

Supporting Information (SI) for:

Bioassay-guided isolation of bioactive compounds from the leaf extract of *Sclerocarya birrea* and their glucose uptake potential in skeletal myocytes

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Table S1 ^1H NMR and ^{13}C NMR data of Myricetin (**1**) in methanol-*d*₄ compared to those reported by (He et al.,2009) in DMSO-*d*₆

| Position | ^1H (ppm, J in Hz) of isolated myricetin in CD ₃ OD | ^1H (ppm, J in Hz) in literature data in DMSO- <i>d</i> ₆ | ^{13}C (ppm) of isolated myricetin in CD ₃ OD | ^{13}C (ppm, J in Hz) literature data in DMSO- <i>d</i> ₆ |
|----------|---|---|---|---|
| 2 | | | 145.5 | 146.8 |
| 3 | | | 136.5 | 135.9 |
| 4 | | | 178.3 | 175.7 |
| 5 | | | 161.8 | 160.7 |
| 6 | 6.22 (1H, d, J=2.09 Hz) | 6.18 (1H, d, J=2.40 Hz) | 98.4 | 98.2 |
| 7 | | | 164.5 | 164.1 |
| 8 | 6.39 (1H, d, J=2.17 Hz) | 6.37 (1H, d, J=1.8 Hz) | 93.3 | 93.2 |
| 9 | | | 157.1 | 156.1 |
| 10 | | | 102.2 | 102.9 |
| 1' | | | 120.5 | 120.7 |
| 2'6' | 6.97 (2H, s) | 7.24 (2H, s) | 108.1 | 107.1 |
| 3' | | | 145.5 | 145.7 |
| 4' | | | 134.9 | 135.8 |
| 5' | | | 145.5 | 145.7 |

Table S2 ^1H NMR and ^{13}C NMR data of Myricetin-3-O- β -D-glucuronide (**2**) in methanol- d_4 compared to those reported by (Granica et al., 2013) in methanol- d_4

| Position | ^1H (ppm, J in Hz) of isolated myricetin-3-O- β -D-glucuronide in CD_3OD | ^1H (ppm, J in Hz) literature data in CD_3OD | ^{13}C (ppm) of isolated myricetin-3-O- β -D-glucuronide in CD_3OD | ^{13}C (ppm, J in Hz) literature data in CD_3OD |
|----------|---|---|---|--|
| 2 | | | 157.5 | 156.2 |
| 3 | | | 134.1 | 133.2 |
| 4 | | | 177.8 | 177.1 |
| 5 | | | 161.7 | 161.2 |
| 6 | 6.21(1H, d, J=2.05 Hz) | 6.20 (1H, d, J=1.90 Hz) | 98.5 | 98.7 |
| 7 | | | 164.6 | 164.2 |
| 8 | 6.39 (1H, d, J=2.00 Hz) | 6.38 (1H, d, J=1.90 Hz) | 93.2 | 93.4 |
| 9 | | | 157.0 | 156.2 |
| 10 | | | 102.7 | 101.1 |
| 1' | | | 120.3 | 119.7 |
| 2' | 7.29 (2H, s) | 7.28 (2H, s) | 108.5 | 108.5 |
| 3' | | | 145.0 | 145.4 |
| 4' | | | 136.7 | 136.8 |
| 5' | | | 145.0 | 145.4 |
| 6' | 7.29 (2H, s) | 7.28 (2H, s) | 108.5 | 108.5 |
| 1'' | 5.39 (1H, d, J=7.88Hz) | 5.36 (1H, d, J=7.60 Hz) | 104.2 | 103.8 |
| 2'' | 3.49-3.61 (3H, m) | 3.66-3.43 (3H, m) | 73.9 | 73.6 |
| 3'' | 3.49-3.61 (3H, m) | 3.66-3.43 (3H, m) | 76.3 | 76.0 |
| 4'' | 3.49-3.61 (3H, m) | 3.66-3.43 (3H, m) | 71.6 | 71.2 |
| 5'' | 3.75 (1H, d, J=9.68 Hz) | 3.77 (1H, d, J=9.50 Hz) | 75.8 | 75.9 |
| 6'' | | | 171.7 | 169.8 |

Table S3 ^1H NMR and ^{13}C NMR data of Quercetin-3-O- β -D-glucuronide (**3**) in methanol- d_4 compared to those reported by (Moon et al., 2001) in methanol- d_4

| Position | ^1H (ppm, J in Hz) of isolated quercetin-3-O- β -D-glucuronide in CD_3OD | ^1H (ppm, J in Hz) literature data in CD_3OD | ^{13}C (ppm) of isolated quercetin-3-O- β -D-glucuronide in CD_3OD | ^{13}C (ppm, J in Hz) literature data in CD_3OD |
|----------|---|---|---|--|
| 2 | | | 161.6 | 159.2 |
| 3 | | | 134.5 | 135.5 |
| 4 | | | 177.9 | 179.4 |
| 5 | | | 164.7 | 163.2 |
| 6 | 6.10 (1H, d, J=2.05Hz) | 6.21 (1H, d, J=2.00Hz) | 101.6 | 100.1 |
| 7 | | | 168.2 | 166.2 |
| 8 | 6.30 (1H, d, J=2.05Hz) | 6.40 (1H, d, J=2.00Hz) | 93.3 | 94.9 |
| 9 | | | 157.7 | 158.6 |
| 10 | | | 104.26 | 105.8 |
| 1' | | | 125.8 | 123.0 |
| 2' | 7.78 (1H, bs) | 7.60-7.67 (2H, m) | 114.7 | 116.2 |
| 3' | | | 144.5 | 146.1 |
| 4' | | | 148.5 | 150.0 |
| 5' | 6.75 (1H, d, J=8.48Hz) | 6.85 (1H, d, J=8.48Hz) | 116.4 | 117.4 |
| 6' | 7.44 (1H, dd) | 7.60-7.67 2H, m) | 121.5 | 123.6 |
| 1'' | 5.32 (1H, d, J=7.57Hz) | 5.34 (1H, d, J=7.40Hz) | 103.9 | 104.4 |
| 2'' | 3.38-3.49(3H, m) | 3.42-3.66 (3H, m) | 74.1 | 75.5 |
| 3'' | 3.38-3.49 | 3.42-3.66 (3H, m) | 76.6 | 77.7 |

| | | | | |
|-----|-----------------------|-------------------------|-------|-------|
| 4'' | 3.38-3.49 | 3.42-3.66 (3H, m) | 73.7 | 73.0 |
| 5'' | 3.55 (1H, d, J=9.7Hz) | 3.75 (1H, d, J=9.60 Hz) | 76.5 | 77.2 |
| 6'' | | | 174.9 | 172.4 |

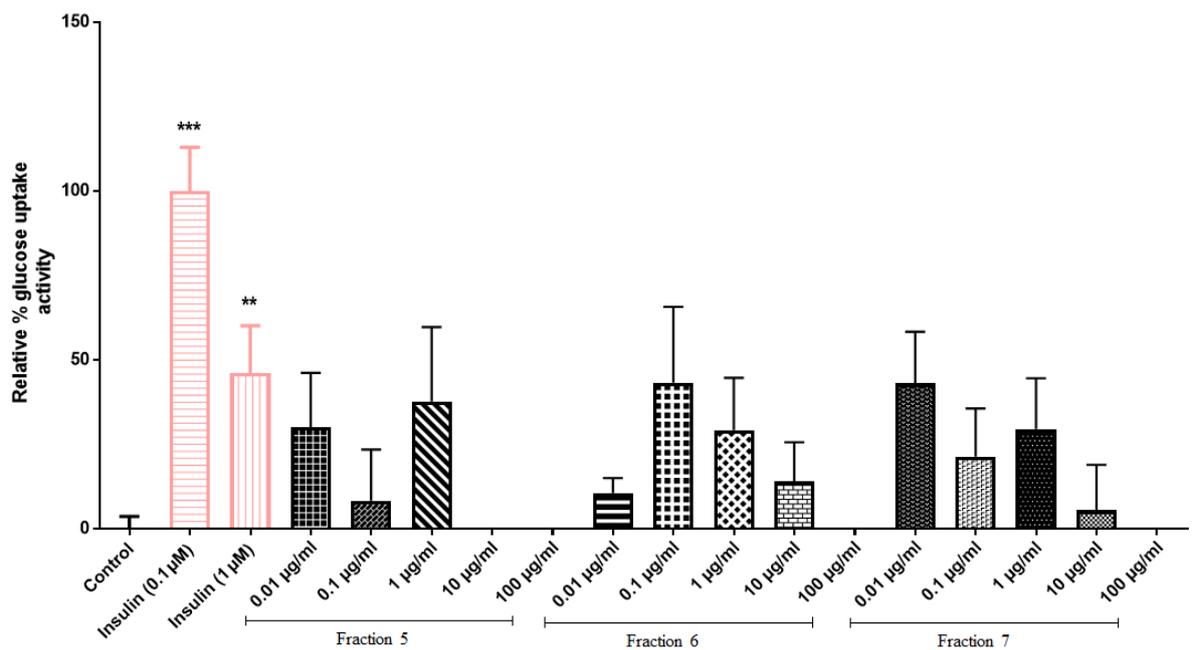
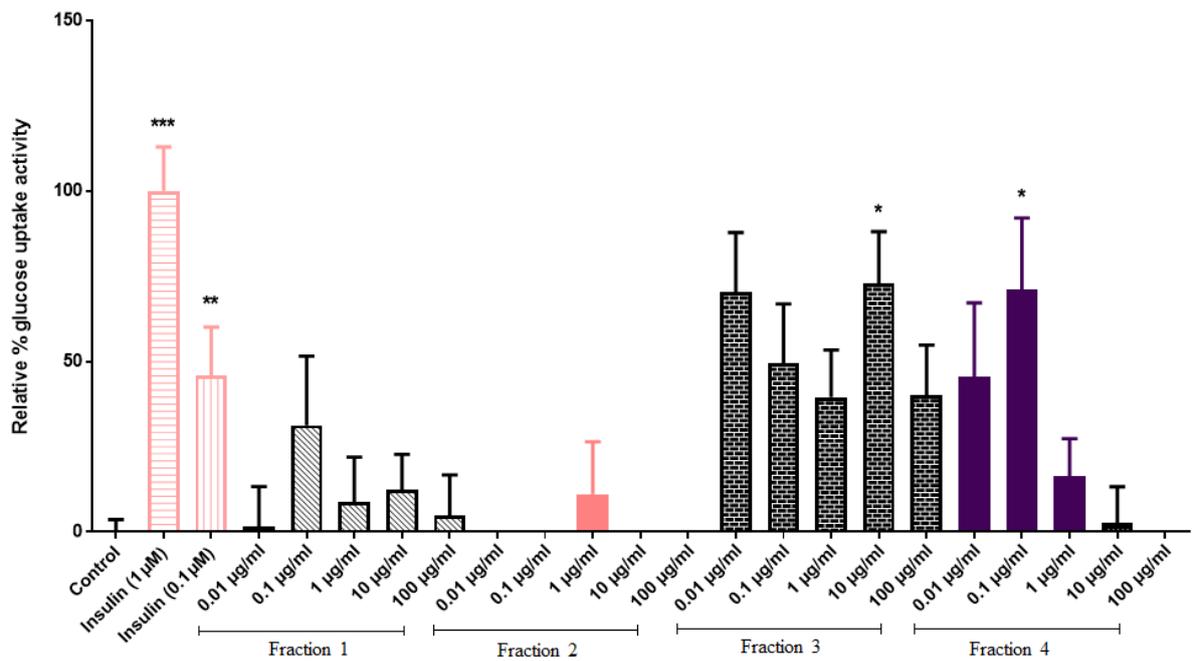


Fig. S4. Relative Glucose uptake activity of Marula fractions in C2C12 myocytes over a range of 0.01-100µg/ml. Activity is expressed relative % to the baseline glucose uptake (control) set at 0% and the positive control insulin (Ins) set at 100%. Active fraction (fraction 3) exhibited comparable potency to Insulin. P value < *p < 0.05, **p < 0.01. ***p < 0.001

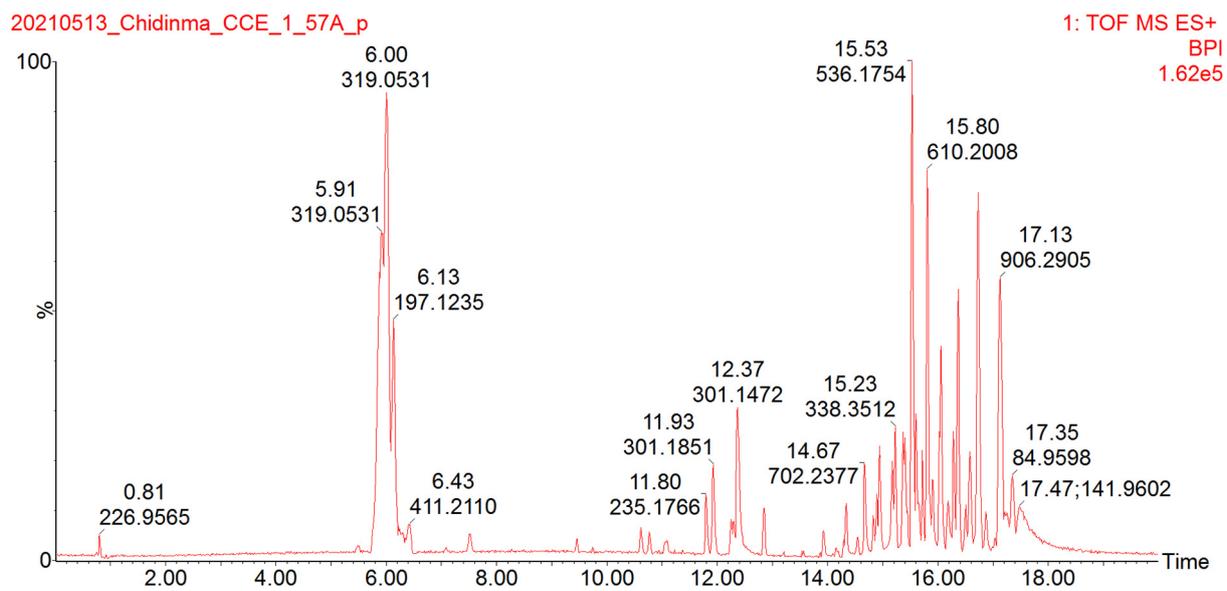


Fig. S5. ESI negative-mode BPI chromatogram of compound **1** (Myricetin) isolated from Fraction 4

20210513_Chidinma_CCE_1_61A_p

1: TOF MS ES+
BPI
1.52e5

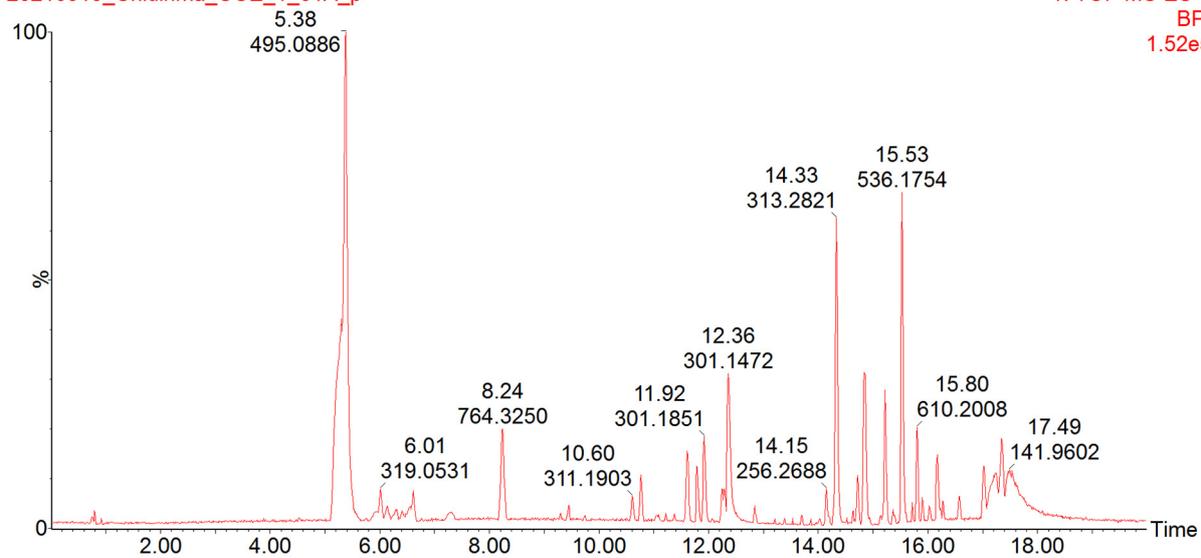


Fig. S6. ESI negative-mode BPI chromatogram of compound **2** (Myricetin-3-O- β -D-glucuronide) isolated from Fraction 3

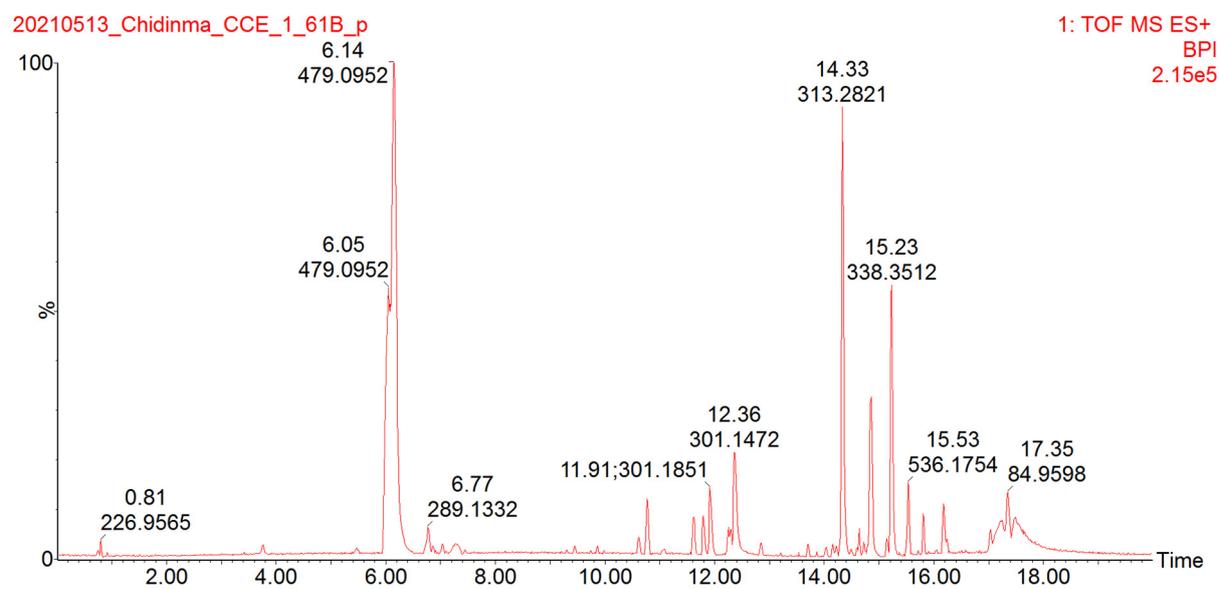


Fig. S7. ESI negative-mode BPI chromatogram of compound **3** (Quercetin-3-O- β -D-glucuronide) isolated from Fraction 3

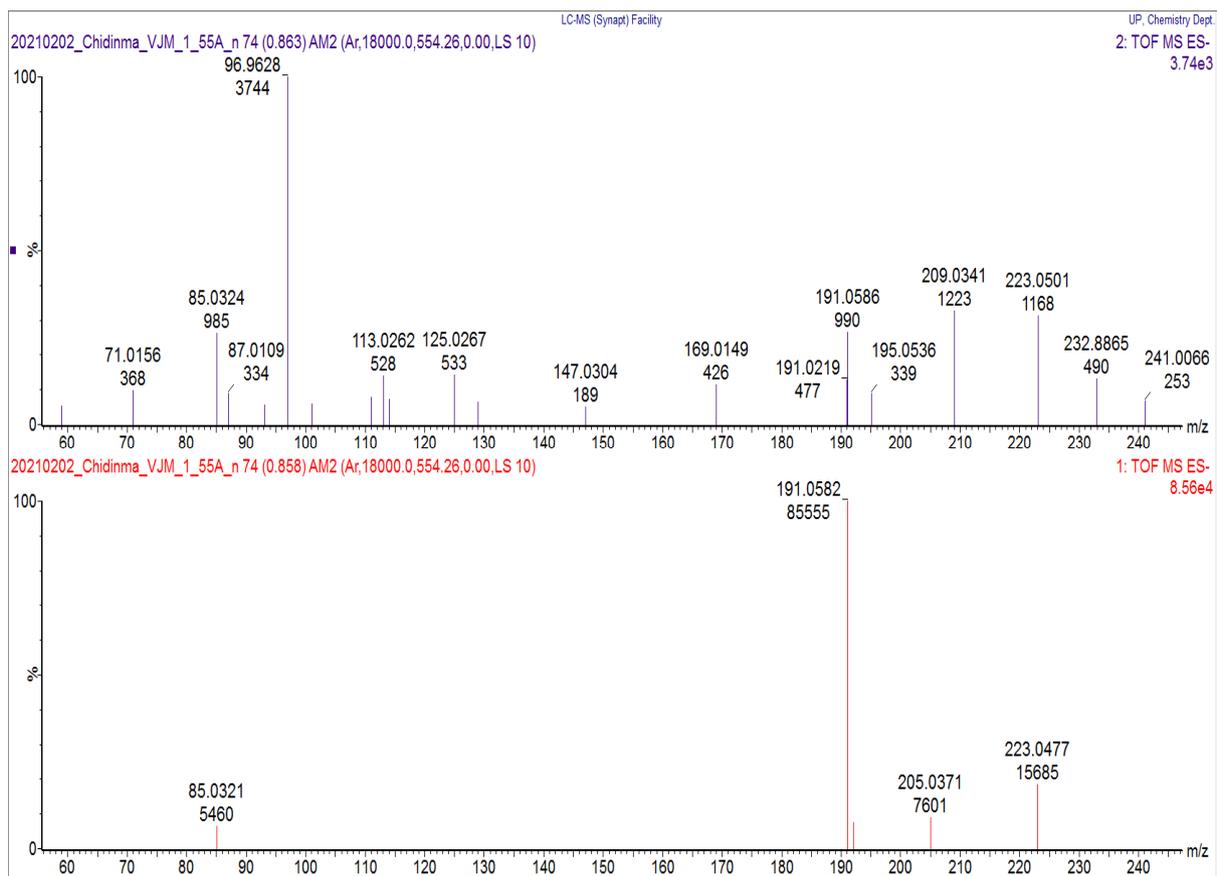


Fig.S8. MS fragmentation pattern of peak 1 overlaid with MSMS fragmentation pattern of peak 1

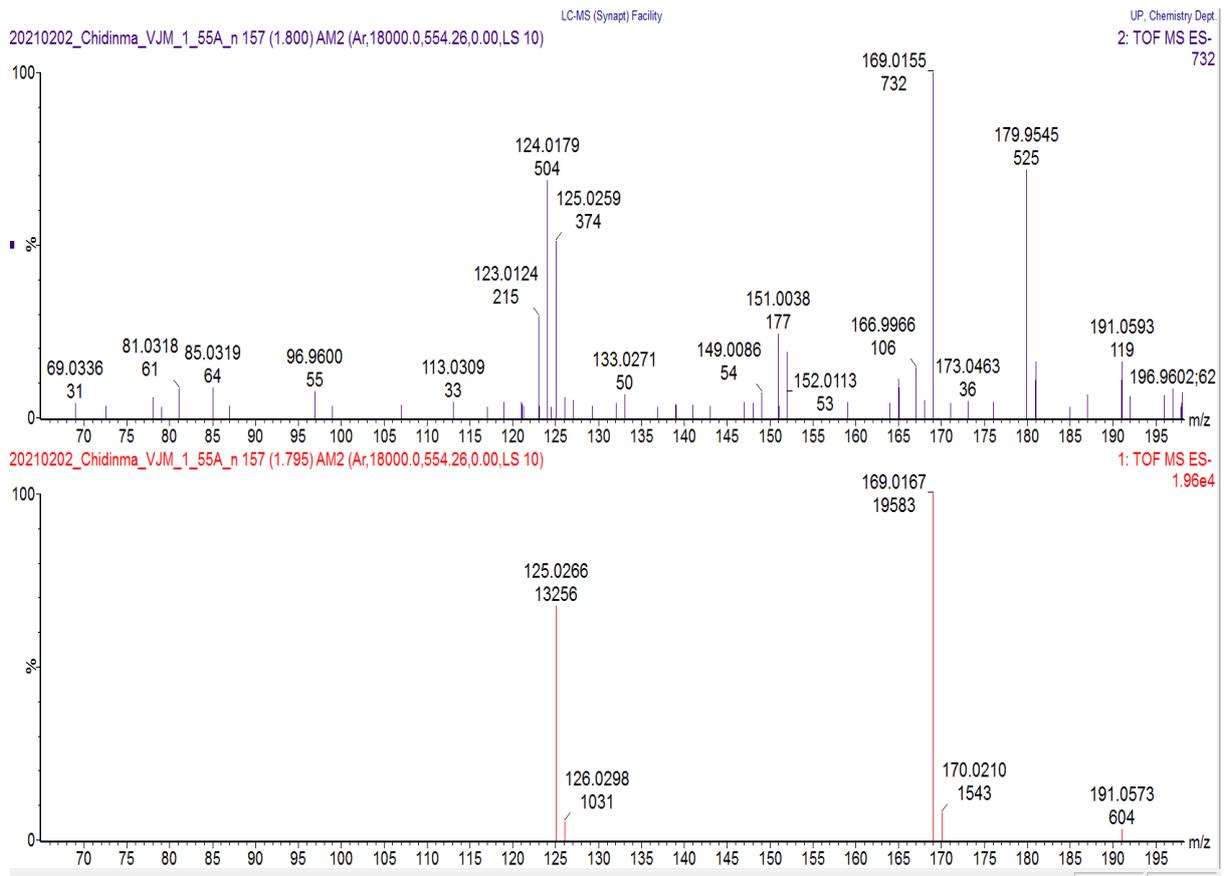


Fig.S9. MS fragmentation pattern of peak 2 overlaid with MSMS fragmentation pattern of peak 2

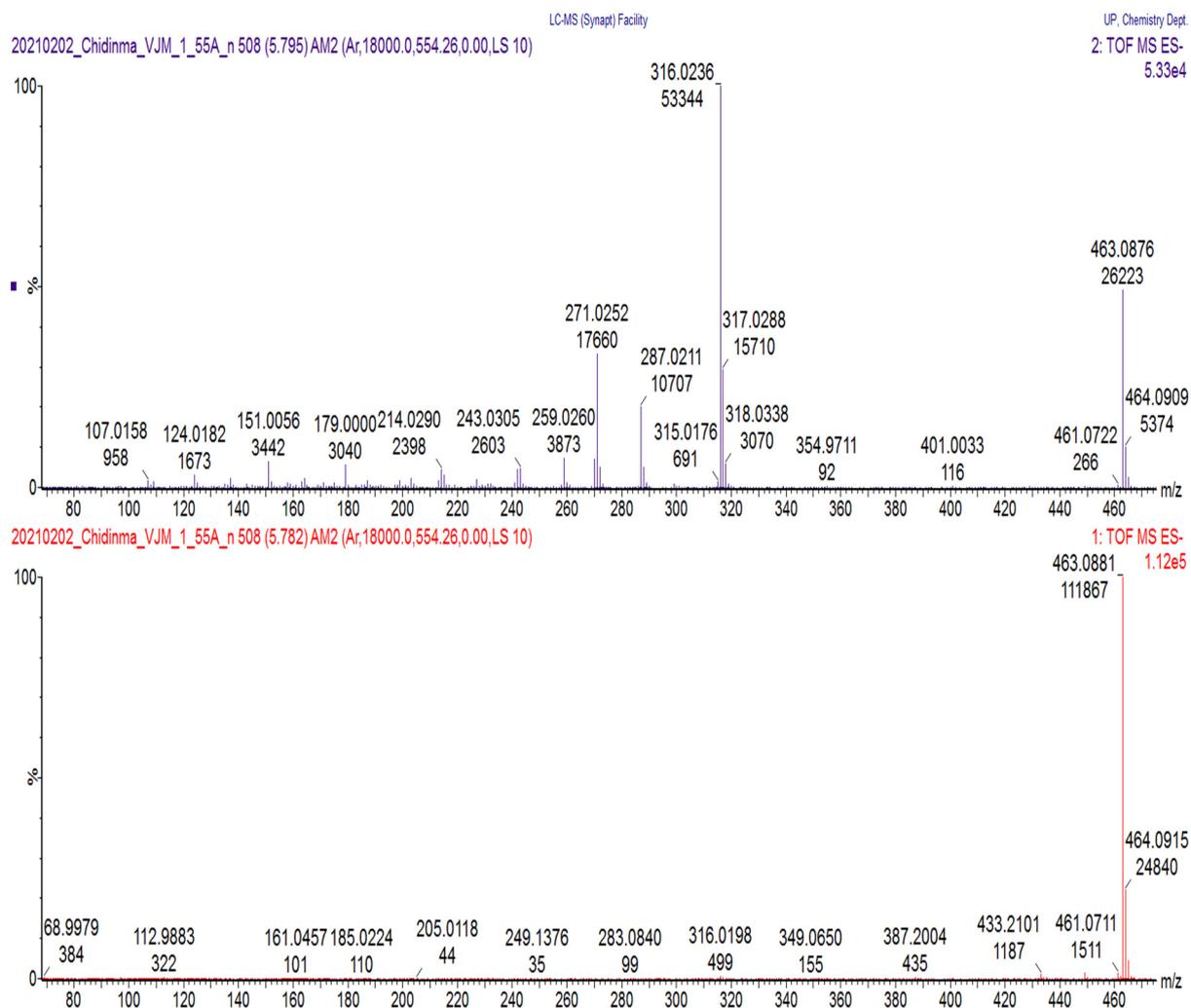


Fig.S10. MS fragmentation pattern of peak 3 overlaid with MSMS fragmentation pattern of peak 3

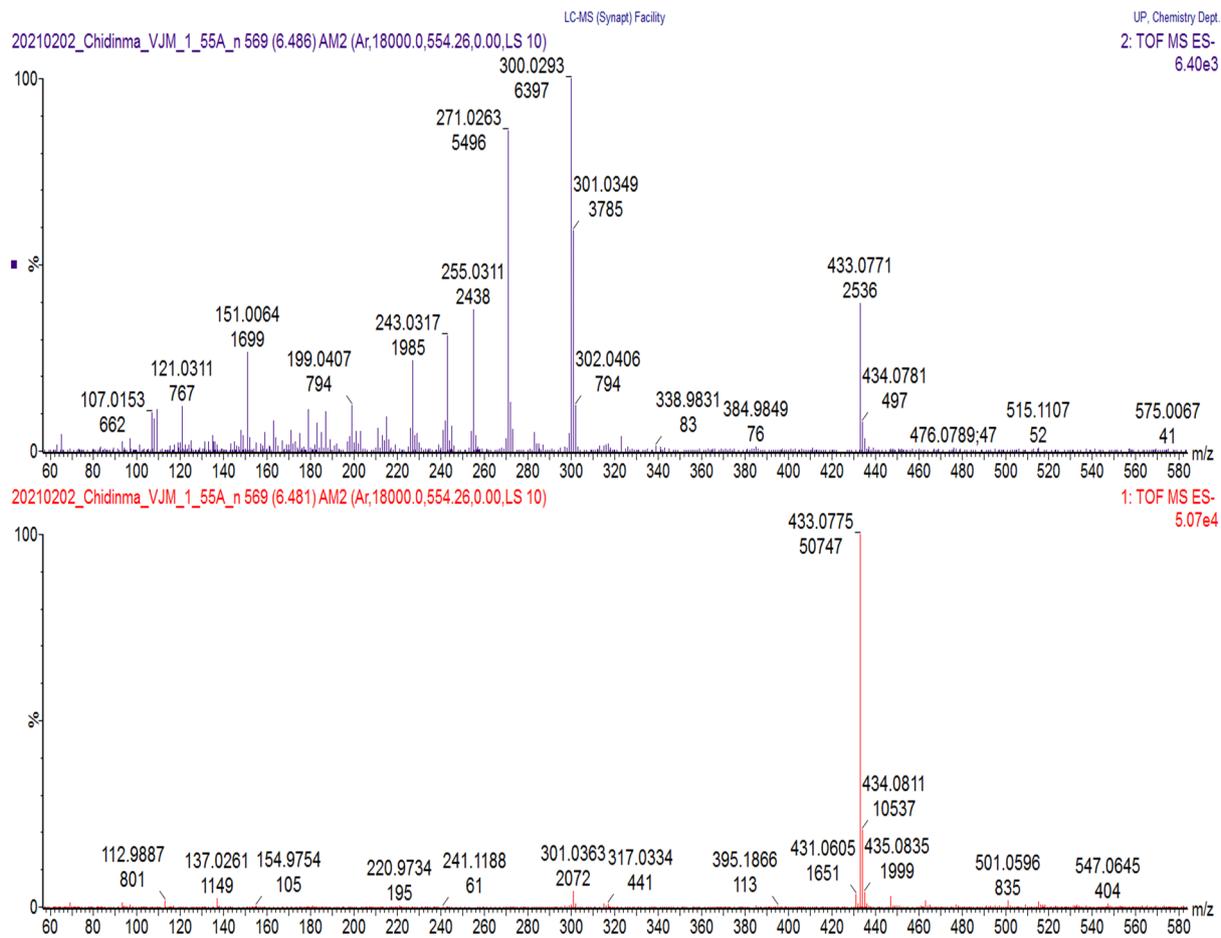


Fig.S11. MS fragmentation pattern of peak 4 overlaid with MSMS fragmentation pattern of peak 4

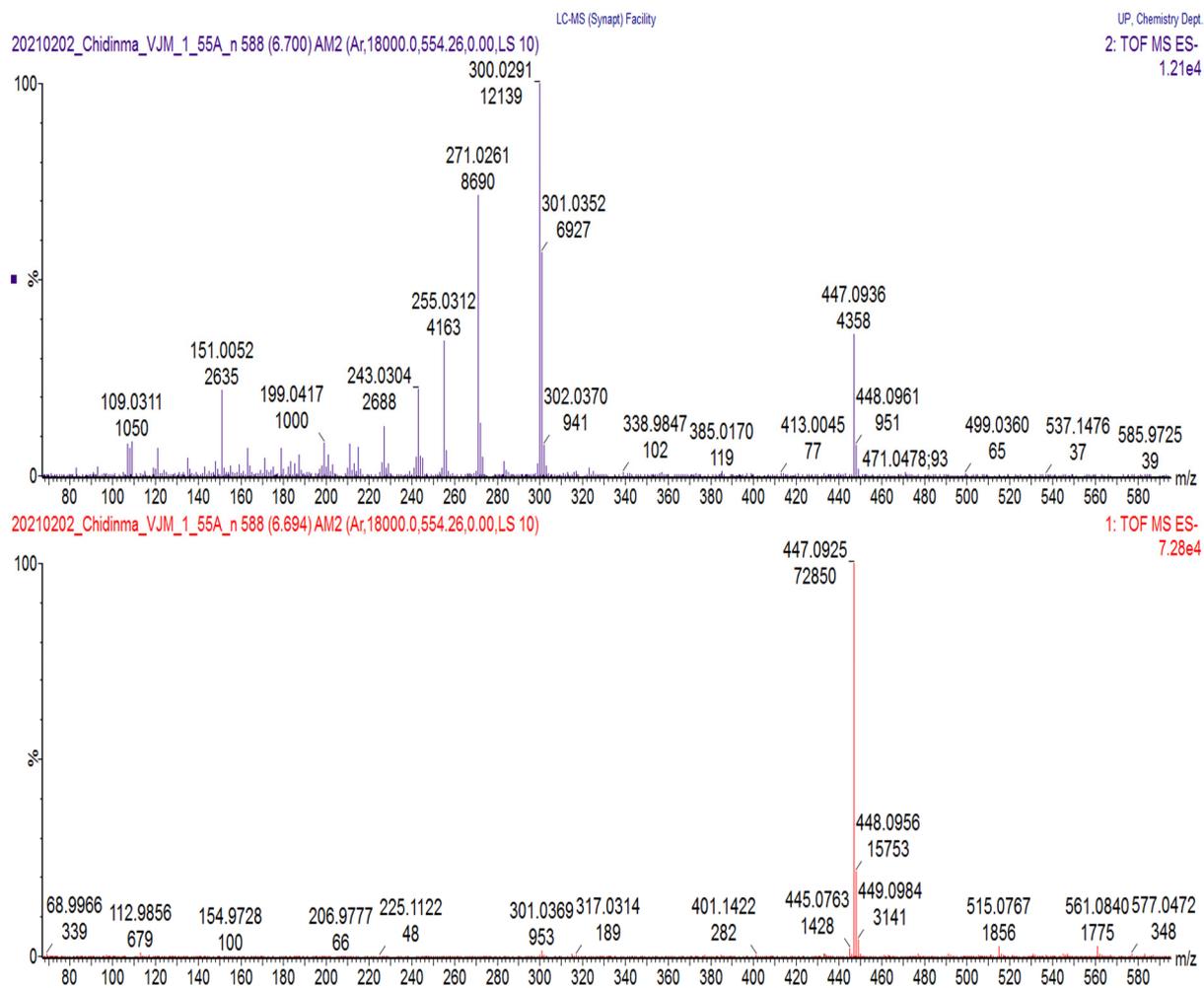


Fig.S12. MS fragmentation pattern of peak 5 overlaid with MSMS fragmentation pattern of peak 5

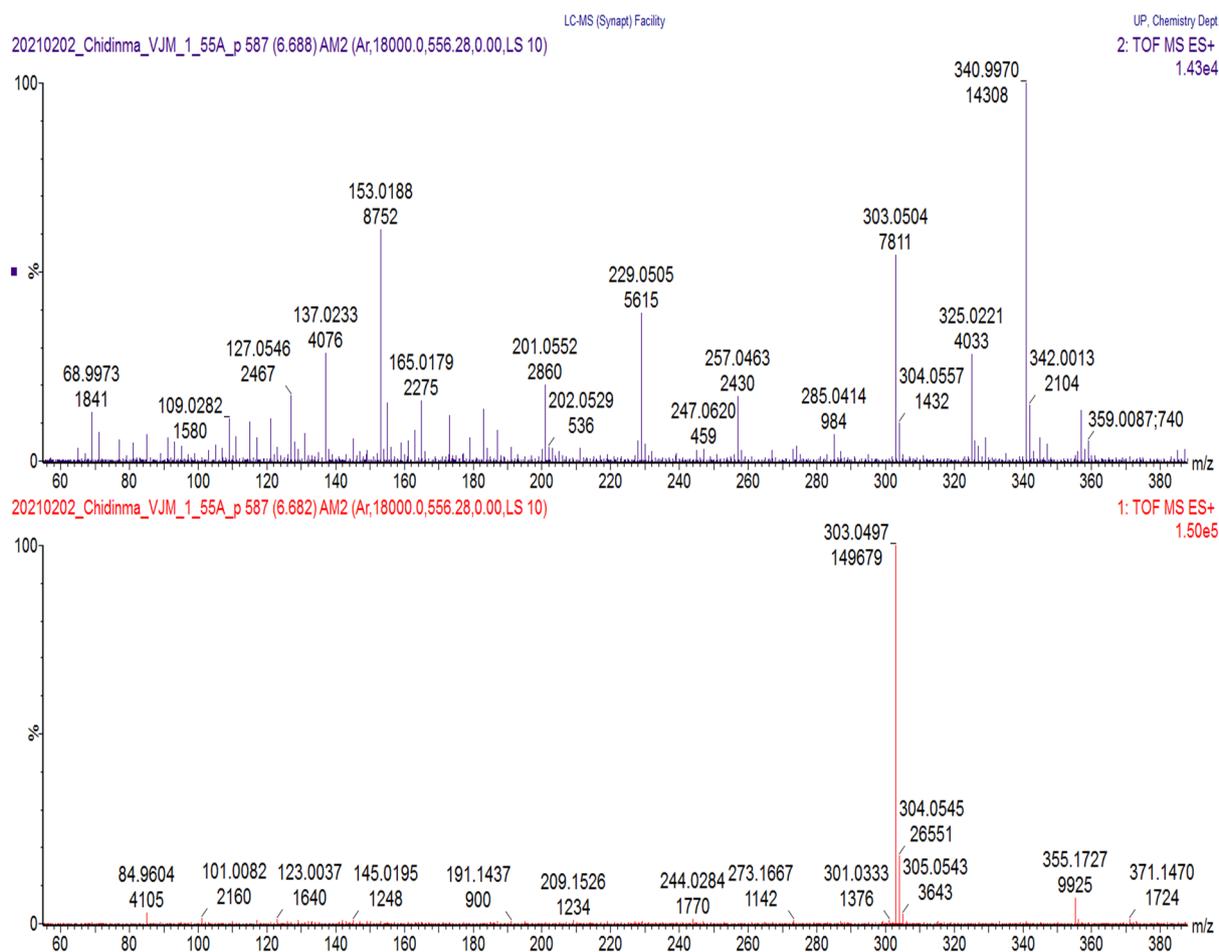


Fig.S13. MS fragmentation pattern of peak 6 overlaid with MSMS fragmentation pattern of peak 6

Isolation of the chemical constituents

Compound **1** was isolated as a light-yellow solid. It had a molecular formula of $C_{15}H_{11}O_8$ as deduced from its monoprotonated molecular ion at m/z 319.0443 (calcd for $[M+H]^+$ m/z 319.0454) based on the QTOF mass spectrum with eleven degrees of unsaturation. The ^{13}C NMR spectroscopic data showed it to have a flavonol skeleton confirming the presence of fifteen carbon atoms, of which four are aromatic methine groups and eleven quaternary carbons (one carbonyl, six O-bearing and four aliphatic) as deduced from its DEPT 135 spectrum. The 1H NMR spectrum showed the presence of four aromatic protons; two protons resonating at δ_H 6.22 (1H, d, $J = 2.09$ Hz, H-6) and δ_H 6.39 (1H, d, $J = 2.17$ Hz, H-8) consistent with the meta coupled protons at H-6 and H-8 positions on the A-ring and a signal at δ_H 6.97 (2H, s, H-2', H-6') indicating presence of two protons at 2' and 6' positions appearing as a singlet due to their para substituted ring B which led to their symmetrical pattern. This indicated that the molecule was a flavonoid, which matched myricetin, previously isolated from black currant³⁸.

Compound **2** was isolated as a light-yellow solid. It had a molecular formula of $C_{21}H_{19}O_{14}$ as deduced from its monoprotonated molecular ion at m/z 495.0886 (calcd for $[M+H]^+$ m/z 495.0775) based on the QTOF mass spectrum with thirteen degrees of unsaturation. The ^{13}C -NMR spectrum showed the presence of nine methine groups (four aromatic methine and five glucuronic acid methine) and twelve quaternary carbons (one carbonyl, one carboxylic acid, six O-bearing, four aliphatic) as deduced from its DEPT 135 spectrum. The 1H NMR spectrum showed the presence of four aromatic protons; two protons resonating at δ_H 6.21 (1H, d, $J =$

2.05 Hz, H-6) and δ_{H} 6.39 (1H, d, $J = 2.00$ Hz, H-8) consistent with the meta coupled protons at H-6 and H-8 positions on the A-ring and a signal at δ_{H} 7.29 (2H, s, H-2', H-6') indicating presence of two protons at 2' and 6' positions appearing as a singlet due to their para substituted ring B which led to their symmetrical pattern. The proposed structure was confirmed by the comparative analysis of the reported myricetin-3-O- β -D-glucuronide to that of compound **2**³⁹.

Compound **3** was isolated as a light-yellow solid. It had a molecular formula of $\text{C}_{21}\text{H}_{18}\text{O}_{13}$ as deduced from its monoprotonated molecular ion at m/z 479.0952 (calcd for $[\text{M}+\text{H}]^+$ m/z 479.0826) based on the QTOF mass spectrum with thirteen degrees of unsaturation. The ^{13}C NMR spectrum showed the presence of ten methine groups (five aromatic methine and five glucuronic acid methine) and eleven quaternary carbons (one carbonyl, one carboxylic acid, five O-bearing and four aliphatic) as deduced from its DEPT 135 spectrum. The ^1H NMR spectrum showed signals corresponding to five aromatic protons (δ_{H} 6.10-7.78). Two proton signals at δ_{H} 6.10 (1H, d, $J = 2.05$ Hz, H-6) and 6.30 (1H, d, $J = 2.05$ Hz, H-8) attributable to the A ring of quercetin which were assigned to H-6 and H-8 positions respectively. Additionally, three proton signals at δ_{H} 7.78 (1H, bs, H-2'), 6.75 (1H, d, $J = 8.48$ Hz, H-5') and 7.44 (1H, dd, $J = 2.15$ and 2.18 Hz, H-6') were assigned to the B ring of quercetin as H-2', H-5' and H-6' respectively. These proton signals confirmed that the structure of the aglycone moiety was quercetin. The proposed structure was finally confirmed by the comparative analysis of the reported quercetin-3-O- β -D-glucuronide to that of compound **3**³⁹.