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CHARACTERISATION OF CRUDE AND PARTIALLY PURIFIED PEPTIDES WITH ANTIMICROBIAL ACTIVITY FROM THE SKIN OF BORNEAN FROGS

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Abstract: Antimicrobial peptides are one of the most promising antibiotic candidates with the effectiveness in killing the microorganisms, can be found largely in the frog skin. In this study, the antimicrobial properties of the crude and partially purified peptides from the frog skin of Bornean frogs; *Chalcorana raniceps, Limnonectes kuhlii, Meristogenys jerboa, Odorrana hosii, Staurois guttatus* and *Limnonectes leporinus* were determined. Crude peptides from the skins of these frogs were partially purified using C18 Sep Pak columns. The antimicrobial activities tested were disc diffusion, minimum inhibitory concentration and minimum bactericidal concentration test. *L. leporinus* and *L. kuhlii* peptides displayed the lowest MIC value against *P. aeruginosa* (62.5 µg/mL) and *S. typhimurium* (125 µg/mL). Moreover, *L. leporinus* peptide showed the lowest MIC value against *S. aureus* (31.25 µg/mL). Both *L. kuhlii* and *L. leporinus* peptides share the lowest MBC value of 125 µg/mL against *S. aureus* and *P. aeruginosa.* Peptides from *L. kuhlii* exhibited the lowest MBC values against MRSA (125 µg/mL), *E. coli* (62.5 µg/mL) and *S. typhimurium* (125 µg/mL). It can be concluded that all extracted skin peptides have antimicrobial activity against the selected bacteria, with the skin peptides from *L. kuhlii* and *L. leporinus* frogs being more potent than other species studied. The antimicrobial characteristics of peptide samples imply that there is a potential of novel AMPs from the frog species of Borneo. For future study, the peptides of frog skin extracts should be further purified.

Keywords: antimicrobial peptides, antimicrobial activities, anuran, bacteria, partially purify

Introduction

Extensive research has been conducted in order to find new antimicrobial agents with novel modes of action and effective in killing target microorganisms (Maróti *et al.,* 2011). Among all, antimicrobial peptides (AMPs) are the new drug candidates with promising structure and variety of function (Mahlapuu *et al.,* 2016). The AMPs are unlikely to promote the emergence of the resistant microorganisms when the peptides have several different mode of actions in affecting prokaryotic cell death (Maróti *et al.,* 2011). In contrast, traditional antimicrobials normally focus on metabolic enzymes thus resulting in resistance among the microorganisms (Sang & Blecha, 2008). Moreover, traditional antimicrobials are normally targeted against bacteria and fungi, in contrast to AMPs which have more usage against many types of microorganisms such as bacteria, fungi, parasites, viruses and even a few types of cancer cells (Sang & Blecha, 2008). Many studies have been conducted globally to find new AMP molecules from frogs (Zhang *et al.,* 2013; Kim *et al.,* 2000; Mashreghi *et al.,* 2013; Dourado *et al.,* 2007). Generally, frog AMPs are relatively small (<10 kDa) (Zare-Zardini *et al.,* 2013) with typically 15−40 amino acid residues in length (Kang & Park, 2014). The AMPs possess amphipathic structure with hydrophobic and hydrophilic residues. Furthermore, the AMPs have an overall charge of +2 and +9, characterized by lysine and arginine positively charged amino acids (Pushpanathan *et al.,* 2013). The peptide consists at least 50% hydrophobic amino acids with a large number of leucine and isoleucine (Conlon & Sonnevend, 2011).

There are more than 180 frog species in Borneo, which is in East Malaysia, Brunei and Kalimantan (Inger *et al.,* 2017). Thus, the wide species diversity may reflect a high potential of new AMPs to be discovered. This has called for the effort to investigate the peptides with potential as antimicrobial among the anuran species of Borneo. As there is a need for alternatives of antimicrobials from other sources, the AMPs from the frog skin give promise in improving human health by treating the infected patients or any other diseases that are related to microorganism infections, without the risk of developing resistance against the AMPs. The aim of our present study was to evaluate the potency of crude and partially purified peptides with antimicrobial activities from skins of frogs found in Borneo.

Methodology

Frog skin extraction

All experiments with live animals were approved by the Animal Ethics Committee, UNIMAS and Sarawak Biodiversity Centre. Frog samples of *Chalcorana raniceps (C. raniceps), Limnonectes kuhlii (L. kuhlii), Meristogenys jerboa (M. jerboa), Odorrana hosii (O. hosii), Staurois guttatus (S. guttatus)* and *Limnonectes leporinus (L. leporinus)* were collected from Kubah National Park and Ranchan Recreation Park, in Kuching, Sarawak. The method used for the frog skin extraction was according to Conlon (2007). The frog was euthanized by an injection of absolute ethanol into its heart. The complete skin was immediately removed, weighted and frozen at 0°C. A mixture of ethanol/0.7 M HCl (3:1 v/v) was added into the frozen skin and homogenized for 10 minutes. The homogenate was stirred for one hour at approximately 0°C and centrifuged at 3000 × g for 20 minutes at 0°C. The ethanol contained in the homogenate was then evaporated and was further centrifuged using at 3000 × g for 20 minutes at 0°C. Equal volume of 0.1% TFA solution was added into the sample before being stored in a 4°C fridge overnight.

Partial purification of frog skin extraction

Partial purification of the frog skin extraction was performed according to a published method by Conlon and Sonnevend (2010). Solvent A (1.2 mL TFA to 1000 mL water) and solvent B (1.0 mL TFA to 300 mL water to 700 mL acetonitrile) were prepared. 100% acetonitrile (2mL per cartridge) were injected manually through eight series of Sep-Pak cartridges. The sample supernatant was loaded onto the Sep-Pak column using plastic syringe. Solvent A was then pumped into the assembles (4mL per cartridge) at a flow rate of 4mL per minute. Subsequently, solvent B was pumped (2mL per cartridge) at a flow rate of 1mL per minute and the eluate was then collected in a tube. The sample was then stored at −20°C until further use.

Tricine-Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

In order to evaluate the purity of the peptides in each sample, Tricine-SDS PAGE was performed. Gel electrophoresis was conducted based on the published method by Jiang *et al.,* (2016). Three different gels were used in each electrophoresis, which were the 2.5 cm stacking gel, (4% T, 3% C), 2 cm spacer gel (10% T, 3% C) and 4 cm separating gel (16.6% T, 3% C). The protein bands were visualized using silver staining according to the protocols by Hashemitabar *et al.* (2014).

Antimicrobial assays

Disk diffusion test was conducted according to Romainor *et al.* (2014). The experiment was conducted against five different bacteria and one fungus, which were *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Salmonella typhimurium* (ATCC 14028) *(S. typhimurium), Pseudomonas aeruginosa* (ATCC 27853) *(P. aeruginosa),* Methicillin resistant *staphylococcus aureus* (MRSA) *and Candida albicans (C. albicans). S. aureus, MRSA, E. coli* and *C. albicans* were the clinical strains obtained from Sarawak General Hospital,

Kuching. Meanwhile, *S. typhimurium* (ATCC 14028) and *P. aeruginosa* (ATCC 27853) were received from the Faculty of Medicine and Health Science, UNIMAS. The pathogens were cultured in the petri dish containing Mueller Hinton Agar (MHA) and incubated for 18-24 hours at 37°C. Twenty microliters of this peptide sample was transferred to the positioned disk on agar in the petri dish. The petri dishes were incubated at 37°C (18-24 hours). As a positive control, antibiotic tetracycline was used. After 18-24 hours, the diameter of zone inhibition was measured using a caliper.

Broth microdilution method was conducted according to Wiegand *et al.* (2008), by incubating peptide samples in Mueller Hinton broth, 50 µL, with an inoculum, 50 µL of 10^6 colony forming units of *E. coli, S. aureus, S. typhimurium* (ATCC 14028), *P. aeruginosa* (ATCC 27853) and MRSA in 96 well microtiter cell culture plates for 18-24 hours at 37°C. Incubations were carried out in parallel with increasing concentration of tetracycline. After incubation, the absorbance value of each well at 620 nm was determined using a microtiter plate. Minimum inhibitory concentration (MIC) was taken as the lowest concentration of peptide sample with no visible growth observed.

Minimum bactericidal concentration (MBC) test was undertaken to determine the minimum concentration of the frog antimicrobial peptides needed to kill the microorganisms. The test was conducted according to Romainor *et al.* (2014). Twenty microliter mixture of peptide samples and inoculum was taken out from 96-well microtiter cell culture plates with no turbidity observed. The mixture were spread onto MHA on a petri dish. The petri dish was then incubated for 18-24 hours at 37°C. After the incubation period, the petri dish was observed for the presence of bacterial colonies. MBC was determined by the lowest concentration of serial dilutions that gives no colony growth after the period of incubation.

Data analysis

In the disk diffusion test, each data point for the inhibition zone diameter represents the mean ± standard deviation of three replicates. Statistical analysis was conducted for the inhibition zone diameter data. The significance of the difference between the means of each group was assessed using Mann-Whitney non-parametric test. The confidence limit for significance was 0.05. Statistical analyses were performed using the SPSS- 17 (SPSS Inc, Chicago, IL, USA).

Results

Electrophoretic profile of the partially purified frog skin peptides

Figure 1 shows all the frog skin extracts contain peptides with 6.5 kDa in size and above. Peptide bands at approximately 14.2 kDa were also detected in all species samples. Furthermore, the profile shows that three out of six frog species possess peptide bands lower than 3.5 kD, as displayed in round box, which are the *C. raniceps, O. hosii* and *M. jerboa.*

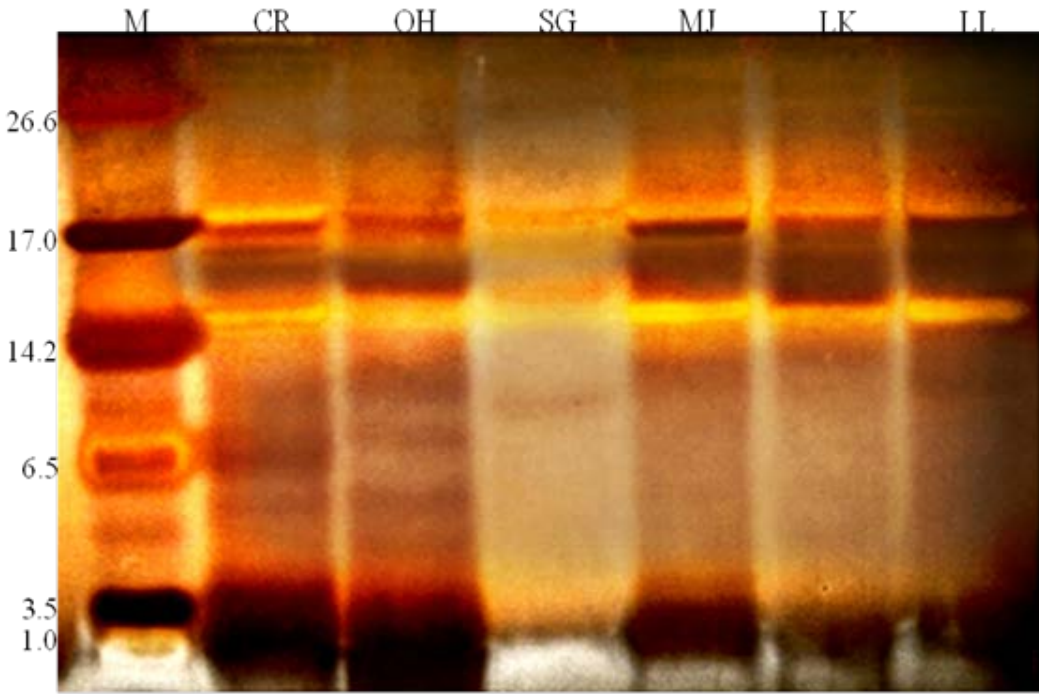

Figure 1: Protein profiling of the partially purified frog peptides by SDS PAGE. Lane (M) denotes protein markers, (CR) denotes *C. raniceps,* lane (OH) denotes *O. hosii,* lane (SG) denotes *S. guttatus,* lane (MJ) denotes *M. jerboa,* lane (LK) denotes *L. kuhlii* and lane (LL) denotes *L. leporinus*. 500 µg/mL of frog peptide samples were loaded into each well. As displayed in round box, CR, OH and MJ lane show peptide bands of approximately 1-3.5 kDa

Antimicrobial assays

Based on the diameter of the inhibition zones, it was observed that the crude and the partially purified peptides demonstrated bacterial growth inhibition against most of the tested bacteria, as shown in Table 1. No inhibition of the fungus growth was detected by all crude peptides and partially purified peptides from any of the six collected Bornean frog skin in disk diffusion test. Hence, further antimicrobial assays to determine the MIC and the MBC against the *C. albicans* was discontinued as the disk diffusion assay employed as the screening tools for the antimicrobial activity of the peptide.

Based on the diameter of the inhibition zones, it was observed that the crude and the partially purified peptides demonstrated bacterial growth inhibition against most of the tested bacteria, as shown in Table 1. The range of inhibition zone sizes were from 6.27 mm to 8.92 mm against *S. aureus,* 6.06 mm to 9.39 mm against MRSA, 8.26 mm to 9.64 mm against *E. coli,* 9.35 mm to 10.76 mm against *P. aeruginosa* and 8.30 mm to 9.58 mm against the *S. typhimurium.* Overall, the diameter of inhibition zone of Gram-negative bacteria; *E. coli, P. aeruginosa* and *S. typhimurium* were larger than that of Gram-positive bacteria; *S. aureus* and MRSA, indicating Gram-negative bacteria are more susceptible to the peptide samples compared to the Gram-positive bacteria.

Partially purified peptides from the skin of *L. kuhlii* are shown to display larger inhibition zone against the MRSA and *P. aeruginosa* growth as compared to the crude peptides. Moreover, partially purified peptides from *M. jerboa* skin exhibited larger inhibition zone

against the MRSA compared to the crude peptides. Apart from that, partially purified peptides from *L. leporinus* also showed bigger inhibition zone against the MRSA and *E. coli* compared to its crude peptides.

However, crude peptides from the skin of *C. raniceps* exhibited larger inhibition zone against Gram-positive MRSA and Gram-negative *S. typhimurium* as compared to the inhibition zones of the partially purified peptides. The same applies to *L. leporinus* crude peptides, which has larger inhibition zone against Gram-positive *S. aureus* and MRSA, and Gram-negative *E. coli* compared to its partially purified peptide samples.

From Table 2, broth microdilution shows that all crude and partially purified peptide samples could inhibit the tested bacterial growth. Peptide crude extraction of *L. leporinus* was shown to have the lowest MIC value against *S. aureus* (31.25 µg/mL), MRSA (62.5 µg/mL). A similar result was observed with peptide crude extraction from *L. kuhlii* and *L. leporinus* against *P. aeruginosa* (62.5 µg/mL) and *S. typhimurium* (125 µg/mL). Meanwhile, partially purified peptide extraction of *S. guttatus* was shown to have the lowest MIC value against *E. coli* (31.25 µg/mL) indicating the efficacy of the peptides in inhibiting the *E. coli* growth.

On the other hand, there are frog species with crude peptides that showed lesser MIC values for against bacteria as compared to their partially purified peptides. These include the peptides from *L. kuhlii* against *P. aeruginosa* and *S. typhimurium, M. jerboa* against *P. aeruginosa, O. hosii* against MRSA and *P. aeruginosa, S. guttatus* against *S. aureus* and *L. leporinus* against *S. aureus,* MRSA, *P. aeruginosa* and *S. typhimurium.* In contrast, the partially purified peptides from frog species exhibited low MIC values as compared to their crude peptides, and these include *C. raniceps* against MRSA and *E. coli, L. kuhlii against E. coli, M. jerboa* against *S. aureus,* and *S. guttatus* against *E. coli.*

Table 3 summarizes the MBC values of crude and partially purified peptides against five different bacterial species. Interestingly, all the crude peptides from the six Bornean frog species possessed bactericidal activity against the tested bacteria. However, for the partially purified peptides, no bactericidal effect against certain Gram-negative bacteria, as seen in the partially purified peptides from *M. jerboa* against *S. typhimurium, O. hosii* against *E. coli* and *P. aeruginosa* and *S. guttatus* against *P. aeruginosa* and *S. typhimurium.*

From Table 3, peptide crude extraction of *L. kuhlii* and *L. leporinus* were shown to have the lowest MBC value against *S. aureus* (125 µg/mL). Furthermore, peptide crude extraction of *L. kuhlii* has the lowest MBC value against MRSA (125 µg/mL) and *S. typhimurium* (125 µg/mL), and showed similar lowest MBC value against *P. aeruginosa* (125 µg/mL) with partially purified peptides of *L. kuhlii* and crude extraction of *L. leporinus.* On the other hand, partially purified *L. kuhlii* share the lowest MBC value with partially purified *C. raniceps* as compared to other protein extracts against *E. coli* (62.5 µg/mL).

Table 1: Comparison of inhibition zones between crude and partially purified peptides on the growth of different bacteria by disk diffusion. Data are expressed as the mean ± standard deviation of three replicates

Species	Peptide sample	*S. aureus* (mm)	MRSA (mm)	*E. coli* (mm)	*P. aeruginosa* (ATCC 27853) (mm)	*S. typhimurium* (ATCC 14028) (mm)
C. raniceps	Crude	6.87 ± 1.50	7.33 ± 0.39*	8.26 ± 0.15	9.54 ± 0.25	9.29 ± 0.19*
	Partially purified	6.77 ± 1.34	6.16 ± 0.17*	8.35 ± 0.32	9.58 ± 0.11	8.66 ± 0.18*
L. kuhlii	Crude	7.00 ± 1.74	6.06 ± 0.11*	8.53± 0.27	9.40 ± 0.04*	8.46 ± 0.54
	Partially purified	7.64 ± 1.83	6.66 ± 0.31*	8.70± 0.18	9.82 ± 0.03*	8.30 ± 0.26
M. jerboa	Crude	6.85 ± 1.47	6.55 ± 0.11*	8.30 ± 0.28	9.48 ± 0.36	9.58 ± 0.18*
	Partially purified	7.80 ± 0.64	7.68 ± 0.21*	8.50 ± 0.18	9.57 ± 0.26	8.98 ± 0.19*
O. hosii	Crude	8.64 ± 0.23	8.57 ± 0.59	9.44 ± 0.39	10.76 ± 0.13	9.48 ± 0.42
	Partially purified	8.83 ± 0.42	8.84 ± 0.12	9.09 ± 0.20	10.43 ± 0.30	9.20 ± 0.18
S. guttatus	Crude	8.92 ± 0.74	8.03 ± 0.38	9.64 ± 0.12	9.47± 0.14	8.72 ± 0.16
	Partially purified	8.27 ± 0.19	7.85 ± 0.51	9.43 ± 0.34	9.35 ± 0.24	8.89 ± 0.13
L. leporinus	Crude	7.27 ± 0.36*	8.25 ± 0.57*	8.94 ± 0.20*	10.69 ± 0.38	9.27 ± 0.12
	Partially purified	6.27 ± 0.21*	9.39 ± 0.23*	9.60 ± 0.17*	10.14 ± 0.30	9.15 ± 0.02

(*) indicate statistically significant differences of inhibition zone diameter between crude and partially purified peptides ($P \leq 0.05$).

Table 2: Comparison of MIC values of crude and partially purified peptides on the growth of different bacteria by broth microdilution

Species	Peptide sample	*S. aureus* (µg/mL)	MRSA (µg/mL)	*E. coli* (µg/mL)	*P. aeruginosa* (ATCC 27853) (µg/mL)	*S. typhimurium* (ATCC 14028) (µg/mL)
C. raniceps	Crude	250	250	250	250	250
	Partially purified	250	125	62.5	250	250
L. kuhlii	Crude	125	125	125	62.5	125
	Partially purified	125	125	62.5	250	250
M. jerboa	Crude	250	125	125	125	250
	Partially purified	125	125	125	250	250
O. hosii	Crude	250	125	125	125	250
	Partially purified	250	250	125	250	250
S. guttatus	Crude	125	125	125	125	250
	Partially purified	250	125	31.25	125	250
L. leporinus	Crude	31.25	62.5	125	62.5	125
	Partially purified	125	125	125	125	250

Table 3: Comparison of MBC values of crude and partially purified peptides on the growth of different bacteria

Species	Peptide sample	*S. aureus* (µg/mL)	MRSA (µg/mL)	*E. coli* (µg/mL)	*P. aeruginosa* (ATCC 27853) (µg/mL)	*S. typhimurium* (ATCC 14028) (µg/mL)
C. raniceps	Crude	250	250	250	250	250
	Partially purified	250	250	62.5	250	250
L. kuhlii	Crude	125	125	125	125	125
	Partially purified	250	250	62.5	125	250
M. jerboa	Crude	250	250	250	250	250
	Partially purified	250	250	250	250	ND
O. hosii	Crude	250	250	250	250	250
	Partially purified	250	250	ND	ND	250
S. guttatus	Crude	250	250	125	250	250
	Partially purified	250	250	250	ND	ND
L. leporinus	Crude	125	250	250	125	250
	Partially purified	250	250	250	250	250

ND= No bactericidal effect detected at or less than 250 µg/mL of peptide concentration

Discussion

In this study, the peptide extractions from all selected Bornean frog species evidently shows protein bands at approximately 6.5 kDa in size (Figure 1). Additionally, protein bands around 1 to 3.5 kDa from *C. raniceps, M. jerboa,* and *O. hosii* protein gels were also observed. These protein bands indicate the presence of the AMP in the frog species. The AMPs from the frog skin were stated to be gene-encoded short molecules (less than 100 amino acids) (Pasupuleti *et al.,* 2012), of low molecular weight, with less than 10 kDa in size (Govender *et al.,* 2012). Furthermore, the AMPs possess amphipathic structure with hydrophobic and hydrophilic residues. The small size and amphipathic characteristics enable the peptides to have effective mechanisms in inhibiting microorganism growth. The first mechanism is by cell membrane permeabilization, and another is by targeting the cellular molecules of the cell (Bahar & Ren, 2013). Both mechanisms require the peptide to bind to the cell membrane.

The AMPs from *O. hosii* studied by Conlon *et al.* (2008) may be genetically similar with the *O. hosii* in the current study as the frog species and the ecosystem where the frogs live are similar. As the published AMPs of *O. hosii* showed molecular sizes of between 1976.1 Da to 4807.6 Da, the protein bands around 1 to 3.5 kDa found in this study from *O. hosii* frog skin sample suggest the presence of AMPs phospholipids of the plasma membrane.

As this study investigates the antimicrobial activity of the crude and partially purified peptides, the antimicrobial activity observed by the peptide samples may be contributed by other defense proteins in the protein extract apart from the AMP. *Bufo andrewsi,* for example, was reported to have no AMP in its skin secretion, but consists of higher levels of other organic molecules e.g., bufadienolides or bufadienolide-like steroids, alkaloids and biogenic amines (Zhao *et al.,* 2006). Hence, *Bufo* toads may use these molecules as its chemical defense against microorganisms. In

addition, lysozyme from skin secretion of *Bufo andrewsi* was able to exert antimicrobial activity against the *S. aureus* and *E. coli* (Zhao *et al.,* 2006). A number of different frog skin proteins from *R. dybowski* were up regulated when exposed to the *S. aureus* and *E. coli* (Xiao *et al.,* 2014). These proteins include stathmin 1a, annexin A1, superoxide dismutase A, C-type lectin, lysozyme, cofilin-1-B, mannose receptor, histone H4, prohormone convertase 1, carbonyl reductase 1 and some components of the Toll-like receptor (TLR) signaling pathway. These proteins were believed to be involved in both innate and adaptive immune systems. Therefore, there are a number of defence proteins present in both the crude and partially purified samples that may act cooperatively in assisting the AMPs, thus resulting in the observed bacteriostatic and bactericidal activities in this study.

The peptide extractions of all studied frog species did not inhibit the growth of *C. albicans*. This finding suggests that the peptides of the selected Bornean frog skin have no antifungal activity. Other AMPs such as pentadactylin from *Leptodactylus pentadactylus* (King *et al.,* 2005) and fallaxin from *Leptodactylus fallax* (Rollins-Smith *et al.,* 2005) were also found to have no fungal activity against *C. albicans*. However, the ability of some AMPs to inhibit bacteria but not fungus is explained by the interaction theory of the hydrophobic and electrostatic forces between AMPs and the prokaryotic bacterial membrane. Zasloff (2002) indicated that the bacterial membrane is more susceptible to damage by the AMPs because of the higher level of phospholipids and other anionic lipids in the outer part of the membrane. The eukaryotic membrane of *C. albicans* fungus, on the other hand, has less anionic charge on its surface. Therefore, the interaction between the AMP and the *C. albicans* membrane is weaker, suggesting a low or no potency of the AMPs against the *C. albicans* fungus.

Despite having a certain similarity in terms of biophysical characteristics, the AMPs rarely have similar peptide sequence between species due to natural evolution occurring since millions of years ago. This lead to specifically and generally acting AMPs that the frogs employ, to avoid the emergence of resistance towards the peptides (Wu *et al.,* 2011). In this study, it was observed that the Bornean frog skin AMP possess antimicrobial activity only on Gram-positive and Gram-negative bacteria, but not against fungus. An AMP with similar antimicrobial activities was esculentin-2JDa, extracted from *Odorrana jingdongesis* found in Jingdong, Yunnan province, China (Liu *et al.,* 2012). The MIC values of esculentin-2JDa were 128 µg/mL and 32 µg/mL against *E. coli* and *S. aureus* respectively, with no detected antifungal activity against *C. albicans*.

Tennessen *et al.* (2009) stated that the observed activity of frog skin peptides was due to selection pressure on populations due to past encounters with microorganisms. This also demonstrates the frog species adaptation to the distinctive microbial environments that the frogs live in (Pasupuleti *et al.,* 2012). *C. raniceps* and *L. kuhlii* frog species, for example, are usually found near the streams. Water from the streams may sometimes be contaminated with *E. coli* and *P. aeruginosa* originating from feces and urine of humans or animals (Centers for Disease Control and Disease, 2013). These frog species may have adapted to the presence of these two bacteria in their habitat, explaining the observation for a lower concentration of partially purified peptides required to inhibit bacterial growth of these two species.

The MIC of skin extracts of *L. kuhlii* peptides in the current study were much higher as compared to the MIC of the purified *L. kuhlii* AMP done by Wang *et al.* (2013). This can be explained by different locations that the frogs live. Even though the species are similar *(L. kuhlii),* the frog individuals were taken from different countries (Borneo, Malaysia and Yunnan, China), hence the peptides produced may be distinct. Study by Tennessen *et al.* (2009), revealed that the pattern of expressed antimicrobial skin peptides of *R. pipiens* found in separate locations of Vermont, Minnesota and Michigan were different.

As this study also looked at bactericidal activities of the samples, the MBC values obtained from the peptide samples range between 62.5 µg/mL-250 µg/mL. One possible mechanism of AMP that promotes bacterial cell death can be explained by temporin, an AMP found in *L. kuhlii* in China which possessed cationic hydrophilic side chains of amphipathic α-helical peptides. The cationic (positive charge) side chains of temporin interacts with the negatively charged phospholipids on the bacterial membrane to form aggregates and later penetrate through the bacterial membrane bilayer to form pores (Mahalka & Kinnunen, 2009).

Results in this study showed that peptide samples from all six tested Bornean frog skins possess antimicrobial activities, not only bacteriostatic but also bactericidal, against the resistant pathogen, MRSA. MRSA is a virulent microbe and is becoming one of the major causes of infections in the hospital and community (Otto, 2012). As the tested protein extracts from the frog skin was found to have antimicrobial activity against MRSA, it may be a possible candidate for antimicrobial therapy in the future.

Conclusion

Chalcorana raniceps, Odorrana hosii and *Meristogenys jerboa* showed peptide bands around 1-3.5kDa. As previous studies reported that most of the AMPs are less than 10 kDa in size, the observed peptide bands with lower molecular size strongly indicate the presence of AMP in the skin of the sampled frog species. Furthermore, the current study shows that the skin peptides from *L. kuhlii* and *L. leporinus* frogs presented the lowest MIC and MBC values against most of the tested bacteria. This implies that the peptides from these two frog species are more potent than other species studied in inducing bacteriostatic and bactericidal activities against the bacteria. The antimicrobial activities observed from the skin peptides give a high potential novel AMPs from the frog species of Borneo. For future study, the peptides of frog skin extracts should be further purified, in order to enhance their activities.

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