

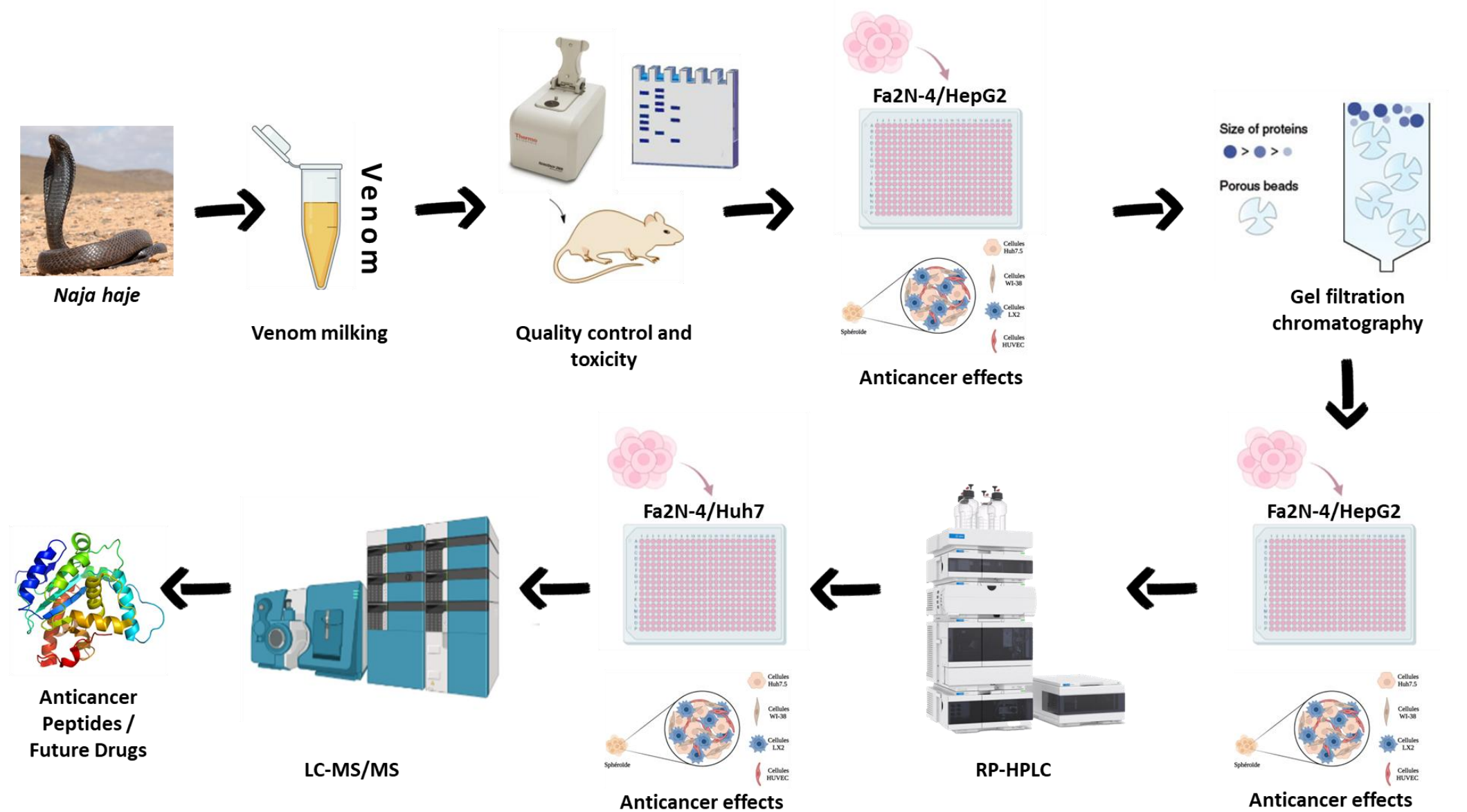
Selective Anticancer Activity of Moroccan *Naja haje* Venom and Purified Cytotoxins Against Hepatocellular Carcinoma in 2D and 3D Tumor ModelsAyoub Lafnoue<sup>1,2</sup>, Nam-Jeong Kim<sup>3</sup>, Su-Yeon Lee<sup>3</sup>, Bouchra Darkaoui<sup>1,2</sup>, Rachida Cadi<sup>2</sup>, David Shum<sup>4</sup>, Haeng Ran Seo<sup>3</sup> and Naoual Oukkache<sup>1</sup><sup>1</sup> Laboratoire des Venins et Toxines, Département de Recherche, Institut Pasteur du Maroc, 1, Place Louis Pasteur, Casablanca 20360, Morocco<sup>2</sup> Laboratoire Physiopathologie, Génétique Moléculaire & Biotechnologie, Faculté des Sciences Ain-Chock, Hassan II University, B.P 5366 Maarif, Casablanca 20000, Morocco<sup>3</sup> Advanced biomedical research lab, Institut Pasteur Korea, 16, Daewangpangyo-ro 712 beon-gil, Bundang-gu, Seongnam-si 13488, Gyeonggi-do, Korea<sup>4</sup> Screening Discovery Platform, Institut Pasteur Korea, 16, Daewangpangyo-ro 712 beon-gil, Bundang-gu, Seongnam-si 13488, Gyeonggi-do, Korea

## INTRODUCTION &amp; AIM

Hepatocellular carcinoma (HCC) is the most prevalent form of primary liver cancer and ranks among the leading causes of cancer-related mortality worldwide. Despite available treatments such as sorafenib, therapeutic outcomes remain limited, with frequent adverse effects. This has driven a growing interest in exploring novel anticancer agents derived from natural sources.

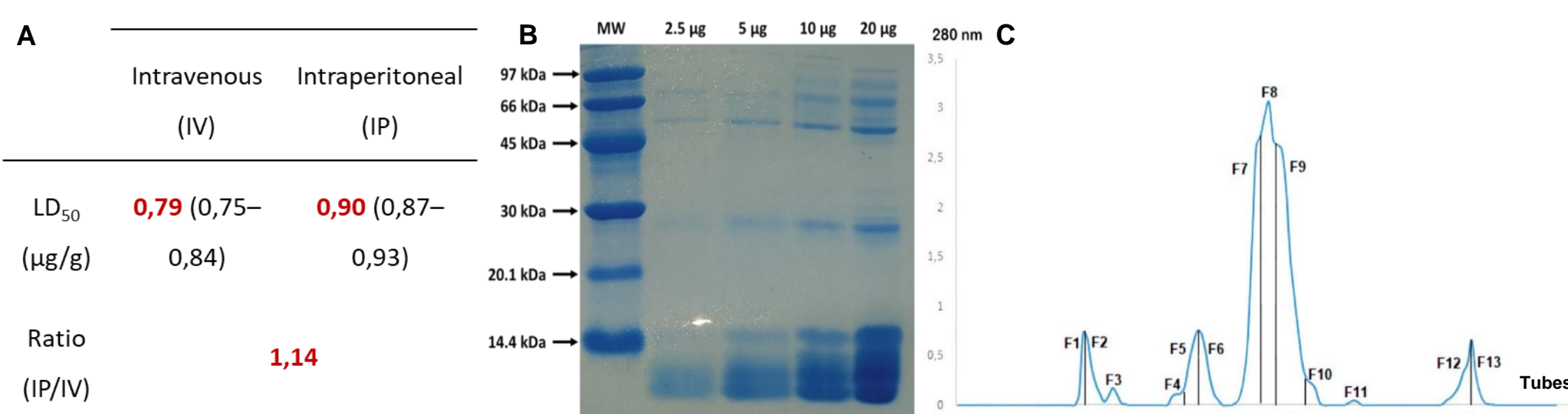
The venom of the cobra *Naja haje* from Morocco has emerged as a promising reservoir of bioactive molecules, particularly cytotoxins from the three-finger toxin (3FTx) family, known for their selective antiproliferative effects on cancer cells. In this study, we investigated the anticancer potential of *Naja haje* crude venom and its fractions purified by gel filtration chromatography, especially the F7 fraction and its subfractions (using RP-HPLC), using both 2D (HepG2, Huh7 cell lines) and 3D (multicellular tumor spheroid) models of HCC. Proteomic analyses by LC-MS/MS further enabled the identification of key cytotoxins involved in the observed anticancer activity.

## METHOD



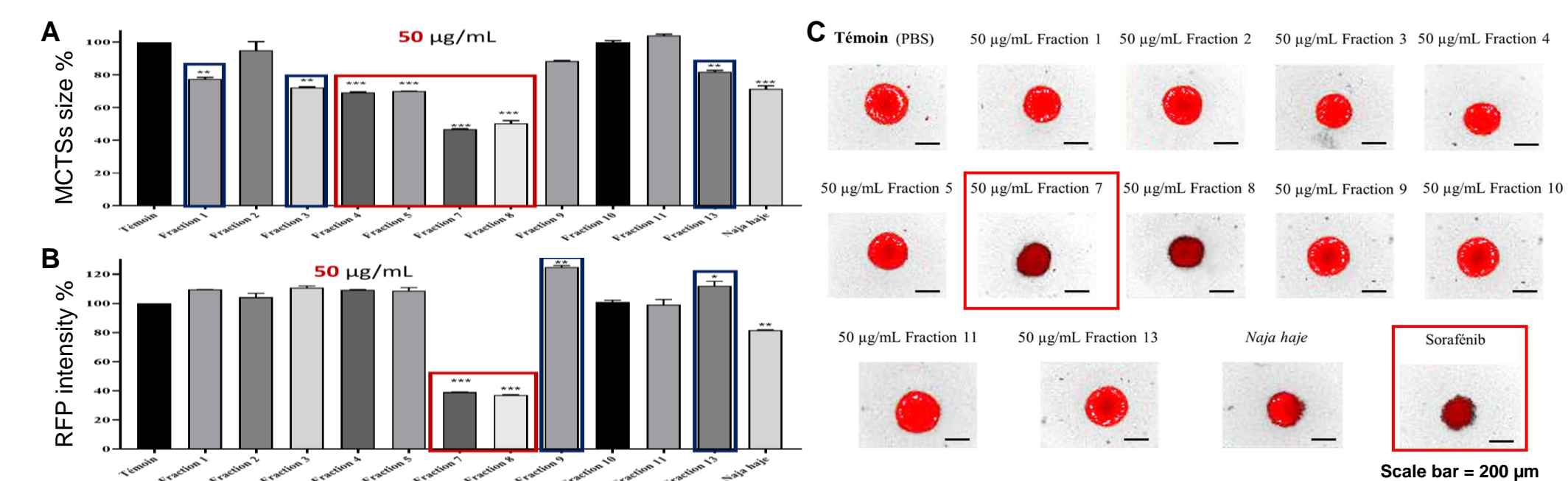
## RESULTS &amp; DISCUSSION

Characterization of *Naja haje* venom: (A) Acute toxicity assessment (LD<sub>50</sub>), (B) Electrophoresis and (C) Gel filtration chromatography.

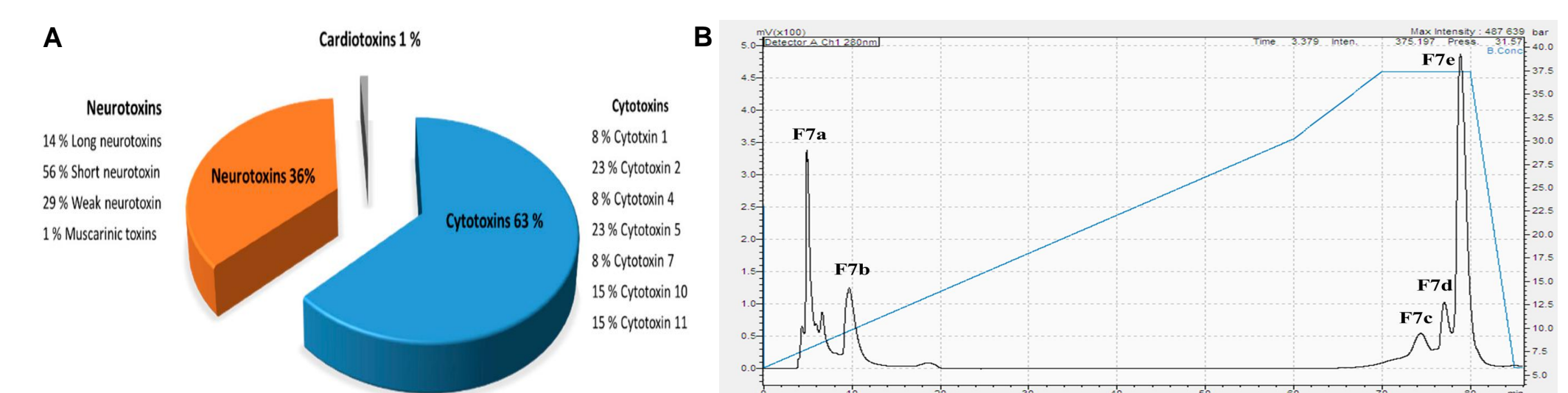


An evaluation of antiproliferative activity was conducted on cancerous hepatocytes (HepG2 cell line) (A), while a cytotoxicity assay was simultaneously performed on normal hepatocytes (Fa2N-4 cell line) (B) to assess the selectivity and safety profile of the compound under investigation.

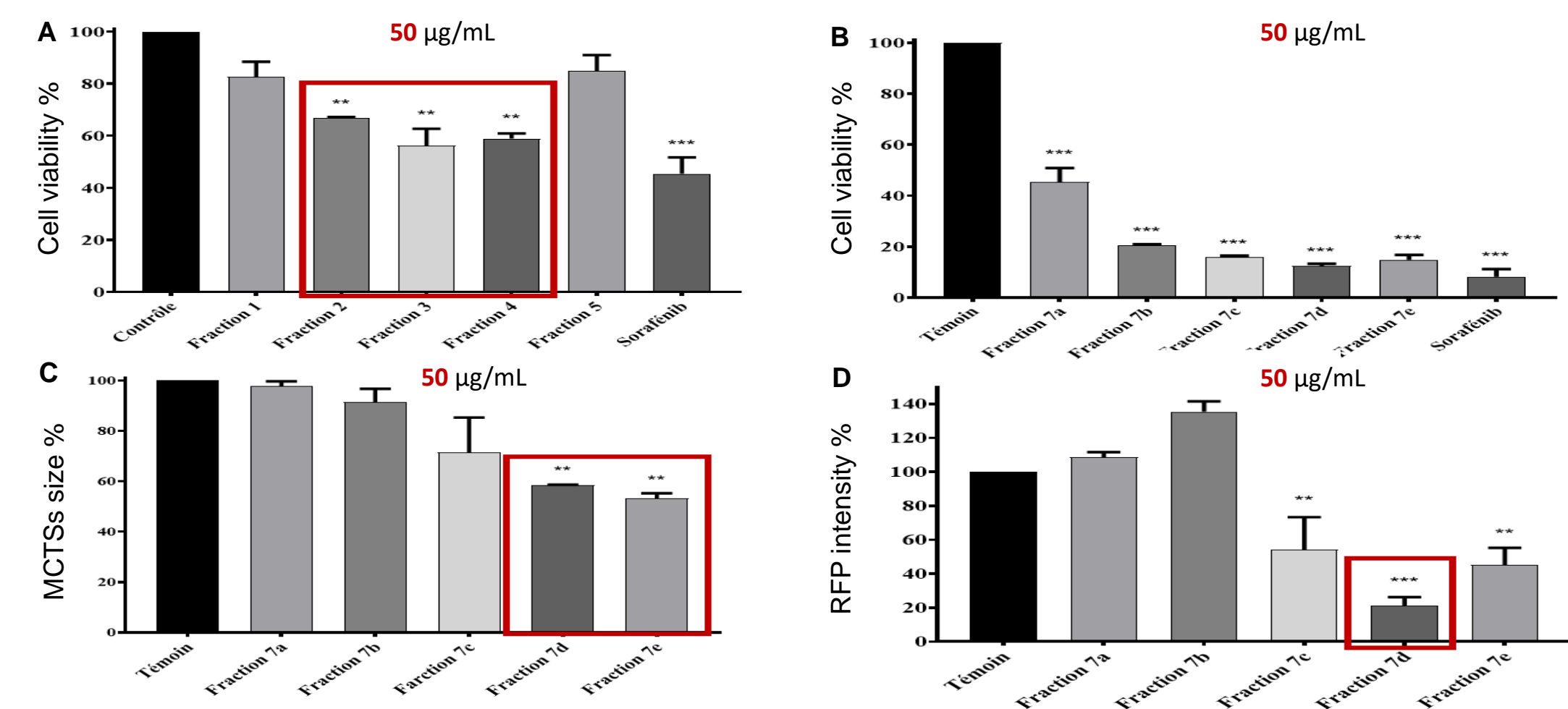
Effect of gel filtration chromatography fractions of *Naja haje* venom on MCTSs: (A) MCTS size, (B) RFP signal intensity, and (C) representative imaging of the spheroids.



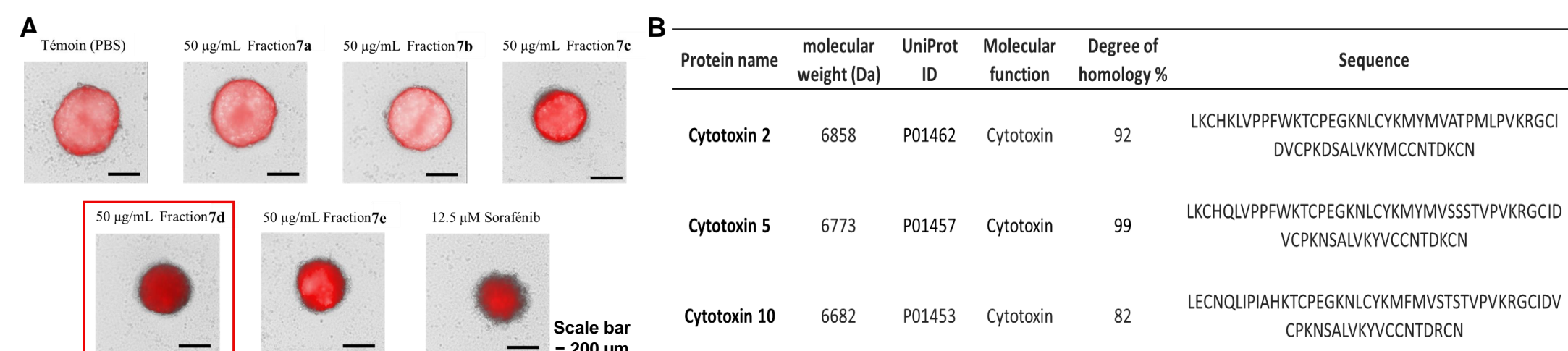
Characterization of fraction 7: (A) Analysis by LC-MS/MS; (B) Further fractionation by RP-HPLC.



Anticancer effects of F7 subfractions: (A) on cancerous hepatocytes (Huh7), (B) on normal hepatocytes (Fa2N-4), (C) on MCTS size, and (D) on RFP signal intensity.



(A) Representative imaging of spheroids treated with F7 subfractions; (B) Characterization of subfraction 7d by LC-MS/MS.



## CONCLUSION

The venom of Moroccan *Naja haje* exhibits strong and selective anticancer activity against hepatocellular carcinoma in both 2D and 3D models. Cytotoxins 2, 5, and 10 are key contributors to this effect, showing minimal toxicity toward normal hepatocytes. These findings highlight their promise as targeted candidates for HCC therapy.

## FUTURE WORK / REFERENCES

- Lafnoue, Ayoub, et al. "Cytotoxic potential of cytotoxins from moroccan *Naja haje* venom against hepatocellular carcinoma in a multicellular tumor spheroid model." *Toxin Reviews* 44.2 (2025): 285-295.
- Lafnoue, Ayoub, et al. "Moroccan *Naja haje* venom and its peptides: *in vivo* toxicity and *in vitro* antiproliferative effect on hepatocellular carcinoma HepG2 cells." *International journal of peptide research and therapeutics* 30.6 (2024): 62.
- Lafnoue, Ayoub, et al. "Anti-cancer effect of Moroccan Cobra *Naja haje* venom and its fractions against hepatocellular carcinoma in 3D cell culture." *Toxins* 13.6 (2021): 402.