SKIN SECRETIONS OF BORNEAN FROGS REVEAL ANTIMICROBIAL PEPTIDES WITH INSULIN RELEASING PROPERTIES IN HIT-T15 CELLS

Abstract: Skin secretion of two Bornean frogs from the Ranidae family, *Pulcharana baramica* and *Hylarana erythraea* were evaluated for their antimicrobial and insulinotropic properties. Comprehensive approach and various techniques were employed to study the characteristics of the antimicrobial peptides (AMPs) isolated from these frogs. Crude skin secretions of the frogs were partially purified using Sep Pak C-18 cartridges, followed by further purification via the HPLC AKTA method. The peptides purity was assessed through SDS-PAGE gel which revealed that the HPLC AKTA method produced clearer and cleaner bands, demonstrating its superior efficacy as compared to Sep-Pak. The disk diffusion assay demonstrated significant zones of inhibition against both *E. coli* and *S. aureus*, underscoring the peptides' ability to effectively impede bacterial growth. The minimum inhibitory concentration (MIC) assay provided specific MIC values for the tested bacteria. The results showed that the AMPs from *Pulchrana baramica* were more potent when compared to *Hylarana erythraea*, with an MIC of 125 µg/ml for *E. coli*. In terms of cytotoxicity, purified AMPs from both Ranid frogs induced over 50% blood cell lysis, suggesting that they may affect the cell membranes integrity of mammalian erythrocytes. This research further investigates the insulinotropic effect of AMPs from Ranidae frogs on HIT-T15 cells. AMPs from both frog species were able to trigger insulin secretion increase from the pancreatic cells. Statistical analysis indicates a significant difference ($p<0.05$) for *P. baramica*, while *H. erythraea's* effect is not significant ($p > 0.05$). The current findings suggest that these multifunctional peptides play an important role in the defence of Ranid frogs from invading pathogenic microorganisms present in their environment and may hold potential future applications as an antidiabetic agent in managing glucose levels and diabetes.

Keywords: Antimicrobial peptides, Bornean amphibians, frog skin secretions, insulin secretion, SDG15

Introduction

Anuran skin plays a vital role in their interactions with the environment, microorganisms, parasites, and more (Varga et al., 2019). Amphibian skin secretions, with their unique chemical diversity, have attracted significant interest for their potential clinical applications (Conlon et al., 2019). Research has focused on bioactive components in amphibian skin secretions, particularly biologically active peptides spanning various families (Indriani et al., 2023). These peptides are stored in specialized glands and can be released in response to stress or injury (Rollins-Smith et al., 2023). Frog skin, a unique and adaptable organ, has evolved to protect against environmental threats (Longo et al., 2017). It comprises an epidermis and dermis, housing crucial components like mucous glands and poison glands (Varga et al., 2019). These mucous glands produce a protective mesh of glycoproteins and proteoglycans, serving as a natural defence barrier (Mauricio et al., 2021). Adaptations in amphibians have led to the development of poison glands in the dermal layer, producing various bioactive compounds. As many of these bioactive peptides have mammalian counterparts such as those in the gastrointestinal tract, increase interests are seen in the research of various peptides isolated from the frog's skin secretion (Wang et al., 2016).

Traditional Chinese medicine, such as venenum bufonis, utilized frog secretions for various ailments (Wei et al., 2019) and used skin extracts from frogs and toads to treat inflammation and infections (Yacoub et al., 2020). Modern research has confirmed the therapeutic potential of frog secretions in treating neglected tropical diseases, arrhythmias, heart diseases, and more (Lewies et al., 2015; Conlon, 2017). Recent research had shown that

AMPs in the frog skin secretions contain biological activity such as anticancer, antibacterial and anti-diabetes (Lin et al., 2021, Casciaro et al 2020, Soltaninejad et al., 2021).

Previous study reported that peptides from frog skin secretion exhibit the capacity to stimulate insulin release *in vitro* from BRIN-BD11 rat clonal β cells at low concentrations, while posing minimal cell toxicity (Owolabi et al., 2016). Remarkably, over 99% of antidiabetic peptides have been sourced from amphibian skin secretions, with a particular emphasis on species belonging to Anura (Soltaninejad et al., 2021). Recent research has uncovered that these peptides, known as AMPs (antimicrobial peptides), exhibit insulin-like properties, which hold great promise in the realm of diabetes treatment (Musala et al., 2021). This revelation has piqued interest in harnessing these peptides for innovative and sustainable diabetes therapies, especially considering the approval of exenatide, a substance derived from lizard venom, for type 2 diabetes treatment (Coulter-Parkhill et al., 2021).

Initially recognized for their role in defending against bacteria, frog skin peptides have surprised researchers by also being capable of releasing insulin (Conlon et al., 2024). Various studies have shown that these frog skin peptides can effectively release insulin, both in controlled laboratory settings and in living organisms, demonstrating their potential utility for managing diabetes (Musale et al., 2019; Ojo et al., 2013). Furthermore, this paper extends its investigation to screen these AMPs for their insulin-like activity, utilizing the HIT-T15 cell line. Employing various analytical techniques such as peptide isolation, purification, and molecular weight characterization, this study aims to shed light on the potential of these frog-derived AMPs to influence insulin-related processes. This study offers a fresh perspective on their possible applications in antibacterial therapies and the development of antidiabetic drugs.

Materials and Methods

Collection of Skin Secretions

The collections of skin secretion were performed via a non-invasive method which did not involve any sacrifice of frogs (Sabri et al., 2018). Individuals from two frog species, *Pulchrana baramica* and *Hylarana erythraea*, were captured during night time around the river areas within the natural forest reserves of Universiti Malaysia Sarawak East Campus. Anhydrous diethyl ether was used to stimulate the secretion from the frogs' skin, followed by rinsing the dorsal part of the frogs with sterile water containing 0.1% TFA. The resulting solutions were centrifuged and freeze-dried for further analysis. Approval by the Universiti Malaysia Sarawak Animal Ethics Committee was sought prior to the commencement of the research (Approval Reference Number: UNIMAS/AEC/T/F07/024).

Purification of Peptides

Partial purification of the crude extracts was carried out using Sep Pak C-18 cartridges according to the previously published method (Sabri et al., 2018). Further purification of the partially purified peptides was conducted using the ÄKTA Pure 25 System (Chemopharm, Malaysia) with a Siliachrom C18 mono HPLC column. Bound peptides were eluted using 70% acetonitrile containing 0.1% TFA. The size and purity of the peptides at each purification step were visualized using SDS-polyacrylamide gel electrophoresis.

Antibacterial Assays

Antimicrobial activities of the isolated peptides against the gram-positive *Staphylococcus aureus* and the gram-negative *Escherichia coli* were evaluated using the disc diffusion and Minimum Inhibitory Concentration (MIC) assays, based on the previously published methods (Khademi et al., 2019; Schadich, 2013).

Hemolysis Assays

Hemolytic activity of the isolated peptides was assessed using human erythrocytes according to the published method (Ju et al., 2021). The erythrocytes, initially washed with phosphate buffer saline (PBS) were incubated at 37°C with frog peptides at several concentrations for 30 minutes. PBS and 1% Triton X-100 were used as negative and positive controls, respectively.

Following a 5-minute centrifugation at 2500 g, the absorbance was measured at 450 nm using the ELISA plate reader.

Cell Culture

The HIT-T15 cells (CRL-1777) were obtained from the ATCC (American Type Culture Collection) from Manassas USA. The cells were cultured in Ham's F12 medium with 100 ml horse serum, 12.5 ml fetal bovine serum (FBS), 100 I.U./ml of penicillin, and 100 µg/L streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. When cells reached 80% confluence, they were sub-cultured using 0.25% trypsin 2.65 mM EDTA, and the medium was changed every 2 days to facilitate optimal growth and confluence.

Cytotoxicity Assay

Evaluation of the AMPs effect on cell viability was performed using the MTT assay kit from Sigma-Aldrich. AMPs concentration ranging from 1µg/ml to 50 µg/ml were used in these assays. The HIT-T15 cells were exposed to the various peptides' concentration for 24 hours, followed by the addition of 1 mg/ml MTT solution and further incubated for 2 hours. Cell viability was assessed quantitatively based on the absorbance at 570nm, using an ELISA plate reader.

Evaluation of Insulin Secretion in HIT-T15 Cells

HIT-T15 cells were cultured at a density of 5 **x** 104 cells per well in a 12-well plate for 48 hours. Subsequently, they were exposed to Krebs-Ringer Buffer (KRB buffer: 1.8 g/L D-glucose, 0.00468 g/L MgCl2, 7.0 g/L NaCl, 0.34 g/L KCl, 0.1 g/L Na2HPO4, 0.18 g/L NaH2PO4) supplemented with test samples for 30 minutes (Min et al., 2019). The conditioned media were collected and centrifuged at 12000 rpm for 10 minutes. Subsequently, the supernatant was analysed for insulin concentration using the Human Insulin ELISA kit (R&D Systems) according to the manufacturer's guidelines.

Results and Discussion

Purifications of Frog Skin Antimicrobial Peptides

Gel electrophoresis of the crude skin secretion from *P. baramica* and *H. erythraea* exhibited almost similar patterns with high-intensity protein bands corresponding to specific molecular weights of 17.0, 6.5, and 3.5 kDa, respectively. Further purification of the secretion mixture successfully eliminated the larger protein bands, revealing sharp and cleaner bands at the bottom of the gels which correspond to smaller peptides with molecular weight between 1 kDa-3.4 kDa. Prior to the purification process, minor differences in both the size and arrangement of protein bands were discernible between these two frog species within the Ranidae family. Partially purified skin secretion of *P. baramica* showed additional protein bands in the range of 6.5 kDa – 3.4 kDa, which are absent in the partially purified secretion obtained from *H. erythraea*. Following further purification via the AKTA PURE, what emerged as particularly noteworthy were the striking parallels between these two frog species, *P. baramica* and *H. erythraea*, as evident in Figure 1. They exhibited notable similarities in the presence of two clear bands at the bottom of the gel which corelate to low molecular weight peptides. These findings align with previous related studies indicating that antimicrobial peptides from frog skin secretion typically possess lower molecular masses, ranging from 1 to 5 kDa (Sabri et al., 2018). Few examples of antimicrobial peptides from Bornean frogs which have been characterized are the Brevinins (2.5 – 3.0 kDa), Esculentins (3.0 – 3.5 kDa) and Temporins (2.0 – 2.5 kDa) (Conlon & Mechkarska 2014; Ong et al., 2021).

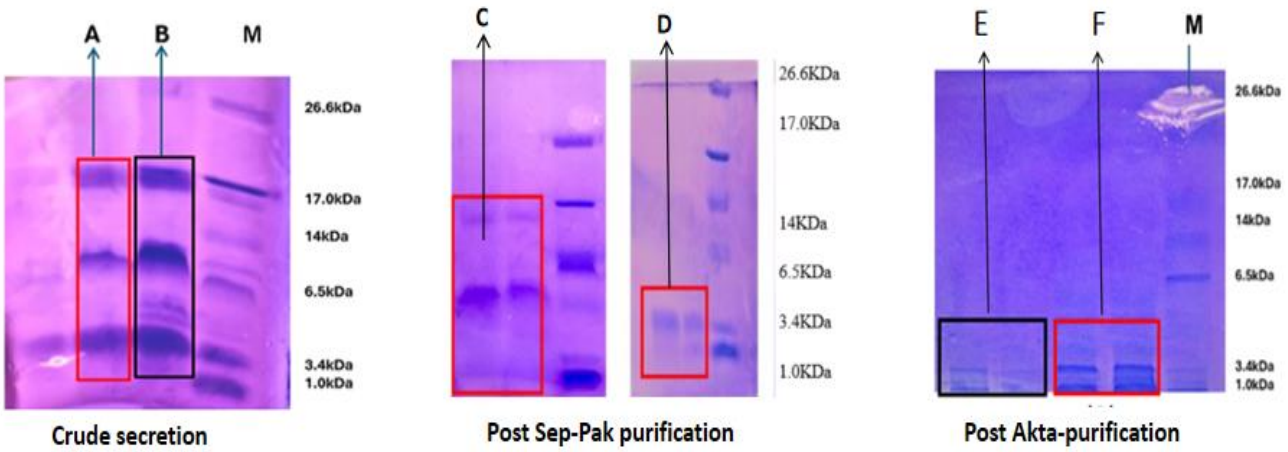

Figure 1: Coomassie blue Tricine SDS-PAGE gel of crude secretion, post Sep-Pak purification and post-Akta Pure purification antimicrobial peptides from the skin secretion of Bornean Ranidae. Lane A: crude secretion from *H. erythraea*; lane B: crude secretion from *P. baramica*; lane C: Sep-Pak purified secretion from *P. baramica* and lane D: Sep-pak-purified secretion from *H. erythraea* Lane E: Akta Pure purified secretion from *H. erythraea*; lane F: Akta-Pure purified secretion from *P. baramica*; Lane M represents protein marker (Sigma Aldrich) ranging from 26.6 to 1.0 kDa

Antimicrobial peptides from the Ranidae family were reported to show conserved amino terminal region with diverse carboxyl terminal segments which corresponds to mature peptides (Conlon et al., 2024). This can be seen in the degree of similarity of the former region among the AMPs from distantly related frog species. However, exposure to different environment elements as well as evolutions or mutations in the pathogens present within their surroundings, have led to diversification of AMPs from these anurans. These variations may also be due to genetic differences in individual frogs of the same species This fact is evidenced from previous findings which showed different protein profiles of the skin secretion of *H. erythraea* from different geographical locations (Zhang et al., 2018; Ong et al., 2021).

Common challenges faced in protein purification is the yield and degree of purity. Protein yield in purification is influenced by a combination of protein-related factors**,** sample source quality, and methodological choices at each purification step. Protein loss increases with increasing number of steps involved in the purification process. In this study, the utilization of a 2-step purification method helps to minimize protein loss during the process.

Table 1 summarizes the protein yield obtained during the purification of peptides from the frogs' skin secretion.

Table (1): Comparison of the concentration and purification yields of peptides in crude, partially purified, and fully purified fractions derived from the skin secretions of *P. baramica* and *H. erythraea*

***Pulchrana baramica* individual**	**Crude protein concentration (µg/mL)**	**Post-Sep Pak protein concentration (µg/mL)**	**Protein recovery percentage (%)**	**Post-ÄKTA (µg/mL)**	**Recovery Post-Akta (%)**
1	1520	1100	72.3	750	49.3%
2	879.3	764	86.8	550	62.6%
3	1020	742	72.7	500	49.0%
***Hylarana erythraea* individual**	**Crude protein concentration (µg/mL)**	**Post-Sep Pak (µg/mL)**	**Protein recovery percentage (%)**	**Post-ÄKTA (µg/mL)**	**Recovery Post-Akta (%)**
1	995.4	580	58.3%	450	45.2%
2	1432	600	41.9%	400	27.9%
3	789	520	65 %	350	44.4%

Antibacterial Properties of Frog Skin Peptides

The isolated frog skin peptides were tested for their antimicrobial activities via the disc diffusion assay, followed by the determination of the MIC against gram positive and gram-negative bacteria. Notably, at a concentration of 1000 µg/ml, *P. baramica* emerged as remarkably potent antimicrobial agent, showcasing a substantial inhibition zone diameter of 11.66 mm against the gram-negative *E. coli.* Conversely, peptides derived from *H. erythraea* demonstrated a comparatively milder effect on *E. coli*, presenting an observed inhibition zone diameter of 7.33 mm.

Skin peptides from both *P. baramica* and *H. erythraea* (at 1000 µg/ml) displayed moderate potency against gram positive bacteria, *S. aureus*, exhibiting inhibition zone diameters of approximately 5.6 mm and 4.6 mm, respectively. These findings highlight the differential antimicrobial effects of the peptides against the tested bacterial strains, emphasizing *P. baramica's* particularly robust activity against *E. coli* as compared to *H. erythraea*. Table 1 provides a summary of the antimicrobial effectiveness exhibited by the purified peptides against both *E. coli* and *S. aureus*, as illustrated by the inhibition zones observed in the disc diffusion test.

Table 1: Inhibition zone diameter of antimicrobial peptides against two strains of bacteria.

Bacteria Strain	***E. coli (mm)***	***S. aureus (mm)***
P. baramica	11.7 ± 3.4	5.6 ± 0.6
H. erythraea	7.3 ± 0.5	4.6 ± 0.6
Positive control	17.6 ± 0.5	15.3 ± 0.6

The antimicrobial potency of the frog peptides was further demonstrated via the determination of the MIC. Figure 2 depicts the MIC of the purified peptides extracted from the

frogs' skin for *E. coli*. As the concentrations of these peptides increase, their antimicrobial effectiveness against both *E. coli* and *S. aureus* also increases. Amongst the frogs tested, peptides from *P. baramica* displayed the most potent antimicrobial activity against *E. coli*, boasting the MIC of 125 µg/ml. Additionally, the MIC values of peptides from both *P. baramica* and *H. erythraea* against *S. aureus* were recorded at 500 µg/ml.

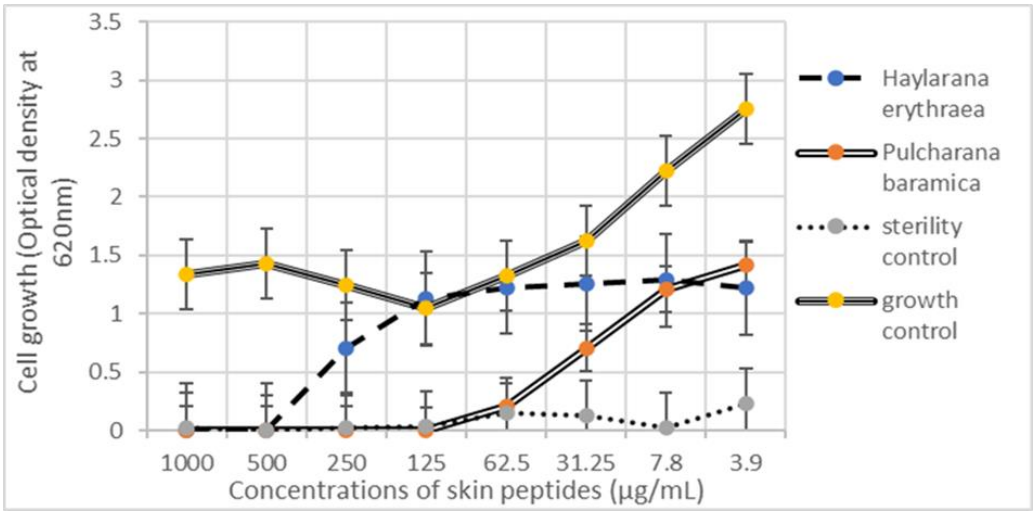

Figure 2: Growth inhibition of *E. coli* by purified peptides from the skin secretion of Bornean Ranidae. Antimicrobial peptides of *P. baramica* and *H. erythraea* at concentrations ranging from 3.9 µg/mL to 1000 µg/mL were incubated with *E. coli* at 1 x 10^6 CFU/ml. After 24 hours, the bacterial growth was analyzed by measuring the optical density at 620 nm using an ELISA plate reader.

Over the last few decades, the skin secretions of amphibian species have yielded over a thousand reported antimicrobial peptides (AMPs) (Ladram et al., 2016). Antimicrobial peptides derived from Ranid frog skin secretions have exhibited significant activity against both gram-positive and gram-negative bacteria (Wang et al., 2016). Skin secretions from *P. baramica* and *H. erythraea* demonstrated significant zone inhibition against *E. coli* and *S. aureus*, with MIC values of 125 µg/ml and 500 µg/ml for the former, and 500 µg/ml and 1000 µg/ml for latter. Table 2 summarizes these results. This finding, supported by research from Chen et al. (2018) underscores the efficacy of these peptides against these bacterial strains. However, the current study indicate that these antimicrobial peptides are more potent against

the gram-negative bacteria as compared to the gram positive bacteria. Similar finding was reported for *P. baramica* in previous study (Sabri et al., 2018). These observations are probably due to several factors such as the differences in cell wall structure between these two groups of bacteria. Gram negative bacteria have an outer membrane composed of lipopolysaccharides (LPS) which may act as a primary target for the AMPs. Studies have reported that an elevated negative charge on bacterial cell membranes may enhance the binding capability of cationic peptides in amphibian skin secretions through electrostatic interactions, potentially resulting in bacterial death (Farhana et al., 2022; Kumar et al., 2018).

Overall, the AMPs isolated from the skin secretion of *P. baramica* show higher potency against both gram-positive and gram-negative bacteria as compared to those from *H. erythreae.* These observations could be due to several factors such as the component of their secondary structures and biochemical properties. AMPs from *P. baramica* may have a higher proportion of cationic residues such as lysine and arginine that enhance electrostatic attraction to negatively charged bacterial membranes. As for their secondary structures, the differences in α-helical or β-sheet structures may improve membrane insertion efficiency. Further study on the structures and physicochemical properties of these peptides are warranted to elucidate their superior antimicrobial actions.

Table 2: Inhibition zone diameter of antimicrobial peptides against *E. coli* and *S. aureus* strains of bacteria.

Frog Species	***E. coli* Skin Secretions (µg/ml)**	***S. aureus* Skin Secretions (µg/ml)**
P. baramica	125	500
H. erythraea	500	1000

In order to strengthen the evidence for therapeutic potential of frogs' AMPs, incorporating clinically relevant resistant strains, such as MRSA, and other multi-drug resistant

microorganisms in future studies is highly recommended. Some AMPs may exhibit stronger activity against resistant strains than against susceptible ones, possibly due to the differences in membrane composition.

Hemolysis Assay

The toxicity assay results reveal that at concentrations below 50 µg/ml, all skin peptides displayed minimal haemolytic activity, causing less than 50% cell lysis. However, at the concentration of 100 µg/ml, the purified peptides obtained from *P. baramica* and *H. erythraea* triggered more than 50% blood cell lysis. It was observed that the peptides from *H. erythraea* caused higher percentage of haemolysis when compared to those of *P. baramica* at concentrations 50 µg/ml and below. At concentration above 50 µg/ml, peptides from *P. baramica* demonstrated higher toxicity towards the human red blood cells. This stark difference highlights the varying effects of the peptides derived from different species of Ranidae on mammalian cell membranes. The finding is parallel to the antimicrobial activity of these two peptides whereby *P. baramica* showed higher potency than *H. erythraea* towards both gram-positive and gram-negative bacteria. These observations indicating their dual nature as potent defenders against pathogens but potentially harmful to red blood cells. It is widely acknowledged that AMPs isolated from frog secretions typically display toxicity toward mammalian cells (Conlon, 2017). Although this study did not investigate the mechanism leading to red blood cell lysis upon AMP interaction, the current findings strongly support the idea that increased AMP toxicity is tied to their higher hydrophobicity and amphiphilic characteristic (Wei et al., 2022). Further research is necessary to understand these dual activities and optimize these peptides for medicinal applications while mitigating their adverse effects. A careful balance between optimizing antimicrobial activity and reducing toxicity to

host cells can be achieved via various methods such as structural modifications and development of analogues.

The cytotoxic and haemolytic properties of the antimicrobial peptides pose one of the major challenges in their adaptations into medicine. In order to introduce these molecules as the potential 'natural antibiotics', several modifications need to be done. Previous research has reported the success in reducing haemolytic activity of the peptides while enhancing the antimicrobial activity and plasma stability via amino acid sequence alteration, conjugation with mPEG and developing peptide dimers (Wang et al., 2021). Encapsulation in nanocarriers, such as liposomes, may also provide an alternative to reduce the cytotoxicity effects of these AMPs.

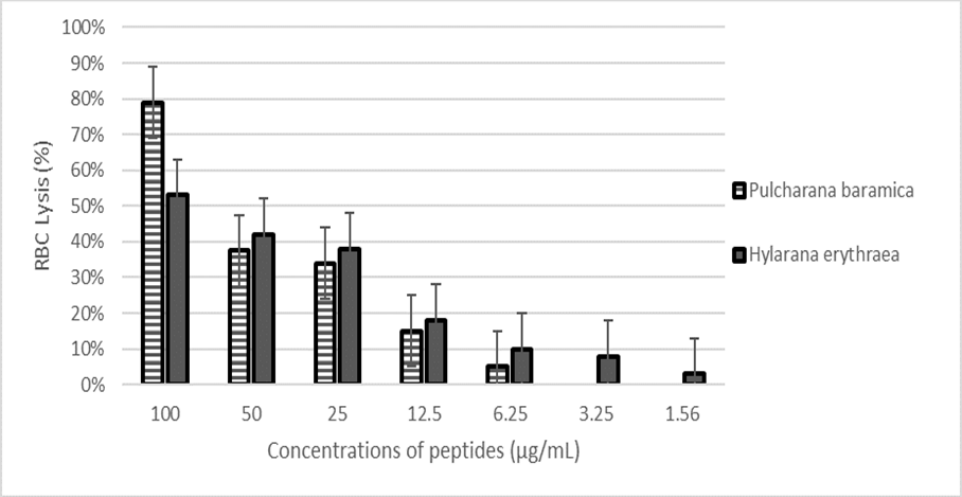

Figure 3: Haemolytic activity of purified peptides from frog skin secretions on human red blood cells. Bar charts showed the percentage of red blood cell lysis, expressed as mean ± SD. The negative control, 0% haemolysis was a sample of red blood cell treated with Phosphate Buffer Saline and positive control, 100 % haemolysis was red blood cell treated with 1 %Tween 20. Values were taken as mean absorbance at 450 nm.

Cell Viability Assay

Cell viability assay was conducted to assess the potential toxicity of the purified peptides on HIT-T15 cells. The results revealed notable differences in cell viability following exposure to peptides from the species studied. *P. baramica* skin secretion exhibited a concentration-dependent effect on cell viability. Incubation of the cell with 50 µg/ml of purified peptides from

P. baramica resulted in a 27% viability. Relatively less toxicity effect was seen with *H. erythraea* peptides at similar concentration. Reducing the concentrations of the skin peptides lead to increase cell viability, as shown in Figure 4. Remarkably, exposure to peptides from *P. baramica* and *H. erythraea* at both 5 µg and 1 µg concentrations consistently maintained approximately 98% - 100% HIT-T15 cells viability.

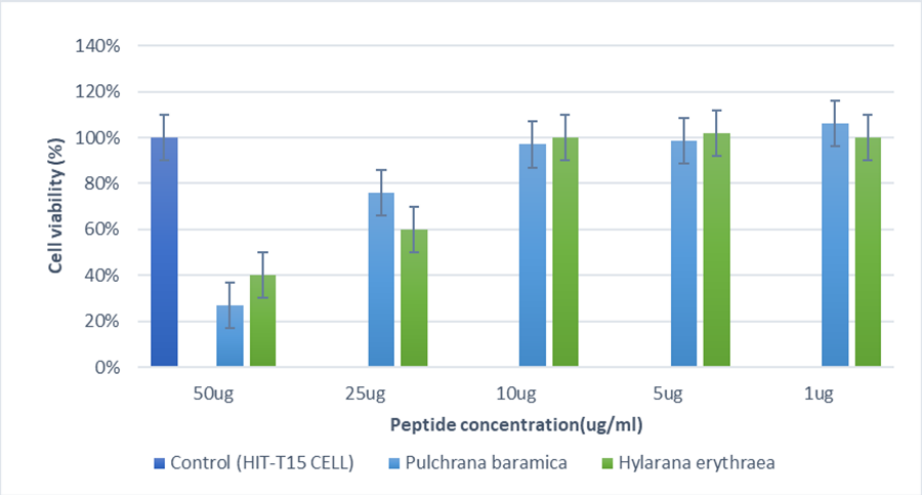

Figure 4: Effects of various concentrations of AMPs from *Pulcharana baramica* and *Hylarana erythraea* on the viability of HIT-T15 cells.

Apart from the AMP concentration, cell viability may also be influenced by the duration of exposure and the peptide structure. Amino acid composition, hydrophobicity, charge and overall structure of the AMP can significantly affect its toxicity. Peptides with higher hydrophobicity or those that form □ helices may interact more strongly with mammalian cell membranes, leading to greater toxicity (Zhang et al., 2021).

Preliminary Evaluation of Insulin Secretion in HIT-T15 Cells

HIT-T15 cells were exposed to antimicrobial peptides (AMPs) sourced from *P. baramica* and *H. erythraea*. Treatment with 5ug/ml of *P. baramica's* AMPs resulted in a significant and dose-dependent elevation in insulin release ($P<0.05$). Specifically, without the exposure to the

antimicrobial peptides, under normal glucose conditions, the baseline insulin release rate measured 10.5 ± 0.34 ng/105 cells/30 min. However, in the presence of 5ug AMPs, this rate increased notably to 11.1 ± 0.37 ng/105 cells/30 min as shown in Figure 5(a). Conversely, *H. erythraea's* AMPs prompted a concentration-dependent surge in insulin secretion at 5ug as shown in Figure 5(b). However, this increase failed to reach statistical significance (P>0.05) when compared to the basal insulin release rate in the presence of glucose alone.

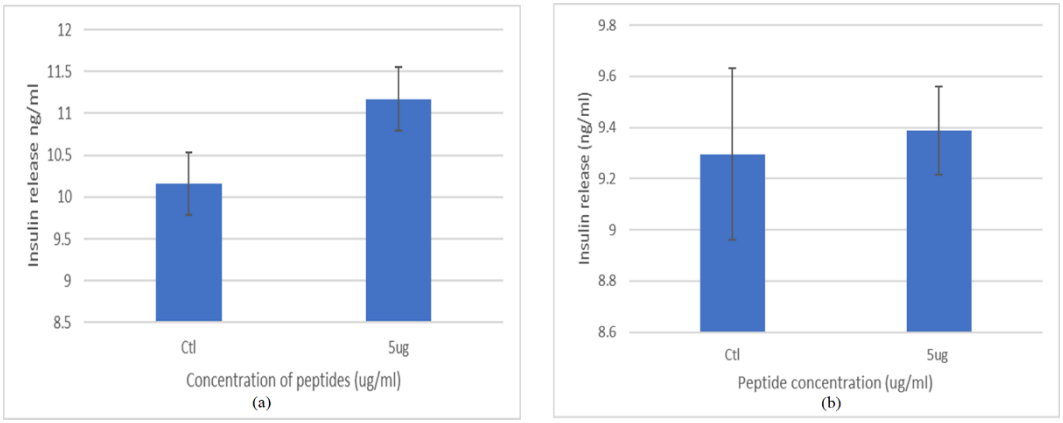

Figure 5: Effects of AMPs from (a) *P. baramica*, and (b) *H. erythraea* on insulin release from HIT-T15 clonal β-cells compared with control. Values show mean ± SEM. $p < 0.05$ compared with (1.8g/L) glucose alone.

The escalating incidence of diabetes presents challenges for strict blood glycemic control and complications management. Peptides derived from frog skin, primarily identified for their antimicrobial activities, have been found to stimulate insulin release, showing potential for development into agents against diabetes (Conlon et al., 2018). In this study, antimicrobial peptides from Ranidae frog skin secretions showed the ability to induce insulin release from HIT-T15 cells. The HIT-T15 cell line, derived from a mouse insulinoma, is widely used to study insulin secretion mechanisms, especially in the context of diabetes and metabolic disorders. The interaction between antimicrobial exposure and insulin secretion in HIT-T15 cells can provide insights into how immune responses and microbial factors influence pancreatic function. The observed rise in insulin release at a specific concentration implies a

potential modulatory effect on pancreatic beta cells, possibly stimulating insulin secretion. However, these in vitro findings necessitate further exploration to unravel the molecular mechanisms behind the peptides' impact on insulin secretion pathways.

The interplay between antimicrobial exposure and insulin secretion in HIT-T15 cells is complex and multifaceted. Certain AMPs have been shown to modulate insulin secretion directly. For example, some AMPs may enhance insulin release by acting on specific receptors on beta cells, potentially influencing calcium dynamics and secretion pathways (Zhang et. al., 2021). Current research has identified compounds in frog secretions, also known as the frog skin peptides, that have the potential to prompt insulin secretion from pancreatic beta cells through specific receptors or signaling pathways (Wang et al., 2018; Long et al., 2018). Some AMPs were shown to have activity similar to sulfonylurea drugs in the modulation of the ATP-sensitive potassium channel (K-ATP channel), leading to insulin release (Musale et al.,2019). Based on the relatively high haemolytic effect of the AMPs observed in this study, it could be hypothesized that the peptides isolated from *P. baramica* may directly depolarize the plasma membrane or forming transient pores in the membrane, leading to calcium ions influx which ultimately results in insulin release.

Comprehensive studies on the primary and secondary structures of the AMPs isolated from these Bornean frogs will offer further understanding in their biochemical properties, which will enable comparative studies with other established AMPs, such as magainin, temporins and tigerinins. The high antimicrobial and insulinotropic activity observed in *P. baramica* secretions may suggest the presence of similarly structured peptides found in the former peptides.

HIT-T15 cells provide a valuable model for understanding insulin dynamics and enable the study on the impact of antimicrobial peptides which underscores the importance of

considering immune responses and inflammation in metabolic research. Further studies could elucidate specific mechanisms by which antimicrobial exposure affects beta-cell function, offering insights into diabetes management and potential therapeutic approaches, whereby these natural peptides hold promise as adjunctive agents for treating type 2 diabetes.

Conclusion

This comprehensive study provided an in-depth exploration into the rich reservoir of proteins and peptides residing within the skin secretions of ranid frogs. The treasure trove of bioactive compounds unearthed from this investigation showcased peptides with a remarkable dual functionality. These peptides demonstrated robust efficacy in combatting a wide spectrum of pathogens, underlining their potent antimicrobial properties. Intriguingly, they also hinted at a potential role in modulating insulin levels This revelation unveils a promising potential for these amphibian-derived peptides beyond their antimicrobial prowess. Understanding the relationship between AMPs and insulin secretion opens new avenues for therapeutic interventions. Enhancing AMP function or mimicking their activity could potentially lead to novel treatments for insulin resistance and type 2 diabetes. Moreover, manipulating the gut microbiome through AMPs might provide another strategy for improving metabolic health. However, despite these promising initial findings, the path forward requires a more nuanced understanding of the specific peptides responsible for these effects. Deeper exploration into the identities and intricate mechanisms of these peptides is indispensable. This pursuit holds the key to unlocking their full therapeutic potential, offering a transformative frontier in medicine. It not only promises to revolutionize approaches to combating infections but also holds the tantalizing prospect of pioneering novel treatments for disorders linked to insulin regulation, heralding a new era of medical innovation and targeted therapeutic interventions.

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