Protein Profiles and Antimicrobial Activity of Common Sunda Toad, *Duttaphrynus Melanostictus* Paratoid Secretions

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***Abstract*—**In this study, the profile and antimicrobial activity of the secretions of *Duttaphrynus melanostictus* were studied. The protein profiles were investigated through SDS-PAGE and the proteins itself has been successfully separated. The antimicrobial activity of the secretions was performed by disk diffusion method and was tested against *Bacillus cereus* ATCC11778, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* IMR S 974/05 B, *Staphylococcus aureus* ATCC 25923, *Staphylococcus eperdimidis* IMR S 384/05 C, *Klebsiella pneumoniae* ATCC 700603 and Methicillin-resistant *Staphylococcus aureus* (MRSA) which had been clinically isolated. According to the findings, the secretions (5mg/ml) have lower antimicrobial activity in comparison to antibiotics used, Tetracycline HCl (120 mg/ml). The secretions however, failed to inhibit the growth of Methicillin-resistant *Staphylococcus aureus*.

***Keywords*—**antimicrobial activity, disk diffusion, protein, SDS-PAGE

I. Introduction

ANIMALS live successfully because they possess the full capacity to function in a variety of ways [8] and adapt to harsh environment. Such cases will always force them to move away from the environment or adapt, tolerate and counter the harsh environment. Organisms from throughout the phylogenetic tree, including animals [3], produce substances for protection against microbes. Many of these substances are known to be peptides and an example of a good source of animals that produces this substance is amphibians.

Amphibians can be further classified into frogs, toads and newts. Their distribution ranging from the tropic region to subarctic regions but most of the species are found in tropical rainforests. Since they are an example of living organisms that went through several evolutionary periods, their immune system are highly evolved from period to another period [13].

Frogs and their skin secretion properties can be found throughout several cultures around the world. Such traditional knowledge has actually diversified into a concept that the amphibian skin may be a treasure of biologically active compounds. A survey of noxious or toxic secretions and the presence of biogenic amines, peptides, bufadienolides, tetrodotoxins and lipophilic alkaloids was described years ago [4] – [15. Other than this, many amphibians' cutaneous peptides have shown strong inhibitory activity against many species of fungi, bacteria, protozoa and tumor cells [10].

Indeed, the peptides derived from amphibian secretions are promising sources of antimicrobial agents [16]. Particularly, the emergence in all regions of strains of pathogenic bacteria and fungi with resistance to commonly used antibiotics constitutes a serious threat to public health [8](J. Michael Conlon et al., 2012). In this study, that pathogenic bacterium chosen was methicillin-resistant *Staphylococcus aureus*. MRSA is now a problem in hospitals worldwide and is increasingly recovered from nursing homes and the community [11]. Therefore, the main focus of this research is to investigate the effectiveness of the toad secretions in inhibiting the growth of MRSA.

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II. Procedure

A. Collection of Secretions

Fresh skin secretions were obtained from the toad by squeezing the parotid glands according to procedure described by [1]. The semi fluid and viscous secretions were not allowed to dry and they were mechanically-sheared with sterile distilled water using a sterile syringe and pooled.

B. Ammonium Sulphate Precipitation

The secretions were pooled for gathering all the protein contents together and the bulk precipitation of proteins was done using ammonium sulphate precipitation method at the saturation level of 30% to 60% at 4ºC temperature. The precipitates were then centrifuged at 10000 rpm and 4°c for 15 minutes. The precipitate formed was dialyzed with phosphate buffer, pH 7.0 at 4°c and left overnight. The dialyzed product was later lyophilized.

C. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

12% polyacrylamide gel electrophoresis containing sodium dodecyl sulfate (SDS-PAGE) was performed using the dialyzed protein sample, in reducing conditions, according to [12].

D. Antimicrobial Activity

In this study, *Bacillus cereus* ATCC11778, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* IMR S 974/05 B, *Staphylococcus aureus* ATCC 25923, *Staphylococcus eperdimidis* IMR S 384/05 C, *Klebsiella pneumoniae* ATCC 700603 and Methicillin-resistant *Staphylococcus aureus* which

was clinically isolated, have been used as bacterial cultures by using Mueller-Hinton agar and Mueller Hinton Broth as media and 0.5 MacFarland standard turbidity is achieved at OD_{625}. In-vitro antimicrobial activity studies were carried out by Agar-Disc Diffusion method. The antibiotic that was used as control was Tetracycline Hydrochloride and dialyzed secretions were implemented into sterile disc at the highest concentration (5mg/ml). Experiments were repeated three times and results were expressed as average values.

III. Results

The secretions presented a profile protein through SDS-PAGE (Figure 1). Even though the protein concentration presence in the sample is low, several proteins appeared to be at its high concentration due to the bright appearance of the bands as in well no three and four. For each well, the proteins from each secretion were pooled together before performing the SDS-PAGE. However, in spite of the broad variation in the relative protein concentration for each well, the amphibian secretions present a general related profile.

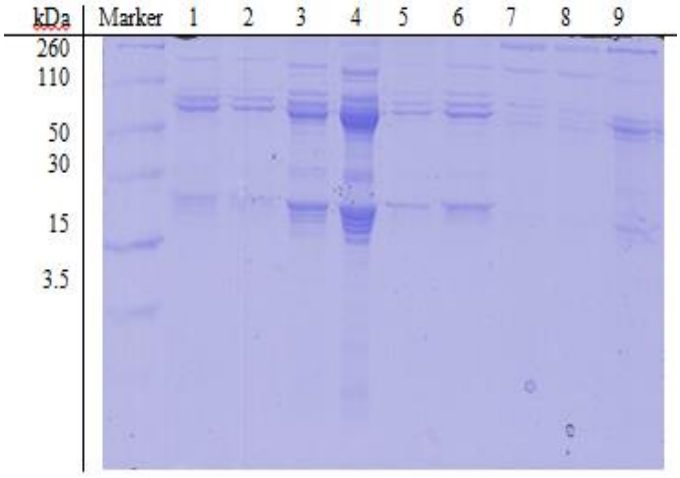

Fig. 1 SDS – PAGE of the toad secretions, *Duttaphrynus melanostictus*

As for the bioactivity of the secretions, the dialyzed protein sample was tested for antimicrobial susceptibility testing through Disc-Diffusion method. It was tested against *Bacillus cereus* ATCC11778, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* IMR S 974/05 B, *Staphylococcus aureus* ATCC 25923, *Staphylococcus eperdimidis* IMR S 384/05 C, *Klebsiella pneumoniae* ATCC 700603 and Methicillin-resistant *Staphylococcus aureus* which was isolated from patient have been used as bacterial cultures. The secretions presented highest zone of inhibition when tested against *Staphylococcus aureus* but no inhibition zone when tested against MRSA. In overall comparison, the secretions activity was less comparable to the antibiotic control used in the experiment, which was Tetracycline Hydrochloride at the dose of 120 mg/ml (Table 1).

Table I
Comparison Of Secretions And Antibiotic Control Against Gram Positive And Gram Negative Bacteria

Bacteria	Secretions	Tetracycline HCl
S. aureus	11 mm	16 mm
MRSA	No Inhibition Zone	15 mm
K.pneumoniae	9 mm	15 mm
B. cereus	10 mm	15 mm
S. eperdimidis	8 mm	14 mm
S. typhimurium	7 mm	16 mm
E. coli	7 mm	16 mm

mm = millimeter, HCl = hydrochloride

IV. Discussions

The secretions presented an expected SDS-PAGE profile (Figure 1). Although the secretions are pooled and the relative protein concentration in each loaded well seems to vary, but several proteins were differ from each other and seemed to appear brightly. This indicates that the protein detected was at high concentration. The proteins profile of the secretions has been successfully separated through SDS-PAGE according to size and they represented general related profiles in terms of protein contents. Such secretions are presumably thought to have an evident dissociation between the secretions pattern into the skin of the toads and their habitat [14].

Any secretions from a single species frequently contain several members of a particular family that are presumed to have arisen from multiple duplications of an ancestral gene. [7]. The SDS-PAGE profile showed a molecular heterogeneity of the secretions within a particular family, and in this case, Bufonidae. However, a peptide from one species rarely being found with an identical amino acid sequence in another, even when those species are quite closely related phylogenetically [5].

The presence of protein bands with identical mobility in the secretions and gland extracts, have indicated the similarity of proteins secreted probably by granular cells of epidermis. The secretary proteins exist as coiled filaments with an epidermal granular cells [17].Other than proteins, many findings have also reported that the alkaloids steroids are also present in the secretions and they are known to be toxic and are of an anti-feeding agent which acts as a major chemical defense strategy against predators [2] – [4] – [9]. In addition to this, the presence of these arrays of proteins in the paratoid gland secretions have suggested that there are more complex role for these secretions than simply to deter predators.

As for the antimicrobial susceptibility test, the results presented here support the hypothesis that amphibians are indeed proven to have their own direct defenses against some opportunistic bacterial which is associated with bacterial dermatosepticemia. Though, the secretions failed to show any inhibition when tested against MRSA, it is proven to inhibit other gram-positive and gram-negative bacteria. Indirectly, this means that the secretion is somehow comparable to those of β-lactam antibiotics because MRSA itself produces an alternative transpeptidase with low affinity for β-lactam antibiotics [7].

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