

Supporting Information

Rec. Nat. Prod. 11:4 (2017) 406-410

Bioassay-Guided Isolation of Antioxidants and α -Glucosidase Inhibitors from the Root of *Cassia sieberiana* D.C. (Fabaceae)

Saidu Jibril^{1,2}, Hasnah Mohd Sirat^{*1} and Norazah Basar¹

¹Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia

²Department of Chemical Sciences, Faculty of Science, Federal University Kashere, P.M.B. 0182, Gombe, Nigeria

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* Corresponding author: E- Mail: hasnah@kimia.fs.utm.my (Sirat HM), Phone +607-55344.

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Experimental Details

Total antioxidant content:

The total antioxidant content was determined according to a previously published method [1] with slight modification. For the total phenolic content, diluted sample (100 μ L) and Folin-Ciocalteu reagent (4.5 mL) were mixed properly. The mixture was allowed to stay for five minutes after which 3.0 mL of Na_2CO_3 (7.5%, w/v) was added. After incubation of the mixture for 60 minutes at room temperature, the absorbance was measured at 765 nm. A standard solution of gallic acid was used to prepare the calibration curve. The total phenolic content was calculated and expressed as mg gallic acid/ g dry extract. For the total flavonoid content, 1.0 mL of test sample was diluted with 4.0 mL of distilled water in a volumetric flask and 0.3 mL of NaNO_2 (5.0%, w/v) was added. After five minutes, 0.3 mL of AlCl_3 (10%, w/v) was added. The mixture was incubated for 6 minutes, followed by addition of 2.0 mL of NaOH (1.0 M) and 2.4 mL of deionized water. The mixture was shaken vigorously and the absorbance was measured against a blank at 510 nm. A standard solution of quercetin was used to prepare a calibration curve. The results were expressed as mg quercetin/ g dry extract.

Antioxidant capacity assay:

The ability of the extract or compound to scavenge DPPH radical and ABTS radical cation was estimated as previously reported in literature [2] with minor modification. The DPPH radical solution was prepared by dissolving 3.9 mg of DPPH in 50 mL MeOH in the dark. The ABTS radical cation was generated by reacting equal volume of the ABTS aqueous solution (7 mM) and $\text{K}_2\text{S}_2\text{O}_8$ (2.45 mM) in the dark and allowed to stay for 12-16 hours. This ABTS radical cation solution was diluted to obtain an absorbance of 0.70 (± 0.02) at 734 nm before use. An aliquot (100 μ L) of each test sample/standards (8 serial dilutions) was added to the DPPH radical (ABTS radical cation) solution (100 μ L) in a 96-well plate. The sample was allowed to react with DPPH radical for 30 mins (ABTS radical cation 6 mins) in dark and the absorbance value (A) was recorded against a blank DPPH at 517 nm (ABTS radical cation at 734 nm) from a spectrophotometer. AA, BHA and BHT were used as positive controls for the DPPH radical while Trolox was used for the ABTS radical cation. The experiment was conducted in triplicate. The inhibition of both radical scavenging activity in percent (I%) was calculated according to the equation below:

$$\text{I\%} = \frac{(\text{A}_{\text{blank}} - \text{A}_{\text{sample}})}{\text{A}_{\text{blank}}} \times 100$$

Where A_{blank} = absorbance of the blank solution (containing radical solution without sample) and A_{sample} = absorbance of a sample solution. The IC_{50} value is the effective concentration that could scavenge 50% of the two radicals against concentration of the samples.

α -Glucosidase Inhibitory activity:

The α -glucosidase inhibitory activity of extracts and isolated compounds was evaluated using quercetin and acarbose as the reference compounds [3]. The tested sample and standard, were dissolved in DMSO and 8 serial dilutions was put in triplicate into a 96-well microtiter plate. The enzyme solution (2 U/mL) was prepared by dissolving α -glucosidase in 50 mM phosphate buffer (pH 6.8). The test sample/standard (20 μ L) and the enzyme solution (10 μ L) were mixed in the well. After 20 min preincubation at 37°C, the substrate solution (50 μ L, 20 mM p-nitrophenyl- α -D-glucopyranoside, pNPG prepared in the same buffer) was added and the mixture was incubated for additional 20 min at 37°C. After the incubation, 50 μ L glycine (2 M, pH 10.0) was added to each well and the absorbance (A) was measured at 405 nm spectrophotometrically. Similarly, a blank experiment was performed without the test sample. The inhibition percentage (I%) was calculated by the equation:

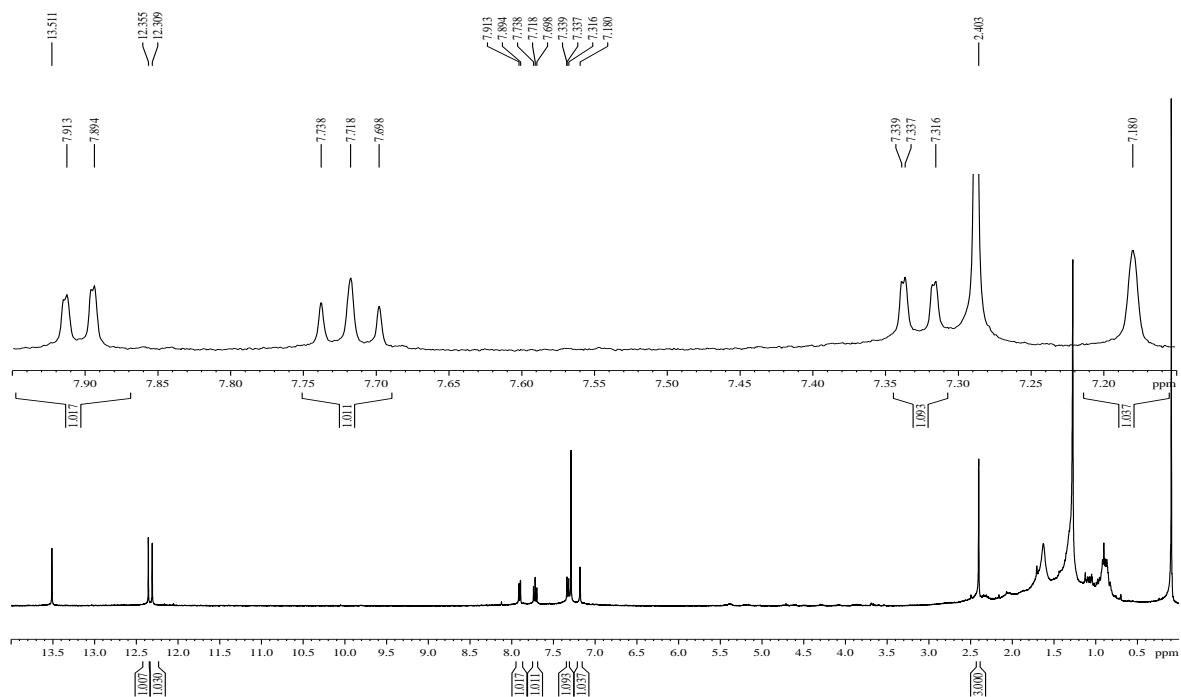
$$\text{Inhibition percentage (I\%)} = [1 - (\text{A}_{\text{sample}}/\text{A}_{\text{control}})] \times 100$$

Statistical Analysis of Data:

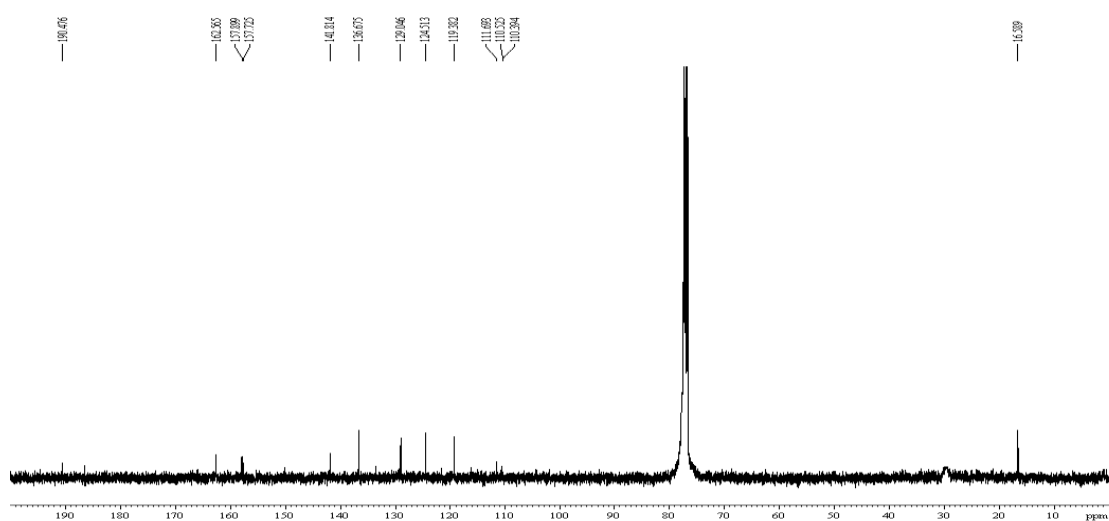
Experiments were performed in three replicates for each sample and statistical analysis values are reported as mean \pm SD. Standard curves were generated and calculation of the 50% inhibitory concentration (IC₅₀) values was carried out using GraphPad Prism for Windows (version 5.02) software. The Student's t-test was performed using SPSS (version 22) software to observe the comparison between treatment of samples and untreated control. A value of $p < 0.05$ was considered significantly different.

Spectral Information

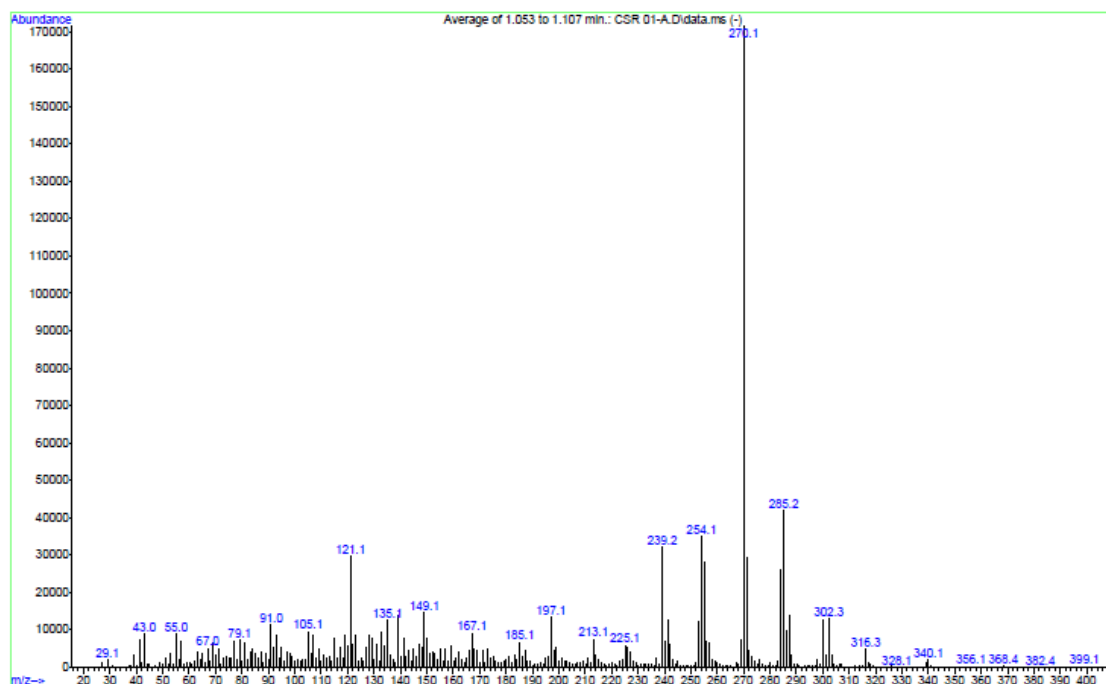
Islandicin (**1**): Reddish powder, IR (KBr, ν_{\max} , cm^{-1}): 3419, 2925, 1732, 1602, 1408; UV (λ_{\max} , MeOH, nm): 205, 225, 250, 289; ^1H NMR data (400 MHz, CDCl_3) δ : 2.40 (3H, s, CH_3), 7.18 (1H, s, H-2), 7.33 (1H, t, $J = 8.4$ Hz, H-7), 7.73 (1H, t, $J = 8.4$ Hz, H-6), 7.91 (1H, d, $J = 8.4$ Hz, H-5), 12.30 (1H, s, OH), 12.35 (1H, s, OH), 13.51 (1H, s, OH). ^{13}C NMR data (100 MHz, CDCl_3) δ : 16.5 (CH_3), 110.1 (C-12), 110.7 (C-13), 116.2 (C-11), 116.5 (C-14), 119.3 (C-5), 124.5 (C-7), 129.0 (C-2), 136.6 (C-6), 141.8 (C-3), 157.7 (C-4), 157.8 (C-1), 162.5 (C-8), 186.8 (C-10), 190.4 (C-9); EIMS (m/z): 270.1 [M] $^+$ ($\text{C}_{15}\text{H}_{10}\text{O}_5$), 254.1, 239.1, 197.1.



S1: ^1H NMR Spectrum of Islandicin (**1**)

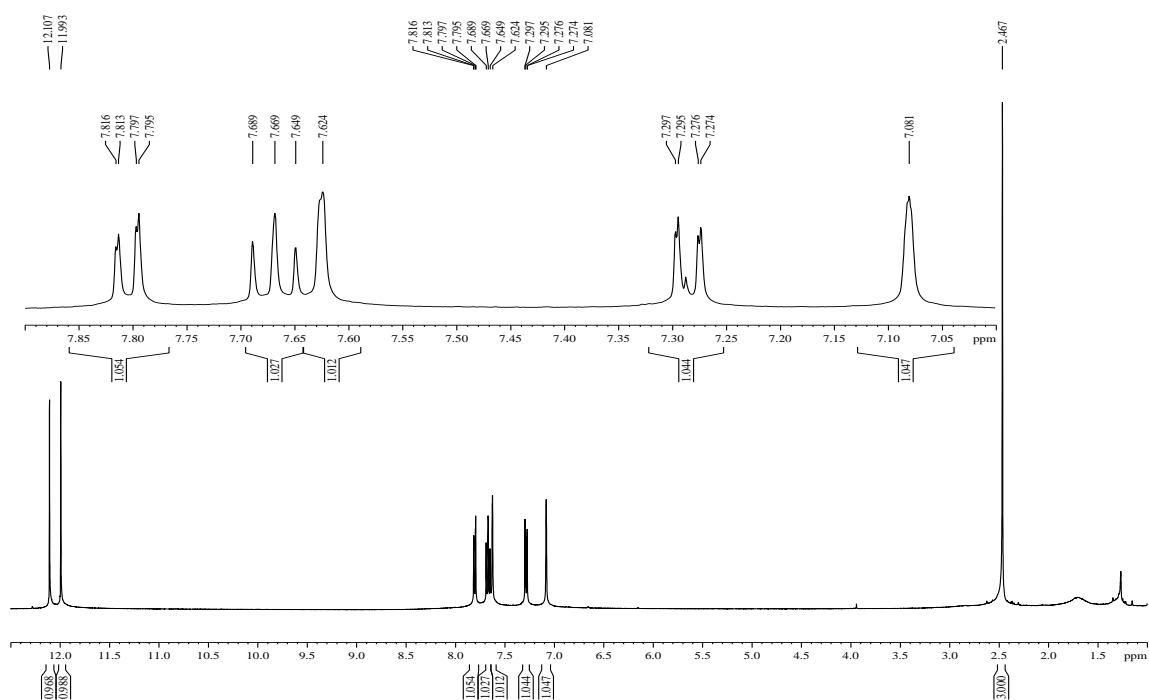


S2: ^{13}C -NMR spectrum of Islandicin (**1**)

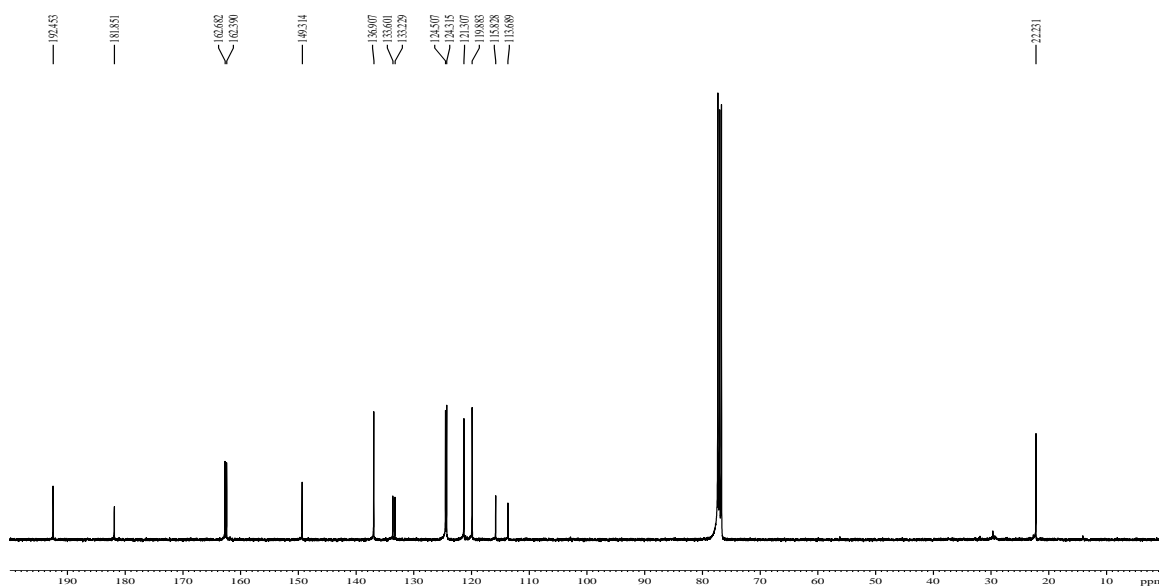


S3: EIMS spectrum of Islandicin (1)

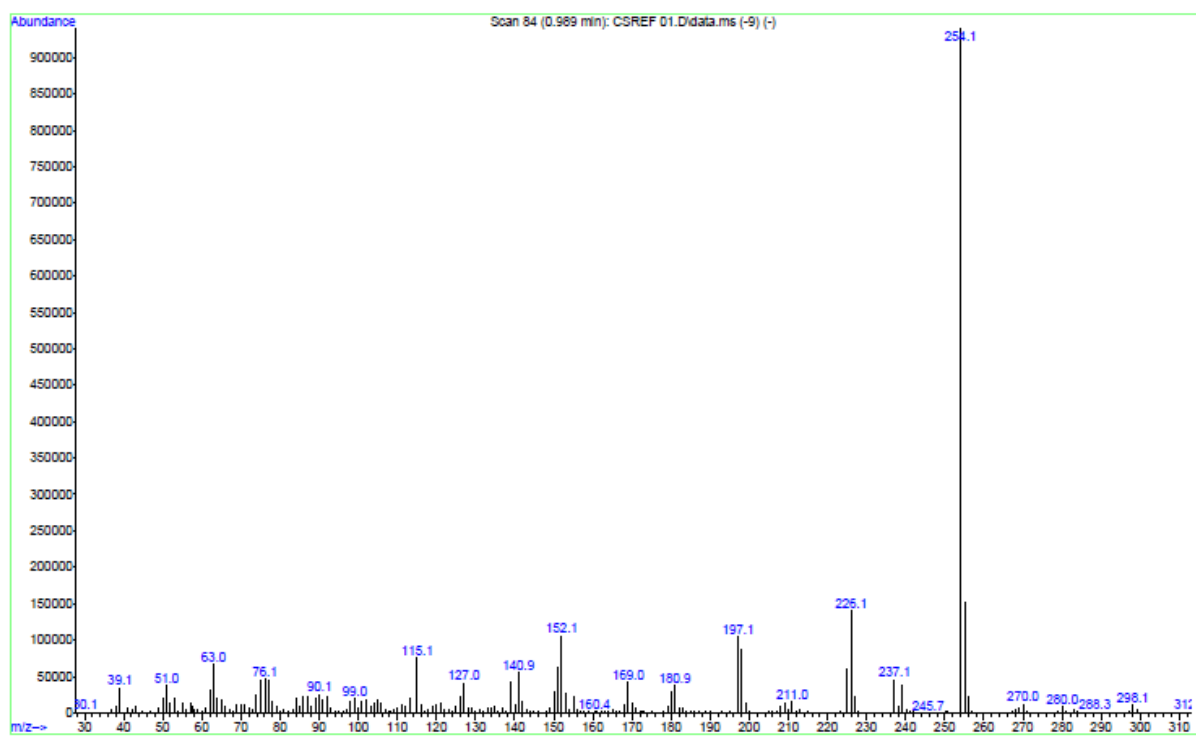
Chrysophanol (2): Yellow solid, IR (KBr, ν_{\max} , cm^{-1}): 3444, 2918, 1677, 1627; UV (λ_{\max} , MeOH, nm): 203, 225, 257, 277, 288; ^1H NMR data (400 MHz, CDCl_3) δ : 7.08 (1H, br.s, H-2), 7.29 (1H, dd, $J = 1.2, 8.4$ Hz, H-7), 7.62 (1H, br.s, H-4), 7.68 (1H, t, $J = 8.4$ Hz, H-6), 7.81 (1H, dd, $J = 1.2, 8.4$ Hz, H-5), 11.99 (1H, s, OH), 12.10 (1H, s, OH); ^{13}C NMR data (100 MHz, CDCl_3) δ : 22.2 (- CH_3), 115.8 (C-12), 113.6 (C-13), 119.8 (C-5), 121.3 (C-4), 124.3 (C-2), 124.5 (C-7), 133.2 (C-14), 133.6 (C-11), 136.9 (C-6), 149.3 (C-3), 162.3 (C-1), 162.6 (C-8), 181.8 (C-10), 192.4 (C-9); EIMS (m/z): 254.1 [M] $^+$ ($\text{C}_{15}\text{H}_{10}\text{O}_4$), 237.1, 226.1, 197.1.



S4: ^1H NMR Spectrum of Chrysophanol (2)

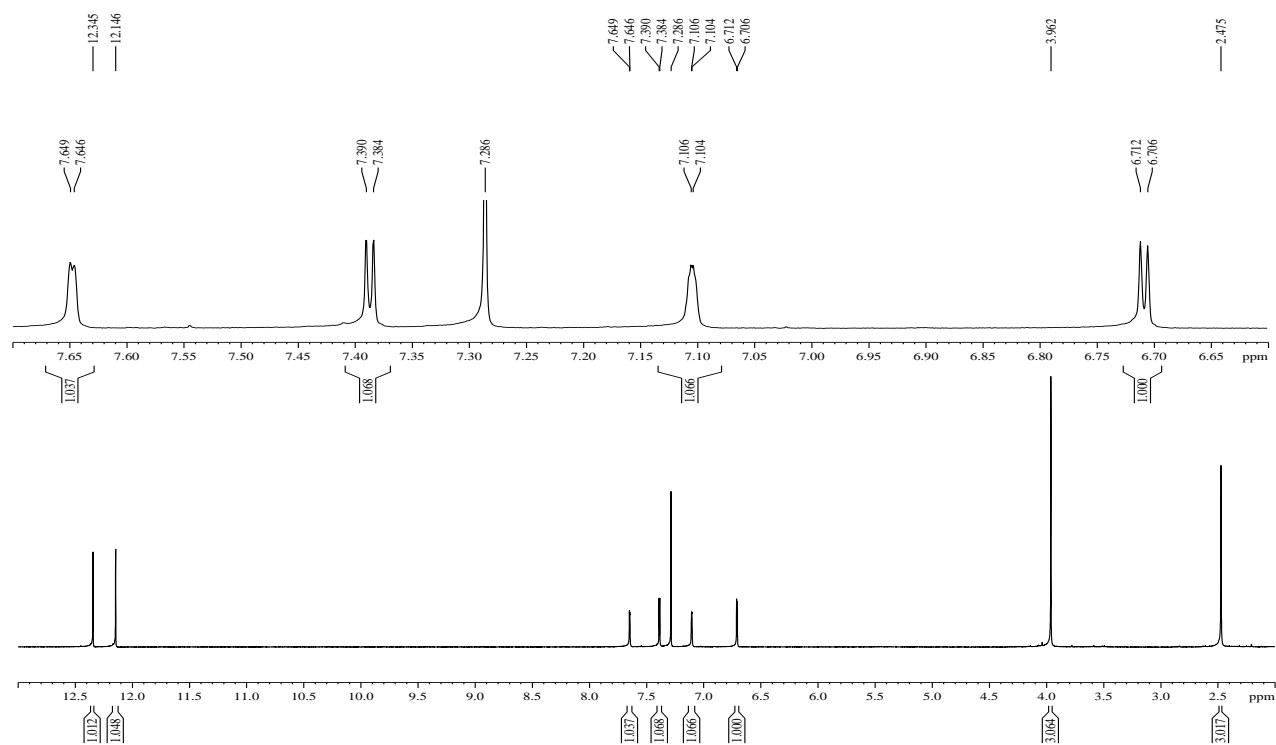


S5: ^{13}C -NMR spectrum of Chrysophanol (2)

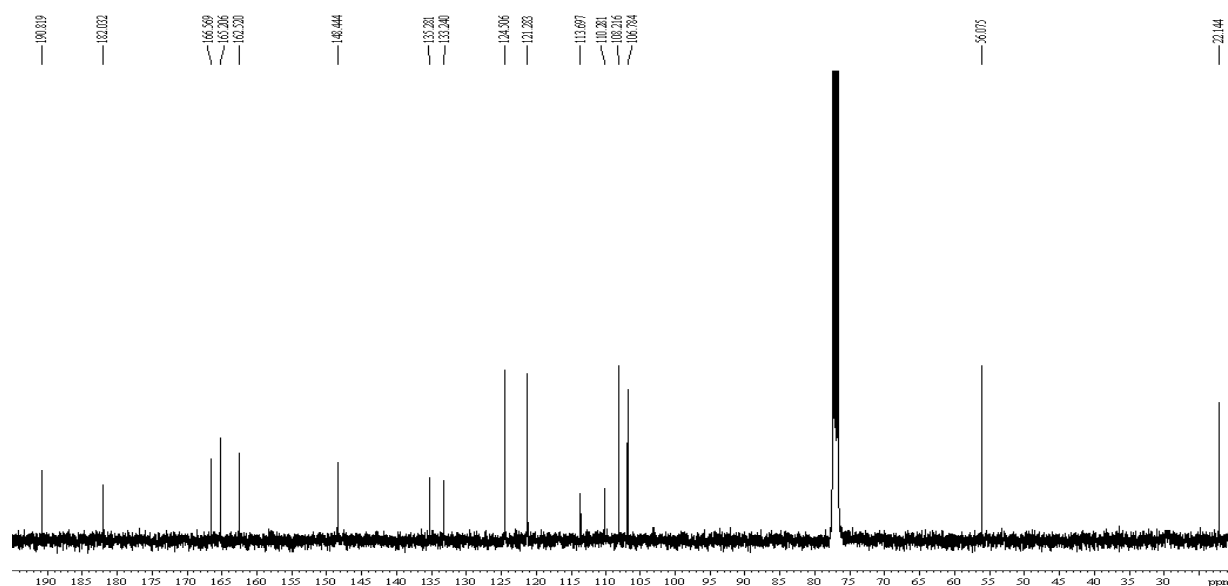


S6: EIMS spectrum of Chrysophanol (2)

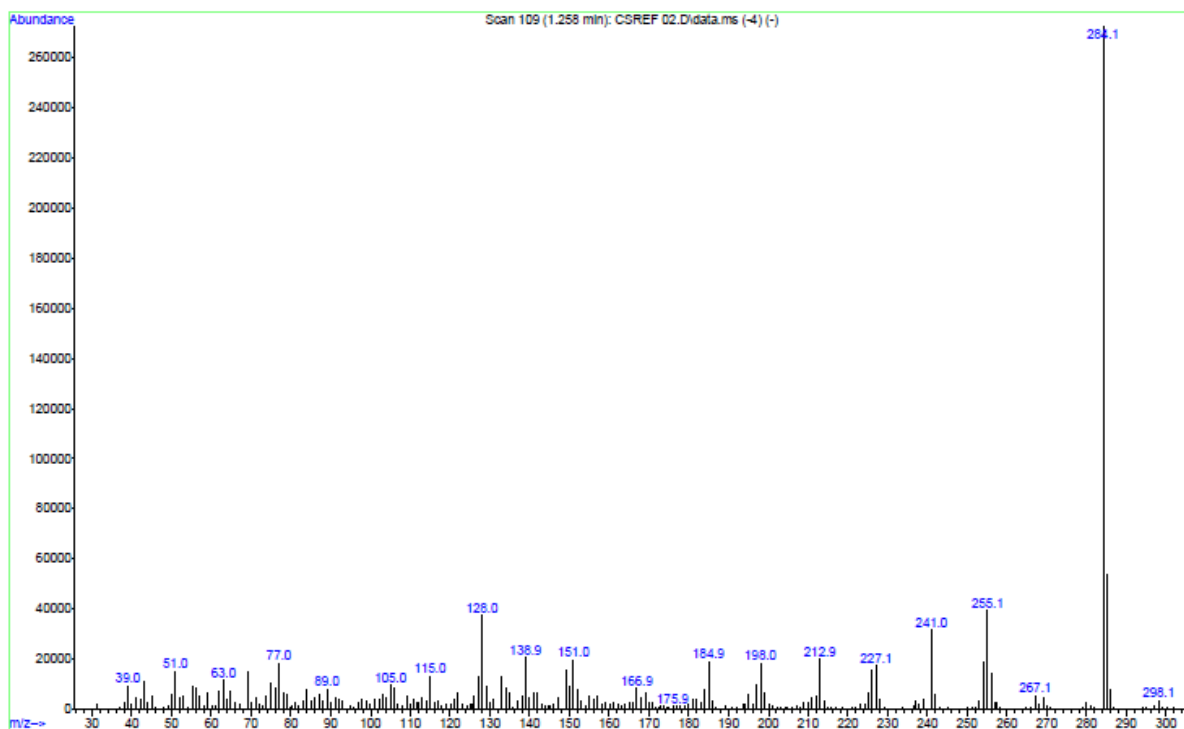
Phycion (**3**): Orange-yellow solid, IR (KBr, ν_{\max} , cm^{-1}): 3428, 2920, 1676, 1628; UV (λ_{\max} , MeOH, nm): 203, 224, 250, 267, 287; ^1H NMR data (400 MHz, CDCl_3): δ 2.47 (3H, s, CH_3), 3.96 (3H, s, OCH_3), 6.71 (1H, d, $J = 2.4$ Hz, H-7), 7.10 (1H, d, $J = 1.2$ Hz, H-2), 7.39 (1H, d, $J = 2.4$ Hz, H-5), 7.64 (1H, d, $J = 1.2$ Hz, H-4), 12.10 (1H, s, -OH), 12.34 (1H, s, -OH); ^{13}C NMR data (100 MHz, CDCl_3) δ : 162.5 (C-1), 114.5 (C-2), 148.4 (C-3), 121.2 (C-4), 108.2 (C-5), 166.5 (C-6), 106.7 (C-7), 165.2 (C-8), 190.8 (C-9), 182.0 (C-10), 135.2 (C-11), 110.2 (C-12), 113.6 (C-13), 133.2 (C-14), 22.1 (CH_3), 56.0 ($-\text{OCH}_3$); EIMS (m/z): 284.1 [M] $^+$ ($\text{C}_{16}\text{H}_{12}\text{O}_5$), 267.1, 255.1, 227.1, 198.0.



S7: ^1H NMR spectrum of Phycion (**3**)

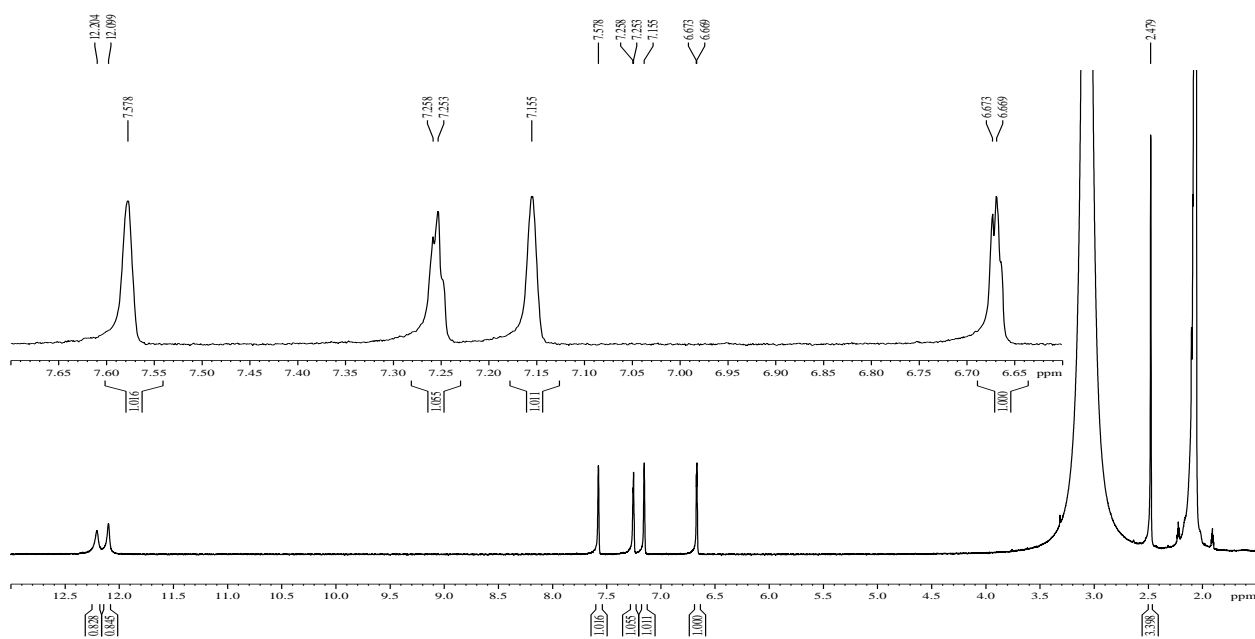


S8: ^{13}C -NMR spectrum of Phycion (**3**)

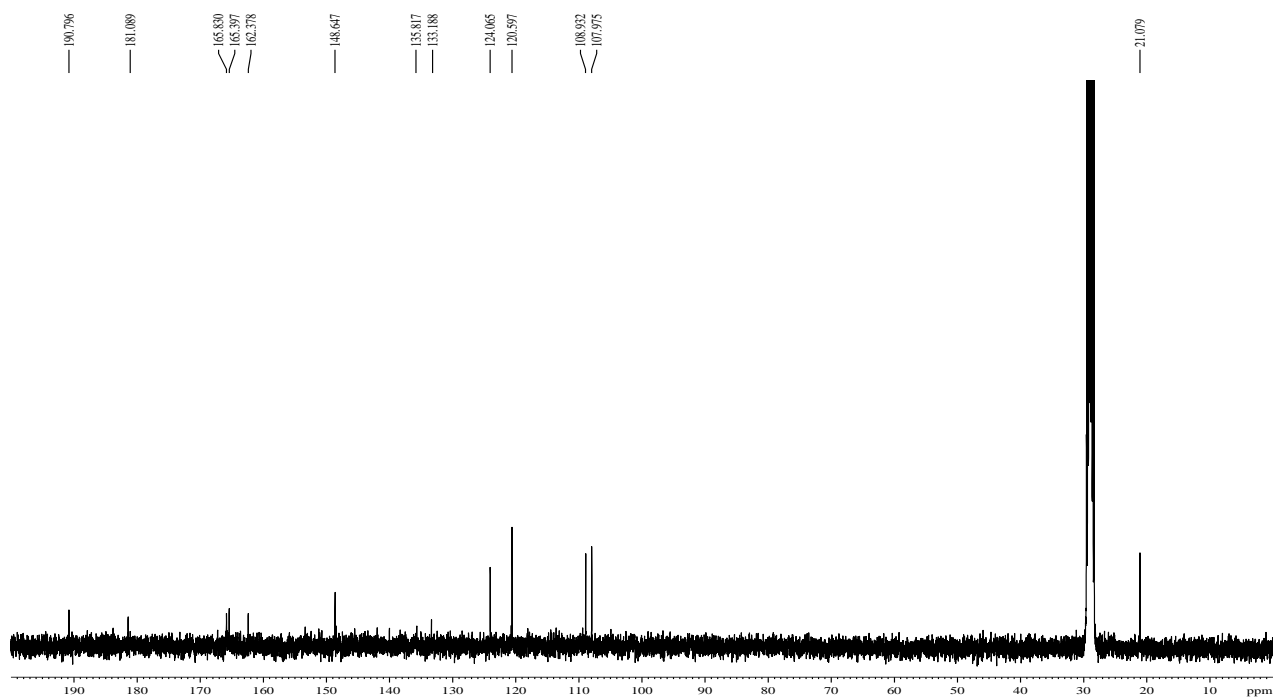


S9: EIMS spectrum of Physcion (**3**)

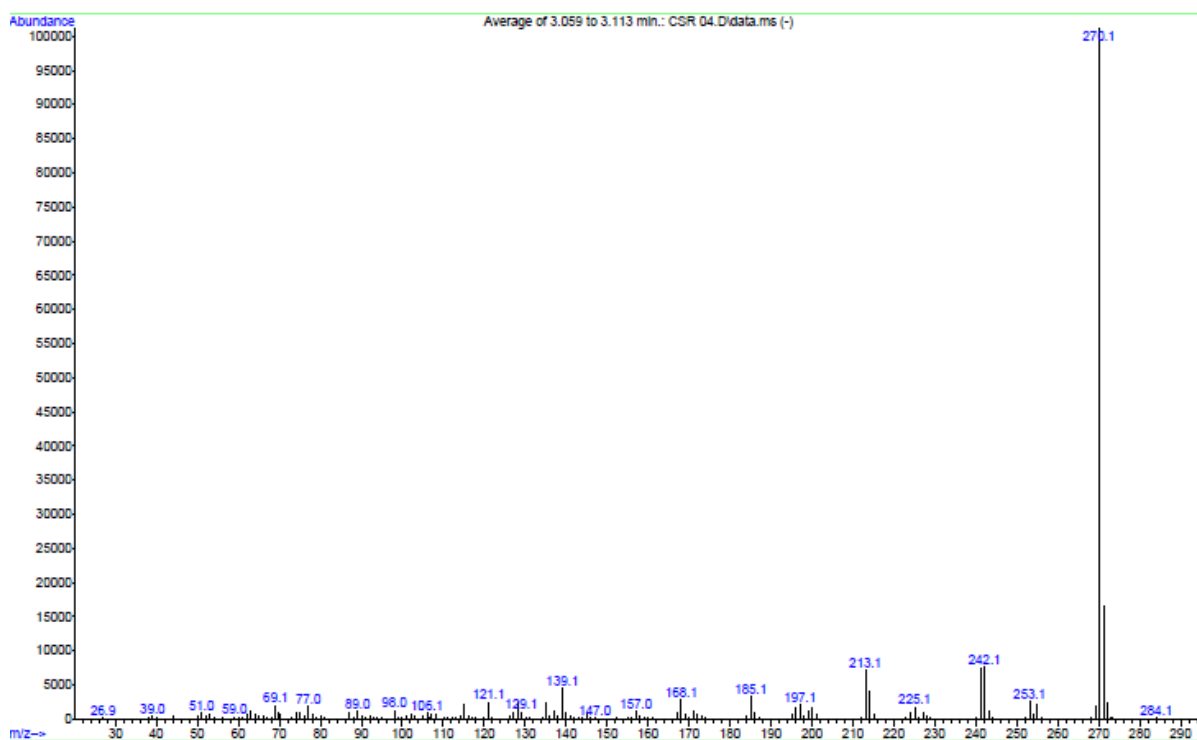
Emodin (**4**): Orange-red solid, IR (KBr) cm^{-1} : 1631, 1675, 3390; UV (λ_{max} , MeOH, nm): 253, 266, 289, 436; ^1H NMR data (400 MHz, CD_3COCD_3) δ : 2.45 (3H, s, CH_3), 6.67 (1H, d, 2.4, H-7), 7.15 (1H, br s, H-5), 7.25 (1H, d, 2.4, H-2), 7.57 (1H, br s, H-4), 12.10 (1H, s, OH), 12.20 (1H, s, OH); ^{13}C NMR data (100 MHz, CD_3COCD_3) δ : 21.0 (CH_3), 107.9 (C-7), 108.9 (C-5), 109.6 (C-13), 113.6 (C-11), 120.6 (C-4), 124.0 (C-2), 133.4 (C-12), 135.7 (C-14), 148.6 (C-3), 162.3 (C-1), 165.4 (C-8); 165.9 (C-6), 181.4 (C-10), 190.7 (C-9); EIMS (m/z): 270.1 [M] $^+$ ($\text{C}_{15}\text{H}_{10}\text{O}_5$), 253.1, 242.1, 225.1, 197.1.



S10: ^1H NMR spectrum of Emodin (**4**)

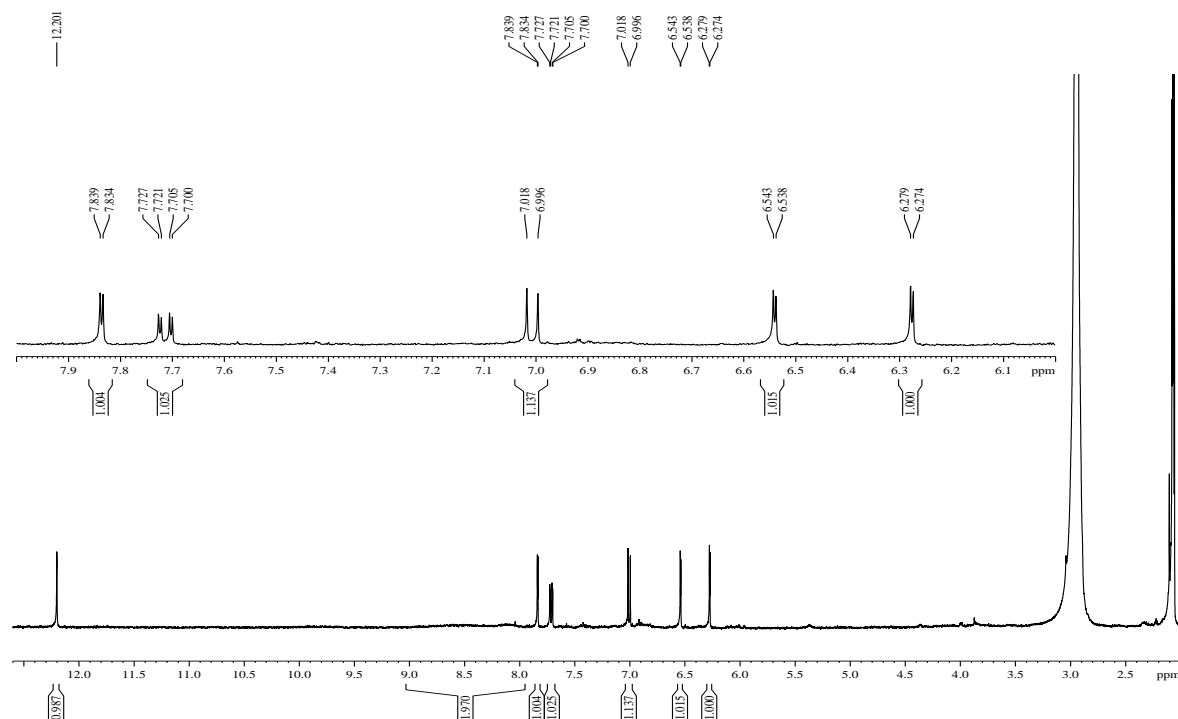


S11: ^{13}C -NMR spectrum of Emodin (**4**)

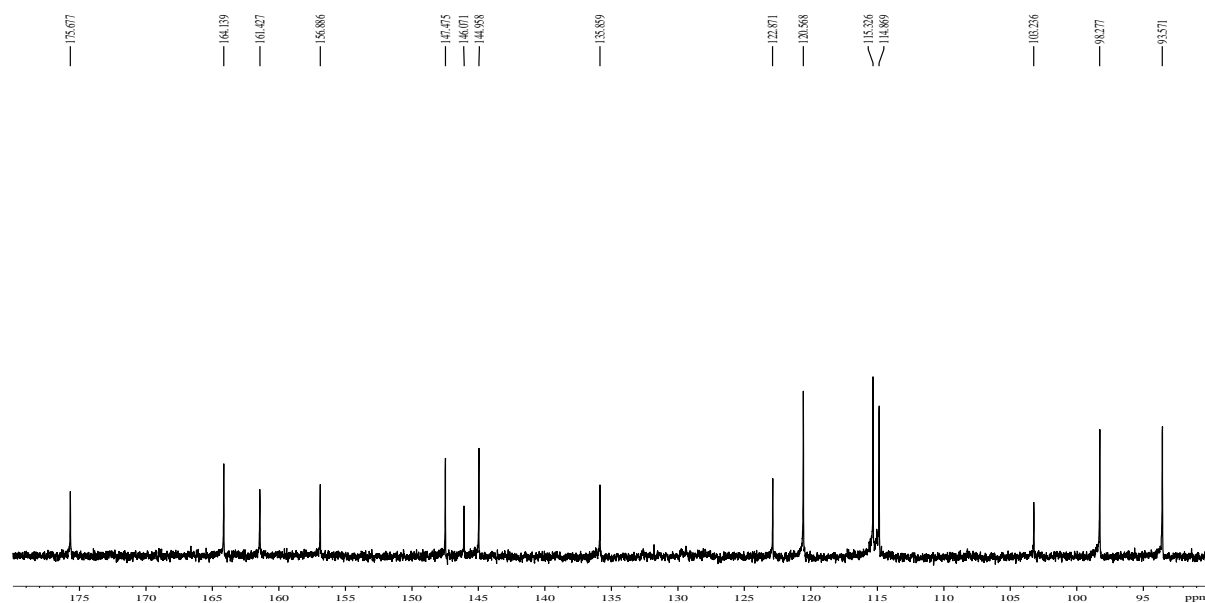


S12: EIMS spectrum of emodin (**4**)

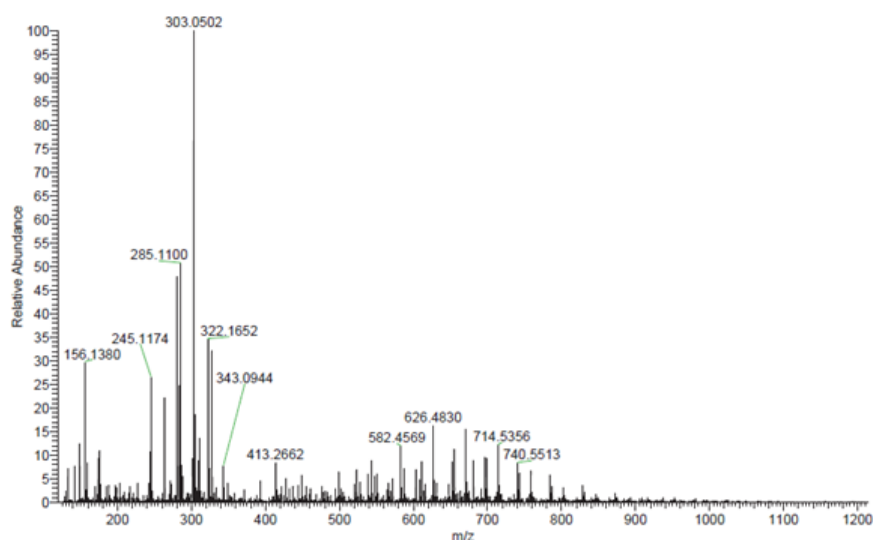
Quercetin (**5**): Yellow powder, IR (KBr) cm^{-1} : 3261, 1661, 1601, 1562; UV (λ_{max} , MeOH, nm): 208, 257, 375 ; ^1H NMR data (400 MHz, CD_3COCD_3) δ : 6.27 (1H, d, 2.0, H-6), 6.54 (1H, d, 2.0, H-8), 6.99 (1H, d, 8.8, H-5'), 7.70 (1H, dd, 2.0, 8.8, H-6'), 7.83 (1H, d, 2.0, H-2'), 12.19 (1H, s, OH); ^{13}C NMR data (100 MHz, CD_3COCD_3) δ : 175.7 (C-4), 164.3 (C-7), 161.3 (C-9), 156.8 (C-5), 147.5 (C-4'), 146.2 (C-2), 145.0 (C-3'), 135.8 (C-3), 122.7 (C-1'), 120.5 (C-6'), 115.2 (C-5'), 114.8 (C-2'), 103.1 (C-10), 98.3 (C-8), 93.5 (C-6); HRESIMS (m/z): 303.0502 $[\text{M}]^+$ ($\text{C}_{15}\text{H}_{10}\text{O}_7$), 285.1100, 245.1174, 156.1380.



S13: ^1H NMR spectrum of Quercetin (**5**)

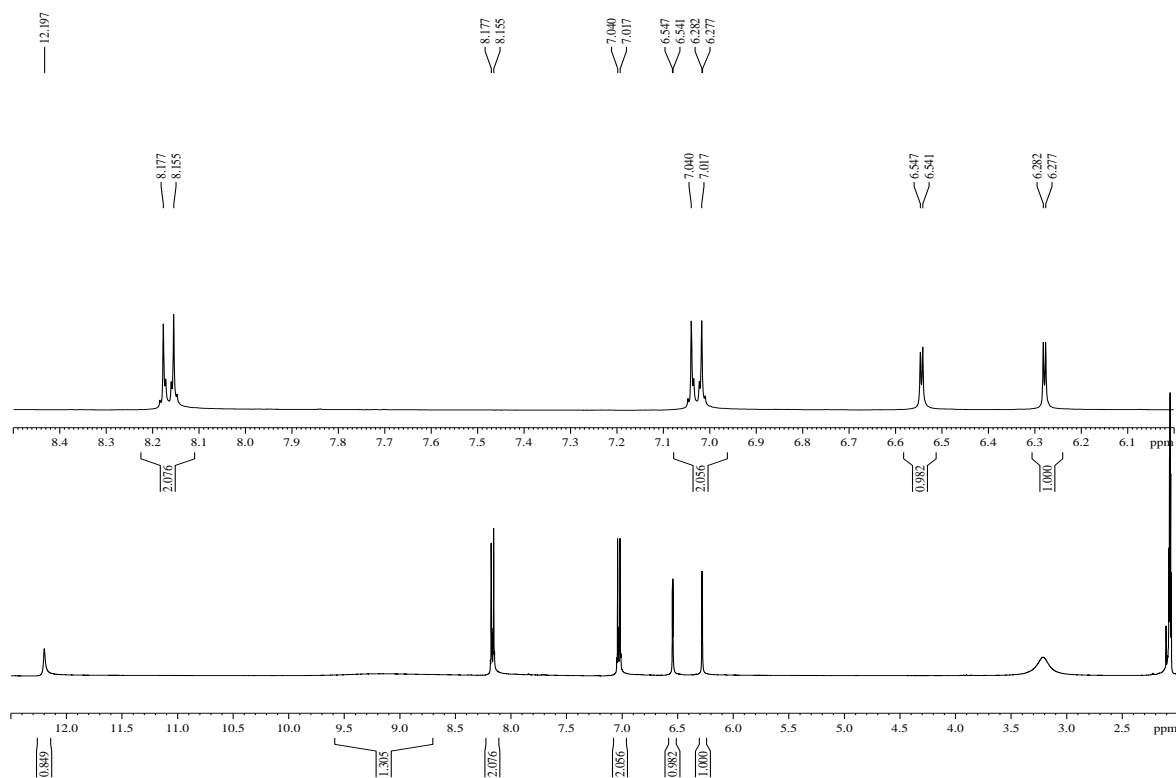


S14: ^{13}C -NMR spectrum of Quercetin (**5**)

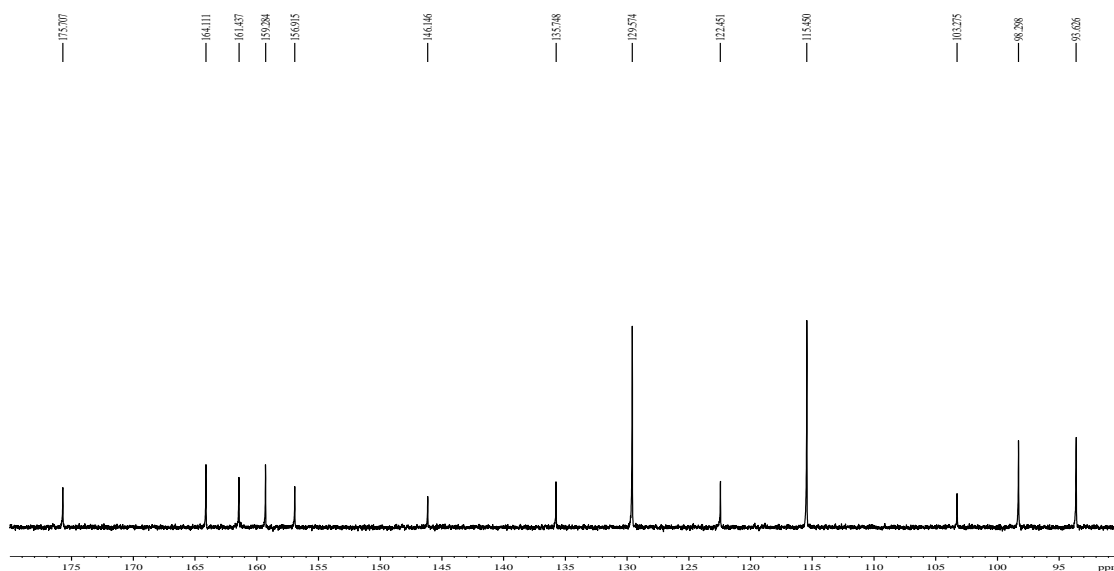


S15: EIMS spectrum of Quercetin (**5**)

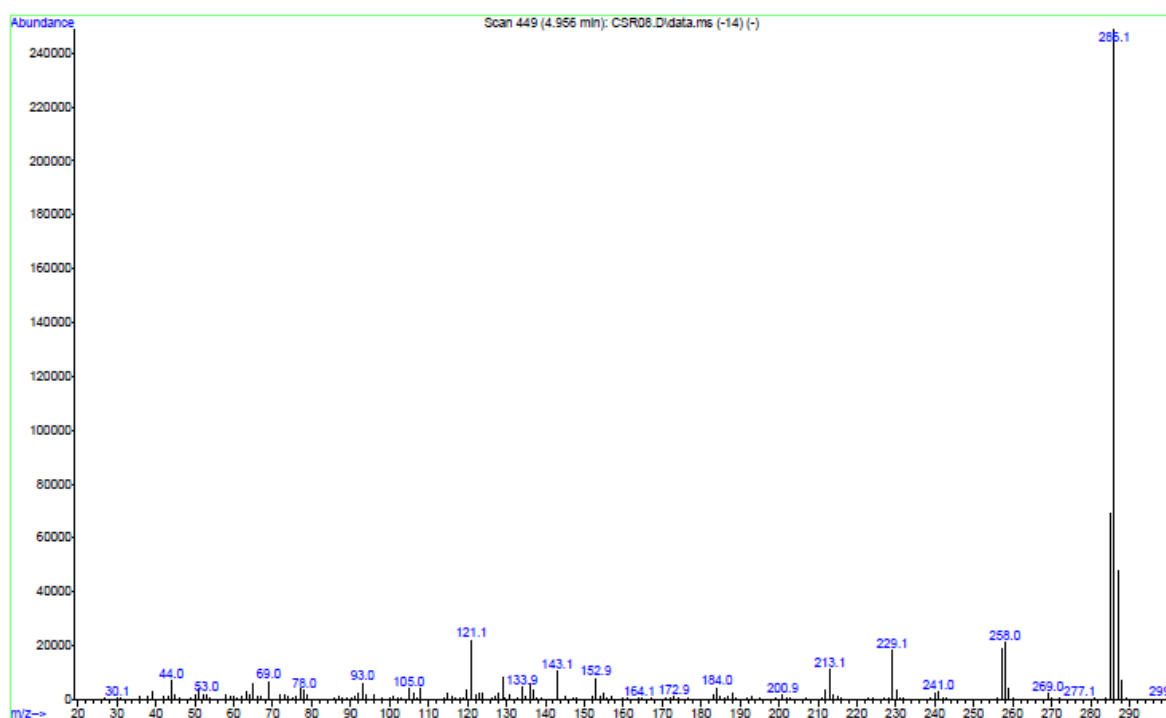
Kaempferol (**6**): Yellow powder, IR (KBr) cm^{-1} : 3310, 1694, 1658, 1611, 1506; UV (λ_{max} , MeOH, nm): 266, 368 ; ^1H NMR data (400 MHz, CD_3COCD_3) δ : 6.28 (1H, d, 2.0, H-6), 6.54 (1H, d, 2.0, H-8), 7.04 (2H, d, 8.8, H-3'/H-5'), 8.17 (2H, d, 8.8, H-2'/H-6'), 12.19 (1H, s, OH); ^{13}C NMR data (100 MHz, CD_3COCD_3) δ : 175.6 (C-4), 164.1 (C-7), 161.4 (C-5), 159.2 (C-8a), 156.8 (C-4'), 146 (C-2), 135.7 (C-3), 129.5 (c-2'/C-6'), 122.4 (C-1), 115.4 (C-3'), 115.4 (C-5), 103.2 (C-4a), 98.2 (C-6), 93.6 (C-8); EIMS (m/z): 286.1 $[\text{M}]^+$ ($\text{C}_{15}\text{H}_{10}\text{O}_6$), 258.0, 229.1, 213.1, 184.0.



S16: ^1H NMR Spectrum of kaempferol (**6**)

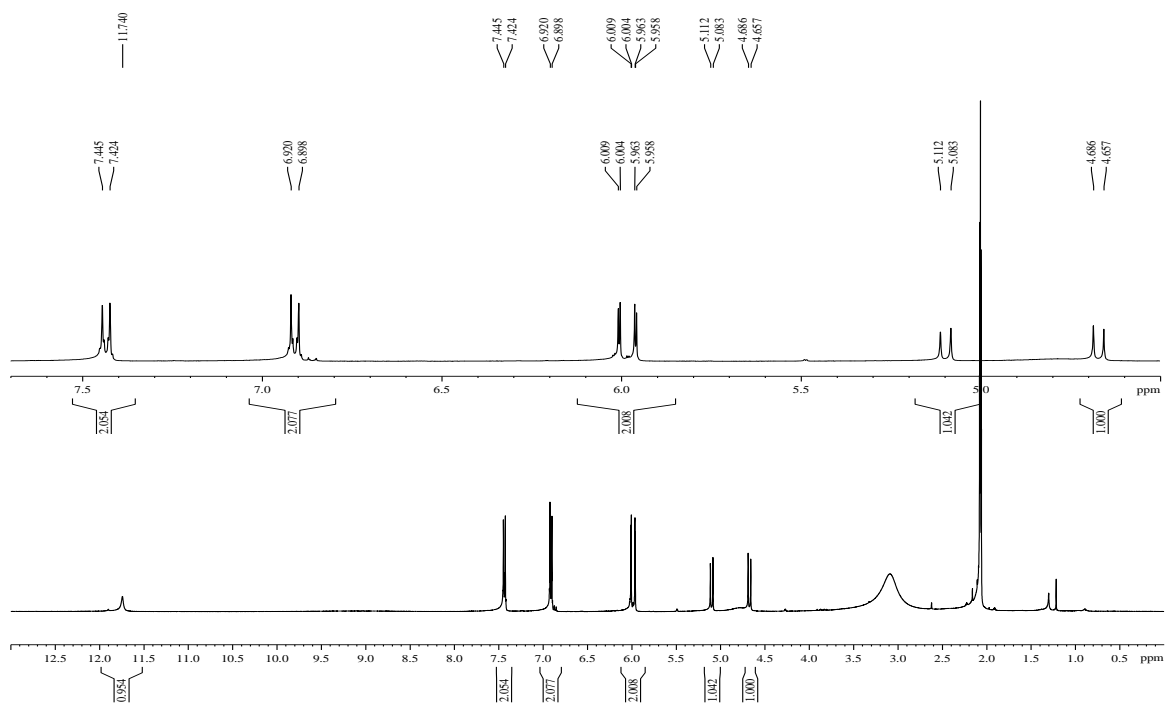


S17: ^{13}C -NMR Spectrum of kaempferol (**6**)

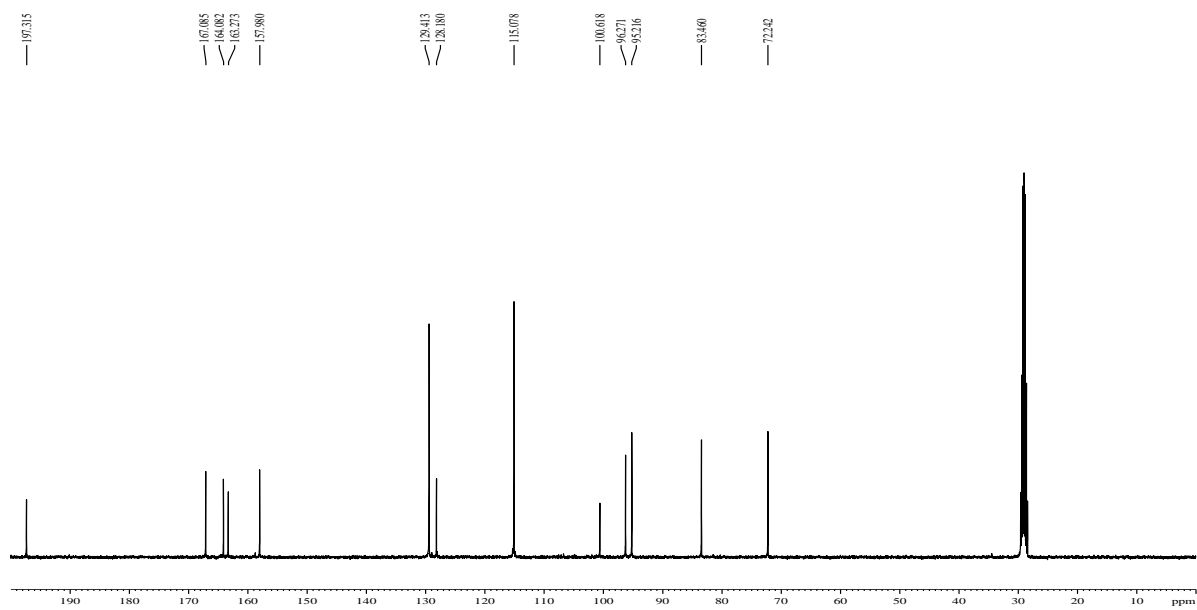


S18: EIMS of kaempferol (**6**)

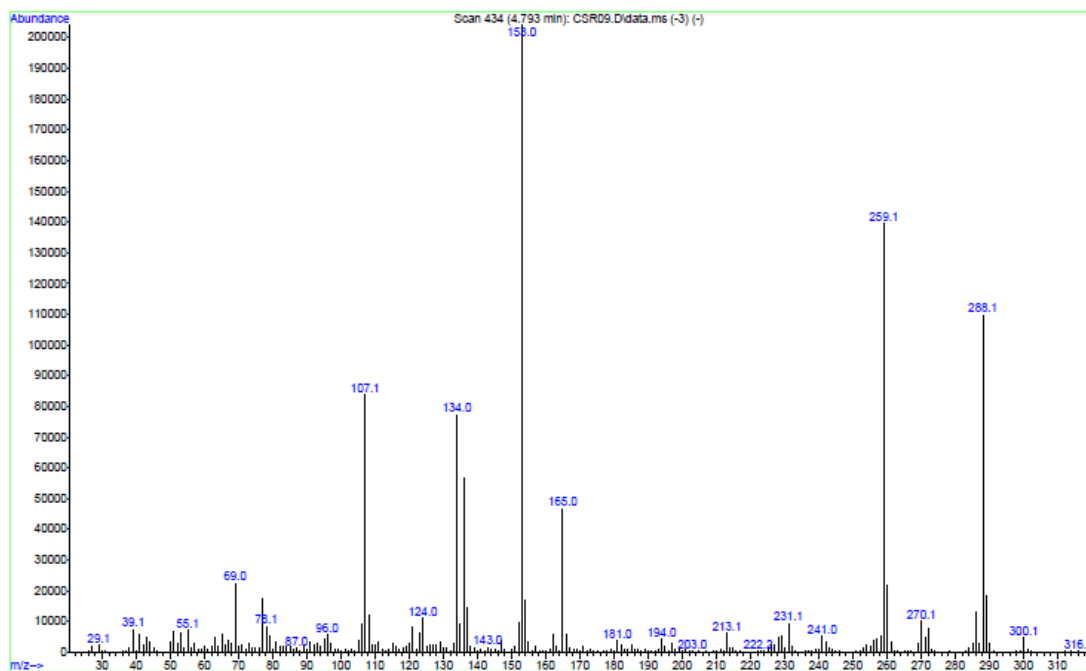
Dihydrokaempferol (**7**): Yellow powder, IR (KBr) cm^{-1} ; 3546, 1610, 1600, 1518 ; UV (λ_{max} , MeOH, nm): 217, 230, 289, 333; ^1H NMR data (400 MHz, CD_3COCD_3) δ : 4.68 (1H, d, 11.6, H-3), 5.10 (1H, d, 11.6, H-2), 5.96 (1H, d, 2.0, H-6), 6.01 (1H, d, 2.0, H-8), 6.92 (1H, d, 8.8, H-3'/H-5'), 7.37 (1H, d, 8.8, H-6'), 7.44 (1H, d, 8.8, H-2'), 11.72 (1H, s, OH); ^{13}C NMR data (100 MHz, CD_3COCD_3) δ : 197.3 (C-4), 167.0 (C-7), 164.0 (C-5), 163.2 (C-9), 157.9 (C-4), 129.4 (C-6'), 129.4 (C-2'), 128.1 (C-1'), 115.0 (C3'), 100.6 (C-10), 96.2 (C-6), 95.2 (C-8), 83.4 (C-2), 72.2 (C-3); EIMS (m/z): 288.1 $[\text{M}]^+$ ($\text{C}_{15}\text{H}_{12}\text{O}_6$), 270.1, 259.1, 231.1, 194.0.



S19: ^1H NMR Spectrum of Dihydrokaempferol (7)

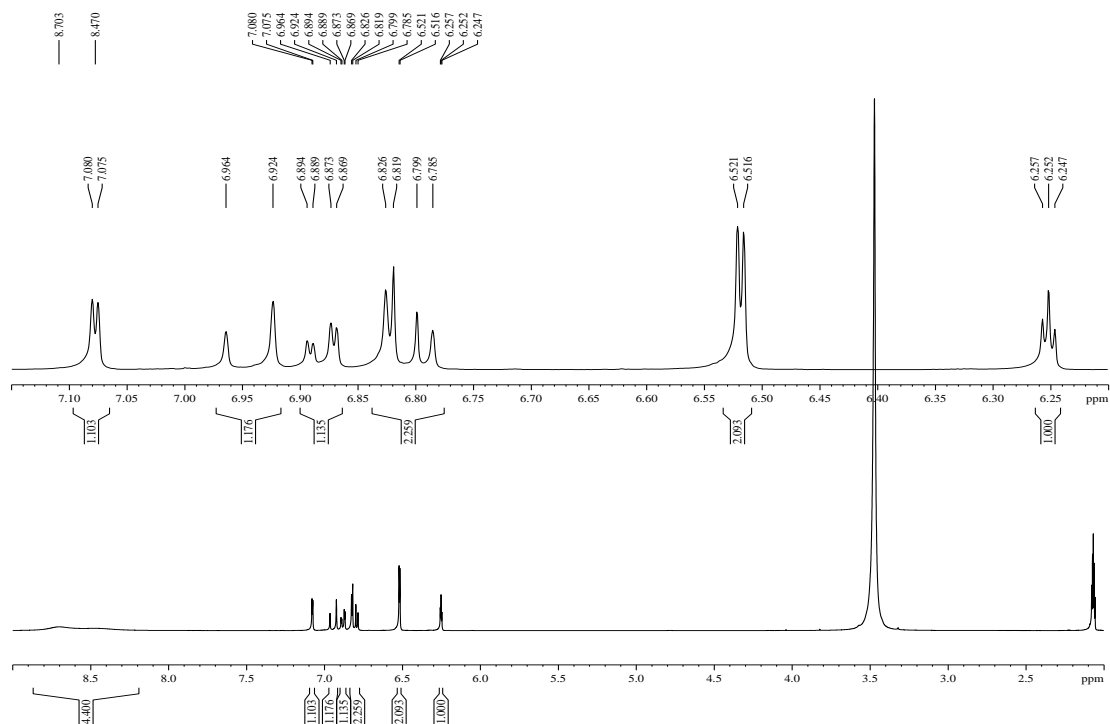


S20: ^{13}C -NMR Spectrum of Dihydrokaempferol (7)

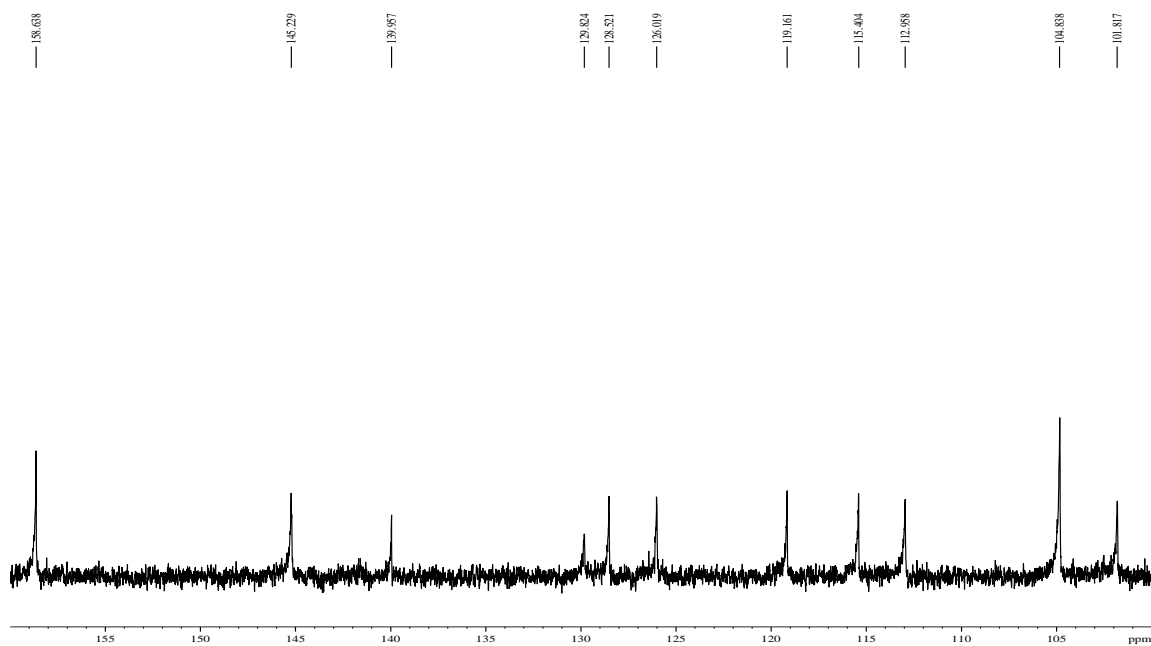


S21: EIMS spectrum of Dihydrokaempferol (**7**)

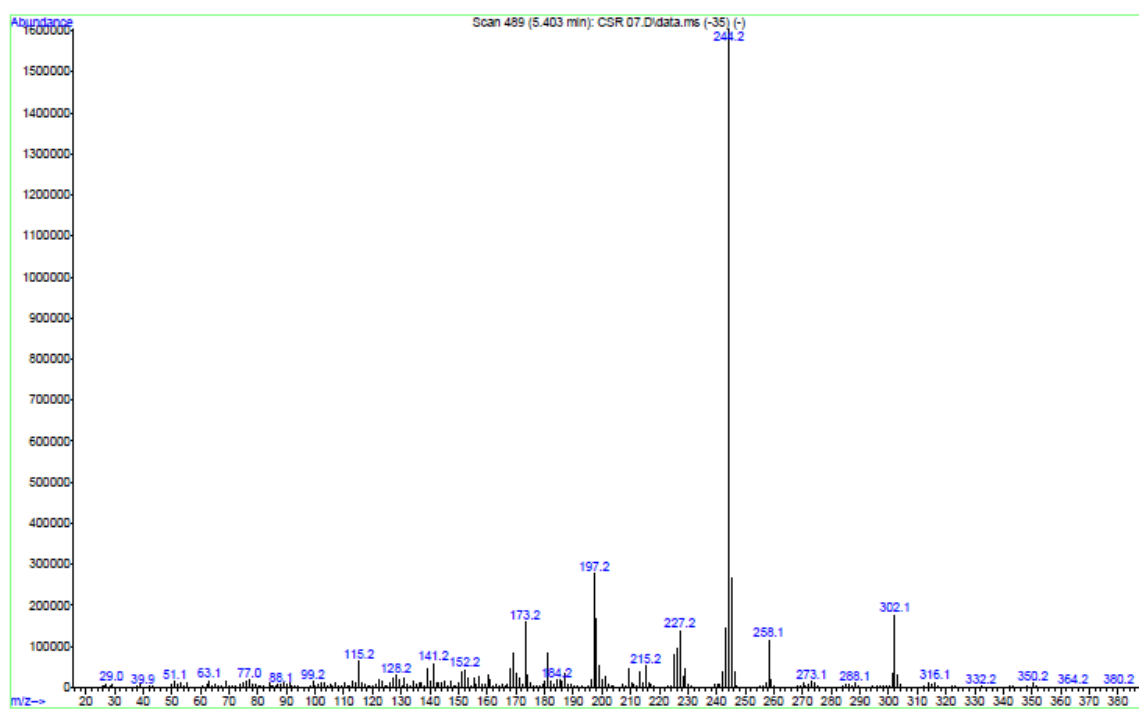
Piceatannol (**8**): Light brown powder, IR (KBr) cm^{-1} : 3291, 1598, 1518, 1443; UV (λ_{max} , MeOH, nm): 221, 327; ^1H NMR data (400 MHz, CD_3COCD_3) δ : 6.26 (1H, t, 2.0, H-4'), 6.53 (1H, d, 2.0, H-6'), 6.79 (1H, d, 16.4, H-7), 6.80 (1H, d, 2.0, H-4), 6.88 (1H, dd, 8.0, H-5), 6.93 (1H, d, 16.4, H-8), 7.08 (1H, d, 2.0, H-6); ^{13}C NMR data (100 MHz, CD_3COCD_3) δ : 158.5 (C-3'), 158.5 (C-5'), 145.3 (C-2), 145.3 (C-3), 139.8 (C-1'), 129.5 (C-1), 128.4 (C-8), 125.8 (C-7), 119.0 (C-5), 115.5 (C-4), 112.9 (C-6), 104.7 (C-2'), 104.7 (C-6'), 101.9 (C-4'); EIMS (m/z): 244.2 $[\text{M}]^+$ ($\text{C}_{14}\text{H}_{12}\text{O}_4$), 227.2, 215.2, 197.2.



S22: ^1H NMR spectrum of Piceatannol (**8**)



S23: ^{13}C -NMR spectrum of Piceatannol (**8**)



S24: EIMS spectrum of Piceatannol (**8**)

References

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