PP12. MANCHURIAN SCORPION TISSUES PROTEIN EXTRACTION WITH CHAOTROPIC SOLUTION AND TANDEM ION-EXCHANGE CHROMATOGRAPHY

Gafurjon T. MAVLONOV[1,2], Ahmidin WALI[1], Amina ARKEN[1], Azimjon A. MAMADRAHIMOV[2], Abulimiti YILI[1], Haji Akber AISA[1]*

[1]Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing South Road 40-1, Urumqi 830011, Xinjiang, P.R. China

[2]Institute of Bioorganic chemistry, Academy of Sciences of Uzbekistan, Tashkent,100125

*Corresponding Author. E-mail: abu@ms.xjb.ac.cn

Manchurian scorpion (*Buthus martensii)* one of high poisonous types for animals and insects. It represent interest of a wide range of researchers as a source of neurotoxic peptides, potentially useful in medicine, including positive impact at vitiligo by melanin synthesis stimulation. The accelerated-simplified extraction and fractionation of venom gland and other tissues of this scorpion species with subsequent screening of bioactivity on murine melanoma cell lines B16 culture was the purpose of the present work.

The cut off scorpion tissues extracted by grinding by means of a mortar and a pestlein 50 mmol Tris-HCl pH 7.8, containing 4% of Triton X-100, 6 M of urea, 5 mmol of EDTA, 2 mmol of a PMSF and 5% of glycerol. A ratio of solid tissue to extracting solvent was 1 g : 5 ml, for efficiency of homogenization quartz sand (2 g tissue : 1 g ofsand) was added into grinding mix. Homogenate was clarified centrifuging at 12000 rpm, 5 min. (Eppendorf, Germany) and the insoluble debris was repeatedly extractedwith the same buffer solution, adhering an initial proportion extracting solution to solid tissue. The pH of combined extract was adjusted to 5.0, than clarified by centrifugation and loaded on a column with Toyopearl CM-650M cation-exchanger equilibrated in 50 mmol Tris-HCl pH 5.0 (buffer "A"). Column washed with buffer A (10 column bed volume) for remove of chaotropic agent and detergent, bonded cationic proteins/peptides eluted with 1.2 M NaCl in buffer A. Eluate desalted by using C18-cartridge: after loading to appropriate size C18-cartridge it washed with 10-15 column bed volume of water and bond protein fraction eluted with 75% ethanol. Resulting desalted fraction concentrated by means of vacuum evaporation and freeze-dried.

Acidic protein/peptides of scorpion tissue extract isolated in the same manner from Toyopearl CM - 650M column unbound fraction. pH of CM-650M column unbound proteins adjusted to 7.8 using 0.1 M Tris –base, clarified by centrifugation and load into column with QAE-sephadex A25. Other procedures as described above for cationic fraction isolation.

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