

UPLC-TOF-MS/MS-based untargeted metabolomics analysis reveals species-specific metabolite compositions in pitchers of *Nepenthes ampullaria*, *Nepenthes rafflesiana*, and their hybrid *Nepenthes* × *hookeriana*

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Supplementary Material

Supplementary Figure 1 | *Nepenthes* pitcher plants in this study. (A-C) *Nepenthes ampullaria*. (D-E) *Nepenthes* × *hookeriana*. (F-H, J, K) *Nepenthes rafflesiana* (A, D, F) Whole pitcher plants. (B) A cluster of *N. ampullaria* pitchers on the ground. (C, E, H) A single pitcher. (G) An aerial pitcher of *N. rafflesiana*. (I) Experimental terrace for pitcher sampling of all three species. (J) Characteristics of a typical *Nepenthes* pitcher. (K) External and internal structures of a *N. rafflesiana* pitcher.

Supplementary Figure 2 | (A) Measurement of pitcher length. (B) Pitcher length at each growth phase. Error bars showing standard errors of six biological replicates.

Supplementary Figure 3 | Mirror cloud plots of differentially accumulated features (DAFs) in pairwise comparisons of (A) *N. ampullaria* and *N. rafflesiana*, (B) *N. ampullaria* and *N. × hookeriana*, and (C) *N. rafflesiana* and *N. × hookeriana*. The numbers of significant ($P < 0.001$, $r_t > 1$) DAFs are shown in parentheses and listed on the side with details in ascending P -value. DAFs are shown for lower (top) or higher (bottom) accumulation in the first species of comparison. Circle size depicts the average fold change. Y-axis indicates the m/z value of the feature; the further away from the origin, the higher the m/z . The superimposed, raw base-peak chromatograms (BPC) of all runs are shown in the background, colored according to different samples. Insets show peak intensity boxplots of selected DAFs with m/z values.

Supplementary Figure 4 | Venn analysis of identified features in XCMS pairwise comparisons of all three species. A: *N. ampullaria*; H: *N. × hookeriana*; R: *N. rafflesiana*.

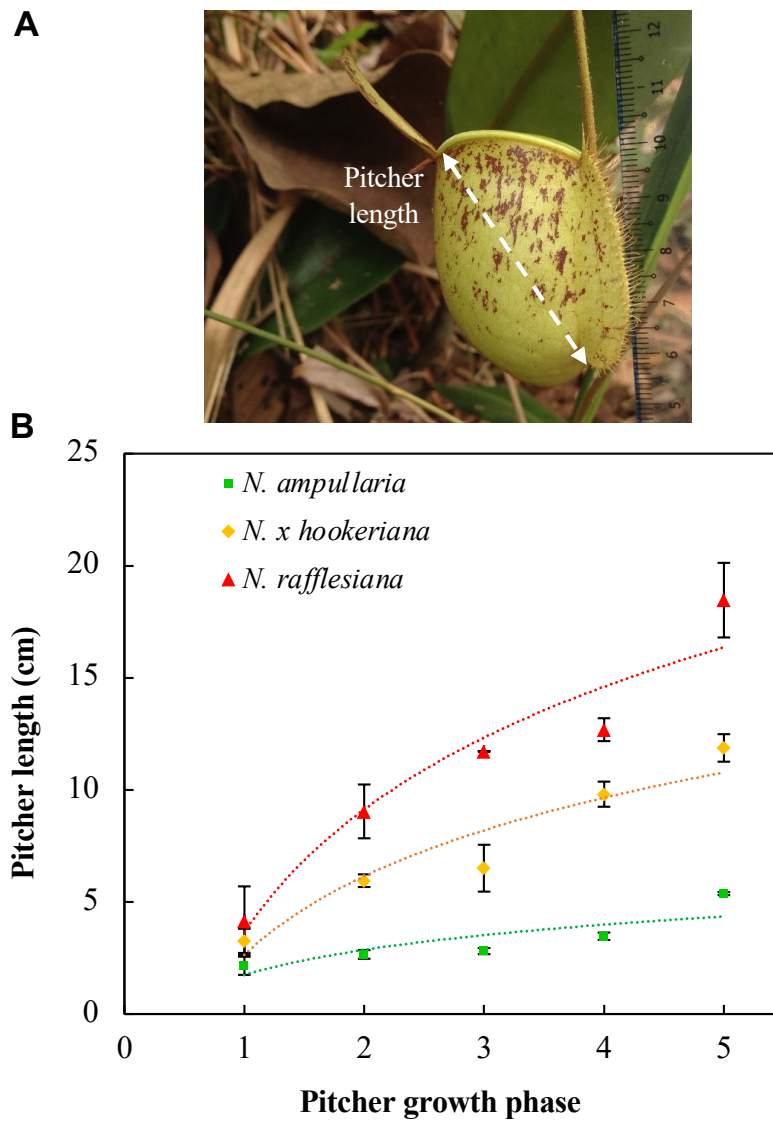
Supplementary Figure 5 | Permutation test of PLS-DA validation model.

Supplementary Figure 6 | PCA score plot analysis based on significant features. Each symbol represents different species of *Nepenthes*, green square: *N. ampullaria*, yellow diamond: *N. × hookeriana*, and red triangle: *N. rafflesiana*.

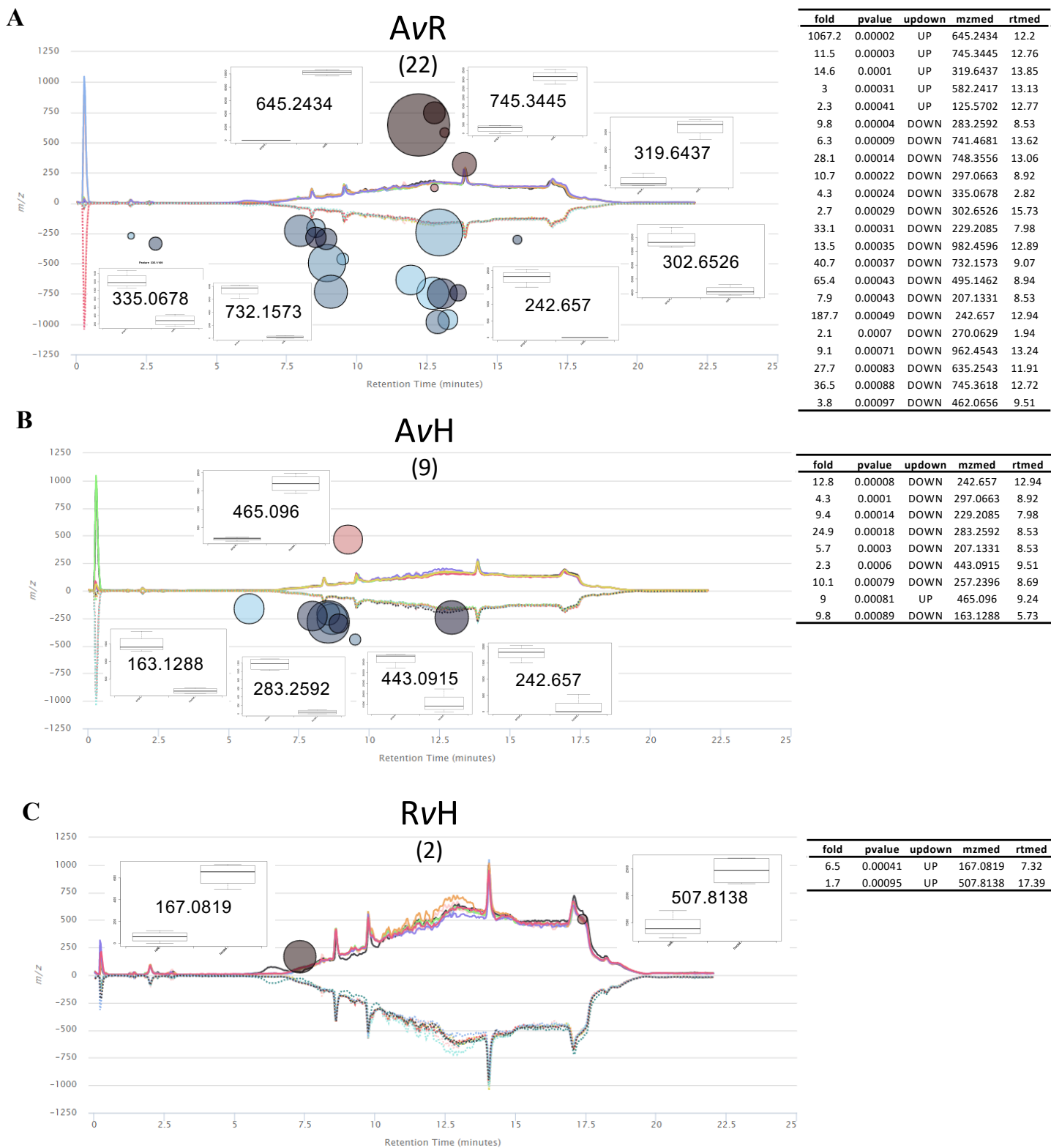
Supplementary Figure 7 | Putative identification of metabolite feature 476.3s:449.102 based on tandem MS data. (A) MS/MS spectra of 476.3s:449.102 with the list of peaks (m/z and intensity). (B) Chemical structure of astragalin and chrysenthemine. Comparison for putative metabolite identification based on (C) METLIN MS/MS spectrum and (D) Fragment Similarity Search. The blue square in (A) shows the precursor ion. For (C), the asterisk indicates the highest peak of precursor ion, while the black and cyan lines represent the MS/MS spectrum of the putative metabolite and the search fragments, respectively.



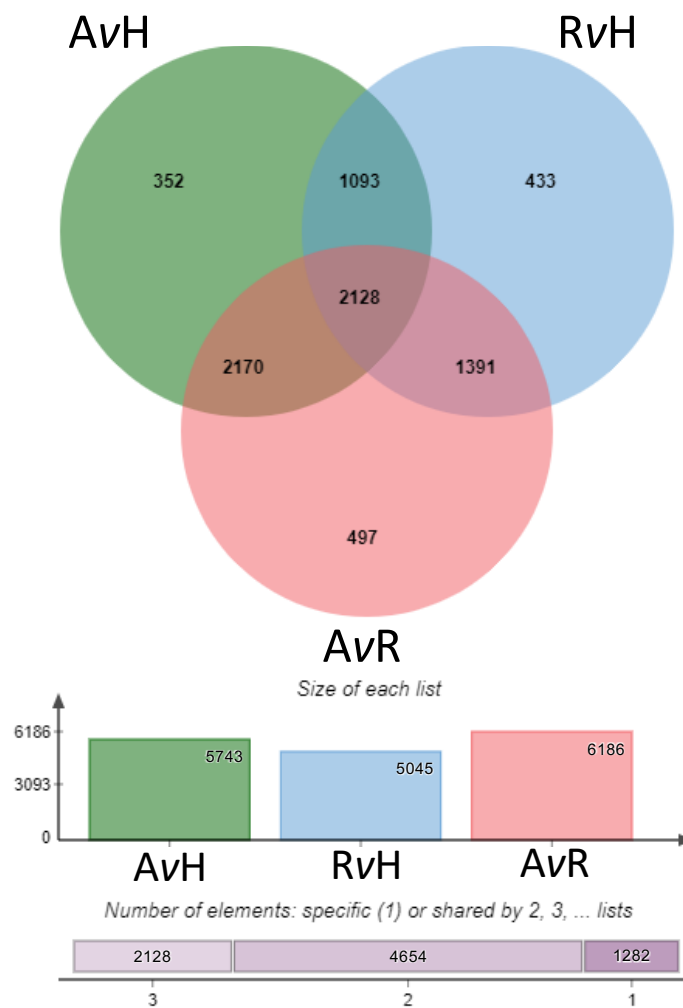
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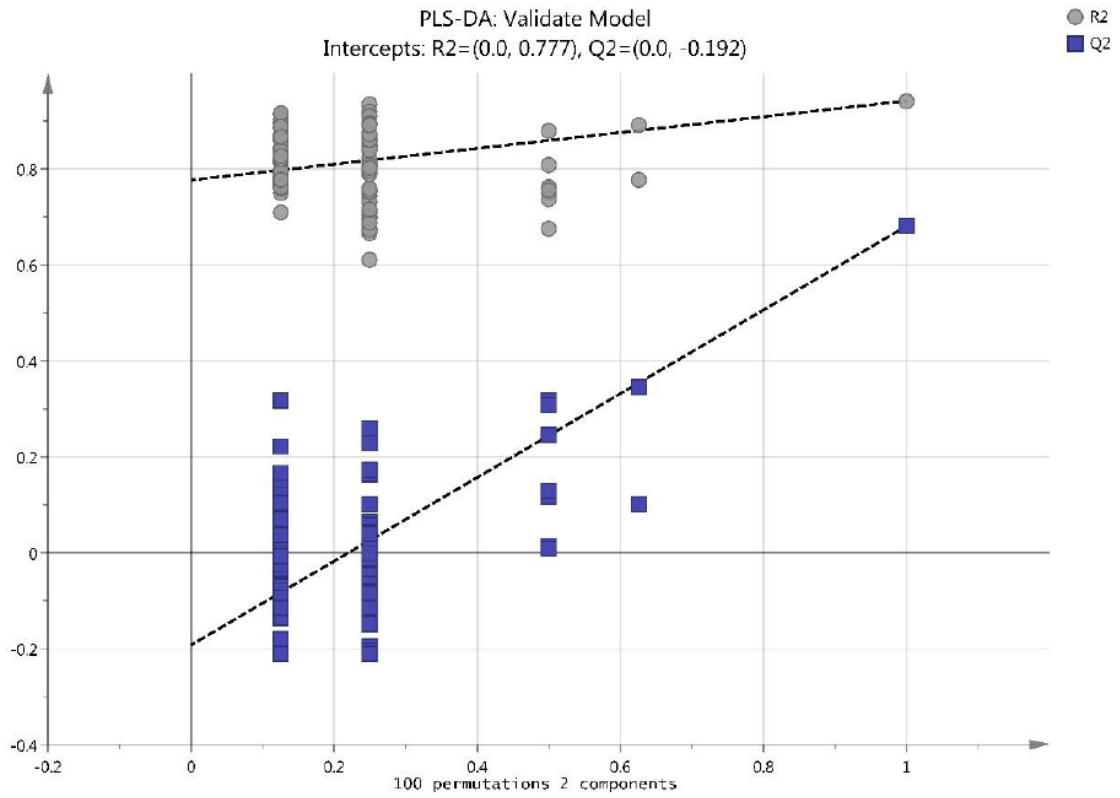
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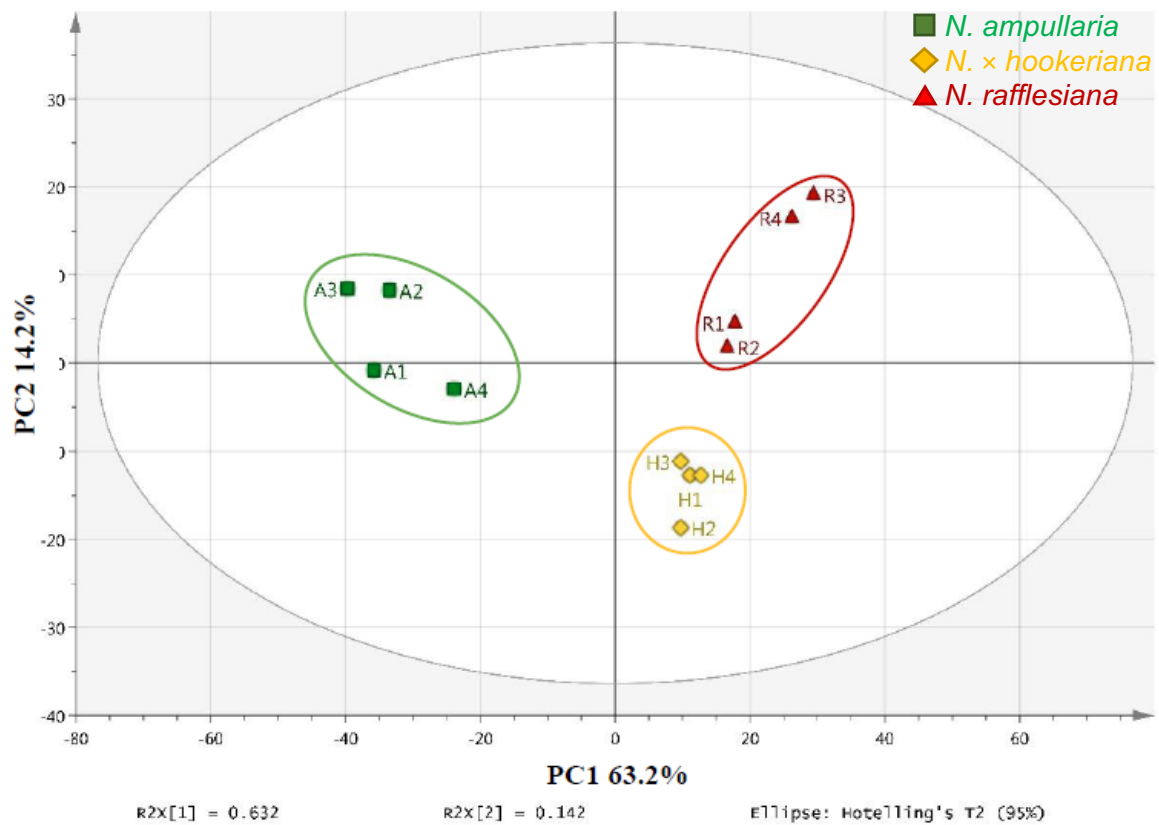
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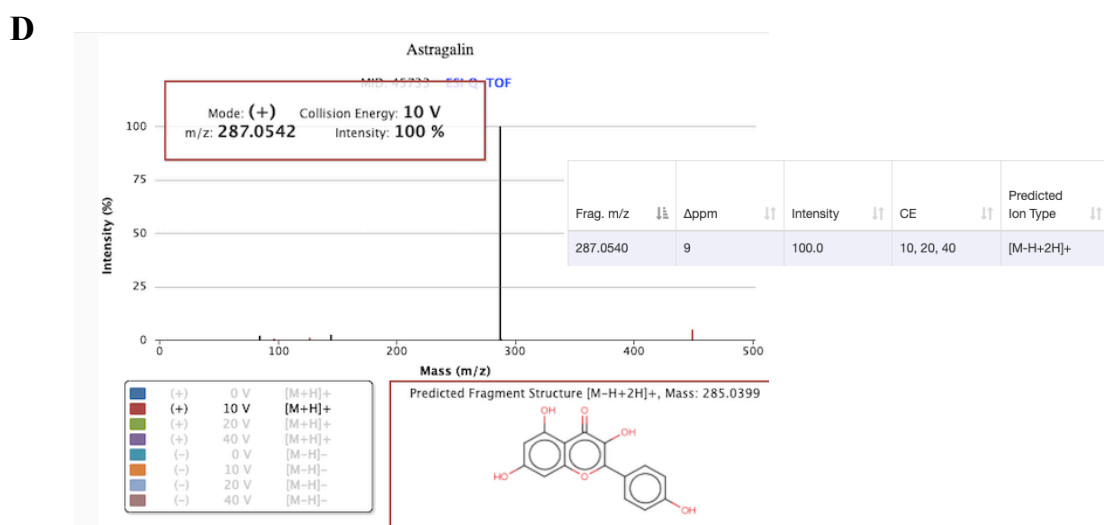
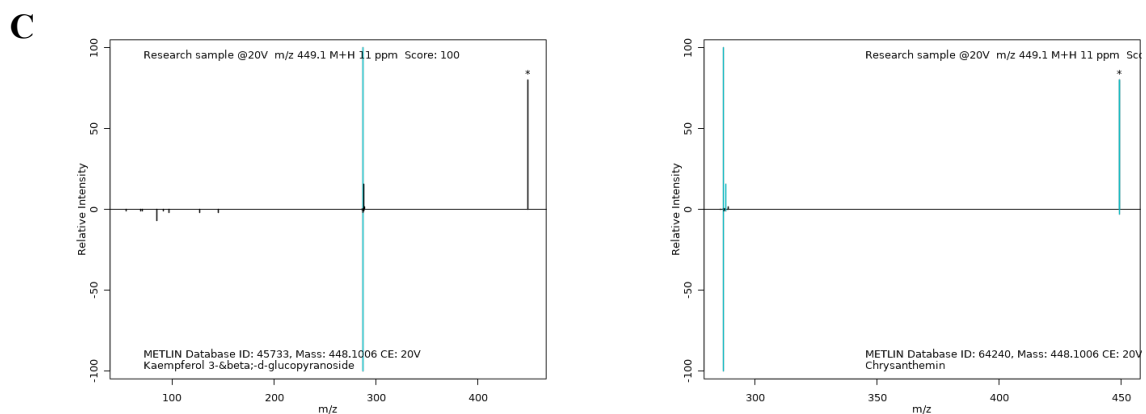
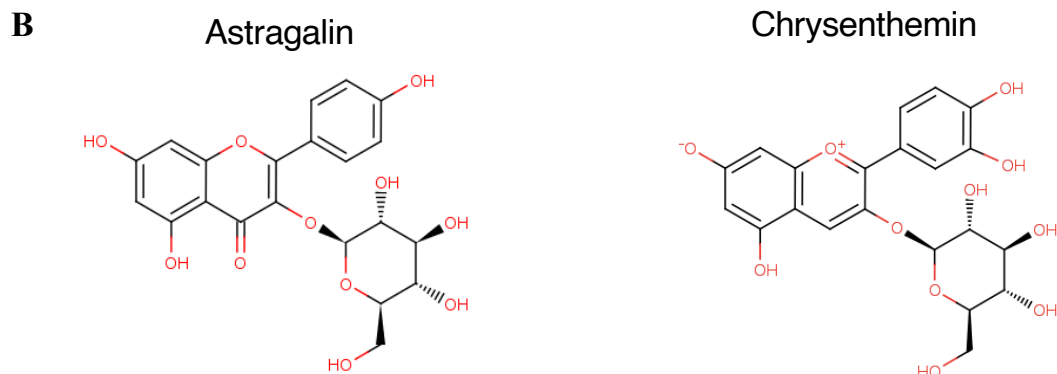
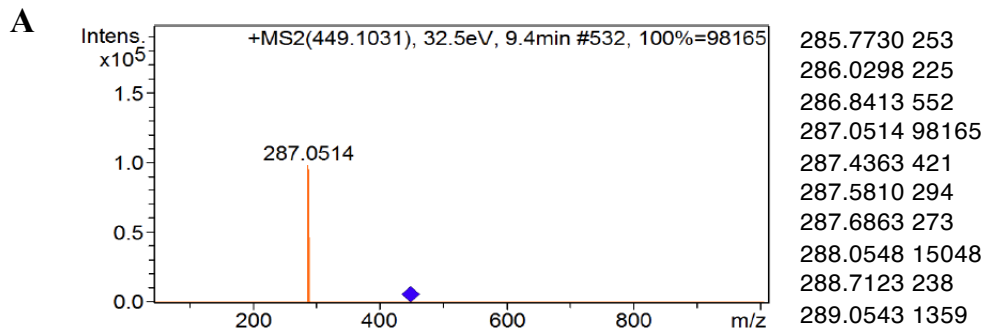
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