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Detection and In Silico Study of Neochlorogenic Acid from *Rhizophora apiculata* Stem Bark Extract as HBV Replication Inhibitors

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Abstract: This study aims to detect and characterize the presence of neochlorogenic acid in *Rhizophora apiculata* stem bark extract and to examine its potential as an inhibitor of Hepatitis B virus (HBV) replication with in silico approach. This explorative descriptive study used liquid chromatography-mass spectrometry (LC-MS/MS) analysis to detect the content of neochlorogenic acid, and an in silico study through molecular docking analysis using the blind docking technique to test its potential to inhibit HBV replication. LCMS/MS results showed that the neochlorogenic acid was present in R. apiculata stem bark extract which was detected with a peak at 3.94 retention time, and fragment ion with a m/z value of 355.1029. The docking analysis results showed that neochlorogenic acid forms a binding site that is relatively similar to the reference ligand to the HBV capsid protein, involving amino acid residues PHE 23, PRO 25, PHE 110, TYR 118, TRP 102, ILE 139, LEU 140, and SER 141, with binding affinity score -6.3 kcal/mol. Therefore, based on the results of this study, it can be concluded that neochlorogenic acid derived from stem bark extract of *R. apiculata* has the potential to be used as an alternative treatment for hepatitis B virus infection.

Keywords: Hepatitis B Replication; Molecular docking; Neochlorogenic acid; *Rhizophora apiculata*.

Introduction

Hepatitis B Virus (HBV) is an infectious agent that has a serious impact on human health globally(Liu, 2020; Pley et al., 2021). HBV infection can lead to Hepatitis B disease, which significantly causes impaired liver function and health problems in infected individuals. This disease not only has an acute impact in the form of symptoms such as fatigue, nausea, and yellowing of the skin and eyes (jaundice), but also has the potential to induce more serious chronic complications. One of the long-term effects of HBV infection is the development of cirrhosis of the liver, a condition characterized by scar tissue replacing healthy liver tissue (Agarwal et al., 2022; Chu, 2000; Khatun & Biswas, 2020). Liver cirrhosis can seriously impair liver function and potentially lead to liver failure, which is a life-threatening condition. In addition, HBV also has a strong link with the development of hepatocellular cancer, which is one of the deadliest forms of cancer worldwide (W. Li, Deng, Liu, Wang, & Sun, 2020).

Although vaccination has helped in the prevention of HBV infection, the main challenge remains in treating individuals who are already infected with this virus. This is due to the ability of HBV to integrate its viral DNA fragments into the host cell genome (Wang & Chen, 2022; Yeh et al., 2023). This process not only makes elimination of the virus in infected individuals difficult, but also has the potential to cause genetic abnormalities in host cells that can trigger the development of chronic diseases or cancer (A. Li et al., 2019; Su et al., 2020). Drugs that have been approved by the American Food and Drug Administration for treating HBV infection are nucleoside analogues (adefovir, telbivudine,

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lamivudine, etc.) and immunomodulators (Fung et al., 2011; Murata et al., 2018). However, long-term use of this drug can result in resistance so that the drug is no longer effective in curing. Therefore, efforts are needed to discover and study new compounds as alternatives that can be used.

Recently, scientific studies have shed greater light on the potentially valuable properties of natural compounds present in various plant species. These compounds have become a major focus in efforts to develop more effective and sustainable anti-HBV drugs. One compound that stands out is neochlorogenic acid, a phenolic compound that has the potential to be developed in alternative medicine for various diseases. Previous research has revealed that neochlorogenic acid displays strong antiviral properties, as well as having the potential to reduce inflammation (Gao et al., 2020; Yu et al., 2021).

Furthermore, in the context of searching for bioactive compounds, *Rhizophora apiculata*, a type of mangrove tree that thrives in tropical and subtropical regions, emerges as a promising candidate in the treatment of various diseases (Alsareii et al., 2022; Saroyo & Saputri, 2021). This plant has extraordinary adaptability to harsh environments, including high tides, high salinity, and low oxygen tension. This may have influenced the development of bioactive compounds in these plants in response to unique environmental challenges (Mitra et al., 2021). Traditionally, people have used parts of this plant such as leaves, roots, flowers, and stem bark in the treatment of various diseases (Sivaperumal et al., 2023; Vinoth et al., 2019). Therefore, investigating the content of bioactive compounds from *R. apiculata*, including the potential for neochlorogenic acid, is an important step in the search for innovative anti-HBV therapeutic alternatives.

This study aims to detect and characterize the presence of neochlorogenic acid in *R. apiculata* stem bark extract and to examine its potential as inhibitor of Hepatitis B virus replication with in silico study. It is hoped that this combined approach can provide new insights in the development of natural compound-based therapies for the treatment of HBV infection.

Method

This research used a descriptive exploratory method that aims to detect the content of neochlorogenic acid in *R. apiculata* stem bark extract and examine in silico the potential of this compound to inhibit the replication of the hepatitis B virus (HBV). The research was carried out in July-August 2023. This research consists of 2 stages, first, the stage of detecting the neochlorogenic acid content from the stem bark extract of R. apiculata, the second stage, the in silico study of the bioactivity of this compound against the HBV capsid protein.

The sample used in this study was *R. apiculata* stem bark obtained from the mangrove forest of Kuala Langsa, Langsa City, Aceh, Indonesia (Figure 1). Samples from the research location were prepared at the Biology Education Laboratory, Syiah Kuala University. In the preparation process, the clean samples were then dried using an oven for 3 days. The dry samples were crushed into simplicia. *R. apiculata* stem bark simplicia was extracted by maceration technique using methanol solvent (Lezoul et al., 2020). The extraction results were then analyzed by liquid chromatography-mass spectrometry (LC-MS/MS) to detect the content of neochlorogenic acid. This analysis was carried out at the Forensic Laboratory Center, the Indonesia National Police Criminal Investigation Agency.

Figure 1. Stem bark *R. apiculata* and it simplicia

The in silico study stage was carried out using molecular dock ing analysis. The technique used is blind docking which aims to get comprehensive results (Ismail et al., 2022). This in silico study was carried out at the Computer Laboratory FKIP UNSAM. Samples for molecular docking analysis were the 3D structures of neochlorogenic acid, reference ligand, and HBV capsid protein obtained from the PubChem compound database and the Protein Data Bank (PDB). The software used in this analysis included PyMol, PyRx, and Biovia Discovery Studio 2019. The stages in this study broadly consisted of the stage of collecting the 3D structures of compounds, reference ligands and target proteins; preparations; molecular docking analysis; and interpretation.

The 3D structure of neochlorogenic acid was obtained from the PubChem database with CID 5280633. In addition, the structure of the reference ligand was also collected from the same database. The reference ligand used was 4-methyl heteroaryldihydropyrimidine (4-methyl HAP), with CID 121488107 (Figure 2) (Khalil et al, 2020; Qiu et al., 2016). Meanwhile, the target protein used in this study was the core protein of HBV capsid whose 3D structure was collected from the PDB database with ID 5GMZ. The 3D structure of this protein has a

resolution of 1.70 Å and is a complex consisting of 5 sequences about 155 amino acids long (Figure 3). For analysis only one complete sequence was used.

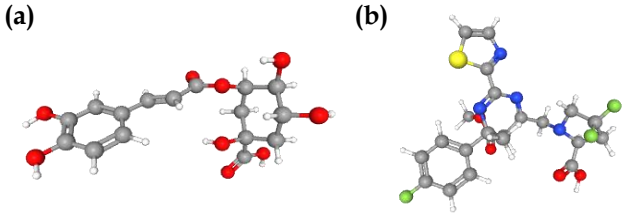

Figure 2. 3D structure of (a) neochlorogenic acid; and (b) 4-methyl HAP

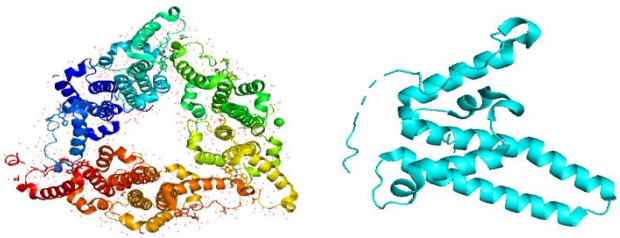

Figure 3. 3D structure of core protein of HBV capsid

Molecular docking analysis uses AutoDock Vina software integrated into PyRx (Trott & Olson, 2009). Visualization of the docking results was carried out using PyMol software version 2.5.4 and Discovery Studio 2019. Interpretation of the results of the analysis was carried out by comparing the binding affinity scores, binding sites, and the interactions between the ligands and receptors that form the complex.

Result and Discussion

Detection of the presence of neochlorogenic acid in *R. apiculata* stem bark extract through liquid chromatography-mass spectrometry (LC-MS/MS) analysis showed that the compound was present in the extract which was detected with a peak at retention time of 3.94 and fragment ion with m/z value of 355.1029 (Figure 4).

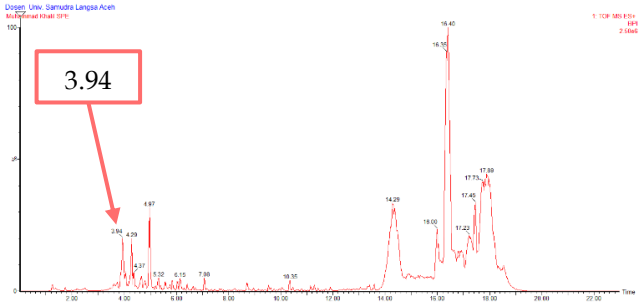

Figure 4. Neochlorogenic acid detected through LC-MS/MS

The neochlorogenic acid was further analyzed with an in silico study regarding the potential of this compound to be used as an alternative candidate in the treatment of hepatitis B virus (HBV) infection. The 2D structures of the neochlorogenic acid and reference ligands used were obtained from the PubChem database (NCBI, 2023). General information of these compounds is shown in Table 1.

Table 1. General profile of the ligands

Compounds	PubChem ID	Chemical formula	Molecular weight (g/mol)	HbD	HbA	nRB	LogP
Neochlorogenic acid	5280633	$C_{16}H_{18}O_9$	354.309	6	9	5	-0.4
4-methyl HAP (reference ligand)	121488107	$C_{22}H_{21}F_3N_4O_4S$	494.5	1	11	7	0.9

An in silico study to examine the potential of neochlorogenic acid compounds was carried out through molecular docking using the blind docking technique (Grasso et al., 2022; Morris & Corte, 2021). computational program to search for the best binding sites on the entire surface of the target protein so that the most optimal binding sites are obtained. The results of the redocking of the reference ligand (4-methyl HAP) showed nine binding modes with binding affinity scores ranging from -6.6 to -5.8 kcal/mol. Whereas the results of docking neochlorogenic acid with the target protein showed nine binding modes with binding affinity scores ranging from -6.3 to -5.7 kcal/mol (Table 2).

Table 2. Binding affinity score

Complex	Mode	Binding Affinity (kcal/mol)	RMSD/ UB (Å)	RMSD/ LB (Å)
Protein capsid HBV and reference ligand	0	-6.6	0	0
	1	-6.3	20.153	17.889
	2	-6.3	17.244	15.255
	3	-6.2	8.621	5.009
	4	-6.1	24.54	21.115
	5	-6.1	19.338	17.064
	6	-6.1	17.109	14.979

Complex	Mode	Binding Affinity (kcal/mol)	RMSD/ UB (Å)	RMSD/ LB (Å)
	7	-5.8	6.069	3.806
	8	-5.8	23.784	21.42
Protein capsid HBV and neochlorogenic acid	0	-6.3	0	0
	1	-6.1	7.877	2.369
	2	-6.1	23.158	18.491
	3	-6	6.079	3.746
	4	-5.9	3.202	1.267
	5	-5.9	7.543	3.215
	6	-5.8	22.814	19.421
	7	-5.8	25.298	21.182
	8	-5.7	2.6	1.681

Visualization of the results of molecular docking is shown in Figure 5. Binding to the HBV virus capsid protein, 4-methyl HAP, in the two compounds analyzed shows that the interactions formed occur at relatively the same binding sites. Theoretically, this indicates that the neochlorogenic acid will have the same bioactivity as the reference ligand attached to the HBV capsid protein.

A comprehensive visualization was also carried out to see how the amino acid residues of the HBV capsid protein interacted with each docked compound (Khalil, 2020). Visualization uses Discovery Studio 2019 software by activating the appearance of ligand and receptor interactions. It is vital to visualize these chemical interactions in order to determine the complex nature of the ligand-target protein and to preserve molecular interactions, stability, and dynamics (Fatchur & Putra, 2020).

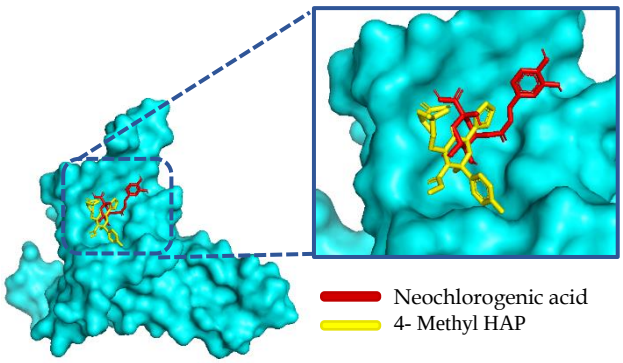

Figure 5. Visualization of binding sites formed between neochlorogenic, l4-methyl HAP, and HBV capsid proteins

The 4-methyl HAP and the HBV capsid protein complex from the results of visualized molecular docking analysis show various kinds of interactions that are formed. This interaction involves 15 amino acid residues of the target protein, namely van der Waals interactions at PHE 23, SER 106, THR 109, and ILE 139, conventional hydrogen bonds at THR 33, TRP 102, LEU 140, SER 141, halogen (fluorine) ASP 29, pi-sulfur at TYR 118, pi-pi t-shaped at TYR 118, alkyl and pi-alkyl at PRO 25, LEU 30, LEU 37, ILE 105, and PHE 110 (Figure 6). Whereas in the neochlorogenic acid and HBV protein capsid complex, the results of the visualized molecular docking analysis showed that the interaction involved 14 amino acid residues of the target protein, namely the van der Waals interaction at PHE 24, PHE 110, TYR 118, TRP 125, PRO 138, LEU 140, SER 141, conventional hydrogen bond on ASP 22, TRP 102, carbon hydrogen bond on PRO 25, ILE 139, pi-pi stacked and pi-pi t-shaped on PHE 23, PHE 122, and pi-alkyl on ALA 137 (Figure 7).

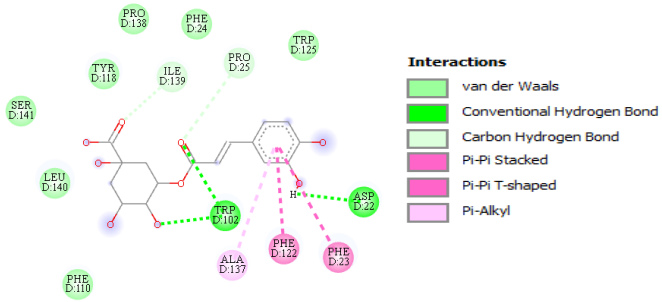

Figure 6. Interaction between 4-methyl HAP and HBV protein capsid

Based on this research, extract from *R. apiculata* stem bark has the potential to be developed as an alternative in the treatment of diseases caused by hepatitis virus infection because it contains neochlorogenic acid. An in silico study using the molecular docking technique has shown that neochlorogenic acid has the lowest binding affinity score -6.3 kcal/mol. The score indicates the amount of Gibbs free energy (ΔG) required to form a bond. The lower the score, the less energy needed to form bonds and more efficient (Bitencourt-Ferreira et al., 2019; Khalil et al., 2023). The binding affinity score between neochlorogenic acid and the HBV capsid protein was still 0.3 kcal/mol higher when compared to the binding affinity score between 4-methyl HAp as the reference ligand and the HBV capsid protein (-6.6 kcal/mol). However, it should be noted that neochlorogenic acid is a natural compound that can be obtained easily, as in the stem bark of *R. apiculata* which has been shown.

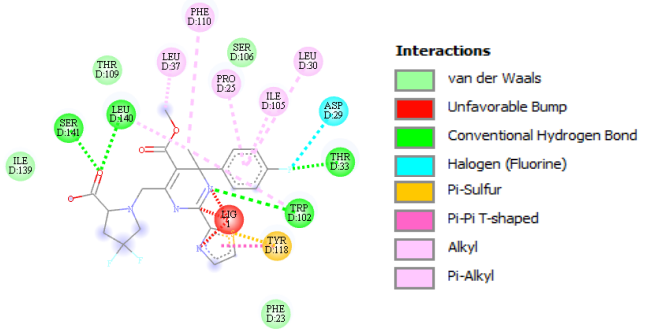

Figure 7. Interaction between neochlorogenic acid and HBV protein capsid

In addition, interaction visualization also showed that the reference ligand and neochlorogenic acid bind to the HBV capsid protein at relatively the same binding sites. There are the same 8 amino acid residues involved in the interaction between the reference ligand and neochlorogenic acid with the target protein, namely PHE 23, PRO 25, PHE 110, TYR 118, TRP 102, ILE 139, LEU 140, and SER 141. This indicates that basically neochlorogenic acid has bioactivity as an inhibitor of hepatitis B virus replication. Binding to the active site involved in the replication pathway is able to suppress the amount of virus developing in an infected host (Dandri, 2020; Hu, Protzer, & Siddiqui, 2019; Khalil, 2023).

Conclusion

Rhizophora apiculata extract contains neochlorogenic acid which has the potential to be used as an alternative in the treatment of hepatitis B virus infection. In silico studies show that neochlorogenic acid has an optimal binding affinity score of -6.3 kcal/mol and forms bonds at binding sites that are relatively the same as the reference ligand, which has the implication that neochlorogenic acid has the potential to inhibit the replication of the virus.

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Author Contributions
Muhammad Khalil: As the head of this research, he is fully responsible for carrying out this research in accordance with the agreement with Universitas Samudra as the party that funded the research.
Yonadiah Dwitya: As a research member and assisting in designing research, conducting research, and compiling research output manuscripts.

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Conflicts of Interest
The authors declare no conflict of interest.

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