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# The recombinant expression and antimicrobial activity determination of Cecropin-like part of Heteroscorpine-1 from *Heterometrus laoticus*

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**Abstract.** *Erviana R, Saengkun Y, Rungsa P, Jangpromma N, Mustofa, Daduang S. 2022. The recombinant expression and antimicrobial activity determination of Cecropin-like part of Heteroscorpine-1 from* Heterometrus laoticus. *Biodiversitas 23: 5646-5653.* Antimicrobial peptides are promising novel antibiotics that hold great potential in combating bacteria, fungi, viruses, and parasites. Recent interest has increased in their potential as new pharmacological agents. Large quantities of antimicrobial peptides are required in order to fulfil the demand of the peptides for scientific research and clinical trials. Gene expression systems for antimicrobial peptides have been developed, which may be utilized efficiently for various antimicrobial peptide-related studies and applications. However, many expression systems that have been developed require many steps that impact to the expression cost. This study established the fast and easy expression system of recombinant Cecropin-like part of Heteroscorpine-1 (CeHS-1) and determined their activity. The gene was chemically synthesized, ligated to the expression vector pET32a, transformed to *Escherichia coli* BL21 (DE3) pLysS competent cell, and induced by 0.2 mM isopropyl β-D-1-thiogalactopyranoside. The induction time optimization determined that the 3 hrs induction resulted in the highest peptides yield. The prolonged induction would decrease the peptides yield, due to the toxicity of the peptides toward the host cells. The peptide purification was facilitated by His tag sequence through purifying affinity chromatographic column of Ni-NTA. The induction was able to express the expected peptides in the soluble fraction. The antimicrobial activity assay showed that the recombinant peptides could inhibit the growth of many bacterial strains. However, their activity was lower compared to the synthetic peptides. This finding demonstrated that the developed expression system in this study might facilitate the easy and feasible expression system for CeHS-1. Additionally, the study revealed that the antimicrobial activity of the expressed peptides could be preserved.

**Keywords:** Antimicrobial, CeHS-1, Heteroscorpine-1, peptides, recombinant expression

## INTRODUCTION

Antimicrobial resistance has become a major threat to global public health in recent decades (Willyard, 2017). It becomes a big problem since conventional antibiotics are extensively used in healthcare systems, farms, and communities (Ventola 2015). Appropriate antibiotic use is not adequate to keep up with antimicrobial resistance. Therefore, alternative antibiotics are needed (Frieri et al. 2017). Antimicrobial peptides are promising due to their advantages, such as rapid and broad-spectrum antibacterial activity, indirect activity, low propensity to develop toxicity, and their ability in avoiding antimicrobial resistance (Luong et al. 2020). Approximately 5000 recorded antimicrobial peptides have been investigated until now, varying in length, charge, and structure (Hazam et al. 2019). They are purified from many sources, including plants, animals, bacteria, archaea, and fungi (Kumar et al. 2018). Since 1981, there are many reports on isolation and identification of antimicrobial peptides from animals, such as cecropin from silk moth *Hyalophora cecropia* hemolymph (plasma and blood) (Steiner et al. 1981), Leucrocin from crocodile *Crocodylus siamensis* white blood cell extracts (Pata et al. 2011), and Magainin from *Xenopus* skin (Matsuzaki et al. 1997).. Antimicrobial peptides can be obtained from direct isolation from natural resources, chemical synthesis, and molecular cloning (Deng et al. 2017). Among these methods, molecular cloning is promising to provide antimicrobial peptides in large quantities effectively. Extracting peptides from natural resources results in very low yield and is not environmentally friendly as it possibly kills many animals. The chemical synthesis requires high cost and potentially produces improper folding for the long peptide chain (Celie et al. 2016).

Many expression systems can be applied for peptide molecular cloning. Many factors should be considered to select the efficient expression system for producing the peptides, such as peptide size, intracellular localization or secretion, proper folding, and glycosylation (Parachin et al. 2012). The expression system that is chosen for antimicrobial peptide expression should be able to maintain

its antimicrobial activities. Therefore it is important to combine the expression host and plasmid to obtain a high yield of antimicrobial peptides with strong activities (Schreiber et al. 2017). The bacterial expression system often remains the preference as it is inexpensive, provides rapid culture time, is easy to handle, and usually produces a high-yield protein (Assenberg et al. 2013). As the most preferred system, the bacterial expression system has abundant supporting equipment that is easily available. There are many resources that help solve the use of this system, such as many different kinds of expression vectors that are suitable for this system (Wibowo and Zhao, 2019). *Escherichia coli* is the most bacterial species, which is widely used among the bacteria in the recombinant protein expression system. *E. coli* is the most attractive system of recombinant protein expression prior to its ability to grow rapidly with the high density in the inexpensive media.

The limitations of the bacterial expression system are the difficulties of expressing some mammalian proteins such as mammalian enzymes and the challenge in the purification process that sometimes the expressed proteins are accumulated in inclusion bodies (Schmidt, 2004). Another challenge in the bacterial expression system for antimicrobial peptides expression is the peptides' toxicity toward the host cells. However, many strategies have been developed to anticipate these limitations, such as designing the fusion tag in the expression. Instead of increasing the solubility of the peptide, in some studies, many expression tags such as the thioredoxin tag and sumo tag have been used to protect against the toxicity of peptides in the host cells (Zhao et al. 2019).

In the recent study, the researchers examined the expression system for recombinant Cecropin-like part of Heteroscorpine-1. Heteroscorpine-1 is an antimicrobial peptide purified from the venom of scorpion *Heterometrus laoticus* (Uawonggul et al. 2007). The previous study determined that Cecropin-like part of Heteroscorpine-1 had high activity against many bacterial strains (Erviana et al. 2021). The researchers attempted to determine the easy and feasible expression system for this peptide by including the fusion tag to the antimicrobial activity assay. This study expressed two peptides. The first peptide was CeHS-1, a cecropin-like part of Heteroscorpine-1; the second one was CeHS-1 GP, the modification of CeHS-1 with higher antimicrobial activity. Due to the peptides' toxicity toward the bacterial host, the expression results of these two peptides were compared.

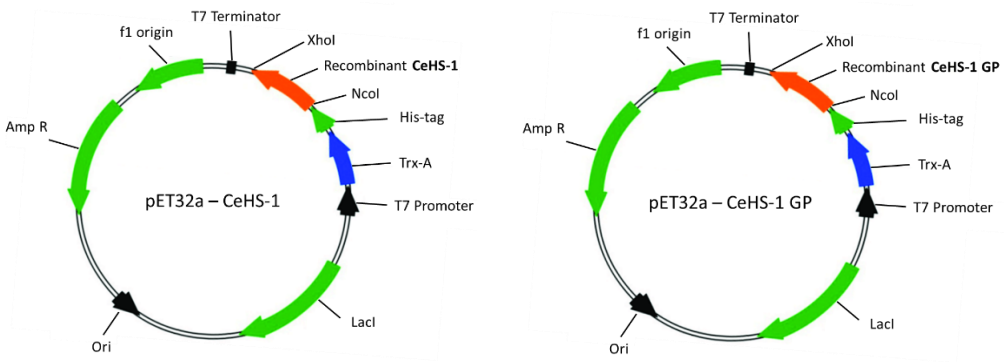
## MATERIALS AND METHODS

### Construction of recombinant expression plasmid

The genes of cecropin-like part of Heteroscorpine-1 (CeHS-1) and CeHS-1 GP were chemically synthesized and built upon the Heteroscorpine-1 gene sequence. CeHS-1 gene was constructed followed the first 38 genes of Heteroscorpine-1 (Uawonggul et al. 2007), while CeHS-1 GP was designed based on the same genes with the modification on the mutation site. The mutation site was composed following the genetic code for each modified amino acid, following the genetic code of cecropin B (Figure 1). In the construction of the expression plasmid, the genes were ligated in the expression vector pET32a vector at the *Nco*I and *Xho*I restriction sites (Figure 2). Before transform to the competent cells, the synthesized genes were stored in -80ºC to maintain their quality.

```
CeHS-1      GGATGGATTAATGAAGAGAAGATACAAAAGAAAATTGACGAAAAAATAGGAAATAATATCCTCGGA
              G  W  I  N  E  E  K  I  Q  K  K  I  D  E  K  I  G  N  N  I  L  G
            GGGATGGCTAAGGCCGTCGTCCACAAACTGGCGAAGGGCGAATTCCAA
              G  M  A  K  A  V  V  H  K  L  A  K  G  E  F  Q
CeHS-1 GP  GGATGGATTAATAAGATACAAAAGAAAATTGAAAAAATAGGAAATAATATCCTCGGAGGGATGGCT
              G  W  I  N  K  I  Q  K  K  I  E  K  I  G  N  N  I  L  G  G  M  A
            AAGGCCGGTCCAGTCGTCCACAAACTGGCGAAGGGCGAATTCCAA
              K  A  G  P  V  V  H  K  L  A  K  G  E  F  Q
```

**Figure 1.** The genes design of cecropin-like part of Heteroscorpine-1 (CeHS-1) and CeHS-1 GP




**A** **B**

**Figure 2**. Construction of recombinant expression plasmid of (A) Heteroscorpine-1 CeHS-1 and (B) CeHS-1 GP

**Recombinant peptides expression**

Amount of 30 ng recombinant expression plasmid pET32a-CeHS-1 and pET32a-CeHS-1 GP were diluted in nuclease-free water. They were then transformed to *E. coli* BL21(DE3)PLysS competent cells using heat shock method. The bacterial colonies from the transformation were cultured in Luria-Bertani (LB) broth with 100 µg/mL of ampicillin overnight at 37ºC and shaken at 180 rpm. The culture was further used for peptides expression. The amount of 5 µL of overnight culture was inoculated to 5 mL LB broth with 100 µg/mL ampicillin and incubated in the same condition until the $OD_{600}$ was 0.6. After reaching the expected $OD_{600}$, the peptides' expression was induced by 0.2 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). The cells were harvested by centrifugation at 10000 g at 4ºC for 5 min. The expressed peptides were determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis to measure the molecular weight of expected peptides.

**Optimizing the induction time**

The induction time was optimized and examined at 3, 6, 9 hrs, and overnight. The system of induction for peptides' expression followed the condition of recombinant expression as previously described. The cells were harvested by centrifugation at 10000 g at 4ºC for 5 min and expressed protein was determined by SDS-PAGE analysis. Furthermore, the optimum time expression was used for the bigger expression scale.

**Peptides' purification**

Peptides' purification was conducted after the bigger scale expression of 1 L culture was obtained. The culture expression cells were harvested by centrifugation at 10,000 rpm, 4ºC, for 5 min, and the supernatant was discarded. The harvested cells were resuspended in 50 mM Tris-HCl, pH 7.4 containing 100mM NaCl, followed by 3 times freeze-taw process for 10 min at -80ºC and 37ºC, continued by incubation on ice for 1-2 hrs. After that, the suspension was sonicated on ice for 1 min ten times with 30-sec pauses. Cell debris was removed by centrifugation at 8000 rpm for 30 min at 4ºC. The supernatant was filtered to remove the remaining cell debris and briefly applied to His Gravitrap column (GE Healthcare) according to the manufacturing protocol. Two mL of supernatant were applied to the column that had been equilibrated with 10 mL of binding buffer (20 mM Tris-HCl pH 7.4, 500 mM NaCl, 20 mM imidazole). After that, the column was washed with 10 mL binding buffer to remove the contaminating protein. Next, the protein was eluted with 2 mL of elution buffer (20 mM Tris-HCl pH 7.4, 500 mM NaCl, 500 mM imidazole).

**Sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis**

The expressed peptide was analyzed by SDS-PAGE system containing 4% of polyacrylamide gel as a stacking gel and 13% of polyacrylamide gel as a separating gel (Anggraeni et al. 2022). The peptide was mixed with 0.5 mM Tris-HCl pH 6.8, 0.5% (w/v) bromophenol blue, 10% (v/v) glycerol, 2% (w/v) SDS, and 10% (v/v) β-mercaptoethanol, heated in boiling water for 5 min, and loaded to the gel 15 µL. The separating condition was set at 150 volts at 1 hr, continued by staining with Coomassie Blue R-250 staining reagent. The protein standard low range marker (Bio-Rad, USA) contained phosphorylase b (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.1 kDa) was used as the protein marker.

**Peptides' sequences analysis**

The sequences of expressed peptides were analysed by liquid chromatography-mass spectrometry (LC-MS/MS). The peptides' band of CeHS-1 and CeHS-1 GP in the SDS-PAGE system were cut, eluted, and loaded to liquid chromatography tandem mass spectrometry (LC-MS/MS) system. The spectra were analyzed using MASCOT MS/MS Ion Search program (www.matrixscience.com). Peptides identification was conducted by searching against the protein database from NCBI with the initial searching parameters; enzyme: trypsin; carbamidomethylation (C): fixed modification; oxidation (HW) and oxidation (M): variable modifications; peptide mass tolerance: 0.5 Da; fragment mass tolerance: 0.5 Da; a peptide charge state: +1,+2, +3; instrument type: ESI-TRAP; and report top: Auto (Janwan et al. 2015).

**Peptides' concentration**

The concentration of the peptides was determined by Bradford Assay (Ernst and Zon 2010). The bovine serum albumin (BSA) at concentration 0, 0.2, 0.4, 0.6, 0.8, and 1 mg/mL was used as protein standard. The standard and the antimicrobial peptides were added to the preformulated Coomassie blue G-250 assay reagent, followed by measuring absorbance at 595 nm.

**Antimicrobial activity assay**

Antimicrobial activity of the peptides was assayed using several species of bacteria: *Bacillus subtilis* TISR 008, *Klebsiella pneumoniae* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. The antimicrobial activity of the peptides was determined by defined minimal inhibitory concentration (MIC). The peptides' MIC was determined triplicated by the liquid microdilution method in the sterile 96-well microplate following the protocol with some modification (Tian et al. 2009). Briefly, test bacteria were inoculated to 5 mL of nutrient broth (NB) media and cultured overnight at 37ºC with the shaking at 180 rpm. The overnight culture was re-cultured into NB media and incubated in the same condition. After the $OD_{600}$ reached approximately 0.6, the culture was diluted until the final concentration reached $5\times10^5$ CFU/mL and used for testing bacteria (Kowalska-Krochmal and Dudek-Wicher 2021). Meanwhile, 100 µL of two-fold serial dilutions of test peptides and antibiotics in the NB media was prepared in the wells. Furthermore, 5 µL of tested bacteria were added to the wells and incubated at 37°C for 16-24 hrs. After incubation, the $OD_{600}$ of each well was determined. The lowest concentration that could inhibit bacterial

growth was defined as MIC. In determining the minimum bactericidal concentration (MBC) of the peptides, the amount of 10 µL of culture with no bacterial growth was spread to the NB agar and incubated at 37ºC for 16-24 hrs. The lowest concentration with no bacterial growth in the plate was defined as MBC (Al-Ani et al. 2015).

## RESULTS AND DISCUSSION

### Recombinant peptides' expression

The product of pET32a-CeHS-1 and pET32a-CeHS-1 GP were overexpressed in *E. coli* BL21(DE3)pLysS through the induction of 0.2 mM IPTG at 37ºC. The overexpressed peptides were displayed by the band size of approximately 22 kDa on the 13% SDS-PAGE analysis (Figure 3). This size corresponded to the theoretical size of pET32a-CeHS-1 and pET32a-CeHS-1 GP as measured by its amino acid chains.

After the induction process, the cell pellet was collected. The recombinant peptides were extracted from the host cells. The extraction of the recombinant peptides from the bacterial body resulted in the recombinant peptides found in the supernatant of the extraction process (Figure 4). It indicated that the recombinant peptides were expressed in the soluble fraction.

Later, using the His Bind Purification Kit, the supernatant that contained recombinant pET32a-CeHS-1 and pET32a-CeHS-1 GP could be separated from other bacterial proteins. The recombinant peptides could be eluted by elution buffer 20 mM Tris-HCl pH 7.4, 500 mM NaCl, 500 mM imidazole and then were collected for further analysis. After the concentration and dialyzation of the eluent, the concentration of fusion CeHS-1 was detected as 0.421 mg/mL, and the concentration of CeHS-1 GP was measured as 0.306 mg/mL.

Instead of SDS-PAGE analysis, the recombinant CeHS-1 and CeHS-1 GP were determined by liquid chromatography–mass spectrometry (LC-MS)/MS analysis. When SDS-PAGE analysis only determined the average molecular weight of the peptides, LC-MS/MS analysis could investigate the peptides sequences. The recombinant peptides were referred to as Heteroscorpine-1 from *Heterometrus laoticus* (Table 1). The LC-MS/MS analysis could identify that the recombinant CeHS-1 and CeHS-1 GP contained thioredoxin 1 from *E. coli*. They also contain the matched sequences with the Heteroscorpine-1 sequences from the database.

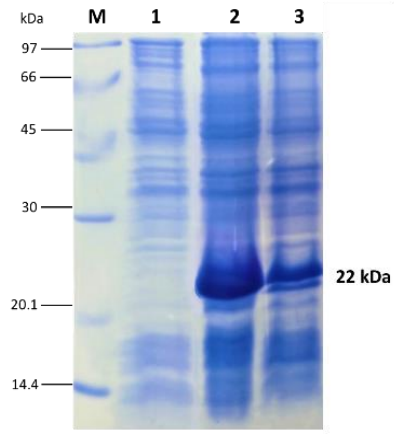



**Figure 3**. The expression of recombinant CeHS-1 and CeHS-1 GP was detected by strong band at approximately 22 kDa (M: Marker, Lane 1: CeHS-1, Lane 2: CeHS-1 GP)

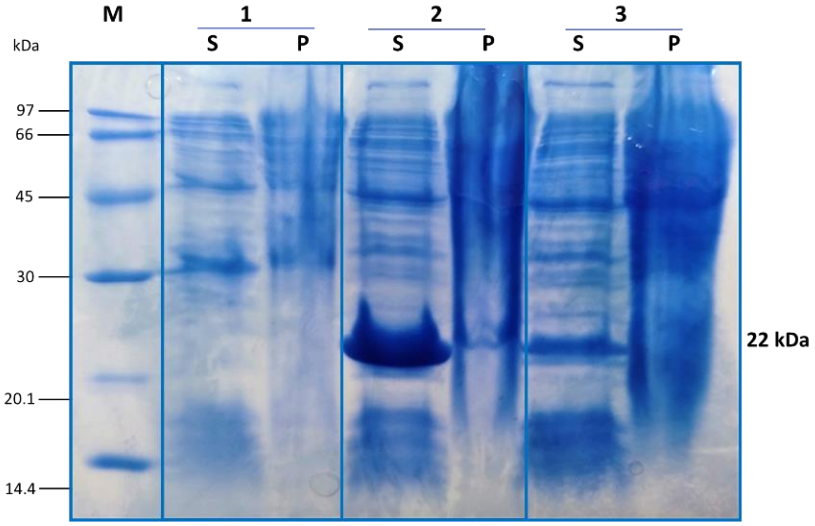



**Figure 4**. Solubility analysis of CeHS-1 and CeHS-1 GP recombinant peptides on 13% SDS-PAGE (M: Marker, Lane 1: Heteroscorpine-1, Lane 2: CeHS-1, Lane 3: CeHS-1 GP, S: supernatant fraction, P: pellet fraction)

**Table 1.** The LC-MS/MS analysis of CeHS-1 and CeHS-1 GP recombinant peptides

| Peptides | Matches | Molecular weight | Score | Sequences |
|---|---|---|---|---|
| CeHS-1 | Thioredoxin 1 | 11913 | 397 | R.GIPTLLLFK.N<br>K.LNIDQNPGTAPK.Y<br>K.IIHLTDDSFDTDVLK.<br>K.MIAPILDEIADEYQGK.L<br>K.ADGAILVDFWAEWCGPCK.M |
| | Heteroscorpine-1 | 10638 | 62 | K.IGNNILGGMAK.A |
| CeHS-1 GP | Thioredoxin 1 | 11913 | 327 | R.GIPTLLLFK.N<br>K.LNIDQNPGTAPK.Y<br>K.IIHLTDDSFDTDVLK.<br>K.MIAPILDEIADEYQGK.L<br>K.ADGAILVDFWAEWCGPCK.M |
| | Heteroscorpine-1 | 10638 | 47 | K.IGNNILGGMAK.A |

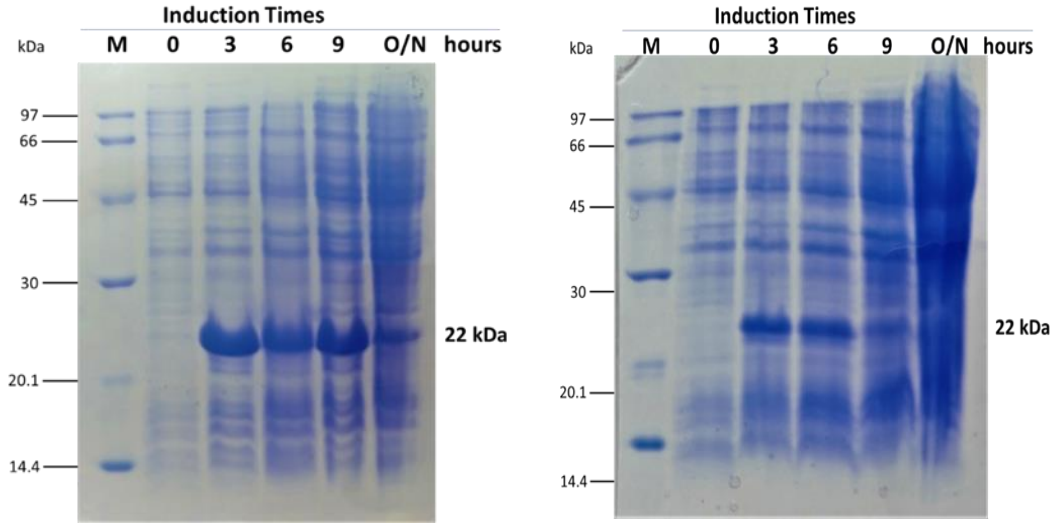



**Figure 5**. The optimization of expression of recombinant peptides resulted that the strongest band was appeared in the induction time of 3 hrs; A. CeHS-1, B. CeHS-1 GP

**Optimization of expression time**

Many factors can influence the expression yield of antimicrobial peptides. The induction time optimization is important to maximize the expression yield (Wulanjati et al. 2021). Moreover, in the antimicrobial peptides expression, time optimization is needed due to the toxicity of the recombinant peptides that may reduce the host cells during the induction process. The time optimization result in this study showed that in the recombinant CeHS-1 expression, there was no significant variation in the induction time of 3, 6, and 9 hrs. In the overnight induction, the natural bacterial peptide was accumulated, so IPTG induction was not useful for overnight induction. The time optimization of recombinant CeHS-1 GP had a different result. The band intensity of recombinant CeHS-1 GP is higher in the induction time of 3 hrs and later decreased in the induction time of 6 and 9 hrs (Figure 5). Like recombinant CeHS-1 induction, the overnight induction was ineffective for recombinant peptides induction due to the accumulation of natural bacterial peptides.

**Antimicrobial activity assay**

In the antimicrobial activity assay, the data showed that recombinant CeHS-1 and CeHS-1 GP was capable of inhibiting the growth of tested bacteria. The recombinant CeHS-1 had the best activity against *S. aureus* with MIC of 64 µg/mL, while the recombinant CeHS-1 GP exhibited the best antimicrobial activity against *K. pneumoniae* with MIC of 32 µg/mL. The comparison between the activity of recombinant CeHS-1 and CeHS-1 GP and the synthetic CeHS-1 and CeHS-1 GP showed that the MIC of the recombinant peptides had a higher MIC than the synthetic ones. It indicated that the antimicrobial activity of recombinant CeHS-1 and CeHS-1 GP was lower than that of the synthetic peptides. In the killing activity assay, the recombinant CeHS-1 and CeHS-1 GPK could not kill the tested bacteria until the highest tested concentration of 128 µg/mL (Table 2). This activity was also lower than the synthetic peptides in most tested bacterial strains. Overall, the recombinant CeHS-1 GP showed higher antimicrobial activity than recombinant CeHS-1. This activity aligned with the activity of synthetic peptides, in which CeHS-1 GP possessed higher activity than CeHS-1.

**Table 2.** The Determination of MIC and MBC of CeHS-1, CeHS-1 GP, and CeHS-1 GPK

| Bacterial strain | rCeHS-1 | | CeHS-1 | | rCeHS-1 GP | | CeHS-1 GP | |
|---|---|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| *K. pneumoniae* ATCC 27853 | 128 | >128 | 64 | >128 | 32 | 64 | 32 | 64 |
| *E. coli* ATCC 25922 | 128 | >128 | 64 | >128 | 64 | >128 | 16 | 16 |
| *B. subtilis* TISR 008 | 128 | >128 | 64 | 64 | 64 | 128 | 32 | 64 |
| *S. aureus* ATCC 25923 | 64 | >128 | 64 | >128 | 64 | >128 | 32 | 128 |
| *P. aeruginosa* ATCC 27853 | >128 | >128 | 64 | 64 | 64 | >128 | 32 | 32 |

Note: MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; MIC and MBC values are determined in μg/mL

## Discussion

Effective and feasible antimicrobial production was needed to fulfill the requirements of antimicrobial peptides for research and clinical purposes. This study introduced a simple and economic system for producing the cecropin-like part of Heteroscorpine-1, which was CeHS-1 and CeHS1-GP. The bacterial expression system was chosen due to its advantages: rapid growth, ease of handling, many established protocols and equipment such as large availability of commercial expression vectors, well-established DNA manipulation protocols, and extensive knowledge regarding its genetics, biochemistry, and physiology (Parachin et al. 2012). Although the bacterial expression system did not involve post-translational modification, the limitation was acceptable. The short sequences of antimicrobial peptides can easily shape their structure as they only need to fold in the secondary structure (Deng et al. 2017).

Among the several options of bacteria host, *E. coli* BL21(DE3)pLysS was chosen for this study due to its chromosomal copy of the gene for T7 RNA polymerase (Rezaei-Moshaei et al. 2021). The host cells were lysogens of bacteriophage DE3 that carried the gene for T7 RNA polymerase under lacUV5 control. After a DE3 lysogen was formed, the lacUV5 promoter was the only promoter that commonly used to direct transcription of the T7 RNA polymerase gene. It was inducible by isopropyl-β-D-thiogalactopyranoside (IPTG) to facilitate the overexpression of the recombinant peptides (Liu and Yang 2012).

The system involved the expression vector pET32a. Among the expression vectors provided, the pET series is the most common expression vector used in antimicrobial peptide expression (Parachin et al. 2012). The expression vector pET32a was chosen since, as a translation vector, it contained a highly efficient ribosome binding site derived from the phage T7 main capsid protein and was used to express target genes without their own ribosomal binding site (Sørensen and Mortensen 2005). Moreover, the pET system is an ideal tool for protein expression since it allows the regulation of peptides expression with the T7 promoter, pLysS hosts, and the addition of IPTG induction (Baneyx, 1999).

The pET32a expression vector contains Thioredoxin Tag, which has many advantages in the expression process. Thioredoxin is a disulfide reductase used to effectively catalyze the forming of disulfide bonds in *E. coli* and exhibit chaperone-like activity. These characteristics make thioredoxin an important carrier protein, aiding the precise creation of disulfide bonds and the proper folding of AMP required for the recombinant peptides (Parachin et al. 2012; Soleimani et al. 2016). Moreover, the tag was also valuable for forcing the expression of the recombinant peptides in the soluble fraction (Zhou et al. 2009). The recombinant CeHS-1 and CeHS-1 GP were expressed in the soluble fraction. This condition was very beneficial since the purification of the peptides from the host cell would be easier. In the expression of recombinant peptides, the formation of inclusion bodies in bacterial hosts poses a significant obstacle. The expression of recombinant peptides in the inclusion bodies requires significant processing, including extraction from the cell, solubilization, refolding, and purification (Singh et al. 2015). The peptides extraction from the inclusion body using high concentrations of chaotropes such as urea and guanidine hydrochloride (GdnHCl) usually results in complete denaturation of the secondary structures of the peptides. These chemicals frequently lead to protein aggregation during refolding process (Bhatwa et al. 2021). This process usually results in a low yield of recombinant peptides due to the loss during extraction. Moreover, the use of many chemicals and many processes will increase the extraction cost.

The pET32a system also contains His Tag fusion. His-tag expression systems were preferred as they had low molecular weight and did not alter the structure and function of the peptides. Therefore, separating the His-tag from the target peptides is unnecessary (Zhao and Huang 2016). Additionally, His-tag could facilitate the separation of the recombinant peptides from other bacterial peptides. The peptide with his tag fusion is easily purified using Ni-NTA affinity resin (Celie et al. 2016).

The challenge of antimicrobial peptides expression using a bacterial expression system was the peptides' toxicity toward the host cell (Wibowo and Zhao 2019). The induction time should be optimized to overcome the limitation in the expression process caused by recombinant peptides toxicity. The expressed recombinant CeHS-1 was highly expressed without any significant difference from 3, 6, and 9 hrs of induction (Figure 4). Meanwhile, recombinant CeHS-1 GP was highly expressed within 3 hours of induction. However, along with an increase in induction time, the yield of the expressed peptides was reduced significantly. By comparing this data with the antimicrobial activity of the peptides, it was revealed that the antimicrobial activity of the peptides influenced their

expression yield. Therefore, with the induction time optimization, a relatively high yield of peptides could still be obtained.

Furthermore, the antimicrobial activity assay showed that recombinant CeHS-1 and CeHS-1 GP were able to inhibit the growth of tested bacteria but unable to show the capability to kill the bacteria. Compared to synthetic peptides, the antimicrobial activity of the recombinant peptides was significantly lower. In this case, many factors, such as the present of fusion tag, can influence the antimicrobial peptide activity of the recombinant peptides. Moreover, the fusion tag also can affect the structure of the peptides. Fusion tags are usually included in the expression system due to their ability to facilitate expression and purification process. It can increase protein solubility and improve the purification process, leading to an increased yield and a decreased production cost. In order to increase the antimicrobial activity of peptides, tag removal can be performed by proteolytic cleavage. However, this process can be challenging for the stability and solubility of the peptides. Other problems include the expensive cleavage enzyme. Moreover, the cleavage process usually causes the loss of recombinant peptides, which also can increase the cost of peptides expression (Tavares et al. 2012). Another study attempting to compare the antimicrobial activity synthetic with recombinant peptides had a similar result, where the recombinant peptides had lower activity than the synthetic peptides (Harnischfeger et al. 2021).

In addition, using *E. coli* as a host in the expression system was advantageous, such as rapid expression, high yields, and low costs. The limitation of the expression of recombinant antimicrobial peptides is the toxicity of the recombinant peptides toward the host cells. The additional tag in the recombinant peptide sequences might be beneficial in increasing the yield of the peptides. Overall, this study generated the expression system for CeHS-1 and CeHS-1 GP that currently had a potential effect against pathogenic bacteria. However, the stability of the expressed peptides needs to be considered since the instability of the peptides at room temperature can cause a reduction in the antimicrobial activity of the peptides (Luong et al. 2020). Further study is needed to determine the optimum condition for the peptides' stability during peptides preparation for research or clinical use. Even though there was a reduction of the antimicrobial activity of the recombinant peptides, a fast, easy, and low-cost expression system is needed and beneficial for antimicrobial peptides production for research and clinical purposes.

In conclusion, bacterial expression system is the system that feasible for the expression of antimicrobial peptides, as it is fast, easy, low cost, and can produce high peptide yield. The expression system for Cecropin-like part of Heteroscorpine-1 using *E. coli* BL21(DE3)PLysS combined with the expression vector pET32a was successfully carried out. The expression was induced by 0.2 mM IPTG with the optimum induction time was 3 hrs. The peptides determination by SDS-PAGE analysis and LC-MS/MS indicated that the peptides was expressed in the size around 22 kDa, linked with thioredoxin tag. The expressed antimicrobial peptides, CeHS-1 and CeHS-1 GP could show their activity against many tested bacterial strains. Similar with the antimicrobial activity of synthetic peptides, CeHS-1 GP had higher antimicrobial activity than CeHS-1.

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