhav, P., Tan, B., Wang, J., 2022. Progress and challenges in targeting the SARS-CoV-2 papain-like protease. *J Med Chem.* 65, 11, 7561–7580. https://doi.org/10.1021/acs.jmedchem.2c00303

Tan, B., Zhang, X., Ansari, A., Jadhav, P., Tan, H., Li, K., Chopra, A., Ford, A., Chi, X., Ruiz, F.X., Arnold, E., Deng, X., Wang, J., 2024. Design of SARS-CoV-2 papain-like protease inhibitor

with antiviral efficacy in a mouse model. *Science.* 383, 1434-1440. https://doi.org/10.1126/science.adm9724

Topless, R., Green, R., Morgan, S.L., Robinson, P., Merriman, T., Gaffo, A.L., 2022. Folic acid and methotrexate use and their association with COVID-19 diagnosis and mortality: a case control analysis from the UK Biobank. *BMJ Open* 12, e062945. https://doi.org/10.1136/bmjopen-2022-062945

Trott, O., Olson, A.J., 2009. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*. 31, 455-461. https://doi.org/10.1002/jcc.21334

Ulfah, M., Mulyawati, L., Riswoko, A., Helianti, I., 2022. Expression of recombinant SARS-CoV-2 papain-like protease (SARS-CoV-2 PLpro) in *Escherichia coli* RIPL. *IOP Conf Ser Earth Environ Sci*. 976, 1-9. https://doi.org/10.1088/1755-1315/976/1/012033

Umar, A.K., Zothantluanga, J.H., Aswin, K., Maulana, S., Sulaiman Zubair, M., Lalhlenmawia, H., Rudrapal, M., Chetia, D., 2022. Antiviral phytocompounds "ellagic acid" and "(+)-sesamin" of *Bridelia retusa* identified as potential inhibitors of SARS-CoV-2 3CLpro using extensive molecular docking, molecular dynamics simulation studies, binding free energy calculations, and bioactivity prediction. *Struct Chem.* 33, 1445–1465. https://doi.org/10.1007/s11224-022-01959-3

Yumni, G., Nuraini, I., Nafis, J., Hasyim, W., 2022.Antioxidant profile and total flavonoid content of water and ethyl acetate extract of Butterfly pea flower (*Clitoria ternatea* L.). *Cendekia Eksakta*. 7, 12–17. http://dx.doi.org/10.31942/ce.v7i1.6547

Yunarto, N., Sulistyaningrum, N., 2017. Quantitative analysis of bioactive compounds in extract and fraction of Star fruit (*Averrhoa carambola* L.) leaves using high performance liquid chromatography. *Jurnal Kefarmasian Indonesia*. 7, 26–33. http://doi.org/10.22435/jki.v7i1.5605.26-33

Zhu, Y., Xie, D.Y., 2020. Docking characterization and *in vitro* inhibitory activity of flavan-3-ols and dimeric proanthocyanidins against the main protease activity of SARS-Cov-2. *Front. Plant Sci*. 11, 601316. DOI:10.3389/fpls.2020.601316