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Research Article

# Screening of Active Biomolecules from the Venom of the Moroccan Viper *Daboia mauritanica*

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## Abstract

*Daboia mauritanica* (*Dm*) is one of the most medically important vipers that can be found in North African countries including Morocco. Although known for the haemorrhagic, anticoagulant, myotoxic and cytotoxic effects that it generates, the venom of *Dm* contains a large variety of active biomolecules whose activity has not yet been explored. The objective of this study is to perform a preliminary proteomic characterization of *Dm* venom in order to detect biomolecules with therapeutic potential. The purification and fractionation of the crude venom were performed by chromatographic techniques (gel filtration, reverse phase HPLC). The fractions obtained by RP-HPLC were then subjected to a trypsin digestion followed by mass spectrometry (QTof LC/MS/MS), the results of which were analyzed by the SwissProt database for the identification of the molecules. The results of the biochemical analysis of the crude venom showed a great complexity in terms of protein composition, the majority of which is of high molecular weight. The analysis by the data bank showed that the proteins of the venom of *Dm* have a great similarity with the proteins of other venoms thus allowing the classification of these proteins in known families. These results have revealed that the venom of *Dm* is composed inter alia of molecules whose therapeutic potential has not yet been explored.

***Keywords:*** *Viper; Daboia mauritanica; Venom; Protein*

## Abbreviations

HPLC: High Performance Liquid Chromatography; *Dm: Daboia mauritanica*; TFA: Trifluoroacetic Acid

## Introduction

*Daboia mauritanica* is one of the 7 species representing the Viperidae family in Morocco and is considered to be the main cause of Ophidian envenomations in Morocco [1].

Viperidae are known for the molecular complexity of their venoms containing both proteins of high molecular weight including enzymes such as metalloproteases and phospholipases A2 but also small molecular weight proteins such as cytotoxins and neurotoxins. These proteins, when released into the body cause systemic damage mainly on the haemostatic system usually causing haemorrhages but can also induce edema or neurological disturbances [2].

It is agreed that the pathological manifestations of ophidian venoms are probably due to a synergistic effect between the various enzymes and toxins they encompass [3]. For this reason, today, these molecules arouse particular interest in their individual mode of action specifically for their potential in the treatment of inflammatory diseases, bacterial and especially in the treatment of cancer.

That said, although there has been some work focused on the composition of the venom of the Moroccan viper *Daboia mauritanica*, its therapeutic potential has not yet been explored.

## Aim of the Study

Our work aims to not only perform an exhaustive characterization of the venom of Daboia mauritanica allowing us to establish an integral proteomic profile, but also to possibly identify molecules that we will be able to test for their therapeutic potential.

## Materials and Methods

### Biological material: Venom

The venom of the viper *Daboia mauritanica* has been extracted at the animal factory of the Institut Pasteur of Morocco at Tit Mellil from 10 adult snakes collected in Guelmim. The batches of venom were then pooled, centrifuged, lyophilized and stored at -20°C until use.

### Gel filtration chromatography

The venom of *Daboia mauritanica* (8 mg) was fractionated using a column-mounted Sephadex G-75 gel (GE Healthcare, 1.6 x 100 cm) equilibrated with ammonium acetate (1 M, pH 7.4). The elution of the proteins was carried out with the same equilibration buffer at a flow rate of 24 ml/h.

### Trypsic digestion

The pure peptides (0.5 mg) were dissolved in 25 µl of ammonium bicarbonate (0.1 M, pH 7.0). 25 µl of TFA and 1µl of DDT were added and incubated at 90°C for 20 minutes. 4 µl of 0.1 M IAM were then added and incubated for 1h at room temperature. 1 µl of DDT was added and incubated for 1 hour at room temperature. The peptides were then diluted with 300 µl of water and 100 µl of 0.1 M ammonium bicarbonate, digested with Trypsin and reduced by adding 1 µl of TFA.

### Liquid Chromatography / Mass Spectrometry

On-line LC / MS analysis of venom samples dissolved in 0.1% TFA at a concentration of 1mg/mL was performed on a C8 analytical column (75 µm x 43 mm, 5 µm, 300 Å) with the solvent A (0.1% TFA) and solvent B (90% acetonitrile in 0.1% TFA) at a flow rate of 0.45µl/min. Solvent delivery and gradient formation were 3% gradient 0 - 80% acetonitrile/0.1% TFA for 19 minutes. The electrospray mass spectra were performed by a PE-SCIEX API 300 LC/MS/MS system with an Ionspray atmospheric pressure ionization source. The samples (1 µl) were manually injected into the LC/MS system and analyzed in positive ion mode.

### Data analysis

The MS/MS analysis and the N-terminal sequence were directly attributed by BLAST analysis to a previously reported protein or a known family of proteins (Http://www.ncbi.nlm.nih.gov/BLAST) [4].

## Results

### Gel filtration chromatography

The analysis of the *Daboia mauritanica* venom by gel filtration chromatography shows a profile with six peaks of different sizes (Figure 1).

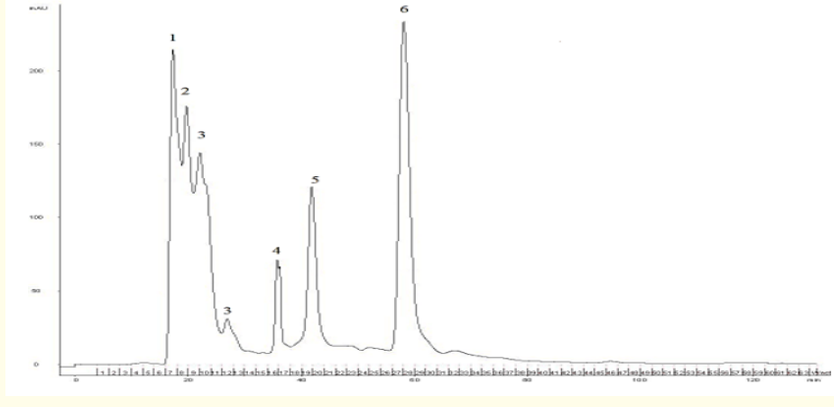


***Figure 1:*** *Gel Filtration chromatogram of the Daboia mauritanica venom (Sephadex G75, Ammonium acetate 1M pH7.4, flow rate of 4 ml/min).*

The majority of the peaks were eluted as early as the first 20 minutes of analysis, which may indicate that the venom contains high molecular weight proteins.

## HPLC fractionation

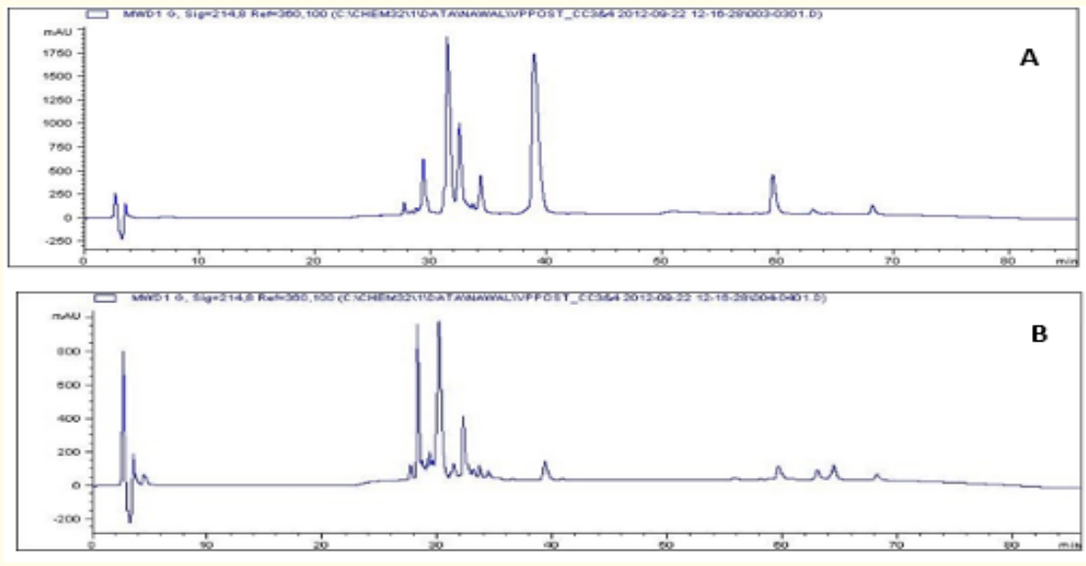


***Figure 2:*** *Reverse-phase chromatogram for Peak 1(A) and 2(B) from size-exclusion chromatography of the Daboia mauritanica crude venom.*

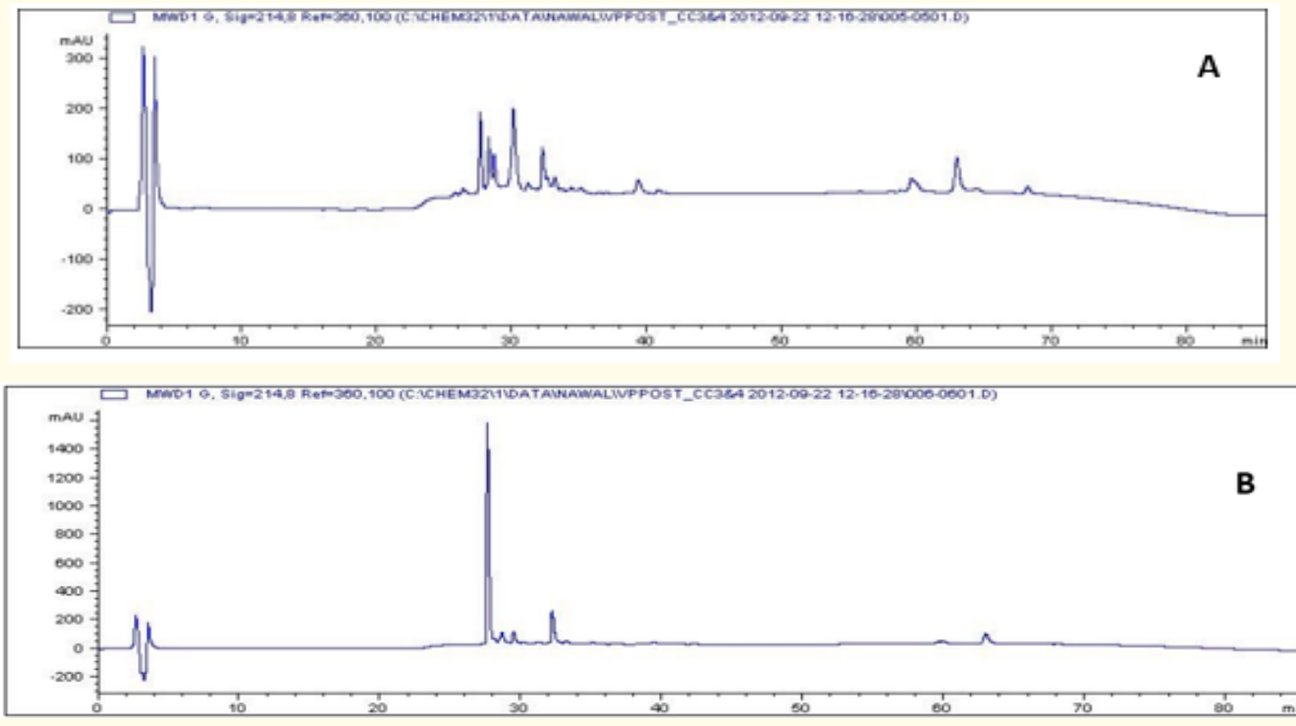


***Figure 3:*** *Reverse-phase chromatogram for Peak 3(A) and 4(B) from size-exclusion chromatography of the Daboia mauritanica crude venom.*

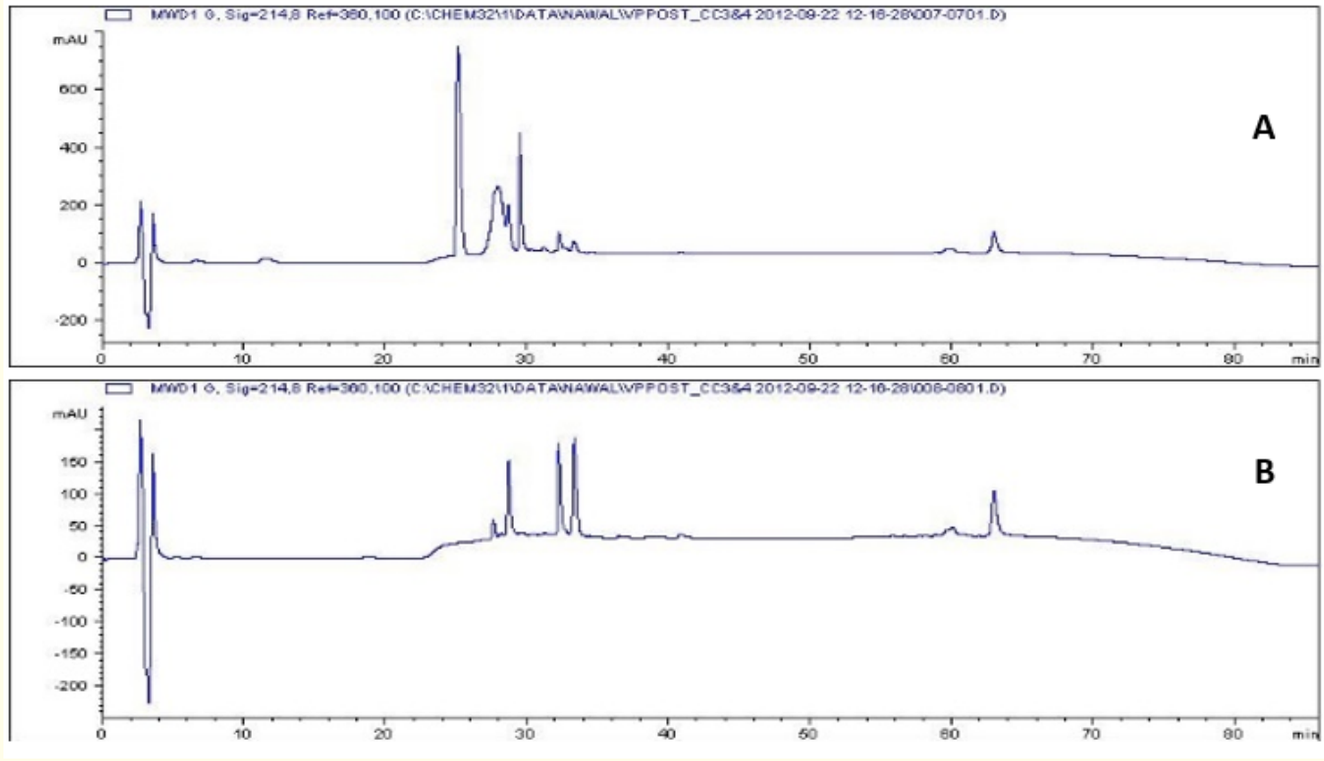


***Figure 4:*** *Reverse-phase chromatogram for Peak 5(A) and 6(B) from size-exclusion of the Daboia mauritanica crude venom.*

### Identification of the molecules of the Daboia mauritanica venom

The results of the mass spectrometry analysis allowed us, after screening in the BLAST database, to determine the protein composition of the venom of *Dm*. The table 1 below gives a non-exhaustive list of the *Dm* venom proteins paired with those of homologous species classified according to the fractions obtained by gel filtration chromatography.

## Discussion and Conclusion

Ophidian venoms are often compared to a molecular cocktail because of their composition in a variety of different molecules in terms of structure and function. This motivated several research projects, including ours, to pursue the purification of ophidian biomolecules for therapeutic interest [5].

The objective of this study was to perform a biochemical characterization of the components of the venom of *Daboia mauritanica* in order to identify molecules with therapeutic properties.

Our results showed that the venom of the Moroccan viper *Daboia mauritanica* presents a great complexity in terms of protein composition in such a way that the majority of proteins found were of a high molecular weight including enzymes such as phospholipases A2 and metalloproteases but also included small non-enzymatic proteins of low molecular weights such as disintegrins and vascular endothelial growth factors.

These proteins have been identified by pairing with homologous species like the Tunisian viper *Macrovipera lebetina* or *Vipera ammodytes, Vipera palaestinae* and *Cerastes vipera.*

| Peak | PM | Name of the homologous protein | Species |
|---|---|---|---|
| 1 | 15432.8 | Phospholipase A2 isozyme acidic | *Vipera palaestinae* |
| 1 | 12574.6 | Snake venom VEGF toxin 2 | *Macrovipera lebetina* |
| 1 | 68710.4 | metalloproteinase-disintegrin | *Macrovipera lebetina* |
| 1 | 53480.5 | Thrombin-like contortrixobin | *Agkistrodon c.contortrix* |
| 1 | 17937.2 | metalloproteinase/disintegrin | *Macrovipera lebetina* |
| 1 | 26570.5 | Thrombin-like enzyme ancrod | *Calloselasma rhodostoma* |
| 1 | 28893.8 | Serine proteinase-like protein 2 | *Macrovipera lebetina* |
| 1 | 22460.1 | Vascular GEF A | *Vipera a. ammodytes* |
| 1 | 22850.8 | Zinc metalloproteinase fibrolase | *Macrovipera lebetina* |
| 2 | 17864.2 | C-type lectin A2 | *Macrovipera lebetina* |
| 2 | 53480.5 | Metalloproteinase/disintegrin | *Macrovipera lebetina* |
| 2 | 12574.6 | Snake venom VEGF toxin 2 | *Macrovipera lebetina* |
| 2 | 12099 | Disintegrin lebein-1-alpha | *Macrovipera lebetina* |
| 2 | 14020.5 | Disintegrin lebein-2-alpha | *Macrovipera lebetina* |
| 2 | 69426.9 | Zinc metalloproteinase-disintegrin | *Echis ocellatus* |
| 2 | 15432.8 | Phospholipase A2 isozyme acidic | *Vipera palaestinae* |
| 2 | 4852.3 | Lebecetin subunit alpha | *Macrovipera lebetina* |
| 3 | 17937.2 | C-type lectin A3 (Fragment) | *Macrovipera lebetina* |
| 3 | 12099 | Disintegrin lebein-1-alpha | *Macrovipera lebetina* |
| 3 | 7019.1 | Disintegrin CV-11-beta | *Cerastes vipera* |
| 3 | 53480.5 | Zinc metalloproteinase/disintegrin | *Macrovipera lebetina* |
| 4 | 17937.2 | C-type lectin A3 (Fragment) | *Macrovipera lebetina* |
| 4 | 12574.6 | Snake venom VEGF toxin 2 | *Macrovipera lebetina* |
| 4 | 11498.5 | Short disintegrin lebestatin | *Macrovipera lebetina* |
| 4 | 4852.3 | Lebecetin subunit alpha | *Macrovipera lebetina* |
| 4 | 7019.1 | Disintegrin CV-11-beta | *Cerastes vipera* |
| 4 | 53480.5 | Zinc metalloproteinase/disintegrin | *Macrovipera lebetina* |
| 4 | 9831.3 | Protease inhibitor 1 | *Vipera ammodytes* |
| 5 | 9831.3 | Protease inhibitor 1 | *Vipera ammodytes* |
| 5 | 17937.2 | C-type lectin A3 (Fragment) | *Macrovipera lebetina* |
| 6 | 17864.2 | C-type lectin A2 | *Macrovipera lebetina* |
| 6 | 9831.3 | Protease inhibitor 1 | *Vipera ammodytes* |
| 6 | 16675.2 | C-type lectin B3/B5 | *Macrovipera lebetina* |
| 6 | 12098.3 | Disintegrin CV-11-alpha | *Cerastes vipera* |

***Table 1:*** *Non-exhaustive list of the proteins of the Daboia mauritanica venom.*

Among these proteins, the disintegrins, for example, known for the haemorrhages that they generate, have been reported in the bibliography to have an anti-cancer action such as the purified contortrostatin of the venom of the viper *Agkistrodon contortrix contortrix* or the Leucurogin of the viper *Bothrops leucurus* showed anti-tumor, anti-angiogenic and growth inhibitory activity in cancer cells [6,7].

Another example would be that of phospholipases A2 such as "BthA-I-PLA2" purified from the viper *Bothrops jararacussu* whose activity has shown an effect on breast adenocarcinoma cells as well as on leukemic cells, or the MVL-PLA2 of the Tunisian viper *Macrovipera lebetina* which showed an anti-tumor effect by inhibition of the angiogenesis of the cancerous cells [8,9].

Metalloproteases have also showed their antitumoral potency as reported by Correa., *et al.* [10], Naumann., *et al.* [11] and Gabriel., *et al.* [12].

That said, the *Dm* venom also contains non-enzymatic proteins such as VEGF, type-C lectins whose therapeutic activity remains to be explored.

## Conflicts of Interest

The authors of this article declare that there is no conflict of interest.

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