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

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ABSTRACT

Skin secretion of two Bornean frogs from the Ranidae family, *Pulcharana baramica* and *Hylarana erythraea* was evaluated for their antimicrobial and insulinotropic properties. A comprehensive approach, including various techniques was employed to characterise the Antimicrobial Peptides (AMPs) isolated from these frogs. Crude skin secretions from the frogs were partially purified using Sep-Pak C-18 cartridges, followed by further purification using the High-Performance Liquid Chromatography (HPLC) AKTA method. The peptide purity was assessed by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE), which revealed that the HPLC AKTA method produced clearer, cleaner bands, demonstrating its superior efficacy compared to Sep-Pak. The disk diffusion assay demonstrated significant zones of inhibition against both *Escherichia coli* and *Staphylococcus 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 demonstrated that the AMPs from *P. baramica* were more potent than those from *H. erythraea*, with an MIC of 125 µg/mL against *E. coli*. Purified AMPs from both Ranid frogs induced over 50% blood cell lysis in terms of cytotoxicity, suggesting that they may affect the integrity of mammalian erythrocyte membranes. This research further investigates the insulinotropic effect of AMPs from Ranidae frogs on HIT-T15 cells. AMPs from both frog species increased insulin secretion from 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 exhibit that these multifunctional peptides play an important role in Ranid frog defence against invading pathogenic microorganisms in their environment and may have future applications as an antidiabetic agent to manage glucose levels and diabetes.

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). Notably, 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 specialised 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). Specifically, it comprises an epidermis and a dermis, housing crucial components such as 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). Moreover, 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, increasing interest is being demonstrated in the research on various peptides isolated from frog skin secretions (Wang *et al*., 2016).

Traditional Chinese medicine such as *Venenum bufonis* utilised 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). Furthermore, 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 has demonstrated that Antimicrobial Peptides (AMPs) in frog skin secretions exhibit biological activities, including anticancer, antibacterial, and antidiabetic (Casciaro *et al*., 2020; Lin *et al*., 2021; Soltaninejad *et al*., 2021).

Additionally, a previous study reported that peptides from frog skin secretions can stimulate insulin release *in vitro* from BRIN-BD11 rat clonal β cells at low concentrations, with minimal cell toxicity (Owolabi *et al*., 2016). Remarkably, over 99% of antidiabetic peptides have been sourced from amphibian skin secretions with particular emphasis on species in the order Anura (Soltaninejad *et al*., 2021). Recent research has revealed that these peptides, known as AMPs, exhibit insulin-like properties and hold great promise for the treatment of diabetes (Musala *et al*., 2021). Therefore, this revelation has sparked interest in harnessing these peptides for innovative and sustainable diabetes therapies, especially given the approval of exenatide, a substance derived from lizard venom, for the treatment of type 2 diabetes (Coulter-Parkhill *et al*., 2021).

Initially recognised for their role in defending against bacteria, frog skin peptides have surprised researchers by also releasing insulin (Conlon *et al*., 2024). Various studies have indicated 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 (Ojo *et al*., 2013; Musale *et al*., 2019). Furthermore, this article extends its investigation to screen these AMPs for insulin-like activity using the HIT-T15 cell line. Employing various analytical techniques, including peptide isolation, purification, and molecular weight characterisation, this study aims to shed light on the potential of these frog-derived AMPs to influence insulin-related processes. Notably, 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 collection of skin secretions was performed using a non-invasive method that did not involve sacrificing frogs (Sabri *et al*., 2018). Individuals of two frog species, *Pulcharana baramica* and *Hylarana erythraea* were captured at night around the river areas within the natural forest reserves of Universiti Malaysia Sarawak East Campus. In this study, 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 performed using Sep-Pak C-18 cartridges according to the previously published method

(Sabri *et al*., 2018). Moreover, the partially purified peptides were further purified using the AKTA Pure 25 System (Chemopharm, Malaysia) with a Siliachrom C18 mono HPLC column. Bound peptides were eluted using 70% acetonitrile containing 0.1% Trifluoroacetic Acid (TFA). The size and purity of the peptides at each purification step were visualised 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, according to previously published methods (Schadich, 2013; Khademi *et al*., 2019).

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-Buffered 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 2,500 g, the absorbance was measured at 450 nm using the Enzyme-Linked Immunosorbent Assay (ELISA) plate reader.

Cell Culture

The HIT-T15 cells (CRL-1777) were obtained from the American Type Culture Collection (ATCC) in Manassas, USA. Subsequently, 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 subcultured using 0.25% trypsin and 2.65 mM Ethylenediaminetetraacetic Acid (EDTA), the medium was changed every two days to facilitate optimal growth and confluence.

Cytotoxicity Assay

Evaluation of the AMPs' effect on cell viability was performed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit from Sigma-Aldrich. AMP concentrations ranging from 1 µg/mL to 50 µg/mL were used in these assays. The HIT-T15 cells were exposed to the various peptide concentrations for 24 hours, followed by the addition of 1 mg/mL MTT solution and incubation for an additional two hours. Cell viability was assessed quantitatively by measuring absorbance at 570 nm using an ELISA plate reader.

Evaluation of Insulin Secretion in HIT-T15 Cells

HIT-T15 cells were cultured at a density of 5×10^4 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 $MgCl_2$, 7.0 g/L NaCl, 0.34 g/L KCl, 0.1 g/L Na_2HPO_4, 0.18 g/L NaH_2PO_4) supplemented with test samples for 30 minutes (Min *et al*., 2019). The conditioned media were collected and centrifuged at 12,000 rpm for 10 minutes. 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. Moreover, further purification of the secretion mixture successfully eliminated the larger protein bands, revealing sharper, cleaner bands at the bottom of the gels, corresponding to smaller peptides with molecular weights between 1 kDa and 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* demonstrated additional protein bands in the range of 6.5 kDa to 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, with two clear bands at the bottom of the gel corresponding to low-molecular-weight peptides. Thus, these findings align with previous studies indicating that AMPs from frog skin secretions typically have lower molecular masses, ranging from 1 kDa to 5 kDa (Sabri *et al*., 2018). Few examples of AMPs from Bornean frogs that have been characterised include the Brevinins (2.5 kDa – 3.0 kDa), Esculentins (3.0 kDa – 3.5 kDa), and Temporins (2.0 kDa – 2.5 kDa) (Conlon & Mechkarska, 2014; Ong *et al*., 2021).

AMPs from the Ranidae family were reported to have a conserved amino-terminal region and diverse carboxyl-terminal segments, which correspond to mature peptides (Conlon *et al*., 2024). Hence, this is reflected in the degree of similarity among AMPs from distantly related frog species. However, exposure to various environmental factors, as well as evolutionary or mutational changes in the pathogens in their surroundings has led to the diversification of AMPs in these anurans. These variations may also be due to genetic differences among individual frogs of the same species. Consequently, this is evidenced by previous findings indicating distinct protein profiles in the skin secretions of *H. erythraea* from different geographical locations (Zhang *et al*., 2018; Ong *et al*., 2021).

Additionally, common challenges in protein purification include low yields and poor purity. Protein yield in purification is influenced by a combination of protein-related factors, sample source quality, and methodological choices at each purification step. On the other hand, protein loss increases with the increasing number of steps involved in the purification process. In this study, the utilisation of a 2-step purification method helps to minimise protein loss during the process. Table 1 summarises the protein yield obtained during the purification of peptides from the frogs' skin secretion.

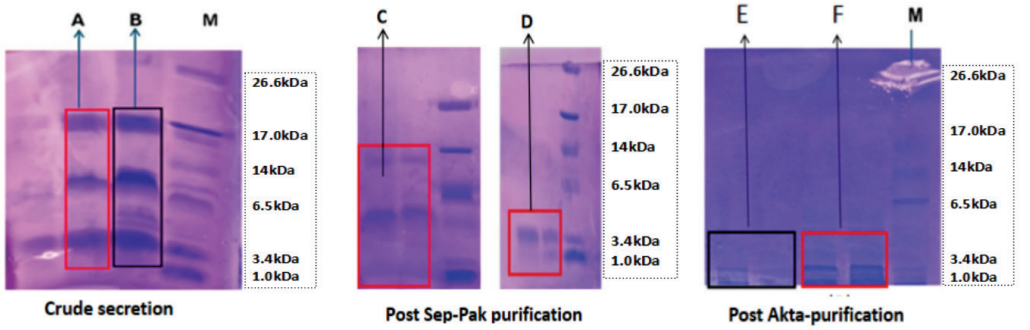

Figure 1: Coomassie blue Tricine SDS-PAGE gel of crude secretion, post Sep-Pak purification, and post-AKTA Pure purification AMPs from the skin secretion of Bornean Ranidae. Lane A: Crude secretion from *H. erythraea*; Lane B: Crude secretion from *P. baramica*; Lane C: Sep-Pak purified secretion from *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 kDa to 1.0 kDa

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 (%)**	**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 (%)**	**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 antimicrobial activity using the disc diffusion assay, followed by MIC determination against gram-positive and gram-negative bacteria. Notably, at a concentration of 1,000 µg/mL, *P. baramica* emerged as a remarkably potent antimicrobial agent, revealing 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 1,000 µg/mL) displayed moderate potency against *S. aureus*, a gram-positive bacterium, with inhibition zone diameters of approximately 5.6 mm and 4.6 mm, respectively. Thus, these findings highlight the differential antimicrobial effects of the peptides against the tested bacterial strains, emphasising *P. baramica*'s particularly robust activity against *E. coli* as compared to *H. erythraea*. Table 2 summarises the antimicrobial activity of the purified peptides against *E. coli* and *S. aureus*, as evidenced by the inhibition zones observed in the disc diffusion test.

The antimicrobial potency of the frog peptides was further demonstrated by determining 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. Among the tested frogs, peptides from *P. baramica* displayed the most potent antimicrobial activity against *E. coli*, with an MIC of 125 µg/mL. Additionally, the MIC values of peptides from both *P. baramica* and *H. erythraea* against *S. aureus* were determined to be 500 µg/mL.

Table 2: Inhibition zone diameter of AMPs 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

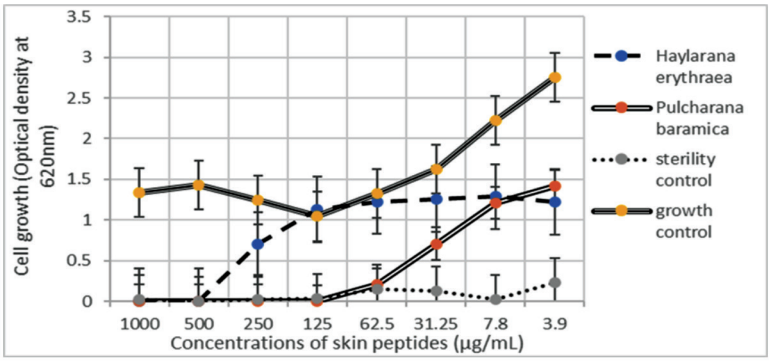

Figure 2: Growth inhibition of *E. coli* by purified peptides from the skin secretion of Bornean Ranidae. AMPs of *P. baramica* and *H. erythraea* at concentrations ranging from 3.9 µg/mL to 1,000 µg/mL were incubated with *E. coli* at 1×10^6 CFU/mL. After 24 hours, the bacterial growth was analysed 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 AMPs (Ladram *et al*., 2016). AMPs derived from Ranid frog skin secretions have exhibited significant activity against both gram-positive and gram-negative bacteria (Wang *et al*., 2016). Furthermore, 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 *P. baramica* and 500 µg/mL and 1,000 µg/mL for *H. erythraea*.

Table 3 summarises 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 indicates that these AMPs are more potent against gram-negative bacteria than against gram-positive bacteria. A similar finding was reported for *P. baramica* in a previous study (Sabri *et al*., 2018). These observations are probably due to several factors, including differences in cell wall structure between the two groups of bacteria. Specifically, gram-negative bacteria have an outer membrane composed of Lipopolysaccharides (LPS), which may serve as a primary target for AMPs. Studies have reported that an elevated negative charge on bacterial cell membranes may enhance the binding of cationic peptides in amphibian skin secretions through electrostatic interactions, potentially leading to bacterial death (Kumar *et al*., 2018; Farhana *et al*., 2022).

Overall, the AMPs isolated from the skin secretion of *P. baramica* demonstrate 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 components of their secondary structures and their biochemical properties. Notably, 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. Thus, further study of the structures and physicochemical properties of these peptides is warranted to elucidate their superior antimicrobial activity.

Table 3: Inhibition zone diameter of AMPs 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	1,000

To strengthen the evidence for the therapeutic potential of frogs' AMPs, incorporating clinically relevant resistant strains such as Methicillin-Resistant Staphylococcus Aureus (MRSA) and other multidrug-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, resulting in 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 a higher percentage of haemolysis when compared to those of *P. baramica* at concentrations of 50 µg/mL and below. At concentrations above 50 µg/mL, peptides from *P. baramica* demonstrated greater toxicity towards human red blood cells. Hence, this stark difference highlights the varying effects of peptides derived from different Ranidae species on mammalian cell membranes.

The finding parallels the antimicrobial activity of these two peptides, in which *P. baramica* indicated higher potency than *H. erythraea* against both gram-positive and gram-negative bacteria. Therefore, these observations suggest a dual nature: Potent defenders against pathogens but potentially harmful to red blood cells. It is widely acknowledged that AMPs isolated from frog secretions are toxic to mammalian cells (Conlon, 2017).

Although this study did not investigate the mechanism underlying red blood cell lysis upon AMP interaction, the current findings strongly support the idea that increased AMP toxicity is linked to their higher hydrophobicity and amphiphilic properties (Wei *et al*., 2022). Thus, additional research is necessary to understand these dual activities and optimise these peptides for medicinal applications while mitigating their adverse effects. A careful balance between optimising antimicrobial activity and reducing toxicity to host cells can be achieved through various methods such as structural modifications and the development of analogues.

Additionally, the cytotoxic and haemolytic properties of AMPs pose a significant challenge to their adaptation for medical use. To introduce these molecules as potential "natural antibiotics", several modifications are needed.

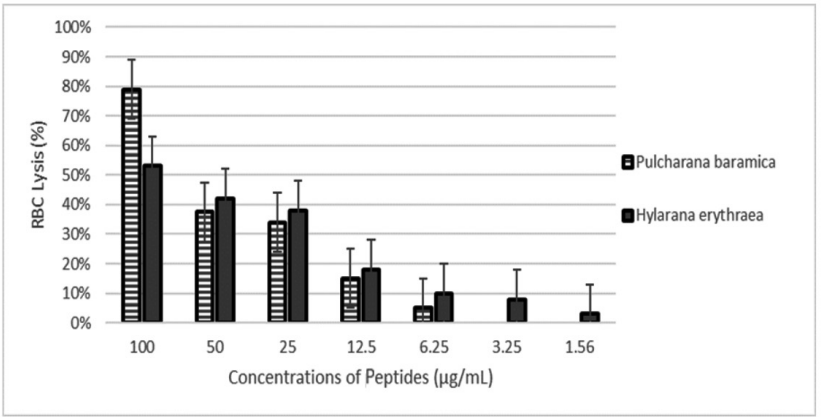

Figure 3: Hemolytic activity of purified peptides from frog skin secretions on human red blood cells. Bar charts demonstrated the percentage of red blood cell lysis, expressed as mean ± SD. The negative control (0% hemolysis) was red blood cells treated with PBS and the positive control (100% hemolysis) was red blood cells treated with 1% Tween 20. Values were taken as the mean absorbance at 450 nm

Previous research has reported success in reducing haemolytic activity while enhancing antimicrobial activity and plasma stability via amino acid sequence alteration, conjugation with methoxy-poly(ethylene glycol) (mPEG), and the development of peptide dimers (Wang *et al*., 2021). Notably, encapsulation in nanocarriers such as liposomes may also provide an alternative to reduce the cytotoxic effects of these AMPs (Figure 3).

Cell Viability Assay

A 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. Moreover, incubation of the cell with 50 µg/mL of purified peptides from *P. baramica* resulted in a 27% viability. A lower toxicity effect was observed with *H. erythraea* peptides at similar concentrations and reducing the concentration of the skin peptides increased cell viability, as presented 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% to 100% viability of HIT-T15 cells.

Nonetheless, apart from AMP concentration, cell viability may also be influenced by exposure duration and peptide structure. The 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, thereby increasing toxicity (Zhang *et al*., 2021).

Preliminary Evaluation of Insulin Secretion in HIT-T15 Cells

HIT-T15 cells were exposed to AMPs sourced from *P. baramica* and *H. erythraea*. Treatment with 5 µg/mL of *P. baramica*'s AMPs resulted in a significant dose-dependent elevation in insulin release ($p < 0.05$). Specifically, without exposure to AMP, under normal glucose conditions, the baseline insulin release rate was 10.5 ± 0.34 ng/105 cells/30 min. However, in the presence of 5 µg AMPs, this rate increased notably to 11.1 ± 0.37 ng/105 cells/30 min, as demonstrated in Figure 5 (a). Conversely, *H. erythraea*'s AMPs prompted a concentration-dependent surge in insulin secretion at 5 µg, as illustrated in Figure 5 (b). Nevertheless, this increase failed to reach statistical significance ($p > 0.05$), compared with basal insulin release in the presence of glucose alone.

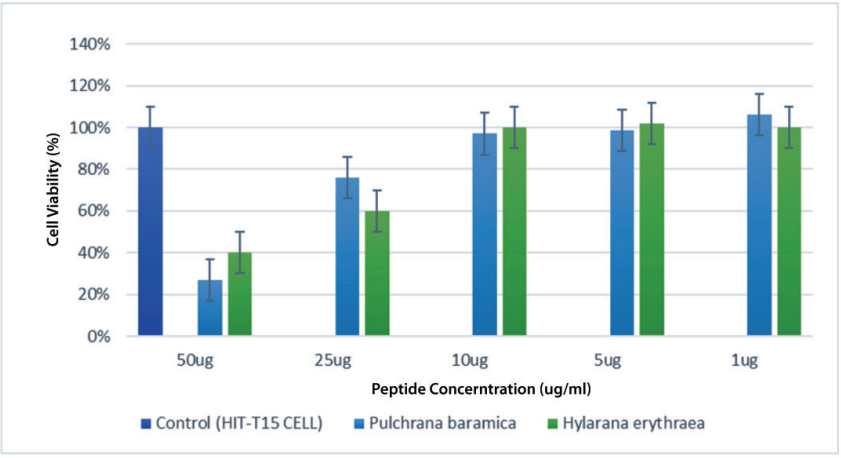

Figure 4: Effects of various concentrations of AMPs from *P. baramica* and *H. erythraea* on the viability of HIT-T15 cells

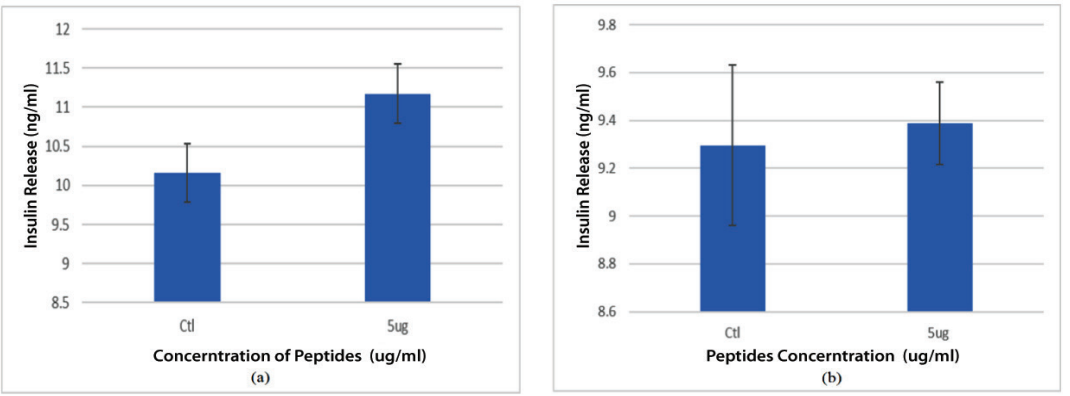

Figure 5: Effects of AMPs from (a) *P. baramica* and (b) *H. erythraea* on insulin release from HIT-T15 clonal β-cells compared with control. Values depict mean ± SEM. $p < 0.05$ compared with (1.8 g/L) glucose alone

The escalating incidence of diabetes presents challenges for strict blood glycemic control and complications management. Peptides derived from frog skin, primarily known for their antimicrobial activities have been identified to stimulate insulin release, suggesting potential as agents for the treatment of diabetes (Conlon *et al*., 2018). In this study, AMPs from Ranidae frog skin secretions were indicated 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.

Furthermore, 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 warrant further investigation to elucidate the molecular mechanisms underlying the peptides' effects on insulin secretion pathways.

Notably, the interplay between antimicrobial exposure and insulin secretion in HIT-T15 cells is complex and multifaceted. Certain AMPs have been demonstrated 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 frog skin peptides that have the potential to stimulate insulin secretion from pancreatic beta cells via specific receptors or signalling pathways (Wang *et al*., 2018; Long *et al*., 2018).

Some AMPs were indicated to have activity similar to sulfonylurea drugs in modulating the adenosine triphosphate, ATP-sensitive potassium channel (K-ATP channel), leading to insulin release (Musale *et al*., 2019). Additionally, given the relatively high haemolytic effect of the AMPs observed in this study, it could be hypothesised that the peptides isolated from *P. baramica* may directly depolarise the plasma membrane or form transient pores, leading to calcium influx and ultimately insulin release.

Conversely, comprehensive studies on the primary and secondary structures of the AMPs isolated from these Bornean frogs will provide further understanding of their biochemical properties, enabling 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 identified in the former peptides.

HIT-T15 cells provide a valuable model for understanding insulin dynamics and enable studies of the impact of AMPs, underscoring the importance of considering immune responses and inflammation in metabolic research. Therefore, further studies could elucidate specific mechanisms by which antimicrobial exposure affects beta-cell function, offering insights into diabetes management and potential therapeutic approaches. Consequently, these natural peptides hold promise as adjunctive agents for the treatment of type 2 diabetes.

Conclusions

This comprehensive study provided an in-depth exploration of the rich protein and peptide repertoire in the skin secretions of ranid frogs. The treasure trove of bioactive compounds unearthed by this investigation included peptides with remarkable dual functionality. As a result, these peptides demonstrated robust efficacy against a broad spectrum of pathogens, underscoring 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. Nonetheless, 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.

Conversely, manipulating the gut microbiome with AMPs could provide another strategy to improve metabolic health. However, despite these promising initial findings, the path forward requires a more nuanced understanding of the specific peptides responsible for these effects. A deeper exploration of the identities and intricate mechanisms of these peptides is indispensable. Thus, this pursuit holds the key to unlocking their full therapeutic potential, offering a transformative frontier in medicine. It promises to revolutionise the way infections are combatted. On the contrary, it also holds the tantalising 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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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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