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Protein Composition and Biomedical Potential of the Skin Secretion of *Hylarana erythraea* (Schlegel, 1837) (Anura: Ranidae) from Langkawi Archipelago, Kedah, Peninsular Malaysia

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Abstract
The skin secretion of amphibians is known for its high content of bioactive compounds. These bioactive compounds are essential for the advancement of biomedical industries. Four wild green paddy frogs, *Hylarana erythraea*, were collected from various habitat types within the Langkawi Archipelago. These frogs' skin secretions were collected, extracted, and analysed for their protein compounds together with biomedical potentials using liquid chromatography–mass spectrometry (LC–MS). The total protein concentration of *H. erythraea* skin secretions was determined as 0.269 mg/mL. Based on the UniProt (Anura) database, we identified 29 proteins. These proteins were categorised as antimicrobial (AMP) (38%), followed by hormone (17%), enzyme (17%), unreviewed proteins (17%), structural proteins (7%), and regulatory proteins (4%). The AMPs identified were from the family of esculentin-1, esculentin-2, brevinin-1, and frenatin-4, while the hormones belonged to the cholecystokinin group. The enzymes detected were adenylate cyclase 9, the suppressor of tumorigenicity 14 protein homolog, and the HGF activator. The structural proteins belonged to toe pad keratin 2 and Krt5.7 proteins, while the single regulatory protein is CCR4-NOT transcription complex subunit 6-like. These proteins have a wide range of biomedical importance, such as wound healings, facilitate digestions, anti-tumours, and anti-cancer effect.

Keywords Biomedical function · Frog · LC–MS/MS · Bioinformatics · Protein profile · Tropical

Introduction

Recently, Oliver et al. (2015) had re-delimited the frog species from the genus of *Hylarana*. Four frog species were identified from *Hylarana* Tschudi's, 1838, which are *H. erythraea*, *H. macrodactylus*, *H. taipehensis*, and *H. tytleri* (Frost 2021). *H. erythraea* and *H. macrodactyla* can be encountered in Peninsular Malaysia (Frost 2021). The typical green paddy frog, *H. erythraea*, was widely distributed in various types of water-bodied, such as streams, ditches, lakes, artificial ponds, and even in disturbed areas (Shahriza et al. 2011; Johana et al. 2016; Inger et al. 2017; Norhayati 2017; Dasi and Shahriza 2020). This medium-sized frogs' snout-vent length (SVL) ranges from 65 to 84 mm for females and 48–93 mm for males (Brown and Alcala 1970; Inger et al. 2017).

Hylarana erythraea is native to Cambodia, Laos, Indonesia, Brunei, Malaysia, Singapore, Thailand, Myanmar, Vietnam, central Nicrobar Islands, and India (Frost 2021). This species can be identified by their bright green dorsal skin, yellowish dorsolateral fold, broad dark stripe on the sides of the head and body, whitish upper lip, and ventral surface. Its toes are webbed to the toe disk's base (Berry 1975). *H. erythraea* is a commensal species. This species can thrive in various habitat types, making it vulnerable to a wide range of pathogens from the environment. Study on protein composition was done in many frog species, especially frogs from the family of Ranidae, since they have the most significant biomedical and pharmaceutical properties (Kumar et al. 2015; Conlon and Leprince 2009; Conlon et al. 2019). Data from protein profiling is vital for biomarkers, drug discovery, vaccine development, evolutionary histories and phylogenetic relationships (Barra and Simaco 1995; Yang and Huang 2007).

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To date, only three studies on skin secretions of frogs from the genus *Hylarana* had been done. Skin secretions of *H. taipenensis* from China are rich in antimicrobial and anti-oxidant properties (Guo et al. 2014). Zhang et al. (2018) documented a Bowman–Birk type chymotrypsin inhibitor peptide from the skin secretion of *H. erythraea*. This peptide showed anti-proliferation activity against multiple human cancer cell lines (Zhang et al. 2018). Al-Ghaferi et al. (2010) identified ten antimicrobial peptides (AMPs) of *H. erythraea* from Vietnam. It consists of four families, including brevinin-1, brevinin-2, esculentin-2, and temporin. These AMPs are effective against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and multidrug-resistant bacteria, *Acetinobacter baumannii*. In this study, the skin secretions of *H. erythraea* were extracted and analysed. Its protein profile and biomedical potentials were also determined.

Materials and Methods

Collection of Frog Skin Secretions

Four medium-sized adult *Hylarana erythraea* were collected from various habitat within Langkawi Island, Kedah, Peninsular Malaysia (Table 1). These habitats include a natural waterfall, paddy field, and artificial pond. The snout-vent length (SVL), tibia length (TL), head width (HW)), and weight was measured using a digital calliper and an electronic balance. The collected specimens were rinsed with distilled water to remove any contaminant from its body. Later, the frog was placed in a plastic bag, with 30–50 mL distilled water and 1% of phenylmethylsulfonyl fluoride (PMSF), depending on the individual's size (Ebran et al. 2000; Leroy et al. 2006; Patel and Brinchmann 2017).

The plastic bag, which consists of a frog, was tied up properly and shaken vigorously for 1 min. The solutions, which contain skin secretions, were collected and kept in a 50 mL falcon tube. The solutions were frozen immediately, held in an icebox and transported to Analytical Biochemistry Research Centre Laboratory at Universiti Sains Malaysia for further analysis. After measured, the alive frogs were released back to their original habitat.

Freeze Drying

The samples were frozen at − 80 °C for 24 h to ensure they were solid for the freeze-dry process. Para film was applied to close the falcon tube gap's opening and piercing with a needle for smaller holes. This step is to minimise loss of samples during suction from the vacuum. The temperature and pressure of the freeze-dried machine (Labconco) were set up at − 46 °C and 0.310 mBar, respectively. Skin secretions samples were freeze-dried for 48 h until they become powder or honeycomb form (dehydrated).

Protein Extraction

One milliliter (mL) of 40 mm Tris–HCl (pH 8.8) were added into one milligram (mg) of freeze-dried skin secretion sample. The samples were left for 20 min at room temperature with frequent vortex, centrifuged at 12,000×*g* for 30 min, and collected the supernatant for subsequent analysis (Shahriza et al. 2017; Kwan et al. 2015; Kwan and Ismail 2018).

Protein Quantification Assay

Total protein concentration for the skin secretion was carried out using Pierce assay. Five microliters (µL) of the samples (supernatants) were mixed up with 125 µL Pierce reagent (Thermo Scientific, USA) in a 96-well plate (triplicate) and incubated at room temperature for 10 min. A standard curve ranging from 0 to 2.0 mg/m; was prepared with Bovine Serum Albumin (BSA) and 40 mM Tris–HCl, pH 8.8, with the absorbance at 660 nm. The sample's total protein concentration was determined and averaged by comparing the absorbance value against the standard curve.

In-solution Protein Digestion

The in-solution digestion method was followed Demesa-Balderrama et al. (2016) and Proaño-Bolaños et al. (2017), with some modifications. 100 µL of each sample was re-solubilised in 100 µL of 6 M urea, 100 mM Tris–HCl buffer, pH 7.8. Five microliters (µL) of 200 mM DTT was added to each sample for the reduction process, and solutions were incubated at room temperature for an hour. Alkylating agent,

Table 1 Sampling location

No.	Locality	Coordinate		Elevation (a.s.l) (m)	Habitat type
		Latitude	Longitude		
1.	Temurun waterfall	6.43283	9.707200	351	Waterfall
2.	Telaga Tujuh waterfall	6.38322	99.67412	587	Waterfall
3.	Kampung Bohor Chempedak	6.35385	99.73215	20	Paddy field
4.	Jetty Kuah	6.30887	99.85408	2	Artificial pond

IAA (200 mM), was then added into the solutions and incubated for another hour at room temperature. 20 µL DTT was added to the samples and incubated for another hour to consume unreacted IAA. Dilution was done by adding 775 µL deionised water and reducing urea concentration (slightly 0.6 M). Later, 100 µL of trypsin solution was added to the samples and left for digestion at 37°C overnight (8 h) in the thermomixer. After incubation, the reaction in sample tubes was stopped by adjusting the pH to less than six by adding concentrated acetic acid. The digested sample was air-dried with a vacuumed concentrator till the volume decreases (almost dry).

LC–MS/MS Analysis

Before LC–MS/MS analysis, the sample was mixed with 50 µL of 0.1% formic acid (FA) in deionised water. The solution was then filtered using a 0.2 µm regenerated cellulose (RC) membrane syringe filter (Sartorius AG, Goettingen, Germany). Liquid Chromatography–Mass Spectrometry (LC–MS/MS) analysis was performed according to Kwan et al. (2015) with some modification. Orbitrap LC–MS/MS detector and Easy-nLC II (Thermo Scientific, USA) system, with a running buffer of 0.1% of FA in deionised water and 0.1% FA in 100% acetonitrile (ACN), were used to analyse the samples. The samples were loaded into Orbitrap for LC–MS/MS analysis, with a flow rate of 0.3 µL/min. Easy column C18 (10 cm, 0.75mm i.d., 3 µm; Thermo Scientific, San Jose, CA, USA) was used as an analytical column, and Easy column C18 (2 cm, 0.1mm i.d., 5 µm; Thermo Scientific, San Jose, CA, USA) was used as pre-column. The pre-column was equilibrated at flow rate of 3 µL/min for 15 µL, and the analytical column at a flow rate of 0.3 µL/min for 4 µL. The sample eluent was sprayed into the mass spectrometer at 220 °C (capillary temperature) and a 2.1 kV voltage source. A full-scan mass analysis from m/z 300 to 2000 was used to detect protein and peptides. At a resolving power of 60,000 (at m/z 400, FWHM; 1-s acquisition) with a data-dependent MS/MS analyses (ITMS). They were triggered by the eight most abundant ions from a parent mass list of predicted peptides, rejecting or unassigned charge states. The fragmentation technique used was the collision-induced dissociation (CID) with a collision energy of 35. Each sample was analysed three times and the data reported was a compilation of the three runs.

Proteins and Peptides Identification (De Novo Sequencing)

The identification of proteins and peptides was made using PEAKS Studio software (PEAKS), version 7.5 (Bioinformatics Solution, Waterloo Canada) under the Anura (order) taxon. This database was last updated in July 2020. From De Novo sequencing, peptide sequences were produced from raw data obtained from LCMS. The standard identification workflow includes the post-translation modification (PTM) parameter of oxidation (M) and carbomethylation (C). These peptide sequences will match with the protein identification from the database- Uniprot (Anura). At the same time, to increase the confidence level, including vital biomedical proteins. The false discovery rate (FDR) of protein and peptide detection was set to less than 0.1% to increase confidence. A significant score of − 10 lg P, greater than 30, was used for protein acceptance.

Results

Four adults of *H. erythraea* were collected from their natural habitat in Langkawi Archipelago, Kedah. One individual was collected at Temurun waterfall, one at Telaga Tujuh waterfall, one in the artificial pool at Jetty Kuah, and one in Kg. Bohor Chempedak paddy field. Based on the eight-point calibration curve, ranging from 0 to 2 mg/mL, the total protein concentration of *H. erythraea* skin secretion was 0.269 mg/mL. A total of 29 proteins were identified from the skin secretions of *H. erythraea*. The entire protein profile is shown in Table 2.

Out of the 29 proteins, AMPs represents the highest number. They contributed 11 peptides (38%), followed by 5 hormones (17%), 5 enzymes (17%), 5 unreviewed proteins (17%), 2 structural proteins (7%), and 1 regulatory protein (4%) (Fig. 1). The AMPs identified were from the family of esculentin-1 (1), esculentin-2 (5), brevinin-1 (4), and frenatin-4 (1). All the hormones belong to the cholecystokinin group. The enzymes are adenylate cyclase 9, suppressor of tumorigenicity, and HGF activator. The single regulatory protein is a CCR4-NOT transcription complex subunit 6-like, while the structural proteins belonged to toe pad keratin 2 and Krt5.7 proteins.

Discussion

Hylarana erythraea can live in various habitats, including natural water bodies (waterfalls, streams) and artificial environments (paddy fields, ditches). Hence, they expose their naked skin to numerous types of pathogens. For protection, their skin's slimy skin produced secretions that act as a defence mechanism towards the environment's various threats. These skin secretions consist of different bioactive compounds, including proteins and peptides with various pharmacological effects (Stebbins and Cohen 1995). Some of the species possess secretions that contain 'super glue' like substance, which can be used to immobilise a predator (Evans and Brodie 1994). The secretions make the

Table 2 List of proteins detected in *H. erythraea* skin secretions

Accession	Protein name	Protein functional category	– 10 lg P	Coverage (%)	#Number of peptides detected	#Unique peptide	Description
ES2L_RANLU	Esculentin-2 L	AMP	78.03	24	1	1	Esculentin-2 L OS = *Rana luteiventris* PE = 1 SV = 1
J9R6K8_RANBO	Esculentin-2BY	AMP	78.03	12	1	1	Esculentin-2BY OS = *Rana boylii* PE = 2 SV = 1
B9W1Q0_9NEOB	Esculentin-2LTa	AMP	78.03	12	1	1	Esculentin-2LTa antimicrobial peptide OS = *Sylvirana latouchii* PE = 2 SV = 1
J9RMY3_9NEOB	Esculentin-2DR	AMP	78.03	12	1	1	Esculentin-2DR OS = *Rana draytonii* PE = 2 SV = 1
E2SN1_SYLSP	Esculentin-2SN1	AMP	74.04	12	1	1	Esculentin-2SN1 OS = *Sylvirana spinulosa* OX = 369,515 PE = 1 SV = 1
M9YMQ0_9NEOB	Toe pad keratin 2	Structural	60.83	3	1	1	Toe pad keratin 2 OS = *Hyla cinerea* PE = 2 SV = 1
A2BDA9_XENLA	LOC100037087 protein	unreviewed	60.83	3	1	1	LOC100037087 protein OS = *Xenopus laevis* GN = LOC100037087 PE = 2 SV = 1
E7EKK5_9NEOB	Brevinin-1TR5	AMP	53.46	13	2	2	Brevinin-1TR5 antimicrobial peptide OS = *Amolops torrentis* PE = 2 SV = 1
FRE4_NYCIN	Frenatin-4	AMP	42.79	29	1	1	Frenatin-4 OS = *Nyctimystes infrafrenatus* OX = 61,195 PE = 1 SV = 1
A7XZR7_SYLVI	Cholecystokinin 3	Hormone	42.28	9	1	1	Cholecystokinin 3 OS = *Sylvirana nigrovittata* OX = 127,021 PE = 2 SV = 1
A7XZR5_SYLVI	Cholecystokinin 2	Hormone	42.28	9	1	1	Cholecystokinin 2 OS = *Sylvirana nigrovittata* OX = 127,021 PE = 2 SV = 1
A7XZR3_SYLVI	Cholecystokinin 1	Hormone	42.28	9	1	1	Cholecystokinin 1 OS = *Sylvirana nigrovittata* OX = 127,021 PE = 2 SV = 1
A7XZR9_SYLVI	Cholecystokinin 4	Hormone	42.28	9	1	1	Cholecystokinin 4 OS = *Sylvirana nigrovittata*OX = 127,021 PE = 2 SV = 1
CCKN_LITCT	Cholecystokinin	Hormone	42.28	9	1	1	Cholecystokinin OS = *Lithobates catesbeiana* GN = CCK PE = 1 SV = 2
A0A1L8FL12_XENLA	Uncharacterised protein	unreviewed	40.14	1	1	1	Uncharacterized protein (Fragment) OS = *Xenopus laevis* OX = 8355 GN = XELAEV_18035255mg PE = 1 SV = 1
Q6GR54_XENLA	Suppressor of tumorigenicity 14 protein homolog	Enzyme	40.14	1	1	1	Suppressor of tumorigenicity 14 protein homolog OS = *Xenopus laevis* GN = st14 PE = 2 SV = 1
Q9DGR1_XENLA	Suppressor of tumorigenicity 14 protein homolog	Enzyme	40.14	1	1	1	Suppressor of tumorigenicity 14 protein homolog OS = *Xenopus laevis* GN = XMT-SP1 PE = 2 SV = 1
Q63ZQ6_XENLA	Suppressor of tumorigenicity 14 protein homolog	Enzyme	40.14	1	1	1	Suppressor of tumorigenicity 14 protein homolog OS = *Xenopus laevis* GN = st14 PE = 2 SV = 1
A0A1L8FFQ3_XENLA	Uncharacterised protein	unreviewed	40.14	1	1	1	Uncharacterized protein OS = *Xenopus laevis* OX = 8355 GN = XELAEV_18037322mg PE = 4 SV = 1
A0A1L8HKX6_XENLA	Uncharacterised protein	unreviewed	40.14	1	1	1	Uncharacterized protein OS = *Xenopus laevis* OX = 8355 GN = XELAEV_18008892mg PE = 4 SV = 1
A0A1L8HT91_XENLA	Uncharacterised protein	unreviewed	40.14	1	1	1	Uncharacterized protein OS = *Xenopus laevis* OX = 8355 GN = hgfac.L PE = 4 SV = 1

Table 2 (continued)

Accession	Protein name	Protein functional category	– 10 lg P	Coverage (%)	#Number of peptides detected	#Unique peptide	Description
A0A6I8QCX8_XENTR	HGF activator	Enzyme	40.14	1	1	1	HGF activator OS = *Xenopus tropicalis* OX = 8364 GN = hgfac PE = 4 SV = 1
F7CEX4_XENTR	Adenylate cyclase 9	Enzyme	40.14	1	1	1	Adenylate cyclase 9 OS = *Xenopus tropicalis* OX = 8364 GN = hgfac PE = 4 SV = 2
E7EKG4_9NEOB	Esculentin-1TP1	AMP	39.72	10	1	1	Esculentin-1TP1 antimicrobial peptide OS = *Hylarana taipehensis* PE = 2 SV = 1
E7EKF8_9NEOB	Brevinin-1TP4	AMP	33.18	10	1	1	Brevinin-1TP4 antimicrobial peptide OS = *Hylarana taipehensis* PE = 2 SV = 1
A0A6B7HYK8_ODOSH	Brevinin-1OS	AMP	33.18	10	1	1	Brevinin-1OS OS = *Odorrana schmackeri* OX = 110,116 PE = 2 SV = 1
E7EKG0_9NEOB	Brevinin-1TP3	AMP	33.18	10	1	1	Brevinin-1TP3 antimicrobial peptide OS = *Hylarana taipehensis* PE = 2 SV = 1
F6UYZ5_XENTR	CCR4-NOT transcriptioncomplex subunit 6-like	Regulatory	28.21	2	1	1	CCR4-NOT transcription complex subunit 6-like OS = *Xenopus tropicalis* OX = 8364 GN = cnot6l PE = 3 SV = 2
B1H1E8_XENTR	Krt5.7 protein (keratin)	Structural	28.21	2	1	1	Krt5.7 protein (Fragment) OS = *Xenopus tropicalis* GN = krt5.7 PE = 2 SV = 1

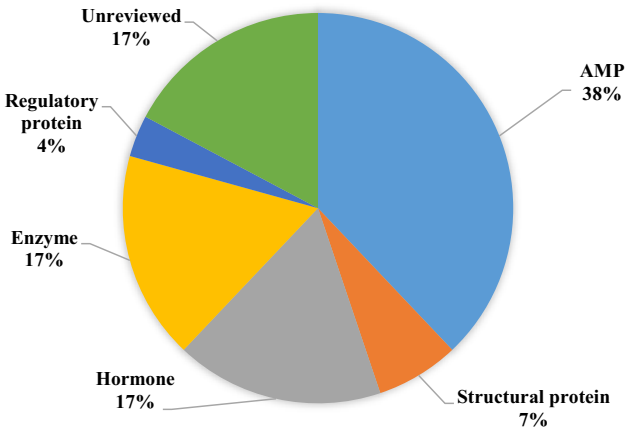

Fig. 1 Protein composition of *H. erythraea* skin secretions

amphibians unpalatable and toxic to their predator (Toledo and Jared 1995). Other than a defence mechanism, the skin secretions also retain moisture for the frog skin.

Various protein types were identified from this study, such as unreviewed, AMP, structural protein, regulatory protein, hormone, and enzymes. In this study, we did a comprehensive research on the protein profile of *H. erythraea* skin secretion from Langkawi Island. However, Al- Ghaferi et al. (2010) only identified and characterised the AMP of *H. erythraea* from Vietnam. All the AMPs described in Al-Ghaferi et al. (2010) were also identified from this study with the addition of frenatin. At the same time, Zhang et al. (2018) revealed the presence of Bowman–Birk type chymotrypsin inhibitor peptide. Unfortunately, this peptide is absent from our study.

Brevinin-2 and temporin are absent from this study because *H. erythraea* from this study was collected in Langkawi's main island, while Al-Ghaferi et al. (2010) collected them in Vietnam. Brevinin-2, temporin, and Bowman–Birk type chymotrypsin inhibitor peptide were absent from this study. This is because frog individuals among these studies were acquired from different localities. For example, Al-Ghaferi et al. (2010) collected *H. erythraea* from Vietnam, whereas in this study, this species was collected in Langkawi Archipelago, Peninsular Malaysia. Therefore, there may be a high genetic difference between these two *H. erythraea* groups from individuals' genetic drift and isolation due to biogeography speciation. For example, Margres et al. (2016) revealed that snake toxins identified vary between mainland and island population. These variations were due to adaptation towards evolutionary selection and slightly altered the individuals' behaviour. Brodin et al. (2013) suggested that the behavioural differences might be influenced by the disperser-dependent founder effect from the isolation-driven environmental filtering between mainland and island populations (Brodin et al. 2013).

The main reason for the absence of Bowman–Birk type chymotrypsin inhibitor peptide identified from Zhang et al. (2018)'s study is that they use a molecular cloning method (genomics) instead of the usual skin secretion protein purification (proteomics). Genomics studies the entire sets of

genes in the genome from a cell, while proteomics focuses on proteins produced by the cell. An individual's genome is constant, whereas proteome is different depending on the external environment and cell processes. Therefore, the protein secreted by frogs of varying environments will be different due to their adaptation.

Amphibian skin secretions possess various types of bioactive compounds. Bioactive compounds characterised from amphibian skins exert multiple functions, including defensive, enzymatic, regulatory, and hormonal (Zhang 2006; Lu et al. 2010; Jiang et al. 2015; Xu and Lai 2015). These bioactive compounds are also a potent therapeutic property against multiple pathophysiological conditions. From this study, AMPs from esculentin-1, esculentin-2, brevinin-2 and frenatin family were identified.

AMP has the highest constituent in the protein composition. AMPs possess a wide range of antimicrobial activities, and contain various biomedical properties (Conlon et al. 2019). For example, esculentin-2 L isolated from North American *Rana luteiventris* is positive against bacteria (gram-positive and gram-negative), fungal, and Chytrid fungus (Goraya et al. 2000; Rollins-Smith et al. 2002). AMP identified by Al-Ghaferi et al. (2010) revealed from *H. erythraea* skin secretion is effective against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and even multidrug-resistant *Acetinobacter baumannii*.

Boparai and Sharma (2020) had reviewed that AMPs derived from amphibian skin are potent in the treatment of local infections caused by multi-drug resistant strains of bacteria. These bacteria include *Candida* sp., *Acinetobacter baumannii* strains, *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* (Migoń et al. 2018; Ong et al. 2021). The current application and potential therapeutic applications of Brevinin had been compiled in Zohrab et al. (2019). Besides antimicrobial properties, Brevinin peptides also promote wound healing, stimulate insulin secretion, and cause dendritic cell maturation (Zohrab et al. 2019).

A significant high uncharacterised protein had been identified from the protein content of these frog species. These findings are expected in species with less research done, resulting in low database entries for these species groups. The information for these species groups is still insufficient because not many studies had been done in Malaysia; only a few species had been done in this group. According to Lubec et al. (2005), uncharacterised protein refers to proteins with high unreviewed protein detection within this sample. This was due to the proteins identified having an unknown and undetectable homology to their known function. These proteins' functions were evident in both the structure and the sequence level (Lubec et al. 2005). Therefore, there are possibilities that they may contribute to biomedical advancement.

The hormones identified were from the cholecystokinin (CCK) family. These hormones are essential as neurotransmitters, intestinal hormones responsible for stimulating fat, and proteins (Rourke et al. 1997; Rehfeld 2017). Various researches had documented that they are vital for human health. Besides facilitating digestion, these proteins function as anti-inflammation and maintain homeostasis by lowering blood pressure and having anti-cancerous properties (Holte et al. 1996; Stewart et al. 2002; Stewart 2003; Wang et al. 2017). CCK also shows a hunger-suppressive effect on mammals (Peikin 1989; Fink et al. 1998). Recent research by Pathak et al. (2018) had proven that CCK is effective in treating obesity and type 2 diabetes.

Enzymes such as adenylate cyclase 9 (AC9), suppressor of tumorigenicity 14 protein homolog, and HGF activator were identified in the skin secretions of *H. erythraea*. AC9 catalyses cyclic AMP formation from ATP, essential in regulation in all cells (Hancock 2017). According to Li et al. (2017), this enzyme's deficiency and loss reduce phosphorylation of Hsp20 and diastolic dysfunction. Moreover, Antoni (2020) highlighted the promising opportunities of AC9 in the treatment of cancer, metabolic syndrome, and autoimmune disorders.

Numerous studies on the suppressor of tumorigenicity 14 proteins and HGF activator proved that these proteins have anti-tumours and anti-cancerous effect (Wang et al. 2009; Kawaguchi and Kataoka 2014; Zheng et al. 2014 ; Owusu et al. 2017; Cotterill 2019). For example, Kosa et al. (2012) reported that this protein is essential in suppressing colitis and colitis-associated colon carcinogenesis. Additionally, the HGF activator also possesses wound healing properties (Conway et al. 2006), used in corneal wound healing treatment (Carrington and Boulton 2005; Miyagi et al. 2018).

The regulatory protein, CCR4-NOT transcription complex subunit 6-like, is vital for many cellular processes, such as transcriptional regulation, mRNA degradation, and miRNA-mediated repression (Geer et al. 2010; Inada and Makino 2014). This protein also allows the efficient and fast adaptation of cellular gene expression in response to environmental conditions and stimulus (Chapat an Corbo 2014). In contrast, the structural protein of the Krt5.7 protein (keratin) plays a role in mechanical stability and regulatory functions. This protein type is essential for wound healing (Kelly 2016; Loan et al. 2016).

Conclusions

Hylarana erythraea, known as a typical green paddy frog, can be found in various freshwater habitats, including natural and artificial habitats. The skin secretions of this frog composed of multiple proteins and peptides, valuable to biomedical and pharmaceutical fields. From this study, a

total of 29 proteins were identified. These included AMP, regulatory proteins, structural proteins, enzymes, hormones, and unreviewed proteins. Eleven AMPs from four families (esculentin-1, esculentin-2, brevinin-1 and frenatin-4) were detected in this work. These proteins have numerous biomedical importance, such as wound healings, facilitating digestions, treating obesity, anti-type 2 diabetes, anti-tumours, and anti-cancerous effect. However, to confirm these biomedical properties, both in-vivo and in-vitro studies are required.

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Author Contributions DO drafted the work, work on the acquisition, analysis, and interpretation of data. SS revised it critically for important intellectual content, approved the version to be published. MNI revised it critically for important intellectual content, made substantial contributions to the conception or design of the work. All agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data Availability All the data and materials comply with field standards.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All applicable institutional guidelines for the care and use of animals were followed. The permission on animal ethical usage was approved by Universiti Sains Malaysia, Institutional Animal Care and Use Committee (IACUC), USM/IACUC/2019/(121)(1039).

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