Untargeted Screening of Plant Metabolites based on Data-independent and Data-dependent Acquisition modes using LC-ESI-QTOF-MS: *Tribulus terrestris* L. as a case study

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**SUPPLEMANTARY DATA**

**Table S-1****.** DIA method Q1 window of 25 and 50 Da

**Table S-2:** All metabolites identified in *Tribulus terrestris* fruits and aerial parts from both regions in positive ion mode.

**Table: S-3.** All metabolites identified in *Tribulus terrestris* fruits and aerial parts from both regions in negative ion mode.

**Figure S-1**. Hierarchical clustering of all metabolites identified in negative ion mode were generated using Perseus software.

**Table S-1.** DIA method Q1 window of 25 and 50 Da.

|  |  |  |  |
| --- | --- | --- | --- |
| **Experiment** | **MS Type** | **Min *m/z*** | **Max *m/z*** |
|  0 | SCAN | 100 | 1200 |
| 1 | SWATH | 99.5 | 150.5 |
| 2 | SWATH | 149.5 | 200.5 |
| 3 | SWATH | 199.5 | 225.5 |
| 4 | SWATH | 224.5 | 250.5 |
| 5 | SWATH | 249.5 | 275.5 |
| 6 | SWATH | 274.5 | 300.5 |
| 7 | SWATH | 299.5 | 325.5 |
| 8 | SWATH | 324.5 | 350.5 |
| 9 | SWATH | 349.5 | 375.5 |
| 10 | SWATH | 374.5 | 400.5 |
| 11 | SWATH | 399.5 | 425.5 |
| 12 | SWATH | 424.5 | 450.5 |
| 13 | SWATH | 449.5 | 475.5 |
| 14 | SWATH | 474.5 | 500.5 |
| 15 | SWATH | 499.5 | 525.5 |
| 16 | SWATH | 524.5 | 550.5 |
| 17 | SWATH | 549.5 | 575.5 |
| 18 | SWATH | 574.5 | 600.5 |
| 19 | SWATH | 599.5 | 625.5 |
| 20 | SWATH | 624.5 | 650.5 |
| 21 | SWATH | 649.5 | 675.5 |
| 22 | SWATH | 674.5 | 700.5 |
| 23 | SWATH | 699.5 | 725.5 |
| 24 | SWATH | 724.5 | 750.5 |
| 25 | SWATH | 749.5 | 775.5 |
| 26 | SWATH | 774.5 | 800.5 |
| 27 | SWATH | 799.5 | 825.5 |
| 28 | SWATH | 824.5 | 850.5 |
| 29 | SWATH | 849.5 | 875.5 |
| 30 | SWATH | 874.5 | 900.5 |
| 31 | SWATH | 899.5 | 950.5 |
| 32 | SWATH | 949.5 | 1,000.50 |
| 33 | SWATH | 999.5 | 1,050.50 |
| 34 | SWATH | 1,049.50 | 1,099.50 |
| 35 | SWATH | 1,049.50 | 1,150.50 |
| 36 | SWATH | 1,199.50 | 1,200.50 |

**Table S-2. All metabolites identified in *Tribulus terrestris* fruits and aerial parts from both regions in positive ion mode.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Metabolite** | **Mol. Formula** | **M+** | **Experimental Precursor *m/z*** | **Reference****Precursor *m/z*** | **Products *m/z*** | **ppm error** | **r/t** |
| 1-Napthalamine | C10H9N | [M+H]+ | 144.0803 | 144.0807 | 143.0731, 115.0565 | 2.7 | 3.7 |
| Xanthine | C5H4N4O2 | [M+H]+ | 153.0405 | 153.0407 | 99.6741, 66.4511 | 1.3 | 1.8 |
| Coumyraldehyde | C9H8O2 | [M+H]+ | 149.0592 | 149.0597 | -- | 3.3 | 3.4 |
| Allantoin | C4H6N4O3 | [M+H]+ | 159.0512 | 159.0500 | 144.0657, 129.1417 | -7.5 | 0.9 |
| Citrulline | C6H13N3O3 | [M+H]+ | 176.1026 | 176.1029 | 159.0767, 142.0709 | 1.7 | 0.8 |
| 4-Pyridoxate | C8H9NO4 | [M+H]+ | 184.0590 | 184.0604 | -- | 7.6 | 5.3 |
| 4-Hydroxy-4-(pyridin-2-yl)butan-2-one | C9H11NO2 | [M+Na]+ | 188.0702 | 188.0700 | 170.0598, 146.0597, 128.4364 | -1.0 | 4.2 |
| Kynurenic acid | C10H7NO3 | [M+H]+ | 190.0495 | 190.0498 | 190.0549, 144.0433 | 1.5 | 5.9 |
| Ligustilide | C12H14O2 | [M+H]+ | 191.1061 | 191.1066 | 177.0528, 173.0963, 109.1020 | 2.6 | 5.7 |
| 6-Methoxy-7-hydroxycoumarin | C10H8O4 | [M+H]+ | 193.0495 | 193.0495 | 178.0273, 150.0310 | 0.0 | 5.9 |
| Vasicinone | C11H10N2O2 | [M+H]+ | 203.0819 | 203.0815 | 185.0622, 156.0646 | 2.4 | 3 |
| NCGC00385243-01{(1R,2R)-2-[(2Z)-5-Hydroxy-2-penten-1-yl]-3-oxocyclopentyl}acetic acid | C12H18O4 | [M-H2O+H]+ | 209.1169 | 209.1170 | 191.1431, 177.1174, 161.0950 | 0.4 | 7 |
| Isofraxidin | C11H10O5 | [M+H]+ | 223.0598 | 223.0601 | 133.0252, 162.0280, 190.0255 | 1.3 | 5.9 |
| Sinapic acid | C11H12O5 | [M+H]+ | 225.0755 | 225.0757 |  153.9595 | 0.8 | 5.4 |
| Methyl jasmonate | C13H20O3 | [M+H]+ | 225.1483 | 225.1499 | 207.1380, 151.1113 | 7.1 | 6.3 |
| Genipin | C11H14O5 | [M+H]+ | 227.0880 | 227.0900 | -- | 8.8 | 6 |
| (E)-2-(2-(5-hydroxypent-2-en-1-yl)-3-oxocyclopentyl)acetic acid | C12H18O4 | [M+H]+ | 227.1270 | 227.1286 | 209.1166, 191.1059, 167.1063 | 7.0 | 5.8 |
| NCGC00385953-01\_(9*E*)-3,6,10-Trimethyl-7,8,11,11a-tetrahydrocyclodeca[b]furan-2,5(4H,6H)-dione | C15H20O3 | [M-H2O+H]+ | 231.1352 | 231.1369 | 213.0688, 200.9741 | 7.3 | 6.4 |
| Threonyllecuine | C10H20N2O4 | [M+H]+ | 233.1490 | 233.1510 | 215.1378, 187.0934 | 8.5 | 3.2 |
| Desmotroposantonin | C15H18O3 | [M+H]+ | 247.1287 | 247.1300 | 173.0955 | 5.2 | 3.7 |
| Isoleucylglutamate | C11H20N2O5 | [M+H]+ | 261.1442 | 261.1442 | 148.0605, 198.1001 | 0.0 | 1.6 |
| 1-(5,8-Dimethoxy-2,2-dimethylchromen-6-yl)ethanone | C15H18O4 | [M+H]+ | 263.1252 | 263.1277 | 245.1385, 227.1270 | 9.5 | 6.5 |
| Oxysophocarpine | C15H22N2O2 | [M+H]+ | 263.1714 | 263.1700 | 247.0379, 229.0815, 205.0014 | -5.3 | 11.2 |
| Huperzine A | C15H18N2O | [M+Na]+ | 265.1307 | 265.1300 | 198.9832, 177.0621 | -2.6 | 4.6 |
| Feruloyl putrescine | C14H20N2O3 | [M+H]+ | 265.1540 | 265.1546 | 248.128, 177.0541 | 2.2 | 4 |
| Heptadecanoic acid | C17H34O2 | [M+H]+ | 271.2625 | 271.2600 | 200.2378 | -9.2 | 11.2 |
| Methylenedioxyprovalerone | C16H21NO3 | [M+H]+ | 276.1589 | 276.1590 | 149.0233, 205.0863 | 0.3 | 4.4 |
| Coumaryl agmatine | C14H20N4O2 | [M+H]+ | 277.1651 | 277.1671 |  147.0925 | 7.2 | 4.2 |
| 6-Shogaol | C17H24O3 | [M+H]+ | 277.1797 | 277.1799 | 137.5460, 241.2922 | 0.7 | 8.5 |
| (5*R*)-*trans*-1,7-Diphenyl-5-hydroxy-6-hepten-3-one | C19H20O2 | [M+H]+ | 281.1488 | 281.1499 | 266.0734, 238.1064 | 3.9 | 3.2 |
| Xanthosine | C10H12N4O6 | [M+H]+ | 285.0827 | 285.0846 | 153.0400 | 6.6 | 3.4 |
| Malonyltryptophan | C14H14N2O5 | [M+H]+ | 291.0970 | 291.0972 | 159.0923, 245.0914 | 0.6 | 6.1 |
| Karanjin | C18H12O4 | [M+H]+ | 293.0817 | 293.0799 | 278.0858 | -6.1 | 9.2 |
| NCGC00380262-01!2,3,5,7-Tetramethoxy-9,10-dihydrophenanthrene | C18H20O4 | [M+H]+ | 301.1404 | 301.1430 | 278.1367, 240.2391 | 8.6 | 4.3 |
| Quercetin | C15H10O7 | [M+H]+ | 303.0499 | 303.0499 | 273.0393 155.0490 | 0.0 | 7.2 |
| NCGC00385659-01\_1-Naphthalenepentanol, decahydro-6-hydroxy-gamma,5,5,8a-tetramethyl-2-methylene- | C20H36O2 | [M+H]+ | 309.2785 | 309.2789 | 277.2121, 237.1503 | 1.2 | 10.7 |
| Feruloyl dehydrotyramine | C18H17NO4 | [M+H]+ | 312.1228 | 312.1247 | 177.0540 | 6.0 | 6 |
| Triptophenolide | C20H24O3 | [M+H]+ | 313.1770 | 313.1799 | 98.2521, 153.2231 | 9.2 | 10.1 |
| Moupinamide | C18H19NO4 | [M+H]+ | 314.1379 | 314.1400 | 240.2438, 177.0538 | 6.6 | 11.4 |
| Progesterone | C21H30O2 | [M+H]+ | 315.2280 | 315.2300 | 109.0644, 283.2649 | 6.3 | 7.9 |
| Dehydrophytosphingosine | C18H37NO3 | [M+H]+ | 316.2837 | 316.2846 | 275.0402 | 2.8 | 7.7 |
| NCGC00381109-01!1,3,6,8-Tetrahydroxy-2-(methoxymethyl)anthracene-9,10-dione | C16H12O7 | [M+H]+ | 317.0651 | 317.0650 | 280.2637, 242.2007 | -0.3 | 7.7 |
| NCGC00380522-01\_1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-7-hydroxy-1,4a-dimethyl-7-(1-methylethyl)-9-oxo- | C20H28O3 | [M+H]+ | 317.2082 | 317.2110 | 299.1982, 257.2461 | 8.8 | 9 |
| Phytosphingosine | C18H39NO3 | [M+H]+ | 318.2997 | 318.2994 | 282.278, 234.1239 | -0.9 | 9.2 |
| NCGC00385530-01!(*E*)-5-(1,2,4a,5-Tetramethyl-7-oxo-3,4,8,8a-tetrahydro-2*H*-naphthalen-1-yl)-3-methylpent-2-enoic acid | C20H30O3 | [M+H]+ | 319.2238 | 319.2260 | 258.2515, 210.9844 | 6.8 | 9.2 |
| NCGC00169469-03\_1-Naphthalenecarboxylic acid, decahydro-5-(5-hydroxy-3-methylpentyl)-1,4a-dimethyl-6-methylene-, (1*R*,4a*S*,5*R*,8a*S*)- | C20H34O3 | [M+H]+ | 323.2550 | 323.2580 | 284.8889, 268.1046 | 9.2 | 10.9 |
| NCGC00381434-01\_2-Methoxy-4-{(2*S*,3*R*)-7-methoxy-3-methyl-5-[(1*E*)-1-propen-1-yl]-2,3-dihydro-1-benzofuran-2-yl}phenol | C20H22O4 | [M+H]+ | 327.1582 | 327.1590 | 312.1582, 295.1311 | 2.4 | 6.5 |
| *N*-Fructosylphenylalanine | C15H21NO7 | [M+H]+ | 328.1386 | 328.1387 | 310.1277, 292.1170 | 0.3 | 3.7 |
| Hydroxygardnutine | C20H22N2O3 | [M+H]+ | 339.1694 | 339.1703 | 305.2111, 280.0919 | 2.6 | 10.3 |
| Kirenol | C20H34O4 | [M+H]+ | 339.2500 | 339.2500 | 269.1121, 191.0708  | 0.0 | 9.1 |
| Yohimbic acid | C20H24N2O3 | [M+H]+ | 341.1855 | 341.1859 | 163.152, 198.6508 | 1.1 | 10.7 |
| NCGC00380426-01\_7,10-Methano-1*H*-benzocycloundecene-4-carboxylic acid, 2,3,4,4a,5,6,9,10,11,12,13,13a-dodecahydro-14-hydroxy-4,13a-dimethyl-9,13-dioxo-, methyl ester, (4*R*,4a*S*,10*R*,13a*R*)- | C20H28O5 | [M+H]+ | 349.1980 | 349.2009 | 330.262, 313.2367 | 8.3 | 8.1 |
| 7-*β*-hydroxylathryol derivative | C20H30O5 | [M+H]+ | 351.2134 | 351.2100 | 329.2498, 312.2267 | -9.6 | 7.9 |
| NCGC00384898-01!1,3,6,8-Tetrahydroxy-2-(1-hydroxyhexyl) anthracene-9,10-dione | C20H20O7 | [M+H]+ | 373.1270 | 373.1279 | 355.117, 325.0518 | 2.4 | 4.9 |
| NCGC00169052-03\_6*H*-Furo[2,3-h]-2-benzopyran-6,8(6aH)-dione, 9-acetyl-3-[(1*E*,3*E*,5*S*)-3,5-dimethyl-1,3-heptadien-1-yl]-9,9a-dihydro-6a-methyl-, (6a*R*,9*R*,9a*R*)- | C23H26O5 | [M+H]+ | 383.1828 | 383.1850 | 365.195, 323.1641 | 5.7 | 7.1 |
| NCGC00347705-02![2-(2-Acetyloxypropan-2-yl)-7-oxo-2,3-dihydrofuro[3,2-g]chromen-3-yl] 3-methylbutanoate | C21H24O7 | [M+H]+ | 389.1575 | 389.1590 | 355.1378, 317.1105 | 3.8 | 5.2 |
| Gitogenin | C27H44O4 | [M+H]+ | 433.3305 | 433.3300 |  279.2177, 158.9212 | -1.1 | 10.4 |
| 5-*O*-Methylvisammoside | C22H28O10 | [M+H]+ | 453.1670 | 453.1700 | 328.1296, 291.0111 | 6.6 | 8.5 |
| 1-Oleoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine | C23H46NO7P | [M+H]+ | 480.3081 | 480.3096 | 436.7828, 339.2859 | 3.1 | 9.9 |
| Aconine | C25H41NO9 | [M+H]+ | 500.2840 | 500.2800 | 482.2660 | -7.9 | 4.5 |
| Kaempferol-3-*O*-glucoside-6-*p*-coumaryl | C30H26O13 | [M+H]+ | 595.1436 | 595.1445 | 309.0962, 147.0433 | 1.5 | 7.2 |
| Tenuifolin | C36H56O12 | [M+H]+ | 681.3830 | 681.3800 | 415.3207, 299.2361 | -4.4 | 8.3 |
| Solanidine | C39H63NO10 | [M+H]+ | 706.4516 | 706.4534 | 631.1512, 566.8934 | 2.5 | 6.7 |
| NCGC00380680-01!5-Hydroxy-3-[(2*S*,3*R*,4*R*,5*S*)-3-hydroxy-5-(hydroxymethyl)-4-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxolan-2-yl]oxy-2-(4-hydroxyphenyl)-7-[(2*S*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxychromen-4-one | C32H38O19 | [M+H]+ | 727.2060 | 727.2080 | 531.348, 365.1871 |  | 6.1 |
| Fumaric acid | C4H4O4 | [M+Na]+ | 139.0023 | 139.0020 | -- | -2.1 | 1.66 |
| 4-Guanidinobutanoic acid | C5H11N3O2 | [M+H]+ | 146.0921 | 146.0923 | 67.1611, 65.3528 | 1.3 | 4.3 |
| Glutamine | C5H10N2O3 | [M+H]+ | 147.0761 | 147.0764 | 57.8212, 84.0834 | 2.0 | 0.8 |
| *cis*-Aconitate | C6H6O6 | [M+H]+ | 175.0236 | 175.0237 | 161.0596 | 0.5 | 1.2 |
| Ferulic acid | C10H10O4 | [M-H2O+H]+ | 177.0543 | 177.0546 | 149.0237 | 1.6 | 7.9 |
| Isocitric acid | C6H8O7 | [M+H]+ | 193.0339 | 193.0342 | 139.0023 | 1.5 | 1.1 |
| Leucylproline | C11H20N2O3 | [M+H]+ | 229.1544 | 229.1551 | 145.0735 | 3.0 | 3.8 |
| Isoleucylvaline | C11H22N2O3 | [M+H]+ | 231.1698 | 231.1707 | 213.0406, 185.1644 | 3.8 | 3.4 |
| Coumaroyl putrescin | C13H18N2O2 | [M+H]+ | 235.1431 | 235.1447 | 218.1207, 204.9814, 189.0077 | 6.8 | 3.3 |
| NCGC00385949-01\_3a-Hydroxy-3,5a,9-trimethyl-3a,5,5a,6,7,9b-hexahydronaphtho[1,2-b]furan-2,8(3*H*,4*H*)-dione | C15H20O4 | [M+H]+ | 265.1429 | 265.1430 | 247.1322, 227.1632, 221.0730 | 0.3 | 5.6 |
| Saccharopine | C11H20N2O6 | [M+H]+ | 277.1407 | 277.1394 | 212.9978, 129.361 | -4.6 | 6.7 |
| Hyoscyamine | C17H23NO3 | [M+H]+ | 290.1743 | 290.1750 | 123.3045, 77.1418 | 2.4 | 4.3 |
| NCGC00380446-01\_11-Hydroxy-11-isopropyl-4-methoxy-8-methyl-10,11-dihydro-6H-oxepino[2,3-d]pyrazino[1,2-a]pyrimidine-6,9(8*H*)-dione | C16H19N3O5 | [M+H]+ | 316.1282 | 316.1290 | 257.2476, 238.0517, 208.0989 | 2.5 | 4.3 |
| 6-Ethyl-3-(4-isopropoxyphenyl)-4-oxo-4H-chromen-7-yl acetate | C22H22O5 | [M+H]+ | 367.1495 | 367.1499 | 349.1382, 331.1298, 308.0637 | 1.0 | 4.3 |
| Dicoumaroyl spermidine | C25H31N3O4 | [M+H]+ | 438.2381 | 438.2398 | 292.4340, 204.1020 | 3.8 | 4.8 |
| 4-Hydroxy-6-methylpyran-2-one | C6H6O3 | [M+NH4]+ | 144.0653 | 144.0655 | ------ | -1.3 | 0.9 |
| Glutamic acid | C5H9NO4 | [M+H]+ | 148.0601 | 148.0604 | 120.5564, 85.3661 | -2.0 | 0.8 |
| Oxypurinol | C5H4N4O2 | [M+H]+ | 153.0403 | 153.0407 | 140.0064, 136.0147, 133.8919 | -2.6 | 3.3 |
| Uric acid | C5H4N4O3 | [M+H]+ | 169.0352 | 169.0356 | 152.0083 | -2.3 | 1.0 |
| Atractylenolide III | C15H20O3 | [M+H]+ | 249.1462 | 249.1485 | 224.4366, 216.9909, 211.1094, 203.0229 | -9.2 | 6.9 |
| NCGC00380646-01!(*E*)-5-(4-Methoxy-5-methyl-6-oxopyran-2-yl)-3-methylhex-4-enoic acid | C14H18O5 | [M+H]+ | 267.1205 | 267.1220 | 231.1226, 218.0801, 193.0835 | -5.6 | 6.8 |
| Oleamide | C18H35NO | [M+H]+ | 282.2787 | 282.2791 | 226.2172, 212.1986, 198.1833 | -1.4 | 10.5 |
| Catechin | C15H14O6 | [M+H]+ | 291.0838 | 291.0863 | 258.9218, 156.8899,  | -8.5 | 5.4 |
| NCGC00169045-03!(2*Z*)-4,6-Dihydroxy-2-[(3,4,5-trihydroxyphenyl)methylidene]-1-benzofuran-3-one | C15H10O7 | [M+H]+ | 303.0498 | 303.0499 | 247.3145, 238.7676, 219.0999, 174.101 | -0.3 | 7.8 |
| NCGC00385697-01!7-Hydroxy-3-(3-hydroxy-4-methoxyphenyl)-6-methoxychromen-4-one | C17H14O6 | [M+H]+ | 315.0838 | 315.0860 | 278.8125, 257.2451, 220.0651 | -6.9 | 6.3 |
| Feruloyl tyramine | C18H19NO4 | [M+H]+ | 314.1381 | 314.1384 | 177.0541 | -0.9 | 7.2 |
| 4,4'-Dimethoxydalbergione | C17H16O4 | [M+K]+ | 323.0730 | 323.0757 | 249.4238 | -8.3 | 4.9 |
| 2-(3,5-Difluorophenyl)-*N*-(((1S,9a*R*)-octahydro-1*H*-quinolizin-1-yl) methyl) acetamide | C18H24F2N2O | [M+H]+ | 323.1950 | 323.1919 | 318.4137, 305.2503, 287.2348, 273.0963 | 9.5 | 10.2 |
| Sanguinarine | C20H14NO4 | [M]+ | 332.0893 | 332.0911 | 261.0783, 289.0637 | -5.4 | 6.3 |
| 9-Hydroxy-10,12-octadecadienoic acid | C18H32O3 | [M+H]+ | 335.1976 | 335.1983 | 317.1782, 296.1268, 281.2343 | -2.0 | 10.4 |
| NCGC00384769-01!6,8-Dihydroxy-2,2,4,4-tetramethyl-5-(2-methylpropanoyl)-9-propan-2-yl-9*H*-xanthene-1,3-dione | C24H30O6 | [M+H]+ | 415.2113 | 415.2110 | 372.5897, 341.1954, 311.2968 | 0.7 | 9.6 |
| Grayanotoxin I | C22H36O7 | [M+K]+ | 451.2118 | 451.2092 | 386.0572, 347.145, 322.1612 | 5.7 | 4.8 |
| Naringin | C27H32O14 | [M+H]+ | 581.1839 | 581.1864 | ----- | -4.3 | 4.8 |
| (4-Dihydroxyphenyl)-3,5-dihydroxy-7-(((3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-((((3*R*,4*S*,5*S*)-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-yl) oxy) methyl) tetrahydro-2*H*-pyran-2-yl) oxy)-4*H*-chromen-4-one | C26H28O16 | [M+H]+ | 597.1558 | 597.1500 | ----- | 9.7 | 6.6 |
| NCGC00380564-01!3-[(2*S*,3*R*,4*S*,5*S*,6*R*)-4,5-Dihydroxy-6-(hydroxymethyl)-3-[(2*S*,3*R,*4*S*,5*R*)-3,4,5-trihydroxyoxan-2-yl]oxyoxan-2-yl]oxy-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxychromen-4-one | C27H30O16 | [M+H]+ | 611.1593 | 611.1599 | 472.1362, 399.1118, 317.0657 | -0.9 | 6.5 |
| 2-(4-(((2*R*,3*R*,4*R*,5*R*,6*R*)-4,5-Dihydroxy-2-(hydroxymethyl)-6-(((2*S*,3*R*,4*S*,5*R*,6*R*)-4,5,6-trihydroxy-2-methyltetrahydro-2*H*-pyran-3-yl)oxy)tetrahydro-2*H*-pyran-3-yl)oxy)-3-hydroxyphenyl)-3,5-dihydroxy-7-(((2*S*, 3*R*,4*R,*5*R*,6*S*)-3,4,5-trihydroxy-6-methyltetrahydro-2*H*-pyr an-2-yl)oxy)-4*H*-chromen-4-one-Cyanidin 3-(2G-glucosylrutinoside) | C33H41O20 | [M+H]+ | 757.2169 | 757.2199 | 716.1713, 662.4353, 623.3477, 317.0655, 164.3791, 131.9166 | -1.4 | 6.6 |
| 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylic acid  | C12H12N2O2 | [M+H]+ | 217.0989 | 217.1006 | 188.037, 164.09, 144.0803 | -7.8 | 4.9 |
| Arachidonic acid | C20H32O2 | [M+H]+ | 305.2475 | 305.2475 | 249.1841, 217.1573, 203.1796 | 0.0 | 9.8 |
| 4-Hydroxyquinoline | C9H7NO | [M+H]+ | 146.0599 | 146.0600 | 77.4044 | 0.6 | 4.7 |
| 1-Benzylimidazole | C10H10N2 | [M+H]+ | 159.0917 | 159.0916 | 142.0656, 105.0763 | 0.6 | 4.2 |
| 3-Hydroxycinnamic acid | C9H8O3 | [M+H]+ | 165.0546 | 165.0546 | 163.122, 148.046 | 0.0 | 1.6 |
| Loliolide | C11H16O3 | [M+H]+ | 197.1168 | 197.1170 | 179.1058, 157.0249 | -1.0 | 5.9 |
| Scoparone | C11H10O4 | [M+H]+ | 207.0498 | 207.0500 | 188.0928, 160.0614 | -0.9 | 1.0 |
| Adenosine | C10H13N5O4 | [M+H]+ | 268.1037 | 268.1040 | 136.0614 | -1.1 | 3.1 |
| Guanosine | C10H13N5O5 | [M+H]+ | 284.0985 | 284.0989 | 150.0424 | -1.4 | 2.6 |
| Coumaroyl tyramine | C17H17NO3 | [M+H]+ | 284.1275 | 284.1285 | 266.1024, 254.0026, 235.0281 | -3.5 | 7.1 |
| Kaempferol | C15H10O6 | [M+H]+ | 287.0548 | 287.0550 |  209.1165, 180.0106 | -0.6 | 7.6 |
| Cortodoxone | C21H30O4 | [M+H]+ | 347.2185 | 347.2210 | 262.226, 240.2326, 216.7003 | -8.9 | 9.0 |
| NCGC00384769-01!6,8-Dihydroxy-2,2,4,4-tetramethyl-5-(2-methylpropanoyl)-9-propan-2-yl-9*H*-xanthene-1,3-dione | C24H30O6 | [M+H]+ | 415.2113 | 415.2110 | 341.1954, 311.2968, 281.1384 | 0.7 | 9.6 |
| NCGC00380595-01!(7*R*,8*S*)-7,8-Dihydroxy-3,7-dimethyl-6-oxo-8*H*-isochromene-5-carbaldehyde | C12H12O5 | [M+H]+ | 237.0756 | 237.0750 | 219.0651, 193.0499, 179.0705 | 2.5 | 5.7 |
| NCGC00381029-01!1-(3-ethyl-2,4-Dihydroxy-6-methoxyphenyl)butan-1-one | C13H18O4 | [M+H]+ | 239.1270 | 239.1280 | 207.138, 192.1011 | -4.1 | 6.5 |
| NCGC00386106-01\_(1*R*,2*E*,6*S*,10*E*,11a*S*,14a*R*)-1-Hydroxy-6-methyl-6,7,8,9,11a,12,14,14a-octahydro-4*H*-cyclopenta[f]oxacyclotridecine-4,13(1*H*)-dione | C16H22O4 | [M+H]+ | 279.1586 | 279.1590 | 239.0022, 201.0454 | -1.4 | 11.7 |
| Asp-Phe | C13H16N2O5 | [M+H]+ | 281.1132 | 281.1131 | 263.102, 246.0752 | 0.3 | 4.2 |
| Glutamyltyrosine | C14H18N2O6 | [M+H]+ | 311.1231 | 311.1239 | 264.0011, 199.1440 | -2.5 | 3.8 |

**Table S-3. All metabolites identified in *Tribulus terrestris* fruit and aerial parts from both regions in negative ion mode.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Metabolite** | **Mol. Formula** | **M-** | **Experimental Precursor *m/z*** | **Reference****Precursor *m/z*** | **Products *m/z*** | **ppm error** | **r/t** |
| Citramalic acid | C5H8O5 | [M-H]- | 147.0301 | 147.0298 | ------ | 2.0 | 1.39 |
| Patulin | C7H6O4 | [M-H]- | 153.0197 | 153.0193 | 151.0401 | 2.6 | 8.1 |
| *m*-Coumaric acid | C9H8O3 | [M-H]- | 163.0400 | 163.0400 | 147.0447 | 0.0 | 6.5 |
| Dehydro ascorbic acid | C6H4O6 | [M-H]- | 173.0092 | 173.0091 | ------ | 0.5 | 3.11 |
| 2-Isopropylanalic acid derivative | C7H12O5 | [M-H]- | 175.0614 | 175.0612 | 157.0503 | 1.1 | 6.03 |
| Kynurenic acid | C10H7NO3 | [M-H]- | 188.0355 | 188.0353 | 144.0459 | 1.0 | 6.5 |
| Acetylglutamic acid | C7H11NO5 | [M-H]- | 188.0562 | 188.0564 | 170.0463, 146.0462 | -1.0 | 1.9 |
| Citric acid | C6H8O7 | [M-H]- | 191.0199 | 191.0197 | 87.6419 | 1.0 | 1.56 |
| 4-Hydroxy-3-methoxycinnamate | C10H10O4 | [M-H]- | 193.0508 | 193.0506 | 178.0304, 161.0257 | 1.0 | 7.06 |
| Pantothanic acid | C9H16NO5 | [M-H]- | 218.1036 | 218.1033 | 175.0182, 153.0196 | 1.3 | 4.83 |
| 3-(8-Hydroxyoctyl)-Phenol | C14H22O2 | [M-H]- | 221.1545 | 221.1540 | ------ | 2.2 | 9.13 |
| (8a*R*,12*S*,12a*R*)-12-Hydroxy-4-methyl-4,5,6,7,8,8a,12,12a-octahydro-1*H*-3-benzoxecine-2,9-dione | C14H20O4 | [M-H]- | 251.1287 | 251.1288 | 154.9740, 189.1292 | -0.3 | 8.27 |
| Palmitic acid | C16H32O2 | [M-H]- | 255.2333 | 255.2329 | 237.2234, 106.7199 | 1.5 | 11.2 |
| 16-Hydroxyhexadecanoic acid | C16H32O3 | [M-H]- | 271.2281 | 271.2278 | 129.2696 | 1.1 | 10.3 |
| Linoleic acid | C18H32O2 | [M-H]- | 279.2328 | 279.2329 | 233.1545, 205.1600 | -0.3 | 10.68 |
| Feruloyl lactate | C13H14O7 | [M-H]- | 281.0668 | 281.0647 | 178.0272, 193.0505 | 7.4 | 6.54 |
| Oleic Acid | C18H34O2 | [M-H]- | 281.2486 | 281.2486 | 232.9328 | 0.0 | 11.37 |
| Guanosine | C10H13N5O5 | [M-H]- | 282.0843 | 282.0840 | 150.0424 | 1.0 | 2.6 |
| Xanthosine | C10H12N4O6 | [M-H]- | 283.0685 | 283.0684 | 153.0400 | 0.3 | 3.97 |
| Kaempferol | C15H10O6 | [M-H]- | 285.0403 | 285.0404 | 151.0042, 227.0359 | -0.3 | 7.93 |
| Methyl-2-ethyl-4-(3*R*,4*R*,5*S*) hydroxy-4,5-dimethyl | C14H22O6 | [M-H]- | 285.1340 | 285.1343 | 241.0353, 197.0456 | -1.0 | 5.12 |
| Delphindin | C15H11ClO7 | [M-H]- | 337.0109 | 337.0111 | 273.0409, 257.0448 | 1.3 | 7.5 |
| Sulfo jasmonate | C12H18O7S | [M-H]- | 305.0699 | 305.0686 | 272.1497, 225.1132 | 4.2 | 7.41 |
| Benzoic acid+20, O-Hex | C13H16O9 | [M-H]- | 315.0723 | 315.0708 | 268.9384, 224.9493 | 4.7 | 4.83 |
| Rhodocladonic acid | C15H10O8 | [M-H]- | 317.0302 | 317.0300 | 179.0353, 163.0055 | 0.6 | 5.86 |
| Protolichesterinic acid | C19H32O4 | [M-H]- | 323.2207 | 323.2210 | 279.2337, 255.2331 | -0.9 | 11.2 |
| Dodecylbenzenesulfonic acid | C18H30O3S | [M-H]- | 325.1845 | 325.1840 | 257.2124, 186.9391 | 0.6 | 9.99 |
| Tranilast (Arthranilic acid) | C18H17NO5 | [M-H]- | 326.1039 | 326.1033 | 282.0887, 267.0657 | 1.8 | 7.06 |
| Laricitrin | C16H12O8 | [M-H]- | 331.0456 | 331.0459 | 282.0778, 145.0962 | -0.9 | 6.37 |
| (2S,3S)-3,5,7-Trihydroxy 6-methoxy-2(3,4,5-trihydroxyphenyl) |  | [M-H]- | 333.0612 | 333.0616 | 315.0502, 284.0311 | -1.2 | 6.72 |
| Coumaroyl + C6H9O8 | C15H16O10 | [M-H]- | 355.0668 | 355.0660 | 320.1629, 308.0704 | 2.2 | 5.69 |
| Humulone | C21H30O5 | [M-H]- | 361.1999 | 361.2020 | 292.2939, 293.7194 | -5.8 | 9.3 |
| Diffractaic acid | C20H22O7 | [M-H]- | 373.1293 | 373.1293 | 283.0486 | 0.0 | 9.1 |
| 4-[4-[Hydroxy-(4-hydroxy-3-methoxyphenyl)methyl]-3-(hydroxymethyl)oxolan-2-yl]-2-methoxyphenol (epoxylignans derivative) | C20H24O7 | [M-H]- | 375.1443 | 375.1449 | 295.085, 262.0638 | -1.5 | 6.3 |
| (*E*)-5- (1*S*,4a*R*,8a*R*) 2-Formyl-5,5,8a | C22H32O5 | [M-H]- | 375.2750 | 375.2764 | 309.1741, 288.8754 | -3.7 | 10.51 |
| NCGC00347775-02!(*Z*)-5-[(1*S*,2*R*,4a*R*,8a*R*)-5-(Hydroxymethyl)-1,2,4a-trimethyl-2,3,4,7,8,8a-hexahydronaphthalen-1-yl]-3-(hydroxymethyl)pent-2-enoic acid | C20H32O4 | [M-H]- | 381.2259 | 381.2280 | 335.2205, 313.2385 | -5.5 | 8.9 |
| NCGC00380272-01!5,8-Dihydroxy-6-methoxy-7-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-2-one | C16H18O11 | [M-H]- | 385.0766 | 385.0780 | 248.9605, 223.0454 | -3.6 | 5.69 |
| NCGC00347834-02\_ 6,10a-Dihydroxy-4-(hydroxymethyl)-4,7,11b-trimethyl-2,3,4,4a,5,6,6a,7,10a,11,11a,11b-dodecahydrophenanthro[3,2-b] furan-9(1*H*)-one | C20H30O5 | [M-H]- | 395.2076 | 395.2080 | 349.1997, 327.2189 | -1.0 | 7.75 |
| Vanillic acid glucoside derivative | C8H8O4 | [M-H]- | 409.0444 | 409.0441 | 373.1402, 242.9975 | 0.7 | 5.52 |
| NCGC00380739-01\_1H-2,6-Dioxacyclopent[cd]inden-1-one, 4-[(acetyloxy)methyl]-5-(beta-D-glucopyranosyloxy)-2a,4a,5,7b-tetrahydro-, (2a*S*,4a*S*,5*S*,7b*S*)- | C18H22O11 | [M-H]- | 459.1145 | 459.1140 | 411.1874, 358.1968 | 1.0 | 5.34 |
| Folinic acid | C20H23N7O7 | [M-H]- | 472.1573 | 472.1586 | 426.1375, 343.1155 | -2.7 | 5 |
| Isorhamnetin-3-galactoside | C22H22O12 | [M-H]- | 477.1069 | 477.1039 | 380.1793, 306.0962 | 6.2 | 5.1 |
| Flavonol base+4*O*, *O*-Hex, 1MeO | C22H22O13 | [M-H]- | 493.1018 | 493.0986 | 447.2231, 413.1461 | 6.4 | 7.92 |
| NCGC00380440-01 2-[[(E)-3-[2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-7-methoxy-2,3-dihydro-1-benzofuran-5-yl]prop-2-enoyl]amino]pentanedioic acid | C25H27NO10 | [M-H]- | 500.1557 | 500.1560 | 470.1458, 438.1557 | -0.5 | 6.72 |
| 7-[4,5-dihydroxy-6-(hydroxymethyl)-3-[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxyoxan-2-yl]oxy-5-hydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one  | C27H32O14 | [M-H]- | 579.1693 | 79.1720 | 500.1559, 470.1454 | -4.6 | 7.06 |
| NCGC00384697-01! [(2*S*,3*R*,4*S*,5*S*,6*R*)-4,5 Dihydroxy -3-[(*E*)-3-(4-hydroxyphenyl) prop-2-enoyl] oxy-6-[[(*E*)-3-(4-hydroxyphenyl) prop-2-enoyl] oxymethyl] oxan-2-yl] 3,4,5-trihydroxybenzoate | C31H28O14 | [M-H]- | 623.1400 | 623.1409 | 460.1762, 315.0510 | -1.4 | 7.58 |
| Furostane base-2H+O-Hex | C33H54O9 | [M-H]- |  639.3750 | 639.3729 | 593.37, 594.3717 | 3.2 | 9.82 |
| Cyanidin-3-*O*-*O*-(2"-*O*-beta- Anthocyanidin-5-*O*-glyco | C32H38O20 | [M-H]- | 741.1877 | 741.1878 | ------ | -0.1 | 6.37 |
| NCGC00169209-03![17-(2,6-Dihydroxy-6-methyl-3-oxoheptan-2-yl)-3-hydroxy-4,4,9,13,14-pentamethyl-2-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-2,3,7,8,10,11,12,15,16,17-decahydro-1*H*-cyclopenta[a]phenanthren-16-yl] acetate | C38H62O12 | [M-H]- | 755.4208 | 755.4220 | 709.4141, 681.4530 | -1.5 | 8.2 |
| NCGC00179940-02\_beta-D-Glucopyranoside, 3,23-dihydroxyspirostan-6-yl 6-deoxy-4-*O*-(6-deoxy-alpha-L-mannopyranosyl)  | C39H64O13 | [M-H]- | 785.4329 | 785.4329 | 740.4306, 577.3739 | 0.0 | 10.16 |
| [(2*R*,3*S*,4*S*,5*R*,6*S*)-6-[(2*S*,3*R*,4*S*,5*R*,6*R*)-6-[[(1*S*,3*R*,4*S*,4a*R*,8a*R*)-4-[(3*S*)-3-[(2*S*,3*R*,4*R*,5*R*,6*R*)-3,4-Dihydroxy-6-methyl-5-[(2*S*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxyoxan-2-yl]oxy-3-methylpent-4-enyl]-3,4,8,8a-tetramethyl-1,2,3,4a,5,6-hexahydronaphthalen-1-yl]oxy]-4,5-dihydroxy-2-methyloxan-3-yl]oxy-3,4,5-trihydroxyoxan-2-yl]methyl acetate | C46H76O20 | [M-H]- | 947.4856 | 947.4857 | 901.4800, 755.4235 | -0.1 | 9.3 |
| Aconitic acid | C6H6O6 | [M-H]- | 173.0088 | 173.0091 | ------ | 1.7 | 2.9 |
| Theophyline | C7H8N4O2 | [M-H]- | 179.056 | 179.0574 | ------ | 7.8 | 0.8 |
| Azelaic Acid | C9H16O4 | [M-H]- | 187.0974 | 187.0976 | 169.0872 | 1.0 | 1.7 |
| Ferulic acid | C10H10O4 | [M-H]- | 193.0490 | 193.0506 | 149.0237 | 3.1 | 6.0 |
| Acetyltryptophane | C13H14N2O3 | [M-H]- | 245.0923 | 245.0931 | 188.0563, 170.0454 | 3.2 | 6 |
| Inosine | C10H12N4O5 | [M-H]- | 267.0727 | 267.0734 | 228.9657 | 2.6 | 1.05 |
| 5,9-Dihydroxy-7-(hydroxymethyl) | C15H22O5 | [M-H]- | 281.1380 | 281.1394 | 237.1491 | 4.9 | 5.5 |
| Oleic acid | C18H34O2 | [M-H]- | 281.2472 | 281.2486 | 232.9307 | 4.9 | 11.3 |
| Luteolin | C15H10O6 | [M-H]- | 285.0393 | 285.0404 | 159.0368, 144.1071 | 3.8 | 7.5 |
| Quercetin-3-*O*-deoxyhexosyl(1-2)Pentoside | C26H28O15 | [M-H]- | 579.1337 | 579.1339 | 417.0071, 301.5500 | 0.3 | 5.1 |
| Flavonal base | C33H54O9 | [M+FA-H]- | 639.3718 | 639.3729 | 591.1338, 387.1054 | 1.3 | 8 |
| *p*-Coumaric acid | C9H8O3 | [M-H]- | 163.0398 | 163.0400 | ------ | 1.2 | 6.5 |
| 3-Hydroxy-4-methoxy cinnamic acid | C10H10O4 | [M-H]- | 193.0506 | 193.0506 | 159.0323 | 0.0 | 6.7 |
| Gluconate | C6H12O7 | [M-H]- | 195.0510 | 195.0511 | 177.0405 | 0.0 | 0.8 |
| D-Saccharic acid | C6H10O8 | [M-H]- | 209.0305 | 209.0300 | 173.0093 | -2.3 | 4.1 |
| Dodecyl Sulfate | C12H26O4S | [M-H]- | 265.1473 | 265.1474 | 170.9472, 232.0816 | 0.3 | 10.4 |
| Stearic acid | C18H36O2 | [M-H]- | 283.2636 | 283.2642 | ------ | 2.1 | 9.4 |
| NCGC00017248-12!2,5-Dihydroxy-3-undecylcyclohexa-2,5-diene-1,4-dione | C17H26O4 | [M-H]- | 293.1784 | 293.1759 | 248.9086, 180.9217 | -8.5 | 4.7 |
| 2,4-Dihydroxyheptadec-16-enyl acetate | C19H36O4 | [M-H]- | 327.2539 | 327.2540 | 299.2585, 253.2525 | 0.3 | 10.16 |
| 5,7-Dihydroxy-2-(4-hydroxy-) (flavanones) | C21H22O6 | [M-H]- | 369.1684 | 369.1685 | 337.1962, 305.2243 | 0.7 | 10.6 |
| Actinonine  | C19H35N3O5 | [M-H]- | 384.2505 | 384.2504 | 338.2441, 248.9601 | -0.2 | 6.2 |
| 5-Hydroxy-6,8-dimethoxy-7-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-2-one | C17H20O11 | [M-H]- | 399.0929 | 399.0932 | 385.0767, 315.0717 | 0.7 | 6.3 |
| Liquiritin | C21H22O9 | [M-H]- | 417.1188 | 417.1191 | 258.0615 | 0.7 | 6.3 |
| N1, N10-Dicoumaroylspermidine | C25H31N3O4 | [M-H]- | 436.2233 | 436.2233 | 205.0775 | 0.0 | 5. |
| Luteolin 6-*C*-glucoside 8-*C*-arabinoside | C27H30O16 | [M-H]- | 609.1447 | 609.1461 | 487.0771, 209.0300 | 2.2 | 6.02 |
| Isorhamnetin 3,4'-diglucoside | C28H32O17 | [M-H]- | 639.1562 | 639.1566 | 447.1303, 327.0873 | 0.6 | 5.8 |
| Tetrasaccharides (Hex-Hex-Hex-Hex) | C24H42O21 | [M-H]- | 711.2194 | 711.2169 | 587.1827, 503.1614 | -3.5 | 2.5 |

The results obtained from DIA negative ion mode were not promising with compared to positive ion mode. We combined the transition lists of fruit samples from both regions (R1 and R2) generated from DDA results in heatmap.



**Figure S1**. Hierarchical clustering of all metabolites identified in negative ion mode were generated using Perseus software. (\*WP stands for whole plant)