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Antibacterial and antibiofilm activity of Bull frog *Hoplobatrachus tigerinus* skin extract

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SUMMARY

Peptides are present in the skin of frogs and toads and having antimicrobial affect. The peptides have been shown effective destructive action on cells in infectious microorganisms. *Hoplobatrachus tigerinus* is recorded from Bangladesh, Pakistan, Nepal, Myanmar, Bhutan, Sri Lanka, Afghanistan, Maldives, Madagascar and India. *H. tigerinus* documented at elevations from 25 to 2,000m above sea level. Skin of toads and frogs are used to treat various ailments like dog bites, depression, seizures, cognitive loss, stroke, Alzheimer's disease, impotency and wounds. So these points were kept in mind and designed this study. Main objectives of the studies were to evaluate antibacterial and antibiofilm activities of skin extract of Bull frog. The extract of Bull frog was used in broth agar well diffusion method to study the antibacterial and antibiofilm activity. In this study the bull frog skin extract showed significant activity of against four tested bacteria. The larger zones of imitation were observed against gram positive and negative bacteria.

Keywords: Antibiofilm, Frog, Skin, Punjab

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INTRODUCTION

The skin secretions of many toads and frogs contain peptides with antimicrobial activity (Conlon and Sonnevend, 2010). These peptides are present in dorsal part of skin (Bowie and Tyler, 2006). The peptides have been shown effective destructive action on cells in infectious microorganisms (Ashcroft *et al.*, 2007), therefore that it is suitable to suggest that they show a part of the innate immunity system that produces host defense for invertebrate and vertebrate species (Hancock *et al.,* 2001).

In any case, the significance of the peptides in the survival procedure of the creature and the degree to which their creation gives a evolutionary favorable position to the species are not obviously understood. Right now, antimicrobial peptides have been distinguished in the skins of frogs from species having a place with the Ranidae, Pipidae, Myobatrachidae, Leptodactylidae, Leiopelmatidae, Hyperoliidae, Hylidae and Bombinatoridae families but many well documented species from the Scaphiopodidae, Rhacophoridae, Pyxicephalidae, Pelobatidae, Microhylidae, Dicroglossidae, Ceratophryidae and Bufonidae families do not show to produce these peptides. Ranidae “true frogs” and Leiopelmatidae “tailed frogs” in North America

have been documented skin secretions peptides. These peptides have not been distinguished in dermal discharges as well as extracts of those species examined having a place with the Eleutherodactylidae, Hylidae, Scaphiopodidae and Bufonidae, families. There doesn't have all the earmarks of being any conspicuous relationship between are the favored environment of the living and its inability or capacity to produce skin peptides. Documentation of these perceptions is dubious however it infers that the blend of peptides may confer favorable position to specific species yet isn't important for life of the organism. This will portray the biological and structural features of peptides present in secretions of dermal having a place with species from three genera: *Rana* (Ranidae), *Lithobates (Ranidae*) and *Ascaphus* (Leiopelmatidae) (Conlon *et al.*, 2004).

Hoplobatrachus tigerinus is recorded from Bangladesh, Pakistan, Nepal, Myanmar, Bhutan, Sri Lanka, Afghanistan, Maldives, Madagascar and India. *H. tigerinus* documented at elevations from 25 to 2,000m above sea level. The from which peptides have been separated that were odentified in the class Rana (Hillis, 2007). Wildlife has been utilized by human being as a food, traditional medicine since the origin of human. This also documented that amphibians are utilized for folk medicinal (Angeletti *et al.*, 1992; Altaf, 2016; Altaf *et al.*, 2020). Skin of toads and frogs are used in materia medica and also used to treat various ailments like dog bites (Costa-Neto, 1999), depression, seizures, cognitive loss, stroke, Alzheimer's disease (Amato, 1992), impotency and wounds (Sai *et al.*, 1995; Altaf *et al.*, 2018). So these points were kept in mind and designed this study. Main objectives of the studies were to evaluate antibacterial and antibiofilm activities of skin extract of Bull frog.

MATERIAL AND METHOD

SAMPLE COLLECTION

The sample was collected in Ali Pur Chattha district Gujranwala, Punjab, Pakistan. The species was identified with help of books name as “The Amphibian and Reptiles of Pakistan” by Khan (2006).

EXTRACT PREPARTION

Take bull frog skin extract was completely cleaned with water to eliminate waste particle and dried region for 1 to about fourteen days. The dried examples in drying stove in “24 hour” when skin remove dried is crushed and changed into powder with the assistance of a grinder weight the powder. For the concentrate planning, “5.05 g” of skin extract was included “100 ml” of refined water, blended for “20 minute” at “60°C”, and later heated. In the wake of heating up the blend was cooled as well as separated with “Whatman paper No. 1”. This filtrate solution was marked.

AGAR WELL DIFFUSION METHOD

The activities of antimicrobe were evaluated with the help of "agar well diffusion method". "Nutrient broth media" (NB-1704) was utilized for "bacterial culture and Nutrient Agar" (NA-1903) was utilized for the antibacterial activities (Seeley Jr and VanDemark, 1962; Hammer *et al.*, 1999).

ANTIBIOFILMASSAY

The "Crystal violet assay" examine was utilized to examine the antibiofilm activities with slight alterations. Microorganisms were filled in tubes having "2 ml" of supplement stock medium alongwith "30 mg/ml" solution of frog extract and integrated nanoparticles for whole night at "37°C". The control tubes have "nutrient broth". Further medium was eliminated and these cells were stained by pouring "125 µl" of a "0.1%" violet. Tubes were brooded at room temperature for "10 to 15 minute" and washed with water to eliminate overabundance unattached cells and colour. Subsequent to staining biofilm, alongwith crystal violet was solubilised by adding 30% acidic corrosive and brooded at normal temperature for "10 to 15 minute". Solubilised crystal violet measured at "550 nm" utilizing a spectrophotometer and "30%" acetic acid in normal water utilized as empty (O'Toole, 2011).

RESULTS AND DISCUSSION

In this study the bull frog skin extract showed significant activity of against four tested bacteria. The bigger zones of imitation were observed in both "gram positive" and "gram negative" bacteria.

In this study, skin extract having their activities against bacterial strains, as represented by the size of zone. Synthesized skin extract showed stronger bactericidal activity. The inhibited size was documented and synthesized skin extract showed activity against pathogens was used. Here four pathogens were used named *aeruginosa, Klebsiela pneumonia, Staphyloccoccus aureus* and *Proteus.* The effective antibacterial activity was shown by extract of *H. tigerinus* against test bacterial pathogens. Highest antibacterial effect Zone of inhibition shown by the extract of *H. tigerinus* was recorded as 15.66mm ± 2.08 mm against *Proteus*. *Klebsiela pneumonia* also showed significant effect recorded as 2.66 mm ± 1.63 mm. *Staphyloccoccus aureus* showed 5.33 mm ±2.30 mm zone of inhibition against *Pseudomonas aeruginosa was* recorded as 16.33 mm ± 1.52 mm. The antibacterial activity was the higher activity of extract in their large surface area, which may enhance contact with selected pathogens (Table 1, Figure 1 and 2).

Table 1: Antibacterial activity skin extract of bull frog.

Test Sample Pathogen	EX of BF	DMSO	Chloramphenicol (10 ug)
Pseudomonas aeruginosa	15.66mm±2.08mm	-	27.0 ±0.0
Klebsiela pneumonia	2.66mm±1.63mm	-	35.0±0.0
Proteus	5.33mm±2.30mm	-	32.0 ± 0.0
Staphylococcus aureus	16.33mm±1.52mm	-	29.0 ± 0.0

The Study was documented to know the ability skin extract to stop biofilm action of bacteria. *P. aeruginosa, S. aureus, K. pneumonia* and *Proteus* are the topmost usual pathogens included in preparation of biofilm. Skin extract have the power to upset the biofilm of many bacteria. In this study, the extract activities were noted utilizing four bacterial separated. Bacteria were used without and with skin extract to prepare a biofilm. The variation in the activities of extract of skin

can be cause of many reasons like extract, the effectiveness of antimicrobial impact (Figure 2).

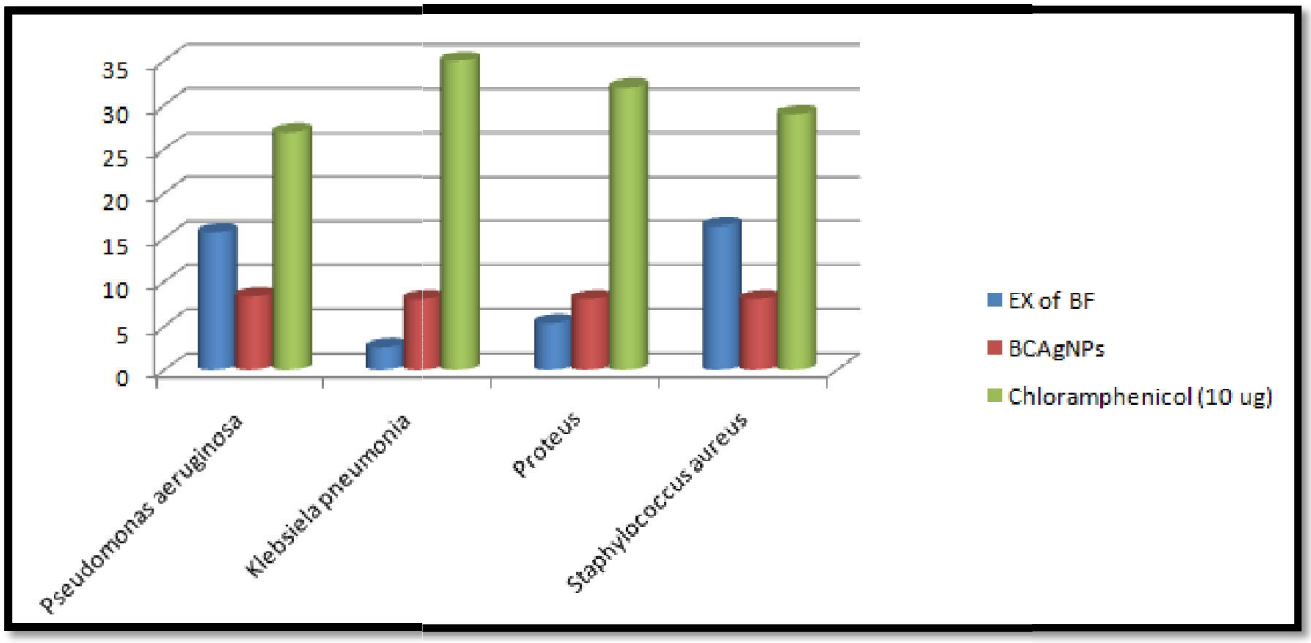

Figure 1: Antibacterial activity of skin extract of bull frog against *p. aeruginsa, S aureus, proteus pneumonia* and *klebsiella pneumonia*.

The higher antimicrobial activity of extract of skin is cause of large area of surface, which may enhance direct contact with the pathogenic bacteri. Antibacterial impact of extracts of *Bergenia ciliata* has been documented (Mazhar-Ul-Islam *et al.*, 2002). Frog skin secretions were previously reported with electrical (Nascimento *et al.*, 2007) and compound activate utilizing norepinephrine chemical (Conlon *et al.*, 2007).

Figure 2: Zones of inhibition formed by aqueous extract of bull frog.

Table 2: Ant biofilm activity of bullfrog skin extract.

Test organism	Extract of bullfrog	Pathogen	Chlp	Control
Pseudomonas aeruginosa	0.432	0.024	0.0193	0.002
Klebsiela pneumonia	0.520	0.066	0.0152	0.002
Staphylococcus aureus	0.140	0.055	0.0186	0.002
Proteus	0.075	0.064	0.0185	0.002

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