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Docking-based workflow and ADME prediction of some compounds in *Curcuma longa* and *Andrographis paniculata* as polymerase PA-PB1 inhibitors of influenza A/H5N1 virus

Sitti Nur Aidah Lya CITRA [1], Arfan ARFAN [1], Armid ALROEM [2], Laode Santiaji BANDE [3], Irnawati IRNAWATI [1], Muhammad ARBA [1]*

1 Department of Pharmacy, Faculty of Pharmacy, Universitas Halu Oleo, Kendari 93232, Indonesia.
2 Department of Chemistry, Faculty of Math and Science, Universitas Halu Oleo, Kendari 93232, Indonesia.
3 Faculty of Agriculture, Universitas Halu Oleo, Kendari 93232, Indonesia.
* Corresponding Author. E-mail: muh.arba@uho.ac.id

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ABSTRACT: Influenza is an infectious disease of the respiratory system caused by the influenza virus. Influenza virus RNA polymerase (RdRp) is essential in viral RNA replication and transcription. This polymerase has acid polymerase (PA) and polymerase basic 1 (PB1) subunits responsible for viral endonuclease and proteolytic activity. This study aims to determine the potential and interactions of the biochemical constituents of turmeric (*Curcuma longa*) and bitter (*Andrographis paniculata*) against the polymerase PA-PB1 (PDB ID: 3CM8) from the influenza A virus. The study was carried out using the molecular docking workflow method with a combination of standard-precision (SP), extra-precision (XP), and induced fit docking (IFD) utilizing the Maestro's Schrodinger. The docking result successfully identified seven compounds in *C. longa* and thirty-four in *A. paniculata*, which had a better docking score than R151785 as a reference ligand. XP docking showed compounds 1 from *C. longa* with a score of -7.555 kcal/mol and 4 from *A. paniculata* with a score of -9.156 kcal/mol. The IFD method results identified compounds 3 from *C. longa* and 5 from *A. paniculata* as potential compounds in inhibiting the activity of the polymerase PA-PB1 with a docking score of -9.979 kcal/mol, and -13.153 kcal/mol, respectively. All the best compounds interacted with critical residues such as Thr7, Gln16, Gln587, Ser594, Lys643, Ser647, Ser659, Ser662, Arg663, and Asn703 of the polymerase PA-PB1 enzyme from influenza A virus. The best compound in turmeric showed an excellent GI absorption profile, and the best compound in bitter showed a good safety profile because it was predicted to only inhibit the CYP3A4 enzyme. All the best compounds from these two plants fulfilled the criteria for oral drugs based on Lipinski's rules. Based on the research, these two plants have a potential antiviral activity to be verified experimentally as a candidate for Influenza A virus inhibitor.

KEYWORDS: *Andrographis paniculata*; *Curcuma longa*; molecular docking; polymerase PA-PB1; Influenza A virus.

1. INTRODUCTION

Influenza is an infectious respiratory disease caused by an influenza virus infection with mild to severe conditions [1]. Influenza viruses are classified into types A, B, C, and D [2]. Types B and C generally cause mild symptoms. Type A and D are the most pathogenic and can cause severe symptoms that can potentially cause an influenza pandemic [3,4]. Influenza is transmitted through droplets containing the virus by coughing or sneezing from an infected individual [1].

The influenza virus can be treated and prevented by vaccinations and antiviral treatment. Vaccination is widely considered to be the most effective for preventing influenza virus infection [5]. However, the current administration of the influenza vaccine still causes significant morbidity and mortality, so it is still necessary to develop antivirals against influenza A [5,6].

RNA-dependent RNA polymerase (RdRp) is an enzyme that can be used as a drug target for the treatment of influenza A virus because it plays an essential role in the replication and transcription of the viral genome [7,8]. RdRp consists of three subunits, namely the acidic polymerase protein (PA), protein polymerase basic 1 (PB1), and protein polymerase basic 2 (PB2) [9]. In addition, residues in the subunits

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(PB1, PB2, and PA) of RdRp are highly conserved in type A viruses, and interestingly RdRp is absent in mammals [10].

These three subunits interact, where PA interacts with PB1 and PB1 interacts with PB2. The C-terminal of PA interacts with the N-terminal of PB1. The PA and PB1 subunits binding involve hydrogen bonding and hydrophobic interactions with the N-terminal residue of PB1, which is located in the PA pocket [10,11]. PB1 and PB2 play a role in viral RNA synthesis and bind to the cap structure of cellular RNA in the viral transcription-initiation process, while PA has functions as RNA cleavage and serine proteinase activity, that are important in the viral life cycle [8,10].

The ability of viruses to mutate can cause antivirals and vaccines to be ineffective due to resistance [12]. Therefore, natural products can be alternative medicine sources [13]. Previous studies have reported that some compounds from turmeric (*C. longa*) and bitter (*A. paniculata*) plants have activity against influenza viruses [14]. Turmeric has activity as an antioxidant, anti-inflammatory, antitumor, and antiviral and also strengthens the immune system [15]. Other studies on curcumin have shown antiviral activity against influenza PR8, H1N1, and H6N1 [16].

Bitter has many therapeutic benefits for treating various illnesses, including influenza, gastrointestinal and respiratory conditions, herpes, fever, and malaria [17]. In another study, it was reported that andrographolide has activity against several viruses such as influenza A virus (IAV), human immunodeficiency virus (HIV), Enterovirus D68 (EV-D68), dengue virus 1(DENV1), and Chikungunya virus (CHIKV) [18,19].

Currently, computational studies are an efficient approach used to design and identify drug candidates by minimizing trial and error in experiments to provide a relatively high confidence level [20,21]. Molecular docking is part of a structure-based drug design in a computational study that predicts a compound's three-dimensional structure and interaction with a therapeutic target as the basis for drug development [22,23]. Based on the description, researchers are interested in studying the molecular docking of a biochemical constituent from turmeric (*C. longa*) and bitter (*A. paniculata*) to inhibit PA-PB1 polymerase from influenza A/H5N1 virus.

According to recent research on PA-PB1 inhibitors of influenza A, the 3-((dibenzylamino)methyl)quinolinone derivative has potent efficacy by preventing the replication of the virus at 50% effective concentrations (EC_{50}) = 0.061-0.226 µM and low toxicity (50% cytotoxic concentration (CC_{50}) >10 µM) [24]. Furthermore, the compounds R160792 and R151785 discovered through screening findings based on the in vitro split luciferase complementation-based assay also demonstrated effective suppression of PA-PB1 from the IAV. In this study, R151785 inhibits PA nuclear localization and viral multiplication and lowers viral RNA and protein levels [9]. The research by Takeshi further demonstrated that baloxavir acid reduces viral RNA transcription by selectively inhibiting Cap-dependent endonuclease in the IAV PA subunit [25]. We expect our discovery to open new avenues for developing PA-PB1 inhibitors of IAV from natural sources.

2. RESULTS AND DISCUSSION

2.1. Docking-based results

Molecular docking of the biochemical constituent in turmeric and bitter was carried out to determine their affinity and interaction with the influenza A virus PA-PB1 polymerase. Here we dock into the polymerase catalytic region following the location of the inhibitor peptide, which has crystallized with polymerase PA-PB1 [26]. In total, this study used 12 compounds from turmeric and 47 compounds from bitter (Table S1-S4). We used three stages of docking, starting with screening with the SP and XP docking procedure and finally with the IFD procedure [27].

In this simulation, the SP and XP procedures used the rigid docking approach, which prevents the receptors from moving throughout the simulation. XP procedure was more precise than SP; hence, it consumed more time during the simulation. This study used SP and XP docking procedures to increase accuracy in assessing compound affinity and conformation by using various precision levels from the docking procedure's scoring function. The compounds with more negative binding energy will be candidates for the next docking stage. Furthermore, the IFD approach was used to analyze the binding energy of the ligand based on applying flexibility to the receptor's residue to clearly define the interaction between the ligand-receptor.

The reference ligand R151785 has binding energies at SP and XP docking of -4.928 kcal/mol and -4.108 kcal/mol, respectively. The simulation results obtained 7 compounds from turmeric and 34 from bitter,

which have better binding energy than the R151785. These results show an increase in the binding energy value of the biochemical constituent of turmeric and bitter when using XP docking with further assessment. Interestingly, the five best compounds from turmeric showed a decrease in binding energy when using XP compared to SP docking (Table 1).

Table 1. The binding energy of the best compounds in turmeric*

Compounds (Structure number)	SP Docking	XP Docking	IFD
R151785	-4.928	-4.108	-6.202
Calebin A (1)	-5.928	-7.555	-8.170
Demethoxycurcumin (2)	-5.062	-5.736	-9.874
bis-Demethoxycurcumin (3)	-4.569	-6.376	-9.979

*All the energies are in kcal/mol.

Compound 1 showed binding energy of -5.928 kcal/mol in the SP procedure and -7.555 kcal/mol in the XP procedure. In the same procedure, compound 2 showed a change in energy from SP to XP of -5,062 kcal/mol to -5,736 kcal/mol. In addition, compounds 3 also showed promising potential with binding energies of -4.569 kcal/mol with SP docking and -6.376 kcal/mol with XP docking procedures to PA-PB1 polymerase. The structures of the three best compounds in turmeric can be seen in Figure 1.

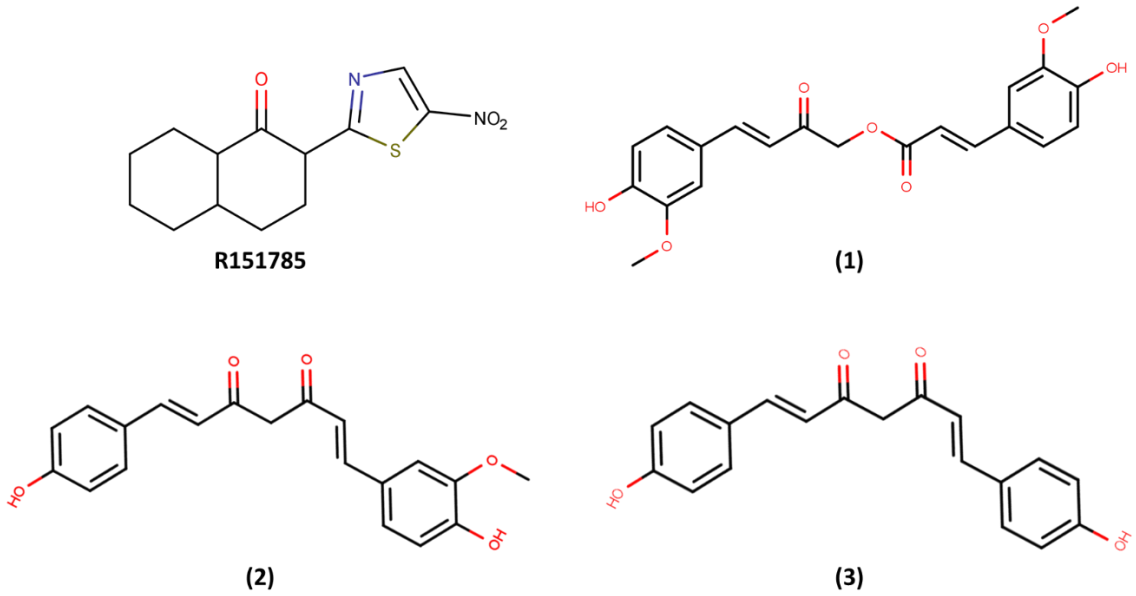

Figure 1. The 2D structure of the reference ligand (R151785) and the best compounds in turmeric

We also applied IFD to the best compounds in turmeric with PA-PB1 polymerase. The results showed that the IFD procedure made the binding energy values increasingly negative. IFD applies flexibility to the receptor-binding domain, which induces conformational changes at the active site upon binding to the ligand [28]. Based on IFD results, compound 3 is predicted to have the lowest docking score, which is -9.979 kcal/mol. Followed by compounds 2, and 1 with energies of -9,874 kcal/mol and -8.170 kcal/mol, respectively.

Interestingly, the docking of compounds from bitter plants generally showed a similar trend in binding energy to those from turmeric plants with SP, XP, and IFD scoring. In bitter plants, the compound with the lowest binding energy was compound 4, which was -7.297 kcal/mol and -9.156 kcal/mol with SP and XP scoring, respectively. Subsequently, compounds 5 and 6 were followed with energies of -6.184 kcal/mol and -7.306 kcal/mol, respectively, with the SP procedure and decreased to -8.646 kcal/mol and -8.532 kcal/mol with the XP procedure. The IFD procedure showed exciting results, where 5 and 6 showed the lowest scores among other compounds. These two compounds had IFD scores of -13.153 kcal/mol and -12.380 kcal/mol. In addition, compound 4 also experienced a decrease in binding energy, which became increasingly negative to -11.320 kcal/mol. The structures and binding energy summary of the best compounds from the bitter plant can be seen in Figure 2 and Table 2.

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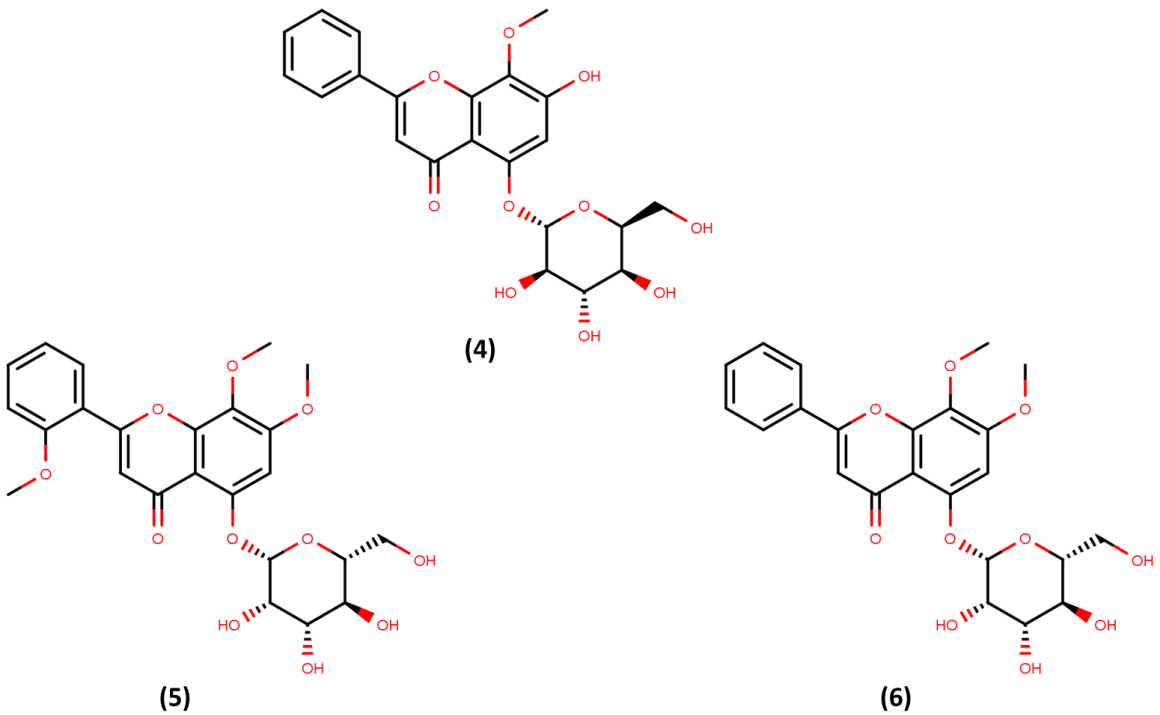

Figure 2. The 2D structures of the best compounds in bitter

Table 2. The binding energy of the best compounds in bitter*

Compounds (Structure number)	SP Docking	XP Docking	IFD
Wogonin 5-glucoside (4)	-7.297	-9.156	-11.320
5-Hydroxy-7,8,2'-trimethoxyflavone 5-glucoside (5)	-6.184	-8.646	-13.153
5-Hydroxy-7,8-dimethoxyflavone 5-glucoside (6)	-7.306	-8.532	-12.380

*All the energies are in kcal/mol.

The interaction analysis from docking results of the R151785 showed the presence of a hydrogen bond (H-bond) contributed by the hydroxy group with the Asn703. R151785 was also observed to form hydrophobic interactions with residues Leu655, Phe658, Trp706, Phe707, Phe10, Leu666, and Leu640 during the simulation. Interaction changes occurred in these compounds when using the IFD procedure. Changes in the interaction of these compounds when using the IFD procedure. The carbonyl group of this compound contributes to the formation of hydrogen bonds with residue Gln591. In addition, new hydrophobic interactions with Leu9, Val13, Pro14, Met595, and Ala598 were observed (Table 3).

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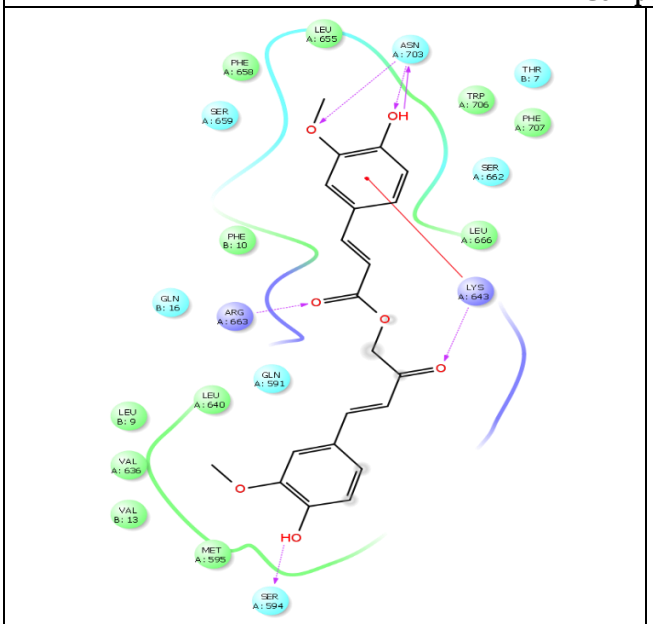
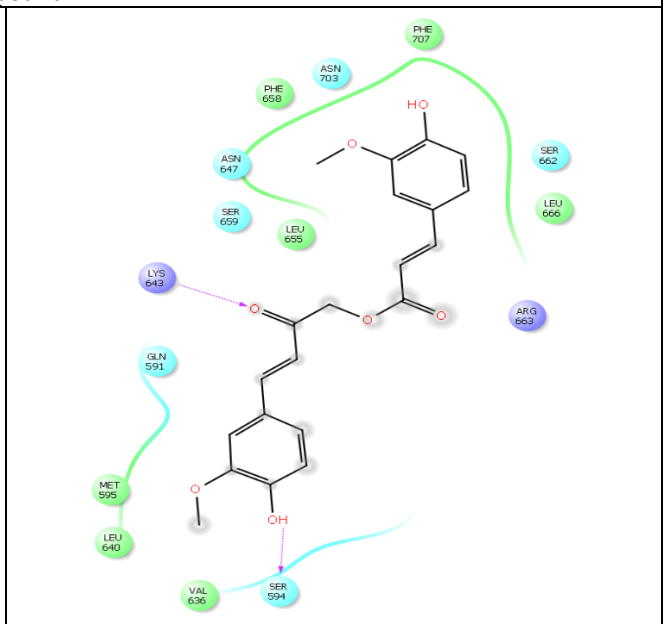
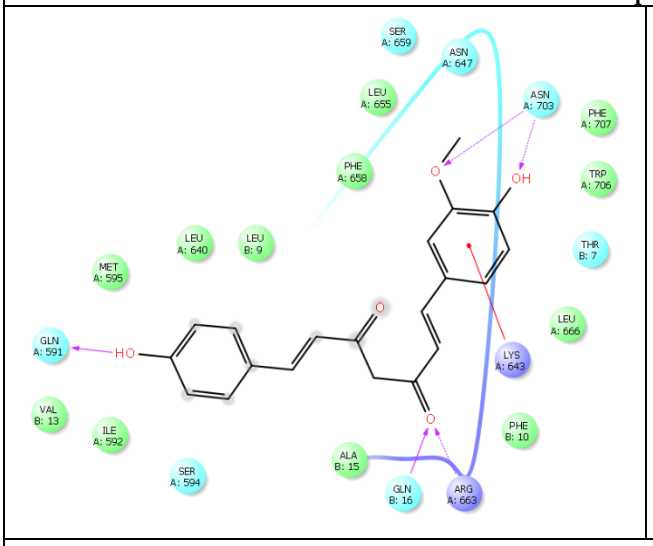
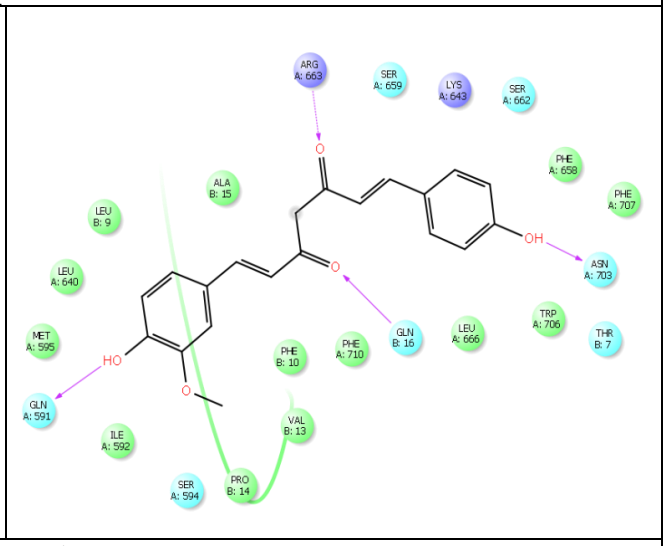
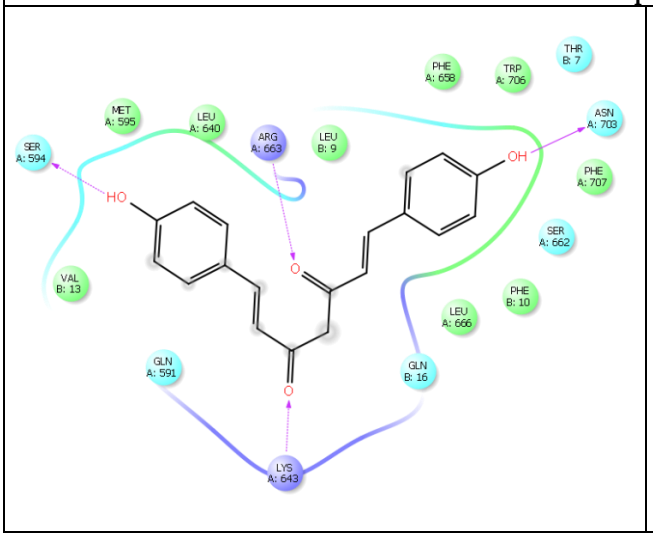
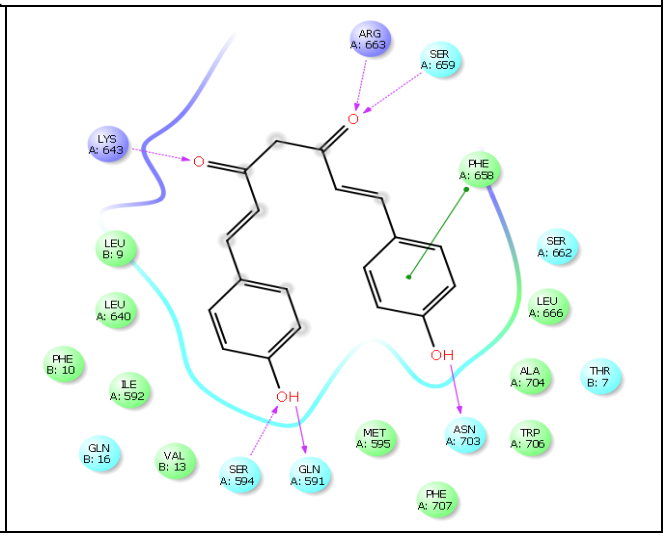
Table 3. The interaction of the best compounds in turmeric

XP Docking	IFD
Compound 1	
	
Compound 2	
	
Compound 3	
	

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During the docking simulation, compounds 1 and 3 showed four H-bonds at residues Asn703, Ser694, Lys643, and Arg663 when applying XP docking. The simulation continued with IFD docking, and the result showed that carbonyl and the hydroxy group from compound 1 maintained their H-bond with Lys643 and Ser594 and the hydroxy group in compound 3 forms two new H-bonds with residues Ser659 and Gln591. The interaction summary of the best compounds in turmeric can be seen in Table 4.

Table 4. The interaction summary of the best compounds in turmeric

Compounds		XP Docking		IFD	
		H-bond	**Hydrophobic**	**H-bond**	**Hydrophobic**
R151785		Asn703	Leu655, Phe658, Trp706, Phe707, Phe10, Leu666, Leu640	Gln591	Leu640, Leu9, Phe10, Val13, Met595, Ala598, Pro14
1		Asn703, Ser594, Lys643, Arg663	Leu666, Trp706, Leu655, Phe658, Phe10, Leu640, Leu9, Val636, Val13, Met959	Ser594, Lys643	Met595, Leu640, Val636, Leu666, Phe707, Phe658, Leu655
2		Asn703, Arg663, Gln16, Gln591	Leu666, Trp706, Leu655, Phe658, Phe10, Leu640, Leu9, Val636, Val13, Met959	Asn703, Arg663, Gln16, Gln591	Met595, Leu640, Leu9, Leu666, Phe707, Phe658, Ala15, Trp706, Phe710, Phe10, Val13, Pro14, Ile592
3		Asn703, Arg663, Ser594, Lys643	Phe707, Trp706, Phe658, Leu666, Leu640, Leu9, Phe10, Met595, Val13	Asn703, Ser659, Lys643, Gln591, Arg663, Ser594	Phe658, Phe707, Phe10, Leu666, Leu640, Val13, Leu9, Met595, Ile592

In compound 2, four hydrogen bonds were observed during docking with the XP procedure. The hydroxy group of the 2-Methoxy-phenol ring forms an H-bond with residue Asn703, and the hydroxy group of the phenyl ring begins an H-bond with residue Gln591. Meanwhile, its carbonyl group includes H-bonds with residues of Arg663 and Gln16. Interestingly in the IFD procedure, these two hydroxy groups exchange H-bond interactions with residues Asn703 and Gln591. Overall, the best compounds exhibited similar hydrophobic interactions in the XP and IFD procedures with Phe658, Leu666, Ala704, Trp706, Phe707, Met595, Val13, Ile529, Phe10, Leu4640, and Leu9 at the binding site of the polymerase PA-PB1 from Influenza A virus. The interaction analysis of the bitter biochemical constituent on polymerase PA-PB1 showed similar H-bonds in compounds 4, and 6 based on the results of XP docking to residues Thr7 and Lys643 from their methoxy and Ser659 groups and Ser662 from their hydroxy groups (Table 5).

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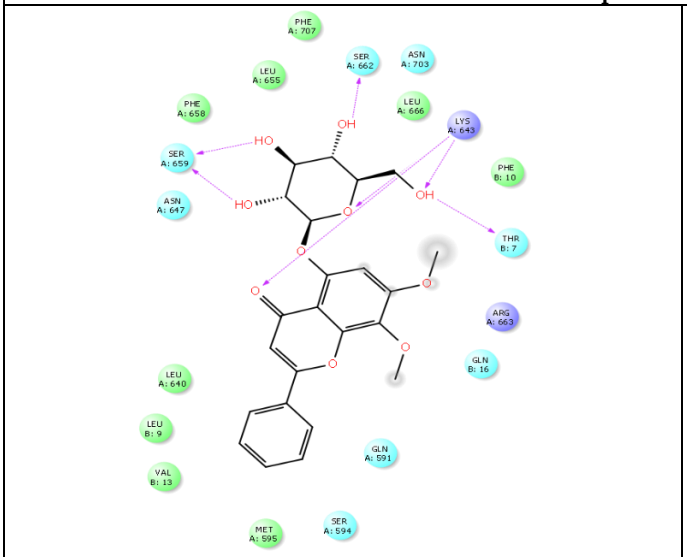
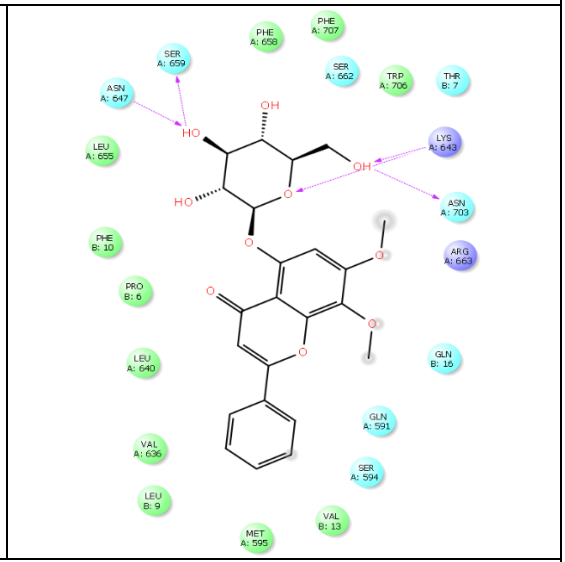
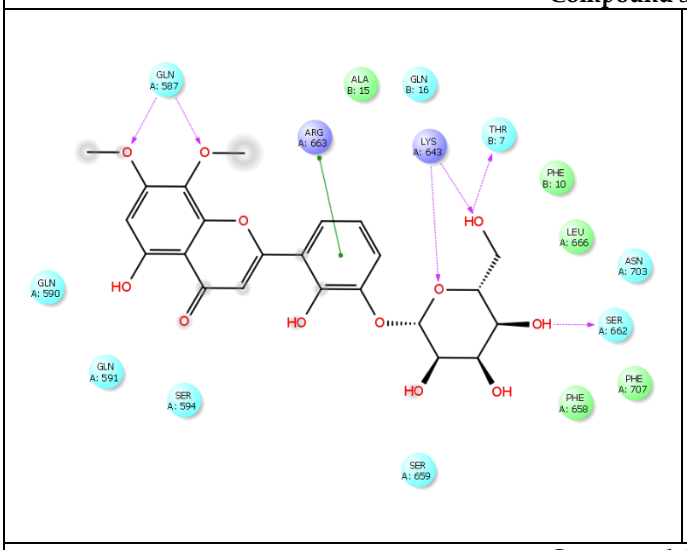
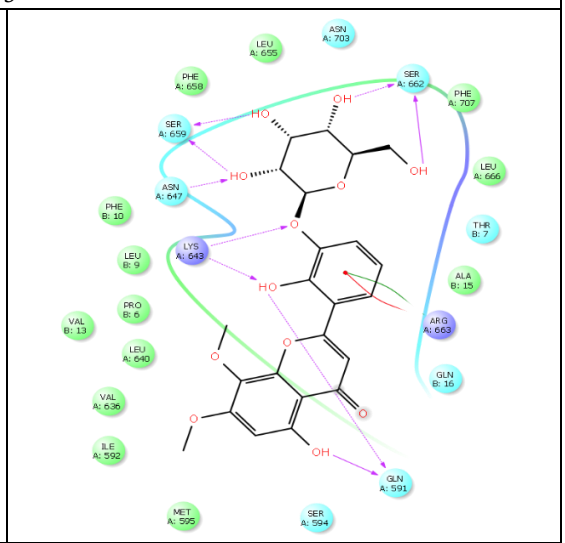
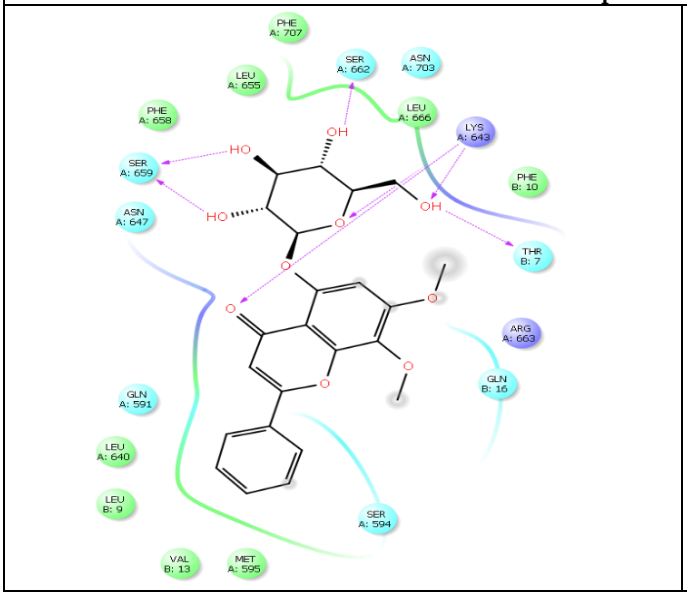
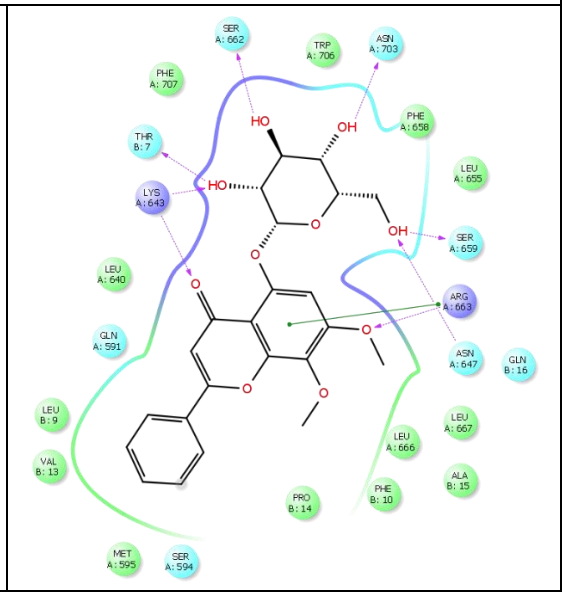
Table 5. The interaction of the best compounds in bitter

XP Docking	IFD
Compound 4	
	
Compound 5	
	
Compound 6	
	

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The two compounds (4 and 6) exhibit new H-bonds with residues Asn701 and Asn647 to the methoxy and hydroxy groups of compound 4 and Gln16 to the methoxy groups of compound 6 when flexibility was applied to the PA-PB1 binding domain by the IFD procedure. The two compounds (4 and 6) exhibit new H-bonds with residues Asn701 and Asn647 to the methoxy and hydroxy groups of compound 4 and Gln16 to the methoxy groups of compound 6 when flexibility was applied to the PA-PB1 binding domain by the IFD procedure. Finally, the methoxy and hydroxy groups of compound 5 form H-bonds with residues Gln587, Lys643, Thr7, and Ser662. The flexibility of the residue on the binding site causes changes in the H-bond interaction to become Ser662, Ser659, Asn647, Lys643, and Gln591. These best compounds formed similar hydrophobic interactions with residues Leu655, Phe658, Phe10, Leu9, Pro6, Val13, Leu640, Val636, Ile592, Met595, Ala15, Leu666, and Phe707. The interaction summary of the best compounds in bitter can be seen in Table 6.

Table 6. The interaction summary of the best compounds in bitter

Compounds		XP Docking		IFD	
		H-bond	Hydrophobic	H-bond	Hydrophobic
4		Thr7, Lys643, Ser662, Ser659	Leu666, Phe707, Leu655, Phe658, Leu640, Leu9, Val13, Met595, Phe10	Asn701, Lys643, Ser659, Asn647	Trp706, Phe707, Phe658, Leu655, Phe10, Pro6, Leu640, Val636, Leu9, Met595, Val13
5		Gln58, Lys643, Thr7, Ser662	Ala15, Phe10, Leu666, Phe707, Phe658	Ser662, Ser659, Asn647, Lys643, Gln591	Leu655, Phe658, Phe10, Leu9, Pro6, Val13, Leu640, Val636, Ile592, Met595, Ala15, Leu666, Phe707
6		Thr7, Lys643, Ser662, Ser659	Leu666, Phe707, Leu655, Phe658, Leu640, Leu9, Val13, Met595, Phe10	Ser662, Thr7, Lys643, Gln16, Ser659	Leu655, Phe658, Phe707, Leu667, Pro6, Leu666, ala15, Phe10, Pro14, Met595, Val13, Ile592, Val636, Leu9, Leu640

2.2. ADME results

We analyzed the ADME prediction results of the best compounds in turmeric and bitter (Table 7). The result shows that compound 2-6 has good solubility (ESOL) (-3.05 to -3.92) except for compound 1, which is predicted to have moderate solubility (-4.01). The compound's solubility strongly supports the drug development process, from the formulation process to its role in drug absorption [29,30]. The best compounds in turmeric showed high GI absorption results compared to compounds in bitter. All of the best compounds were thought not to inhibit the activity of CYP2C19 and CYP2D6 enzymes but had the potential to inhibit CYP3A4 enzymes. The CYP isoforms are metabolizing enzyme that plays a vital role in drug elimination. Inhibition of this enzyme can cause toxicity and even cause unwanted effects [31,32]. Based on the analysis of data similarity of the drug properties with Lipinski's criteria, it is known that all the best compounds have potential as drug candidates (Table 8). In contrast to the others, compound 5 violates one of Lipinski's rules because it has more than ten hydrogen bond acceptors. Compounds that fulfill Lipinski's criteria have the opportunity to be developed as oral drug candidates and have an impact on increasing the bioavailability of these compounds [33].

Table 7. The ADME properties of the best compounds in turmeric and bitter

Compounds	Log S (ESOL)	GI Absorption	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
1	-4.01; Moderately soluble	High	No	No	Yes	No	Yes
2	-3.9; Soluble	High	Yes	No	Yes	No	Yes
3	-3.80; Soluble	High	Yes	No	Yes	No	Yes
4	-3.05; Soluble	Low	No	No	No	No	Yes
5	-3.34; Soluble	Low	No	No	No	No	Yes
6	-3.26; Soluble	Low	No	No	No	No	Yes

Table 8. Lipinski's rule estimation of the best compounds in turmeric and bitter

Compounds	Molecular Weight (≤ 500) (g/mol)	MLOGP (≤ 4.15) (log $P_{o/w}$)	Num. H-bond Acceptors (≤ 10)	Num. H-bond Donors (≤ 5)	Lipinski Rule
1	384.38	1.49	7	2	Yes; 0 violation
2	338.35	1.80	5	2	Yes: 0 violation
3	308.33	2.13	4	2	Yes: 0 violation
4	446.40	-1.39	10	5	Yes: 0 violation
5	490.46	-1.46	11	4	Yes; 1 violation: NorO > 10
6	460.43	-1.18	10	4	Yes; 0 violation

3. CONCLUSION

Molecular docking identified seven compounds from turmeric (C. longa) and 34 compounds from bitter (A. paniculata) that were predicted to have better affinity than R151785 as the reference ligand. The results of docking by the XP method showed the lowest docking values for compounds 1 in turmeric and 4 in bitter, with a value of -7.555 kcal/mol and -9.156 kcal/mol, respectively. IFD docking showed that compounds 3 in turmeric and 5 in bitter had the lowest docking values, with a value of -9.979 kcal/mol and -13.153 kcal/mol, respectively. This study also showed a similar interaction in the binding of the biochemical component to the polymerase PA-PB1 of IAV. Finally, based on the docking strategy, this study successfully identified compounds 4 and 6 that have the potential to inhibit the PA-PB1 enzyme. These compounds showed a good safety profile, inhibiting only the CYP3A4 enzyme and meeting the criteria of an oral drug according to the Lipinski rule.

4. MATERIALS AND METHODS

The PDB structure of the PA-PB1 polymerase enzyme from the influenza A virus was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/) with PDB ID 3CM8. The structure of the enzyme was prepared using Maestro's Protein Preparation Wizard with the default procedure [34].

KNApSAcK-3D was used to collect the 3D structure of the compounds contained in turmeric and bitter (http://knapsack3d.sakura.ne.jp). R151785 was chosen as the reference ligand because it is known by in vitro experimental to inhibit the PA-PB1 polymerase of the influenza A virus [9]. All structures were prepared by ionizing at pH 7 using Epik's pKa calculation, optimized, and minimized in the Maestro's Ligand preparation [34]. All ligands were then docked to polymerase PA-PB1 using standard-precision (SP) and extra-precision (XP) [35], and the top five compounds from turmeric and bitter will be docked using the induced fit docking (IFD) procedures [36] on the maestro's glide [37,38].

Prediction of the ADME properties of the best compounds in turmeric and bitter based on the multilevel docking result was carried out using the swissADME webserver [39].

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