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Peptide Based Hepatitis C Vaccine Design From RNA-dependent RNA polymerase (RdRp) NS5B: Immunoinformatics Approach

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ABSTRACT: Hepatitis is an inflammatory disease of the liver that can be caused by various factors. A common cause of hepatitis is a viral infection. Until now, there is no effective vaccine for Hepatitis C Virus infection so it is still a health problem in the world. This study aims to obtain a peptide-based vaccine design that acts as an antigen, does not cause an allergen, is not toxic, and is not homologous to human cells. This study used NS5B RNA-dependent RNA polymerase (RdRp) samples taken from PDB. All stages of the analysis are carried out using the appropriate web server and software. Sequence analysis obtained selected T-cell epitopes, namely LSAFSLHSY, VLDDHYRDV, YLFNWAVKT, TLTCYLKASAACRAA, NTLTCYLKASAACRA, and LTCYLKASAACRAAK. Meanwhile, the selected B-cell epitopes were FCVQPEKGGGRK and DSTVTENDIRV. Which was given the connectors EAAAK, AAY, GPGPG, and KK on each of the corresponding epitope connectors. Then, the vaccine candidate is docked and visualized in 2D and 3D. The research shows that the design of the hepatitis C vaccine using the NS5B protein is stable and not homologous to human cells so it can be used as a vaccine candidate.

KEYWORDS: Epitope, Hepatitis C, Immunoinformatics, Vaccine Design

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I. INTRODUCTION

Hepatitis was a liver inflammation disease that could be caused by various factors such as genetic factors, viral infections, alcohol, and drugs. The common cause of hepatitis was a viral infection. The Hepatitis C virus (HCV) was one of the viruses that caused hepatitis and were considered the most dangerous among other hepatitis viruses. Most patients who were infected with the hepatitis C virus did not show any symptoms. Thus, many were unaware that they had been infected with the hepatitis C virus until liver damage appeared (Alhawaris 2019).

According to data from the World Health Organization (WHO), an estimated 58 million people worldwide have been infected with chronic hepatitis C, with about 15.2 million people being infected with chronic hepatitis C each year. There are also 3.2 million adolescents and children with chronic hepatitis C infection. In Indonesia, it is estimated that 6.6-7 million people have been infected with hepatitis C, with a prevalence that varies greatly from 0.5% to 3.37%. In 2019, WHO estimated that about 290,000 people died from hepatitis C, mainly due to cirrhosis and hepatocellular carcinoma (Prasetya, Nugroho, and Triloka 2022).

Until now, there has been no effective vaccine to prevent the spread of Hepatitis C virus infection. Therefore, it is important to design new vaccines that can be used as vaccine candidates to control the spread of hepatitis C virus infection (Pradana et al. 2021).

Technology has developed in line with the genomic era so that genetic information can be used for vaccine development. Currently, the discovery of vaccine candidates using the immunoinformatics approach has been developed. Immunoinformatics is a branch of bioinformatics that deals with the computational analysis of immunology data. The immunoinformatics approach has been used to design vaccines against several infectious diseases. By predicting the appropriate antigen, epitope, carrier, and adjuvant for vaccine candidates, the immunoinformatics can efficiently save time and costs in vaccine development (Ahammad and Sultana 2020).

The principle of immunoinformatics is to predict peptide binding with MHC (Major Histocompatibility Complex). Peptide-based vaccines do not use whole microbes, making them safe to use. The vaccine that can be developed using the immunoinformatics approach is the peptide vaccine. The peptide vaccine is a vaccine that

consists of several amino acid residues in the form of epitopes, and proteins are the main compounds of peptide vaccine candidates. The immunoinformatics approach can be used to design peptide vaccines against the hepatitis C virus using the virus peptide sequence (Rezaldi et al. 2021).

The RNA genome of hepatitis C virus encodes a polyprotein that is processed to generate at least 10 viral proteins, including structural and non-structural proteins. NS5B protein is an RNA-dependent RNA polymerase and plays a key role in the replication of hepatitis C virus, making NS5B an attractive target for the hepatitis C vaccine discovery (Polamreddy, Vishwakarma, and Saxena 2018).

This research aims to discover a peptide-based hepatitis C vaccine candidate from NS5B protein using an immunoinformatics approach. This study is expected to assist in the process of discovering more effective and efficient hepatitis C vaccine candidates, which could be beneficial for vaccine development in Indonesia, especially for hepatitis C disease.

II. MATERIALS AND METHODS

2.1 NS5B Sequence Data Retrieval

The virus sequence data was obtained from the Protein Data Bank (PDB) web server: (www.rcsb.org). The sequence of the NS5B protein was extracted from the FASTA format and further analyzed (Shin and Cho 2005)

2.2 Antigen analysis and allergen analysis

Antigen analysis was performed on the web server: (<http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html>) with the target organism: Virus and Threshold: 0.4. Allergen analysis was conducted online using the web server: (<https://www.ddgpharmfac.net/AllerTOP/>) (Alom et al. 2021).

2.3 MHC-I Epitope Analysis

Analysis MHC was performed on the IEDB web server (<http://iedb.org>). MHC-I using identification from Net MHC pan EL 4.1 and allele codes HLA-A01:01 and HLA-A02:01 with a 9-amino acid length. The analysis results were obtained with a percentile rank value of less than 1.00 (Alom et al. 2021; Fadaka et al. 2021; Sharma et al., 2021).

2.4 MHC-II Epitope Analysis

MHC-II epitope analysis was performed by analyzing the sequence using the IEDB recommended 2.22 method and allele code DRB1*01:01, with a 15-amino acid length. The analysis results were obtained with a percentile rank value of less than 10.00 (Alizadeh et al. 2022; Alom et al. 2021; Fadaka et al. 2021).

2.5 B-Cell Epitope Analysis

B-cell epitope analysis was also performed on the IEDB web server: (<http://iedb.org>). The analysis that B-cells were epitope-specific with a peptide length ranging from 10-30 amino acids (Alizadeh et al. 2022).

2.6 Selected Toxin & Homolog Epitope Analysis

Toxin analysis was performed on the web server: (<http://webs.iitd.edu.in/raghava/toxinpred/design.php>) using the SVM-based method (support vector method). Homology analysis was conducted on the NCBI BLAST on the web server: (<http://blast.ncbi.nlm.nih.gov>), and if the E-value is greater than 0.05, the epitope is considered non-homolog (Alom et al. 2021).

2.7 Final Vaccine Candidate Design

The method used to design the vaccine candidate was the design with linker and adjuvant. The vaccine candidate was formulated as follows: Adjuvant-EAAAK-(MHC-I)-AAY-(MHC-I)- GPGPG-(MHC-II)-KK-(B-cell epitope) with the appropriate linkers for each component (Alom et al. 2021).

2.8 Physicochemical analysis

Physicochemical analysis, including molecular weight, aliphatic index, amino acid composition, GRAVY, and instability index were performed using the ExPasy web server (<https://web.expasy.org/protparam/>). Solubility analysis using the Protein-sol web server (<https://protein-sol.manchester.ac.uk/>) (Alom et al., 2021).

2.8 Secondary Structure Prediction of Final Vaccine

The next is to visualize the vaccine design to see the vaccine's secondary structure using the PSIPRED v4.0 web server (PSI-blast based secondary structure prediction): (<http://bioinf.cs.ucl.ac.uk/psipred/>) (Alom et al., 2021).

2.9 Vaccine Candidate 3D Visualization

The results of the vaccine candidate designs are visualized on the webserver page: (<http://galaxy.seoklab.org/>) → galaxy TBM (Alom et al., 2021). Further refinement of the predicted 3D structure with galaxy refine on the webserver: (<http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE>) (Alom et al., 2021; Ikram et al., 2018).

3.0 Molecular Docking

Docking of vaccine candidates was performed using ligands and TLR4 receptors extracted from PDB data (www.rcsb.org). Haddock web server (<https://wenmr.science.uu.nl/haddock2.4/>) (Alom et al., 2021).

3.1 Visualization 2D & 3D Vaccine Candidates

The results of docking the vaccine candidates were then visualized using the Ligplot software to observe 2D visualization as well as the interactions and bonds between the vaccine and the TLR4 receptor. Meanwhile, the 3D visualization of the vaccine was examined using the Yasara software (Balupuri and Cho 2013).

III. RESULT AND DISCUSSION

The adaptive immune, whether humoral or cellular, was very important in fighting virus infections that entered the human body. Humoral immunity, which was mediated by antibodies produced by B-cells, bound to the virus and could prevent the virus from entering cells. Cellular immunity, which was mediated by T-cells, killed virus-infected cells, thus preventing the spread of the infection to other cells (Gustiananda et al. 2021).

With the advancement of bioinformatics technology, it became easy to find data and information about the genomics of various infectious pathogens. Bioinformatics played a crucial role in predicting potential vaccine candidates, accelerating vaccine development, and cost-saving simultaneously. This immunoinformatics technique has been used to design epitope-based vaccines for various viral, bacterial, fungal, and parasitic infections (Alom et al., 2021).

3.1 The NS5B sequence data

The sequence data used for this study was the RNA-dependent RNA polymerase (RdRp) protein NS5B from Hepatitis C virus, which was obtained from the protein data bank with the code (PDB ID: 2GIQ) (Le Pogam et al., 2006). The sequence analysis showed that the predicted antigenicity of the sequence had a score of 0.4028 using Vaxijen v2.0, with a threshold score of 0.4. This value was determined to evaluate the accuracy and sensitivity of the test. Additionally, the sequence was predicted not to cause any allergies, according to the results of the AllerTOP prediction described in Table 1 (Alom et al., 2021).

Table 1. Results of Protein Sequence Analysis

Protein	Sequence	Antigen	Allergen
RNA-dependent RNA polymerase (RdRp) NS5B	MHHHHHMSYTWGTGALITPCAAEESKLPINALSNSLLRHHNMVYATTSRSAG LRQKKVTFDRLQVLDDHYRDVLEKEMKAKASTVKAKLLSVEEACKLTPPHSA KSKFGYGAKDVRNLSSKAVNHIHSVWKDLEDVTPIDTTIMAKNEVFCVQPE KGGRRKPARLIVFPDLGVRVCEKMALYDVVSTLPQVVMGSSYGFQYSPGQRVE FLVNTWKSCKNPMGFSDYTRCFDSTVTENDIRVEESIQCCDLAPPEARQAIKSL TERLYIGGPLTNSKGNQCYRRCRASGVLTTSCGNTLTCYLKASAACRAAKL QDCTMLVNGDDLTVICESAGTQEDAASLRVFTEAMTRYSAPPDPPQPEYDL ELITSCSSNVSVAHDASGKR VYYL TRDPTPLARA AWETARHTPVNSWLGNI MYAPTLWARMILMTHFFSILLAQEQLKALDCQIYGACYSIEPLDLPQIIERLH GLSAFSLHSYSPGEINRVASCLRKLGVPPLRVWRHRARSVRARLLSQGGRAAT CGKYLFWAVKTKLKLTPIPAASRLDLSGWVAVAGYSGGDIYH	0.4028	Non Allergen
Webserver	PDB	VaxiJen v2.0	AllerTOP v2.0

3.2 T-Cell Epitopes Analysis

T-Cell is an important agent of cell-mediated immunity. T-Cell helper (Th) recognizes virus peptides associated with MHC-II proteins, while Sel-T cytotoxic (Tc) recognizes virus peptides associated with MHC-I proteins (Bhatnager et al. 2021).

Table 2. T-Cell Sequence Analysis Results (MHC-I and MHC-II)

	Epitope	Allele	Antigen	Allergen	Toxin	Homolog	Percentile Rank
MHC-I	LSAFSLHSY	HLA-A*01:01	Yes	Non	Non	Non	0.07
	ALYDVVSTL	HLA-A*02:01	Non	Yes	Non	Non	0.01
	KLQDCTMLV	HLA-A*02:01	Non	Yes	Non	Non	0.06
	VLDDHYRDV	HLA-A*02:01	Yes	Non	Non	Non	0.15
	YLFNWAVKT	HLA-A*02:01	Yes	Non	Non	Non	0.17
MHC-II	HSASKSFGY	HLA-A*01:01	Yes	Yes	Non	Non	0.17
	TLTCYLKASAACRAA	Allel DRB1*01:01	Yes	Non	Non	Non	2.30
	NLTCYLKASAACRAA	Allel DRB1*01:01	Yes	Non	Non	Non	2.50
	AKDVRNLSSKAVNHI	Allel DRB1*01:01	Yes	Yes	Non	Non	3.30
	LTCYLKASAACRAAK	Allel DRB1*01:01	Yes	Non	Non	Non	3.30
MHC-II	TCYLKASAACRAAKL	Allel DRB1*01:01	Yes	Yes	Non	Non	3.30
	KDVRNLSSKAVNHIH	Allel DRB1*01:01	Yes	Yes	Non	Non	3.90

The MHC-I analysis was performed using the allele codes HLA-A01:01 and HLA-A02:01 with a 9-amino acid length and a percentile rank of less than 1.00, resulting in several selected epitopes. Meanwhile, the MHC-II epitope analysis using Alel DRB1*01:01 code resulted in several selected epitopes with a 15-amino acid length and a percentile rank of less than 10.00, as shown in Table 2 (Alom et al., 2021; Fadaka et al., 2021; Sharma et al., 2021).

All selected Sel-T epitopes (MHC-I and MHC-II) have undergone further analysis, some of which meet the epitope requirements, where the epitope must be antigenic, non-allergenic, non-toxic, and non-homologous to humans, thus making them suitable candidates for vaccine development (Sanami et al. 2022).

3.3 B-Cell Epitopes Analysis

B-Cell plays a crucial role in vaccine development as it is the main component of humoral immunity. Antibodies produced by B-cell play a vital role in preventing the spread of viral infections. Linear B-cell epitopes are responsible for producing specific antigenic antibodies (Alizadeh et al., 2022). The B-cell analysis was performed using the BepiPred 2.0 method, which resulted in several epitopes that have been described in Table 3.

Table 3. B-Cell Sequence Analysis Results

Epitope	Antigen	Allergen	Toxin	Homolog
FCVQPEKGGRK	Yes	Non	Non	Non
PCAAEESKLPINALSNSLLR	Non	Yes	Non	Non
STLPQVVMGSSYGFQYSPGQ	Non	Non	Non	Non
DSTVTENDIRV	Yes	Non	Non	Non

The accuracy of the BepiPred method for predicting B cell epitopes reaches 80%. The BepiPred method predicts the location of B-cell linear epitopes (Simarmata et al. 2022). Figure 1 shows that the yellow area with a score above the threshold of 0.50 is the B cell epitope with a positive prediction in yellow, while the negative prediction is in the green area (Sinha, Grewal, and Roy 2020).

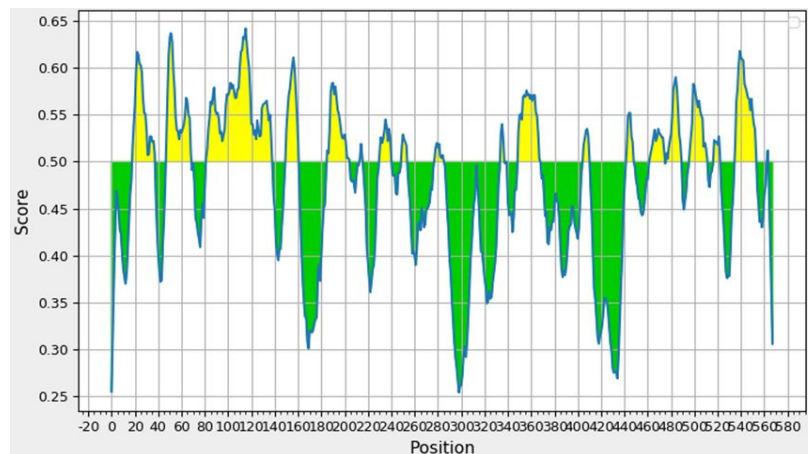


Figure 1. Graph of B Cell Epitope Prediction From NS5B Protein

3.4 Analysis of Selected Epitopes

All of the analyzed epitopes above (MHC-I, MHC-II & Sel B) have met the requirements as final vaccine candidates. After analyzing antigens, allergens, toxins, and homologs, several epitopes were obtained as vaccine candidates from MHC-I, MHC-II, and Sel B analyses, as shown in Table 4.

Table 4. Selected Epitopes

	Epitop	Antigen	Alergen	Toksin	Homolog
MHC-I	LSAFSLHSY	Yes	Non	Non	Non
	VLDDHYRDV	Yes	Non	Non	Non
	YLFNWAVKT	Yes	Non	Non	Non
MHC-II	TLTCYLKASAACRAA	Yes	Non	Non	Non
	NLTCYLKASAACRA	Yes	Non	Non	Non
Sel-B	LTCYLKASAACRAAK	Yes	Non	Non	Non
	FCVQPEKGGRK	Yes	Non	Non	Non
	DSTVTENDIRV	Yes	Non	Non	Non

All selected epitopes were used as final vaccine candidates. Antigenicity is the ability of an antigen to stimulate the formation of specific antibodies. Meanwhile, allergenicity is the ability of a substance to cause an allergic reaction. And not homologous with humans means that the peptide vaccine protein does not show similarity to the human genome, so the vaccine does not elicit an autoimmune response when used as a component of a peptide vaccine (Rezaldi et al. 2021).

3.5 Final Vaccine Candidate Design

In the past, Human β -defensin HBD3 was chosen as an adjuvant to enhance the effectiveness of the vaccine by stimulating a strong immune response. EAAAK (Glu-Ala-Ala-Ala-Lys), AAY (Ala-Ala-Tyr), GPGPG (Gly-Pro-Gly-Pro-Gly), and KK (bi-lysine) were used as linkers to connect the vaccine sequences (Alizadeh et al., 2022; Alom et al., 2021). As described in Figure 2. Linkers that have been designed as in Table 5 are used as the final vaccine candidate sequence.

Linkers play an important role in minimizing functional immunogenicity and also in maintaining the identity of each epitope during the processing of the vaccine in cells thereby ensuring the immunogenicity of each epitope. The EAAAK linker is a rigid α -helix peptide linker that provides efficient separation of functional domains in fusion proteins, thus enhancing antigenicity and immunogenicity. Epitopes fused using the AAY linker are effectively separated within the cell, thereby reducing junctional immunogenicity and increasing epitope presentation to enhance protein stability (Ayyagari et al. 2022).

The GPGPG linker has been proven to be able to induce a highly important Th lymphocyte response for vaccines. The GPGPG linker can stimulate MHC-II response and enhance conformation-dependent immunogenicity as well as antibody epitopes. The KK linker also played an important role in reducing junctional immunogenicity by avoiding the induction of antibodies to peptide sequences that can be formed by each epitope when combined linearly (Ayyagari et al. 2022).

Table 5. Design Vaccine with Adjuvant and Linkers

Design Vaccine with Adjuvant and Linkers	Jumlah Asam Amino
GIINTLQKYYCRVRGGRCVLSCLPKKEEQIGKCSTRGRKCCRRK KEAAAKLSAFSLHSYAAAYVLDDHYRDVAAYYLFNWAVKTGP GPGTLTCYLKASAAACRAAGPGPGNTLTCYLKASAAACRAGPGP GLTCYLKASAAACRAAKKFKCVQPEKGGRRKKKDDSTVTENDIRV	169

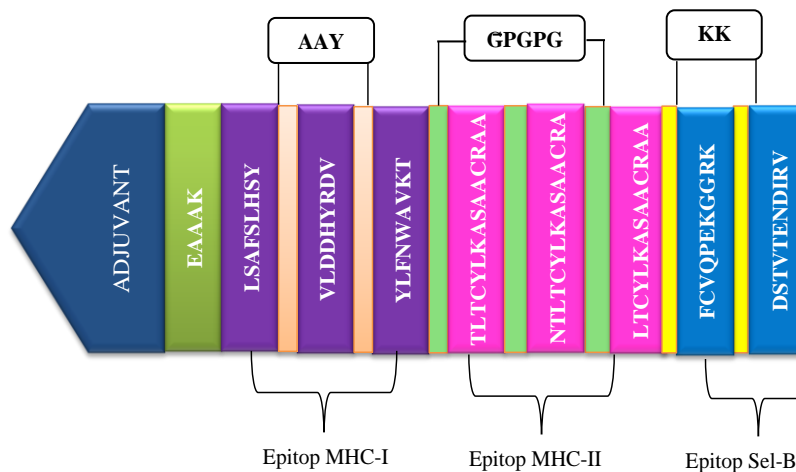


Figure 2. Graphic of Final Vaccine Candidate Designs (From Left to Right)

In this study, β -defensin was used as an adjuvant because β -defensin is known to be an effective adjuvant when conjugated with an antigen. Vaccines containing β -defensin as an adjuvant have been studied both in vivo and in vitro to activate the primary innate antiviral immune response and mediate other immunomodulatory activities against various viruses (Behmard et al., 2020). Based on this literature, the vaccine adjuvant design in this study used Human β -defensin HBD3 with the adjuvant code: GIINTLQKYYCRVRGGRCVLSCLPKKEEQIGKCSTRGRKCCRRKK (Ikram et al. 2018).

3.6 Physicochemical analysis

The final result of the vaccine candidate design was re-analyzed and it was found that the designed vaccine candidate had an antigenic score of 0.6408, consistent with what was predicted by VaxiJen v2.0.

Additionally, the vaccine candidate was predicted to not cause allergies when used, according to AllerTOP v2.0. as shown in Table 5 (Alom et al., 2021).

The physicochemical properties analysis presented in Table 6 indicated that the vaccine's molecular weight is 18199.18 g/mol, with a molecular formula of C₇₉₄H₁₂₈₆N₂₃₈O₂₂₆S₁₃. Its theoretical pI is 9.69, identifying it as a basic property. Furthermore, the vaccine's stability index is 31.05, indicating that the vaccine protein complex is stable. The aliphatic index is 67.16, indicating that the peptide vaccine is thermally stable. The total number of negatively charged residues (Asp+Glu) is 10, and the total number of positively charged residues (Arg+Lys) is 31 (Ahmad and Komari 2022; Panda and Chandra 2012).

The average hydropathicity score (GRAVY) is -0.336. A positive GRAVY value indicates hydrophobic behavior, while a negative value indicates hydrophilic behavior. This average hydropathicity score indicates that overall, the protein is hydrophilic and can interact better with water molecules in its surroundings (Ahmad & Komari, 2022). And for the solubility analysis, with a score of 0.773 according to the protein-sol prediction. The scaled solubility value (QuerySol) is the solubility predicted with the population average for the experimental dataset (PopAvrSol), which is 0.45.

Table 6. Physicochemical Analysis, Antigen, Allergen and Vaccine Candidate Solubility

Parameter	Database	Value
Number of amino acids	ProtParam	169
Molecular weight	ProtParam	18199.18
Molecular formula	ProtParam	C ₇₉₄ H ₁₂₈₆ N ₂₃₈ O ₂₂₆ S ₁₃
Theoretical pI	ProtParam	9.69
Number of negatively charged residues (Asp+Glu)	ProtParam	10
Number of positively charged residues (Arg+Lys)	ProtParam	31
Stability index	ProtParam	31.05
Aliphatic index	ProtParam	67.16
Grand average of hydropathicity (GRAVY)	ProtParam	-0.336
Antigenicity	VaxiJen v.2.0	0.6408
Allergenicity	AllerTOP v2.0	Non-Allergen
Solubility	Protein-sol	0.773

3.7 Secondary Structure Prediction of Final Vaccine

The next step involved predicting the secondary structure of the vaccine candidate using the web server for PRISPPRED v4.0 analysis of the vaccine construct sequence. The results of the analysis are shown in Figure 3, indicating the presence of helix, strand, and coil structures (Akter et al. 2022).

The helix structure generally describes the characteristics of transmembrane proteins because the hydrogen bonds of the helix structure form the backbone of the molecule. The coil structure functions in flexibility and conformational changes, where the peptide bonds in the coil are not involved in the hydrogen bonding of the protein (Ruslin, Putri, and Arba 2019).

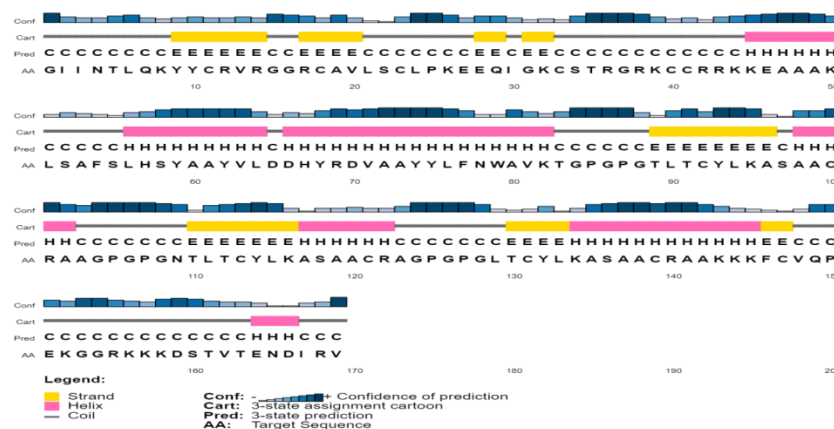


Figure 3. Secondary Structure Prediction of Final Vaccine

3.8 Vaccine Candidate 3D Visualization

The 3D structure visualization of the vaccine was modeled through GalaxyWEB. The 3D prediction visualization was used for evaluation, molecular docking with receptors, and further refinement. The refine model was used for evaluation and further refinement (Alom et al., 2021).

The GalaxyRefine server produced a refined vaccine candidate model, and the result of the GalaxyRefine visualization is shown in Figure 4. Next, the 3D visualization results were used for molecular docking, which will be combined with the receptor to become the final vaccine (Ikram *et al.*, 2018).

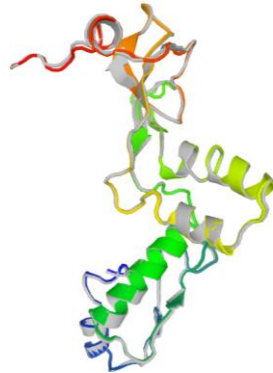


Figure 4. Vaccine 3D Structure Enhanced By Galaxyrefine

3.9 Molecular Docking

Molecular docking of the vaccine candidate was performed to combine the TLR4 receptor and ligand from the 3D visualization results of GalaxyRefine. The receptor used was TLR4, which was taken from the PDB database (PDBID: 4G8A). TLR4 is a pathogen recognition receptor on the surface of immune cells that plays an important role in the maturation process of each cell. TLR4 is used to enhance the immune response in the body (Alom *et al.*, 2021).

The receptor was prepared first using Yasara software. The docking was performed on the Haddock web server, which combines the TLR4 receptor and the vaccine protein from GalaxyRefine. Ten best docking models were obtained in Table 7, and the docking result with the lowest score was chosen. Cluster 1 was chosen because it had the smallest score of -83.0 +/- 1.8 (Ikram *et al.*, 2018).

Table 6. Skor Haddock Result

Model	Skor Haddock
Cluster 1	-83.0 +/- 1.8
Cluster 7	-74.8 +/- 7.6
Cluster 10	-64.2 +/- 12.8
Cluster 4	-58.5 +/- 7.0
Cluster 11	-55.0 +/- 12.0

The best docking result is the one with the lowest score among the other docking results. The negative sign in the docking score indicates a strong binding affinity between the ligand and the enzyme, which suggests the formation of a stable complex (Tambunan and Alamudi 2010). The molecular docking results were visualized using Yasara software, as shown in Figure 5. The vaccine binding is indicated in red, while the TLR4 receptor structure is shown in green.

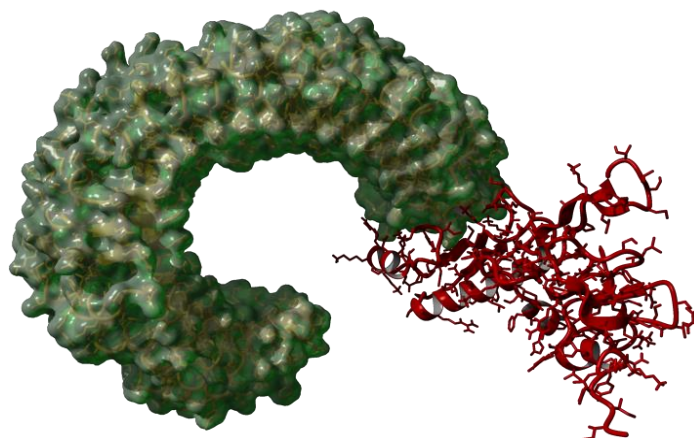


Figure 5. TLR4 (Green) and Vaccine (Red) Molecular Docking Results, 3D Visualization

The amino acids that interact between the TLR4 receptor and the vaccine in the docking results were visualized in 2D using DIMPLOT in the LigPlot application, as shown in Figure 6. There are hydrogen bonds and hydrophobic interactions between the TLR4 receptor and the vaccine. The interactions that are formed

indicate the formation of ten hydrogen bonds, between nine residues of the TLR4 receptor and six residues of the vaccine (Bouzari and Savar 2014).

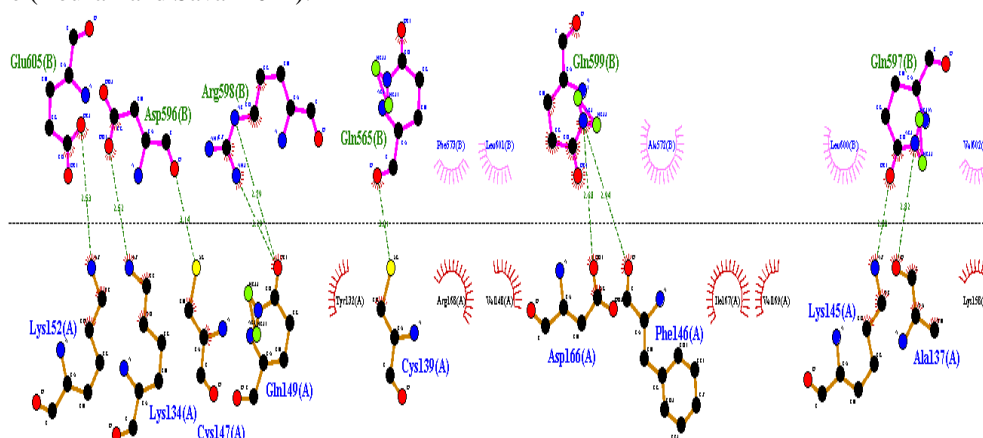


Figure 6. Interaction Between Amino Acid Chain A (TLR4) And Chain B (Vaccines), 2D Visualization

The dotted green lines indicate the hydrogen bonds between the amino acid residues of the receptor and the amino acid residues of the vaccine. Meanwhile, drawing circular, curved red lines, easy-to-curve red lines, and pink circular lines indicate the amino acid residues that form hydrophobic interactions around the amino acid residues (Rezaldi et al., 2021).

From the results and discussion above, it can be concluded that the hepatitis C vaccine candidate from the RNA-dependent RNA polymerase (RdRp) NS5B protein with TLR4 receptor binding is antigenic, non-allergenic, non-toxic, and non-homologous to humans. The vaccine candidate is predicted to have suitable physicochemical characteristics such as solubility and stability. The 2D and 3D structure of the vaccine candidate shows good binding and interactions with the receptor to enhance immune response. Therefore, this study suggests that the vaccine candidate can be used as a vaccine to combat hepatitis C infection. However, the results of this study need to be validated by further research both in vitro and in vivo.

IV. CONCLUSION

Based on the research results, the peptide sequence that has the potential as a hepatitis C vaccine candidate is GIINTLQKYCRVRRGRCVLSCLPKKEEQIGKCSRGRKCCRRKKEAAKLSAFSLHSY AAYVLDDHYRDVAAYYLFNWAVKTGPGPGTLTCYLKASAACRAAGPGPGNTLTCYLKASAACRAGP GPLTTCYLKASAACRAAKKFKCVQPEKGGRRKKDSTVTENDIRV. It also has physicochemical properties that indicate the hepatitis C vaccine candidate in this study has stable stability index, antigenic properties and does not cause allergic reactions. Homologous vaccine design analysis also showed that the vaccine candidate is non-homologous to human cells, making the vaccine design a potential Hepatitis C vaccine candidate.

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**YAYASAN PERGURUAN CIKINI
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SURAT PENUGASAN TENAGA PENDIDIK
Nomor : 48 /03.1-H/IX/2023
SEMESTER GANJIL TAHUN AKADEMIK 2023/2024

Nama : Apt. Erwi Putri Setyaningsih, M.Si. **Status** : Tetap.
Nik : 01.201519 **Program Sarjana Prodi Farmasi**
Jabatan Akademik : AA

Untuk melaksanakan tugas sebagai berikut:

Bidang	Perincian Kegiatan	Tempat	Jam/ Minggu	Kredit (SKS)	Keterangan
I PENDIDIKAN DAN PENGAJARAN	MENGAJAR DI KELAS (KULIAH/RESPONSI DAN LABORATORIUM)				
	Analisa Farmasi 2 (D)	Ruang HC-9		1	Jumat, 08:00-09:40
	Biokimia (D)	Ruang HC-7		1,5	Jumat, 15:20-17:00
	Biokimia (L)	Ruang HC-10		1,5	Sabtu, 17:00-19:40
	Kimia Medisinal (B)	Ruang HC-9		1,5	Selasa 16:00-18:40
	Kimia Medisinal (D)	Ruang HC-9		1,5	Selasa 10:00-11:40
	Praktikum Kimia Analisis (A)	Laboratorium		1	Senin, 15:00-18:00
	Praktikum Kimia Organik (A)	Laboratorium		1	Selasa, 10:00-13:00
	Bimbingan Skripsi		3 Jam/Minggu	1	
	Menguji Tugas Akhir		3 Jam/Minggu	1	
	Gugus Kendali Mutu Fakultas (Struktural)		6 Jam/Minggu	2	
II PENELITIAN	Penulisan Karya Ilmiah		3 Jam/Minggu	1	
III PENGABDIAN DAN MASYARAKAT	Pelathan dan Penyuluhan		3 Jam/Minggu	1	
IV UNSUR UNSUR PENUNJANG	Pertemuan Ilmiah		3 Jam/Minggu	1	
	Jumlah Total			16	

Kepada yang bersangkutan akan diberikan gaji/honorarium sesuai dengan peraturan penggajian yang berlaku di Institut Sains dan Teknologi Nasional
Penugasan ini berlaku dari tanggal 01 September 2023 sampai dengan tanggal 28 Februari 2024

Tembusan :

1. Direktur Akademik - ISTN
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4. Kepala Program Studi Farmasi Fak. Farmasi
5. Arsip



Etnobotani Tanaman Obat Sebagai Imunomodulator di Desa Adi Luhur Kecamatan Panca Jaya Provinsi Lampung

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ABSTRAK

Kajian etnobotani dilakukan untuk melihat interaksi masyarakat setempat dengan lingkungannya dan mempelajari hubungan manusia dengan tanaman di sekitarnya. Masyarakat Desa Adi Luhur telah memanfaatkan tanaman obat tradisional sejak lama yang dibuktikan dengan adanya kelompok tani wanita di Desa Adi Luhur. Setiap rumah memiliki tanaman obat keluarga untuk memenuhi kebutuhan obat herbal yang dapat digunakan untuk mencegah atau mengobati penyakit. Penelitian ini bertujuan untuk mengetahui jenis tanaman imunomodulator yang dimanfaatkan oleh masyarakat di desa Adi Luhur Kecamatan Panca Jaya Provinsi Lampung. Jenis penelitian yang dilakukan secara observasional menggunakan metode penelitian kualitatif yang bersifat deskriptif. Pengambilan sampel dilakukan pada masyarakat Desa Adi Luhur, Kecamatan Panca Jaya, Provinsi Lampung secara *purposive sampling*. Sampel ditentukan berdasarkan ciri-ciri yang sesuai dengan kriteria inklusi yaitu masyarakat Desa Adi Luhur yang bersedia menjadi responden dan mengisi kuesioner, masyarakat yang mengonsumsi jamu 1 bulan terakhir, dan masyarakat dalam rentang usia 20-75 tahun. Pengisian kuesioner melibatkan responden yang berjumlah 110 orang. Penentuan jumlah sampel berdasarkan perhitungan *Lamshow* dari jumlah total populasi 1.989 orang. Berdasarkan hasil penelitian diperoleh informasi bahwa di Desa Adi Luhur terdapat 5 tanaman yang dibudidayakan dan dapat digunakan sebagai imunomodulator yaitu sambiloto, jahe, kunyit, lempuyang dan temu kunci yang dikonsumsi dengan cara direbus lalu diminum. Bagian tanaman yang diambil daunnya adalah sambiloto, sedangkan yang diambil bagian rimpangnya adalah jahe, kunyit, lempuyang dan temu kunci.

Kata Kunci: *Desa Adi Luhur, etnobotani, imunomodulator, tanaman obat*

Ethnobotany of Traditional Medicinal Plants as Imunomodulators in Adi Luhur Village, Panca Jaya District, Lampung Province

ABSTRACT

An ethnobotanical study was conducted to see the interaction of local people with their environment and to study the relationship between humans and their surrounding plants. The people in Adi Luhur Village have used traditional medicinal plants for a long time as evidenced by the existence of a women's farmer group in Adi Luhur Village. Each house has a family of medicinal plants to meet the needs of herbal medicines that can be used to prevent or treat disease. This study aims to determine the types of immunomodulatory plants used by the people in Adi Luhur Village, Panca Jaya District, Lampung Province. The type of research was observational using descriptive qualitative research methods. Sampling was carried out in the community of Adi Luhur Village, Panca Jaya District, Lampung Province by purposive sampling. The sample was determined based on the similarity between characteristics and inclusion criteria including the people of Adi Luhur Village who are willing to be respondents and fill out a questionnaire, people who have consumed herbal medicine in the last one month, and people in the age range of 20-75 years. Filling out the questionnaire involved a respondent of 110 people. Determination of the number of samples based on Lamshow calculations of the total population of 1.989 people. Based on the results of the study, information was obtained that in Adi Luhur Village 5 plants were cultivated and could be used as immunomodulators, namely sambiloto, turmeric, ginger, lempuyang, and finger root which are consumed by boiling and then drinking. The part of the plant that was taken for the leaves is sambiloto, while the parts for the rhizome were ginger, turmeric, lempuyang, and finger root.

Keywords: *Adi Luhur Village, ethnobotany, herbal medicine, immunomodulator*

PENDAHULUAN

Etnobotani merupakan ilmu yang mempelajari hubungan antara manusia dengan tanaman. Diketahui bahwa manfaat tanaman untuk kehidupan manusia sangat beragam. Pengetahuan mengenai kandungan serta manfaat dari tanaman terus berkembang seiring dengan banyaknya penelitian-penelitian yang dilakukan oleh ilmuwan. Kekayaan alam Indonesia meliputi 30.000 spesies tumbuhan dari total 40.000 spesies tumbuhan dunia, dan sebanyak 940 spesies di antaranya merupakan tanaman berkhasiat obat (Masyhud, 2010). Tanaman obat merupakan tanaman berkhasiat yang dapat digunakan untuk mengobati berbagai penyakit, membunuh bibit penyakit, memperbaiki organ yang rusak, dan meningkatkan daya tahan tubuh (Saputri *et al.*, 2021). Saat ini penelitian mengenai tanaman obat telah banyak dilakukan, sehingga obat yang dihasilkan dari zat aktif tanaman dapat melawan sejumlah penyakit, baik dalam bentuk sederhana dari bagian tanaman atau bagian yang lebih kompleks (Mussafi *et al.*, 2017). Tanaman mengandung banyak komponen senyawa kimia atau zat aktif seperti diterpenoid, flavonoid, gingerol, dan senyawa-senyawa lain yang dapat dimanfaatkan untuk aksi preventif maupun kuratif terhadap penyakit, salah satunya sebagai imunomodulator (Alkandahri *et al.*, 2018). Menurut Siagian (2020), sistem pertahanan tubuh dapat diaktifkan dengan memberikan imunomodulator yang dapat meningkatkan respons imun seseorang. Sistem imun sangat berperan dalam patogenesis suatu penyakit. Hampir semua penyakit berhubungan dengan sistem imun, apakah itu suatu infeksi, inflamasi, penyakit autoimun, maupun keganasan. Hal ini menandakan sistem imun bisa berubah, dan sekaligus juga dapat dimodulasi dengan berbagai cara, yaitu dengan menggunakan imunomodulator. Oleh sebab itu, saat ini imunomodulator mempunyai posisi yang cukup prospektif dalam pengobatan suatu penyakit.

Imunomodulator adalah zat atau substansi yang dapat mengembalikan sistem imun yang terganggu. Imunitas merupakan suatu sistem pertahanan tubuh yang sangat penting, apabila terjadi penurunan imunitas maka tubuh akan mudah terpapar virus atau bakteri. Sebagian orang mudah mengalami gangguan sistem imun, imunomodulator bekerja untuk mengembalikan sistem imun yang berperan dalam pertahanan tubuh sehingga memberikan manfaat yang besar untuk tubuh. Mekanisme imunomodulator yaitu mengembalikan fungsi imun yang terganggu (imunorestorasi), memperbaiki fungsi sistem imun (imunostimulasi) dan menekan respons imun (imunopresi) (Aldi *et al.*, 2016).

Pada penelitian Lestari & Susanti (2020), terdapat 40 jenis dari 30 famili tumbuhan yang berpotensi sebagai imunomodulator, di antaranya yaitu famili Zingiberaceae dan Euphorbiaceae. Penelitian yang dilakukan oleh Aziz *et al.*, (2020), terdapat 18 jenis tanaman yang dimanfaatkan dalam penanganan COVID-19 sebagai imunomodulator. Tanaman lokal Indonesia sudah terbukti secara turun-temurun mampu bertindak sebagai

imunomodulator, mencegah tindakan antagonis pada stres oksidatif, dan memerangi penyakit yang disebabkan oleh infeksi virus. Mekanisme aktivitas imunomodulator terjadi terutama melalui stimulasi fagositosis, aktivasi makrofag, efek imunostimulan pada peritoneal makrofag, dan stimulasi sel limfoid (Aziz *et al.*, 2020).

Pemanfaatan tanaman sebagai imunomodulator salah satunya dilakukan oleh masyarakat Desa Adi Luhur, Kecamatan Panca Jaya, Provinsi Lampung. Kelompok Tani Wanita Desa Adi Luhur dibentuk dengan tujuan untuk meningkatkan dan mengembangkan kemampuan di bidang pertanian. Hasil budidaya tanaman dikelola oleh Kelompok Tani Wanita pada lahan khusus, kemudian akan dibagikan kepada setiap warga Desa Adi Luhur. Tanaman yang paling banyak dibudidayakan oleh Kelompok Tani Wanita dan dimanfaatkan oleh masyarakat Desa Adi Luhur adalah sambiloto, kunyit, jahe, lempuyang dan temu kunci. Tanaman obat keluarga adalah hasil budidaya rumahan yang berkhasiat obat. Setiap rumah diharapkan memiliki tanaman obat keluarga untuk memenuhi kebutuhan obat herbal yang dapat digunakan untuk aksi preventif ataupun terapi komplementer berbagai penyakit akut hingga kronis dan juga dapat dimanfaatkan sebagai bumbu pelengkap masakan.

METODOLOGI PENELITIAN

Tempat dan Waktu Penelitian Penelitian dilakukan di Desa Adi Luhur Kecamatan Panca Jaya, Kabupaten Mesuji, Provinsi Lampung pada Januari 2021. Di desa ini masih banyak program Tanaman Obat Keluarga (TOGA) yang dilakukan sebagai salah satu program desa, sehingga tanaman obat dapat dijumpai di setiap rumah warga atau lahan khusus untuk tanaman obat keluarga.

Alat dan Bahan Penelitian. Alat dan bahan yang digunakan di antaranya daftar pertanyaan atau kuesioner, buku daftar tumbuhan obat Indonesia untuk identifikasi jenis tumbuhan obat, alat tulis untuk mencatat data yang diperoleh di lapangan, dan kamera untuk dokumentasi.

Metode. Jenis penelitian menggunakan metode pendekatan kualitatif yang bersifat deskriptif. Pengambilan sampel dilakukan secara *purposive sampling*. Teknik pengumpulan data dilakukan dengan kuesioner yang telah dilakukan uji validitas dan reliabilitas. Uji validitas dan reliabilitas dilakukan dengan menggunakan program SPSS versi 20. Data angket yang diperoleh diberikan skor 1 untuk jawaban “setuju” dan 0 untuk jawaban “tidak setuju”. Tabulasi data angket dalam bentuk *Microsoft Excel* atau *file doc* dan dimasukkan ke dalam program SPSS lalu memilih menu *Analyze*, kemudian sub menu *Correlate*, lalu *Bivariate*. Setelah itu, akan muncul kotak baru lalu semua variabel dimasukkan ke dalam kotak *Variables*, setelah di klik *OK* selanjutnya akan muncul *Output* hasil.

Sebanyak 20 item pertanyaan yang diajukan berupa pilihan dengan jawaban “setuju” atau “tidak setuju”. Bagi responden yang menjawab “setuju” akan

mendapatkan skor 1, sedangkan responden yang menjawab “tidak setuju” akan mendapatkan skor 0. Skala yang di gunakan adalah skala Guttman agar mendapatkan jawaban yang tegas terhadap suatu permasalahan yang ditanyakan.

Kriteria inklusi yang ditetapkan pada penelitian ini diantaranya adalah masyarakat Desa Adi Luhur yang bersedia menjadi responden dan mengisi kuesioner, masyarakat yang mengonsumsi jamu 1 bulan terakhir, dan masyarakat dalam rentang usia 20-75 tahun. Jumlah populasi yang memenuhi kriteria inklusi dalam penelitian ini adalah 1.989 orang dengan menggunakan perhitungan *Lameshow* menurut Riyanto & Hatmawan (2020) dibawah ini:

$$n = \frac{Z^2 \cdot P(1 - P) \cdot N}{d^2(N - 1) + Z^2 \cdot P(1 - P)}$$

Keterangan :

n = jumlah sampel

Z = skor Z pada derajat kepercayaan 95% = 1,96

p = maksimal estimasi, ditetapkan 50% (0,50)

d = tingkat kesalahan : 10% (0,10), 5% (0,05) atau 1% (0,01)

N= jumlah populasi yang memenuhi kriteria inklusi (1.989)

Berdasarkan rumus tersebut, diperoleh jumlah sampel sebanyak 92 orang, lalu digunakan penambahan nilai presisi 20% sehingga dibutuhkan 110 responden.

HASIL DAN PEMBAHASAN

Karakteristik Responden

Jumlah responden dalam penelitian ini diperoleh sebanyak 110 orang yang telah memenuhi kriteria inklusi serta dianggap memiliki pengetahuan tentang pemanfaatan tumbuhan sebagai pengobatan. Karakteristik responden seperti pada **Tabel 1** menunjukkan bahwa jumlah responden laki-laki (68,18%) lebih banyak dibandingkan dengan jumlah responden perempuan (31,82%). Hal ini dikarenakan laki-laki memiliki aktivitas fisik yang lebih banyak dan pekerjaan yang lebih berat. Aktivitas fisik yang berat dapat menyebabkan daya tahan tubuh menurun. Daya tahan tubuh dapat ditingkatkan dengan mengatur pola makan, istirahat cukup, dan mengonsumsi tanaman berkhasiat obat seperti jahe dan kunyit (Pamadyo & Mujahid, 2014).

Tabel 1. Karakteristik responden

Karakteristik Responden		Persentase
Jenis Kelamin	Laki – laki	68,18%
	Perempuan	31,82%
Usia	20-49 tahun	31,90%
	50-75 tahun	68,10%
Pendidikan	SD	10,91%
	SMP	20,00%
	SMA	43,64%
	Sarjana	25,45%
Pekerjaan	Ibu rumah tangga	9,09%
	Karyawan swasta	42,73%
	Mahasiswa	9,09%
	Pedagang	13,64%
	PNS	25,45%

Pada penelitian ini, usia dibagi menjadi dua kategori, yaitu 20-49 tahun dan 50-75 tahun. Responden usia 50-75 tahun sebanyak 75 orang (68,1%), sedangkan responden usia 20-49 tahun sebanyak 35 orang (31,90%) yang menggunakan tanaman tradisional untuk menjaga kesehatan. Usia berkaitan dengan *immunosenescence* dimana sistem kekebalan tubuh lupa cara menangkal serangan penyakit dari bakteri maupun virus. Seiring bertambahnya usia, tubuh juga mengalami perubahan sel individu dan seluruh organ tubuh yang menyebabkan terjadinya perubahan fungsi pada tubuh, sel T yang terbentuk akan lebih sedikit karena *thymus* menyusut sehingga terjadi penurunan daya tahan tubuh. Perubahan fungsi dalam tubuh menyebabkan lansia rentan terkena gangguan kesehatan (Mulyadi *et al.*, 2017).

Berdasarkan tingkat pendidikan, responden dengan tingkat pendidikan SMA (43,64%) lebih banyak dibandingkan dengan responden dengan tingkat pendidikan sarjana (25,45%), SMP (20,00%), dan SD (10,91%). Tingkat pendidikan dapat memengaruhi sikap dan respons seseorang terhadap suatu jenis pengobatan. Seseorang yang berpendidikan tinggi akan mendapatkan informasi pengobatan lebih banyak baik dari orang lain maupun media massa. Semakin banyak informasi yang diperoleh maka semakin banyak pengetahuan yang didapat, sehingga seseorang dengan pendidikan tinggi akan memiliki lebih banyak pilihan jenis pengobatan (Febrianti *et al.*, 2018).

Pekerjaan responden di Desa Adi Luhur tergolong bervariasi. Pekerjaan paling banyak adalah karyawan swasta sebanyak 47 orang (42,73%). Jenis pekerjaan berhubungan dengan tingkat pendapatan seseorang. Tingkat pendapatan memengaruhi pilihan pengobatan. Jamu yang berasal dari tanaman tradisional digunakan sebagai alternatif dari penggunaan obat modern, karena jamu lebih mudah didapatkan dan lebih murah (Andriati & Wahjudi, 2016). Selain dipengaruhi oleh karakteristik responden, pengaruh tradisi dan budaya, kearifan lokal, serta kepercayaan masyarakat juga berperan penting dalam penggunaan tanaman obat (Agustina *et al.*, 2022).

Tumbuhan obat yang digunakan oleh masyarakat Desa Adi Luhur

Pengumpulan data menggunakan kuesioner tentang nama dan bagian tumbuhan yang digunakan, cara pengolahan, serta cara penggunaannya. Berdasarkan hasil penelitian, ditemukan sebanyak 5 (lima) tanaman obat yang digunakan oleh masyarakat desa Adi Luhur yaitu jahe, kunyit, lempuyang, sambiloto, dan temu kunci (Tabel 2). Tanaman jahe, kunyit, lempuyang, dan

temu kunci merupakan tanaman famili Zingiberaceae, sedangkan sambiloto merupakan tanaman famili Acanthaceae. Tanaman tersebut secara empiris dipercaya dapat menyembuhkan penyakit, sebagai imunomodulator dan beberapa diantaranya seperti kunyit, jahe, dan temu kunci dapat digunakan sebagai bahan tambahan masakan dalam keseharian masyarakat (Hidayah & Indradi, 2020).

Tabel 2. Tumbuhan obat yang digunakan oleh masyarakat Desa Adi Luhur

Nama Umum	Nama Ilmiah	Famili	Bagian yang Digunakan	Cara Pengolahan	Cara Penggunaan	Khasiat empiris
Jahe	<i>Zingiber officinale</i>	Zingiberaceae	Rimpang	Direbus	Diminum	Masuk angin
Kunyit	<i>Curcuma domestica</i>	Zingiberaceae	Rimpang	Diparut	Diminum	Maag
Lempuyang	<i>Zingiber zerumbet</i>	Zingiberaceae	Rimpang	Direbus	Diminum	Pegal linu
Sambiloto	<i>Andrographis paniculata</i>	Acanthaceae	Daun	Direbus	Diminum	Demam
Temu kunci	<i>Boesenbergia rotunda</i>	Zingiberaceae	Rimpang	Direbus	Diminum	Diare

Zingiberaceae merupakan famili tanaman terbesar dan terdistribusi secara luas di wilayah Asia. Keberagaman famili tersebut dapat disebabkan oleh beberapa faktor seperti musim, suhu, dan intensitas hujan. Pada suhu tropis famili Zingiberaceae lebih mudah tumbuh bahkan dengan perawatan yang minimal (Paramita *et al.*, 2019). Selain digunakan sebagai bumbu masak, Zingiberaceae juga digunakan sebagai obat tradisional untuk mengatasi masalah batuk, sakit tenggorokan, demam, dan lain-lain (Hidayah & Indradi, 2020). Acanthaceae merupakan famili tanaman terbesar kedua yang banyak diteliti untuk tujuan pengobatan. Sambiloto merupakan salah satu tanaman famili Acanthaceae yang sering dikonsumsi oleh masyarakat untuk memberikan sensasi hangat pada tubuh, menurunkan demam, dan mencegah gejala flu (Agustina *et al.*, 2022). Penelitian mengenai pemanfaatan tanaman famili Acanthaceae sebagai tanaman obat juga telah dilakukan oleh Fongod *et al.*, (2013) di Kamerun yang menyatakan bahwa pemanfaatan tanaman famili Acanthaceae sangat erat kaitannya dengan budaya lokal dan kepercayaan masyarakat setempat.

Bagian tanaman yang lebih banyak digunakan adalah bagian rimpang. Hasil penelitian serupa juga menyatakan bahwa jenis tanaman yang banyak digunakan adalah golongan rimpang-rimpangan terutama kunyit yang digunakan sebagai upaya pengobatan tambahan (Puspareni *et al.*, 2020). Namun ada pula penelitian serupa yang menyatakan bahwa bagian daun lebih banyak digunakan untuk pengobatan tradisional. Masyarakat percaya bahwa pada bagian daun terdapat zat berkhasiat bagi tubuh (Mulyani *et al.*, 2020).

Cara penggunaan tanaman sebagai obat yang paling tinggi adalah dengan cara diminum. Responden menyatakan bahwa diminum adalah cara penggunaan yang mudah dilakukan dan paling efektif. Masyarakat

lebih sering menjadikan tumbuhan dijadikan sebagai air rebusan dibandingkan mengonsumsi secara langsung. Selain itu, proses penyembuhannya menjadi lebih cepat karena langsung diproses dalam metabolisme tubuh (Gunarti, 2021). Hal ini sejalan dengan penelitian Suwardi *et al.*, (2021) yang menyatakan bahwa pengolahan dengan cara direbus kemudian diminum merupakan cara pengolahan yang paling banyak dilakukan. Cara pengolahan direbus dapat diaplikasikan pada semua bagian tanaman seperti daun, rimpang, akar, buah, dan bunga. Selain itu, pengolahan dengan cara direbus juga dipercaya dapat menjaga senyawa aktif tanaman dan menghilangkan racun yang terdapat dalam tanaman (Chaachouay *et al.*, 2022).

Tumbuhan obat yang digunakan oleh masyarakat desa Adi Luhur telah dibuktikan khasiatnya secara ilmiah. Jahe (*Zingiber officinale*) memiliki kandungan senyawa gingerol yang dapat dimanfaatkan sebagai imunomodulator. Selain imunomodulator, jahe juga dapat digunakan sebagai imunostimulan dan immunosupresan sebagai agen antiinflamasi, salah satunya adalah dengan menghambat enzim siklooksigenase (COX-1 dan COX-2). Uji secara *in vitro* pada sel T manusia selama 48 jam dengan pemberian 6-gingerol, 8-gingerol, dan 10-gingerol, terdapat peningkatan sitokin inflamasi. (Schoenkecht *et al.*, 2016). Aktivitas imunostimulan jahe juga dapat menurunkan tingkat infeksi dari *Ichthyophthirius* pada ikan *grass carp* (*Ctenopharygodon idellus*) (Lin *et al.*, 2016).

Kunyit (*Curcuma domestica*) dikenal sebagai bahan yang digunakan untuk mengobati beberapa penyakit. Kurkumin merupakan salah satu senyawa aktif pada kunyit yang memiliki aktivitas sebagai imunostimulan yang dapat menstimulasi sistem imun dengan meningkatkan aktivitas komponen sistem imun untuk melawan infeksi dan penyakit (Yusuf *et al.*, 2023).

Obat tradisional atau obat herbal yang mengandung kunyit telah banyak didaftarkan dengan klaim membantu memelihara kesehatan tubuh dan membantu memperbaiki nafsu makan. Aktivitas imunomodulator secara *in vivo* dengan menggunakan *poly d,l-lactic-co-glycolic acid entrapped curcumin nanoparticle* pada mencit albino yang diinduksi dengan sel darah merah kambing (SRBCs) memperlihatkan bahwa *nano curcumin* dengan dosis 5 mg/kg dan 10 mg/kg meningkatkan respons imun yang memediasi respons awal sel dibandingkan kontrol. Hasil yang sama juga terjadi pada antibodi humoral sekunder, di mana produksi sel darah putih dan berat organ limfoid juga meningkat pada kelompok yang diberi 10 mg/kg *nano curcumin* (Afolayan *et al.*, 2018).

Rimpang tanaman lempuyang dapat digunakan untuk mengatasi gangguan imun. Lempuyang merupakan tanaman dari genus *Zingiber*. Senyawa yang terkandung pada lempuyang adalah *zerumbone*. Lempuyang dapat menurunkan proses inflamasi, sehingga dapat dikatakan bahwa komponen dari lempuyang memiliki aktivitas sebagai immunosupresan. Hasil penelitian sebelumnya menunjukkan adanya penurunan ekspresi CD18 terhadap penurunan proses inflamasi dengan pemberian *zerumbone* pada *polymorphonuclear neutrophils* yang diinduksi oleh lipopolisakarida secara *in vitro* (Akhtar *et al.*, 2019). Ekstrak etanol lempuyang juga terbukti memiliki efek immunosupresan pada kelompok tikus yang diberikan ekstrak dengan dosis 200 dan 400 mg/kg BB dibandingkan dengan kelompok kontrol yang hanya diberikan Tween 20 (Ghezalee *et al.*, 2019).

Sambiloto memiliki aktivitas sebagai immunomodulator, karena adanya kandungan senyawa aktif berupa andrografolid, deoksiandrografolid, neoandrografolid, 14-deoksi-11, 12-didehidroandrografolid, homoandrografolid, diterpenoid, dan flavonoid (Rahayu, 2015). Sambiloto juga digunakan sebagai immunostimulator yang dapat meningkatkan respons imun saat kekebalan tubuh menurun, dan menjadi immunosupresor yang dapat menurunkan respons kekebalan tubuh saat sistem kekebalan tubuh meningkat melebihi kondisi tubuh normal. Selain sebagai immunomodulator, sambiloto juga mampu menormalkan kondisi tubuh saat terjadi infeksi (Alkandahri *et al.*, 2018).

Temu kunci memiliki kandungan senyawa flavanon (inostrobin, pinosembrin, alpinetin, dan 5,7-dimetoksiflavanon), flavon (dimetoksinflavon dan 3,4,5,7-tetra-metoksiflavanon), kalkon (2,6-dihidroksi-4-metoksikalkon, Panduratin A panduratin B, cardamonin, boesenbergin A, boesenbergin B, dan rubranin), monoterpene (garnial dan neral), dan diterpene (asam pimaric) (Hati *et al.*, 2019). Hasil penelitian menunjukkan bahwa senyawa dalam tanaman temu kunci dapat digunakan sebagai immunomodulator dengan adanya kandungan panduratin yang bersifat kompleks sehingga dapat menstimulasi pembentukan antibodi. Kandungan panduratin A menjadi faktor utama yang bertindak sebagai immunomodulator (Choi *et al.*, 2012).

KESIMPULAN

Terdapat lima jenis tanaman (sambiloto, jahe, kunyit, lempuyang, dan temu kunci) dari dua famili (Acanthaceae dan Zingiberaceae) yang digunakan sebagai tumbuhan obat di Desa Adi Luhur. Bagian tanaman yang paling banyak digunakan sebagai obat adalah rimpang dengan cara direbus lalu diminum.

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