Community Acquired Infection

ORIGINAL ARTICLE

In silico identification of a *Lactobacillus plantarum* metabolic product that potentially inhibits three essential human immunodeficiency virus-2 enzymes

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ABSTRACT

Background: Human immunodeficiency virus (HIV)-2 does not spread as rapidly as HIV-1; however, it poses a threat because it has a higher mutation rate and is resistant to existing antiretroviral drugs. Hence, it is vital to design inhibitors that specifically target HIV-2 to reduce the occurrence of HIV-2 infections. **Methods:** In this study, we focused on natural products from a probiotic, namely plantaricins D, BN, JLA-9, and W from *Lactobacillus plantarum*, due to their reported ability to inhibit viral replication and trigger an enhanced immune response in viral infections, and the lack of resistance to these compounds. We screened these compounds for anti-HIV-2 activity using an in silico methodology, which consisted of collecting and preparing data from online databases, identifying ligand-binding sites *via* docking simulations, performing molecular interactions analyses, visualizing the three-dimensional structures of the ligand-target complexes and determining their stability, and calculating the translocation probability of the ligand. **Results:** The results showed that plantaricin JLA-9 from *L. plantarum* may inhibit three essential HIV-2 enzymes: protease, reverse transcriptase, and integrase. **Conclusion:** This *in silico* study establishes plantaricin JLA-9 as a highly promising multi-target candidate for HIV-2 inhibitor development, demonstrating effective binding to protease, reverse transcriptase, and integrase. Its natural origin and predicted efficacy against multiple drug targets make it a standout lead compound. We therefore advocate for its prioritization in subsequent *in*

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vitro and *in vivo* validation studies to accelerate the development of specific HIV-2 therapeutics.

Key words: antiviral, bioinformatics, human immunodeficiency virus-2, *Lactobacillus plantarum*, plantaricin

INTRODUCTION

Despite the development of effective antiviral drugs, human immunodeficiency virus (HIV)-1 continues to pose a threat to human health.[1] In addition, an estimated one to two million people are infected with HIV-2 worldwide.[2] HIV-2 spreads less rapidly than HIV-1; however, its high mortality rate and resistance to antiretroviral drugs contribute to the persistence of HIV-2 infections.[3,4] HIV-2 is highly resistant to current antiretroviral drugs, including protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and integrase strand transfer inhibitors.[5,6] Furthermore, the use of antiretroviral drug combinations has been shown to result in adverse reactions and other issues.[7–9]

Several studies have revealed that probiotics have antiviral effects.[10–12] Thus, they may have potential as anti-HIV-2 agents. Probiotics are bacteria that are beneficial to the host, and they have been shown to have a range of effects. For example, probiotics have been shown to stimulate the immune system to respond effectively to pathogens.[13] More specifically, probiotics help fight viral infections by changing the host cell membrane permeability, modulating the immune system, and generating metabolic products that inhibit viral replication.[14] In addition, probiotics have potential as therapeutic agents due to the positive effects some of their metabolic products have on diseases such as dermatitis, cancer, and inflammatory bowel disease.[13] *Lactobacillus plantarum* is a probiotic, rod-shaped Gram-positive species found in foods and beverages generated *via* fermentation that provides various pharmacological benefits.[15]

Several studies have shown that lactic acid bacteria (LAB), such as *Lactiplantibacillus plantarum* KAU007, can reduce the viral load of H1N1 influenza A and have low cytotoxicity.[16] It was also shown that administering a mix of LAB (*L. plantarum*, *Bifidobacterium longum*, and *Lactococcus lactis* spp.) could induce anti-coronavirus disease 2019 (COVID-19) cytokines, such as interleukin-18 (IL-18), IL-1β, tumor necrosis factor-α (TNF-α), IL-8, and IL-6.[17] *L. plantarum* Probio-88 strain P88-CFS was found to hinder Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection by inhibiting viral replication and reducing the levels of reactive oxygen species and proinflammatory cytokines (*e.g.*, IL-6, interferon-α [IFN-α], and IFN-β).[18,19] *L. plantarum* strain SN35N isolated from pear fruit reportedly has anti-inflammatory activity and antiviral activity against influenza A and feline calicivirus.[20] While evidence suggests that *L. plantarum* has anti-HIV-1 activity, with a slight increase in the cluster of differentiation (CD) 4^+/$CD8^+$ T-cell ratio reported after exposure to *L. plantarum*, further research is needed to confirm this finding.[21]

In terms of the specific *L. plantarum* compounds responsible for the observed antiviral activity of this probiotic, plantaricins D, BN, JLA-9, and W have been identified as exhibiting anti-SARS-CoV-2 activity. Using an *in silico* approach, these plantaricins were determined to have potential as anti-COVID-19 agents.[11] Previous studies have not shown the potential of plantaricin for anti-HIV-2.

Given that other studies have shown that *L. plantarum* may be an effective anti-HIV-1 agent and that its plantaricins D, BN, JLA-9, and W may have anti-SARS-CoV-2 activity, we aimed to (1) determine whether these plantaricins can inhibit three essential HIV-2 enzymes and thus prevent viral replication, (2) identify the molecular mechanisms underlying the antiviral activity, and (3) evaluate their potential as antiretroviral compounds. To achieve these objectives, we adopted an *in silico* approach.

MATERIALS AND METHODS

Ligand retrieval and preparation

Data on *L. plantarum* plantaricin W (CID 139586573), plantaricin JLA-9 (CID 132535900), plantaricin D (CID 139586697), and plantaricin BN (CID 380907) were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).[11] These compounds were used as ligands. Energy minimization, in preparation for the docking experiments, was performed using OpenBabel integrated with PyRx v.1.1.0 (The Scripps Research Institute, La Jolla, CA, USA) with an academic license and the following parameters: force field = uff, optimization algorithm = conjugate gradient, total number of steps = 200, number of steps for update = 1, and stop if energy difference is less than = 0.1.[22,23]

Target retrieval and preparation

Data on three HIV-2 enzymes were retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (PDB) database (https://www.rcsb.o

rg/): protease (PDB ID: 3EBZ),[24] reverse transcriptase (PDB ID: 1MU2),[25] and integrase (PDB ID: 3F9K).[26] These proteins were used as targets. Native ligands and water molecules were removed from the targets using PyMol v.2.5.0 (Schrödinger, LLC, Cambridge, MA, USA; https://pymol.org/) with an academic license for molecular docking optimization.[27]

Molecular docking simulations

Molecular docking experiments are performed to determine the strength of interactions within ligand-target complexes *via* binding affinity values.[28] We performed molecular docking simulations to determine the strength with which the *L. plantarum* plantaricins bound to the target enzymes and their capacity to inhibit enzymatic activity. We utilized the screening docking method, which involved directing the docking grid to cover the entire target area. This method was also used to identify new binding regions for antiretroviral candidates on the target enzymes and to avoid mutation domains on the targets.[11] The molecular docking simulations were performed using PyRx v.1.1.0 software (with an academic license).[29]

Identification of molecular interactions

Molecular interactions within ligand-target complexes were identified using LigPlot$^+$ v2.2.4 software (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/). Hydrogen bonds and hydrophobic interactions are relatively weak interactions; however, they contribute to the stability of ligand-target complexes and can mediate ligand-induced biological responses. These types of interactions can be detected using LigPlot$^+$ v2.2.4.[30] Molecular interaction data can be used to identify strategic ligand-binding domains.[31]

Visualization of the ligand, target, and complex structures

The three-dimensional (3D) structures of the ligands, targets, and ligand-target complexes were visualized using PyMol v2.5 software (https://pymol.org/). The structural elements and colors used to visualize the compounds were selected to ensure alignment with publication standards (*i.e.*, use of cartoons, surfaces, and sticks).[32]

Molecular dynamics simulations

The molecular docking results were validated via molecular dynamics simulations performed using the CABS-flex v2.0 server (https://biocomp.chem.uw.edu.pl/CABSflex2/index). Each root mean square fluctuation (RMSF) value (down to < 3 Å[10^{-10} m]) was reviewed to determine the stability of the associated interaction hotspot. Ligands involved in stable interactions are predicted to trigger specific biological activities *via* the target.[33]

Membrane translocation probability

The CELLPM tool (https://cellpm.org/) was used to simulate passive translocation through the cell membrane. The methodology reflected naturally occurring membrane-peptide interactions with biological responses, such as penetration due to polarity and molecular charge factors without harming the cell membrane.[34]

Toxicity prediction

The toxicity of the ligands was evaluated using ProTox v3.0 (https://tox.charite. de/protox3/). Computational methods are often used to estimate the toxicity of compounds to reduce the need for animal experimentation. The median lethal dose (LD_{50}), class, accuracy, query similarity, and toxicity type of compounds can all be estimated using computational methods.[35]

RESULTS

Structural properties of the L. plantarum plantaricins and HIV-2 enzymes

L. plantarum is a probiotic species with many health benefits, including antiviral activity.[15] Among its secondary metabolites are plantaricins W, JLA-9, D, and BN,[11] which were used as ligands in this study. Information on each plantaricin was retrieved from the PubChem database, including the compound name, CID, molecular weight, formula, canonical simplified molecular input line entry system (SMILE), and 3D structure (Table 1). Two-dimensional images obtained from the database were edited using Canva (https://www.canva.com/) and downloaded in portable network graphics (PNG) format to obtain the representative images shown in Figure 1.

The targets consisted of HIV-2 protease (PDB ID: 3EBZ), integrase (PDB ID: 3F9K), and reverse transcriptase (PDB ID: 1MU2). The 3D protein structures were obtained from the RCSB PDB database and visualized using PyMol v2.5.0 software (Figure 2).

Binding affinity of plantaricins for HIV-2 enzymes

Plantaricins W, JLA-9, D, and BN were found to interact with HIV-2 protease, reverse transcriptase, and integrase, with binding affinity values below –6.0 kcal/mol (Table 2). Among these, plantaricin JLA-9 exhibited the strongest binding to all three enzymes, with affinity values of –9.0, –9.8, and –7.4 kcal/mol for HIV-2 protease, reverse transcriptase, and integrase, respectively. Based on this result, which indicated that plantaricin JLA-9 bound with the highest affinity to

Table 1: Details of the *L. plantarum* plantaricins included in this study

Ligand	CID	Molecular weight (g/mol)	Formula	Canonical SMILE
Plantaricin W	139586573	1751.1	$C_{75}H_{131}N_{25}O_{21}S$	CCC (C) C (C (= O) NC (C) C (= O) NC (CC[= O] N) C (= O) NC (CC1= CC= C (C= C1]O) C (= O) NC (CC (C]C) C= O) NC (= O) C (C) NC (= O) C (CC (= O]N) NC (= O) C (CCCCN) NC (= O) C (CCCN= C [N] N) NC (= O) C (CCCN= C (N]N) NC (= O) C (CO) NC (= O) C (C (C]O) NC (= O) C (CCCCN) NC (= O) C (C [C] O) NC (= O) C (CCSC) *N*
Plantaricin JLA-9	132535900	1044.2	$C_{51}H_{69}N_{11}O_{11}S$	CC (C[= O] O) NC (= O) C (CC1= CC= CC= C1) NC (= O) C (CO) NC (= O) C (CCSC) NC (= O) C (CCCCN) NC (= O) C (CCC (= O] N) NC (= O) C (CC2= CNC3= CC= CC= C32) NC (= O) C (CC4= CC= CC= C4) *N*
Plantaricin D	139586697	946.1	$C_{40}H_{71}N_{11}O_{13}S$	CCC (C) C (C= O) NC (= O) C (C) NC (= O) C (C (C] O) NC (= O) C (C) NC (= O) CNC (= O) C (CCSC) NC (= O) C (CCC (= O]N) NC (= O) C (CC [C] C) NC (= O) C (CO) NC (= O) C (C) *N*
Plantaricin BN	380907	484.5	$C_{24}H_{36}O_{10}$	C1CCCCC (C (= O) C (= O) C (CCC1) (CCC[= O] O) CCC (= O) O) (CCC[= O] O) CCC (= O) O

SMILE, simplified molecular input line entry system.

Table 2: Binding affinity between each plantaricin (ligand) and HIV-2 enzyme (target)

Ligand	CID	Minimization energy (kcal/mol)	Binding affinity (kcal/mol)		
			Protease (PDB ID: 3EBZ)	Reverse transcriptase (PDB ID: 1MU2)	Integrase (PDB ID: 3F9K)
Plantaricin W	139586573	+1751.06	-6.1	-6.7	-6.7
Plantaricin JLA-9	132535900	+1044.23	-9.0	-9.8	-7.4
Plantaricin D	139586697	+946.12	-7.3	-6.6	-7.2
Plantaricin BN	380907	+484.54	-6.5	-7.5	-7.1

PDB, Protein Data Bank.

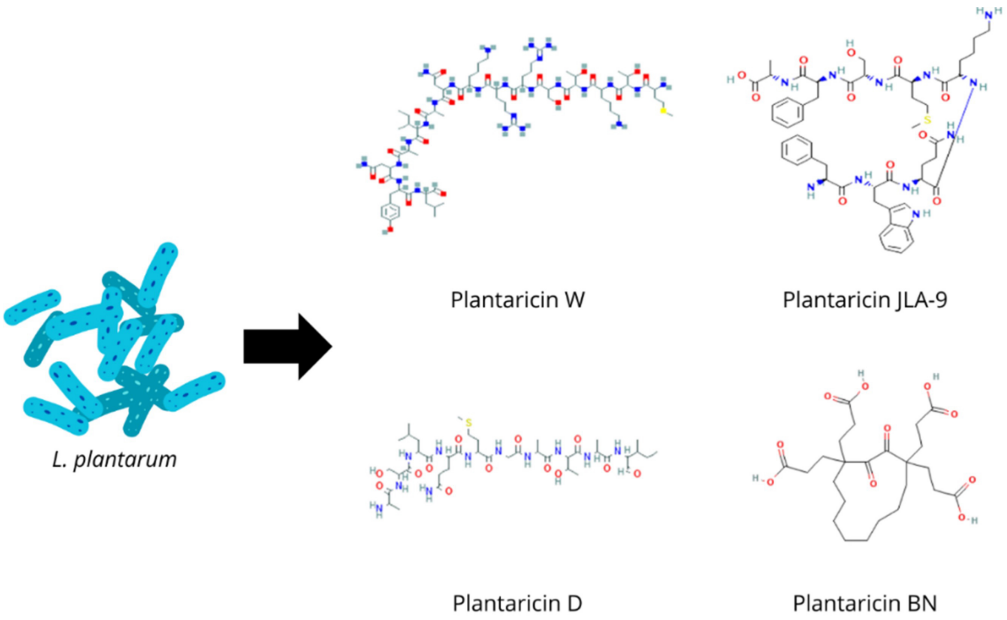

Figure 1. Structures of plantaricins W, JLA-9, D, and BN produced by *L. plantarum*.

HIV-2 enzymes, we selected this ligand for all subsequent experiments, while excluding the others, to focus on the most promising candidate. The structures of the ligand-target complexes involving plantaricin JLA-9 generated from the molecular docking results are displayed in Figure 3. These results support those of a previous *in silico* study, which showed plantaricins from probiotics could potentially inhibit SARS-CoV-2 replication,[11] and those of a study in which probiotics were found to inhibit influenza virus infection by

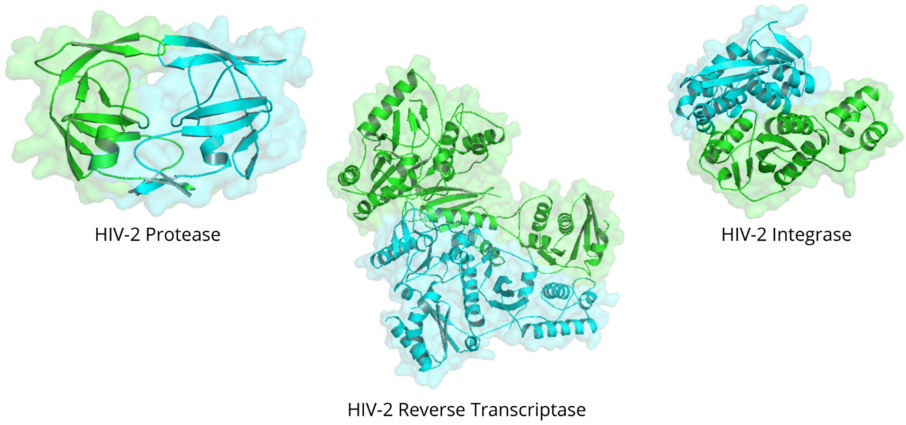

Figure 2. Visualization of the 3D structures of the HIV-2 enzymes used as targets in this study. Chain A: green; chain B: cyan. HIV-2, human immunodeficiency virus.

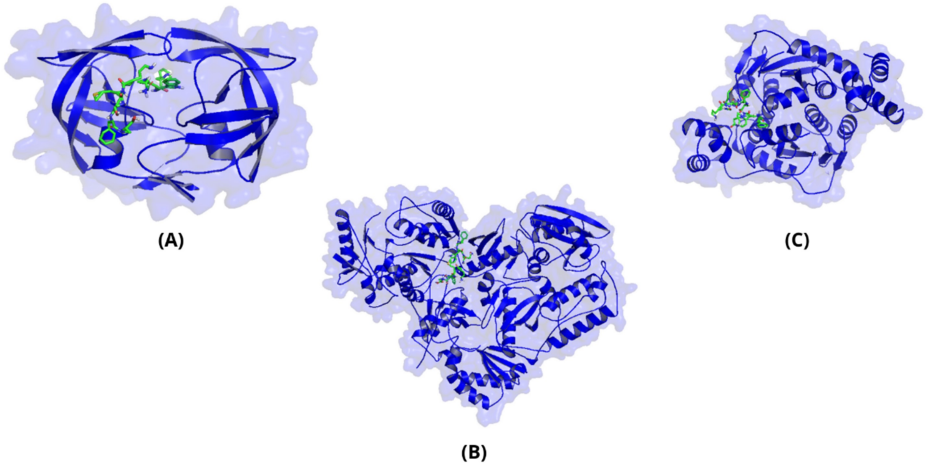

Figure 3. Visualization of the ligand-target complex structures involving plantaricin JLA-9 generated from the molecular docking results. (A) Plantaricin JLA-9 and protease, (B) plantaricin JLA-9 and reverse transcriptase, and (C) plantaricin JLA-9 and integrase. The ligand is shown with a green backbone, and the targets are shown in blue.

enhancing the immune response.[36]

Molecular interactions and dynamics of the ligand-target complexes involving plantaricin JLA-9

Weak molecular interactions, such as hydrogen bonds and hydrophobic contacts, play a critical role in stabilizing ligand–target complexes and mediating biological activity.[37–39] To better understand how plantaricin JLA-9 interacts with HIV-2 enzymes, we analyzed its molecular interactions and dynamic behavior when bound to protease, reverse transcriptase, and integrase. Our analysis focused on hydrogen bonding and hydrophobic interactions occurring at domains with RMSF values < 3 Å (Table 3). The molecular interaction plots are presented in Figure 4, and the stability of the amino acids within the identified interaction hotspots is shown in Figure 5.

Membrane translocation probability and toxicity of plantaricin JLA-9

In membrane translocation prediction studies, compounds with a log permeability coefficient (logPcalc) value greater than -5 are generally predicted to cross the cell membrane when their surface area is approximately 10 Å.[40,41] In this study, plantaricin JLA-9 had a logPcalc value of -22.56 (Figure 6), which indicates that it possesses the ability to cross the cell membrane.

When the toxicity of plantaricin JLA-9 was evaluated, it was predicted to have an LD50 value of 5000 mg/kg and was categorized in toxicity class 5, with an average similarity value of 57.62% and a prediction accuracy of 67.38%. Organ-level toxicities were predicted, including hepatotoxicity, neurotoxicity, and cardiotoxicity, whereas

Table 3: Molecular interaction sites and RMSF values of ligand-target complexes formed with plantaricin JLA-9

Natural product	Interaction hotspots and RMSF		
	Protease (PDB ID: 3EBZ)	Reverse transcriptase (PDB ID: 1MU2)	Integrase (PDB ID: 3F9K)
Plantaricin JLA-9	Hydrogen bonds: Gly48, Asp29, Arg87 Hydrophobic interactions: Arg108, Glu58, Lys45, Lys107, Asp30, Trp106, Thr91, Asn88 RMSF (Å): 1.134	Hydrogen bonds: Arg22, Leu92, Glu139, Ser134 Hydrophobic interactions: Trp24, Val380, Asn136, Pro133, Leu26, Lys376, Ile31, Asn137, Pro140, Gly141, Gln23, Val135, Pro25, Leu379 RMSF (Å): 0.887	Hydrogen bonds: Lys25, Gly134, His181, Ile133 Hydrophobic interactions: Thr111, Ile110, Lys186, Leu58, Pro109, Gly59 RMSF (Å): 0.897

PDB, Protein Data Bank.

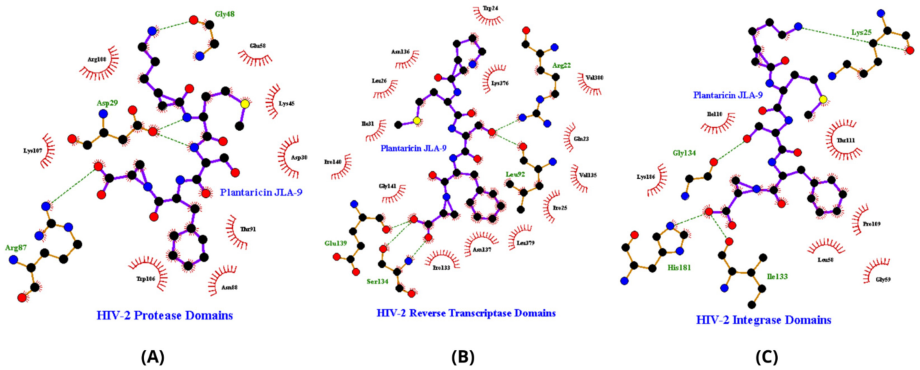

Figure 4. Two-dimensional molecular interaction plots of the ligand-target complexes involving plantaricin JLA-9. (A) Plantaricin JLA-9 and protease, (B) plantaricin JLA-9 and reverse transcriptase, and (C) plantaricin JLA-9 and integrase. Hydrogen bonds are shown as green lines, hydrophobic interactions are shown as eyelash-like structures, and the amino acids of the target are labeled.

nephrotoxicity was not predicted. The compound was also predicted to have carcinogenic, immunotoxic, mutagenic, and cytotoxic potential. No effect on the blood–brain barrier (BBB) was predicted, and the compound was not expected to be ecotoxic. This preliminary information may help guide wet-lab experiments in animal models.[42]

DISCUSSION

Probiotics are known to have multiple health benefits. For example, they have been shown to inhibit viral infection, induce an enhanced mucosal immune response to support viral elimination without acute inflammation, and produce compounds that inhibit essential viral proteins.[43] In this study, we identified a natural product of a probiotic that may inhibit the activity of three essential HIV-2 enzymes, as well as its potential mechanism of action. Given that existing antiretroviral drugs have limited effects on HIV-2, it is of paramount importance to identify and develop compounds that are active against HIV-2.[44] Previous studies have shown that integrase inhibitors disrupt HIV replication by forming hydrogen bonds in the integrase catalytic domain,[45–47] and that NNRTIs participate in hydrophobic interactions in the active site of HIV reverse transcriptase, inhibiting its activity.[48,49]

In this study, plantaricins W, JLA-9, D, and BN were found to bind to HIV-2 protease, reverse transcriptase, and integrase, with the strongest binding affinities observed between plantaricin JLA-9 and protease (-9.0 kcal/mol), reverse transcriptase (-9.8 kcal/mol), and integrase (-7.4 kcal/mol). Since these binding affinity values were all below -6.0 kcal/mol, it is predicted that plantaricin JLA-9 has an effect on each of these enzymes.[10] Our results align with those of previous studies that found that the binding affinity values of candidate HIV inhibitors ranged from -10.3 kcal/mol to -6.4 kcal/mol.[50] The HIV-1 PI amprenavir was found to have a binding affinity value of -9.0 kcal/mol, which is similar to that of plantaricin JLA-9 in our study; however, plantaricin JLA-9 had a more negative binding affinity value than nevirapine, an NNTRI.[51] It has been reported that the optimal binding affinity value for HIV-1 integrase inhibitors is below -7.0 kcal/mol.[52]

Our molecular dynamics simulations showed that plantaricin JLA-9 likely participates in hydrogen bonds and hydrophobic interactions at all interaction sites with RMSF values < 3 Å. Thus, it is proposed that plantaricin JLA-9 inhibits the activity of all the target enzymes by

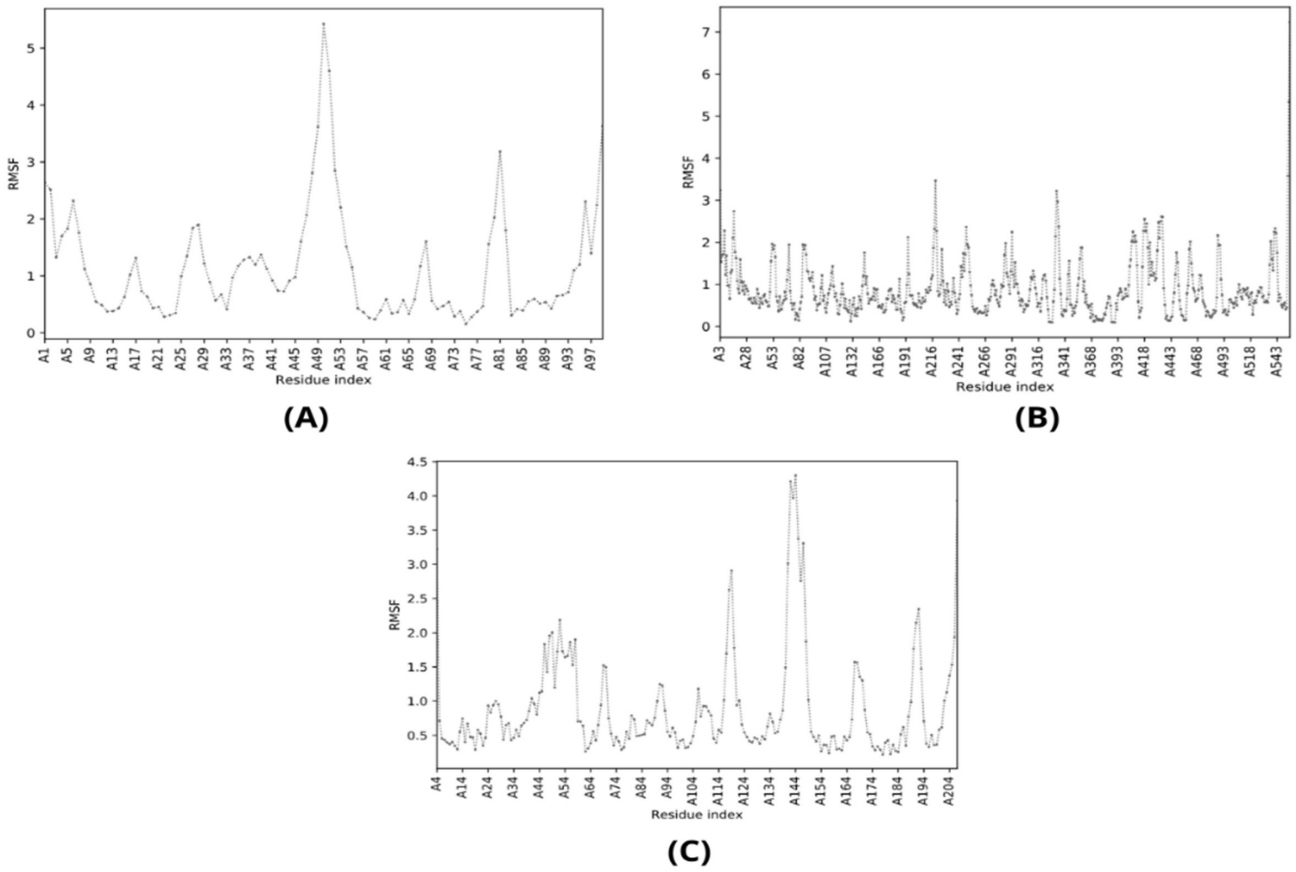

Figure 5. Dynamic fluctuation plots of the ligand-target complexes formed with plantaricin JLA-9. (A) Plantaricin JLA-9 and protease, (B) plantaricin JLA-9 and reverse transcriptase, and (C) plantaricin JLA-9 and integrase. RMSF, root mean square fluctuation.

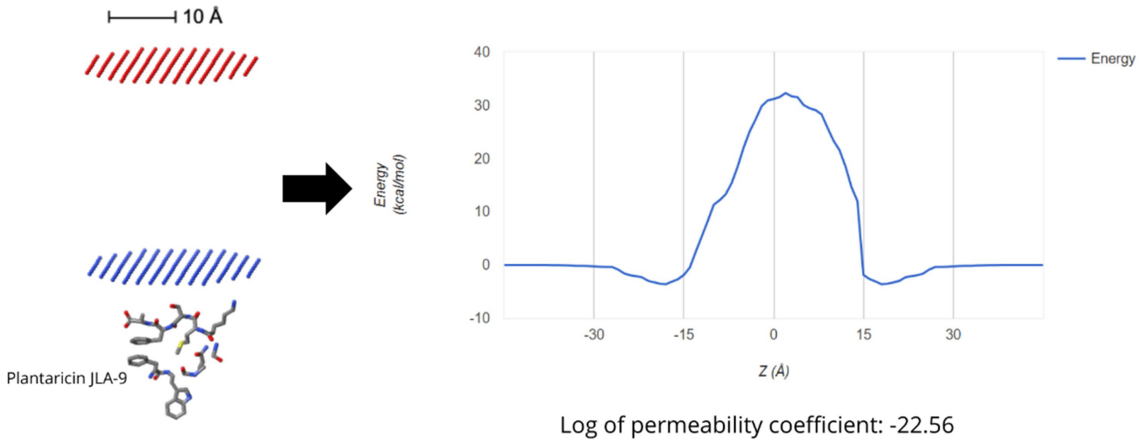

Figure 6. Dynamic fluctuation of the ligand-target complexes formed with plantaricin JLA-9.

forming stable ligand-target complexes *via* weak interactions. In computational studies, the presence of weak interactions is taken into account when determining the effectiveness of candidate antiviral compounds.[53] In addition, our results indicate that plantaricin JLA-9 can translocate across the cell membrane ($logP_{calc}$ value > -5) and thereby access the target enzymes.

Thus, our findings indicate that plantaricin JLA-9 has several advantages over other antiviral compounds. First, it is predicted to have low toxicity and few side effects.

Second, it is a natural product of probiotic bacteria. Third, it likely forms stable ligand-target complexes with the target enzymes through weak interactions. Fourth, there is a high probability that it can translocate across the cell membrane. Finally, based on our results, plantaricin JLA-9 may inhibit three HIV-2 enzymes.

CONCLUSION

In this in silico study, *L. plantarum* plantaricin JLA-9 was found to have potential as an inhibitor of HIV-2 protease, reverse transcriptase, and integrase. Its strong binding affinity for the target enzymes is likely mediated by hydrogen bonds and hydrophobic interactions, and it is predicted to form stable ligand-target complexes. Our results indicate that plantaricin JLA-9 has low toxicity and can translocate across the cell membrane to reach the target enzymes. Hence, plantaricin JLA-9 is a candidate triple inhibitor of HIV-2.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

DECLARATION

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Author contributions
Kharisma VD: Conceptualization, Methodology, Data Curation, Writing—Original Draft Preparation. Sahadewa S: Investigation, Formal Analysis, Visualization. Fara Disa Durry: Resources, Data Curation, Validation. Wiradana PA: Software, Formal Analysis, Visualization. Turista DDR: Supervision, Writing—Review & Editing, Project Administration. Jakhmola V: Validation, Resources, Writing—Review & Editing. Rebezov M: Funding Acquisition, Project Administration, Writing—Review & Editing. Khairullah AR: Supervision, Writing—Review & Editing, Project Administration. Ansori ANM: Conceptualization, Supervision, Writing—Review & Editing, Final Approval of Manuscript. All authors have read and agreed to the published version of the manuscript.

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Ethics approval
Not applicable.

Conflicts of interest
The authors declare no conflicts of interest in any capacity, including competing or financial interests.

Use of large language models, AI and machine learning tools
None declared.

Data availability statement
No additional data.

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