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Antibacterial activity of skin secretion of toad *Bufo melanostictus and* frog *Rana cyanophylictitus*

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Abstract
Many disease initiated by the Bacteria in mammals, therefore they may effect on immune system, that's why it is important to search for new types of antibacterial agents. The main purpose of this study is to test antibacterial activity of skin secretion derived from two species of anurans endemic found in India (Sirohi), *Bufo melanostictus* and *Rana cyanophylictitus*. The toads and frogs were induced to secret skin secretion without using any stimulant. The collected skin secretion was filter and sterilized and finally subjected to antibacterial assay. Results showed that skin secretion of some toads and frogs has antibacterial activity against the *Clarithromycin*. Some chemical compounds with high potential activity were detected in skin secretion include fatty acids, steroids and amines. Therefore, the toad and frog skin secretion has the potential to be developed as a source of antibacterial agents.

Keywords: antibacterial agents, *bufo melanostictus* and *rana cyanophylictitus*, *clarithromycin*, bacteria

Introduction
The global decline in amphibian's population is increasingly day by day. Habitat destruction, introduction of predators and direct exposure to toxins has been implicated in this decline [8-11]. Bacteria is an important cause of human, animal and plant disease. Treatment of bacteria disease is quite possible due to lack of antibacterial agents [15-17]. In stress condition amphibians secrete some specific chemicals from granular glands, which are usually dispersed throughout the dorsal surface [18-21]. These chemicals include alkaloids, biogenic amines, peptides and some specific proteins. These chemicals provide protection against many microorganisms [1-5]. In this way chemical secretion of toad and frog skin might also be as benefit to human health with its antibacterial, antiprotozoal and other therapeutic properties. Skin secretion of many anurans (frogs and toads) contains peptides with antibacterial activity [6-8]. now a day; scientists are now exploring the therapeutic potential of various toad and frog skin secretions and extracts. India is country with diverse geographical variation [9-12].

Materials and Methods
Animals were collected from Pindwara, Sirohi, India. Frogs and Toads were transferred first in a container full of water. No stimulators were given to the experimental animal. A simplest method was used to remove peptide or antibacterial skin material from animals. Animals were taken in hand and one by one their upper skin part was rubbed along with water. The processes were repeated 3-4 times with each animal. Water with skin extract was preserved in 50ml flask. It was followed by autoclave before use. The antibacterial activity assay was carried out using a modified method. As much as 50ml of each bacteria culture was mixed with warm Mueller Hinston agar medium (30.0% beet infusion,1.75%casein hydrolyses, 0.15% starch,1.7%agar, pH 7.0) followed by incubation until the medium solidified. Formation of clear zone around treated area shows the antibacterial activity of each sample. Ranges of diameter were observed around each sample it shows the antibacterial activity[21-24]. Holes were made to which the toad or frog skin secretion was applied[22-24]. 0.4mg (40ml), 1ml (40ml) was used as a control. The culture plates were put in incubator for 24h at 28℃.

Results and Discussion
We tested specimens of *Bufo melanostitus* and *Rana cyanophylictitu.* The average snout Vent Length (SVL) of the *Buf omelanostitus* was 22.3±4.8mm, with average body mass of 4.9±0.9g. The average SVL of the *Rana cyanophylictitus* was 39.7±1.8mm average body mass was 6.9±1.2g.
The average dry weight of skin secretion of *Bufo melanostictus* will be 264±266mg. On the other hand, the average dry weight of *Rana cyanophylictitus* skin secretion were 123±171mg.
Antibacterial Activity of Toad and Frog Skin Secretion
Antibacterial activity was shown by some skin secretions of *Bufo melanostitus* and *Rana cyanophylictitus*. The average diameter of the clear zone of frog skin secretions of *Bufo melanostitus*against fungus *Clarithromycin* was 12.5±2.9mm, while that of Rana cyanophylictitus was 8.9±4.3mm.
There are many methods to collect the skin secretion of frog, but the frog and toad were sacrificed for their skin. The components of the skin secretion are required for bioactivity assay and chemical analysis and not every component of toad or frog integument. Frog skin secret different types of compounds such as fatty acids, amines, peptides, proteins, alkaloids and steroids. Fatty acids and their derivatives have

been reported to possess antibacterial activity. Fatty acids target the cell membrane of bacteria. Fatty acids cause an increase in membrane fluidity that leads to conformational changes in membrane proteins, the release of intracellular components, cytoplasmic disorder, cell disintegration and eventually cell death.

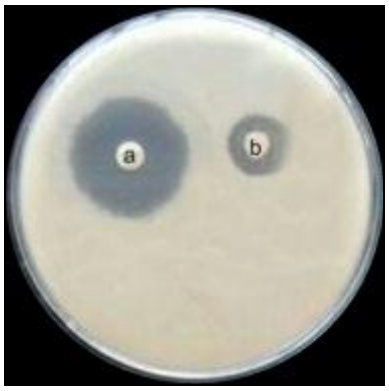

Fig 1: The skin secretions of frog were compared A=*Clarithromycin*, B= *Buf omelanostitus.*The formation of clear zone around the treated area indicated antibacterial activity.

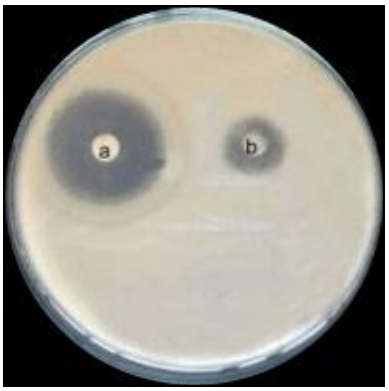

Fig 2: The skin secretions of frog was compared A=*Clarithromycin*, B=*Ran acyanophylictitus.* The formation of clear zone around the treated area indicated antibacterial activity.

Fig 3: The skin secretions of frog was compared A=*Bufo melanostitus.* B=*Rana cyanophylictitus.* The formation of clear zone around the treated area indicated antibacterial activity. Toad skin (*Bufo melanostitus)* showed the highest antibacterial activity as compared to frog (*Rana cyanophylictitus).*

Conclusion

We conclude that release of skin secretion of toad, *Bufo melanostictus* and *Rana cyanophylictitus* has the potential to develop as a source of antibacterial agents.

References

1. Levy S. Antibiotic resistance: Origins, evolution, selection, and spread. New York: John Wiley, 1997.
2. Chicarelli-Robinson M, Gibbons S, McNicholas C. Plants and microbes as complementary sources of chemical diversity for drug discovery. In: Wrigley S, Hayes M, Thomas R, Chrystal E, eds. Phytochemical diversity: A source of new industrial products. London: Royal Society of Chemistry, 1997; 57-83.
3. Georgopapadakou NH, Walsh TJ. Antibacterial agents: Chemotherapeutic targets and immunologic strategies. Antimicrob Agents Chemother. 1996; 40(2):279-291.
4. Walsh C, Wright G. Introduction: Antibiotic resistance. Chem Rev. 2005; 105(2):391-394.
5. Harris A, Torres-Viera C, Venkataraman L, DeGirolami P, Samore M, Carmeli Y. Epidemiology and clinical outcomes of patients with multiresistant Pseudomonas aeruginosa. Clin Infect Dis. 1999; 28:1128-1133.
6. World Health Organization (WHO). The world health report 2000: Life in the 21st century. A vision for all. Geneva: WHO, 2000.
7. Angeletti L, Agrimi U, Curia C, French D, Mariani-Costantini R. Healing rituals and sacred serpents. Lancet. 1992; 340:223-225.
8. Hancock R, Patrzykat A. Clinical development of cationic antimicrobial peptides: From natural to novel antibiotics. Curr Drug Targets Infect Disord. 2002; 2:79-83.
9. Tyler MJ, Stone DJM, Bowie JH.A novel method for the release and collection of dermal, glandular secretions from the skin of frogs. J Pharmacol Toxicol Methods. 1992; 28(4):199-200.
10. Nicolas P, Mor A. Peptides as weapons against microorganisms in the chemical defense system of vertebrates. AnnuRev Microbiol. 1995; 49:277-304.
11. Clarke B. The natural history of amphibian skin secretions, their normal functioning and potential medical applications.Biol Rev CambPhilos Soc. 1997; 72:365-379.
12. Lazarus L, Attila M. The toad, ugly and venomous, wears yet a precious jewel in his skin. ProgNeurobiol. 1993; 41:473-507.
13. Simmaco M, Mangoni M, Boman A, Barra D. Experimental infections in Rana esculenta with Aeromonas hydrophila: A molecular mechanism for the control of the natural flora. Scand J Immunol. 1998; 48:357-363.
14. Conlon J. The therapeutic potential of antimicrobial peptides from frog skin. Rev Microbiol. 2004; 15:17-25.
15. Che Q, Zhou Y, Yang H, Li J, Xu X, Lai R. A novel antimicrobial peptide from amphibian skin secretions of Odorrana grahami. Peptides. 2008; 29(4):529-535.
16. Mangoni ML, Miele R, Renda TG, Barra D, Simmaco M. The synthesis of antimicrobial peptides in the skin of

Rana esculenta is stimulated by microorganisms. FASEB J. 2001; 15:1431-1432.
17. Pal T, Abraham B, Sonnevend A, Dimitrov T, John A. Brevinin-1BYa: A naturally occurring peptide from frog skin with broad-spectrum antibacterial and antifungal properties. Int J Antimicrob Agents. 2006; 27:525-529.
18. Conlon JM, Kolodziejek J, Nowotny N. Antimicrobial peptides from ranid frogs: Taxonomic and phylogenetic markers and a potential source of new therapeutic agents. Biochim Biophys Acta. 2004; 1696(1):1-14.
19. Clark D, Durell S, Maloy W, Zasloff M. Ranalexin, a novel antimicrobial peptide from bullfrog (Rana catesbeiana) skin, structurally related to the bacterial antibiotic, polymyxin. J Biochem. 1994; 269:10849-10855.
20. Gabay JE. Ubiquitous natural antibiotics. Science. 1994; 264(5157):373-374.
21. Kim JB, Iwamuro S, Knoop FC, Conlon JM. Antimicrobial peptides from the skin of the Japanese mountain brown frog, Rana ornativentris. J Pept Res. 2001; 58(5):349-356.
22. Isaacson T, Soto A, Iwamuro S, Knoop FC, Conlon JM. Antimicrobial peptides with atypical structural features from the skin of the Japanese brown frog Rana japonica. Peptides. 2002; 23(3):419-425.
23. Ng TB, Cheung RCF, Wong JH, Wang Y, IP DTM, Wan DCC, Xia J. Antiviral activities of whey proteins. Appl. Microbiol. Biotechnol, 2015, 6997-7008.