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Anti-Bacterial Activity of Parotoid Gland Secretion and It's Extract of the Toad *Bufo melanostictus*

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Abstract

The present study was to investigate and assess the antibacterial activity of granular gland secretion and its extract of Indian toad *Bufo melanostictus* (Schneider). Amphibians like toads secrete antimicrobial secretions from outside their body into their environment, through skin pores and parotoid glands. The parotoid gland secretions and its extracts were collected from *Bufo melanostictus*. The parotoid glands were press gentle to release the secretions with the help of sterile forceps. The collected parotoid gland secretions and its extract was filtered-sterilized, freeze dried and subjected to antibacterial assay through well diffusion technique. In this method, on Asthana Hawkers agar medium, the plates were seeded with selectived bacterial strains i.e., *Escherichia coli*, *Klebsilla pneumonia*, *Staphylococcus aerius* and *Protious vulgaris* and Zone of Inhibition (ZOI) was measured. The zone of inhibition in gland secretion was maximum for *K. pneumonia* (38mm) and minimum against *E.coli* (24mm). An inhibition zone in parotoid gland extrac was observed maximum for *P. vaulgaris* (34mm) and minimum against *K. pneumonia* (28mm). An inhibition was observed with 40µl/ ml of parotoid gland secretion and its extract have showed nearest inhibition zones i.e. viz., 38mm, 32mm; 29mm, 24mm; 34mm, 33mm; 30mm and 28mm pathogenic bacteria respectively. Therefore we conclude that the toad parotoid gland secretion and its extract have the potential to be developed as a potent source for the development antibacterial agents used in the treatment of infectious diseases.

Keywords: *Antibacterial activity, B. melanostictus, parotoid gland, Zone of Inhibition, Klebsilla pneumonia, Escherecia coli, Staphylococcus aerius and Proteus vulgaris.*

1. Introduction:

Amphibians are most diversified vertebrates with environmental dependant body temperature and having glandular glands and moist skin. The skin of amphibians (frogs and toads) characterized by the presence of a pair of parotoid glands located between eyes and tympanum [1]. These gland secretions generally associated with chemical defense and against predators and microbial infection i.e. fungal and bacterial strains to protect themselves [2-6]. These gland secretions contain rich components like biogenic amines, peptides which have the ability to inhibit the growth of pathogenic microorganisms.

Bacteria are an important cause of human, animal and plant diseases. The treatment of bacterial diseases is quite not possible due to lack of potent bactericidal agents [7-10]. The toad parotoid gland secretions contain peptides which exhibit antimicrobial activity against gram posstive (+ve), gram negative (-ve) bacteria and as well as against yeast and protozoans too [12-15]. Many host defense peptides show high potency against bacteria and other potent biological activity [16-17]. The secretions of toad granular gland might also be of benefit to human health with its antibacterial, antiprotozoal, antidiabetic and other therapeutic properties [18-20].

2. Material & Methods:

The toads (7.0cm to 10 cm in length, weighed about 50 to 75 gm) were collected from the vicinity of hostel buildings of Kakatiya University. The parotoid glands were gently pressed to release the secretion with the help of sterile forceps. The secretions were collected into ice-jacketed containers. After collecting secretions, the gland was dissected out, blotted to free from blood clots and other adherent tissues and weighed to the

nearest milligram and processed for further analysis by the combination with methanol. The gland secretions and its extract were filtered-sterilized, freeze dried and subjected to antibacterial assay. All the qualitative and quantitative chemicals were supplied by Himeda Laboratories Pvt. Ltd. Mumbai.

3. Anti bacterial activity:

For antibacterial activity of four bacterial strains gram (–ve) bacteria and gram (+ve) bacteria (*Escherichia coli, Klebsiella pneumonia, Staphylococcus aerus* and *Proteous vulgaris*) are used. Bacterial subculture was swabbed on the surface of the solidified Asthana Hawkers agar medium (Mueller J. H. and Hinton J., 1941&1959) [21,22]. Now wells of 5mm diameter were made into agar medium with borer. Toad parotoid gland secretion and its extract (40µL) were filled in the wells. The culture was then incubated at 28^0C for 24h at room temperature until the bacterial growth in the petridish was homogeneous. The antibacterial activity was measured from the formation of clearing zone due to inhibition of parotid gland secretion & its extract around the treated area. Zone of inhibition was measured with Himedia antibiotic scale and experiment was repeated with three replicates. Inhibition zones were measured and tabulated.

4. Results:

The results obtained from the present investigation are presented in tab.1., plate.1 & 2, fig.1. The gland secretion showed highest inhibition (ZOI) against *K. pneumonia* (38mm) and lowest inhibition *E. coli* (24mm). The parotoid gland extract showed highest activity (ZOI) against *P. vulgarius* (34mm) and minimum zone of inhibition for *K. pneumonia* (28mm). While nearly similar activity of *E. coli* was observed showing lowest inhibition among four different species with gland secretion. *S. aureus* showed minor variation with gland secretion and its extracts (32mm & 30mm). *P. vulgaris* has shown highest inhibition among four bacterial species but less inhibited by gland secretion compared with gland extract. Both parotoid gland secretion and its extract have shown inhibition zones with nearest values like viz., 38mm, 28mm; 32mm, 30mm; 29mm, 34mm and 24mm, 33mm respectively against *Klebsilla pneumonia, Staphylococcus aerus, Proteus vulgaris* and *Escherichia coli*. Thus, from our present investigation it is evident that both the parotoid gland secretion and its extract have potency to inhibit the growth of all bacterial strains tested.

Tab.1.Antibacterial activity of parotoid gland secretion and its extract of *Bufo melanostictus*

Bacterial strain	**ZOI of Gland secretion**	**ZOI of Gland extract**
Escherichia coli	24mm	33mm
Klebsilla pneumoniae	38mm	28mm
Staphylococcus aerius	32mm	30mm
Proteus vulgaris	29mm	34mm

ZOI= Zone of Inhibition

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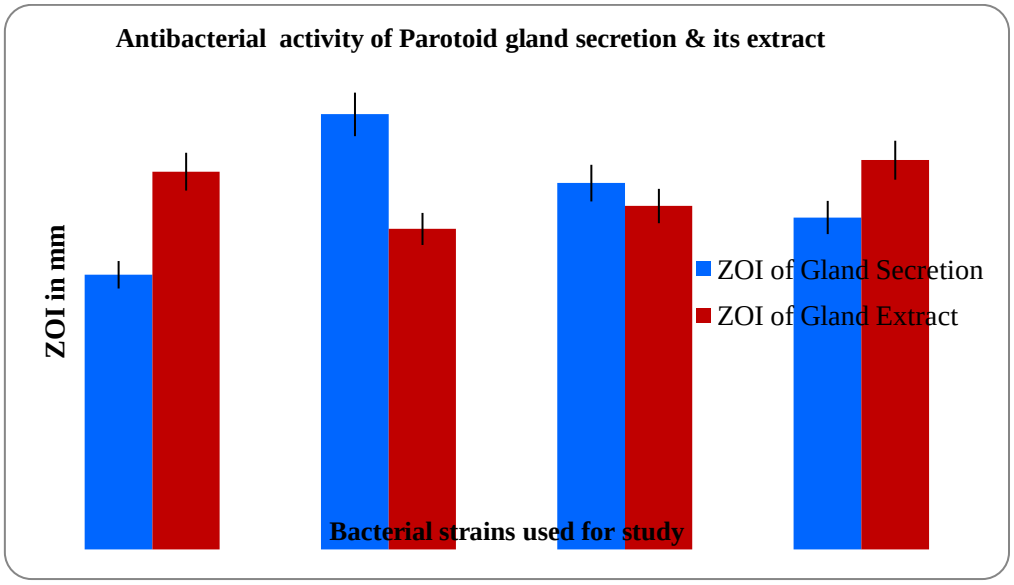

Fig.1. Antibacterial activity of parotoid gland secretion & its extract of *Bufo melanostictus*

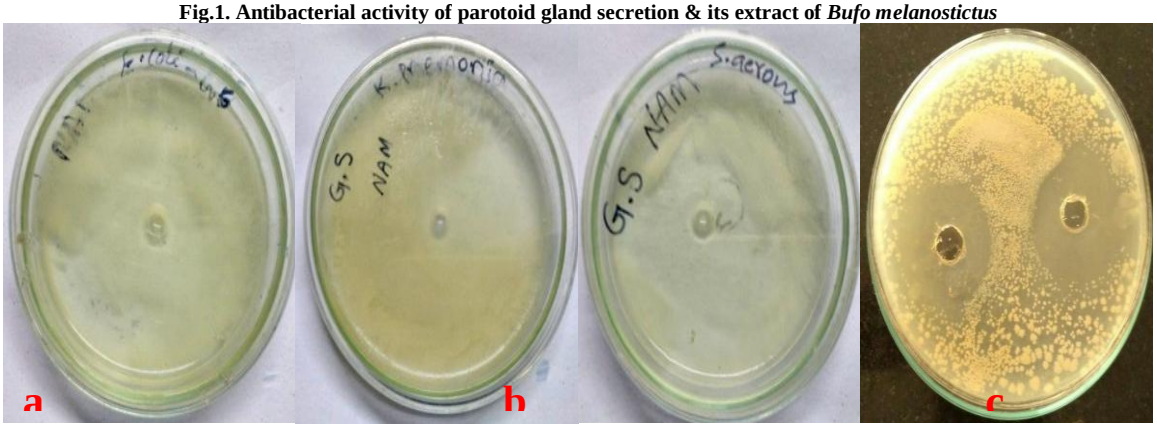

Plate.1. Antibacterial activity of parotoid gland secretion against a. *E. coli*, b. *K.*

Plate.2. Antibacterial activity of parotoid gland extract against a. *E. coli*, b. *K.*

5. Discussion:

Amphibians exist in microorganism-rich environment, and as a result they produce potent antimicrobial peptides as a defense [23]. Potential to be used as a source of antibacterial agents [04]. Amphibian skin is a remarkable organ that serves the multiple roles of fluid, balanced respiration and

transport of essentials for survival of individual and population. The antimicrobial peptides can protect the skin from skin-invasive pathogens is an important goal from a conservation biology of view in order to predict the survival of individual species of amphibian population decline [24]. The antibiotic peptide is synthesized in the gland before being cleaved by a protease releasing the inactive spacer peptide. When the animal is attacked, stimulated or sick, a second protease removes the spacer and the active peptide is secreted onto the skin or into the gut as required [25]. The antibiotic and anticancer activity is a result of the active peptide inducing alterations in the hydrophobic–hydrophilic seal of the cell membrane, effecting lysis of the bacterial or cancer cell [26-33].

A number of studies have also reported that the antimicrobial activity of various skin secretions derived from different anuran amphibians [04, 09-11, 19, 34]. Artika *et al*., (2015) [35] reported the chemical compounds like fatty acids tri, acontane, hepadecane, pregnane and myrtenol of the toad and frog skin secretions, having antibacterial activity and it was also reported that long chain unsaturated fatty acids found in frog skin secretion inhibited the activity of bacterial enoyl-acyl carrier protein reductase which is an essential component of bacterial fatty acid synthesis. Some skin secretions of amphibians like African clawed frog have powerful antibiotic properties; they help in healing cut and bruises and tropical poison dart frog can produce a pain killer stronger than morphin [36].

Thus our present investigation suggests that the antimicrobial peptides produced in the skin are indeed an important defense against skin pathogens and do affect survival of toad & frog populations.

6. Conclusion:

From our results it can be concluded that the parotoid gland secretion and its extracts of Indian common toad *B. melanostictus* 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.

Conflict of Interest:

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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