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NARINGIN'S POTENTIAL AS A HEPATITIS B VIRUS REPLICATION INHIBITOR: AN IN-SILICO STUDY OF SECONDARY METABOLITE COMPOUND

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ABSTRACT
Naringin is a secondary metabolite compound of the flavonoid group which is generally found in plants that are consumed and traditionally used as medicine. The aim of this study was to examine the potential of naringin as a candidate for hepatitis B virus replication inhibitor using an in-silico approach. This research uses exploratory descriptive method with molecular docking analysis was carried out using the blind docking technique. The 3D structures of naringin and reference ligands were collected from the PubChem database, and the 3D structures of target proteins were collected from the PDB database. The target protein used is the hepatitis B virus capsid protein with PDB ID: 5GMZ. Docking analysis was performed using AutoDock Vina which is integrated into PyRx. Docking results were visualized using the PyMol software and Biovia Discovery Studio 2019. The results of the analysis showed that the binding affinity of all simulation models between naringin and the HBV capsid protein ranged from -7.1 to -7.9 kcal/mol. The binding site formed between naringin and the receptor corresponds to the reference ligand, involving the same 12 amino acid residues, namely PHE 23, PRO 25, LEU 30, THR 33, TRP 102, ILE 105, SER 106, PHE 110, TYR 118, ILE 139, LEU 140, and SER 141. Based on these results, it can be concluded that the naringin compound has the same bioactivity as the reference ligand in inhibiting viral replication, so that naringin has the potential as a candidate for hepatitis B virus replication inhibitor

Keywords: Antivirus; Hepatitis; Molecular docking; Naringin; Natural compounds

1. INTRODUCTION

Hepatitis B virus infection (HBV) is a health problem that causes acute and chronic liver disease that infects one in three human populations in the world, where around 360 million individuals are at risk of developing chronic hepatitis B disease, cirrhosis and liver cancer (Chuang et al., 2009; Kanda et al, 2019). Diseases due to HBV infection in Indonesia itself are still classified as moderate to high with a prevalence percentage of between 2.5-10% (Muljono, 2017). Based on data findings released by the World Health Organization (WHO) in 2020 regarding the prevalence of hepatitis B in the ASEAN, the prevalence of hepatitis B in children under five years of age in Indonesia is the highest in ASEAN, which is 1.3% (Ministry of Health of the Republic of Indonesia, 2021).

HBV classified into the genus Othohepadnavirus which is a member of the hepadnaviridae virus family with a genome in the form of circular double helix DNA with a length of about 3.2

kb (Li et al., 2020; Littlejohn et al, 2016). HBV uses reverse transcriptase in its replication cycle, which has the ability to replicate like some retroviruses found in animals and pararetroviruses found in plants (Jones & Hu, 2013).

The Food and Drug Administration (FDA) has approved two types of HBV drugs, namely immunomodulators/interferons (IFN-α-2b and peg-IFN-α-2a) and nucleoside analogues (Hu et al., 2019; Tseng et al., 2014), but long-term use of this drug can cause resistance and specific mutations in HBV so that the drug's efficacy decreases (Firdayani et al., 2017; Khalil et al., 2020). One of the efforts made is to use the capsid protein of HBV as a drug target because it has an important role in the HBV replication cycle (Chen et al., 2021; Liu, et al., 2021). Previous studies showed that the interaction between 4-methyl heteroaryldihydropyrimidine (4-methyl HAP) was able to inhibit HBV replication (Qiu et al., 2016b). The study of the use of natural compounds from plants that have medicinal properties is also one of the efforts that can be implemented to deal with this problem.

Naringin is a compound with the molecular formula C27H32O14 with a molecular weight of 580.5 g/mol (NCBI, 2022). Compounds belonging to the flavonoid class can be found as secondary metabolites in plants which are generally consumed and also used as traditional medicines (such as: Drynaria fortunei, Citrus aurantium and Citrus medica) (Zhang et al., 2014). Consuming foods that contain naringin can reduce the risk of various diseases, including cancer and cardiovascular diseases (Fadholly et al., 2020). Naringin also exhibits bioactivity as an anti-inflammatory (Chtourou et al., 2016; Jain & Parmar, 2011), as well as an antiviral (Roy et al., 2022). Therefore, this study was conducted to examine the potential of naringin compounds as candidates for HBV replication inhibitors using an in-silico approach.

2. METHODS

2.1. Study Area

The study used a descriptive exploratory method that examined the potential of naringin compounds in inhibiting hepatitis B virus (HBV) replication activity using an in-silico approach through molecular docking techniques. The research was carried out in July-November 2022 at the computer laboratory of the Faculty of Teacher Training and Education, Universitas Samudra. This in silico study used the PubChem compound database and the Protein Data Bank (PDB) as data sources, as well as PyMol, PyRx, and Biovia Discovery Studio 2019 software for the docking and visualization analysis stages.

2.2. Collection of Ligands and Receptor

The 3D structure of the naringin compound was obtained from the PubChem database with CID 442428 (NCBI, 2022). The 3D structure of naringin was downloaded in *.sdf format (**Figure 1a**). In addition, the reference ligand structures are also collected from the same database. The reference ligand used was 4-methyl heteroaryldihydropyrimidine (4-methyl HAP) (Khalil et al., 2020; Qiu et al., 2016a), with CID 121488107 (NCBI, 2022) (**Figure 1b**).

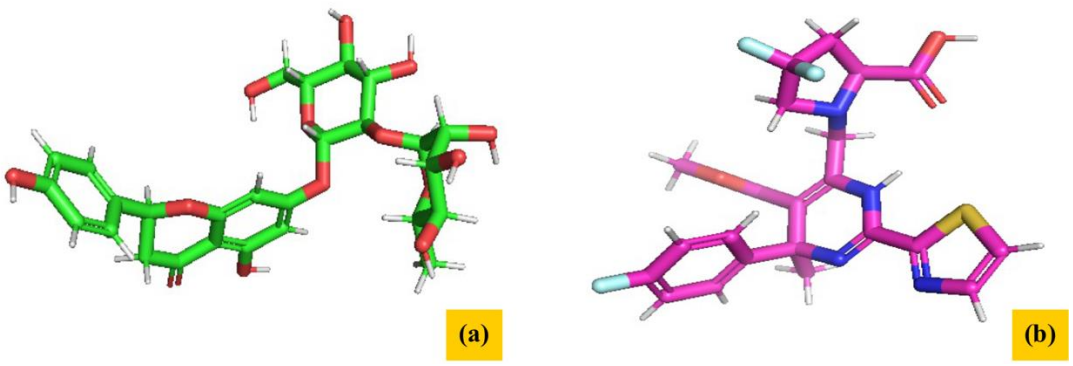

Figure 1. 3D structure of (a) naringin compound; and (b) reference ligand

As for the receptor used in this study, the core protein of the HBV capsid was collected from a protein database, namely PDB. The 3D structure of the protein used has an ID of 5GMZ with a resolution of 1.70 Å (**Figure 2a**). The 3D structure of the HBV core protein was obtained using the x-ray diffraction method, the structure consists of six chains with a sequence length of 155. The HBV capsid protein forms a complex with 4-methyl HAP (ligand ID: 6XU), which is the reference ligand that will be used in molecular docking analysis. Then the 3D structure is downloaded in *.pdb format and saved for further analysis.

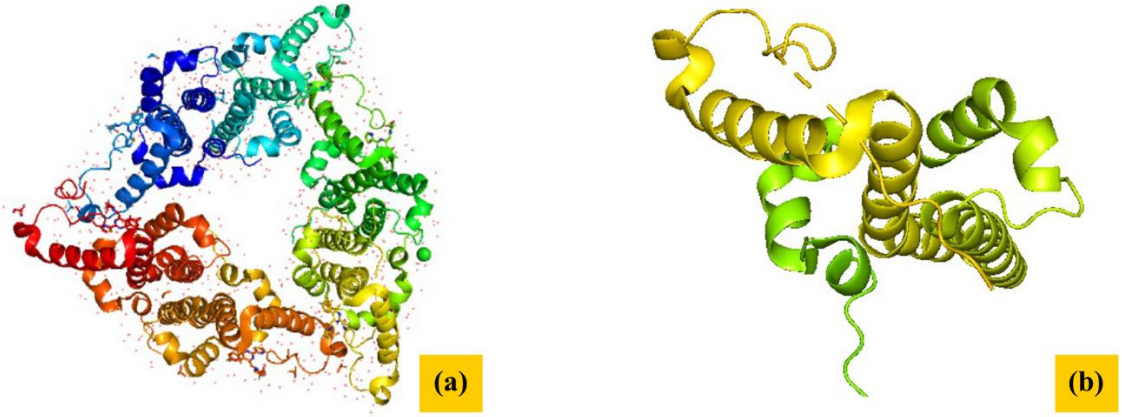

Figure 2. 3D structure of (a) HBV capsid protein; and (b) HBV capsid chains were prepared

2.3. Ligands and Receptor Preparation

The naringin compound and the reference ligand obtained from PubChem were then prepared by optimizing and converting the format. Optimization and conversion of naringin compounds and reference ligands were carried out using the Open babel software which has been integrated with PyRx (Husain et al., 2022). Optimization is carried out to minimize the energy used by the compound and make the ligand stable (Hanif et al., 2020). Conversion of compounds and ligands was carried out to change the format from *.sdf to *.pdb so that it can be read by PyRx software for molecular docking analysis.

HBV capsid protein receptors were prepared using the PyMol software. The preparation was carried out by selecting a chain with the most complete amino acid from the six chains available in the 3D structure that was previously downloaded. This study used the D chain from the available sequences which is the chain with the most complete sequence compared to the other five chains. The chain is then cleaned of contaminating ligands and water molecules and stored in *.pdb format (**Figure 2b**).

2.4. Analysis and Interpretation

Naringin compounds, reference ligands, and HBV capsid proteins were analyzed by molecular docking technique in the form of blind docking. This blind docking strategy is used so that the program runs the search for the most optimal binding sites on all sides of the protein with the lowest binding energy (binding affinity) (Du et al., 2016; Yan et al, 2016). Docking using AutoDock Vina software integrated with PyRx (Trott & Olson, 2009).

Binding affinity data obtained from molecular docking analysis are then interpreted by comparing the scores between the tested compounds and the reference ligands used. The smaller the score formed indicates that the bond formed is more optimal (Atkovska et al., 2014; Pantsar & Poso, 2018). Data analysis results were also visualized using PyMol and Biovia Discovery Studio 2019 (Kusumawati et al., 2022), so that binding sites were obtained and interactions formed in the docking complex between the naringin compound and the HBV capsid protein and between the reference ligand and the HBV capsid protein (Seeliger & de Groot, 2010). The binding site data and the interactions that are formed can be used as an indicator showing that the tested compound has relatively the same bioactivity as the reference compound used in the study.

3. RESULTS AND DISCUSSION

3.1. General Properties of Naringin and Reference Ligand

Treatment with the use of herbal plants is an alternative effort whose trend is currently popular in curing various diseases. The use of herbal plants has been passed down from generation to generation and has provided guidance in the selection, preparation and application of herbal formulations to control and treat various diseases. Healing using herbal plants has been carried out for endocrine and metabolic, immune, neurodegenerative diseases, and even for curing diseases caused by bacterial and viral infections (Khan & Ahmad, 2019)

Herbal plants contain various secondary metabolite compounds which are natural products found in plants. Naringin is an example of a secondary metabolite compound found in plant extracts. The naringin compound belongs to the flavonoid group which is widely found in medicinal plants and fruits (Rivoira et al., 2021). Naringin is a secondary metabolite compound that is commonly found in plants such as tomatoes, grapefruits, plants of the Citrus genus, and in herbal plants that are commonly used for traditional medicine such as *Hydrocotile sibthorpioides* (Hazarika et al., 2021; Husin al., 2015)

Detailed information regarding the reference ligands and natural compounds used are presents in **Table 1** and their 2D structures are presented in **Figure 3**. The table presents PubChem ID, chemical formula, molecular weight, hydrogen bond donor (HbD), hydrogen bond acceptor (HbA), number of rotational bonds (nRB), partition coefficient (LogP), and also **Figure 3** show the 2D structures of the ligands and compounds used in this study. This information is obtained from the PubChem database.

Table 1. General information regarding reference ligands and naringin compounds

Compounds	PubChem ID	Chemical formula	Molecular weight (g/mol)	HbD	HbA	nRB	LogP
Naringin	442428	$C_{27}H_{32}O_{14}$	580.5	8	14	6	-0.5
4-methyl HAP (reference ligand)	7121488107	$C_{22}H_{21}F_3N_4O_4S$	494.5	1	11	7	0.9

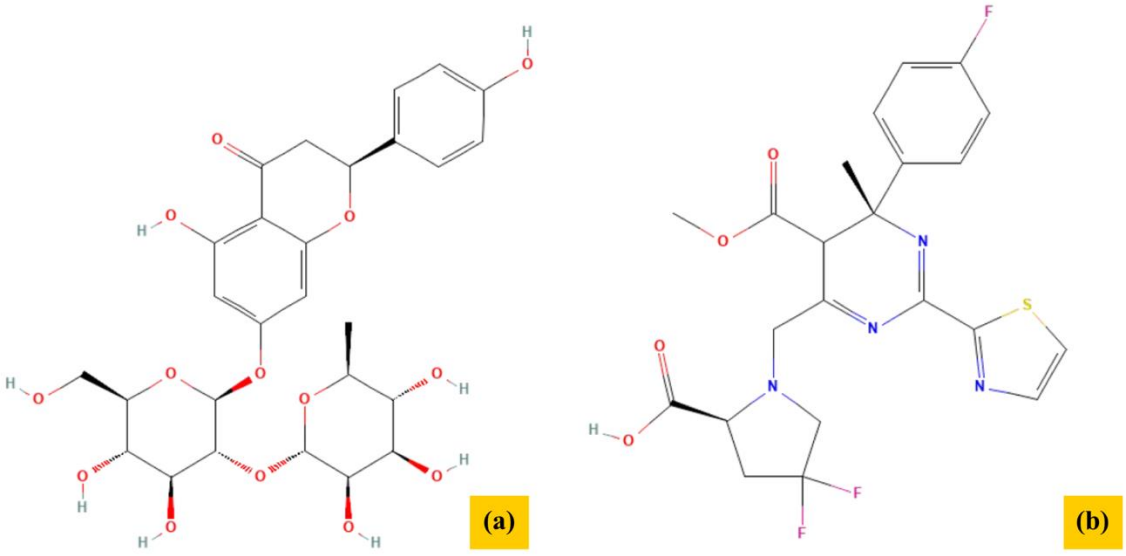

Figure 3. 2D structure regarding reference ligands and naringin compounds

3.2. Docking Analysis

The molecular docking analysis carried out in this study used the blind docking technique. This technique is used for the detection of possible binding sites and modes of peptide ligands by

scanning the entire surface of protein targets, in order to obtain comprehensive docking results. The grid box is set to cover the entire protein so that the AutoDock Vina program algorithm will look for the most optimal ligand binding locations in all parts of the target protein (Sharma et al., 2018). The results of the analysis show nine tethering model simulations for each interaction. The docking results between the reference ligand and the HBV capsid protein showed nine simulation models with binding affinity scores ranging from -6.2 to -6.6 kcal/mol. Meanwhile, the model that successfully simulated the binding between the naringin compound and the HBV capsid protein showed a binding affinity score between -7.3 to -7.9 kcal/mol (**Table 2**).

Table 2. Binding affinity score between ligand and receptor

Ligand	Mode	Binding Affinity (kcal/mol)	RMSD/UB (Å)	RMSD/LB (Å)
5gmz—reference ligand	0	-6.6	0	0
	1	-6.3	7.242	3.273
	2	-6.3	6.239	3.999
	3	-6.3	8.618	4.958
	4	-6.3	20.348	18.03
	5	-6.3	17.443	15.403
	6	-6.2	22.277	18.212
	7	-6.2	17.48	15.146
	8	-6.2	24.706	21.227
5gmz—naringin	0	-7.9	0	0
	1	-7.5	20.467	16.789
	2	-7.5	20.879	17.633
	3	-7.4	6.033	3.017
	4	-7.3	8.567	2.691
	5	-7.1	1.592	1.354
	6	-7.1	19.77	16.376
	7	-7.1	21.606	18.033
	8	-7.1	25.838	21.88

The results of redocking the reference ligand, 4-methyl heteroaryldihydropyrimidine (4-methyl HAP), with the structure of the HBV capsid protein showed that the interaction between the two formed a complex that had the highest binding affinity score of -6.6 kcal/mol in the mode 0 simulation results. Meanwhile, the docking results of naringin with the structure of the HBV capsid protein produces a complex that interacts with a binding affinity score in mode 0 of -7.9 kcal/mol the naringin complex are more optimal. The binding affinity score is a value that indicates the number of Gibbs free energy (ΔG) of binding. The smaller the value, the less energy is required to form an optimal bond (Bitencourt-Ferreira et al., 2019; Shen et al., 2020).

3.3. Binding Site and Interactiom Formed to HBV Capsid Protein

The 3D structures of naringin, reference ligand, and HBV capsid protein formed were visualized with PyMol to study binding sites. The visualization results show that naringin and the reference ligand form a complex with the HBV capsid protein at relatively the same binding site (**Figure 4**).

Further visualization analysis was carried out to see the interactions formed between the ligand and the receptor using the Biovia Discovery Studio 2019 software. The visualization was carried out by activating the display of the interaction of the ligand and the amino acid residues of the receptor. Visualization of these chemical interactions is necessary to determine the complex character of the ligand target protein and maintain molecular interactions, stability, and dynamics (Fatchur & Putra, 2020). The results of visualization of the reference ligand complex with amino acid residues from the HBV capsid protein show various interactions that are formed, namely van der Waals interactions on amino acid residues PHE 23, SER 106, THR 109, ILE 139, SER 141, and THR 142, Conventional Hydrogen bond on amino acid residues THR 33 and TRP 102,

halogen (fluorine) interactions on ASP 29, pi-sulfur interactions on TYR 118, pi-pi T-shaped interactions on TYR 118, and alkyl and pi-alkyl interactions involving PRO 25 amino acids , LEU 30, LEU 37, TRP 102, ILE 105, PHE 110, and LEU 140 (**Figure 5**).

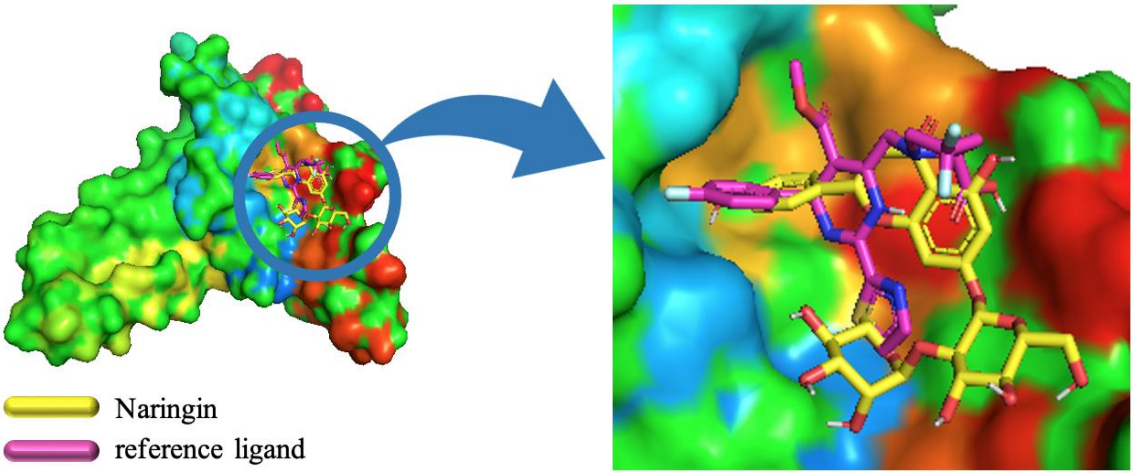

Figure 4. Visualization of binding sites between naringin, reference ligand, and HBV capsid protein docked

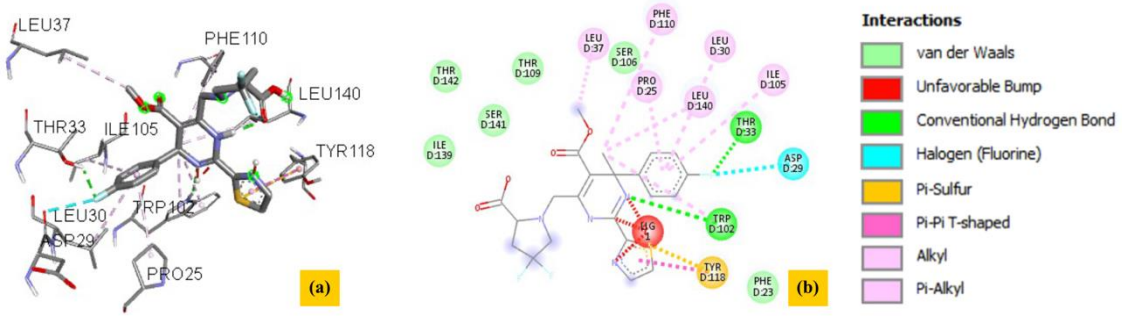

Figure 5. Visualization of reference ligand interactions (a) 3D structure; and (b) 2D structure

While the results of visualization of the naringin compound complex with amino acid residues from the HBV capsid protein show interactions that are formed, including van der Waals interactions on amino acid residues ASP 22, PRO 25, LEU 30, THR 33, ILE 105, SER 106, PHE 110, PHE 122, ASN 136, ALA 137, PRO 138, and SER 141, conventional hydrogen bond interactions at amino acid residues PHE 23 and TYR 118, carbon hydrogen bond interactions and pi-donor hydrogen bonds at ILE 139 and LEU 140, and alkyl interactions and pi-alkyl at amino acid residues PHE 23, TRP 102, TYR 118, ILE 139 and LEU 140 (**Figure 6**).

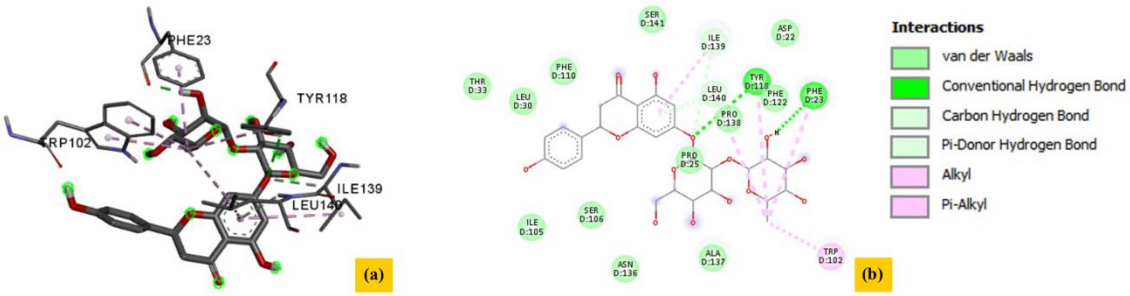

Figure 6. Visualization of the interaction of naringin compounds (a) 3D structure; and (b) 2D structure

The visualization performed on the two docking complexes showed that the naringin compound formed a binding site that was relatively the same when compared to the reference ligand used. The interaction between 4-methyl HAP and HBV capsid protein involves a total of

16 amino acid residues from the capsid protein structure, namely PHE 23, PRO 25, ASP 29, LEU 30, THR 33, LEU 37, TRP 102, ILE 105, SER 106, THR 109, PHE 110, TYR 118, ILE 139, LEU 140, SER 141, and THR 142 by van der Waals interaction, conventional hydrogen bond, halogen (fluorine), pi-sulfur, pi-pi T-shaped, alkyl and pi- alkyl. Meanwhile, the interaction between the naringin complex and the HBV capsid protein involves 17 amino acid residues, namely ASP 22, PHE 23, PRO 25, LEU 30, THR 33, TRP 102, ILE 105, SER 106, PHE 110, TYR 118, PHE 122, ASN 136, ALA 137, PRO 138, ILE 139, LEU 140, and SER 141 with van der Waals interactions, conventional hydrogen bonds, carbon hydrogen bonds and pi-donor hydrogen bonds, alkyl and pi-alkyl. Naringin and 4-methyl HAP bind to the HBV capsid protein at sites involving the exact same 12 amino acid residues, namely PHE 23, PRO 25, LEU 30, THR 33, TRP 102, ILE 105, SER 106, PHE 110, TYR 118, ILE 139, LEU 140, and SER 141. The reference ligand has formed a conventional hydrogen bond on the THR 33 and TRP 102 amino acid residues of the target protein. Whereas in the target naringin-protein complex, the conventional hydrogen bonds that are formed are PHE 23 and TYR 118. 4-methyl HAP has bioactivity in interrupting the normal capsid formation of HBV so that it can block HBV replication (Cole, 2016; Qiu et al., 2017, 2016b).

Based on data from in silico analysis in this study with indicators of comparison of binding affinity scores, binding sites, and interactions formed between the tested compound and reference ligands on viral proteins, naringin has bioactivity inhibiting HBV replication, so this compound has the potential to be used as a candidate.

4. CONCLUSION

It can be concluded that the naringin compound has potential as an inhibitor of hepatitis B virus replication based on the results of molecular docking. The results of the analysis showed that naringin and HBV capsid protein formed a complex with a binding affinity score of -7.9 kcal/mol (<-6.6 kcal/mol). Naringin binds to the binding site according to the reference ligand and forms the same interaction with the 12 amino acid residues of the structure of the HBV capsid protein complex and the reference ligand. Therefore, naringin has the same potential as the reference ligand so that it can be developed as a candidate for HBV replication inhibitor.

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6. CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

7. REFERENCES

Ahmad Khan, M. S., & Ahmad, I. (2019). Herbal medicine: current trends and future prospects. In *New Look to Phytomedicine* (pp. 3–13). https://doi.org/10.1016/B978-0-12-814619-4.00001-X

Atkovska, K., Samsonov, S., Paszkowski-Rogacz, M., & Pisabarro, M. (2014). Multipose Binding in Molecular Docking. *International Journal of Molecular Sciences*, *15*(2), 2622–2645. https://doi.org/10.3390/ijms15022622

Bitencourt-Ferreira, G., Pintro, V. O., & de Azevedo, W. F. (2019). *Docking with AutoDock4*. https://doi.org/10.1007/978-1-4939-9752-7_9

Chen, L., Liu, W.-G., Xiong, F., Ma, C., Sun, C., Zhu, Y.-R., … Wang, Z.-H. (2021). 3D-QSAR, molecular docking and molecular dynamics simulations analyses of a series of heteroaryldihydropyrimidine derivatives as hepatitis B virus capsid assembly inhibitors. *New Journal of Chemistry*, *45*(47), 22062–22076. https://doi.org/10.1039/D1NJ02542B

Chtourou, Y., Aouey, B., Aroui, S., Kebieche, M., & Fetoui, H. (2016). Anti-apoptotic and anti-inflammatory effects of naringin on cisplatin-induced renal injury in the rat. *Chemico-Biological Interactions*, *243*, 1–9. https://doi.org/10.1016/j.cbi.2015.11.019

Chuang, S.-C., Vecchia, C. La, & Boffetta, P. (2009). Liver cancer: Descriptive epidemiology and risk factors other than HBV and HCV infection. *Cancer Letters*, *286*(1), 9–14. https://doi.org/10.1016/j.canlet.2008.10.040

Cole, A. G. (2016). Modulators of HBV capsid assembly as an approach to treating hepatitis B virus infection. *Current Opinion in Pharmacology*, *30*, 131–137. https://doi.org/10.1016/j.coph.2016.08.004

Du, X., Li, Y., Xia, Y. L., Ai, S. M., Liang, J., Sang, P.,Liu, S. Q. (2016). Insights into protein–ligand interactions: Mechanisms, models, and methods. *International Journal of Molecular Sciences*, *17*(2), 1–34. https://doi.org/10.3390/ijms17020144

Fadholly, A., Ansori, A. N. M., & Sucipto, T. H. (2020). An overview of naringin: Potential anticancer compound of Citrus fruits. *Research Journal of Pharmacy and Technology*, *13*(11), 5613–5619. https://doi.org/https://doi.org/10.5958/0974-360X.2020.00979.8

Fatchur, R., & Putra, W. E. (2020). Short Communication: The bioinformatics perspective of Foeniculum vulgare fruit's bioactive compounds as natural anti-hyperglycemic against alpha-glucosidase. *Biodiversitas Journal of Biological Diversity*, *22*(1). https://doi.org/10.13057/biodiv/d220111

Firdayani, Kusumaningrum, S., & Miranti, Y. R. (2017). Potensi Senyawa Bioaktif Tanaman Genus Phyllanthus Sebagai Inhibitor Replikasi Virus Hepatitis B. *Jurnal Bioteknologi & Biosains Indonesia (JBBI)*, *4*(2), 85. Retrieved from http://ejurnal.bppt.go.id/index.php/JBBI/article/view/2589

Hanif, A. U., Lukis, P. A., & Fadlan, A. (2020). Pengaruh Minimisasi Energi MMFF94 dengan MarvinSketch dan Open Babel PyRx pada Penambatan Molekular Turunan Oksindola Tersubstitusi. *Alchemy*, *8*(2), 33–40. https://doi.org/10.18860/al.v8i2.10481

Hazarika, I., Mukundan, G. K., Sundari, P. S., & Laloo, D. (2021). Journey of Hydrocotyle sibthorpioides Lam.: From traditional utilization to modern therapeutics—A review. *Phytotherapy Research*, *35*(4), 1847–1871. https://doi.org/10.1002/ptr.6924

Heidary Moghaddam, R., Samimi, Z., Moradi, S. Z., Little, P. J., Xu, S., & Farzaei, M. H. (2020). Naringenin and naringin in cardiovascular disease prevention: A preclinical review. *European Journal of Pharmacology*, *887*, 173535. https://doi.org/10.1016/j.ejphar.2020.173535

Hu, Y., Ye, Y.-Z., Ye, L.-J., Wang, X.-H., & Yu, H. (2019). Efficacy and safety of interferon alpha-2b versus pegylated interferon alpha-2a monotherapy in children with chronic hepatitis B: a real-life cohort study from Shanghai, China. *World Journal of Pediatrics*, *15*(6), 595–600. https://doi.org/10.1007/s12519-019-00303-w

Husain, D. R., Wardhani, R., & Erviani, A. E. (2022). Antibacterial activity of bacteria isolated from earthworm (Pheretima sp.) gut against Salmonella typhi and Staphylococcus aureus: in vitro experiments supported by computational docking. *Biodiversitas Journal of Biological Diversity*, *23*(2). https://doi.org/10.13057/biodiv/d230257

Husin, F., Chan, Y. Y., Gan, S. H., Sulaiman, S. A., & Shueb, R. H. (2015). The Effect of Hydrocotyle sibthorpioides Lam. Extracts on In Vitro Dengue Replication. *Evidence-Based Complementary and Alternative Medicine*, *2015*, 1–9. https://doi.org/10.1155/2015/596109

Jain, M., & Parmar, H. S. (2011). Evaluation of antioxidative and anti-inflammatory potential of hesperidin and naringin on the rat air pouch model of inflammation. *Inflammation Research*, *60*(5), 483–491. https://doi.org/10.1007/s00011-010-0295-0

Jones, S. A., & Hu, J. (2013). Hepatitis B virus reverse transcriptase: diverse functions as classical and emerging targets for antiviral intervention. *Emerging Microbes & Infections*, *2*(1), 1–11. https://doi.org/10.1038/emi.2013.56

Kanda, T., Goto, T., Hirotsu, Y., Moriyama, M., & Omata, M. (2019). Molecular Mechanisms Driving Progression of Liver Cirrhosis towards Hepatocellular Carcinoma in Chronic Hepatitis B and C Infections: A Review. *International Journal of Molecular Sciences*, *20*(6),

1358. https://doi.org/10.3390/ijms20061358
Khalil, M., Amin, M., & Lukiati, B. (2020). Analisis potensi senyawa repensol sebagai kandidat inhibitor replikasi virus hepatitis B secara in silico. *In Prosiding Seminar Nasional Biotik*, *8*(1), 358–363. https://doi.org/https://doi.org/10.22373/pbio.v8i2.9669.
Khalil, M., Rosadha, R. S., Saputra, A. R., Akbar, M. N., Azmi, U., & Hapsari, W. K. (2020). Potensi Senyawa Bioaktif B-Carotene Sebagai Kandidat Antivirus Hepatitis B. *Jurnal Geuthèë: Penelitian Multidisiplin*, *3*(1), 393–399. https://doi.org/10.52626/jg.v3i1.73
Kusumawati, D., Widyarti, S., Maftuch, M., & Rahayu, S. (2022). In Silico Study of Haematococcus pluvialis Biomarker Compound as Supplement to Fish Bone Remodelling. *HAYATI Journal of Biosciences*, *29*(3), 330–342. https://doi.org/10.4308/hjb.29.3.330-342
Li, H., Yan, L., Shi, Y., Lv, D., Shang, J., Bai, L., & Tang, H. (2020). *Hepatitis B Virus Infection: Overview*. https://doi.org/10.1007/978-981-13-9151-4_1
Littlejohn, M., Locarnini, S., & Yuen, L. (2016). Origins and Evolution of Hepatitis B Virus and Hepatitis D Virus. *Cold Spring Harbor Perspectives in Medicine*, *6*(1), a021360. https://doi.org/10.1101/cshperspect.a021360
Liu, H., Xi, J., & Hu, J. (2021). Regulation of Hepatitis B Virus Replication by Cyclin Docking Motifs in Core Protein. *Journal of Virology*, *95*(12). https://doi.org/10.1128/JVI.00230-21
Ministry of Health of the Republic of Indonesia. *Guide to Commemoration of World Hepatitis Day XII July 28 2021*. (2021).
Muljono, D. H. (2017). Epidemiology of hepatitis B and C in Republic of Indonesia. *Euroasian Journal of Hepato-Gastroenterology*, *7*(1), 55–59. https://doi.org/https://doi.org/10.5005%2Fjp-journals-l0018-1212.
National Center for Biotechnology Information. (n.d.-a). PubChem Compound Summary for CID 121488107. Retrieved December 6, 2022, from https://pubchem.ncbi.nlm.nih.gov/compound/71739468
National Center for Biotechnology Information. (n.d.-b). PubChem Compound Summary for CID 442428, Naringin. Retrieved December 7, 2022, from https://pubchem.ncbi.nlm.nih.gov/compound/Naringin
Pantsar, T., & Poso, A. (2018). Binding Affinity via Docking: Fact and Fiction. *Molecules (Basel, Switzerland)*, *23*(8), 1–11. https://doi.org/10.3390/molecules23081899
Qiu, Z., Lin, X., Zhang, W., Zhou, M., Guo, L., Kocer, B., Zhu, W. (2017). Discovery and Pre-Clinical Characterization of Third-Generation 4-H Heteroaryldihydropyrimidine (HAP) Analogues as Hepatitis B Virus (HBV) Capsid Inhibitors. *Journal of Medicinal Chemistry*, *60*(8), 3352–3371. https://doi.org/10.1021/acs.jmedchem.7b00083
Qiu, Z., Lin, X., Zhou, M., Liu, Y., Zhu, W., Chen, W.,Tang, G. (2016a). Design and synthesis of orally bioavailable 4-methyl heteroaryldihydropyrimidine based hepatitis B virus (HBV) capsid inhibitors. *Journal of Medicinal Chemistry*, *59*(16), 7651–7666. https://doi.org/10.1021/acs.jmedchem.6b00879
Qiu, Z., Lin, X., Zhou, M., Liu, Y., Zhu, W., Chen, W.,Tang, G. (2016b). Design and Synthesis of Orally Bioavailable 4-Methyl Heteroaryldihydropyrimidine Based Hepatitis B Virus (HBV) Capsid Inhibitors. *Journal of Medicinal Chemistry*, *59*(16), 7651–7666. https://doi.org/10.1021/acs.jmedchem.6b00879
Rivoira, M. A., Rodriguez, V., Talamoni, G., & Tolosa de Talamoni, N. (2021). New Perspectives in the Pharmacological Potential of Naringin in Medicine. *Current Medicinal Chemistry*, *28*(10), 1987–2007. https://doi.org/10.2174/0929867327666200604171351
Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B. S., Babalghith, A. O., Islam, M. R. (2022). Flavonoids a Bioactive Compound from Medicinal Plants and Its Therapeutic Applications. *BioMed Research International*, *2022*, 1–9. https://doi.org/10.1155/2022/5445291
Seeliger, D., & de Groot, B. L. (2010). Ligand docking and binding site analysis with PyMOL and Autodock/Vina. *Journal of Computer-Aided Molecular Design*, *24*(5), 417–422. https://doi.org/10.1007/s10822-010-9352-6
Sharma, S., Garg, I., Kumar, B., & Ashraf, M. Z. (2018). Comparative analysis of blind docking reproducibility. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, *4*(3), 211–222. https://doi.org/https://doi.org/10.26479/2018.0403.18
Shen, C., Ding, J., Wang, Z., Cao, D., Ding, X., & Hou, T. (2020). From machine learning to deep learning: Advances in scoring functions for protein–ligand docking. *WIREs Computational Molecular Science*, *10*(1). https://doi.org/10.1002/wcms.1429

Trott, O., & Olson, A. J. (2009). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, NA-NA. https://doi.org/10.1002/jcc.21334

Tseng, T.-C., Kao, J.-H., & Chen, D.-S. (2014). Peginterferon α in the treatment of chronic hepatitis B. *Expert Opinion on Biological Therapy*, *14*(7), 995–1006. https://doi.org/10.1517/14712598.2014.907784

Yan, C., Xu, X., & Zou, X. (2016). Fully Blind Docking at the Atomic Level for Protein-Peptide Complex Structure Prediction. *Structure*, *24*(10), 1842–1853. https://doi.org/10.1016/j.str.2016.07.021

Zhang, J., Gao, W., Liu, Z., Zhang, Z., & Liu, C. (2014). Systematic Analysis of Main Constituents in Rat Biological Samples after Oral Administration of the Methanol Extract of Fructus Aurantii by HPLC-ESI-MS/MS. *Iranian Journal of Pharmaceutical Research : IJPR*, *13*(2), 493–503. Retrieved from https://pubmed.ncbi.nlm.nih.gov/25237344.