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Original Article

Bioinformatic analysis of lucifensin potential as a nutraceutical source for livestock

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Abstrak

Tujuan: Meningkatnya kasus resistensi antibiotik pada ternak akan berdampak terhadap meningkatnya kasus infeksi oleh mikroba. Penggunaan antibiotik sintetik saat ini telah dibatasi, salah satu alternatif yang dapat digunakan untuk treatment antibiotik dengan memanfaatkan makanan yang mengandung efek obat (*nutraceutical*) dalam hal ini adalah kandungan antibiotik. Penggunaan pakan ternak bersumber dari larva ordo diptera belakangan ini semakin marak, hal ini selain dikarenakan kandungan protein yang lebih tinggi, terdapat kandungan nutraceutical yang dapat berfungsi sebagai peptida antimikrobial dengan bahan aktif salah satunya adalah lucifensin dalam larva *lucilia sp*. Penelitian ini bertujuan untuk mengetahui potensi lucifensin sebagai produk *nutraceutical* ternak dengan kajian bioinformatik.
Metode: Penelitian ini merupakan penelitian deskriptif, data protein lucifensin didapatkan dari protein data bank (PDB), selanjutnya dilakukan analisis kekerabatan gen menggunakan blast-NCBI dan dilakukan analisis lipinski rules of 5 (Ro5) untuk mengetahui potensinya sebagai obat oral/ oral *bioavailability*.
Hasil: Hasil analisis kekerabatan menunjukkan protein lucifensin 2LLD memiliki kekerabatan 2 protein dari ordo diptera lainnya seperti phormicin dan sapecin, termasuk dalam keluarga protein defensin yang gen pengkodenya terkonservasi hingga tingkat ordo. Hasil Ro5 menunjukkan bahwa lucifensin memiliki massa sebesar 312 dalton, highlippopolycity -0.5, hydrogen bound donor 5, hydrogen bound aceptor 6 dan molar refractivity sebesar 77.14 yang menandakan oral bioavailabilitynya baik. Uji toksisitas juga menunjukkan protein ini tergolong komponen yang aman, serta hasil docking menggambarkan ikatan energi yang kuat yang berperan penting dalam aktivitas antimikroba.
Kesimpulan: Disimpulkan bahwa protein lucifensin dalam larva *Lucilia sp.* berpotensi untuk digunakan sebagai produk nutraceutical ternak yang dapat berperan sebagai peptida antimikroba.

Kata Kunci: Bioinformatik; *Lucilia sp*.; Lucifensin; *Nutraceutical*; Peptide antimikrobial

Abstract

Objective: High number of antibiotic resistance cases in livestock will impact on increasing cases of microbe's infection. On the other hand, the use of synthetic antibiotics is currently limited. A possible alternative can be used is the utilization of foods that possess medicinal effects (nutraceuticals). The use of animal feed sourced from Diptera larvae has been increasing widely, due to its high protein content. In addition, it is comprised of nutraceuticals as antimicrobial peptides like lucifensin. This study aimed at determining the potential of lucifensin as a nutraceutical product for livestock based on bioinformatics study.

Methods: This study is descriptive research. The lucifensin protein data was obtained from the Protein Data Bank (PDB). The protein relationship analysis was performed using Blast-NCBI and lipinski Rules of 5 (Ro5) analysis to determine its potential as an oral drug (bioavailability). The molecular docking was used Swiss Docking online with lucifensin as target protein and Lipoteichoic Acid (LTAs) of *Staphyllococus aureus* as ligand.

Results: The relationship analysis results showed that the lucifensin protein was related to two proteins of the other orders of diptera such as phormicin and sapecin. This protein belongs to the defensin protein family in which its coding genes are conserved until the order level. The Ro5 results showed that the mass of lucifensin protein was 312 daltons, its high lippopolycity was -0.5, it has five hydrogen bond donors, six hydrogen bond acceptors, and its molar refractivity was 77.14. Toxicity test also showed that this protein is classified as a safe component, and the docking results illustrate a strong energy bond that plays an important role in antimicrobial activity.

Conclusions: In conclusion, lucifensin protein in the *Lucilia sp.* larvae is potential to be used as a livestock nutraceutical source that acts as an antimicrobial peptide.

Keywords: Antimicrobial peptide; Bioinformatic; *Lucilia sp*.; Lucifensin; Nutraceutical

INTRODUCTION

The increase of resistance cases to synthetic antibiotics will bring a negative impact, one of which is by increasing microbial infections [1–3]. Consequence, human health will also be influenced [4,5]. This due to the high transmission rate which may occur as human interact with livestock [6]. The use of antibiotics as growth hormones and feed additives has now been regulated and limited to reduce the further resistance impact [7,8]. Current researchers have developed many animal feeds containing antimicrobial peptide (AMP) then called nutraceuticals [9–11]. Generally, the development of nutraceutical products is made from plants [12,13]. Meanwhile, nutraceuticals derived from animals, especially insects, have not been widely developed [14]. One of the nutraceutical antimicrobial peptide sources is lucifensin which is obtained from *Lucilia sp*. (Diptera order) [15,16].

Lucifensin is a peptide secreted by Lucilia sp.'s larvae of which has the potential to be used as an antibiotic [17]. Lucifensin peptides are generally 4-6 kDa in size. It forms cyclic peptides in which the folds form a mixed -helical/β-sheet structure. This peptide acts as microbicidal and cytolytic. Lucifensin is generally effective in killing gram-positive bacteria and some types of fungi [18]. Moreover, some research that investigated lucifensin obtained from the *Lucilia sp*.'s larvae concluded that this compound is potential as an antibacterial for open wound treatment [10,11],[20,21]. However, the research on activity and effectiveness of lucifensin of *Lucilia sp*.'s larvae to be utilized as nutraceutical animal feed and replace the role of synthetic antibiotics to reduce antibiotic resistance cases in livestock that has never been done. The purpose of this study was to determine the potential of lucifensin, obtained from *Lucilia sp*.'s larva, as animal feed based on bioinformatics studies. The result of this research is expected to contribute as basic information for further researches which develop nutraceutical sources derived from the larvae of *Lucilia sp*. containing lucifensin for animal feed.

MATERIALS AND METHODS

Protein structure analysis

This descriptive research employed bioinformatics approach to analyze the lucifensin defensin peptide. The lucifensin protein data was obtained from the Protein

Data Bank (PDB) (https://www.rcsb.org/structure/2LLD). The lucifensin protein was named 2LLD on the PDB website. Furthermore, the protein data was analyzed the 3D structure, protein composition and also to observe the bond of each protein by using Pymol software.

Kinship analysis of protein

The phylogenetic tree was constructed by using Blast protein analysis. The protein data was copied into the website of Blast protein, this was fundamental step to determine lucifensin protein relationship with other proteins from several genera.

Bioavailability analysis

The bioavalability and also potentiality of lucifensin protein as neutraceutical were observed by Lipinski 5 of rules website. This analysis was also carried out to determine the protein mass, lippopolycity, hydrogen bond donors, and molar refractivity.

Prediction of toxicity

The toxicity prediction was performed by using *pkCSM Online Tools* https://biosig.unimelb.edu.aupkcsm/prediction. The objective of this step was to analyze the mutagenic potential, Oral rat acute toxicity/ Lethal Doses (LD 50) and skin sensitization from the lucifensin compound.

Molecular docking and amino acid residues analysis

The ligand was chosen based on the information that lucifensin had powerful impact as an antibiotic for Gram-positive bacteria, one of the bacteria as gram positive is *Staphylococus aureus* (*S. aureus*). One of the ligands from *S. aureus* as membrane protein is Lipoteichoic Acid Synthesis (LTAs) with PDB ID 2W5T. The Lucifensin protein with PDB ID 2LLD was converted to canonical smile first by using online PDB converter (https://cactus.nci.nih.gov/translate/) and protein docking between Lucifensin protein and LTAs of *S. aureus* was analyzed by using online swiss docking (http://www.swissdock.ch/).

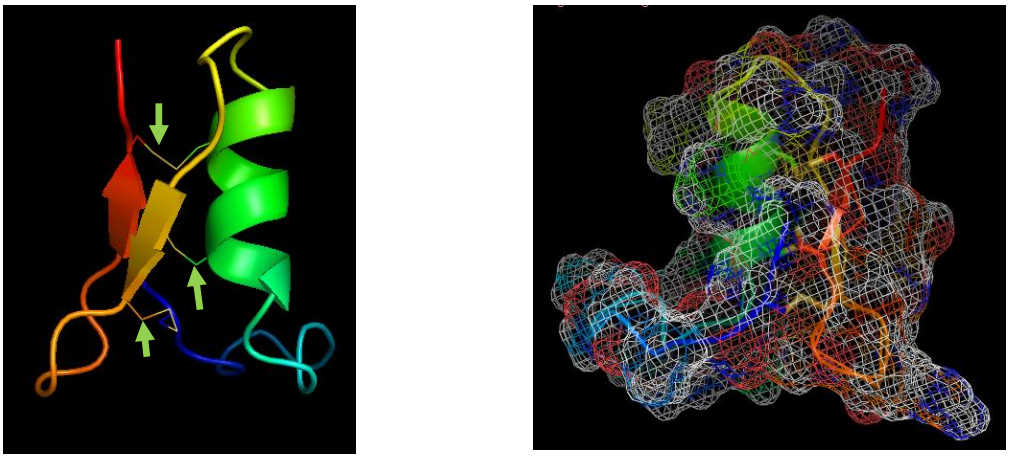
RESULTS

Lucifensin protein structure

Based on the data searching process in PDB, the data were obtained for lucifensin protein (*Lucilia serricata*) with the protein code of 2LLD. The structure of the lucifensin protein was rebuild by using the pymol software to explore the 3D structure and also the folding way of the protein with the bond structure, which can be seen below (Figure 1).

Kinship of lucifensin protein

The analysis of the phylogenetic tree of lucifensin protein to observe the kindship of this protein with other protein from the diptera ordo. The result showed that this protein was only specific produced by the *Lucilia sericata* and conserved only in the *Lucilia* genus, moreover the other genus that have the lowest distance are bractocera, ceratitis, and zeugodacus (Figure 2).



A B

Figure 1. The illustration of lucifensin peptide model (2LLD) using pymol: (A) lucifensin structure with 3 disulfide bonds marked with arrows; (B) 3-dimensional lucifensin structure

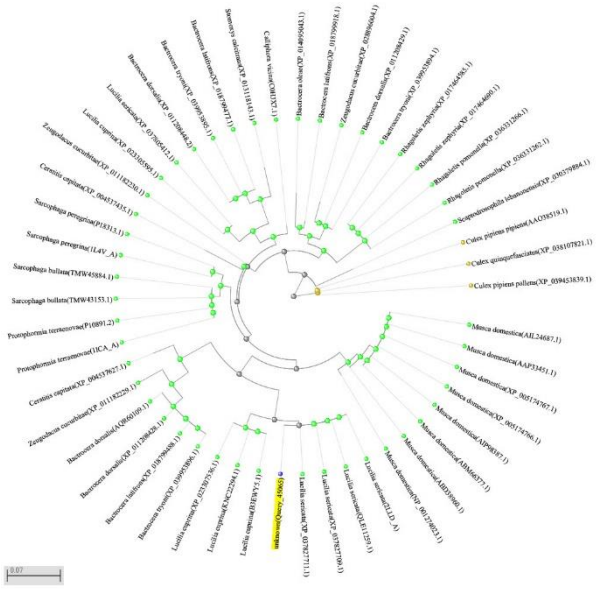
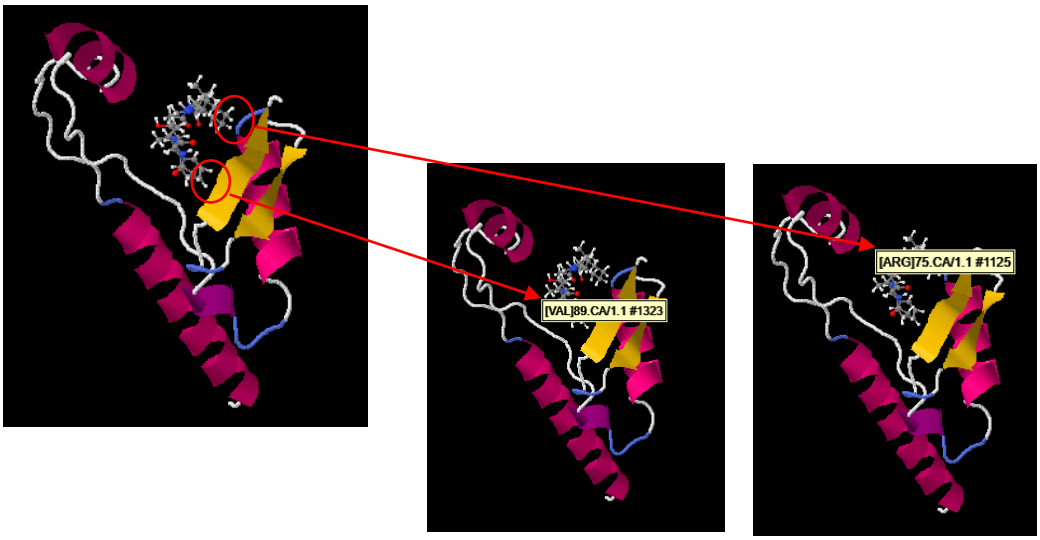


Figure 2. Construction of the lucifensin peptide relationship using Blast-NCBI



(A1) (A2)

Figure 3. ligand interaction with amino acid residue of lucifensin; (A1) Valine amino acid as the residue that bind to the ligand of LTAs *S. aureus*; (A2) Arginine amino acid as residue that bind to the ligand of LTAs *S. aureus*

Bioavailability

Bioavailability analysis of lucifensin protein can be observed bellow (Table 1). Based on the table that all the criteria such as protein mass, lippopolycity, hydrogen bound donors, and molar refractivity were still on the good value for bioavailability.

Prediction of toxicity

Lucifensin toxicity prediction showed negative for Ames toxicity (mutagenic), the LD 50 categorized as moderately toxic (2.482), and indicated as negative for skin sensitization.

Molecular docking and amino acid residues analysis

There are 33 clusters based on the binding energy between lucifensin and the ligand of *S. aureus*, with the highest score being -565.63 and the lowest score being -530.87. The amino acid interaction with ligand can be seen in 3D (Figure 3).

Table 1. The analysis results of Lipinski rules of 5 (Ro5)

Information	Result	Rules
Molecular Mass	312.000000	Less than 500 daltons
High lipophilicity (Log P)	-0.053101	Less than 5
Hydrogen bond donors	5	Less than 5
Hydrogen bond acceptors	6	Less than 10
Molar Refractivity	77.145782	should be between 40-130

DISCUSSION

The lucifensin protein has a peptide length of 40 amino acids and belongs to the scorpion toxin-like knottin superfamily and secreted by arthropod family of defensins [22]. Generally, defensins are secretory peptides in the form of toxins in several types of insects such as *Dolopus genitalis* [23]. Nygaard [22] reported that lucifensin peptide isolated from the cDNA libraries of the salivary glands of *L. serricata* larvae has been reported to be potential as a bactericide against *Stapylococus camosus*, *Streptococcus pyogenese*, *Streptococcus pneumoniae*, and *Staphylococus aureus*. Lucifensin is expressed for 5-6 hours after hatching, with maximum expression after 24 hours of hatching and will persist until the 3rd larval phase, meanwhile, its 4th larval phase the production will decrease [19].

The protein structure of lucifensin is composed by six cysteine and three disulfide bridges at the C3-30, C15-C36, and C-20-38 positions [24]. By predicting using Pymol software, the structure of lucifensin can be seen in Figure 1. Lucifensin is a cationic peptide which has a +4 net positive charge which will interact with the negative charge phosphatase group of the membrane phospholipid bilayer of gram-positive bacteria, which, in turn, will cause bacterial cell lysis [17,22,24] (Figure 1.a).

Figure 2 shows the results of the Lucifensin 2LLD peptide relationship analysis using Blast. There were three defensin proteins from 16 genera which show close relationship. The three proteins were defensins, sapecin, and phormicin. While the genus consisted of *lucilia, zeugodacus, sarcophaga, musca, rhagoletis, bactrocera, protophormia, ceratitis, calliphora, stomoxys, scaptodrosophila, culex, aedes, camponotus, drosophila,* and *lasius.* This finding shows that the gene coding for the lucifensin protein which belongs to the defensin family is conserved at the ordo level (Diptera) which generally acts as a self-defense weapon [18,25]. This family protein is the secreted protein, usually the maggot would release the lucifensin protein to the environment as their mechanism of maggot debridement process.

The result of bioavailability (Table 1) shows the lucifensin potential as a nutraceutical (medicinal food). The results of this analysis indicate that lucifensin met several criteria of the Lipinski rules of 5 (Ro5). The Ro5 analysis includes several aspects such as drug-like physicochemical features, drug-like structural features, and a comparison between drug-like and non-drug-like in the analysis of new drug candidates [26]. The analysis of new drug candidates depends on how the drug will be used either orally or intramuscularly. Oral use of the drug candidates with active ingredients must comply with physicochemical parameter ranges *i.e*. the molecular mass must be below 500 daltons, high lipopolycity (log P) less than or equal to 5, hydrogen bond donors less than equal to 5 and, hydrogen bond acceptors must be equal with 10 and less, and the molar refractivity is in the fit range [27, 28, 29]. Bioavailability test of lucifensin would be strengthen by the toxicity prediction for better understanding of the toxicity status.

The toxicity prediction of lucifensin compound consist of AMES Toxicity for carcinogenic potential, oral Lethal Doses (LD 50) for rat, and skin sensitization. The AMES Toxicity showed that this compound was a non-mutagenic chemmical. This analysis was strongly recommended by Zhang et al. [28] due to the mutagenic compound could pose a toxic risk to humans and animal. The result of Lethal Doses analyses also showed that lucifensin has moderately toxic since the valus of LD 50 is 2.482. Thus, besides lucifencin potential agent as antimicrobes also less toxic for human and animals, this is supported by Kausar et al. [29] that the higher LD50 value indicated the less

toxicity of compound. Skin sensitization test showed there is no effect can be caused by lucifensin or it means this compound is safe. These test are required by chemical regulation authorities due to protection from health hazards and risk associated with allergic responses caused by chemicals product (33).

The molecular docking result revealed the potential lucifensin as an antimicrobial for gram-positive bacteria. This is indicated by the cluster of binding energy between lucifensin and LTAs *S. aureus* has 33 clusters with the highest binding energy reaching -565.63 kcal/mol and the lowest score is -530.87. Protein-ligand binding energy plays an important role in biological processes, such as immune response, signal transduction, and cell regulation [30]. Figure 3 showed the result of docking protein and ligand with the highest bonding energy, there are two amino acid residues that binding to the ligand surface. Native ligand establishes the hydrogen bonds to the Valine (Val 89), and Arginine (Arg 75) of lucifensin proteins. This finding support Cerovsky et al. [17] that there is two specific amino acid that plays an important role in peptides' antimicrobial activity as the result a mass spectrophotometry test. Further more, the tertiary structure of lucifensin has strong folding caused the two cysteine disulfide bridges between α-helix and β-helix or known as cysteine-stabilized $\alpha\beta$ motif wich is important for antimicrobial [18]. The peptide lucifensin has met the criteria for bioavailability, toxicity, and molecular docking as the first stage in the drug candidate test, hence, lucifensin has the potential to be continued into phase II of clinical testing phases.

CONCLUSION

The lucifensin protein prossesses close relationship to sapecin and phormicin proteins. Lucifensin protein belongs to defensin protein family with the conserved coding genes until its order level. Ro5 analysis showed that lucifensin has good oral bioavailability. The toxicity test also showed that this protein is classified as a safe component, and the docking results illustrate a strong energy bond that plays an important role in antimicrobial activity. Thus, lucifensin is potential to be used as a livestock nutraceutical product in terms of as an antimicrobial peptide.

CONFLICT OF INTEREST

The authors declare there are no conflicts of interest with any financial organization regarding the material discussed in the manuscript.

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