

Untargeted and targeted LC-MS/MS based metabolomics study on *in vitro* culture of *Phaeoacremonium* species

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Supplementary material

Figure S1. (a) XIC of MRM transitions of scytalone (1); (b) XIC of MRM transitions of isosclerone (2); (c) selected MRM transitions for scytalone (1); (d) selected MRM transitions for isosclerone (2).

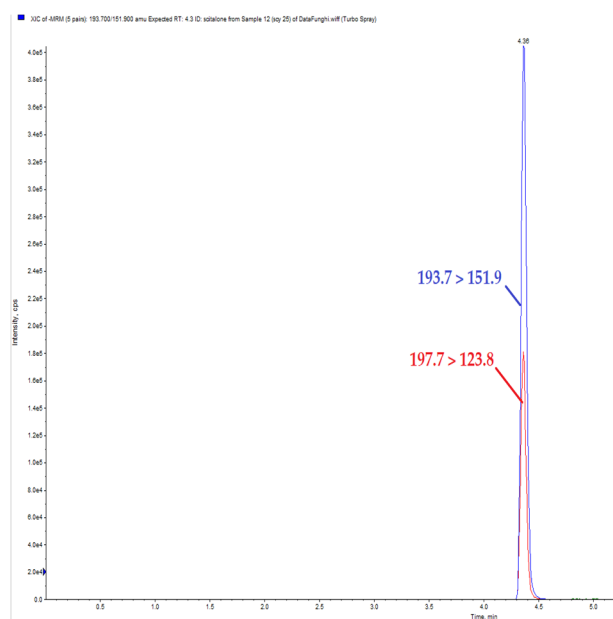
Figure S2. Hierarchical Clustering result shown as dendrogram. In red the extracts at unmodified pH (UpH), in green the extracts after acidification (pH 2) with formic acid.

Table S1. Calibration curves, correlation coefficient (r), imprecision, inaccuracy, LOD and LOQ value for scytalone (1) and isosclerone (2) quantification.

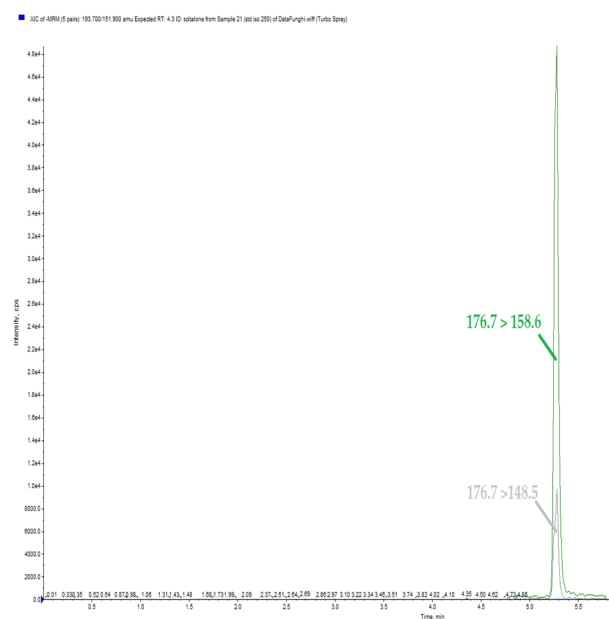
Table S2. Within-day imprecision (CV%) and inaccuracy (%) of results obtained by measuring two QC levels 6 times within one day (n=6).

Table S3. Between-day imprecision (CV%) and inaccuracy (%) of results obtained by measuring two QC levels once a day for five days (n=5).

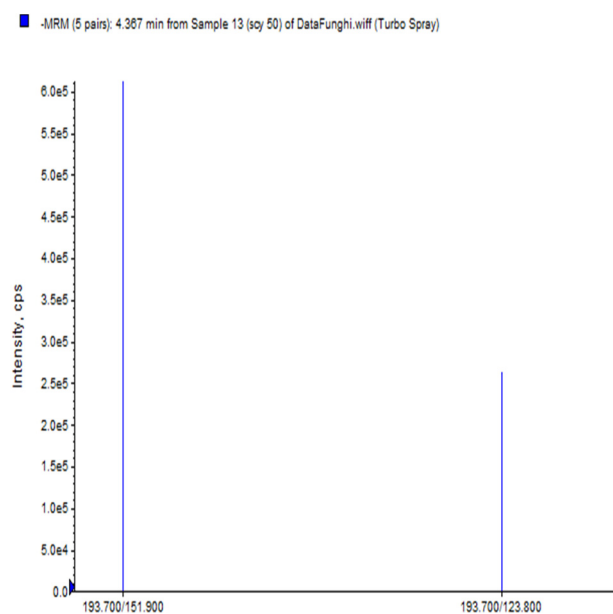
Table S4. Workflow parameters Mzmine 2.



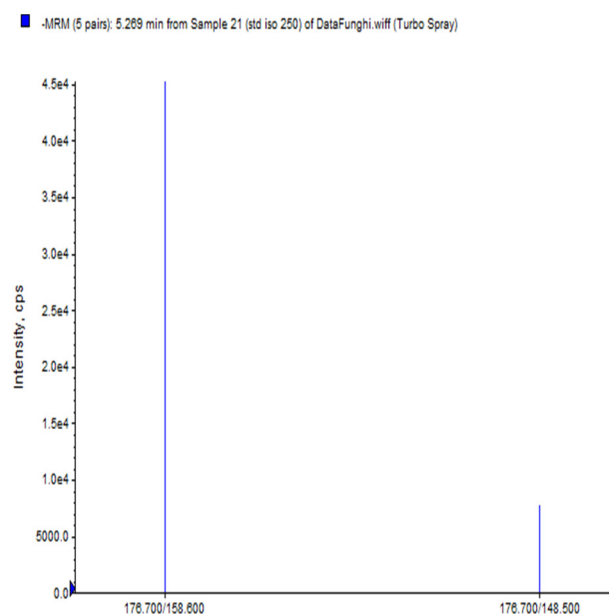
(a)



(b)



(a)



(b)

Figure S1. (a) XIC of MRM transitions of scytalone (1); (b) XIC of MRM transitions of isosclerone (2); (c) selected MRM transitions for scytalone (1); (d) selected MRM transitions for isosclerone (2).

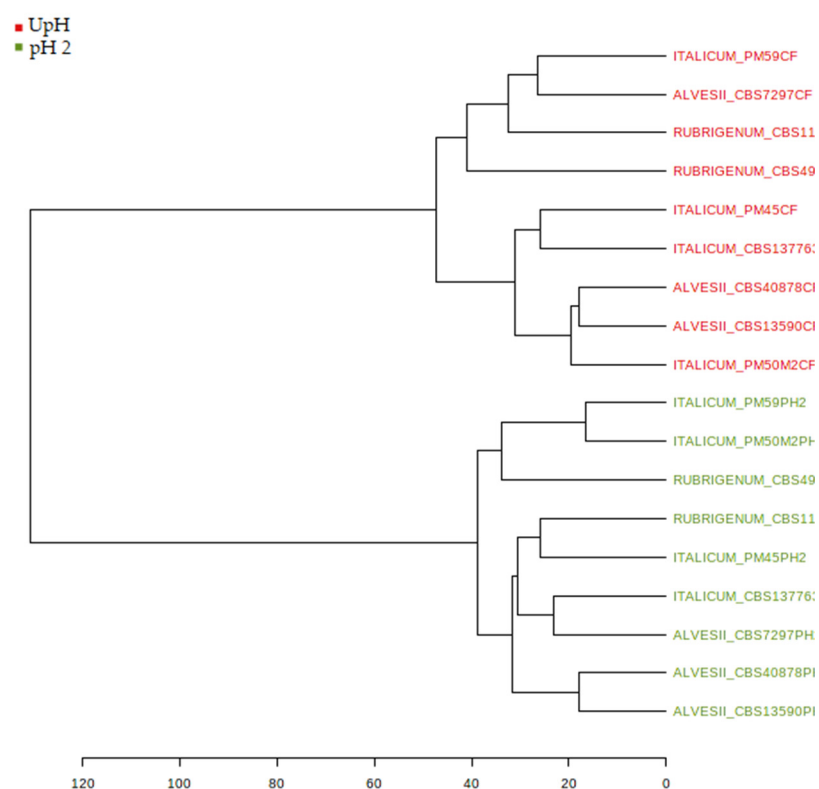


Figure S2. Hierarchical Clustering result shown as dendrogram. In red the extracts at unmodified pH (UpH), in green the extracts after acidification (pH 2) with formic acid

Table S1. Calibration curves, correlation coefficient (r), imprecision, inaccuracy, LOD and LOQ value for scytalone (1) and isosclerone (2) quantification.

| P | LOD | LOQ | Standard concentrations (µg/mL) | 1 | 5 | 10 | 15 | 25 |
|---|------|-------|------------------------------------|-------|-------|-------|--------|--------|
| scytalone (1) $y = 0.23380x + 0.02331$ $r = 0.99904$ | 0.01 | 0.52 | Mean | 0.97 | 5.16 | 11.10 | 15.49 | 24.87 |
| | | | Imprecision ^a | 0.9 | 9.5 | 3.0 | 1.5 | 5.7 |
| | | | Inaccuracy ^b | -2.6 | 3.2 | 11.0 | 3.3 | -0.5 |
| | | | Standard concentrations (µg/mL) | 15 | 30 | 60 | 150 | 250 |
| isosclerone (2) $y = 650.00401x - 4755.59055$ $r = 0.99376$ | 3.99 | 10.50 | Mean | 15.82 | 29.34 | 59.17 | 134.15 | 266.03 |
| | | | Imprecision ^a | 7.7 | 4.1 | 6.2 | 7.9 | 5.6 |
| | | | Inaccuracy ^b | 5.4 | -2.2 | -1.4 | -10.6 | -6.4 |

a Expressed as coefficient of variation (CV%): (standard deviation/mean) x 100. b Expressed as % difference: [(concentration calculated – concentration expected)/ concentration expected] x 100.

Table S2. Within-day imprecision (CV%) and inaccuracy (%) of results obtained by measuring two QC levels 6 times within one day (n=6).

| Peptide | Expected Concentration (µg/mL) | Calculated Concentration (µg/mL) | Imprecision (CV%) | Inaccuracy (%) |
|-----------------|--------------------------------------|--|----------------------|-------------------|
| scytalone (1) | 7 | 6.6 | 4.2 | -5.7 |
| | 20 | 21.1 | 2.2 | 5.5 |
| isosclerone (2) | 35 | 34.7 | 3.1 | -0.8 |
| | 100 | 99.8 | 1.9 | -0.2 |

Table S3. Between-day imprecision (CV%) and inaccuracy (%) of results obtained by measuring two QC levels once a day for five days (n=5).

| Peptide | Expected Concentration (µg/mL) | Calculated Concentration (µg/mL) | Imprecision (CV%) | Inaccuracy (%) |
|--------------------|--------------------------------------|--|----------------------|-------------------|
| scytalone (1) | 7 | 7.1 | 5.6 | 1.4 |
| | 20 | 20.35 | 7.6 | 1.75 |
| isosclerone (2) | 35 | 35.8 | 6.3 | 2.3 |
| | 100 | 101.8 | 2.0 | 1.8 |

Table S4. Workflow parameters Mzmine 2.

| Mass detection | Parameters |
|-----------------------------------|---------------------|
| mass detector | exact mass |
| noise level | 1.0x10 ³ |
| ADAP chromatogram builder | Parameters |
| minimum group size | 5 |
| group intensity threshold | 1.0x10 ⁴ |
| minimum highest intensity | 1.0x10 ³ |
| m/z tolerance | 0.001 m/z - 5 ppm |
| Smoothing | Parameters |
| Filter width | 5 |
| Chromatogram deconvolution | Parameters |
| algorithm | Wavelets (ADAP) |
| m/z centre calculation | AUTO |
| S/N threshold | 5 |
| S/N estimator | Intensity window SN |
| Min feature height | 1.0x10 ³ |
| Coefficient/area threshold | 50 |
| Peak duration range | 0.05 - 10 |
| RT wavelet range | 0.00 – 0.5 |
| Deisotoping | |
| m/z tolerance | 0.001 m/z - 5 ppm |
| RT tolerance | 0.1 absolute (min) |
| maximum charge | 2 |
| representative isotope | most intense |
| RANSAC Aligner | Parameters |
| m/z tolerance | 0.001 m/z -5 ppm |
| RT tolerance | 1 min |
| RT tolerance after correction | 0.20 |
| RANSAC iteration | 0 |
| Minimum number of points | 50% |
| Threshold value | 1 |
| Gap filling | Parameters |
| m/z tolerance | 0.001 m/z - 5 ppm |
| intensity tolerance | 1% |
| Retention time tolerance | 0.20 min |