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Lucilia sericata Lucifensin as a Therapeutic Inhibitor: Protein Structure Predictions and Effects on JAK Kinase and PDE-4 in Atopic Dermatitis

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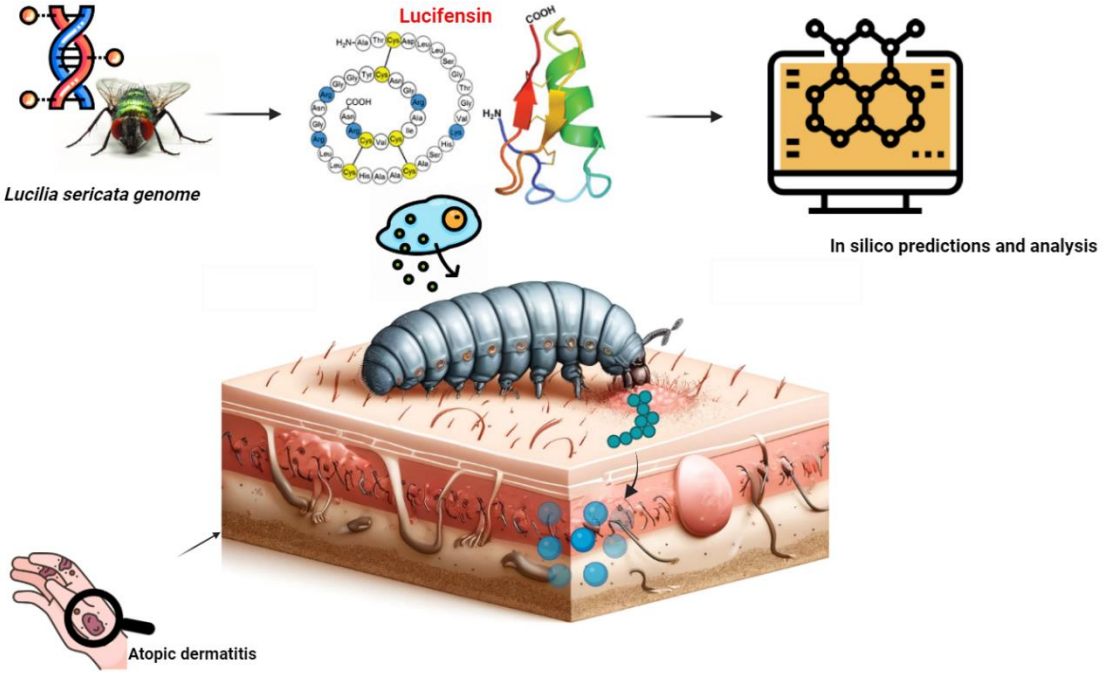
Grafical Abstract



Abstract: Lucifensin is an antimicrobial component structurally located in Lucilia sericata and functions as a defensin. Lucilia sericata larvae are used in the treatment of chronic wounds which are named Maggot Debridement Therapy. While the larvae perform debridement at the wound site, larval secretions also exhibit antimicrobial effects. Lucifensin is believed to be the key component underlying the mentioned debridement feature. This study aims to enhance the protein structure prediction accuracy of lucifensin through in silico approaches and to reveal its potential inhibitory effects on structures involved in atopic dermatitis, a chronic inflammatory disease. The genome mining of Lucilia sericata has revealed the structural proteins it secretes. Simultaneously, the three-dimensional structures of lucifensin were modeled, validated, and its active regions predicted. Through protein-protein docking and molecular dynamics, antagonist effects on JAK-Kinase 1 and Phosphodiesterase 4, which are involved in atopic dermatitis, were identified with high binding affinity. The structural dynamics observed through the analyses indicate that the target protein-lucifensin complexes maintained consistent binding with minimal changes in their backbones over a 500 ps simulation

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period, potentially enabling inhibition within the protein's active pockets through various chemical bonds. These findings reveal that this lucifensin holds promise as a potential lead compound for developing new drugs targeting proteins associated with atopic dermatitis. The in silico analyses highlight the significant potential biochemical mechanisms underlying the investigation of lucifensin as a drug molecule, laying a foundation for further research and in vitro exploration, ultimately aiming to advance drug discovery strategies for the treatment of atopic dermatitis and other dermal diseases.

Keywords: Lucifensin, Lucilia sericata, in silico, molecular docking, molecular dynamics

Highlight:

- Lucifensin, derived from *Lucilia sericata*, is a potent antimicrobial component used in chronic wound treatment.
- The structure of lucifensin was modeled and its active regions were identified using in silico methods.
- Lucifensin strongly inhibited JAK-Kinase 1 and Phosphodiesterase 4, key proteins in atopic dermatitis.
- This study reveals lucifensin’s therapeutic potential beyond its antimicrobial effects.

1. Introduction

Lucilia genus flies belong to the Cyclorrhapha suborder, which infest the tissues and organs of humans and animals in their larval stages, causing myiasis. L. sericata is one of these fly organisms that undergoes egg, larva, pupa, and adult stages [1]. They lay their eggs in moist areas associated with necrotic tissue in live and dead vertebrates. L. sericata larvae feeds on necrotic organic matter, passing through three stages before maturing [2]. As a result of extracellular digestion, the larvae secrete digestive enzymes into the substrate to pre-digest the tissue and cease feeding before undergoing metamorphosis into adult flies, typically leaving the host. Today, they are one of the medicinal methods used in the treatment of chronic wounds due to their ability to consume necrotic tissue and the high biotherapeutic effects contained in their secretions [3]. The wound healing properties of the larvae primarily occur through debridement and disinfection. The larvae also produce secretions that significantly reduce the quantity of particularly Gram-positive bacteria [4]. Among these secretions is lucifensin, a peptide known to have antimicrobial properties, which contains 40 amino acid residues [5]. This peptide has a high sequence similarity with insect defensins obtained from other insects and structurally serves as a defense mechanism in L. sericata. The exploration role of lucifensin in larval therapy is crucial for expanding its scope of effects and investigating potential for various diseases. Although it has been structurally studied in protein databases, reanalyzing the structure through different servers and algorithms is expected to contribute to existing studies [6]. Another important feature of using larvae in chronic wounds is their anti-inflammatory effect, highlighting the significance of exploring the potential use of lucifensin in inflammatory dermatological diseases [7]. Among the inflammatory skin diseases mentioned, atopic dermatitis is a common condition that leads to chronic inflammation of the skin. This disease, which often begins in childhood, causes symptoms such as intense itching, redness, dryness, and crusting of the skin. Atopic dermatitis is fundamentally characterized by an overreaction of the immune system and a weakened skin barrier. This condition results in a loss of the skin's protective functions, making it easily affected by environmental allergens, irritants, or microorganisms. Researching innovative treatment strategies for the disease and discovering biotherapeutic agents are of great importance because atopic dermatitis can severely diminish patients' quality of life and lead to permanent skin damage. Appropriate therapeutic agents play a critical role in alleviating symptoms, controlling inflammation, and strengthening the skin barrier [8]. JAK kinase-1 and Phosphodiesterase 4 (PDE4) were selected as inhibition targets for their well-documented roles in the pathogenesis of atopic dermatitis. JAK kinase-1 is a critical component of the Janus kinase-signal transducer and activator of

transcription (JAK-STAT) pathway, which regulates the signaling of various cytokines implicated in inflammation and immune dysregulation, both of which are hallmarks of atopic dermatitis [9]. Targeting JAK kinase-1 has been shown to effectively reduce inflammatory cytokine signaling, thereby alleviating symptoms of the disease. Similarly, PDE4 is an enzyme responsible for breaking down cyclic adenosine monophosphate (cAMP), a molecule that suppresses inflammation [10]. Inhibition of PDE4 increases intracellular cAMP levels, which leads to the downregulation of pro-inflammatory mediators. The present study aimed to identify, by in silico approaches, lucifensin secreted by L. sericata and potentials on atopic dermatitis inhibitor biomarkers JAK kinase-1 and (PDE4).

2. Computational Method

2.1. Analysis of the proteins secreted by *L. sericata*

The genomes of *L. sericata* were obtained from the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov) database. The number and content of potentially secreted proteins from the genome were also determined using the analysis tools of the same database. Following this step, lucifensin was specifically selected from the structure, and the corresponding genomic region was obtained. The protein structure was recorded and stored in FASTA format for further analyses. To ensure the accuracy of the selected sequence, multiple sequence alignment was performed using Clustal Omega to compare homologous sequences across related species. Additionally, physicochemical properties such as molecular weight and hydrophobicity were calculated using ExPASy ProtParam to ensure structural accuracy and perform necessary validations [11].

2.2. Target selection, molecular modeling, and validation

Molecular docking and simulation analyses preceded the lucifensin protein sequence obtained was cross validated using UniProt (https://www.uniprot.org). To determine the degree of similarity with human homologs, a similarity analysis against the human genome was conducted using the protein Blast web-based tool available at NCBI. Subsequently, predictions regarding the three-dimensional structure of the lucifensin protein were obtained using I-TASSER, Alpha-Fold, and ModWeb [12]. Evaluating the integrity of the protein is crucial for the protein structure prediction procedure. Since protein is not frequently studied in literature, evaluations were performed through multiple tools to ensure the three-dimensional structure. Analysis of the Ramachandran plot and energy minimization studies for suitable positions were done using the UCSF Chimera package program [13]. To identify the active regions of the obtained protein structure for further analysis, the DoGSiteScorer tool was used, and the data were predicted and evaluated using the Chimera [14]. In addition to the Ramachandran plots, an evaluation and analysis were performed using the ModWeb Server tool, which assesses other factors such as contact analysis of all atoms to evaluate the quality of the generated three dimension (3D) models. The ProSA-web tool was used to predict the Z-score, a measure of the quality of the 3D models [15]. The predicted Z-score value is visualized and evaluated in a graph containing the Z-scores of all proteins experimentally determined in the PDB to determine if it falls within the range of scores typically found in natural proteins of similar size.

2.3. Protein-protein docking and molecular dynamic simulations

The ClusPro web server (https://cluspro.org) was utilized for conducting protein-protein docking analyses. ClusPro server employs a hybrid strategy to predict protein-protein (or peptide) binding sites [16]. The effects of lucifensin, secreted by *L. sericata*, on human JAK1 kinase (PDB: 6GGH) and PDE4 (PDB: 4NW7), known to play roles in atopic dermatitis and targeted by various inhibitors, were elucidated through these binding interactions. Emphasis was placed on specific amino acid residues, particularly ARG26 and ARG39, in the identified active regions of lucifensin, with site-specific investigations focused on these residues. Coefficient weight scores were calculated by the Equation 1 that provided below [16-17],

$$E = 0.4\text{Erep} + (-0.4\text{Eatt}) + 600\text{Elec} + \text{EDARS} \quad (1)$$

The energy function E consists of several terms, including the repulsive (E_{rep}) and attractive (E_{att}) components of the van der Waals energy, an electrostatic interaction term (E_{lec}) representing Coulombic forces and a structure-based solvation term derived from the Decoys as the Reference State (DARS) approach, denoted as E_{DARS} [18]. The best positions selected from the results were exported for conducting molecular dynamics simulations. The MD simulation was performed using the AMBER99SB force field with the BioBB-Wfs-based MD algorithm [19]. Proteins and complexes were solved in octahedral boxes using the TIP3P model. All structures were neutralized by adding counterions at a concentration of 0.05. Subsequently, all systems were equilibrated for 1 ns each under NVT and NPT conditions. NVT equilibration was conducted at 300 K and 1.0 bar pressure. A 500 ps MD simulation was performed, and the trajectory was analyzed using parameters of the root mean square deviation (RMSD), radius of gyration (Rg), and GROMACS energies.

3. Results and discussion

To define the lucifensin protein secreted by L. sericata, an in silico workflow summarizing the bioinformatics tools is presented below.

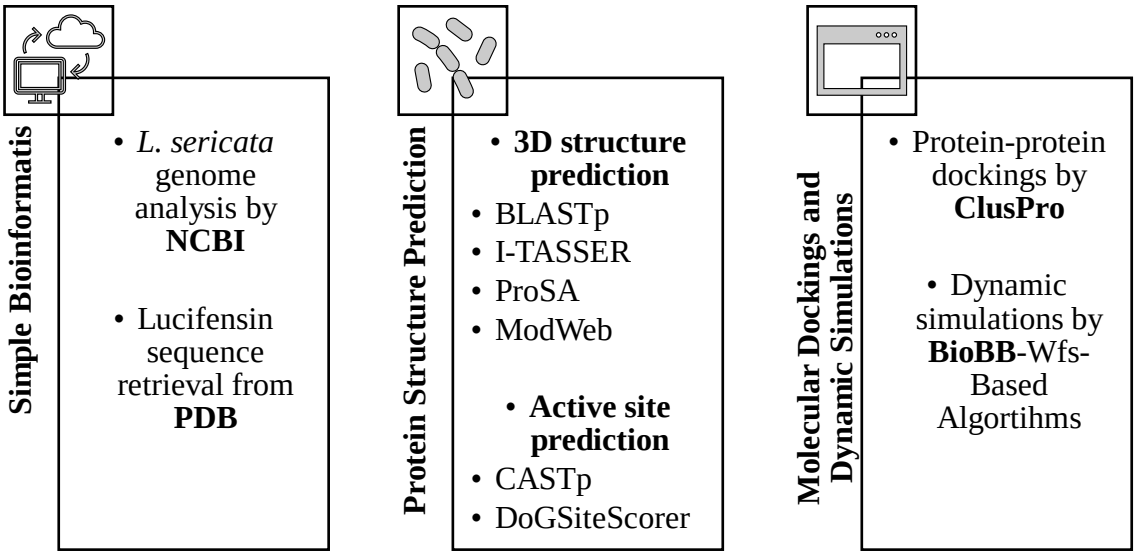

Figure 1. Basic workflow of the study

Table 1. The specific characterized features of lucifensin and the target proteins to be used in in silico docking studies

Species	Protein ID	Name	Length (aa)	Molecular mass (kDa)	Protein family	Biological process
L. sericata	XP_046808737.1	Lucifensin	40	4.13	invertebrate defensin family	Defense response to fungus and bacteria
Target Protein						
H. sapiens	P23458 (UniProt)	Tyrosine-protein kinase JAK1	282	33.8	protein kinase superfamily	growth hormone receptor signaling pathway via JAK-STAT
H. sapiens	Q07343 (UniProt)	Phosphodiesterase 4B	372	43.7	Phosphodiesterase family	Hydrolyzing cyclic adenosine monophosphate to inactive form

3.1. Protein selection and molecular validation

The protein sequence and other structural data were obtained from the Protein Data Bank (PDB Code: 2LLD) before advanced molecular analyses were conducted. These data were then cross validated through the UniProt database using the code

A0A7D5FFX3. The Blastp algorithm revealed significant similarities with 100 sequences from various fly and mosquito species, with empirical analysis total scores ranging from 84 to 67.8 for lucifensin. The results indicate the presence of 53,716 protein structures in the species. Additionally, information regarding the target proteins to be used in the study is presented in Table 1.

A series of web tools were utilized to generate the best 3D model for the lucifensin protein and to work on it. The associated results, along with the Z-score, are presented in Table 2. Also, additional Ramachandran plots and 3D model view generated through Chimera are depicted in Figure 2. PROSA algorithms were employed to analyze the integrity and structural quality of the protein. Based on Table 2, it has been determined that PROSA and ModWeb offer higher accuracy and stability in protein structure prediction compared to I-TASSER. Notably, the Z-score of PROSA and the high residue percentage in the most favored regions indicate that its structure is of high quality, enhancing the usability of the predicted structure [20]. Additionally, Ramachandran analysis indicates that 27 residues (84.4%) are in the most favored regions (red area) [21]. This suggests stability and high accuracy in the prediction of protein structure of lucifensin. Furthermore, the absence of amino acid residues in the white areas, known as disallowed regions, demonstrates that the protein does not consist of undesirable structures, indicating that the predicted 3D structure can be utilized in computational molecular analyses [22].

3.2. Prediction of binding site amino acid residues

To determine the binding sites, CASTp and DoGSiteScorer servers were utilized, and the results are presented in Table 3. CASTp identified 13 potential binding sites, while DoGSiteScorer results predicted approximately 14 binding sites. The CASTp results will be considered for the subsequent stages of the study.

Table 2. The quality and accuracy assessment of the best lucifensin model for in silico docking studies with Z-scores obtained from various servers and Ramachandran plot analysis

Prediction servers	Z-score	Residues in the most favored regions (%)	Residues in allowed regions (%)	Residues in generously allowed regions (%)	Residues in disallowed regions (%)
I-TASSER	**-1.57**	**74.8**	**24.3**	**0.1**	**0.8**
PROSA	**-6.99**	**84.4**	**15.6**	**0**	**0**
ModWeb	**0.4**	**84.2**	**14.7**	**0.2**	**0.9**

Table 3. Binding site amino acid residues prediction results

Method	Pocket	Area (SA) Å2	Volume (SA) Å	Residues within the pocket
CASTp	**1**	**73.917**	**36.402**	**ALA1, THR2, CYS3, ASP4, LEU5, LEU6, SER7, THR9, GLY10, HIS13, ALA15, CYS16, HIS19, ARG26**
DoGSiteScorer	**1**	**70.65**	**31.4**	ALA1, THR2, CYS3, ASP4, LEU5, LEU6, SER7, GLY8 THR9, GLY10, HIS13, ALA15, HIS19, CYS20

3.3. Molecular docking interactions and MD analysis

The ClusPro web-based server was utilized to analyze the antagonist activity of lucifensin against human JAK1 kinase and PDE4 targets at the molecular level. The results revealed that the binding affinity of inhibitory activity towards the protein targets was -45.27 and -50.19 kcal/mol, respectively. Comparative molecular docking analysis with positive control groups emphasized that the binding affinity results were higher compared to drug molecules like Baricitinib and

Upadacitinib [23]. In a study conducted on JAK1 kinase focusing on protein-ligand interactions, hydrogen bonds were predominantly reported at the ARG1008, HIS885, and ASP1003 amino acid residues within the protein pockets, highlighting the high binding energy associated with these interactions [24]. On the other hand, in an in silico research study conducted on PDE4, where various ligands were compared with the control molecule Rolipram, the binding scores of inhibitor-potential compounds were reported in the range of -16 to -24 kcal/mol [25]. The study emphasized the pocket region located between the PHE414 and PHE506 residues. Subsequently, molecular interactions were further evaluated using MD analysis based on docking scores and other criteria. In the molecular dynamics performed using the BioBB-Wfs-based MD algorithm, the rigidity of the docking complexes and thus their resistance to molecular change were observed. Eigenvalues related to different modes of analysis represent the stiffness of motion. The values obtained from MD analyses are directly proportional to the energy required for structural deformation, the smaller the eigenvalue, the easier the molecular deformation [26]. The highest eigenvalues for JAK1 kinase and PDE4 were determined to be 7.75628e-05 and 3.9178e-06, respectively. In addition to the protein-protein molecular docking analysis, the bonds and types of interactions between the proteins were visualized and analyzed using Discovery Studio Visualizer. The results demonstrated that lucifensin showed a propensity to bind to and activate the ARG33, ILE35, and ARG39 amino acid residues. Salt bridges and charge interactions were identified for both protein targets, and the results are provided in Table 4, Figure 3 and Figure 4. All these findings highlight stable and tight interactions between the proteins, despite minor fluctuations in their interactions. Literature studies highlight the importance and necessity of high stability in ligand-protein binding regions, demonstrating that different types of binding interactions have a direct impact on scores and overall interactions. Furthermore, they reveal that the background algorithms of in silico prediction analyses are fundamentally dependent on these factors [27-28].

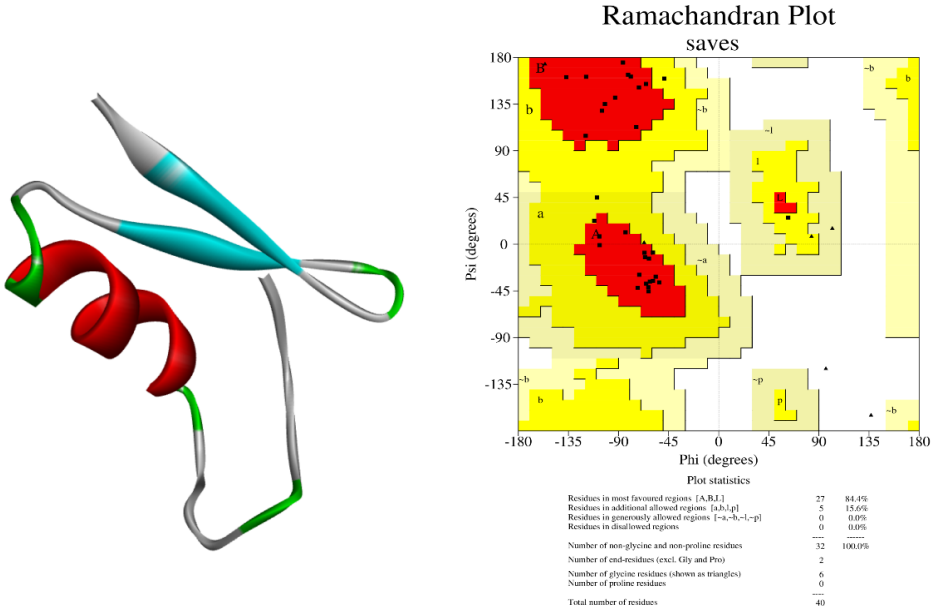

Figure 2. Three-dimensional structure and Ramachandran plot of lucifensin

Table 4. Molecular docking interactions between lucifensin and target proteins

Ligand Residue	Target Residue	Distance (Å)	Interaction Type
JAK Kinase-1			
ARG33	ASP1003	1.93	H bond
ARG23	GLN1098	1.88	H bond
ASN31	HIS 885	1.89	H bond
ARG33	ASP1042	1.86	H bond
CYS20	CYS36	3.19	Sulfur X
ARG33	ASP1003	2.69	Salt bridges
ARG33	ASP1042	3.05	Salt bridges
ARG39	ARG1007	4.59	Pi-Cation
ARG39	ARG1008	4.22	Pi-Cation
CYS3	GLY1023	3.88	pi-stacked
TYR29	LEU1024	4.02	pi-stacked
ASP4	PRO1044	4.42	pi-stacked
HIS7	CYS1052	4.31	pi-stacked
CYS3	MET1054	4.22	Alkyl interaction
PDE-4			
ARG39	SER420	2.18	H bond
ASN40	GLN419	1.95	H bond
GLY32	ASN272	2.3	H bond
ALA34	ASP335	2.59	H bond
ARG33	ASP370	2.09	Salt bridges
ARG33	ASP264	2.59	Salt bridges
ARG23	ASP264	2.59	Salt bridges
ARG33	ASP370	1.93	Charge-Charge interaction
CYS36	HIS19	4.59	pi sulfur interaction
ILE35	HIS339	5.24	pi alkyl interaction
ILE35	MET336	5.07	pi alkyl interaction
ILE35	PHE340	5.11	pi alkyl interaction

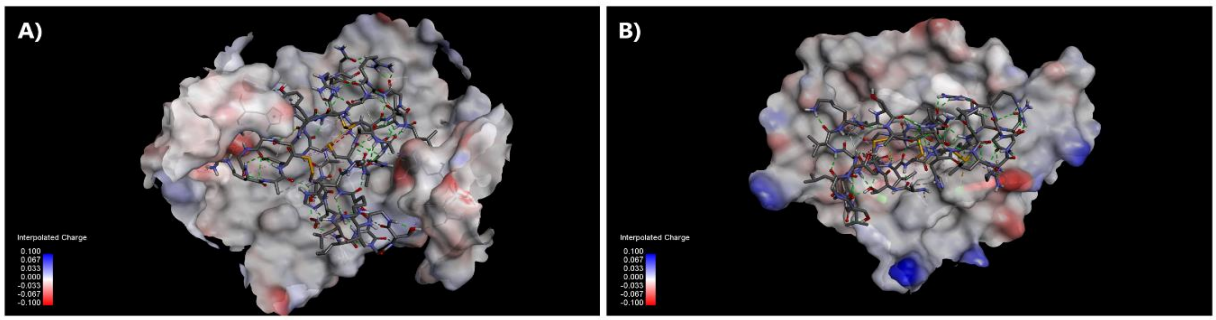

Figure 3. Three-dimensional structure and Ramachandran plot of lucifensin A) JAK kinase-1 and B) PDE-4

In the molecular docking analyses of lucifensin, the formation of hydrogen bonds at very short distances (1.86-1.93 Å) with key residues such as ASP1003, GLN1098 [29], and ASP1042 at the ARG33 residue indicates that the ligand is highly stable in the active site [30]. The interactions, especially with acidic residues like ASP1003 and ASP1042, suggest that they could affect important catalytic regions, thereby potentially inhibiting kinase activity. Lucifensin's formation of salt bridges with both protein targets reveals that it is electrostatically stabilized, enhancing binding

stability in the functional regions by maintaining the correct conformation [31]. As seen in the charge regions of the 3D protein structures shown in Figure 2, lucifensin tends to interact with negatively charged regions (red areas), which is a natural consequence of lucifensin being a cationic peptide.

Figure 4. MD simulations of lucifensin with receptor proteins A) JAK kinase-1 and B) PDE-4

It has been determined that the potential and total energy of JAK kinase-1 remained stable during the simulation, indicating that the reaction proceeded in a steady state. The potential energy was found to be approximately -1050 kJ/mol. The negative energy value indicates that attractive interactions between molecules dominate over repulsive interactions [32]. The average Rg value was found to be around 0.9 nm, with no significant fluctuations observed throughout the simulation [33]. This suggests that the binding between the ligand and the protein maintained a compact form. Although there were increases in RMSD values between 300-500 ps, the overall profile indicates that the structure did not

deviate significantly from its initial conformation. In contrast, the results of the PDE-4 simulation were quite different. Similar to the other interactions, the potential and total energy remained stable, with the energy value being approximately -600 kJ/mol. While the simulation was stable, the energy obtained was considerably high. The Rg value for the PDE-4 protein interactions was found to be around 1.8 nm, indicating that this ligand-protein complex is larger in structure compared to JAK kinase-1. In the RMSD graph, it was observed that the PDE-4 protein exhibited some flexibility compared to its initial structure but eventually stabilized [34].

4. Conclusions

The potential application of L. sericata lucifensin as a therapeutic seems to go far beyond their antimicrobial activity, since they seem to be also able to act as an agent for dermatological diseases. Furthermore, the 3D structure of lucifensin was predicted using various intelligent algorithms, and the most suitable model was utilized in computational molecular analyses to evaluate the molecular utilization and to gain insights into its potential therapeutic applications for dermatological diseases. The simple in silico workflow used in the study can be applied to the genomes of other Lucilia species to find secreted proteins and also for structure predictions. Computational and structural research on proteins are the first steps into the development of novel therapeutic agents. This study aimed to explore the potential of lucifensin as a novel therapeutic agent targeting key proteins, such as JAK1 kinase and PDE4, for the treatment of atopic dermatitis. The findings lay the groundwork for further experimental validation and drug development strategies to address unmet medical needs in inflammatory diseases. Further in vitro, in vivo, and in silico studies are required to determine the various potential applications of lucifensin.

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