<u>**Supplementary file**</u>

# The distribution of glucosinolates in different phenotypes of *Lepidium peruvianum* and their role as acetyl- and butyrylcholinesterase inhibitors - *in silico* and *in vitro* studies

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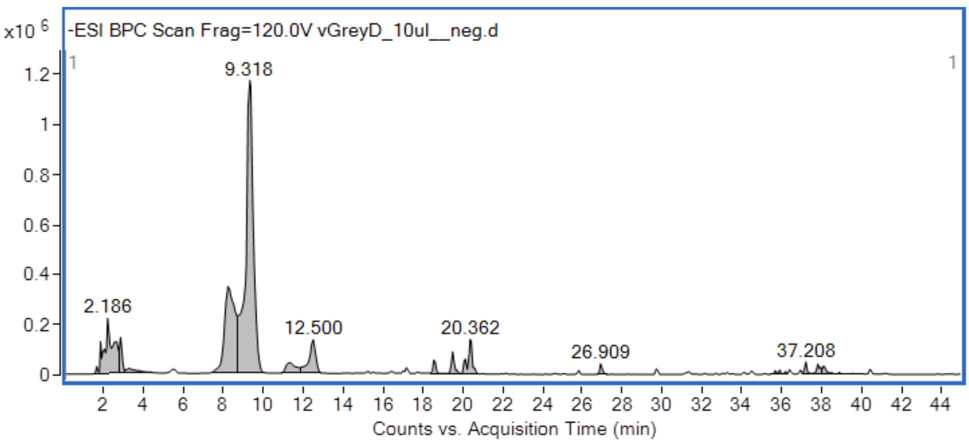


Figure S1. The composition of the 50% EtOH (v/v) extract from black A variety of *Lepidium peruvianum* in the negative ionization mode

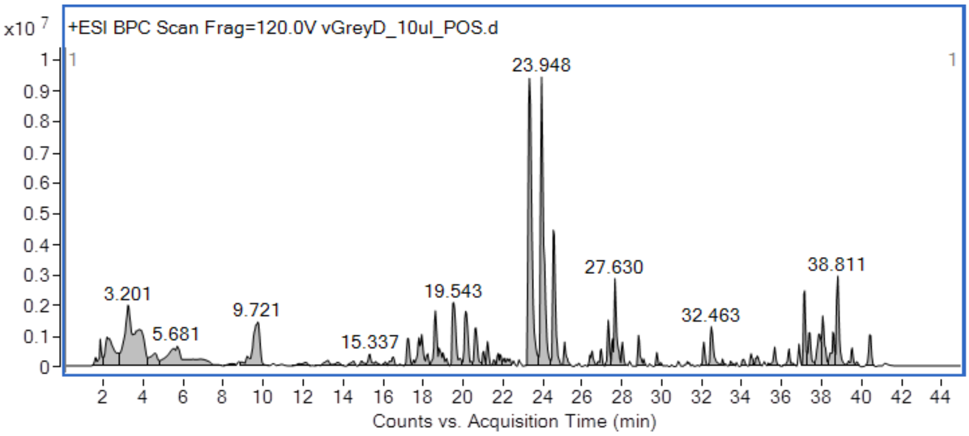



Figure S2. The composition of the 50% EtOH (v/v) extract from black A variety of *Lepidium peruvianum* in the positive ionization mode

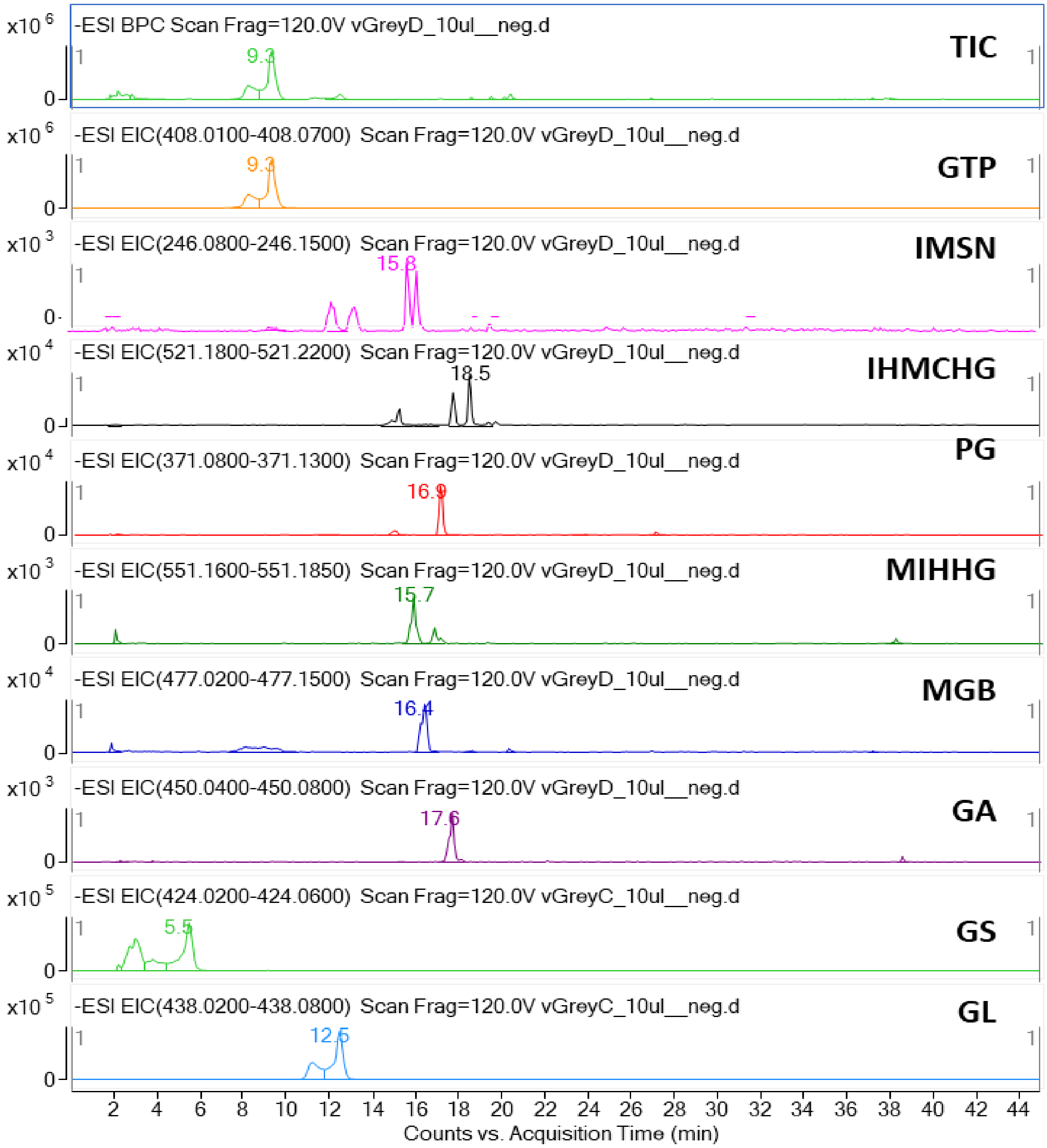
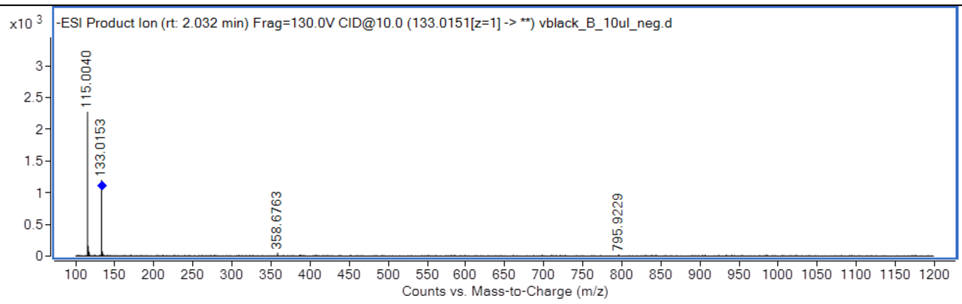
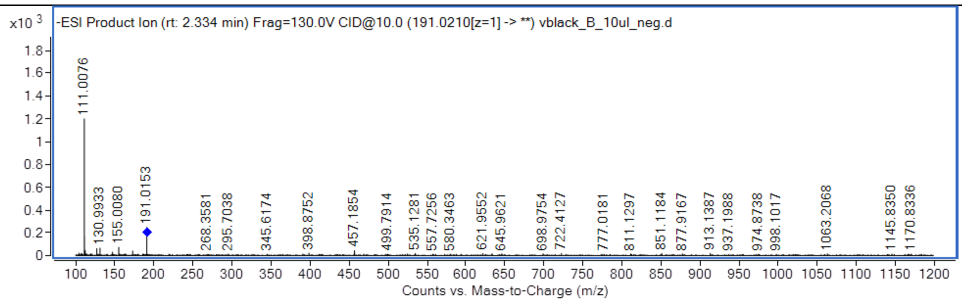
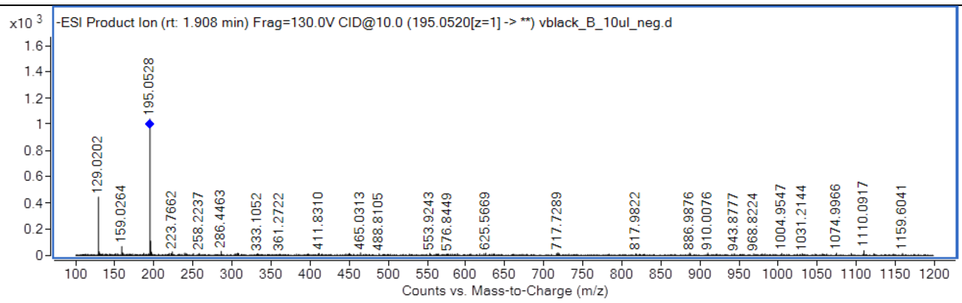
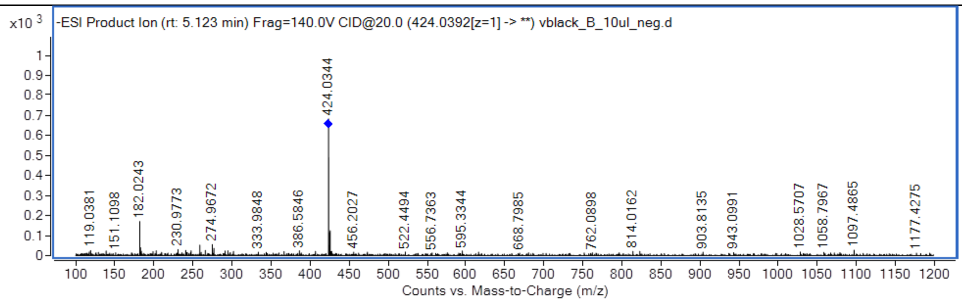
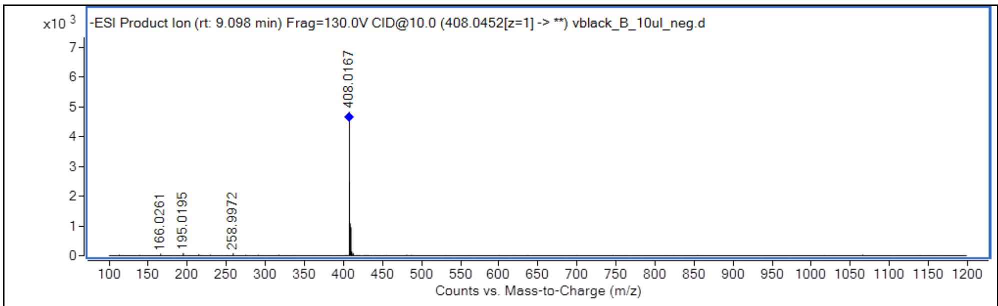


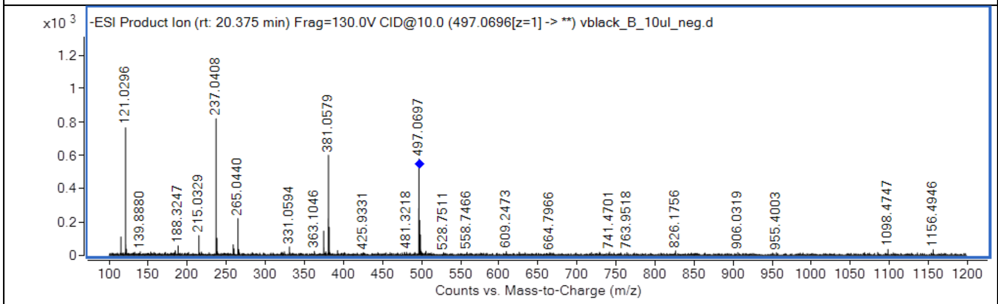

Figure S3. The total ion chromatogram (TIC) recorded in the negative ionization mode for the extract from powdered tubers of grey maca size D together with extracted ion chromatograms (EIC) indicating the presence of glucosinolates (GTP=**G2** - Glucotropaeolin, IMSN=**G6** - 1-isothiocyanato-9-methanesulfinylnonane, IHMCHG=**G9** - indolyl-3-hexyl-4-methyl-cyclohexaneglucosinolate, PG=**G7** - pent-4-enylglucosinolate, MIHHG=**G5** - 4-methoxyindolyl-3-hexylhydroxyglucosinolate, MGB=**G4** - 4-methoxyindolyl-3-methyloxyglucosinolate, GA=**G8** - glucoalyssin, GS=**G1** - glucosinalbin, GL=**G3** - glucolimnantin)

Table S1. The MS/MS spectra of the identified components of the extract visible in the negative ionization mode

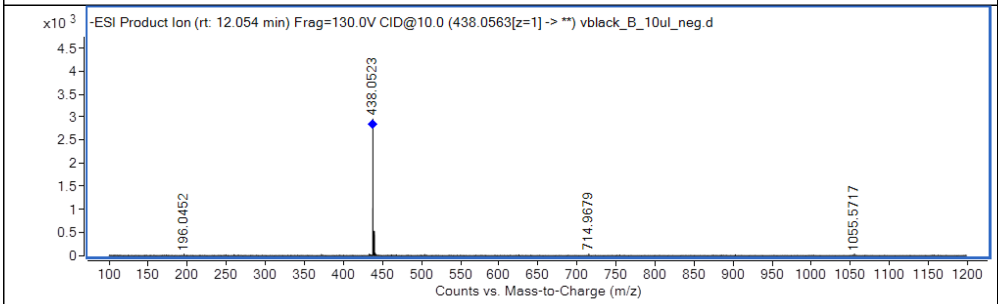
| MS/MS spectra | Identified compound |
|---|---|
|  | Malic acid |
|  | Citric Acid |
|  | Gluconic acid |
|  | Glucosinalbin |



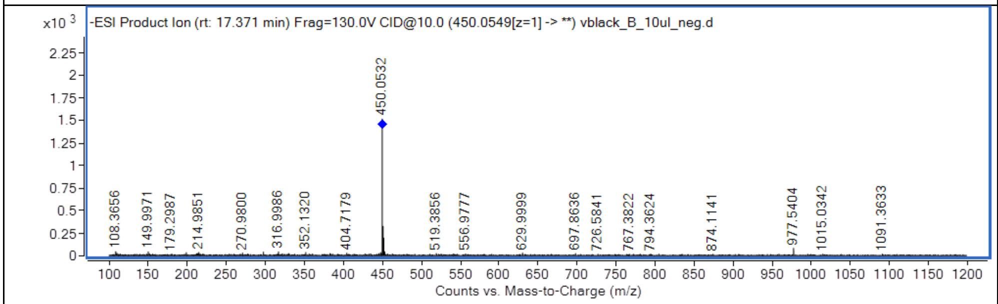

Glucotropaeolin



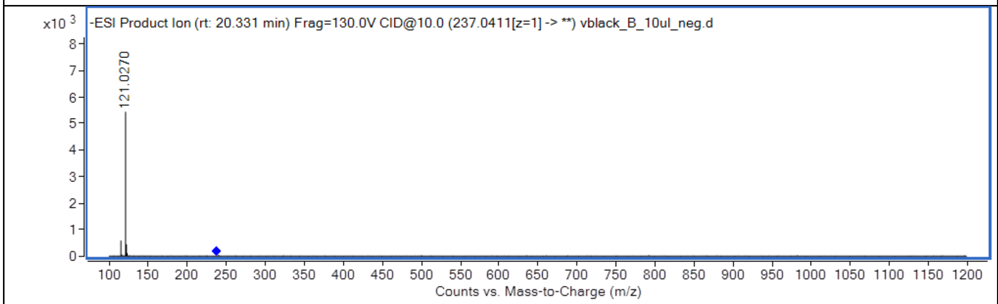

Benzoic acid derivative/ Fucodiphlorethol




Glucolimnathin




Glucoalyssin




Malic acid benzoate

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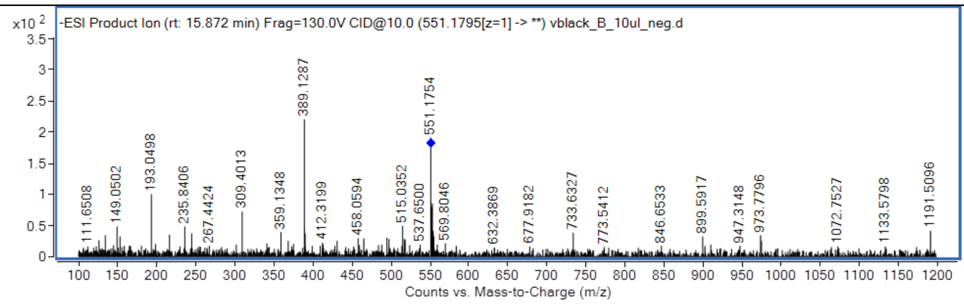

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<td>4-methoxyindolyl-3-hexylhydroxyglucosinolate</td>
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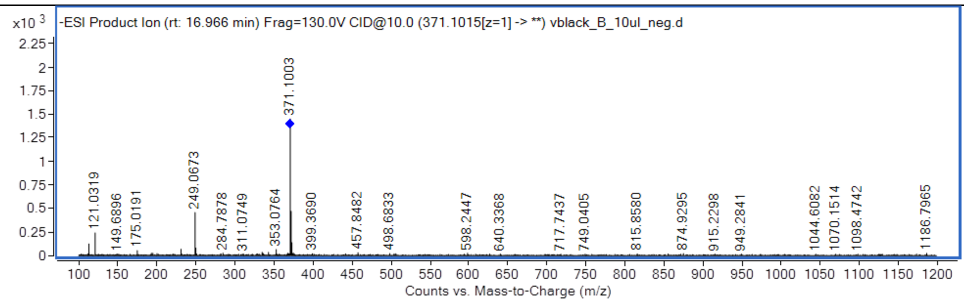

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<td>Pent-4-enylglucosinolate</td>
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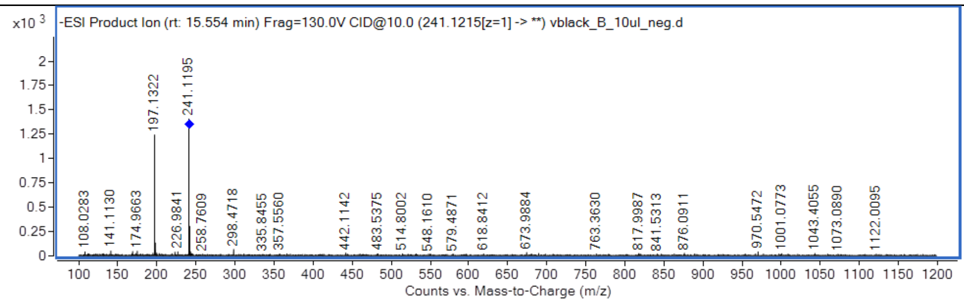

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<td>Pyroglutamylleucine</td>
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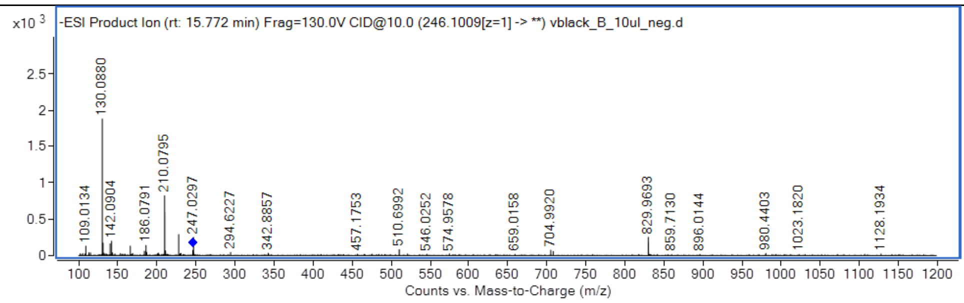

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<td>1-isothiocyanato-9-methanesulfinylnonane</td>
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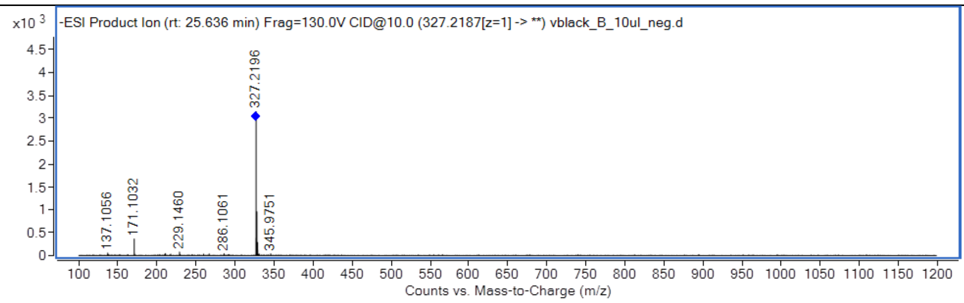


</td>
<td>Trihydroxy -octadecadienoic acid</td>
</tr>
</table>

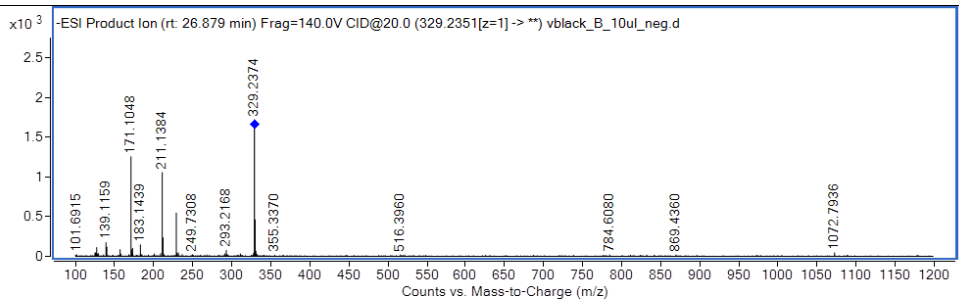
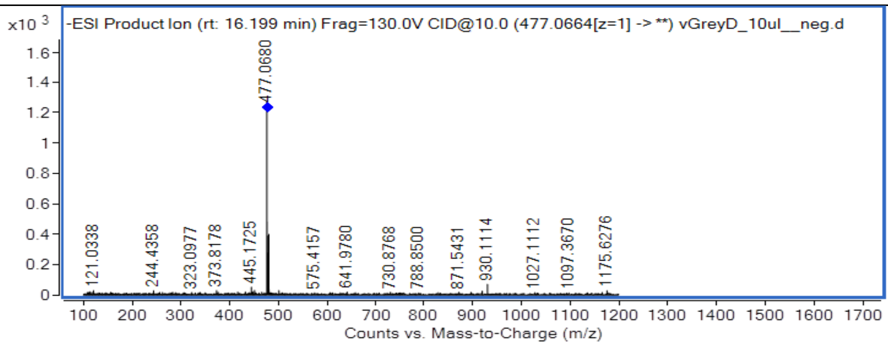
| | |
|---|---|
|  | Pinellic acid (9(S),12(S),13(S)-Trihydroxy-10(E)-octadecenoic acid) |
|  | 4-methoxyglucobrassicacin |

Table S2. The ‘absolute’ approach data table (**ABS**).

| Sample code | No. | G2 | G6 | G9 | G7 | G5 | G4 | G8 | G1 | G3 |
|---|---|---|---|---|---|---|---|---|---|---|
| BL_A | 1 | 2.92E+08 | 1741743 | 3586 | 265387.5 | 40576 | 6815.5 | 943560.5 | 2434883 | 32855380 |
| BL_B | 2 | 1.9E+08 | 1173372 | 18473 | 377483 | 60131 | 4480 | 697770 | 972315.5 | 20095215 |
| BL_C | 3 | 1.21E+08 | 1595676 | 28712 | 806816.5 | 116475 | 34405 | 312405.5 | 1476665 | 13966368 |
| BL_D | 4 | 1.74E+08 | 1155251 | 54747 | 282929.5 | 105605 | 43103 | 473277.5 | 2283710 | 16642125 |
| BL_X | 5 | 53850129 | 984394 | 6577 | 240704.5 | 33288.5 | 32449.5 | 121107.5 | 547056.5 | 8978421 |
| BL_Z | 6 | 65762830 | 33448.5 | 239883 | 124071 | 78230 | 519072.5 | 48084.5 | 627747 | 6723647 |
| CH | 7 | 15716274 | 1236353 | 65882 | 708966 | 194031.5 | 0 | 56395.5 | 205589 | 3906229 |
| BL_gel | 8 | 1.09E+08 | 1588586 | 21737.5 | 726558 | 57375 | 9915.5 | 244623.5 | 1045382 | 14577931 |
| MIX_gel | 9 | 0 | 400425 | 104117 | 487568 | 56482.5 | 5616 | 4139.5 | 281205 | 113469 |
| BL_Bp | 10 | 1.92E+08 | 871312.5 | 18646.5 | 424903 | 58770 | 61212.5 | 415736 | 1521886 | 26517616 |
| RE_Bp | 11 | 1.84E+08 | 261557 | 46610.5 | 334317 | 56864 | 35883.5 | 304908 | 1841570 | 28773780 |
| RE_LA | 12 | 68575801 | 302112 | 25851.5 | 136213.5 | 63850.5 | 18318.5 | 171619 | 612997 | 10755339 |
| RE_LAgel | 13 | 11498005 | 328208.5 | 24486.5 | 346130.5 | 74161 | 9340.5 | 55641.5 | 101765.5 | 1959255 |
| RE_gel | 14 | 20509823 | 1784042 | 17617 | 541020 | 104006.5 | 0 | 82320.5 | 405566.5 | 3440318 |
| RE_SM | 15 | 48300215 | 347838 | 25211.5 | 384182.5 | 62380.5 | 6452.5 | 98554.5 | 430109 | 8400278 |
| GR_A | 16 | 1.03E+08 | 1365563 | 10793 | 343642.5 | 52080 | 9585.5 | 404851 | 941037 | 14585134 |
| GR_B1 | 17 | 2.02E+08 | 1344030 | 12335.5 | 379528.5 | 50289.5 | 34812 | 628505.5 | 2042833 | 28862835 |
| GR_B2 | 18 | 1.55E+08 | 2132562 | 11595.5 | 404428.5 | 54583.5 | 12044.5 | 652672.5 | 1596182 | 25550725 |
| GR_C | 19 | 2.92E+08 | 1011538 | 16022.5 | 380733.5 | 68446.5 | 50739 | 987584.5 | 2241915 | 33895805 |
| GR_D | 20 | 2.54E+08 | 139570.5 | 147189 | 512590.5 | 219374.5 | 386324 | 312464.5 | 2484093 | 37314344 |
| GR_X | 21 | 1.27E+08 | 1151411 | 29020.5 | 211754.5 | 133748 | 30924 | 269724.5 | 1835665 | 19550278 |
| GR_Z | 22 | 2.67E+08 | 1133910 | 127932 | 335446 | 363497.5 | 1056874 | 360230 | 2928251 | 29546764 |
| PU_A | 23 | 42631007 | 538174.5 | 150308 | 266243 | 314251 | 14873 | 103249.5 | 368624.5 | 5626706 |
| PU_B1 | 24 | 15076461 | 478001.5 | 120739 | 342281.5 | 337776 | 23856 | 65109.5 | 219084.5 | 3396035 |
| PU_B2 | 25 | 47717650 | 67074.5 | 225697.5 | 150964 | 205991.5 | 15030.5 | 64843 | 483534.5 | 11676492 |
| PU_C | 26 | 3.03E+08 | 1422338 | 56896 | 244695 | 220862 | 50295 | 795752 | 3100773 | 35375445 |
| PU_D | 27 | 2.2E+08 | 177048 | 45092.5 | 137814 | 113009.5 | 0 | 579161 | 2401185 | 37622653 |
| GR_JU | 28 | 0 | 475671 | 4359.5 | 174295 | 331915 | 0 | 0 | 0 | 0 |
| PU_JU | 29 | 0 | 426615 | 3828 | 168153 | 289435.5 | 0 | 0 | 0 | 0 |
| BL_JU | 30 | 0 | 130904.5 | 7094.5 | 53938.5 | 470675.5 | 0 | 0 | 0 | 0 |
| YE_A | 31 | 1.49E+08 | 656856 | 26178.5 | 370031 | 81799 | 24137.5 | 376578.5 | 1328340 | 20106477 |
| YE_B | 32 | 1.69E+08 | 505266.5 | 108134.5 | 431920.5 | 492392 | 40361.5 | 387046.5 | 1266754 | 21052593 |
| YE_D | 33 | 94599733 | 530182.5 | 76089.5 | 263986 | 485417.5 | 40667.5 | 92946 | 1124159 | 14261539 |
| YE_JU | 34 | 0 | 482571.5 | 1625 | 112279 | 286380 | 0 | 0 | 0 | 0 |
| YE_Z | 35 | 2.38E+08 | 401178 | 89252.5 | 273368 | 394113 | 30222 | 726126.5 | 1769694 | 17970082 |
| YE_B2 | 36 | 1.69E+08 | 505266.5 | 108134.5 | 431920.5 | 492392 | 40361.5 | 387046.5 | 1266754 | 21052593 |

Table S3. Eigenvalues and variance explained by the principal components resulting from the **ABS** analysis.

| PC | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Eigenvalue | 3.997722 | 2.062326 | 1.119657 | 0.741948 | 0.567376 | 0.320099 | 0.131897 | 0.049876 | 0.009099 |
| % of variance | 44.42 | 22.91 | 12.44 | 8.24 | 6.30 | 3.56 | 1.47 | 0.55 | 0.10 |
| sum % of variance | 44.42 | 67.33 | 79.77 | 88.02 | 94.32 | 97.88 | 99.34 | 99.90 | 100.00 |

Table S4. Loadings of the original variables in the space of the first three varivectors (dimensions) resulting from the **ABS** analysis. One can calculate the coordinates of the samples in space of the first three varivectors by multiplying matrix presented in Table S3 by the autoscaled version of the matrix presented in Table S1.

| | **Dim1** | **Dim2** | **Dim3** |
|---|---|---|---|
| *G2* | 0.981 | 0.100 | 0.087 |

| | | | |
|---|---|---|---|
| *G6* | 0.306 | -0.313 | 0.750 |
| *G9* | -0.068 | 0.840 | -0.207 |
| *G7* | -0.034 | 0.119 | 0.892 |
| *G5* | -0.142 | 0.396 | -0.474 |
| *G4* | 0.250 | 0.805 | 0.052 |
| *G8* | 0.909 | -0.234 | 0.134 |
| *G1* | 0.949 | 0.159 | 0.129 |
| *G3* | 0.956 | 0.075 | 0.085 |

**Table S5.** The ‘relative’ approach data table (**REL**). Each row sums to 1.

| Sample code | No. | G2 | G6 | G9 | G7 | G5 | G4 | G8 | G1 | G3 |
|---|---|---|---|---|---|---|---|---|---|---|
| BL_A | 1 | 0.884024 | 0.005275 | 1.09E-05 | 0.000804 | 0.000123 | 2.06E-05 | 0.002858 | 0.007375 | 0.09951 |
| BL_B | 2 | 0.890427 | 0.005495 | 8.65E-05 | 0.001768 | 0.000282 | 2.10E-05 | 0.003267 | 0.004553 | 0.094101 |
| BL_C | 3 | 0.868339 | 0.011457 | 0.000206 | 0.005793 | 0.000836 | 0.000247 | 0.002243 | 0.010602 | 0.100277 |
| BL_D | 4 | 0.892366 | 0.00591 | 0.00028 | 0.001447 | 0.00054 | 0.00022 | 0.002421 | 0.011682 | 0.085133 |
| BL_X | 5 | 0.831096 | 0.015193 | 0.000102 | 0.003715 | 0.000514 | 0.000501 | 0.001869 | 0.008443 | 0.138568 |
| BL_Z | 6 | 0.886805 | 0.000451 | 0.003235 | 0.001673 | 0.001055 | 0.007 | 0.000648 | 0.008465 | 0.090668 |
| CH | 7 | 0.711475 | 0.05597 | 0.002982 | 0.032095 | 0.008784 | 0 | 0.002553 | 0.009307 | 0.176835 |
| BL_gel | 8 | 0.856703 | 0.012458 | 0.00017 | 0.005698 | 0.00045 | 7.78E-05 | 0.001918 | 0.008198 | 0.114325 |
| MIX_gel | 9 | 0 | 0.275581 | 0.071655 | 0.335554 | 0.038872 | 0.003865 | 0.002849 | 0.193531 | 0.078092 |
| BL_Bp | 10 | 0.865583 | 0.003918 | 8.39E-05 | 0.001911 | 0.000264 | 0.000275 | 0.00187 | 0.006844 | 0.119251 |
| RE_Bp | 11 | 0.853295 | 0.001212 | 0.000216 | 0.001549 | 0.000264 | 0.000166 | 0.001413 | 0.008535 | 0.13335 |
| RE_LA | 12 | 0.850161 | 0.003745 | 0.00032 | 0.001689 | 0.000792 | 0.000227 | 0.002128 | 0.0076 | 0.133338 |
| RE_LAgel | 13 | 0.798639 | 0.022797 | 0.001701 | 0.024042 | 0.005151 | 0.000649 | 0.003865 | 0.007069 | 0.136088 |
| RE_gel | 14 | 0.76288 | 0.066359 | 0.000655 | 0.020124 | 0.003869 | 0 | 0.003062 | 0.015085 | 0.127966 |
| RE_SM | 15 | 0.83197 | 0.005992 | 0.000434 | 0.006618 | 0.001075 | 0.000111 | 0.001698 | 0.007409 | 0.144695 |
| GR_A | 16 | 0.853698 | 0.011279 | 8.91E-05 | 0.002838 | 0.00043 | 7.92E-05 | 0.003344 | 0.007773 | 0.12047 |
| GR_B1 | 17 | 0.858464 | 0.005703 | 5.23E-05 | 0.00161 | 0.000213 | 0.000148 | 0.002667 | 0.008668 | 0.122473 |
| GR_B2 | 18 | 0.836266 | 0.01148 | 6.24E-05 | 0.002177 | 0.000294 | 6.48E-05 | 0.003514 | 0.008593 | 0.137549 |
| GR_C | 19 | 0.882981 | 0.003062 | 4.85E-05 | 0.001153 | 0.000207 | 0.000154 | 0.00299 | 0.006787 | 0.102618 |
| GR_D | 20 | 0.859674 | 0.000472 | 0.000498 | 0.001733 | 0.000741 | 0.001306 | 0.001056 | 0.008396 | 0.126124 |
| GR_X | 21 | 0.845759 | 0.007651 | 0.000193 | 0.001407 | 0.000889 | 0.000205 | 0.001792 | 0.012198 | 0.129907 |
| GR_Z | 22 | 0.881456 | 0.003749 | 0.000423 | 0.001109 | 0.001202 | 0.003494 | 0.001191 | 0.009682 | 0.097693 |
| PU_A | 23 | 0.852391 | 0.010761 | 0.003005 | 0.005323 | 0.006283 | 0.000297 | 0.002064 | 0.007371 | 0.112504 |
| PU_B1 | 24 | 0.751593 | 0.023829 | 0.006019 | 0.017063 | 0.016839 | 0.001189 | 0.003246 | 0.010922 | 0.169299 |
| PU_B2 | 25 | 0.787325 | 0.001107 | 0.003724 | 0.002491 | 0.003399 | 0.000248 | 0.00107 | 0.007978 | 0.192658 |
| PU_C | 26 | 0.880289 | 0.004126 | 0.000165 | 0.00071 | 0.000641 | 0.000146 | 0.002308 | 0.008995 | 0.10262 |
| PU_D | 27 | 0.842887 | 0.000677 | 0.000172 | 0.000527 | 0.000432 | 0 | 0.002215 | 0.009184 | 0.143904 |
| GR_JU | 28 | 0 | 0.482307 | 0.00442 | 0.176727 | 0.336546 | 0 | 0 | 0 | 0 |
| PU_JU | 29 | 0 | 0.480405 | 0.004311 | 0.189355 | 0.325929 | 0 | 0 | 0 | 0 |
| BL_JU | 30 | 0 | 0.197558 | 0.010707 | 0.081403 | 0.710332 | 0 | 0 | 0 | 0 |
| YE_A | 31 | 0.866741 | 0.003811 | 0.000152 | 0.002147 | 0.000475 | 0.00014 | 0.002185 | 0.007706 | 0.116645 |
| YE_B | 32 | 0.874656 | 0.002608 | 0.000558 | 0.002229 | 0.002541 | 0.000208 | 0.001998 | 0.006538 | 0.108663 |
| YE_D | 33 | 0.848621 | 0.004756 | 0.000683 | 0.002368 | 0.004355 | 0.000365 | 0.000834 | 0.010084 | 0.127935 |
| YE_JU | 34 | 0 | 0.546603 | 0.001841 | 0.127177 | 0.324379 | 0 | 0 | 0 | 0 |
| YE_Z | 35 | 0.916672 | 0.001544 | 0.000343 | 0.001052 | 0.001517 | 0.000116 | 0.002794 | 0.00681 | 0.069152 |
| YE_B2 | 36 | 0.874656 | 0.002608 | 0.000558 | 0.002229 | 0.002541 | 0.000208 | 0.001998 | 0.006538 | 0.108663 |

Table S6. Eigenvalues and variance explained by the principal components resulting from the REL analysis.

| PC | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Eigenvalue | 4.744784 | 2.428596 | 0.972154 | 0.372068 | 0.276201 | 0.186960 | 0.015201 | 0.004036 | 1.49E-31 |
| % of variance | 52.72 | 26.98 | 10.80 | 4.13 | 3.07 | 2.08 | 0.17 | 0.04 | 0.00 |
| sum % of variance | 52.72 | 79.70 | 90.51 | 94.64 | 97.71 | 99.79 | 99.96 | 100.00 | 100.00 |

Table S7. Loadings of the original variables in the space of the first three varivectors (dimensions) resulting from the **REL** analysis. One can calculate the coordinates of the samples in space of the first three varivectors by multiplying matrix presented in Table S4 by the autoscaled version of the matrix presented in Table S6.

| | Dim1 | Dim2 | Dim3 |
|---|---|---|---|
| *G2* | -0.885 | -0.433 | 0.064 |
| *G6* | 0.897 | 0.281 | -0.096 |
| *G9* | 0.137 | 0.960 | 0.171 |
| *G7* | 0.627 | 0.760 | 0.020 |
| *G5* | 0.912 | -0.023 | -0.093 |
| *G4* | -0.057 | 0.300 | 0.922 |
| *G8* | -0.764 | 0.272 | -0.402 |
| *G1* | -0.062 | 0.969 | 0.167 |
| *G3* | -0.871 | -0.073 | -0.029 |

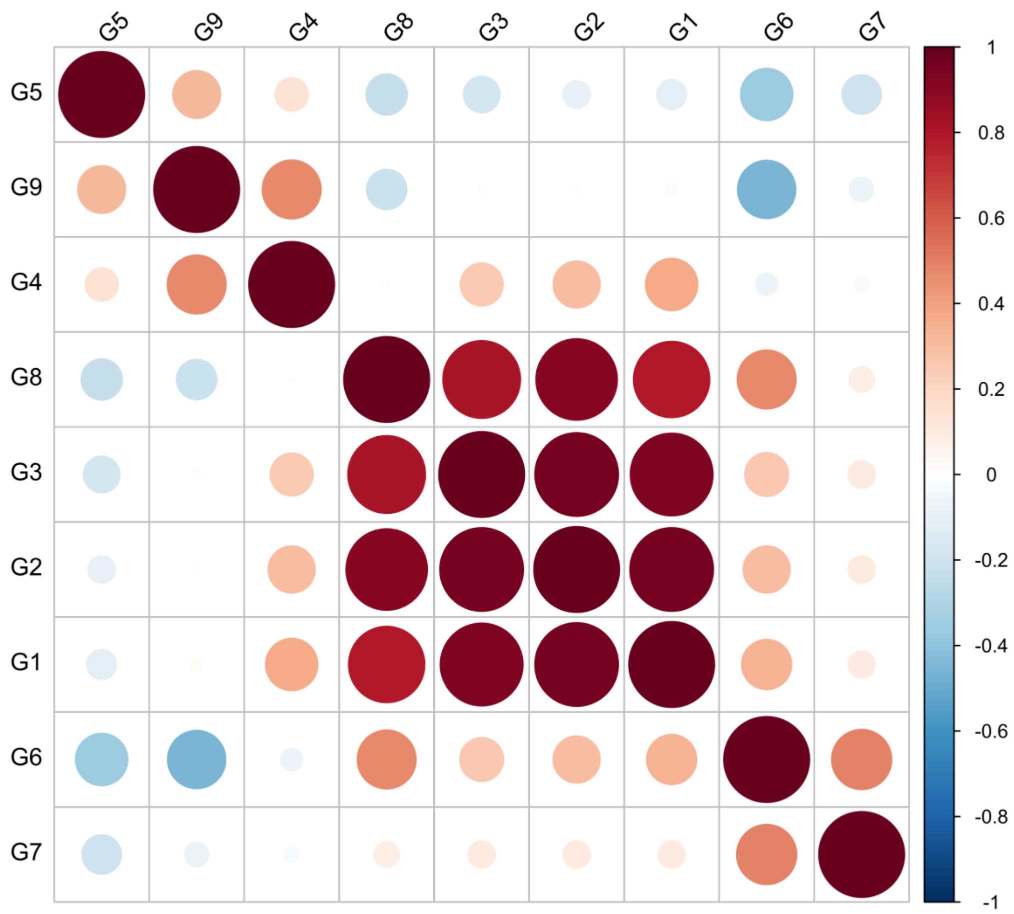



Figure S4. Graphical representation of the correlation coefficients (R) for the ABS dataset.

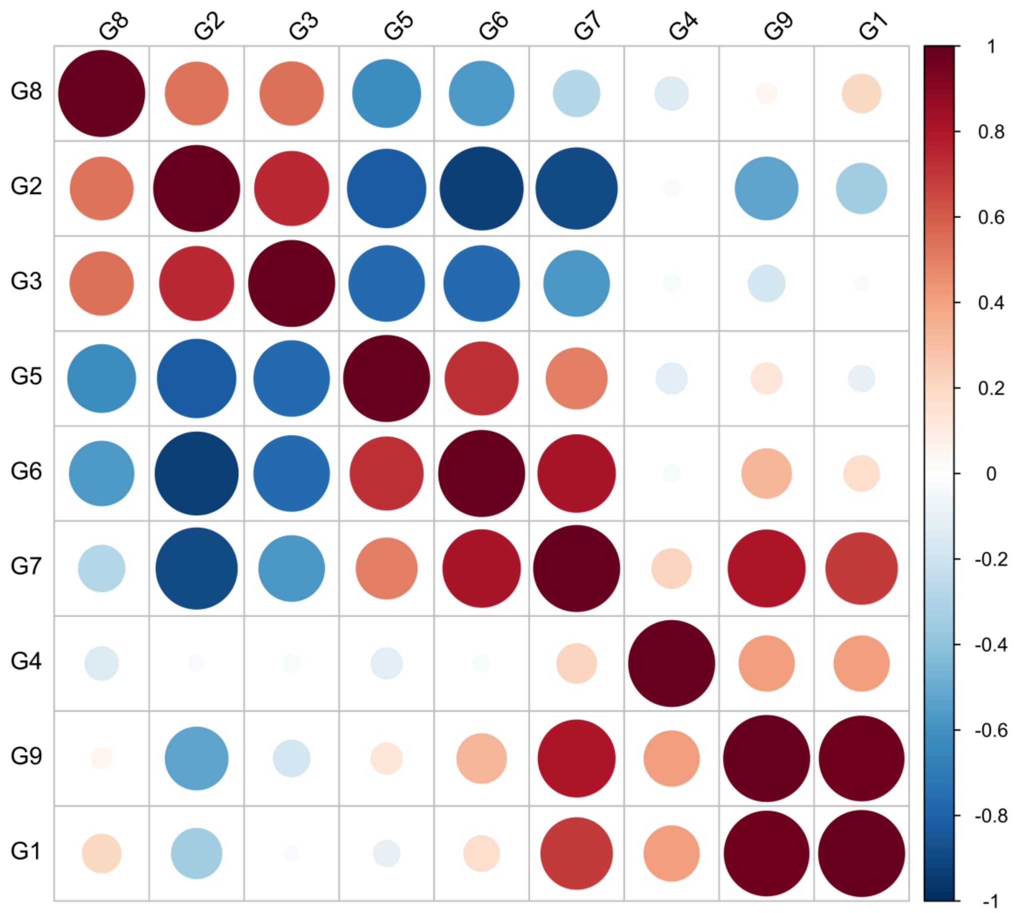



Figure S5. Graphical representation of the correlation coefficients (R) for the REL dataset.