**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 permg extract****(%)** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 1a | 0.89 | 203.0526 5365.1054 5527.1586 5707.2219 5215.0328 6377.0856 6539.1390 6719.2019 6 | 0.2-0.1 | SaccharidesC6H12O6 + (C6H10O5)xTentative | OC1C(O)C(OCC2OC(OCC3OC(O)C(O)C(O)C3O)C(O)C(O)C2O)OC(CO)C1O | 29,000110(11%) | HRMS supports molecular formulaeMS/MS supports structure(s) via mzCloud online database. 4Saccharide-based structures (i.e., sugars) are common to botanicals.The chirality cannot be determined. |
| 1b | 338.1448- | -0.0- | Sugar + C7H13NO4C13H23NO9Partial | OC1C(O)C(CO)OC(OC#CC#CC#CC)C1O.O[N+]([O-])=O.[HH].[HH].[HH].[HH] | HRMS supports molecular formulaMS/MS supports structure with loss of sugar, but the aglycone cannot be determined.  |
| 2a | 0.96 | 142.1226 | -0.5 | Tropine OR β-tropineC8H15NO(120-29-6135-97-7)Reference | CN1C2CCC1CC(O)C2 | 75,000280(28%) | HRMS supports molecular formulaMS/MS supports structure via reference standard.Retention time matches reference standard. |
| 2b | 203.0526 5365.1054 5527.1586 5707.2219 5215.0328 6377.0856 6539.1390 6719.2019 6 | 0.2-0.1 | SaccharidesC6H12O6 + (C6H10O5)xTentative | OC1C(O)C(OCC2OC(OCC3OC(O)C(O)C(O)C3O)C(O)C(O)C2O)OC(CO)C1O | HRMS supports molecular formulaeMS/MS supports structure(s) via mzCloud online database. 4Saccharide-based structures (i.e., sugars) are common to botanicals.The chirality cannot be determined. |
| 3a | 0.99 | 142.1226- | -0.5 | Tropine OR β-tropineC8H15NO(120-29-6135-97-7)Reference | CN1C2CCC1CC(O)C2 | 150,000550(55%) | HRMS supports molecular formulaMS/MS supports structure via reference standard.Retention time matches reference standard. |
| 3b | 203.0526 5365.1054 5527.1586 5707.2219 5215.0328 6377.0856 6539.1390 6719.2019 6 | 0.2-0.1 | SaccharidesC6H12O6 + (C6H10O5)xTentative | OC1C(O)C(OCC2OC(OCC3OC(O)C(O)C(O)C3O)C(O)C(O)C2O)OC(CO)C1O | HRMS supports molecular formulaeMS/MS supports structure(s) via mzCloud online database. 4Saccharide-based structures (i.e., sugars) are common to botanicals.The chirality cannot be determined. |
| 4 | 1.59 | 527.1583 5503.1620 | 0.00.5 | 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 | 1400.54 ± 0.3(0.054%) | HRMS supports molecular formulaMS/MS supports structure via mzCloud online database. 4Saccharide-based structures (i.e., sugars) are common to botanicals.The exact connectivity and chirality cannot be determined. |
| 5 | 2.01 | 130.0498128.0353 | -0.5-0.4 | Pyroglutamic acidC5H7O3N(98-79-3)Matched | O=C1NC(C(O)=O)CC1 | 1500.55 ± 0.3(0.055%) | HRMS supports molecular formulaMS/MS supports structure via mzCloud online database. 4Amino acid-based structures are common to botanicals. |
| 6 | 2.25 | 190.0710188.0564 | -0.2-0.9 | Acetylglutamic acidC7H11O5N(1188-37-0)Matched | OC(CCC(NC(C)=O)C(O)=O)=O | 1600.58 ± 0.3(0.058%) | HRMS supports molecular formulaMS/MS supports structure via mzCloud online database. 4Amino 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-PiperidineethanolC8H17ON(496-47-9OR934-90-7)Tentative | CN1CCCC1CC(C)OOC(C)CC1CCCCN1 | HRMS supports molecular formulaMS/MS supports structure via manual interpretation.No literature/database available to match. |
| 7 | 3.42 | 142.1225- | -0.1- | Hygrine OR PelletierineC8H15ON(496-49-1OR2858-66-4)Tentative | CN1CCCC1CC(C)=OO=C(C)CC1NCCCC1 | 1000.39 ± 0.2(0.039%) | HRMS supports molecular formulaMS/MS supports structure via literature [1]. |
| 8 | 4.28 | 144.1381- | -1.4- | HygrolineORSedridineC8H17ON(496-47-9OR501-83-7)Tentative | CN1CCCC1CC(C)OOC(C)CC1NCCCC1 | 2000.76 ± 0.4(0.076%) | HRMS supports molecular formulaMS/MS supports structure via manual interpretation.No literature/database available to match. |
| 9 | 7.90 | 184.1331 | -3.6 | AcetyltropineC10H18O2N(3423-27-6)Tentative | CN1C2CCC1CC(OC(C)=O)C2 | 1500.56 ± 0.2(0.056%) | HRMS supports molecular formulaMS/MS supports structure via manual interpretation.No literature/database available to match. |
| 10a | 20.29 | 474.2596472.2454 | -0.60.2 | N1,N8-Bis(dihydrocaffeoyl) spermidineC25H35O6N3(132194-39-9)Tentative | OC1=CC(CCC(NCCCNCCCCNC(CCC2=CC(O)=C(O)C=C2)=O)=O)=CC=C1O | 910.34 ± 0.3(0.034%) | HRMS supports molecular formulaMS/MS supports structure via literature.Literature contained fragmentation but was NOT related to *Withania [2-3]*. |
| 10b | 636.3127634.2984 | 0.10.4 | Lycibarbarspermidines H or IC31H45O11N3(1884356-81-3 or1884356-82-4)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 formulaMS/MS supports structure via literature.Literature contained fragmentation but was NOT related to *Withania [2-3]*. |
| 11a | 20.41 | 474.2596472.2454 | -0.60.2 | N1,N8-Bis(dihydrocaffeoyl) spermidineC25H35O6N3(132194-39-9)Tentative | OC1=CC(CCC(NCCCNCCCCNC(CCC2=CC(O)=C(O)C=C2)=O)=O)=CC=C1O | 1200.46 ± 0.1(0.046%) | HRMS supports molecular formulaMS/MS supports structure via literature.Literature contained fragmentation but was NOT related to *Withania [2-3]*. |
| 11b | 636.3127634.2984 | 0.10.4 | Lycibarbarspermidines H or IC31H45O11N3(1884356-81-3 or1884356-82-4)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 formulaMS/MS supports structure via literature.Literature contained fragmentation but was NOT related to *Withania [2-3]*. |
| 12 | 22.82 | 224.1644- | -0.4- | TropiglineC13H21O2N(533-08-4)Tentative | CN1C2CCC1CC(OC(/C(C)=C/C)=O)C2 | 1400.51 ± 0.07(0.051%) | HRMS supports molecular formulaMS/MS supports structure via manual interpretation.No literature/database available to match. |
| 13 | 30.95 | 815.4060813.3914 | 0.0-0.1 | Withagenin A DiglucosideC40H62O17(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 | 990.37 ± 0.1(0.037%) | HRMS supports molecular formulaMS/MS supports structure via literature.Literature contained fragmentation and was related to *W. somnifera* fruits [4]. |
| 14 | 31.22 | 1107.52141105.5068 | -0.4-0.4 | Withanoside IXC52H82O25(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 | 5402.0 ± 0.3(0.2%) | HRMS supports molecular formulaMS/MS supports structure via literature.Literature contained fragmentation and was related to *W. somnifera* fruits [4]. |
| 14b | 1095.55691093.5427 | -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-​GlucopyranosideC52H86O24(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 formulaMS/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 | 933.5050931.4907 | -0.4 | C46H76O19Tentative | 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 | 920.35 ± 0.06(0.035%) | HRMS supports molecular formulaMS/MS supports structure via manual interpretation.Literature only contained closely related analogues and was related to *W. somnifera* seeds [5]. |
| 15b | 945.4682989.4596 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 formulaMS/MS supports structure via literature.Literature contained fragmentation and was related to *W. somnifera* fruits [4] and roots [6]. |
| 16a | 32.67 | 653.3533697.3440 7 | 0.2-0.1 | Withanoside IIIC34H52O12(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 | 1000.39 ± 0.06(0.039%) | HRMS supports molecular formulaMS/MS supports structure via literature.Literature contained fragmentation and was related to *W. somnifera* fruits [4]. |
| 16b | 1241.61751239.6013 | 1.2-0.8 | C58H96O28Partial | 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 formulaMS/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 | 945.4682943.4540 | -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 | 3401.3 ± 0.3(0.13%) | HRMS supports molecular formulaMS/MS supports structure via literature.Literature contained fragmentation and was related to *W. somnifera* fruits [4] and roots [6]. |
| 17b | 1079.56321077.5485 | -0.10.9 | Sterol with four sugar moietiesC52H86O23Partial | 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 formulaMS/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 | 933.5050931.4902 | -0.4-0.6 | Sterol with three sugar moietiesC46H76O19Partial | 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 | 1100.42 ± 0.1(0.042%) | HRMS supports molecular formulaMS/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 | 950.4037964.4191 | -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 | 1300.49 ± 0.08(0.049%) | HRMS supports molecular formulaMS/MS supports structure via literature.Literature contained fragmentation but was NOT related to *Withania* [7]. |
| 20 | 33.96 | 1079.56291077.5483 | -0.40.6 | C52H86O23Tentative | 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 | 2100.78 ± 0.2(0.078%) | HRMS supports molecular formulaMS/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 | 569.2412567.2269 | -0.5-0.2 | 2,3-Dihydro-3-β-O-sulfate withaferin AC28H40O10S(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 | 1100.42 ± 0.1(0.042%) | HRMS supports molecular formulaMS/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 isomerC40H62O15Matched | 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 | 1300.47 ± 0.1(0.047%) | HRMS supports molecular formulaMS/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 | 569.2412567.2269 | -0.5-0.2 | 2,3-Dihydro-3-β-O-sulfate withaferin AC28H40O10S(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 | 1800.67 ± 0.4(0.067%) | HRMS supports molecular formulaLiterature 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 | 553.2466551.2325 | 0.10.7 | 2,3-Dihydro-3-β-O-sulfate withanolide B C28H40O9SPartial | 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 formulaMS/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 | 771.4524769.4382 | -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-glucopyranosideC40H66O14(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 formulaLiterature 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 | 897.3877895.3741 | -3.0-1.9 | C51H60O12SPartial | 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] | 3001.1 ± 0.2(0.11%) | HRMS supports molecular formulaIn-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 | 569.2412567.2269 | -0.5-0.2 | 2,3-Dihydro-3-β-O-sulfate withaferin AC28H40O10S(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 | 1000.38 ± 0.1(0.038%) | HRMS supports molecular formulaMS/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 | 783.4155827.4065 7 | -0.9-0.7 | Withanoside IVC40H62O15(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 | 1,8006.8(0.68%) | HRMS supports molecular formulaMS/MS supports structure via reference standard.Retention time matches reference standard. |
| 26b | 489.2853533.2760 7 | -0.00.7 | Viscosalactone Bor related isomerC28H40O7(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 formulaMS/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 | 489.2845533.2760 7 | -0.40.7 | Viscosalactone Bor related isomerC28H40O7(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 | 1200.43 ± 0.08(0.043%) | HRMS supports molecular formulaMS/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 | 489.2845533.2760 7 | -0.40.7 | Viscosalactone Bor related isomerC28H40O7(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 | 1300.48 ± 0.1(0.048%) | HRMS supports molecular formulaMS/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 | 783.4155827.4062 7 | -0.9 | Withanoside VIC40H62O15(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 | 6802.5 ± 0.5(0.25%) | HRMS supports molecular formulaMS/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 | 799.4110797.3962 | -0.1-0.4 | Withanoside IIC40H62O16(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 | 7002.6 ± 0.5(0.26%) | HRMS supports molecular formulaMS/MS supports structure via literature.Literature contained fragmentation and was related to *W. somnifera* fruits [4]. |
| 31b | 40.14 | 473.2897517.2813 7 | -0.10.7 | 23, 24 Dihydrowithaferin AC28H40O6(5589-41-3)Matched | OC1C=CC(O)C23C1(C)C4C(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC4)CC2O3 | 930.35 ± 0.1(0.035%) | HRMS supports molecular formulaMS/MS supports structure via literature.Literature contained fragmentation and was related to *W. somnifera* fruits [4]. |
| 31b | 351.2143 5327.2175 | 0.0-0.5 | 9,12,13-Trihydroxy-10,15-octadecadienoic acidC18H32O5(51146-90-8)Tentative | OC(CCCCCCCC(O)/C=C/C(O)C(O)C/C=C\CC)=O | HRMS supports molecular formulaMS/MS supports structure via literature.Literature does not contain references to *W. somnifera [10].* |
| 31c | 823.4975 5799.5003 | 0.00.2 | Unidentified(Potential Artifact)C46H72O11Partial |  | HRMS supports molecular formulaInterestingly, 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 | 976.4554974.4415 | -0.7-0.9 | C51H61O11N9Partial |  | 690.26 ± 0.08(0.026%) | HRMS supports molecular formula.Isotope ratios support molecular formula. |
| 33 | 40.49 | 621.3634665.3540 7 | 0.1-0.4 | Coagulin QC34H52O10(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 | 1500.55 ± 0.2(0.055%) | HRMS supports molecular formulaMS/MS supports structure via literature.Literature contained fragmentation and was related to *W. somnifera* fruits [4]. |
| 34 | 41.38 | 637.3585681.3489 7 | 0.4-0.4 | Withanoside IC34H52O11(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 | 860.32 ± 0.04(0.032%) | HRMS supports molecular formulaMS/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 | 471.2740515.2653 7 | -0.20.5 | Withaferin AC28H38O6(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 | 1,4005.2 ± 0.8(0.52%) | HRMS supports molecular formulaMS/MS supports structure via reference standard.Retention time matches reference standard. |
| 36 | 43.31 | 353.2298 5329.2332 | -0.2-0.4 | 9,10,13-trihydroxy octadecenoic acidC18H34O5(29907-56-0, 61911-67-9, etc.)Tentative | OC(CCCCCCCC(O)Ccc(O)c(O)cccCC)=O | 1700.63 ± 0.1(0.063%) | HRMS supports molecular formulaMS/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 | 505.2616503.2476 | -0.50.6 | SulfonatedWithaferin AC28H40O6S(2354327-03-8OR2354326-93-3)Tentative | O=C1CC(S)C(O)C23C1(C)C4C(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC4)CC2O3O=C1CC2C(O)C3(O)C1(C)C4C(C(CCC5C(C)C6OC(C(CO)=C(C)C6)=O)C5(C)CC4)CC3S2 | 830.31 ± 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 | 471.2740515.2653 7 | -0.20.5 | 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 | 3701.4 ± 0.2(0.14%) | HRMS supports molecular formulaMS/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 | 507.2506505.2365 | -0.30.5 | 6α-Chloro-5β-hydroxywithaferin AC28H39O6Cl52329-20-1Tentative | O=C1C=CC(O)C2(O)C1(C)C3C(C(CCC4C(C)C5OC(C(CO)=C(C)C5)=O)C4(C)CC3)CC2Cl | 3001.1 ± 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 | 471.2740515.2653 7 | -0.20.5 | Withanolide AC28H38O6(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 | 5502.1 ± 0.4(0.21%) | HRMS supports molecular formulaMS/MS supports structure via reference standard.Retention time matches reference standard. |
| 41 | 45.62 | 767.4203765.4062 | -1.2-0.7 | Withanoside VC40H62O14(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 | 1,1003.9(0.39%) | HRMS supports molecular formulaMS/MS supports structure via reference standard.Retention time matches reference standard. |
| 42 | 47.18 | 517.3157561.3069 7 | -0.50.0 | (20S,22R)-3-acetate δ-lactone 6α,7α-epoxy-1α,3β,5,22-tetrahydroxy-5α-Ergost-24-en-26-oic acidC30H44O7(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 | 870.33 ± 0.05(0.033%) | HRMS supports molecular formulaMS/MS supports structure via manual interpretation.Literature only makes mention that this is a known constituent of *W. somnifera [15]*. |
| 43a | 48.39 | 605.3684649.3591 7 | 0.0-0.4 | (1α,​3β,​22R)​-δ-​lactone 3-​(β-​D-​glucopyranosyloxy)​-​1,​22-​dihydroxy Ergosta-​5,​24-​dien-​26-​oic acidC34H52O9(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 | 870.33 ± 0.04(0.033%) | HRMS supports molecular formulaMS/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 acidC28H40O6S2(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 | 975.5280973.5132 | -0.7-0.9 | AshwagandhanolideC56H78O12S(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 | 1200.43(0.043%) | HRMS supports molecular formulaMS/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 | 1600.61(0.061%) | HRMS supports molecular formulaMS/MS supports structure via reference standard.Retention time matches reference standard. |
| 46a | 76.77 | 256.2638- | 0.2- | HexadecanamideC16H33NO(629-54-9)Matched | NC(CCCCCCCCCCCCCCC)=O | 1100.40(0.040%) | HRMS supports molecular formulaMS/MS supports structure via mzCloud online database. 4Alkylamides are common to botanicals. |
| 46b | 319.1946 8279.2330 | 0.40.0 | Linoleic acidC18H32O2(60-33-3)Tentative | OC(CCCCCCC/C=C\C/C=C\CCCCC)=O | HRMS supports molecular formulaMS/MS supports structure via mzCloud online database. 4Fatty 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.