

# ***Laguncularia racemosa* metabolite profiling by three-phase solvent system step-gradient using high-performance countercurrent chromatography with *off-line* ESI-MS/MS detection**

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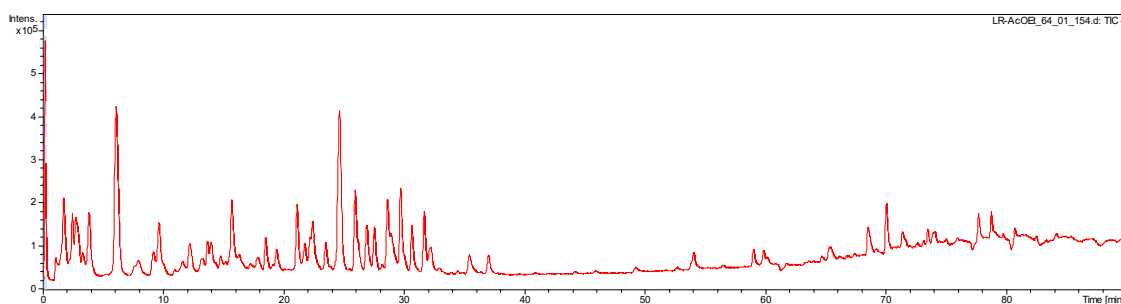
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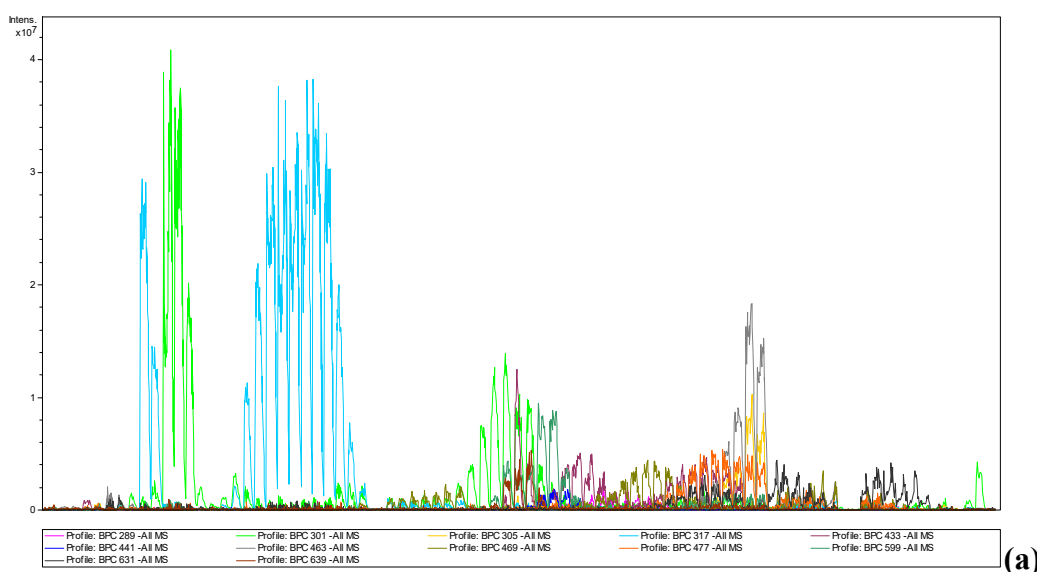
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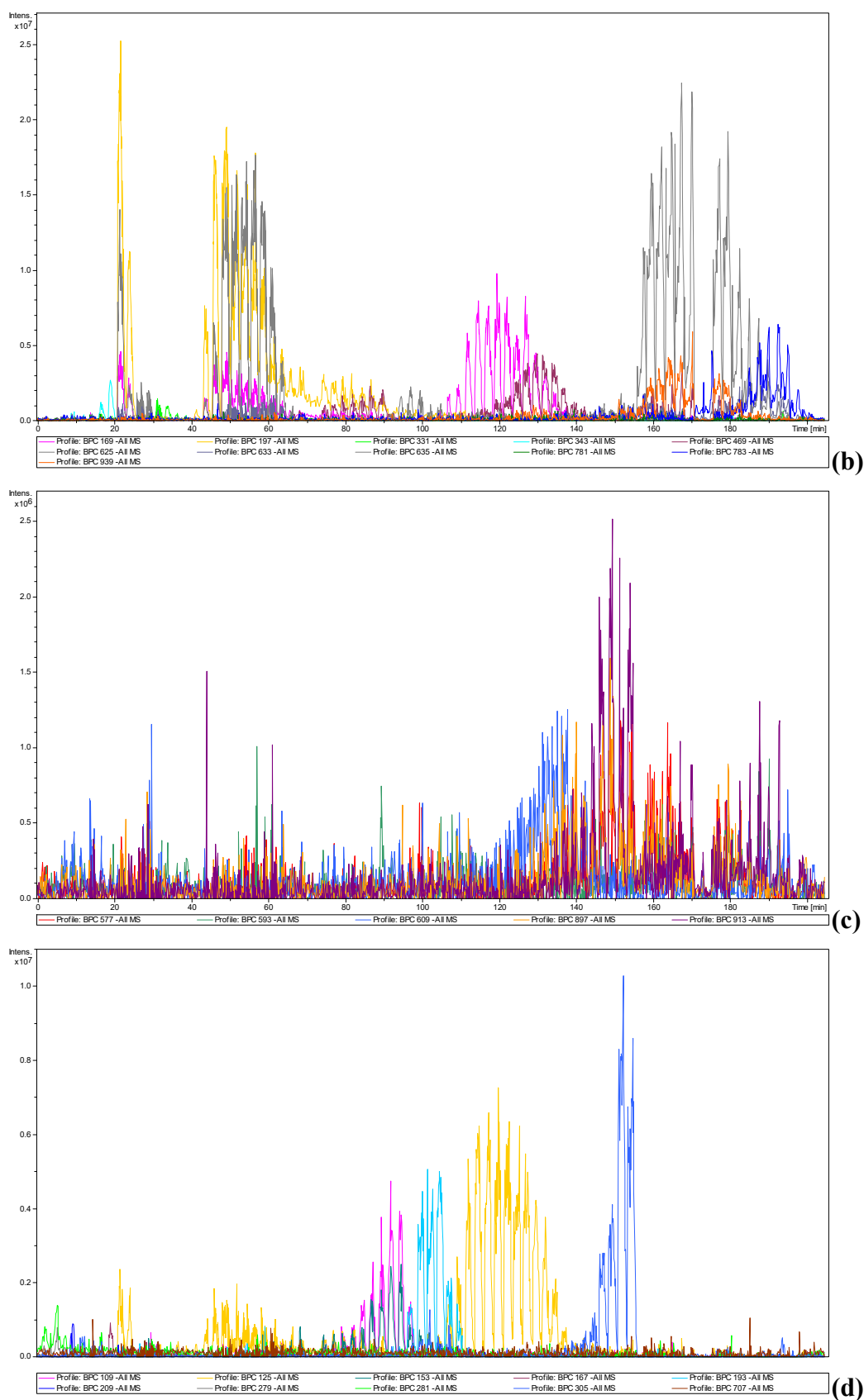
## **Supplementary Material**



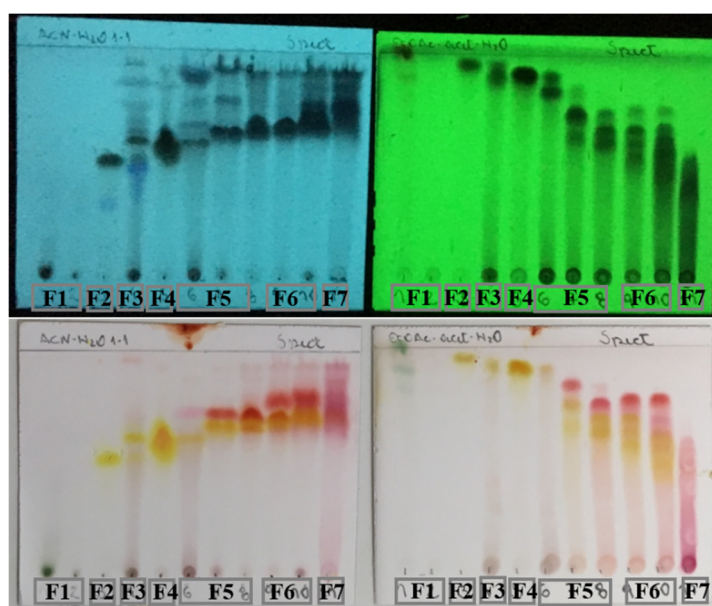
**Supplementary Figure S1.** EtOAcPart preliminary analysis by LC-ESI-TOF-MS. The mobile phase was methanol (B) and water (A) containing 0.05% (v/v) formic acid. The linear gradient elution was set from 10% to 100% of B in 90 min.



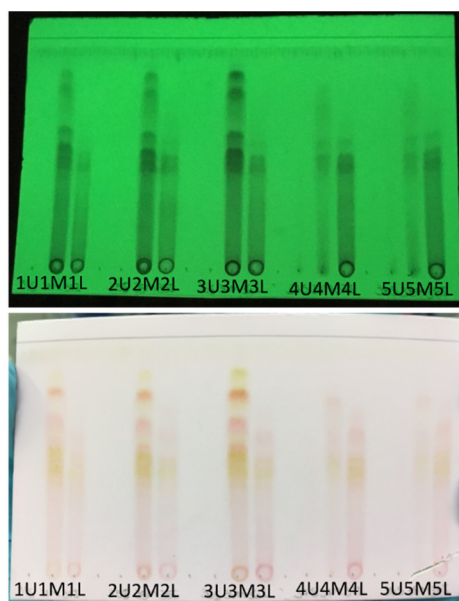
**(a)**



**Supplementary Figure S2.** HPLC off-line injection ESI-MS/MS profile of the the EtOAc Part by use of selected single ion traces for target compounds or classes. (a) Flavonoids, (b) Hydrolysable tannins, (c) Condensed tannins and (d) other compounds.



**Supplementary Figure S3.** TLC analysis of *L. racemosa* CCC combined fractions. On the left: reversed phase silica gel TLC plates developed with acetonitrile-H<sub>2</sub>O 1:1 (v/v). On the right: Normal phase silica gel TLC plates developed with EtOAc-acetone-H<sub>2</sub>O 25:15:10 (v/v/v). Visualization was done using  $\lambda$  254 nm UV light and spray-reagent H<sub>2</sub>SO<sub>4</sub> 10% and vanillin 5%. F means a group of jointed fractions according to TLC similarity.



**Supplementary Figure S4.** Three-phase solvent system test by TLC. HPLC solvent system: *n*-hexane-*tert*.butyl-methyl ether-ACN-H<sub>2</sub>O (1) 1-1-2-1, (2) 2-1-3-2, (3) 2-2-3-2, (4) 2-3-3-2 and (5) 3-5-5-2 (v/v); (U) upper, (M) middle and (L) lower phases. Normal

phase silica gel TLC plates developed with EtOAc-acetone-H<sub>2</sub>O 25:15:10 (v/v/v), visualized using  $\lambda$  254 nm UV light and spray-reagent H<sub>2</sub>SO<sub>4</sub> 10% and vanillin 5%.