Antimicrobial Activity in the Skin Secretion of *Bufo viridis* (Laurenti, 1768)

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Abstract. - In this study, antimicrobial activity of various extracts prepared from *Bufo viridis* skin secretion were tested against the microorganisms by disk diffusion method. *Escherichia coli* ATTC 10536, *Listeria monocytogenes* ATCC 19117, *Klebsiella pneumoniae* UC57, *Salmonella typhi* ATCC 19430, *Staphylococcus aureus* ATCC 6538P, *Mycobacterium smegmatis* CCM 2067, *Rhodotorula rubra* and *Saccharomyces cerevisae* ATCC 9763 were used. According to our results, the extracts prepared from *Bufo viridis* skin secretion have high antimicrobial activity against the tested microorganisms.

Key words. - *Bufo viridis*, Amphibia, antimicrobial activity, skin secretion.

Introduction

Amphibians have skin glands producing mucous and poison. Amphibians have been studied and have attracted special attention from a toxicological point of view. Various substances with antimicrobial activity have been isolated from skin secretions of amphibian species (Dapson, 1970; Croce et al., 1973; Dapson et al., 1973; Preusser et al., 1975; Cevikbas, 1978). Several toxins in amphibian poisons have been used as experimental tools and contributed to significant progress in physiology. Some toxins (Batrachotoxins) specifically block the inactivation of the voltage regulated Na^+ channels in nerve and muscle cells, which causes a massive inflow of Na^+. The cells become irreversibly depolarized, which, among other things, produces heart arrhythmia and respiratory failure and finally cardiac insufficiency. In humans, some amphibian toxins (Bufotenin) produce symptoms similar to those of LSD (Lutz, 1971; Edstrom, 1992). In previous studies, some skin secretions showed remarkable cytotoxic activity against eukaryotic cells (Kolbe et al., 1993; Sanna et al. 1993).
The aim of the this study is to test the antimicrobial activity of *Bufo viridis* skin secretions against Gram-positive, Gram-negative bacteria and yeast cultures for future possible use in providing pharmacological tools for the study of new drugs and aid in benefitting human health.

Materials and Methods

Specimens of *Bufo viridis* were collected from different regions in Bursa, Turkey in March 1998. Collected frogs were brought to the laboratory and kept in an aquarium. Before experimentation, the frogs were washed first with tap water and then with distilled water. They were placed for 3-5 minutes in a glass jar containing a piece of cotton soaked with ether to stimulate skin secretions. The secretion accumulated on the skin was obtained by scraping the body of the animals with a spatula. The foamy secretion thus obtained was placed in a tube, left in an 80°C water bath for 30 min and centrifuged at 5,500 rev/min for 30 min. After centrifugation, the precipitate was used in the experiments. Before using in the experiments, the precipitate was diluted with distilled water 0.1 M HCl, 0.1 M NH_4OH, and 1 M phosphate buffers (pH: 4 and pH: 7).

In this study, *Escherichia coli* ATTC 10536, *Listeria monocytogenes* ATCC 19117, *Klebsiella pneumoniae* UC57, *Salmonella typhi* ATCC 19430, *Staphylococcus aureus* ATCC 6538P, *Mycobacterium smegmatis* CCM 2067 bacteria cultures and *Rhodotorula rubra* and *Saccharomyces cerevisae* ATCC 9763 yeast cultures were used.

In vitro antimicrobial activity studies were carried out by the Agar-Disc Diffusion Method. Mueller Hinton Agar (Oxoid) was preferred as the most suitable medium for antimicrobial activity studies. Each extract was implemented into a sterile disc in varying concentrations starting from 20 µl. Each disc was 6 mm in diameter.

Bacteria and yeast cultures were suspended in 4-5 ml Brain Heart Infusion Broth (Oxoid) and Malt Extract Broth (Difco). Bacteria were incubated in 37°C for 2-5 hours. Yeast cultures were incubated in 30°C for 5-7 hours. A visible turbidity was obtained at the end of this time. The turbidity of bacterial suspension was adjusted

Table 1. Antimicrobial activity of various extracts of *Bufo viridis* skin secretions on microorganisms.

Microorganisms / solvents	0.1 N HCl	0.1 N NH_4OH	Phosphate Buffer pH: 4	Phosphate Buffer pH: 7	Distilled water
Escherichia coli ATCC 10356	++	+++	(+)	(+)	++
Listeria monocytogenes ATCC 19117	+++	+++	++	++	++
Staphylococcus aureus ATCC 6538P	+++	++	++	++	+++
Klebsiella pneumoniae UC57	+++	++	+++	++	+++
Salmonella thyphi ATCC 19430	+++	++	+++	++	++
Mycobacterium smegmatis CCM 2067	+++	+++	++	++	+++
Rhodotorula rubra	+++	+++	+++	+++	++
Saccharomyces cerevisiae ATCC 9730	+++	+++	+++	+++	+++

(+) : Inhibition zone less than 1 mm surrounding the 6 mm paper disk.
+ : Inhibition less than
++ : Inhibition comparable to
+++: Inhibition more than 10 µg penicillin or sulconazole / disk; Inhibition zones of references : 12-16 mm diameter.

according to Macfarland Standard Tube [0,5] with physiologic serum and inoculation performed. Prepared bacterial suspension was mixed with a sterile applicator and excess fluid of applicator was removed by rotating the applicator to one side of the tube. We streaked the entire Mueller Hinton Agar surface in three different directions by rotating the plate 60° angles after each streaking. Yeast cultures were inoculated into Muller Hinton Agar (10^2 cfu/ml). All petri dishes after inoculation were allowed to dry for 15-20 min at room temperature (bacteria at 35°C and yeast at 30°C). Inhibition zone diameters were measured after 24-48 hours (Collins et al. 1987, NCCLS 1993). In addition, continued only solvent was used as negative control disc and antibiotic penicillin and sulcanozole discs were used as references. Experiments were repeated three times and results were expressed as average values.

Results and Discussion

Antimicrobial activity effects of five different extracts, which were prepared by using distilled water, 0.1 N HCl, 0.1 N NH_4OH, 1 M phosphate buffers (pH: 4 and pH: 7), were obtained from the skin secretions of *Bufo viridis* against bacteria and yeast cultures, results are given in Table 1.

According to our findings, all the extracts of skin secretion against the yeast cultures exhibit higher antimicrobial activity than that of a compared antibiotic. The 0.1 M HCl extract shows more effect than that of the other extracts against bacteria. 1 M phosphate buffer (pH: 4 and 7) extracts exhibited minor effects against *Escherichia coli*. However, phosphate buffer (pH: 4 and 7) extracts exhibited strong effects against the other bacteria. It can be said that the active substance obtained from *Bufo viridis* skin secretion dissolves easily in the 0.1 M HCl and has high antimicrobial activity as a consequence. It has been reported that sensitivity of the microorganisms to the chemotherapeutic agents changes from strain to strain (Cetin et al., 1989). Our results are in agreement with the other authors' results.

Inhibition zone diameters around the control disc were measured as 0-1 mm. In this study, antimicrobial effects of the prepared extracts on the tested microorganisms were determined by using different solvents.

Croce et al. (1973) investigated antimicrobial activity of skin secretions from *Bombina variegata pachypus*. They homogenized skin secretion with phosphate buffers (pH: 4 and 7) 1 M HCl, 1 M NH_4OH and distilled water. These homogenates show high antimicrobial activity against *Staphylococcus aureus* but they do not show any antimicrobial effect against *Aspergillus niger*, *Trichophyton mentagrophytes* ATCC 8757 and *Candida albicans*.

Cevikbas (1978) examined antibacterial activity in the skin secretions of *Rana ridibunda*. The author reported that skin secretion of *Rana ridibunda* shows antibacterial activity at different levels. However, in our

present study, skin secretions of *Bufo viridis* against the yeast cultures shows more antimicrobial activity than that of the bacterial cultures. Our findings parallel those reported in the above studies. In Amphibia, antimicrobial activity of skin secretions differ at both the generic and specific levels.

Although the antimicrobial activity of skin secretions from *Bufo viridis*, *Bufo vulgaris*, *Salamandra maculosa* and *Salamandra atra* were determined (Pavan, 1962; Pavan and Nascimbene, 1948), antimicrobial activity of skin secretion from *Bufo marinus*, *Triturus,* and *Xenopus* were not observed (Preusser et al., 1975; Kolbe et al., 1993; Ozeti and Yilmaz, 1994). Antiyeast activity observed in our study was not observed in Croce's et al. study (1973). Our results show that skin secretion components from *Bufo viridis* may be different from *Bombina variegata pachymus*.

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