# Enrichment of major bioavailable molecule glucuronated flavone TMMG in *Spinacia oleracea* ameliorates cartilage degeneration at a lower dose in ACLT-induced osteoarthritis

Geeta Dhaniya[a], Vallabh Mulay[b], Priyanka Kothari[a], Anirban Sardar[a,d], Kunal Chutani[a,d], Parul[c], Shikha Verma[a,d], Shubha Shukla[c,d], Lal Hingorani[b], Ritu Trivedi[a,d,*]

[a] Endocrinology Division, CSIR-Central Drug Research Institute, Lucknow, 226031, India

[b] Pharmanza Herbal Pvt Ltd, Anand, Gujarat, 388435, India

[c] Division of Neuroscience and Ageing Biology, CSIR-Central Drug Research Institute, Lucknow, 226031, India

[d] Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, 201002, India

*Corresponding Author- Dr. Ritu Trivedi

Endocrinology Division. CSIR-Central Drug Research Institute, Sector 10, Jankipuram Extension, Sitapur Road, Lucknow, 226031, India Phone: +91-522-2771940-42 (Ext. 4397) Email: ritu_trivedi@cdri.res.in ORCID ID: 0000-0002-9524-2541

# Supplementary information

**Compound:** 5, 3', 4'-trihydroxy-3-methoxy-6, 7-methylenedioxy-flavone 4’glucuronide

**IUPAC Name-** 3,4,5-trihydroxy-6-(2-hydroxy-4-(9-hydroxy-7-methoxy-8-oxo-8H-[1,3]dioxolo[4,5-g]chromen-6-yl)phenoxy)tetrahydro-2H-pyran-2-carboxylic acid

$^{1}$H NMR[1]: (DMSO-$d_6$, 400MHz) δ: 3.81 (3H,s, MeO), 4.2-4.9 (4H, m, H on sugar moiety), 5.82 (1H, d, J=8Hz anomeric H), 6.17 (2H, s, -O-$CH_2$-O-), 6.94 (1H, s, H-8), 7.25 (3H, d, J=8.56), 7.54 (3H, d, J=8.6, H-2’, 5’, 6’). $^{13}$C NMR[1]: (DMSO-$d_6$, 100MHz) δ:140.4 (C-2), 138.2 (C-3), 178.5 (C-4), 151.8 (C-5), 129.2 (C-6), 154.0 (C-7), 89.7 (C-8), 155.4 (C-9), 107.2 (C-10), 123.9 (C-1’), 115.7 (C-2’), 146.8 (C-3’ or C-4’), 147.6 (C-4’ or C-3’), 116.1 (C-5’ or C-2’), 120.1 (C-6’), 102.8 (-O-$CH_2$-O-), 59.7 (MeO-3), 101.1 (C-1’’), 73.0 (C-2’’), 75.6 (C-3’’), 71.8 (C-4’’), 74.6 (C-5’’), 171.0 (C-6’’).

**Chemical Structure:**

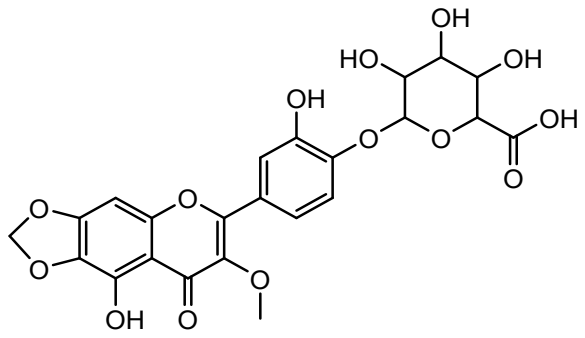
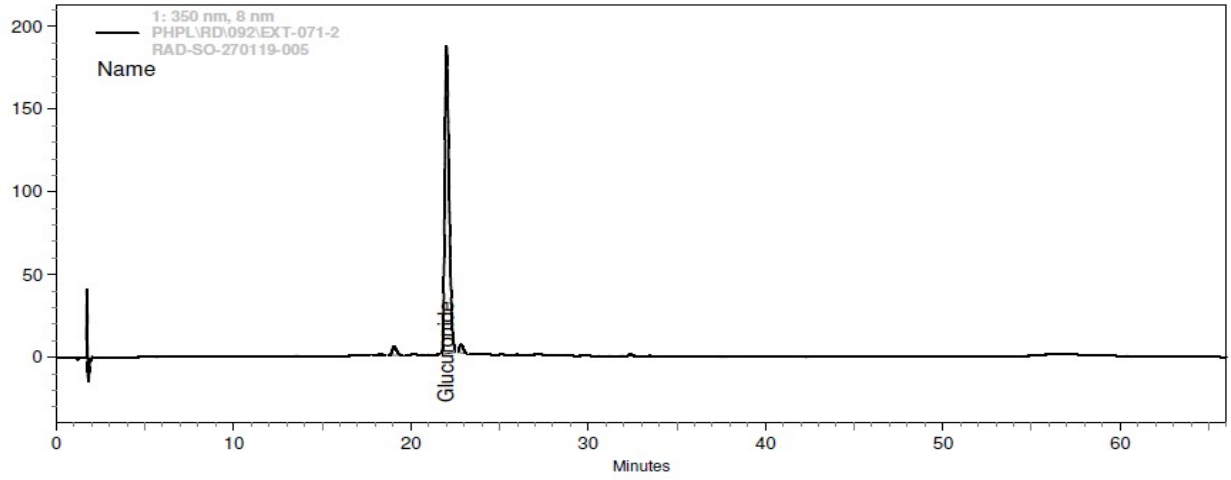



**Figure Supplementary 1: Structure of 5, 3', 4'-trihydroxy-3-methoxy-6, 7-methylenedioxy-flavone 4’glucuronide (TMMG).**

**Physical and chemical properties:**

| Sr. No | Parameters | Reported | Observed |
|---|---|---|---|
| 1 | Colour | Pale yellow colour | Pale yellow to brown |
| 2 | Melting Point | 197 –198 $^0$C | 198 $^0$C |
| 3 | Lambda max | 274, 336 nm | 275, 339 nm |
| 4 | Molecular weight | 520 g/mol | 520 g/mol |

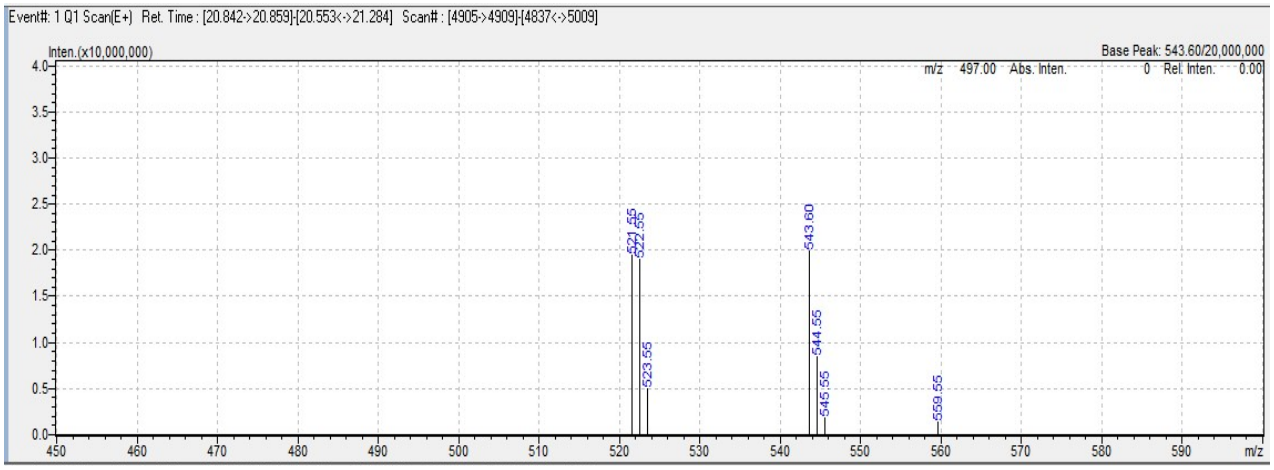
**HPLC Chromatogram:**



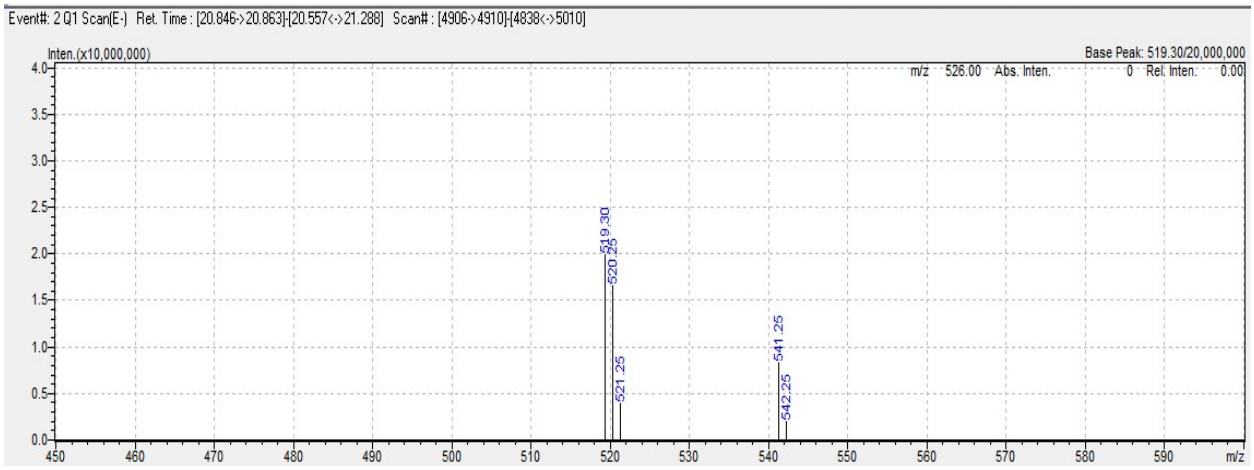

**Supplementary Figure 2: HPLC Chromatogram of isolated 5, 3', 4'-trihydroxy-3-methoxy-6, 7-methylenedioxy-flavone 4'glucuronide.**

**Mass data:**

Q1 Scan (E+)  m/z [M+H] = 521.5



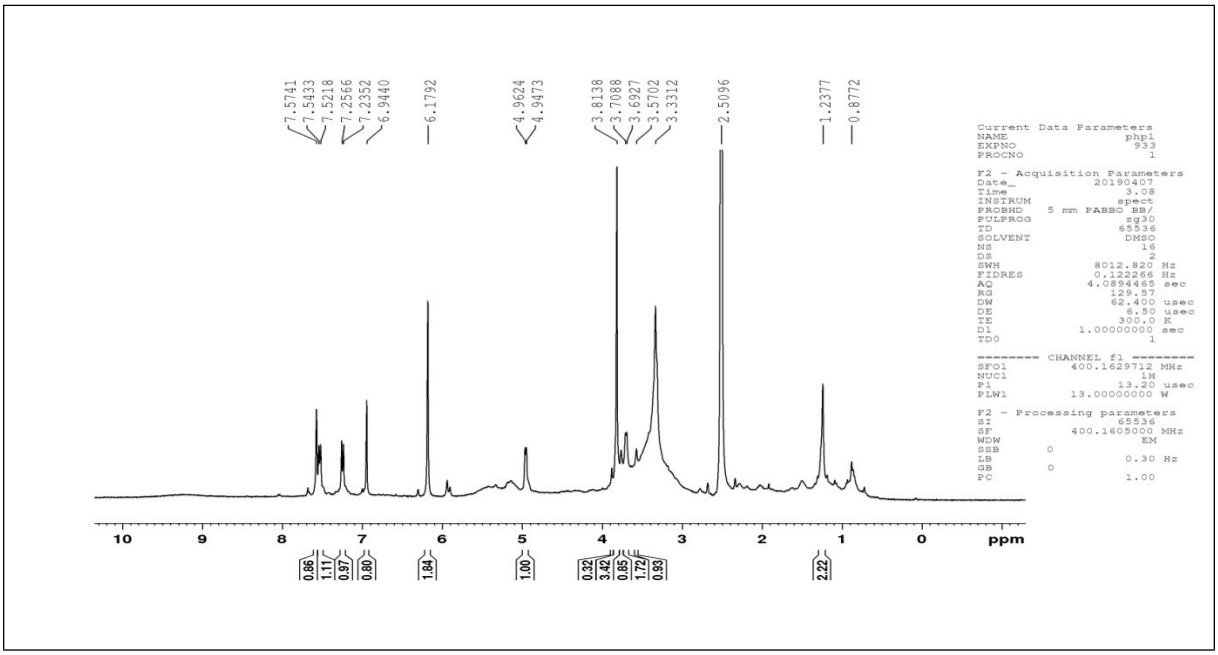

Q1 Scan(E-)  *m/z* [M-H] = 519.3




**Supplementary Figure 3: ESI-MS spectrum of isolated compound of 5, 3', 4'-trihydroxy-3-methoxy-6, 7-methylenedioxy-flavone 4'glucuronide.**

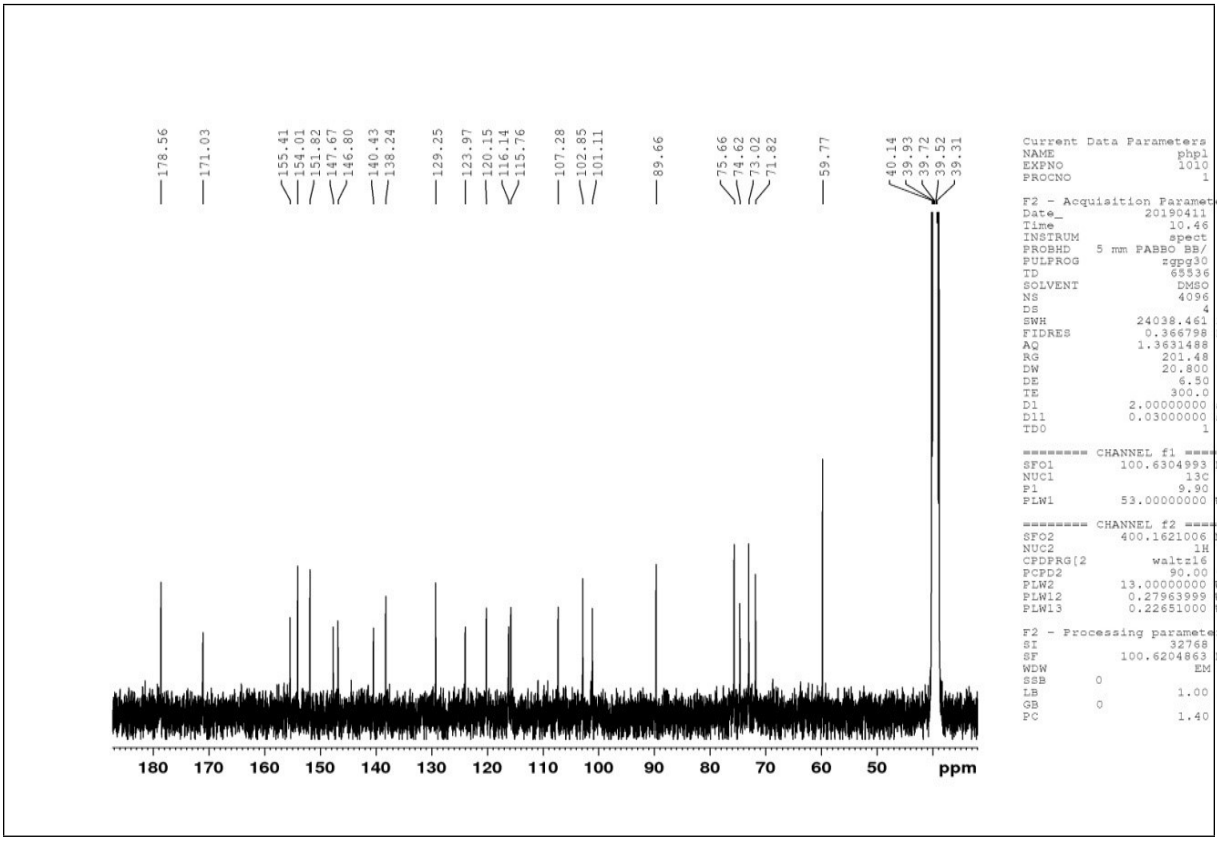
**NMR Data:**

**$^{1}$H NMR[1]:**




**Supplementary Figure 4: $^{1}$H NMR[1] spectrum of 5, 3', 4'-trihydroxy-3-methoxy-6, 7-methylenedioxy-flavone 4'glucuronide in DMSO-$d_6$**

**$^{13}$C NMR[1]:**




**Supplementary Figure 5: $^{13}$C NMR[1] spectrum of 5, 3', 4'-trihydroxy-3-methoxy-6, 7-methylenedioxy-flavone 4'glucuronide in DMSO-$d_6$.**

**References:**

(1) Aritomi, M. and Kawasaki, T., 1984. Three highly oxygenated flavone glucuronides in leaves of Spinacia oleracea. *Phytochemistry*, *23*(9), pp.2043-2047.