**Table 1.** Identifications of components producing CAD peaks in the UHPLC-PDA-CAD-HRMS analysis of Ashwagandha Root (*Withania sominifera*) extract.

| **CAD**  **Peak**1 | **RT**  **(min)**2 | **Exp. *m/z***7  **+, top**  **-, bottom** | **Mass Acc.**  **+, top**  **-, bottom** | **Proposed ID**  **Molecular Formula**  **(CAS)**  **Confidence**3 | **Structure** | **Amt. µg per day**  **µg constituent per mg extract**  **(%)** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 1a | 0.89 | 203.0526 5  365.1054 5  527.1586 5  707.2219 5  215.0328 6  377.0856 6  539.1390 6  719.2019 6 | 0.2  -0.1 | Saccharides  C6H12O6 + (C6H10O5)x  Tentative | OC1C(O)C(OCC2OC(OCC3OC(O)C(O)C(O)C3O)C(O)C(O)C2O)OC(CO)C1O | 29,000  110  (11%) | HRMS supports molecular formulae  MS/MS supports structure(s) via mzCloud online database. 4  Saccharide-based structures (i.e., sugars) are common to botanicals.  The chirality cannot be determined. |
| 1b | 338.1448  - | -0.0  - | Sugar + C7H13NO4  C13H23NO9  Partial | OC1C(O)C(CO)OC(OC#CC#CC#CC)C1O.O[N+]([O-])=O.[HH].[HH].[HH].[HH] | HRMS supports molecular formula  MS/MS supports structure with loss of sugar, but the aglycone cannot be determined. |
| 2a | 0.96 | 142.1226 | -0.5 | Tropine OR β-tropine  C8H15NO  (120-29-6  135-97-7)  Reference | CN1C2CCC1CC(O)C2 | 75,000  280  (28%) | HRMS supports molecular formula  MS/MS supports structure via reference standard.  Retention time matches reference standard. |
| 2b | 203.0526 5  365.1054 5  527.1586 5  707.2219 5  215.0328 6  377.0856 6  539.1390 6  719.2019 6 | 0.2  -0.1 | Saccharides  C6H12O6 + (C6H10O5)x  Tentative | OC1C(O)C(OCC2OC(OCC3OC(O)C(O)C(O)C3O)C(O)C(O)C2O)OC(CO)C1O | HRMS supports molecular formulae  MS/MS supports structure(s) via mzCloud online database. 4  Saccharide-based structures (i.e., sugars) are common to botanicals.  The chirality cannot be determined. |
| 3a | 0.99 | 142.1226  - | -0.5 | Tropine OR β-tropine  C8H15NO  (120-29-6  135-97-7)  Reference | CN1C2CCC1CC(O)C2 | 150,000  550  (55%) | HRMS supports molecular formula  MS/MS supports structure via reference standard.  Retention time matches reference standard. |
| 3b | 203.0526 5  365.1054 5  527.1586 5  707.2219 5  215.0328 6  377.0856 6  539.1390 6  719.2019 6 | 0.2  -0.1 | Saccharides  C6H12O6 + (C6H10O5)x  Tentative | OC1C(O)C(OCC2OC(OCC3OC(O)C(O)C(O)C3O)C(O)C(O)C2O)OC(CO)C1O | HRMS supports molecular formulae  MS/MS supports structure(s) via mzCloud online database. 4  Saccharide-based structures (i.e., sugars) are common to botanicals.  The chirality cannot be determined. |
| 4 | 1.59 | 527.1583 5  503.1620 | 0.0  0.5 | Trisaccharide  (e.g., raffinose)  C18H32O16  (e.g., 512-69-6)  Matched | OCC1OC(OCC2OC(OC3(CO)OC(CO)C(O)C3O)C(O)C(O)C2O)C(O)C(O)C1O | 140  0.54 ± 0.3  (0.054%) | HRMS supports molecular formula  MS/MS supports structure via mzCloud online database. 4  Saccharide-based structures (i.e., sugars) are common to botanicals.  The exact connectivity and chirality cannot be determined. |
| 5 | 2.01 | 130.0498  128.0353 | -0.5  -0.4 | Pyroglutamic acid  C5H7O3N  (98-79-3)  Matched | O=C1NC(C(O)=O)CC1 | 150  0.55 ± 0.3  (0.055%) | HRMS supports molecular formula  MS/MS supports structure via mzCloud online database. 4  Amino acid-based structures are common to botanicals. |
| 6 | 2.25 | 190.0710  188.0564 | -0.2  -0.9 | Acetylglutamic acid  C7H11O5N  (1188-37-0)  Matched | OC(CCC(NC(C)=O)C(O)=O)=O | 160  0.58 ± 0.3  (0.058%) | HRMS supports molecular formula  MS/MS supports structure via mzCloud online database. 4  Amino acid-based structures are common to botanicals.  This is the only major signal that appears in both positive and negative mode. |
| 6b | 144.1381  - | -1.5  - | Hygroline  OR  1-α-methyl-Piperidineethanol  C8H17ON  (496-47-9  OR  934-90-7)  Tentative | CN1CCCC1CC(C)O    OC(C)CC1CCCCN1 | HRMS supports molecular formula  MS/MS supports structure via manual interpretation.  No literature/database available to match. |
| 7 | 3.42 | 142.1225  - | -0.1  - | Hygrine OR Pelletierine  C8H15ON  (496-49-1  OR  2858-66-4)  Tentative | CN1CCCC1CC(C)=O  O=C(C)CC1NCCCC1 | 100  0.39 ± 0.2  (0.039%) | HRMS supports molecular formula  MS/MS supports structure via literature [1]. |
| 8 | 4.28 | 144.1381  - | -1.4  - | Hygroline  OR  Sedridine  C8H17ON  (496-47-9  OR  501-83-7)  Tentative | CN1CCCC1CC(C)O  OC(C)CC1NCCCC1 | 200  0.76 ± 0.4  (0.076%) | HRMS supports molecular formula  MS/MS supports structure via manual interpretation.  No literature/database available to match. |
| 9 | 7.90 | 184.1331 | -3.6 | Acetyltropine  C10H18O2N  (3423-27-6)  Tentative | CN1C2CCC1CC(OC(C)=O)C2 | 150  0.56 ± 0.2  (0.056%) | HRMS supports molecular formula  MS/MS supports structure via manual interpretation.  No literature/database available to match. |
| 10a | 20.29 | 474.2596  472.2454 | -0.6  0.2 | N1,N8-Bis(dihydrocaffeoyl) spermidine  C25H35O6N3  (132194-39-9)  Tentative | OC1=CC(CCC(NCCCNCCCCNC(CCC2=CC(O)=C(O)C=C2)=O)=O)=CC=C1O | 91  0.34 ± 0.3  (0.034%) | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation but was NOT related to *Withania [2-3]*. |
| 10b | 636.3127  634.2984 | 0.1  0.4 | Lycibarbarspermidines H or I  C31H45O11N3  (1884356-81-3 or  1884356-82-4)  Tentative | OC1=CC(CCC(NCCCNCCCCNC(CCC2=CC(O)=C(O)C=C2)=O)=O)=CC=C1OC3C(O)C(O)C(O)C(CO)O3 | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation but was NOT related to *Withania [2-3]*. |
| 11a | 20.41 | 474.2596  472.2454 | -0.6  0.2 | N1,N8-Bis(dihydrocaffeoyl) spermidine  C25H35O6N3  (132194-39-9)  Tentative | OC1=CC(CCC(NCCCNCCCCNC(CCC2=CC(O)=C(O)C=C2)=O)=O)=CC=C1O | 120  0.46 ± 0.1  (0.046%) | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation but was NOT related to *Withania [2-3]*. |
| 11b | 636.3127  634.2984 | 0.1  0.4 | Lycibarbarspermidines H or I  C31H45O11N3  (1884356-81-3 or  1884356-82-4)  Tentative | OC1=CC(CCC(NCCCNCCCCNC(CCC2=CC(O)=C(O)C=C2)=O)=O)=CC=C1OC3C(O)C(O)C(O)C(CO)O3 | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation but was NOT related to *Withania [2-3]*. |
| 12 | 22.82 | 224.1644  - | -0.4  - | Tropigline  C13H21O2N  (533-08-4)  Tentative | CN1C2CCC1CC(OC(/C(C)=C/C)=O)C2 | 140  0.51 ± 0.07  (0.051%) | HRMS supports molecular formula  MS/MS supports structure via manual interpretation.  No literature/database available to match. |
| 13 | 30.95 | 815.4060  813.3914 | 0.0  -0.1 | Withagenin A Diglucoside  C40H62O17  (1379595-75-1)  Matched | OC1CC(OC2C(O)C(O)C(O)C(COC3C(O)C(O)C(O)C(CO)O3)O2)CC4(O)C1(C)C5C(C(CCC6C(C)C7OC(C(CO)=C(C)C7)=O)C6(C)CC5)C8C4O8 | 99  0.37 ± 0.1  (0.037%) | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation and was related to *W. somnifera* fruits [4]. |
| 14 | 31.22 | 1107.5214  1105.5068 | -0.4  -0.4 | Withanoside IX  C52H82O25  (500721-40-4)  Matched | OC1CC(OC2C(O)C(O)C(O)C(COC3C(O)C(O)C(O)C(CO)O3)O2)CC4=CCC(C(CCC5C(C)C6OC(C(COC7C(O)C(O)C(O)C(COC8C(O)C(O)C(O)C(CO)O8)O7)=C(C)C6)=O)C5(C)CC9)C9C41C | 540  2.0 ± 0.3  (0.2%) | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation and was related to *W. somnifera* fruits [4]. |
| 14b | 1095.5569  1093.5427 | -1.2  -0.9 | (1α,​3β,​22*R*,​24*Z*)​-​1,​22-​dihydroxyergosta-​5,​24-​diene-​3,​26-​diyl bis[6-​*O*-​β-​D-​glucopyranosyl-β-​D-​Glucopyranoside  C52H86O24  (2411842-88-9)  Tentative | OC(C/C(C)=C(COC1C(O)C(O)C(O)C(COC2C(O)C(O)C(O)C(CO)O2)O1)\C)C(C)C3CCC4C5CC=C6CC(OC7C(O)C(O)C(O)C(COC8C(O)C(O)C(O)C(CO)O8)O7)CC(O)C6(C)C5CCC43C | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature only contained HRMS and NMR (i.e., no fragmentation), but was related to *W. somnifera* seeds [5]. |
| 15a | 32.38 | 933.5050  931.4907 | -0.4 | C46H76O19  Tentative | OC(C/C(C)=C(C)/CO)C(C)C1CCC2C3CC=C4CC(O)CC(O)C4(C)C3CCC21C.OC5C(CO)OC(O)C(O)C5O.OC6C(CO)OC(O)C(O)C6O.OC7C(CO)OC(O)C(O)C7O | 92  0.35 ± 0.06  (0.035%) | HRMS supports molecular formula  MS/MS supports structure via manual interpretation.  Literature only contained closely related analogues and was related to *W. somnifera* seeds [5]. |
| 15b | 945.4682  989.4596 7 | -0.8 | Sominone Triglucoside  (Withanoside VIII)  C46H72O20  (519186-57-3)  Matched | OC(C1)CC(O)C2(C)C1=CCC3C2CCC4(C)C3CCC4C(C)C5OC(C(CO)=C(C)C5)=O.OC6C(CO)OC(O)C(O)C6O.OC7C(CO)OC(O)C(O)C7O.OC8C(CO)OC(O)C(O)C8O | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation and was related to *W. somnifera* fruits [4] and roots [6]. |
| 16a | 32.67 | 653.3533  697.3440 7 | 0.2  -0.1 | Withanoside III  C34H52O12  (362472-80-8)  Matched | OC1CC(OC2C(O)C(O)C(O)C(CO)O2)CC3(O)C1(C)C4C(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC4)C7C3O7 | 100  0.39 ± 0.06  (0.039%) | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation and was related to *W. somnifera* fruits [4]. |
| 16b | 1241.6175  1239.6013 | 1.2  -0.8 | C58H96O28  Partial | C/C(CO)=C(C)\CCC(C)C1CCC2C3CC=C4CC(O)CC(O)C4(C)C3CCC21C.OC5C(CO)OC(O)C(O)C5O.OC6C(CO)OC(O)C(O)C6O.OC7C(CO)OC(O)C(O)C7O.OC8C(CO)OC(O)C(O)C8O.OC9C(CO)OC(O)C(O)C9O | HRMS supports molecular formula  MS/MS largely contains losses of sugar moieties. There are several, albeit structurally similar, compounds in literature that would be supported by the HRMS and MS/MS.  None of these options have been reported to come from Withania, but there is high confidence that it is some sterol with four sugar moieties attached. The backbone of the sterol proposed is consistent with those reported in literature.  Molecular formula and MS/MS matches peak 23c with an additional sugar moieties. |
| 17a | 33.07 | 945.4682  943.4540 | -0.8  -0.4 | Sominone Triglucoside  (Withanoside VIII)  C46H72O20  (500722-19-0)  Matched | OC(C1)CC(O)C2(C)C1=CCC3C2CCC4(C)C3CCC4C(C)C5OC(C(CO)=C(C)C5)=O.OC6C(CO)OC(O)C(O)C6O.OC7C(CO)OC(O)C(O)C7O.OC8C(CO)OC(O)C(O)C8O | 340  1.3 ± 0.3  (0.13%) | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation and was related to *W. somnifera* fruits [4] and roots [6]. |
| 17b | 1079.5632  1077.5485 | -0.1  0.9 | Sterol with four sugar moieties  C52H86O23  Partial | OC(C1)CCC2(C)C1=CCC3C2CCC4(C)C3CC5C4C(C)C(OC)(CCC(C)CO)O5.OC6C(CO)OC(O)C(O)C6O.OC7C(CO)OC(O)C(O)C7O.OC8C(CO)OC(O)C(O)C8O.OC9C(CO)OC(O)C(O)C9O | HRMS supports molecular formula  MS/MS largely contains losses of sugar moieties. There are several, albeit structurally similar, compounds in literature that would be supported by the HRMS and MS/MS.  None of these options have been reported to come from Withania, but there is high confidence that it is some sterol with four sugar moieties attached. The backbone of the sterol proposed is consistent with those reported in literature.  Fragmentation is similar to the isomer at Peak 20. |
| 18 | 33.17 | 933.5050  931.4902 | -0.4  -0.6 | Sterol with three sugar moieties  C46H76O19  Partial | CC1(C(O)CCC2)C2=CCC3C1CCC4(C)C3CC5C4C(C)C(OC)(CCC(C)CO)O5.OC6C(CO)OC(O)C(O)C6O.OC7C(CO)OC(O)C(O)C7O.OC8C(CO)OC(O)C(O)C8O | 110  0.42 ± 0.1  (0.042%) | HRMS supports molecular formula  MS/MS largely contains losses of sugar moieties. There are several, albeit structurally similar, compounds in literature that would be supported by the HRMS and MS/MS.  None of these options have been reported to come from Withania, but there is high confidence that it is some sterol with four sugar moieties attached. The backbone of the sterol proposed is consistent with those reported in literature.  Molecular formula and MS/MS matches peak 23c with an additional sugar moiety. |
| 19 | 33.70 | 950.4037  964.4191 | -0.9 | Lyciumin C  C49H57O12N9  (150394-23-3)Tentative | O=C1NC(C(N2C(C(NC(C(NC3N4C=C(CC(C(O)=O)NC(C(NC(C(NC(C(C(C)C)NC3=O)=O)CC5=CC=CC=C5)=O)CO)=O)C6=C4C=CC=C6)=O)CC7=CC=C(O)C=C7)=O)CCC2)=O)CC1 | 130  0.49 ± 0.08  (0.049%) | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation but was NOT related to *Withania* [7]. |
| 20 | 33.96 | 1079.5629  1077.5483 | -0.4  0.6 | C52H86O23  Tentative | OC(C1)CCC2(C)C1=CCC3C2CCC4(C)C3CC5C4C(C)C(OC)(CCC(C)CO)O5.OC6C(CO)OC(O)C(O)C6O.OC7C(CO)OC(O)C(O)C7O.OC8C(CO)OC(O)C(O)C8O.OC9C(CO)OC(O)C(O)C9O | 210  0.78 ± 0.2  (0.078%) | HRMS supports molecular formula  MS/MS largely contains losses of sugar moieties. There are several, albeit structurally similar, compounds in literature that would be supported by the HRMS and MS/MS.  None of these options have been reported to come from Withania, but there is high confidence that it is some sterol with four sugar moieties attached. The backbone of the sterol proposed is consistent with those reported in literature.  Fragmentation is similar to the isomer at Peak 17b. |
| 21 | 34.14 | 569.2412  567.2269 | -0.5  -0.2 | 2,3-Dihydro-3-β-O-sulfate withaferin A  C28H40O10S  (1159096-16-8)  Matched | O=C1CC(OS(O)(=O)=O)C(O)C23C1(C)C4C(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC4)CC2O3 | 110  0.42 ± 0.1  (0.042%) | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature only contained HRMS and NMR (i.e., no fragmentation), but was related to *W. somnifera* leaves [8] and roots [9].  The molecular formula matches withaferin A with a sulfate. |
| 22 | 34.30 | 783.4158 | -0.4 | Withanoside IV isomer  C40H62O15  Matched | OC(C1)CC(O)C2(C)C1=CCC3C2CCC4(C)C3CCC4C(C)C5OC(C(CO)=C(C)C5)=O.OC6C(CO)OC(O)C(O)C6O.OC7C(CO)OC(O)C(O)C7O | 130  0.47 ± 0.1  (0.047%) | HRMS supports molecular formula  MS/MS supports structure via literature (isomer of Withanoside IV).  Literature contained fragmentation of Withanoside IV only (not other isomers) and was related to *W. somnifera* fruits [4]. |
| 23a | 34.53 | 569.2412  567.2269 | -0.5  -0.2 | 2,3-Dihydro-3-β-O-sulfate withaferin A  C28H40O10S  (871949-35-8)  Matched | O=C1CC(OS(O)(=O)=O)CC2(O)C1(C)C3C(C(CCC4(O)C(C)C5OC(C(C)=C(C)C5)=O)C4(C)CC3)C6C2O6 | 180  0.67 ± 0.4  (0.067%) | HRMS supports molecular formula  Literature only contained HRMS and NMR (i.e., no fragmentation), but was related to *W. somnifera* leaves [8] and roots [9].  MS/MS is similar, though slightly different, than Peak 25.  The molecular formula matches withaferin A with a sulfate. |
| 23b | 553.2466  551.2325 | 0.1  0.7 | 2,3-Dihydro-3-β-O-sulfate withanolide B  C28H40O9S  Partial | O=C1CC(OS(O)(=O)=O)CC2(O)C1(C)C3C(C(CCC4C(C)C5OC(C(C)=C(C)C5)=O)C4(C)CC3)C6C2O6 | HRMS supports molecular formula  MS/MS is similar to the constituent at peak 25 (less one oxygen) but there is no literature for this structure (nor anything with this formula).  The molecular formula matches withanolide B with a sulfate. |
| 23c | 771.4524  769.4382 | -0.2  -0.2 | (1α,3β,22*R*,24*Z*)-1,3,22-trihydroxy ergosta-5,24-dien-26-yl 6-*O*-β-D-gluco pyranosyl-β-D-glucopyranoside  C40H66O14  (2411842-87-8)  Tentative | OC(C1)CC(O)C2(C)C1=CCC3C2CCC4(C)C3CCC4C(C)C(O)C/C(C)=C(C)\CO.OC5C(CO)OC(O)C(O)C5O.OC6C(CO)OC(O)C(O)C6O | HRMS supports molecular formula  Literature only contained HRMS and NMR (i.e., no fragmentation), but was related to *W. somnifera* seeds [5].  Molecular formula and MS/MS matches several other constituents in this sample with varying number of additional sugar moieties. |
| 24 | 34.65 | 897.3877  895.3741 | -3.0  -1.9 | C51H60O12S  Partial | O=C1CC(OS(=O)(O)=O)C(O)C23C1(C)C4C(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC4)CC2O3.C#CC#CC#CC#CC#CC#CC#CC#CC#CC#CC#CC.O=O.[HH].[HH].[HH].[HH].[HH].[HH].[HH].[HH] | 300  1.1 ± 0.2  (0.11%) | HRMS supports molecular formula  In-source fragments and MS/MS support it’s related to peaks 23 and 25 with an addition of C23H20O2. The structure of this addition is unknown.  MS/MS is similar, though slightly different, than Peak 23a. |
| 25 | 35.46 | 569.2412  567.2269 | -0.5  -0.2 | 2,3-Dihydro-3-β-O-sulfate withaferin A  C28H40O10S  (1159096-16-8)  Tentative | O=C1CC(OS(=O)(O)=O)C(O)C23C1(C)C4C(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC4)CC2O3 | 100  0.38 ± 0.1  (0.038%) | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature only contained HRMS and NMR (i.e., no fragmentation), but was related to *W. somnifera* leaves [8] and roots [9].  MS/MS is similar, though not exact, to Peak 23a.  The molecular formula matches withaferin A with a sulfate. |
| 26a | 37.15 | 783.4155  827.4065 7 | -0.9  -0.7 | Withanoside IV  C40H62O15  (362472-81-9)  Reference | OC1CC(OC2C(O)C(O)C(O)C(COC3C(O)C(O)C(O)C(CO)O3)O2)CC4=CCC(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC7)C7C41C | 1,800  6.8  (0.68%) | HRMS supports molecular formula  MS/MS supports structure via reference standard.  Retention time matches reference standard. |
| 26b | 489.2853  533.2760 7 | -0.0  0.7 | Viscosalactone B or related isomer  C28H40O7  (76938-46-0)  Matched | O=C1CC(O)C(O)C23C1(C)C4C(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC4)CC2O3 | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation of Viscosalactone B only (not other isomers) and was related to *W. somnifera* fruits [4].  Retention time is shifted and likely not a major contributor to mass. |
| 27 | 37.50 | 489.2845  533.2760 7 | -0.4  0.7 | Viscosalactone B or related isomer  C28H40O7  (76938-46-0)  Matched | O=C1CC(O)C(O)C23C1(C)C4C(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC4)CC2O3 | 120  0.43 ± 0.08  (0.043%) | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation of Viscosalactone B only (not other isomers) and was related to *W. somnifera* fruits [4]. |
| 28 | 37.76 | 489.2845  533.2760 7 | -0.4  0.7 | Viscosalactone B or related isomer  C28H40O7  (76938-46-0)  Matched | O=C1CC(O)C(O)C23C1(C)C4C(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC4)CC2O3 | 130  0.48 ± 0.1  (0.048%) | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation of Viscosalactone B only (not other isomers) and was related to *W. somnifera* fruits [4]. |
| 29 | 38.46 | 783.4155  827.4062 7 | -0.9 | Withanoside VI  C40H62O15  (362472-81-9)  Matched | OC1CC(OC2C(O)C(O)C(O)C(COC3C(O)C(O)C(O)C(CO)O3)O2)CC4=CCC(C(CCC5C(C)(O)C6OC(C(C)=C(C)C6)=O)C5(C)CC7)C7C41C | 680  2.5 ± 0.5  (0.25%) | HRMS supports molecular formula  MS/MS supports structure via literature (isomer of Withanoside IV).  Literature contained fragmentation of Withanoside IV only (not other isomers) and was related to *W. somnifera* fruits [4]. |
| 30 | 38.99 | 799.4110  797.3962 | -0.1  -0.4 | Withanoside II  C40H62O16  (362472-79-5P)  Matched | OC1CC(OC2C(O)C(O)C(O)C(COC3C(O)C(O)C(O)C(CO)O3)O2)CC4(O)C1(C)C5C(C(CCC6C(C)C7OC(C(C)=C(C)C7)=O)C6(C)CC5)C8C4O8 | 700  2.6 ± 0.5  (0.26%) | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation and was related to *W. somnifera* fruits [4]. |
| 31b | 40.14 | 473.2897  517.2813 7 | -0.1  0.7 | 23, 24 Dihydrowithaferin A  C28H40O6  (5589-41-3)  Matched | OC1C=CC(O)C23C1(C)C4C(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC4)CC2O3 | 93  0.35 ± 0.1  (0.035%) | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation and was related to *W. somnifera* fruits [4]. |
| 31b | 351.2143 5  327.2175 | 0.0  -0.5 | 9,12,13-Trihydroxy-10,15-octadecadienoic acid  C18H32O5  (51146-90-8)  Tentative | OC(CCCCCCCC(O)/C=C/C(O)C(O)C/C=C\CC)=O | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature does not contain references to *W. somnifera [10].* |
| 31c | 823.4975 5  799.5003 | 0.0  0.2 | Unidentified  (Potential Artifact)  C46H72O11  Partial |  | HRMS supports molecular formula  Interestingly, MS/MS fragments includes 31a ([M+Na]+) and 31b ([M+H]+). Further investigation to determine if this is an in-source product or if the constituent is those two constituents connected. |
| 32 | 40.33 | 976.4554  974.4415 | -0.7  -0.9 | C51H61O11N9  Partial |  | 69  0.26 ± 0.08  (0.026%) | HRMS supports molecular formula.  Isotope ratios support molecular formula. |
| 33 | 40.49 | 621.3634  665.3540 7 | 0.1  -0.4 | Coagulin Q  C34H52O10  (261637-26-7)  Matched | OC1CC(OC2C(O)C(O)C(O)C(CO)O2)C=C3C1(C)C4C(C(CCC5C(C)(O)C6OC(C(C)=C(C)C6)=O)C5(C)CC4)CC3 | 150  0.55 ± 0.2  (0.055%) | HRMS supports molecular formula  MS/MS supports structure via literature.  Literature contained fragmentation and was related to *W. somnifera* fruits [4]. |
| 34 | 41.38 | 637.3585  681.3489 7 | 0.4  -0.4 | Withanoside I  C34H52O11  (362472-78-4)  Matched | OC1CC(OC2C(O)C(O)C(O)C(CO)O2)CC3(O)C1(C)C4C(C(CCC5C(C)(O)C6OC(C(C)=C(C)C6)=O)C5(C)CC4)C7C3O7 | 86  0.32 ± 0.04  (0.032%) | HRMS supports molecular formula  MS/MS supports structure via literature.\*  Literature contained fragmentation and was related to *W. somnifera* fruits [4].  \*Fragmentation was on [M+H-H2O]+ due to low abundance of [M+H]+. |
| 35 | 42.95 | 471.2740  515.2653 7 | -0.2  0.5 | Withaferin A  C28H38O6  (5119-48-2)  Reference | O=C1C=CC(O)C23C1(C)C4C(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC4)CC2O3 | 1,400  5.2 ± 0.8  (0.52%) | HRMS supports molecular formula  MS/MS supports structure via reference standard.  Retention time matches reference standard. |
| 36 | 43.31 | 353.2298 5  329.2332 | -0.2  -0.4 | 9,10,13-trihydroxy octadecenoic acid  C18H34O5  (29907-56-0, 61911-67-9, etc.)  Tentative | OC(CCCCCCCC(O)Ccc(O)c(O)cccCC)=O | 170  0.63 ± 0.1  (0.063%) | HRMS supports molecular formula  MS/MS supports structure via mzCloud4 and literature, but relative intensities differ. Placement of double bond cannot be confirmed but matches to literature/database were highlighted. [11] |
| 37 | 43.44 | 505.2616  503.2476 | -0.5  0.6 | Sulfonated  Withaferin A  C28H40O6S  (2354327-03-8  OR  2354326-93-3)  Tentative | O=C1CC(S)C(O)C23C1(C)C4C(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC4)CC2O3  O=C1CC2C(O)C3(O)C1(C)C4C(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC4)CC3S2 | 83  0.31 ± 0.04  (0.031%) | HRMS supports molecular formula.  MS/MS supports structure via manual interpretation.  No literature/database fragmentation available to match. Constituent only reported as synthetic product of withaferin A. [12] |
| 38 | 43.92 | 471.2740  515.2653 7 | -0.2  0.5 | Withanone isomer  (Withanolide D)  C28H38O6  (30655-48-2)  Tentative | O=C1C=CCC2(O)C1(C)C3C(C(CCC4(O)C(C)C5OC(C(C)=C(C)C5)=O)C4(C)CC3)C6C2O6 | 370  1.4 ± 0.2  (0.14%) | HRMS supports molecular formula  MS/MS is similar to other isomers.  Retention time does not match reference standard for withanone but matches with an impurity in the withanone standard. Can’t determine which isomer without standard.  Withanolide D is a possibility based on it’s reported abundance in literature [13]. |
| 39 | 44.26 | 507.2506  505.2365 | -0.3  0.5 | 6α-Chloro-5β-hydroxywithaferin A  C28H39O6Cl  52329-20-1  Tentative | O=C1C=CC(O)C2(O)C1(C)C3C(C(CCC4C(C)C5OC(C(CO)=C(C)C5)=O)C4(C)CC3)CC2Cl | 300  1.1 ± 0.2  (0.11%) | HRMS supports molecular formula. [14]  MS/MS supports structure via manual interpretation.  No literature/database fragmentation available to match. |
| 40 | 45.07 | 471.2740  515.2653 7 | -0.2  0.5 | Withanolide A  C28H38O6  (32911-62-9)  Reference | O=C1C=CCC2(O)C1(C)C3C(C(CCC4C(O)(C)C5OC(C(C)=C(C)C5)=O)C4(C)CC3)C6C2O6 | 550  2.1 ± 0.4  (0.21%) | HRMS supports molecular formula  MS/MS supports structure via reference standard.  Retention time matches reference standard. |
| 41 | 45.62 | 767.4203  765.4062 | -1.2  -0.7 | Withanoside V  C40H62O14  (256520-90-8)  Reference | OC1CC(OC2C(O)C(O)C(O)C(COC3C(O)C(O)C(O)C(CO)O3)O2)CC4=CCC(C(CCC5C(C)C6OC(C(C)=C(C)C6)=O)C5(C)CC7)C7C41C | 1,100  3.9  (0.39%) | HRMS supports molecular formula  MS/MS supports structure via reference standard.  Retention time matches reference standard. |
| 42 | 47.18 | 517.3157  561.3069 7 | -0.5  0.0 | (20S,22R)-3-acetate δ-lactone 6α,7α-epoxy-1α,3β,5,22-tetrahydroxy-5α-Ergost-24-en-26-oic acid  C30H44O7  (33903-25-2)  Tentative | OC1CC(OC(C)=O)CC2(O)C1(C)C3C(C(CCC4C(C)C5OC(C(C)=C(C)C5)=O)C4(C)CC3)C6C2O6 | 87  0.33 ± 0.05  (0.033%) | HRMS supports molecular formula  MS/MS supports structure via manual interpretation.  Literature only makes mention that this is a known constituent of *W. somnifera [15]*. |
| 43a | 48.39 | 605.3684  649.3591 7 | 0.0  -0.4 | (1α,​3β,​22R)​-δ-​lactone 3-​(β-​D-​glucopyranosyloxy)​-​1,​22-​dihydroxy Ergosta-​5,​24-​dien-​26-​oic acid  C34H52O9  (1567812-88-7)  Tentative | OC1CC(OC2C(O)C(O)C(O)C(CO)O2)CC3=CCC(C(CCC4C(C)C5OC(C(C)=C(C)C5)=O)C4(C)CC6)C6C31C | 87  0.33 ± 0.04  (0.033%) | HRMS supports molecular formula  MS/MS supports structure via manual interpretation.  Literature only makes mention that this is a known constituent of *W. somnifera* seeds [5]. |
| 43b | 535.2199 | 1.0 | (3α,​4β,​5β,​6α,​22*R*)​- δ-​lactone 3,​6-​epidithio-​4,​5,​22,​27-​tetrahydroxy-​1-​oxo- Ergost-​24-​en-​26-​oic acid  C28H40O6S2  (2354326-97-7)  Partial | O=C1CC2C(O)C3(O)C1(C)C4C(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC4)CC3SS2 | HRMS supports molecular formula.  MS/MS supports structure via manual interpretation.  No literature/database fragmentation available to match. Constituent only reported as synthetic product of withaferin A. [12] |
| 44 | 52.21 | 975.5280  973.5132 | -0.7  -0.9 | Ashwagandhanolide  C56H78O12S  (919478-81-2)  Matched | O=C1C=CC(O)C2(O)C1(C)C3C(C(CCC4C(C)C5OC(C(CO)=C(C)C5)=O)C4(C)CC3)CC2SC6C(C7(C)C8C(C(CCC9C(C)C%10OC(C(CO)=C(C)C%10)=O)C9(C)CC8)C6)(O)C(O)C=CC7=O | 120  0.43  (0.043%) | HRMS supports molecular formula  MS/MS supports structure via manual interpretation.  Literature only contained HRMS and NMR (i.e., no fragmentation), but was related to *W. somnifera* roots [16]. |
| 45 | 54.18 | 455.2791 | -0.2 | Withanolide B  C28H38O5  (56973-41-2)  Reference | O=C1C=CCC2(O)C1(C)C3C(C(CCC4C(C)C5OC(C(C)=C(C)C5)=O)C4(C)CC3)C6C2O6 | 160  0.61  (0.061%) | HRMS supports molecular formula  MS/MS supports structure via reference standard.  Retention time matches reference standard. |
| 46a | 76.77 | 256.2638  - | 0.2  - | Hexadecanamide  C16H33NO  (629-54-9)  Matched | NC(CCCCCCCCCCCCCCC)=O | 110  0.40  (0.040%) | HRMS supports molecular formula  MS/MS supports structure via mzCloud online database. 4  Alkylamides are common to botanicals. |
| 46b | 319.1946 8  279.2330 | 0.4  0.0 | Linoleic acid  C18H32O2  (60-33-3)  Tentative | OC(CCCCCCC/C=C\C/C=C\CCCCC)=O | HRMS supports molecular formula  MS/MS supports structure via mzCloud online database. 4  Fatty acids are common to botanicals. |
| 1. P&G CAD peak numbers reflect the sequential order of peaks detected and integrated in the UHPLC-CAD chromatogram of the extracted sample. If multiple components (determined from MS data) eluted within the integration window of the CAD peak, sequential letter designations were appended to the peak number. 2. Retention times listed are based on the time the constituent(s) was detected by the CAD detector. The retention times for the HRMS were approximately 0.1 min later. 3. Confidence level assignments from lowest confidence to highest confidence based on TAC Standard Laboratory Practice SLP-MVSA-1010:    1. **Partial** molecular formula derived from exact mass measurements    2. **Tentative** molecular formula derived from exact mass measurements and proposed structure supported by either MS/MS spectrum or UV spectrum or literature data reporting the structure in plants of the same genus (*Withania*) as *Withania somnifera*.    3. **Matched** molecular formula derived from exact mass measurements and proposed structure supported by MS/MS (manual interpretation) and literature data reporting the structure in *Withania somnifera* or proposed structure supported by MS/MS (match with database) and compound reasonably likely to be present in plant material.    4. **Reference** same criteria as a “Matched” identification and there is a retention time and MS/MS match to an authentic reference standard. 4. Databases for reference:    1. **mzCloud**: An online (https://www.mzcloud.org) curated mass spectral database containing high resolution/accurate mass fragmentation spectra. 5. Sodiated adduct was measured (i.e., [M+Na]+). 6. Chlorinated adduct was measured (i.e., [M+Cl]-). 7. Formate adduct was measured (i.e., [M-H+CH2O2]-). 8. Calcium adduct was measured (i.e., [M-H+Ca]+). | | | | | | | |

[1] *Journal of Analtyical Toxicology* **2019,** *43*, 196-202.

[2] *Journal of Separation Science* **2019,** *42*, 1163-1173.

[3] *Biomedical Chromatography* **2020,** *34*, e4914.

[4] *Rapid Communications in Mass Spectrometry* **2012,** *26*, 1277-1290.

[5] *Natural Product Research* **2019**, 1-6.

[6] *Chemical and Pharmaceutical Bulletin (Tokyo)* **2002,** *50*, 760-765.

[7] *Analytical Methods* **2015,** *7*, 7511-7526.

[8] *Phytochemistry* **2005,** *66*, 2702-2707.

[9] *Chemical Biodiversity* **2017,** *14*.

[10] *Food Chemistry* **2021,** *364*, 130362.

[11] *Phytochemical Analysis* **2018,** *29*, 398-405.

[12] *ChemistrySelect* **2017,** *2*, 10470-10475.

[13] *Fitoterapia* **2003,** *74*, 68-76.

[14] *Phytochemistry Letters* **2011,** *4*, 411-414.

[15] *Journal of the Chemical Society C: Organic* **1971**, 2032-2044.

[16] *Journal of Natural Products* **2006,** *69*, 1790-1792.