High-resolution snake venom inhibition profiling combined with HPLC-HRMS-SPE-NMR for identification of antivenomous constituents in Clausena excavata

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Background
On average 100,000 persons are bitten by venomous snakes in China each year, with a mortality rate of 5-10%. According to the clinical report, Gloydius blomhoffi brevicaudus, Deinagkistrodon acutus, Naja naja atra and Trimeresurus stejnegeri bites are most common.\textsuperscript{1,5}

As for the treatment of snakebite patients, usually the first choice is the corresponding antiserum. Despite antiserum can alleviate the lethal effect of venom to some extent, it has minimal preventive effect on local tissue damage caused by snakebite which could even result in physical disability. The venom components in the venom in the crude ethanol extract of Deinagkistrodon acutus, Naja naja and Trimeresurus stejnegeri were analyzed by HPLC-HRMS-SPE-NMR.\textsuperscript{6}

High-resolution snake venom profiling
Crude ethanol extract of Clausena excavata showed 99.5% inhibition against Deinagkistrodon acutus venom in the hyaluronidase assay, and was therefore subjected to high-resolution hyaluronidase profiling. The biochromatogram is shown below the HPLC trace in Figure 3, and allowed pinpointing of peak 6 as an active constituent. The active hump between 5 and 13 min is presumably due to the presence of tannins.\textsuperscript{5}

Perspectives and concluding remarks
Compounds 1-6 were purified by preparative scale HPLC and subjected to the activity test in the hyaluronidase assay against Deinagkistrodon acutus venom. Compounds 1-5 showed no activity in the test. The IC\textsubscript{50} value of lansiumamide B (6) was very close to the value of the standard hyaluronidase inhibitor aristolochic acid, which indicate lansiumamide B might be a promising inhibitor against snakebite of Deinagkistrodon acutus.

In conclusion, this study showed:
\begin{itemize}
  \item High-resolution snake venom inhibition profiling allowed fast pinpointing of a bioactivity constituent, i.e., lansiumamide B (6).
  \item High-resolution hyaluronidase assay allows subsequent HPLC-HRMS-SPE-NMR analysis to be targeted bioactive constituents only.
\end{itemize}

References: