Exploring Snake Venoms For Novel Antimicrobial Agents

Maria Tosounidou[1],Vicky Nicolaidou[2], Yiannis Sarigiannis[1], Tolis Panayi[1&2]
[1]Department of Health Sciences, School of Life and Health Sciences, University of Nicosia, Cyprus
[2]Department of Life Sciences, School of Life and Health Sciences, University of Nicosia, Cyprus

Introduction/objectives/aim

Antibiotic resistance is developing into a major health issue worldwide. As a result, alternative antimicrobials are being investigated. Snake venoms are a complex cocktail of peptides and proteins, with remarkable potential for novel discoveries in this field. Here we present some preliminary results for the antimicrobial activity of crude snake venoms against clinically relevant bacterial genera.

Results

Screening for antimicrobial activity

Lyophilised Venom from the following snake species was resuspended in Phosphate Buffered saline (PBS): *Naja Pallida, Pseudohaje Goldii, Naja Katiensis, Naja Melanoleuca, Naja Nigricincta, Gaboon Viper, Macrovipera Lebetina Lebetina.* These were screened against the bacterial species *Escherichia coli* MG1655, ATCC-700926 *Staphylococcus epidermidis* and ATCC-14990 *Neisseria subflava* ATCC-49275. Bacterial lawns were created, the crude venom spotted on at different concentrations, incubated at appropriate conditions and evaluated for growth inhibition (**Figure 1**).

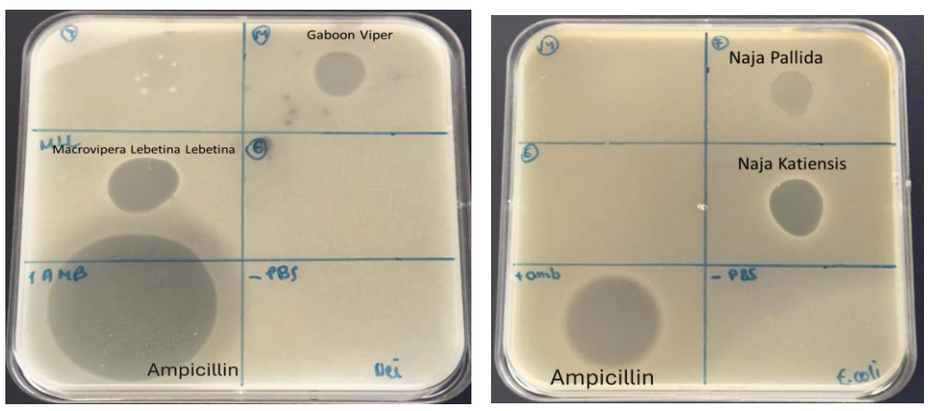

Figure 1: The antimicrobial effects of venoms studied using the spot method on bacterial lawns

This initial screening of the venom effect indicated antimicrobial activity in four out of the seven venoms against all three bacterial strains at 325-675µg per spot. Ampicillin at a concentration of 100µg/ml was used as positive control (**Table 1**).

Table 1: Minimum inhibitory concentration using Spot tests per 50µl/spot

Snake Venom	*E.coli*	*S. epidermidis*	*N. sublava*
Naja Melanoleuca	n/a	n/a	n/a
Naja Pallida	325µg	325µg	325µg
Naja Katiensis	325µg	325µg	325µg
Naja Nigricincta	n/a	n/a	n/a
Pseudohaje Goldii	n/a	n/a	n/a
Gaboon Viper	675µg	675µg	675µg
Macrovipera Lebetina Lebetina	375µg	375µg	375µg

Well diffusion

Antimicrobial activity was also assessed via the well method. Bacterial lawns were created on solid media, 6mm wells punched into them and venom subsequently added and observed following incubation. Interestingly, with this method, only the venoms of *Gaboon Viper* and *Macrovipera Lebetina Lebetina* showed antimicrobial action with the same concentrations previously used (**Figure 2**).

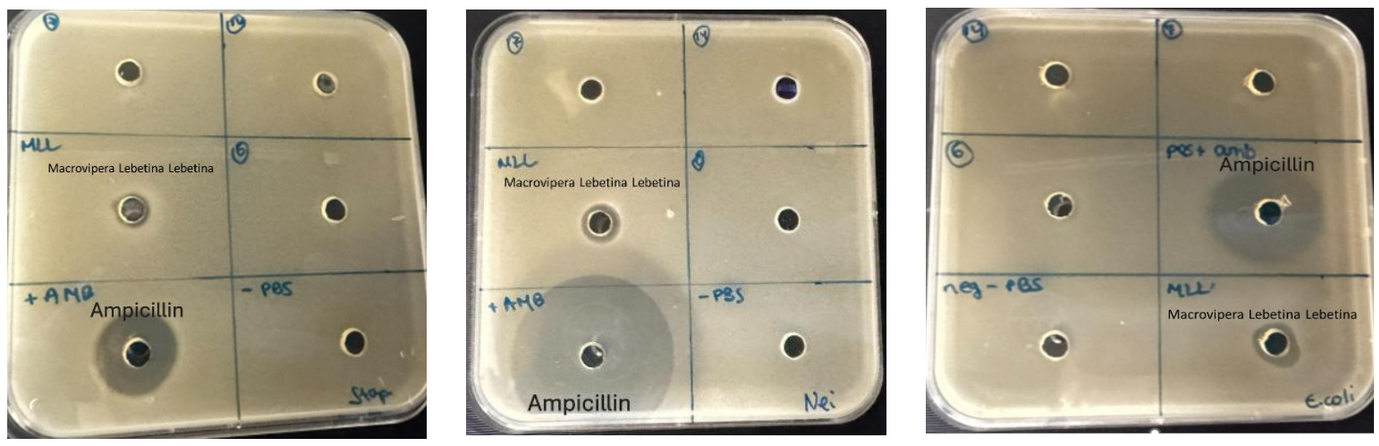

Figure 2: The antimicrobial effects of venoms against bacterial species using well diffusion.

Figure 2 depicts the antimicrobial activity of the venom of *Macrovipera Lebetina Lebetina* at a concentration of 375µg per well which was able to create inhibition zones 4-6mm in diameter in *S. epidermidis* (left), *N. subflava* (middle) and *E. coli* (right). The venom of *Gaboon Viper* was also able to create zones of inhibition of 4mm against *N. subflava* at a concentration of 625µg per well. Ampicillin at a concentration of 100µg/ml was used as positive control.

Microdilutions

The antimicrobial activity of the venoms was also assessed via the microdilution method. Crude venom was added at various concentrations in exponentially growing cultures in a 96-well plate and incubated at 37°C and then the bacterial growth measured via plate reader at 600nm (**Figure 3**).

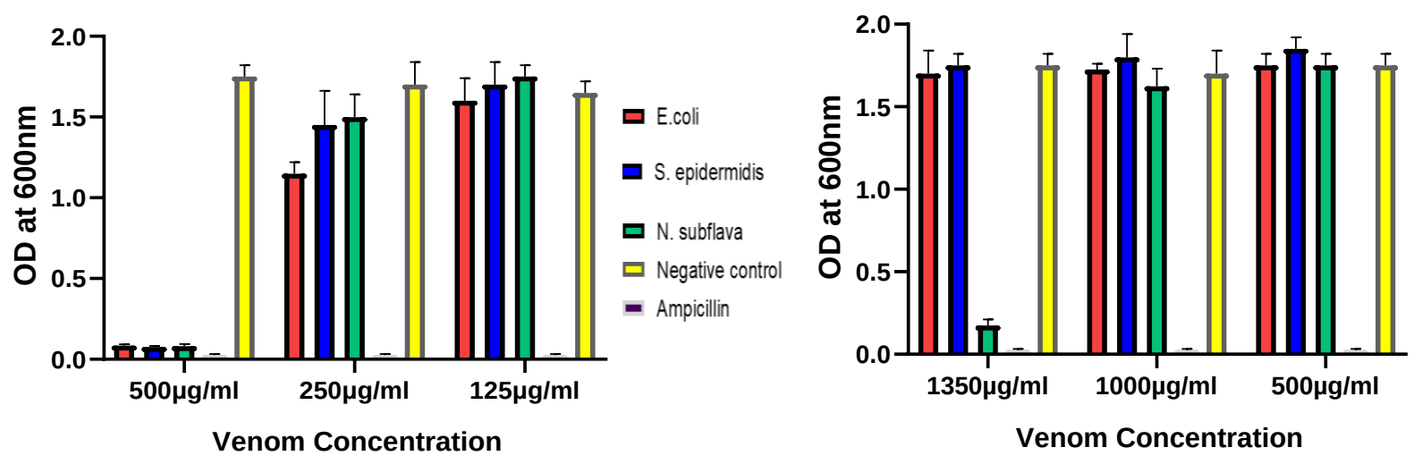

Figure 3: The antimicrobial effects of venoms against bacterial species using the microdilution method

Figure 3 presents that the venom of *Macrovipera Lebetina Lebetina* was able to cause inhibition of all three bacterial species at 500ug/mL. *Gaboon Viper* venom displayed inhibitory zone of 4mm only against *N. subflava*. In all cases ampicillin was used at a concentration of 100µg/ml as a positive control.

Conclusions

- The initial screening indicated **that 4 of the 7 venoms screened exhibited antimicrobial activity**.
- However, screening using the well diffusion method, very commonly used to assess venom antimicrobial action, indicated a narrower antimicrobial profile.
- This can be attributed to the fact that crude snake venom is a cocktail of peptides and proteins with various polarities, sizes, and shapes, and some of these components **might be unable** to diffuse through the pores of the agar and exhibit any antimicrobial activity.
- In addition, the venoms mostly appear to be less effective in liquid cultures **in contrast with the venom of *Macrovipera Lebetina Lebetina***.
- It has been reported that the composition of growth media, in combination with the bacterial metabolites can deactivate AMP components present in venom and therefore **some venoms tend to exhibit higher antimicrobial activity in solid media**.
- Therefore, further investigation is needed for the venoms that exhibited higher antimicrobial potential.

References

Ahmad, M. and Khan, A.U. (2019). Global economic impact of antibiotic resistance: A review. *Journal of Global Antimicrobial Resistance*, 19. doi:https://doi.org/10.1016/j.jgar.2019.05.024

Alam, Md.I., R, O., MA, A., H, Q. and O, A. (2019). Therapeutic potential of snake venoms as antimicrobial agents. *Frontiers in Drug, Chemistry and Clinical Research*, 2(4). doi:https://doi.org/10.15761/fdccr.1000136

Bocuertani, Awatef, Chloé Mollet, Yasmine Boughanmi, Harold de Pomyers, Amor Mosbah, Hadda-Imene Ouzari, Ameur Cherif, Didier Gigmes, Marc Maresca, et Kamel Mabrouk. « Screening of antimicrobial activity in venom: Exploring key parameters ». Toxicon 251 (28 novembre 2024): 108135. https://doi.org/10.1016/j.toxicon.2024.108135ian, A. and Hus, K.K. (2019). Antibacterial properties of snake venom components. *Chemical Papers*, 74(2), pp.407–419. doi:https://doi.org/10.1007/s11696-019-00939-y.

 Contact information: panayi.t@unic.ac.cy