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

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Abstract
Many disease initiated by the Fungal in mammals, therefore they may effect on immune system, that's why it is important to search for new types of antifungal agents. The main purpose of this study is to test antifungal activity of skin secretion derived from two species of anurans endemic found in India (sirohi), *Bufo melanostictus* and *Ran acyanophylictitus*. 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 antifungal assay. Results showed that skin secretion of some toads and frogs has antifungal activity against the *Penicillium*. 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 antifungal agents.

Keywords: antifungal agents, *bufomelanostictus* and *ranacyanophylictitus*, *penicillium* fungal

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. Fungal is an important cause of human, animal and plant disease. Treatment of fungal disease is quite possible due to lack of antifungal agents. In stress condition amphibians secrete some specific chemicals from granular glands, which are usually dispersed throughout the dorsal surface. 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, antifungal, antiprotozoal and other therapeutic properties. Skin secretion of many anurans (frogs and toads) contains peptides with antifungal 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 stimulant was given to the experimental animals. The simplest method was used to remove peptide or antifungal skin material from animals. Animals were taken in hand one by one and 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 antifungal activity assay was carried out using a modified method. As much as 50ml of each fungal culture was mixed with warm Mueller Hinston agar medium (30.0% beet infusion,1.75%casein hydrolysate, 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 antifungal activity of each sample. Ranges of diameter were observed around each sample it shows the antifungal activity. Holes were made to which the toad or frog skin secretion was applied. 0.4mg (40ml), 1ml (40ml) was used as a control. The culture plates were put in incubator for 24h at 28℃.

Results and Discussion
Toad and Frog Skin Secretion
We tested specimens of *Bufomelanostitus* and *Ranacyanophylictitu.* The average snout Vent Lenth(SVL) of the *Bufomelanostitus* was 28.3±5.8mm, with average body mass of 5.9±0.6g. The average SVL of the *Ranacyanophylictitus* was 44.7±1.5mm average body mass was 7.9±0.6g.
The average dry weight of skin secretion of *Bufomelanostictus* will be 264±266mg. On the other hand, the average dry weight of *Ranacyanophylictitus* skin secretion was 148±221mg.
Antifungal Activity of Toad and Frog Skin Secretion
Antifungal 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 *Penicillium* was 14.5±2.9mm, while that of *Rana cyanophlyctis* was 9.9±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 antifungal activity. Fatty acids target the cell membrane of fungal. 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 frogwere compared A=*Penicillium*, C= *Bufomelanostitus.*The formation of clear zone around the treated area indicated antifungal activity.

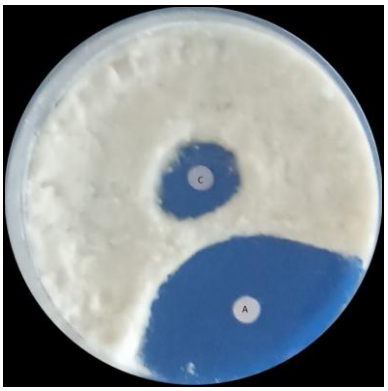

Fig.2: The skin secretions of frog were compared A=*Penicillium*, C=*Ranacyanophylictitus.*The formation of clear zone around the treated area indicated antifungal activity.

Fig 3: The skin secretions of frog and toad were compared B=*Bufomelanostitus.* C=*Ranacyanophylictitus.*The formation of clear zone around the treated area indicated antifungal activity. Toad skin secretion (*Bufomelanostitus)* showed the highest antifungal activity as compared to frog skin secretion (*Ranacyanophylictitus).*

Conclusion

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

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