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ANTIBACTERIAL ACTIVITY OF SKIN SECRETION AND ITS EXTRACTION FROM THE TOAD *Bufo melanostictus*

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ABSTRACT

Skin secretion of Amphibians generally contain multiple antimicrobial peptides with distinct spectra of activity and it has been speculated that the animal is protected from invasion by a wide array of different microorganisms. The objective of the present study was to assess the antimicrobial properties of skin secretion and its extract from Indian toad *Bufo melanostictus* (Schneider). The collected skin secretion was filter-sterilized, freeze-dried and subjected to antibacterial assay. Antibacterial activity of toad skin secretion was tested on Asthana Hawkers agar medium plates seeded with the species of *Escherichia coli, Staphylococcus aureus, Proteus vulgaris* and *Klebseilla pneumoniae* through well diffusion technique by using Zone of inhibition (ZOI) method. An inhibition was observed with 40µl/ml of frog skin secretion. Results showed that the skin secretion of toad has antibacterial activity against four strains. All the skin secretion and extraction have showed nearest inhibition zones i.e., viz., 30mm, 27mm, 28mm,33mm,19mm, 24mm, 19mm and 25mm.Hence,we conclude that the skin secretion and extract can be employed in the development of potent antibacterial agents used to treat infectious diseases.

KEY WORDS: Skin secretion, Asthana Hawkers agar medium, Zone of inhibition, *Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Klebseilla pneumoniae.*

INTRODUCTION

In the last few years, data revealed that occurrence of very few antimicrobial peptides characterized from a wide range of organisms. Antibiotics have been termed as the single most significant discovery in medicine. Consequently, there have been efforts in for the production of novel antimicrobial agents.[1] Amphibian skin is a morphologically, biochemically and physiologically complex organ, which helps not only in respiration, osmoregulation, excretion, temperature control, reproduction but also in anti predator activity and antimicrobial defense mechanism.[2] The skin of toad acts as a barrier for entry of bacteria, fungi and other invades. We partially known the origin and function of poisonous and noxious substances of Amphibians such as biogenic amines and their derivatives found in skin of frogs and toads.[3,4]

Toads and Salamanders have been considered noxious creatures for containing noxious and poisonous substances in their skin secretions.[3,5] These secretions contain peptides which have the ability to inhibit the growth of pathogenic microorganisms.[6] These secretions contain peptides which exhibit anti-microbial activity against gram positive, gram negative bacteria and some yeast and protozoans also.[7,8,9,10] Many host defense peptides show high potency against bacteria[11] and other potent biological activities too.[12] The secretions of frog and toad may benefit human health with its antibacterial, antifungal, antiprotozoal, antidiabetic and other therapeutic properties.[13,14,2] In view of the above, we have undertaken the present investigation to screen the antibacterial activity of the skin secretion and its extract to find out the *B. melanostictus*.

MATERIAL AND METHODS

Collection of toad and toad skin extract (TSE) preparation

Adult live toad (40-50 gm) *B. melanostictus* were collected from the vicinity of Kakatiya University hostels buildings and maintained in well ventilated glass box, some insects were given as feeding. Animals were pithed and their skin was separated from the body except parotid gland. The skin was kept in methanol at room temperature for 30 days. The supernatant was centrifuged and was pooled. It was evaporated to dryness by rotary evaporator and the extract was kept at RT (28°C) in a desiccator. Then toad skin extract was dissolved at definite concentration in normal saline (0.9%) until use.[15]

Collection of toad skin secretion

The skin secretions were obtained from the toads by gentle electrical stimulation (4-ms pulse width, 50Hz, 5V) using platinum electrodes rubbed over the moistened dorsal skin surface for 10s. Secretions were washed off into a glass beaker, using distilled water.[16] The resultant secretions were freeze dried in a freeze dryer. Approximately 50 mg, dry weight of skin secretion was obtained. Bacterial suspension was swabbed on the surface of the solidified Asthana Hawkers agar medium. Now 40µl of each extract was added into the wells and plates were incubated at 28^0 C for 24 hours. Zone of inhibition was measured with Hi-media antibiotic scale and experiment was repeated three replicates. Inhibition zones were measured and tabulated. All the qualitative and quantitative chemicals were supplied by Himeda Laboratories Pvt. Ltd. Mumbai.

Anti bacterial activity

For antibacterial activity four bacterial strains gram – ve bacteria and gram + ve bacteria such as *Escherchia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*, *Proteous vulgaris* were used respectively. Bacterial subculture was swabbed on the surface of the solidified Asthana Hawkers agar medium. Now Holes of 5mm diameter were made into which the toad skin secretion (40µL) was applied. The culture was then incubated for 24h at room temperature until the bacterial growth in the petri dish was homogeneous. The antibacterial activity was measured from the formation of clearing zone due to inhibition around the treated area.

RESULTS AND DISCUSSION

Tab. 1: Anti-bacterial activity of Toad Skin Secretion (TSS) and its Extraction(TSE).

MicroOrganism	Skin Secretion (ZOI in mm)	Skin Extraction (ZOI in mm)
Escherichia coli	25mm	19mm
Klebsiella pneumoniae	19mm	24mm
Staphylococcus aureus	30mm	27mm
Proteus vulgaris	28mm	33mm

Gram – ve bacteria: *Escherichia coli* and *Klebsiella pneumoniae;*
Gram + ve bacteria: *Staphylococcus aureus* and *Proteus vulgaris*
ZOI= Zone of Inhibition

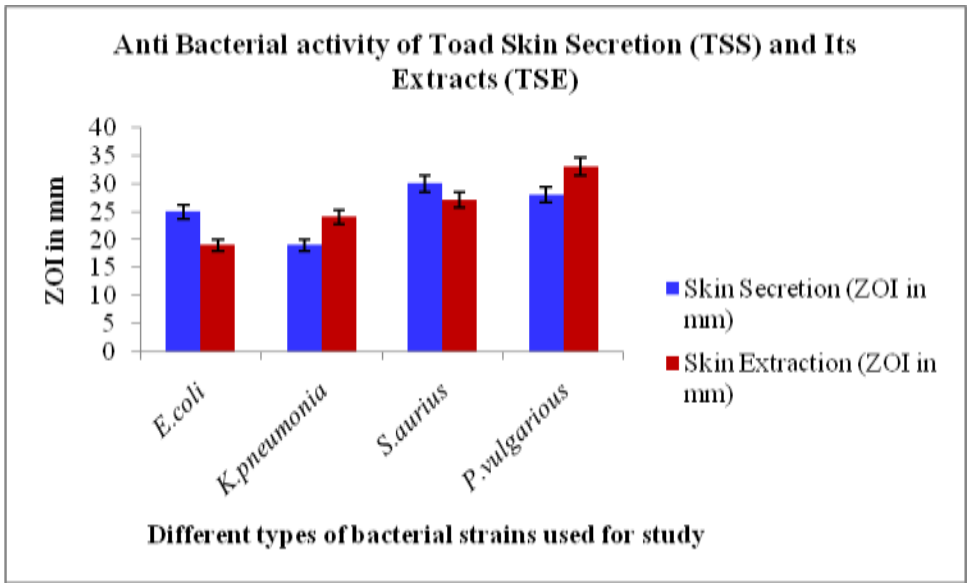
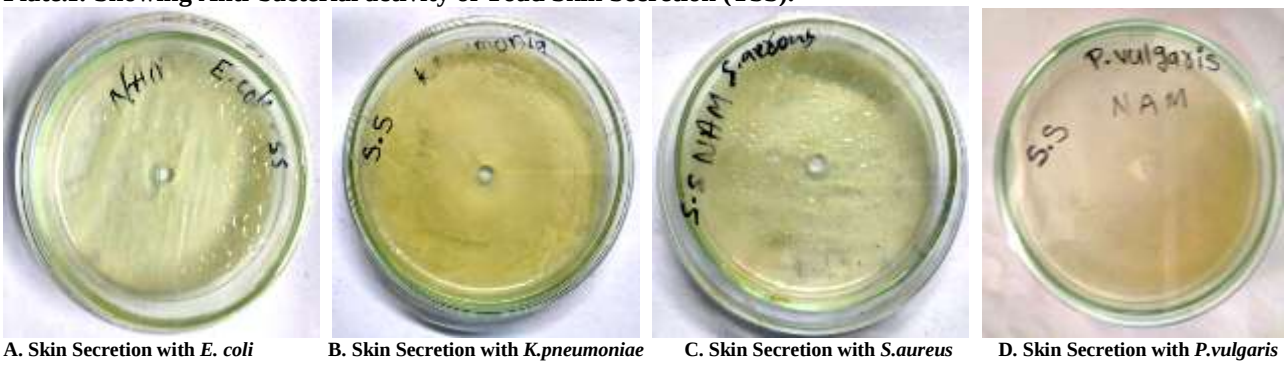


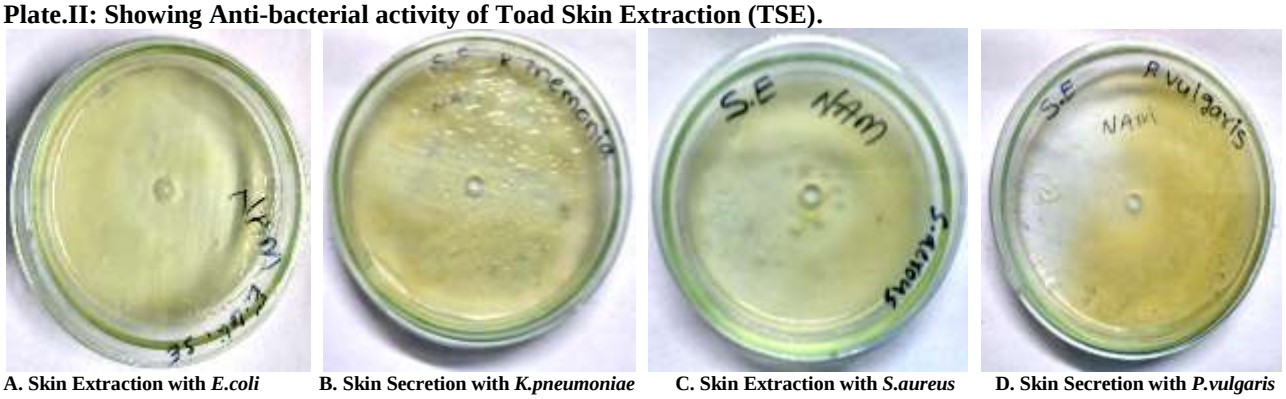
Fig.1: Anti-bacterial activity of Toad Skin Secretion (TSS) and its Extraction (TSE).

Plate.I: Showing Anti-bacterial activity of Toad Skin Secretion (TSS).



A. Skin Secretion with *E. coli* **B. Skin Secretion with *K.pneumoniae*** **C. Skin Secretion with *S.aureus*** **D. Skin Secretion with *P.vulgaris***

Plate.II: Showing Anti-bacterial activity of Toad Skin Extraction (TSE).



A. Skin Extraction with *E.coli* **B. Skin Secretion with *K.pneumoniae*** **C. Skin Extraction with *S.aureus*** **D. Skin Secretion with *P.vulgaris***

Results obtained from present investigation are presented in table.1, Plate I and II and fig.1. The highest inhibition zone against *staphylococcus aureus* (30mm) and lowest activity towards *Klebsilla* species was shown by skin secretion. Skin extract showed highest activity against *P. vulgaris* and minimum inhibition for *E. coli*, while nearly similar activity (28, 25mm) by skin secretion as well as skin extraction was observed against *K. pneumoniae* and *S. aureus* with minor variations in ZOI such as 24 mm, 27mm. Both skin secretion and extraction have showed inhibition zones with nearest values like viz., 30mm, 27mm, 28mm, 33mm, 19mm, 24mm and 19mm, 25mm respectively. Thus from our present investigation it is evident that both the skin secretion and its extract have potency to inhibit the growth of all bacterial strains tested.

Amphibians exist in microorganism-rich environment, and as a result they produce potent antimicrobial peptides as a defense.[17] Over the past two decades, Amphibian skin secretions have become a pivotal model for the discovery of new bioactive molecules with potential therapeutic activities, such as antimicrobial, antidiabetic, antineoplastic, analgesic and sleep-inducing properties, thus, attention has been increasingly focused upon Amphibian skin peptides as potential therapeutic agents. In general during injuries adrenergic receptors are stimulated by sympathetic nervous system and release various antimicrobial peptides (AMP) on to the surface of the skin.[18] The high levels of vasco-active peptides serve in defense against predators and antimicrobial activity against microorganisms.[4] Antibacterial sequences data base have been described more than 750 of eukaryotic peptides were isolated from amphibians and other organisms.[19,20]

It has been reported by some workers that the presence of cationic peptides in Amphibian skin secretions with their positive charges and amphipathic features may enhance the binding capability towards bacterial cells by electrostatic interactions and hence lead to the death of the bacteria.[22,21] Some skin secretions of Amphibian like African clawed frog have powerful antibiotic properties, they help in healing cuts and bruises and tropical poison dart frog can produce a pain killer stronger than morphine.[23]

Thus, from our present investigation our results report that the skin secretion and its extract have also got therapeutic properties as they have shown antibacterial property.

CONCLUSION

From our results it can be concluded that the Amphibian skin secretions have powerful antibacterial properties. The secretions of toads and frogs might also be of benefit to human health with its antibacterial properties. Further studies like purification and sequencing of these peptides are worthy and of immense importance.

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