ABSTRACT

Antibiotic-resistant pathogens are now becoming a global problem in public health. Resistant microbes are difficult to treat and give impact to the nations, both economically and medically. Thus, this crisis necessitates the needs for alternative antimicrobial agents in which the bacteria has never been exposed to. Such alternative has been made possible by the discovery of antimicrobial peptides (AMPs) as the novel therapeutic agents which are found to confer a broad spectrum antibacterial activity. Recent discoveries of AMPs from frog skin secretions have become increasingly relevant to overcome the antibiotic resistance issue. The effectiveness of frog's AMPs against microorganisms have made the frog peptides as a potential source for the development of novel antimicrobial agents. In this current research, the isolation, purification, identification and characterization of AMPs from the skin secretions of Bornean frog's species were conducted. The species studied include *Pulchrana glandulosa*, *Pulchrana signata*, *Pulchrana baramica*, *Odorrana hosii*, *Meristogenys jerboa* and *Chalcorana raniceps* that inhabit the south-eastern region of Sarawak. Results of the preliminary study using disk diffusion test revealed encouraging antimicrobial activity in all of the tested species. Secretions were then partially purified and fractionated to near homogeneity using the high performance liquid chromatography (HPLC) technique. Microdilution steps were used to investigate the AMPs' potency against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. Further analysis by CD spectrum and protein sequencing were then undertaken to elucidate the primary and secondary structures of the purified peptides. The results showed that the tested species possessed encouraging antimicrobial and antifungal activities. The activity of skin secretions' peptides from *M. jerboa* are most potent with MIC= 12.5 µg/mL against *E.coli* isolates and MIC = 25 µg/mL against *S. aureus* isolates. Nonetheless, *Pulchrana glandulosa*, *Pulchrana signata*, *Odorrnana hosii*, *Pulchrana baramica* and *Chalcorana*

raniceps shown promising results with MIC = 25, 50, 25, 125 and 100 µg/mL respectively against *E.coli* isolates. *P. signata, C. raniceps, O. hosii* and *P. baramica* inhibit *S. aureus* isolates at a minimum concentration of within 50 to 1000 µg/mL. All peptides showed moderate haemolytic activity at the concentration between 1.56 µg/mL to 12.5 µg/mL. Incubation of frog skin secretions in human red blood cell (RBC) for 1 hour at a concentration of more than 25 µg/mL demonstrated 50 percent lysis of the RBC, thus suggesting that AMPs, although not all, has moderate toxicity at the concentrations effective to kill bacteria. The CD spectrum of the peptides from all frog species showed that the dominant secondary structures are in the forms of β-pleated sheets and random coils with low composition of α-helices. Mass spectrometry analysis revealed the peptides assigned for *P. signata*, *C. raniceps* and *M. jerboa* as Ranateurin-2 (GLFVQLVDDDPAYFDAK, Mr = 1911 Da), Distinctin (NLVTLATATTG, Mr = 1060 Da) and Magainin-2 (GIGMHLIAGIAK, Mr = 1195 Da) respectively. Two AMPs were isolated from *O. hosii* which are Odorannain-1 (VYSEVNSLSR, Mr = 1152 Da) and Odorannain-B (AAGVAAWLPLAR, Mr = 1194 Da). The peptide isolated from *P. baramica* is similar to a hypothetical protein (FPNDTAAR) found in *Xenopous tropicalis* whereas a peptide related to the cationic trypsin was isolated from *P. glandulosa* skin secretion.

Keywords: Antimicrobial peptides, skin secretions, purification, peptides

Pencirian Peptida Antimikrobial daripada Rembesan Kulit Katak dari famili Ranidae yang Terdapat di Borneo

ABSTRAK

Mikroorganisma yang rentan terhadap antibiotik merupakan masalah global dalam kesihatan awam. Mikroorganisma yang tahan kepada antibiotik susah dirawat dan memberi impak kepada bidang ekonomi dan perubatan, lalu memerlukan ubatan alternatif yang tidak pernah didedahkan kepada mikroorganisma. Alternatif tersebut boleh dimungkinkan melalui penemuan antimikrobial peptida (AMPs) sebagai ejen terapeutik yang novel serta menunjukkan aktiviti antibakteria yang luas. Baru-baru ini, penemuan antimikrobial peptida dari rembesan kulit katak telah menjadi perkaitan semasa untuk mengatasi rintangan antibiotik. Kadar AMP yang tinggi di dalam rembesan kulit katak menunjukkan keberkesanan peptida dari katak sebagai sumber utama untuk pembuatan peptida yang novel. Oleh itu, kajian ini melakukan pengasingan, pengenalpastian dan pencirian AMP dari kulit katak yang berasal daripada kepulauan Borneo seperti Pulchrana glandulosa, Pulchrana signata, Pulchrana baramica, Odorrana hosii, Meristogenys jerboa *dan* Chalcorana raniceps *yang menduduki rantau Timur-Selatan Sarawak. Keptusan preliminari menggunakan ujian penyebaran cakera menunjukkan aktiviti antibakteria yang signifikan di dalam semua spesies yang dikaji. Seterusnya, rembesan katak separuh dipurifikasi dan diasingkan sehingga sepenuhnya melalui kromatografi cecair prestasi tinggi (HPLC). Teknik pencairan mikro digunakan untuk menyiasat potensi AMPs terhadap* Escherichia coli, Staphylococcus aureus *dan* Candida albicans. *Analisis lanjut menggunakan spektra 'circular dichroism' (CD) dan penjujukan protein dilakukan untuk pencirian struktur AMP. Keputusan menunjukkan aktiviti antimikrobial dan antifungal yang meyakinkan. Aktiviti dari rembesan kulit* Meristogenys jerboa *adalah paling kuat dengan mencatatkan kosentrasi inhibitori minimum (MIC = 12.5 µg/mL) terhadap* E. coli

dan MIC = 25 µg/mL terhadap S. aureus. *Namun begitu,* P. glandulosa, P. signata, *O. hosii, P. baramica dan C. raniceps menunjukkan keputsan yang signifikan degan masing masing mencatat MIC= 25, 50, 25, 125 dan 100 µg/mL. Apbila diuji terhadap* S. aureus, P. signata, C. raniceps, O. hosii *and* P. baramica *menghalang pertumbuhan bakteria dalam lingkungan minima di antara 50 sehingga 1000 µg/mL. Semua peptida menunjukkan aktiviti hemolitik yang sederhana pada kepekatan di antara 1.56 µg/mL sehingga 12.5 µg/mL. Inkubasi lendir katak di dalam sel darah merah manusia (RBC) pada kepekatan lebih daripada 25 µg/mL menunjukkan 50 peratus lysis dalam tempoh satu jam yang menunjukkan AMP, walaupun tidak semua, mempunyai ketoksikan sederhana pada kepekatan yang effektif terhadap bakteria. Spektrum CD menunjukkan struktur sekondari yang dominan untuk semua peptide adalah putaran rawak dan lembaran pelapisan β. Spektrometri jisim menunjukkan peptida untuk* P. signata, C. raniceps *dan* M. jerboa *sebagai Ranateurin-2 (GLFVQLVDDDPAYFDAK, Mr = 1911 Da), Distinctin (NLVTLATATTG, Mr = 1060 Da) dan Magainin-2 (GIGMHLIAGIAK, Mr = 1195 Da). Dua AMP diasingkan daripada* O. hosii *iaitu Odorannain-1 (VYSEVNSLSR, Mr = 1152 Da) dan Odorranain-B (AAGVAAWLPLAR, Mr = 1194 Da).* P. baramica *menunjukkan peptida yang menyerupai 'hypothetical protein (FPNDTAAR) seperti ditemui di rembesan kulit katak* Xenopous tropicalis *manakala trypsin kationik diasingkan daripada* P. glandulosa.

Kata kunci: *Antimikrobial pepida, rembesan skulit katak, purifikasi, peptida*