HPLC–NMR-Based Chemical Profiling of *Matricaria pubescens* (Desf.) Schultz and *Matricaria recutita* and their Protective Effects on UVA-Exposed Fibroblasts

Supplementary Data

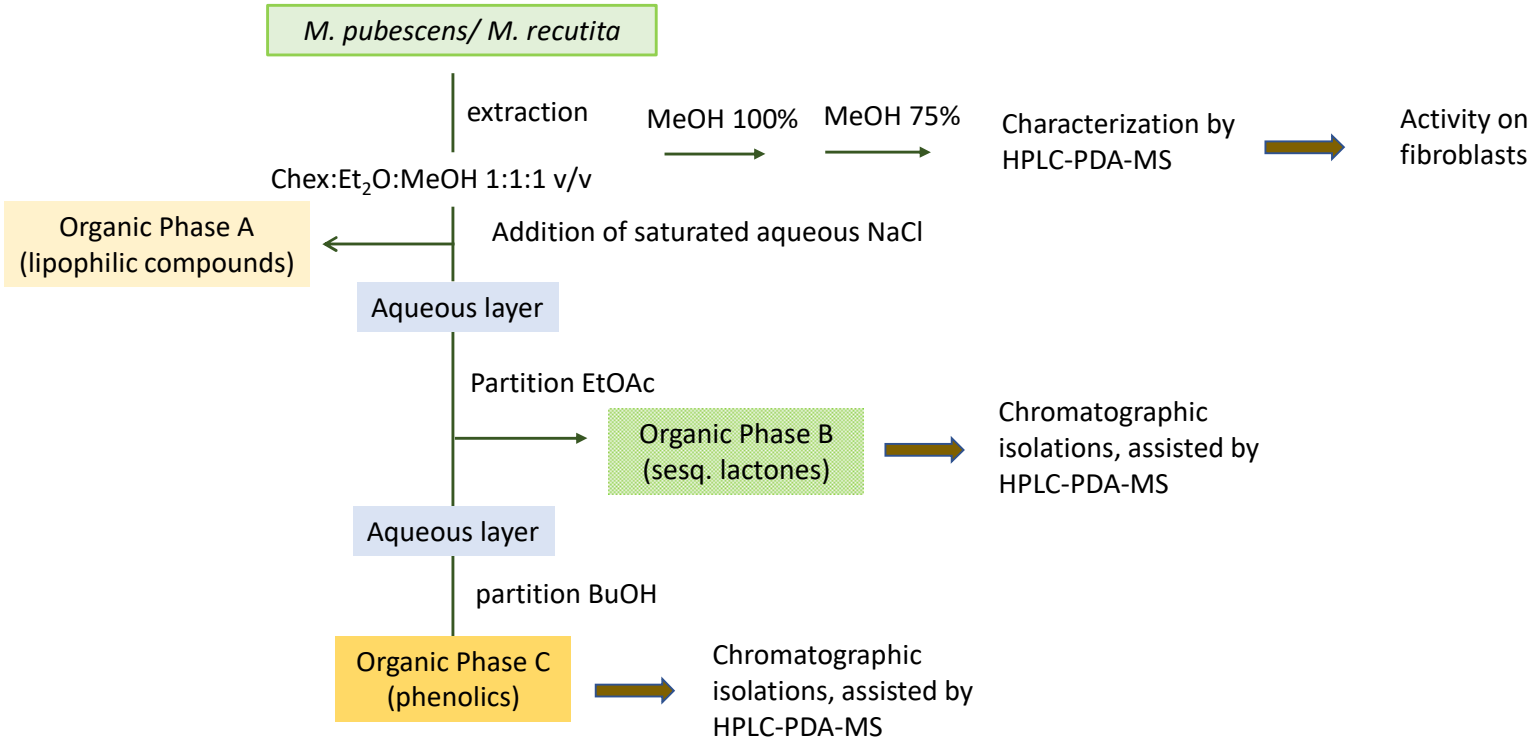


Figure S1. Scheme of the whole extraction protocol, analysis and biological assays. The extraction protocol with cyclohexane:diethylether:methanol 1:1:1 was described by Zdero and Bohlmann [Zdero, C.; Bohlmann, F. Sesquiterpene lactones and other terpenes from *Geigeria* species. *Phytochemistry* **1989**, *28*, 3105-3120.]

PART A: Spectra of fractions studied by a combination of NMR and HPLC-PDA-MS

Subfraction ALG-CQ

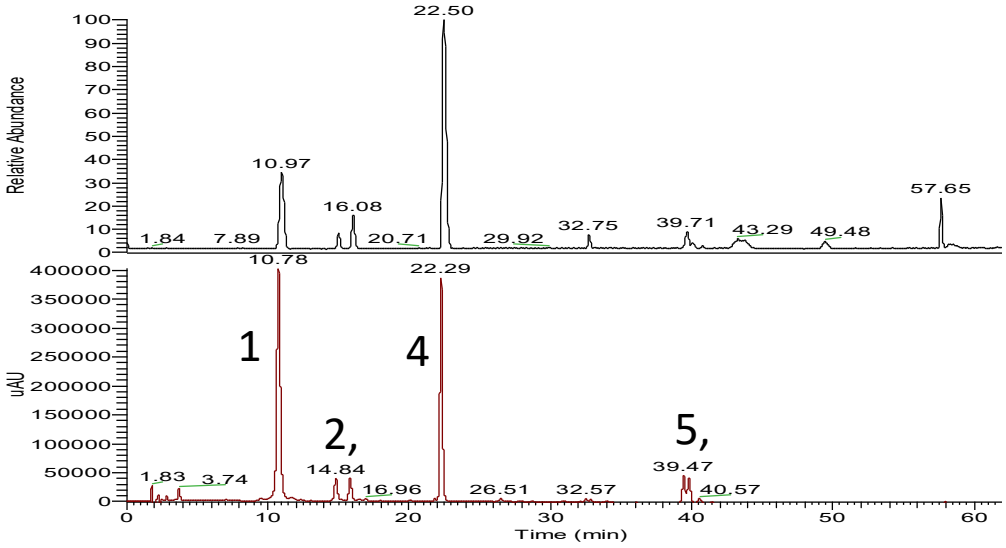


Figure S2. HPLC-PDA MS chromatogram of fraction ALG-CQ

HPLC-PDA-MS chromatogram of **subfraction ALG-CQ** exhibited two main peaks (**Fig. S2**), one of which belonged to the previously isolated luteolin-4′-O-glucoside (**13**). Furthermore, apigenin and hispidulin were detected in small amounts. Subtraction of the signals of **13** from ^{1}H-NMR, COSY and HSQC spectra, showed unambiguous signals of an ABX aromatic system belonging to a flavonol (259, 274sh, 356nm) bearing only one proton at δ 6.90 (δ_C 95.6). From the chemical shifts it is attributed to H-8 of the flavonol moiety, therefore the peak was attributed to a quercetagenin skeleton. HSQC-TOCSY spectra of the mixture confirmed the presence of a glucopyranose group which was placed on position 3 based on the HMBC (H-1′′/C-3) and UV data (356 nm). Therefore, the compound was identified as quercetagenin-3-O-glucopyarnoside and is detected for the first time in *Matricaria* spp. Its NMR data are also described here for the first time. Two minor peaks in the HPLC-PDA-MS chromatogram were identified as quercetin-7-O-glucoside (**6**) and 6-hydroxykaempferol-3-hexoside (**24**). Comparison of the retention time, UV and MS data of quercetin-7-O-glucoside and quercetin-3-O-glucoside, confirmed the identity of compound **6**, whereas compound **24** has been reported several times in plants of the Asteraceae family [1-2]. Finally, in the ^{1}H-NMR and COSY spectra a remaining AA'BB' system was evident at low fields at δ 8.1/132 ppm, indicating a flavonol unit. Indeed, in the HPLC-PDA-MS chromatogram a minor peak with maxima 274 and 346nm, suggested the

presence of a 3-O-substituted flavonol and especially a kaempferol moiety. From the fragment at *m/z* = 300.9 an oxygenated kaempferol derivative was suggested, while the UV absorption at 274nm indicated that the hydroxylation site was carbon 6. The placement of the sugar moiety on position 3 explains the hypsochromic shift at 346 nm, observable for kaempferol derivatives [3]. In the case of a quercetin derivative absorption maxima at ~354nm would have been observed [3].

References

[1] Bhave A.; Schulzová, V.; Mrnka, L.; Hajšlová, J. Influence of Harvest Date and Postharvest Treatment on Carotenoid and Flavonoid Composition in French Marigold Flowers. *Journal of Agricultural and Food Chemistry* **2020** *68,* 7880-7889.

[2] Gong, Y.; Liu, X.; He, WH.; Xu, HG.; Yuan, F.; Gao, YX. Investigation into the antioxidant activity and chemical composition of alcoholic extracts from defatted marigold (*Tagetes erecta* L.) residue. *Fitoterapia* 2012, 83, 481-489.

[3] Mabry, T.J.; Markham, K.R.; Thomas, M.B. The Ultraviolet Spectra of Flavones and Flavonols. In The Systematic Identification of Flavonoids, Mabry, T.J., Markham, K.R., Thomas, M.B., Eds.: Springer, Berlin, Heidelberg, 1970; pp.41-164.

Table T1. MS fragmentation and UV-vis absorption data of fraction ALG-CQ

	Rt (min)	UV	ESI-MS(-)	Identification
1	10.8	259, 274sh, 356	316.9 $[A-H]^-$, 478.9 $[M-H]^-$	quercetagenin-3-O-glucopyranoside
2	14.8	255, 370	300.9$[A-H]^-$, 462.8 $[M-H]^-$	quercetin-7-O-glucopyranoside
3	15.8	257sh, 274, 345	300.9 $[A-H]^-$, 462.9 $[M-H]^-$	6-hydroxykaempferol-3-hexoside
4	22.3	248sh, 268, 338	284.9 $[A-H]^-$, 447.1 $[M-H]^-$	luteolin -4'-O-glucoside
5	39.5	267, 336	268.9 $[M-H]^-$	apigenin
6	39.8	273, 334	283.9, 298.8 $[M-H]^-$	hispidulin

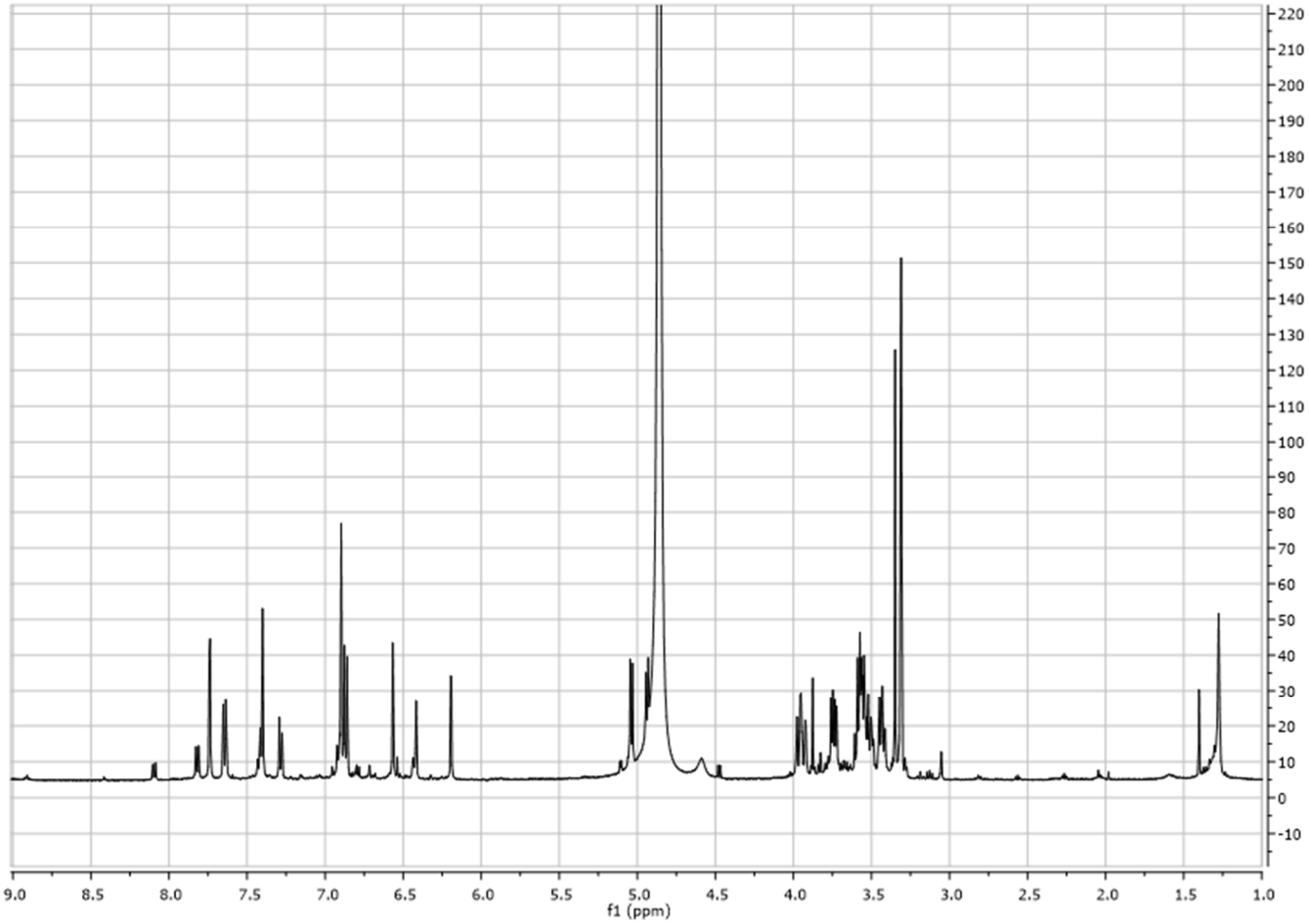


Figure S3. ^{1}H-NMR spectrum (CD_3OD, 500 MHz) of subfraction ALG-CQ, *Matricaria pubescens*: mixture of luteolin-4'-glucopyranoside and quercetagenin-3-O-glucopyranoside

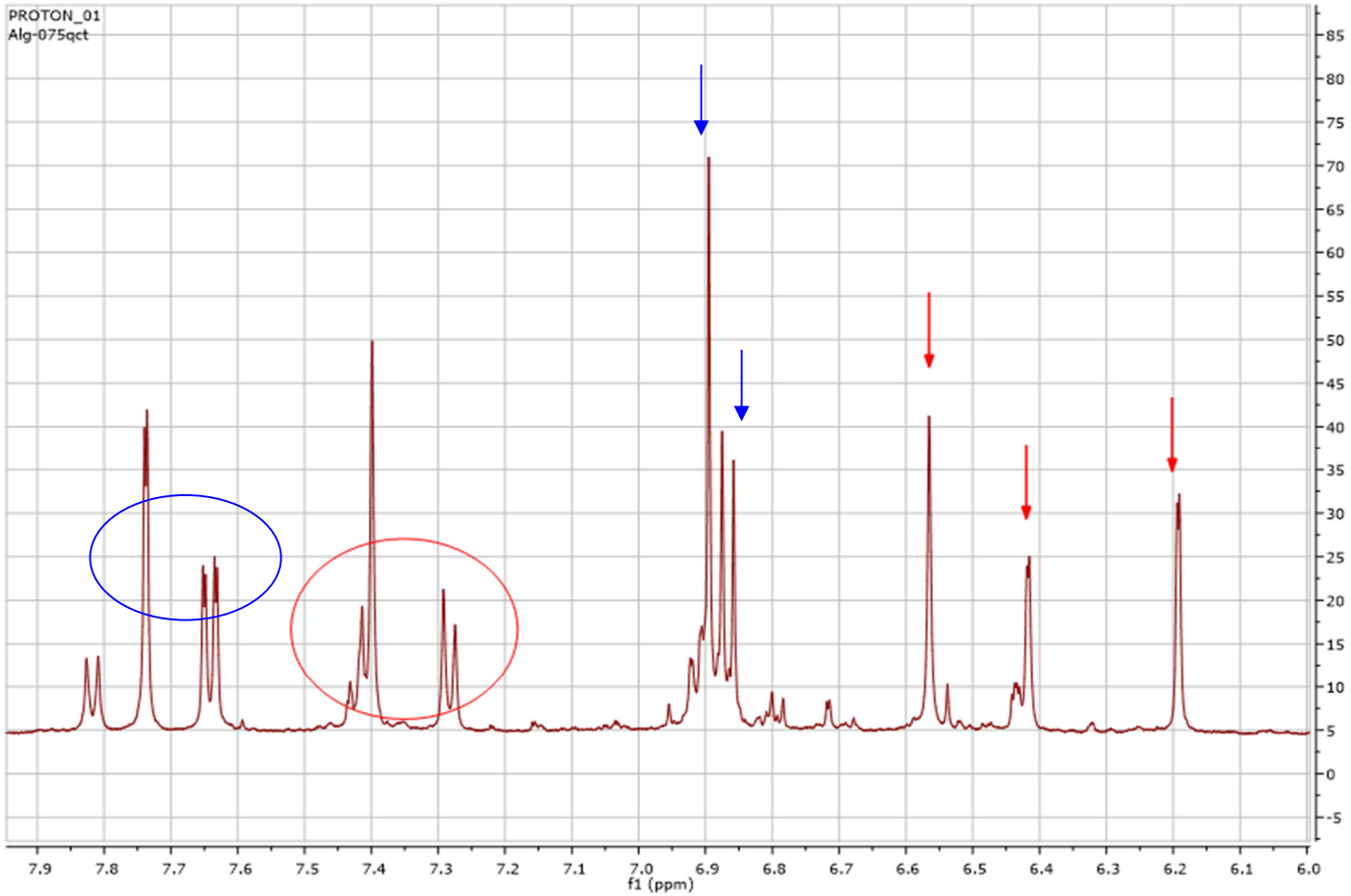


Figure S4. ^{1}H-NMR spectrum (CD_3OD, 500 MHz): in the aromatic area the characteristic signals of luteolin-4'-glucopyranoside (in red) and quercetagenin-3-O-glucopyranoside (in blue) can be observed

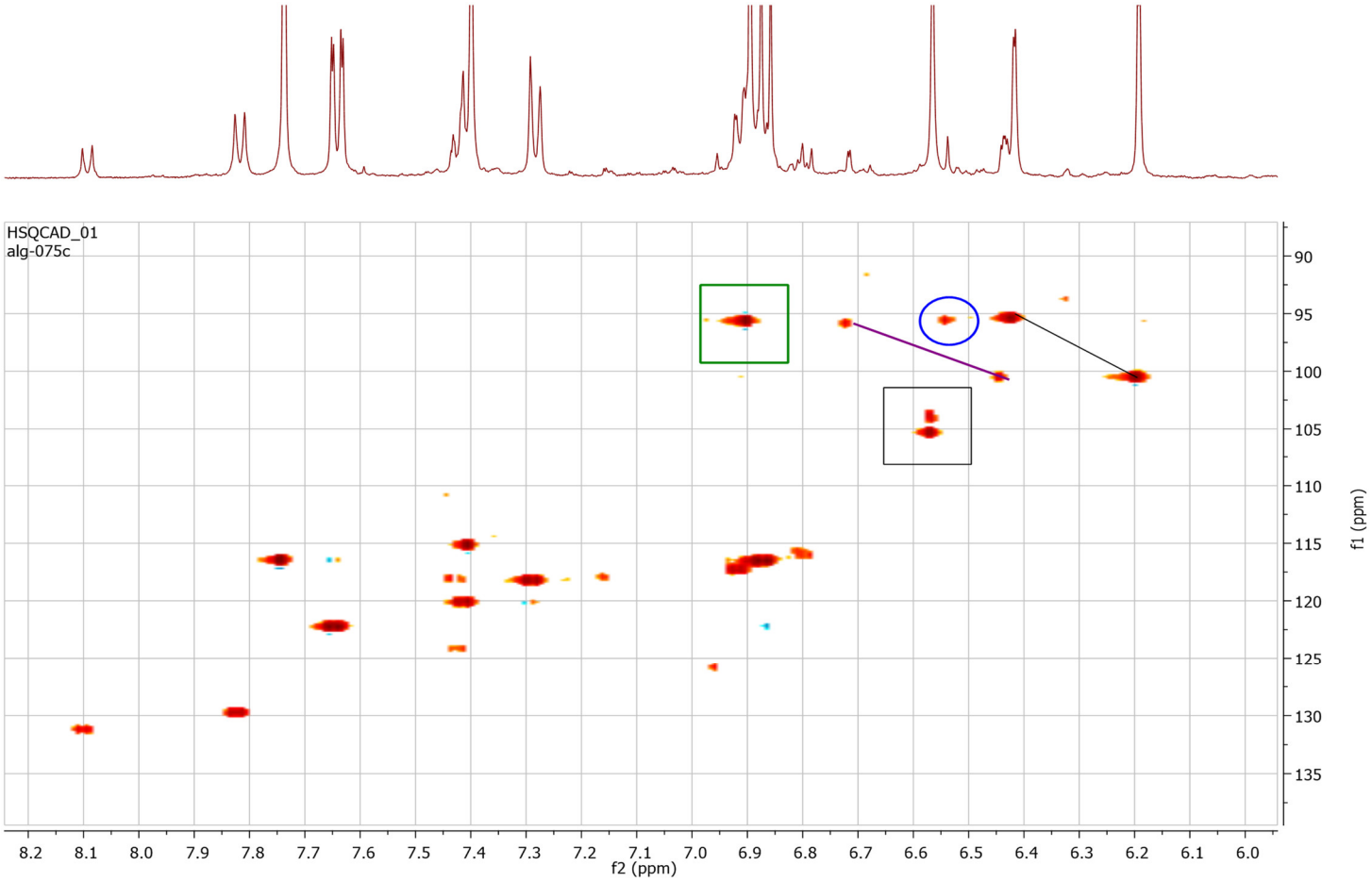


Figure S5. HSQC spectrum (CD_3OD, 500 MHz): in green color H8 of quercetagenin-3-O-glucopyranoside is indicated, in blue H8 of hispidulin, in black H3 luteolin-4'-glucopyranoside along with H3 of the aglycons (apigenin and hispidulin) and in violet H8 and H6 of quercetin-7-O-glucoside. The signals of deshielded protons at ~ 8.1/132 ppm are attributed to protons H-2''/H-6'' (AA'BB' system) of the 6-hydroxy-kaempferol-3-O-glucoside.

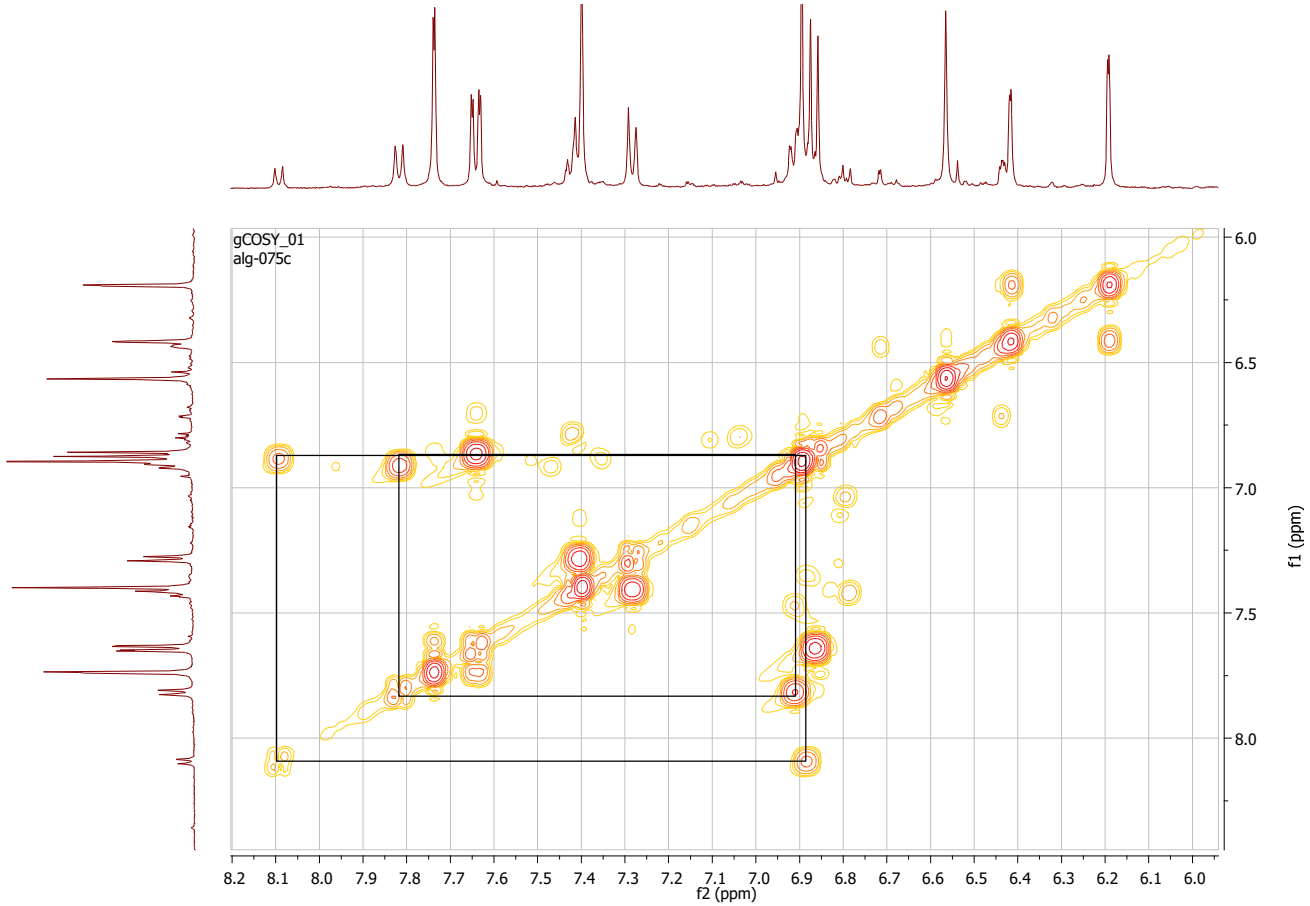
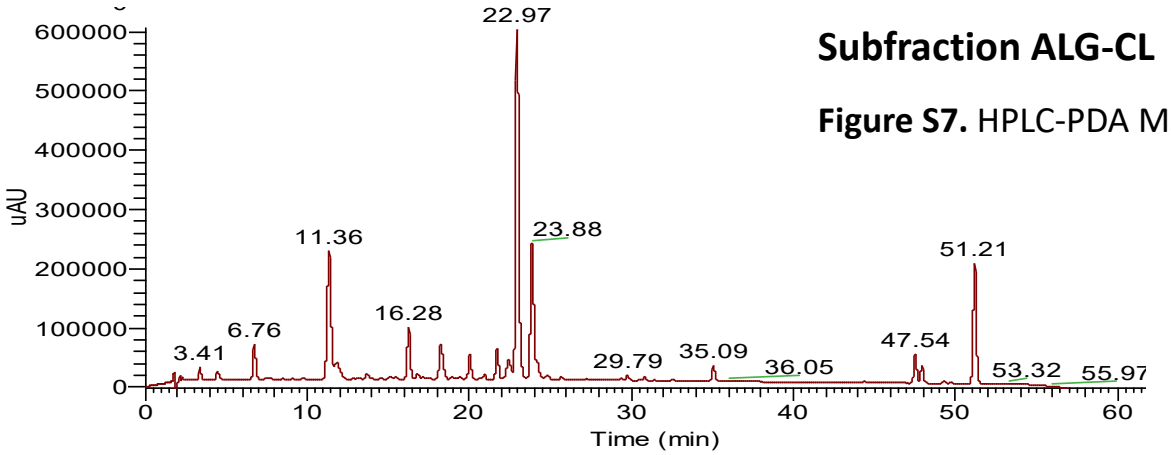


Figure S6. COSY spectrum of subfraction Alg-CQ (CD_3OD, 500 MHz): the two AA'BB' systems of apigenin and 6-hydroxykaempferol-3-O-glucoside are observed.



Subfraction ALG-CL

Figure S7. HPLC-PDA MS chromatogram of fraction ALG-CL

	Rt (min)	UV	ESI-MS(-)	Identification
1	**11.36**	**256sh, 269, 349**	**327.0 [M-120-H]$^-$, 357.1 [M-90-H]$^-$, 447.1 [M-H]$^-$**	**luteolin-6-C-glucopyranoside**
2	16.28	270, 337	283.0, 310.9 [M-120-H]$^-$, 340.9 [M-90-H]$^-$, 431.0 [M-H]$^-$	Apigenin-7-C-glucoside
3	18.2	282, 334	284.0 [A-2H]$^-$, 447.1 [M-H]$^-$	6-hydroxyapigenin-7-hexoside or scutellarein-7-O-hexoside (Ravn et al., 1990)
4	20.1	256, 271, 344	298.9 [A-CH_3-H]$^-$, 315.0 [A-H]$^-$, 477.0 [M-H]$^-$	6-methoxykaempferol-3-hexoside, tentatively
5	21.94	254, 266, 350	314.0 [A-2H]$^-$, 477.0 [M-H]$^-$	isorhamnetin-3-hexoside
6	**22.97**	**267, 333**	**268.9 [A-H]$^-$, 431.1 [M-H]$^-$**	**apigenin-7-O-glucoside**
7	23.88	267, 336	269.0[A-H]$^-$, 445.0 [M-H]$^-$	apigenin-7-O-glucuronide
8	35.09	267, 337	268.0 [A-2H]$^-$, 473.0 [M-H]$^-$	apigenin-7-O-acetylhexoside
9	51.21	298, 308	785 [M-H]$^-$	Acylated spermine derivative

Subfraction ALG-CL

Table T2. MS fragmentation and Uv-vis absorption data of fraction ALG-CL

ALG-CL was analyzed in a similar manner. Apart from apigenin-7-O-glucopyranoside which was easily confirmed by a reference standard, luteolin-6-C-glucopyranoside (isoorientin) (**3**) was identified by 2D NMR spectra. The anomeric carbon at δ_C 75.4 was diagnostic of a C-heteroside, while the remaining protonated carbon at δ_C 95.3 (C-8) suggested that 6 was the glycosylation site. An HSQC-TOCSY proved the beta glucose moiety.

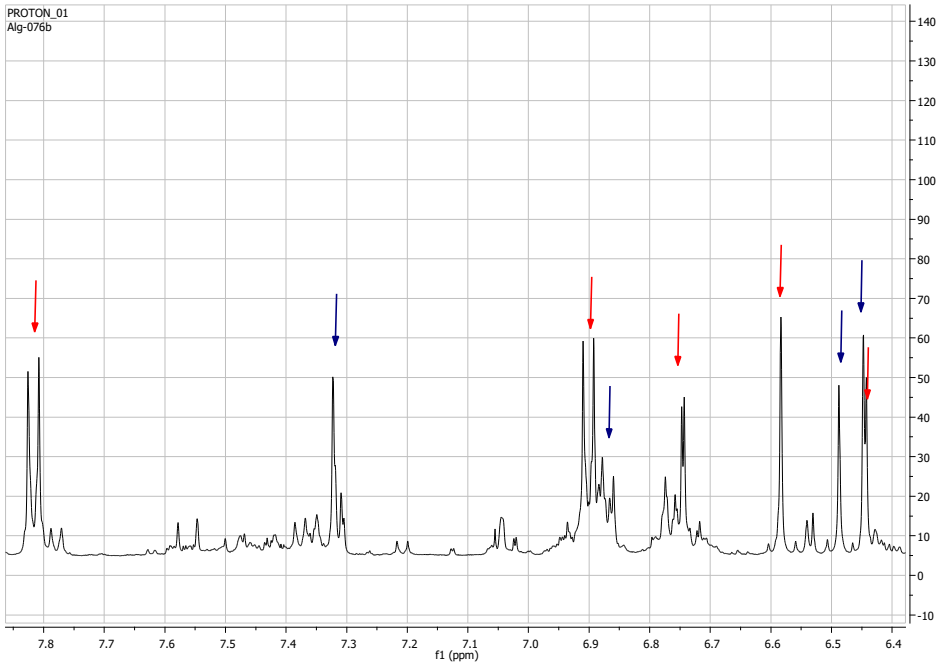


Figure S8. ^{1}H-NMR spectrum (CD_3OD, 500 MHz) of subfraction ALG-CL, *Matricaria pubescens*: mixture of apigenin-7-O-glucopyranoside in red color (AA'BB') and luteolin-6-C-glucopyranoside, in blue color (ABX system).

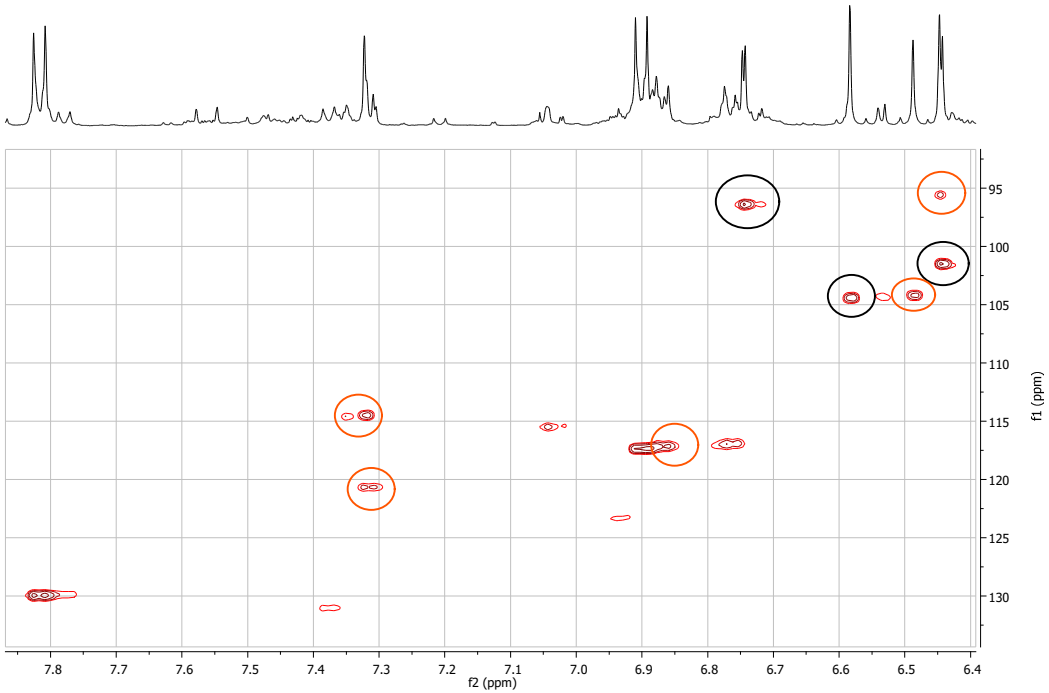


Figure S9. HSQC spectrum (CD_3OD, 500 MHz) of subfraction ALG-CL, *Matricaria pubescens,* containing apigenin-7-O-glucopyranoside (in black color) and luteolin-6-C-glucopyranoside (in red color).

Subfraction ALG-CP

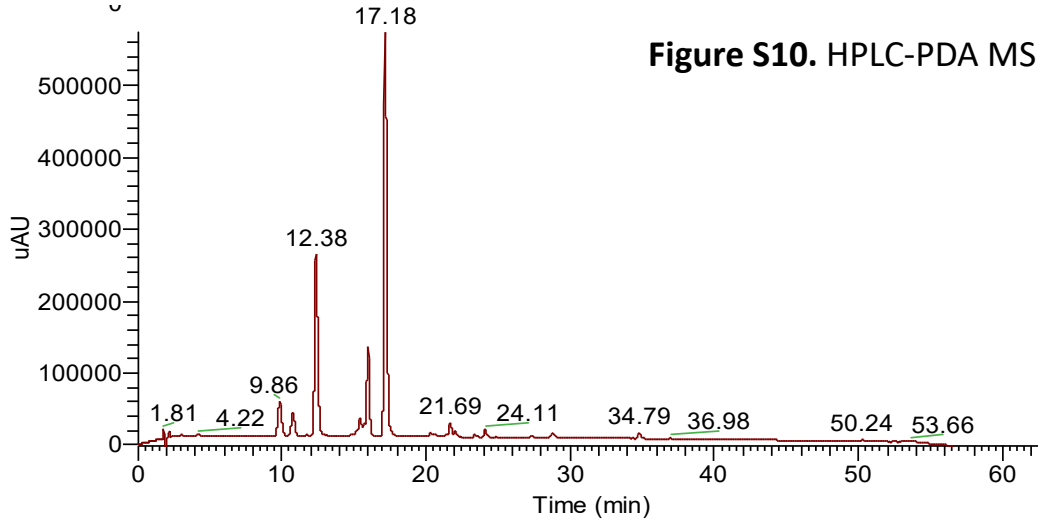


Figure S10. HPLC-PDA MS chromatogram of fraction ALG-CP

Table T3. MS fragmentation and UV-vis absorption data of fraction ALG-CP

	Rt (min)	UV	ESI-MS(-)	Identification
1	12.4	281, 344	300.9 $[A\text{-}H]^-$, 463.0 $[M\text{-}H]^-$	6-hydroxyluteolin-7-O-glucoside
2	17.2	254, 263sh, 348	284.9 $[A\text{-}H]^-$, 447.1$[M\text{-}H]^-$	luteolin-7-O-glucoside

Finally, ALG-CP contained luteolin-7-O-glucopyranoside (**10**), confirmed by both NMR and a reference standard, whereas the remaining signals in the NMR spectra were attributed to 6-hydroxyluteolin-7-O-glucopyranoside (**5**). Diagnostic signals for compound **5**, were the ABX system along with two single protons which resonated at δ 6.96 (δ_C at 95.7, C-8) and at δ 6.53 (δ_C at 103.8, C-3) while an extra oxygenated carbon at δ_C 132.1

was attributed to C-6. All UV, MS and retention time data were in accordance with this hypothesis. The presence of 6-hydroxyluteolin-7-O-glucopyranoside is considered characteristic of the European chamomile *M. recutita* [4].

[4] Greger, H. Laubblatt-Flavonoide und Systematik bei *Matricaria* und *Tripleurospermum (Asteraceae-Anthemideae)*. *Pl Syst Evol* **1975**, *124*, 35–55.

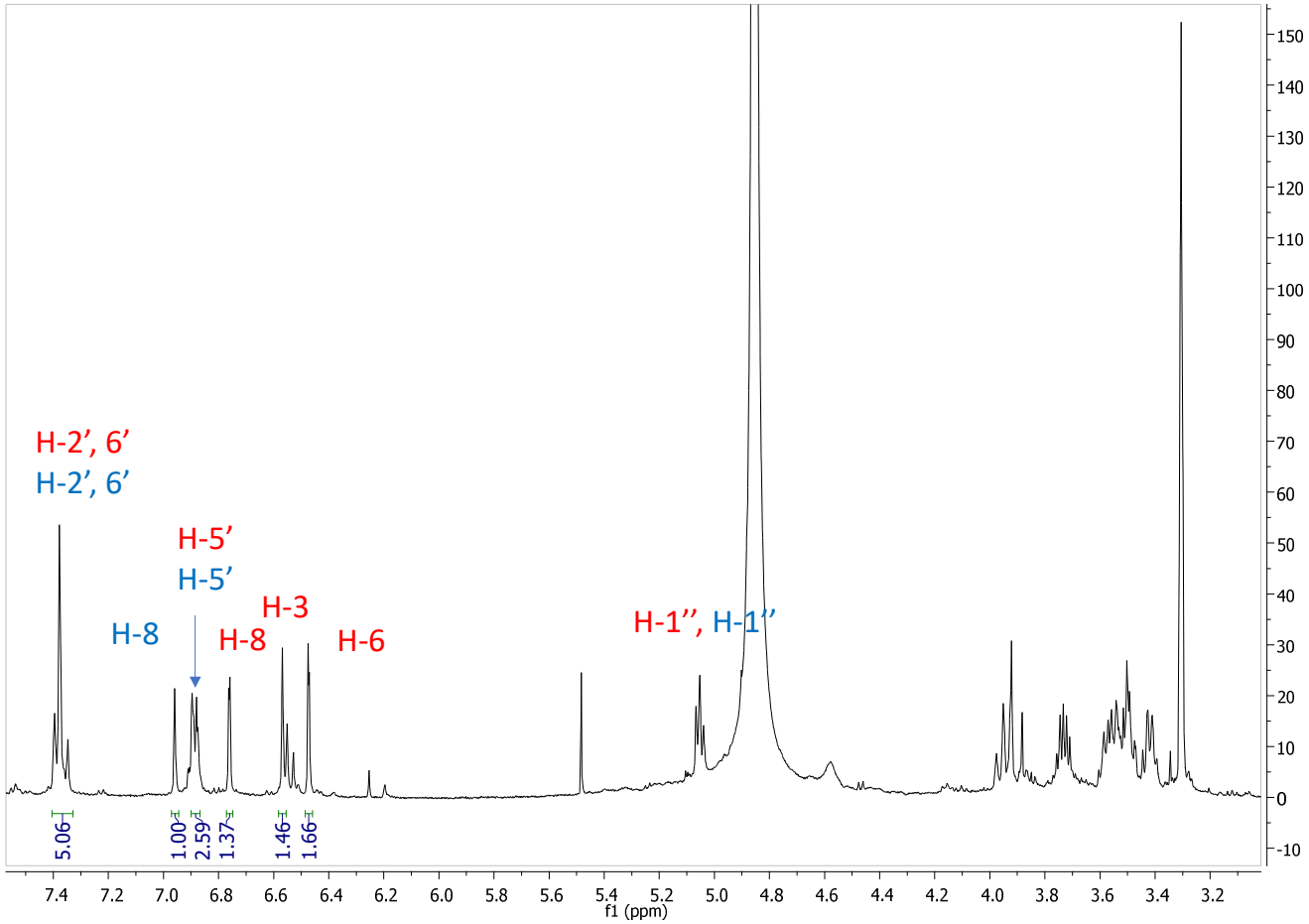


Figure S11. ^{1}H-NMR spectrum (CD_3OD, 500 MHz) of subfraction ALG-CP, *Matricaria pubescens*: mixture of luteolin-7-O-glucopyranoside (in red color and 6-hydroxyluteolin-7-O-glucopyranoside, in blue color.

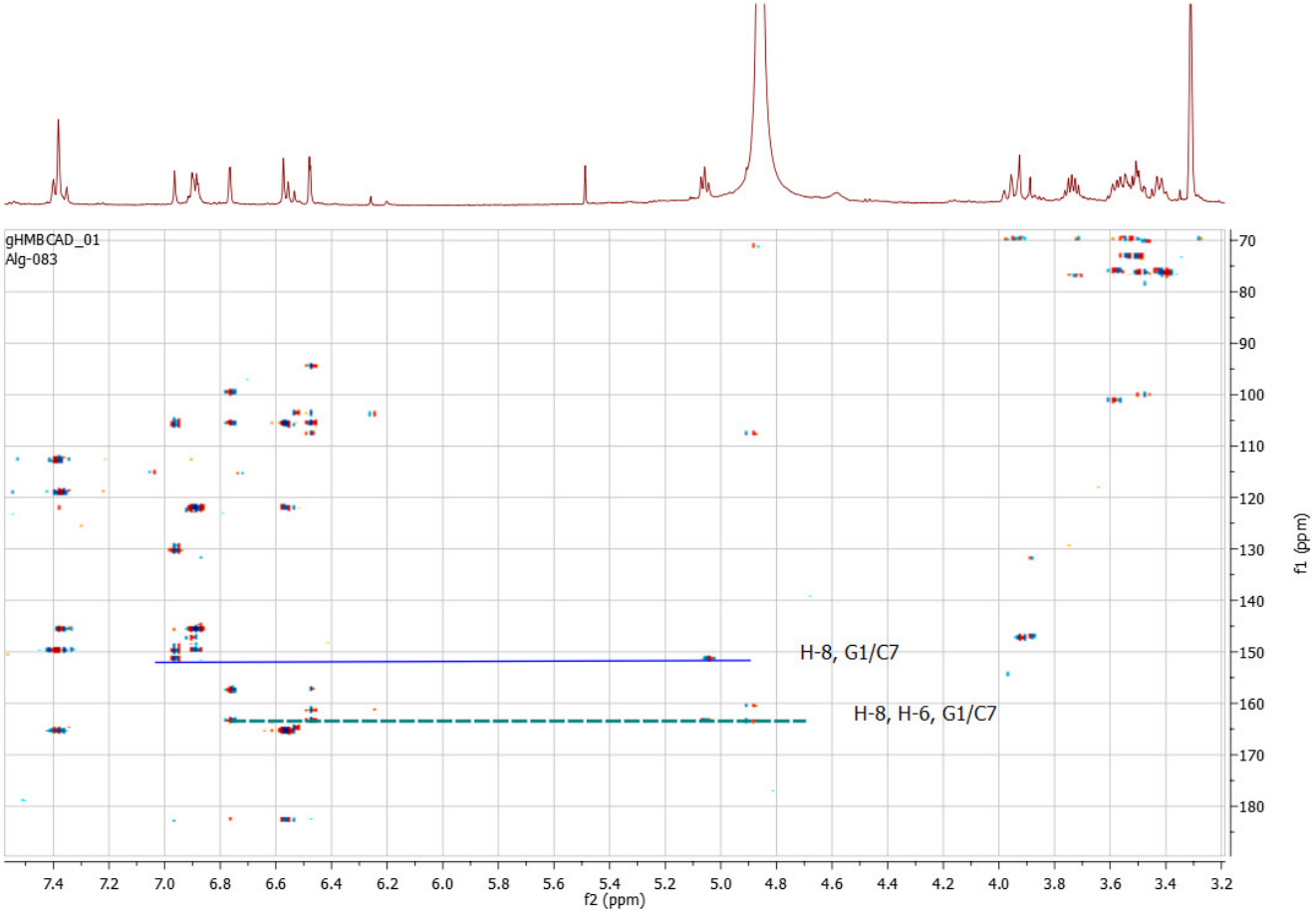


Figure S12. HMBC spectrum (CD_3OD, 500 MHz) of subfraction ALG-CP, *Matricaria pubescens*: crosspeaks of H-8, G1/C-7 of 6-hydroxyluteolin-7-O-glucopyranoside (in blue color) and crosspeaks between H-8, H-6, G1/C-7 of luteolin-7-O-glucopyranoside, in green color.

PART B: HPLC-PDA-MS Chromatograms and Tables of identification of extracts of *M. pubescens* and *M. recutita* by HPLC-PDA-MS

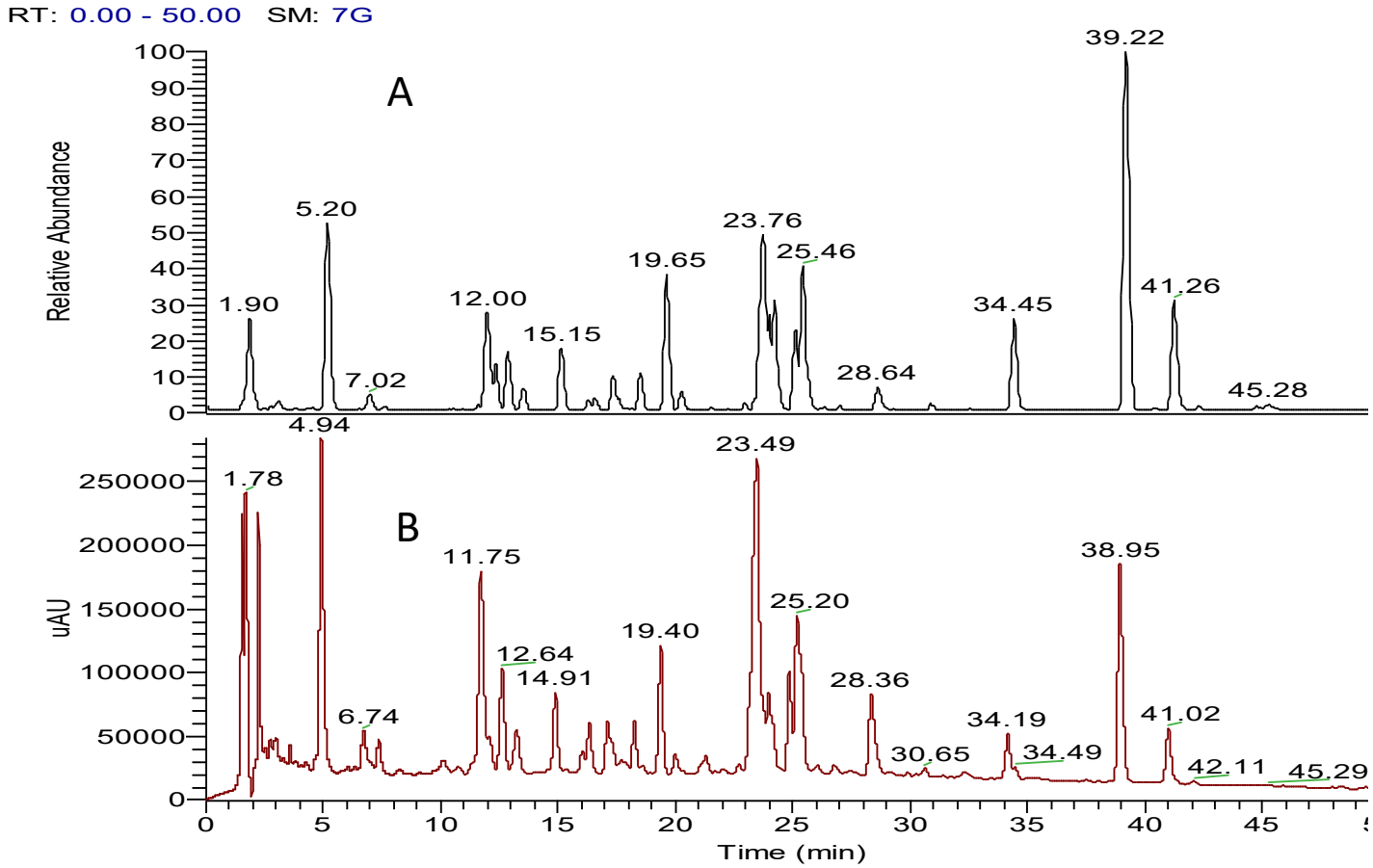


Figure S13. HPLC-PDA-MS chromatogram of the MeOH 50% extract of *Matricaria pubescens* flowers. A: MS chromatogram; B: UV chromatogram at 330 nm.

Table T4. MS fragmentation and UV-vis absorption data of the compounds detected in the MeOH:H_2O 50:50 extract of *Matricaria pubescens* flowers.

Rt	UV(nm)	MS-ESI(-)	Identification
4.94	297, 326	191 $[quinic\ acid\text{-}H]^-$, 353 $[M\text{-}H]^-$	Chlorogenic acid
11.75	259, 275, 356	317 $[A\text{-}H]^-$, 479 $[M\text{-}H]^-$	quercetagenin-3-O-glucoside
12.64	255, 269, 349	327 $[M\text{-}120\text{-}H]^-$, 357 $[M\text{-}90\text{-}H]^-$, 447 $[M\text{-}H]^-$	Isoorientin (luteolin-6C-glucopyranoside)
13.31	252, 269, 340	327 $[M\text{-}120\text{-}H]^-$, 357 $[M\text{-}90\text{-}H]^-$, 447 $[M\text{-}H]^-$	Orientin (luteolin-8C-glucopyranoside)
14.91	281, 344	301 $[A\text{-}H]^-$, 463 $[M\text{-}H]^-$	6-hydroxyluteolin-7-O-glucoside
17.11	278, 323	301 $[A\text{-}H]^-$, 463 $[M\text{-}H]^-$	6/8-hydroxyluteolin-4'-O-glucoside
17.59	269, 333	269 $[A\text{-}H]^-$, 311, 341, 431 $[M\text{-}H]^-$	isovitexin
18.26	259, 276sh, 352	331, 493 $[M\text{-}H]^-$	patuletin-3-O-glucoside
19.40	254, 266, 347	285 $[A\text{-}H]^-$, 447 $[M\text{-}H]^-$	luteolin-7-O-glucoside
23.49	245, 298, 328	179 $[caffeic\ acid\text{-}H]^-$, 191 $[quinic\ acid\text{-}H]^-$, 353 $[M\text{-}caffeoyl\ group\text{-}H]^-$, 515 $[M\text{-}H]^-$	3,5-O-dicaffeoylquinic acid
23.77	268, 334	285 $[A\text{-}H]^-$, 447 $[M\text{-}H]^-$	luteolin-4'-O-glucoside

23.98	267, 333	269 $[A-H]^-$, 431 $[M-H]^-$	apigenin-7-O-glucoside
24.87	267, 336	269 $[A-H]^-$, 445 $[M-H]^-$	apigenin-7-O-glucuronide
25.20	298, 327	161, 179, 381, 543 $[M-H]^-$	derivative of caffeic acid
34.19	266, 291, 346	285 $[M-H]^-$	luteolin
38.95	297, 326	543, 705 $[M-H]^-$	polyamin derivative -not identified
41.02	267, 336	269 [M-H]-	apigenin

A: aglycon

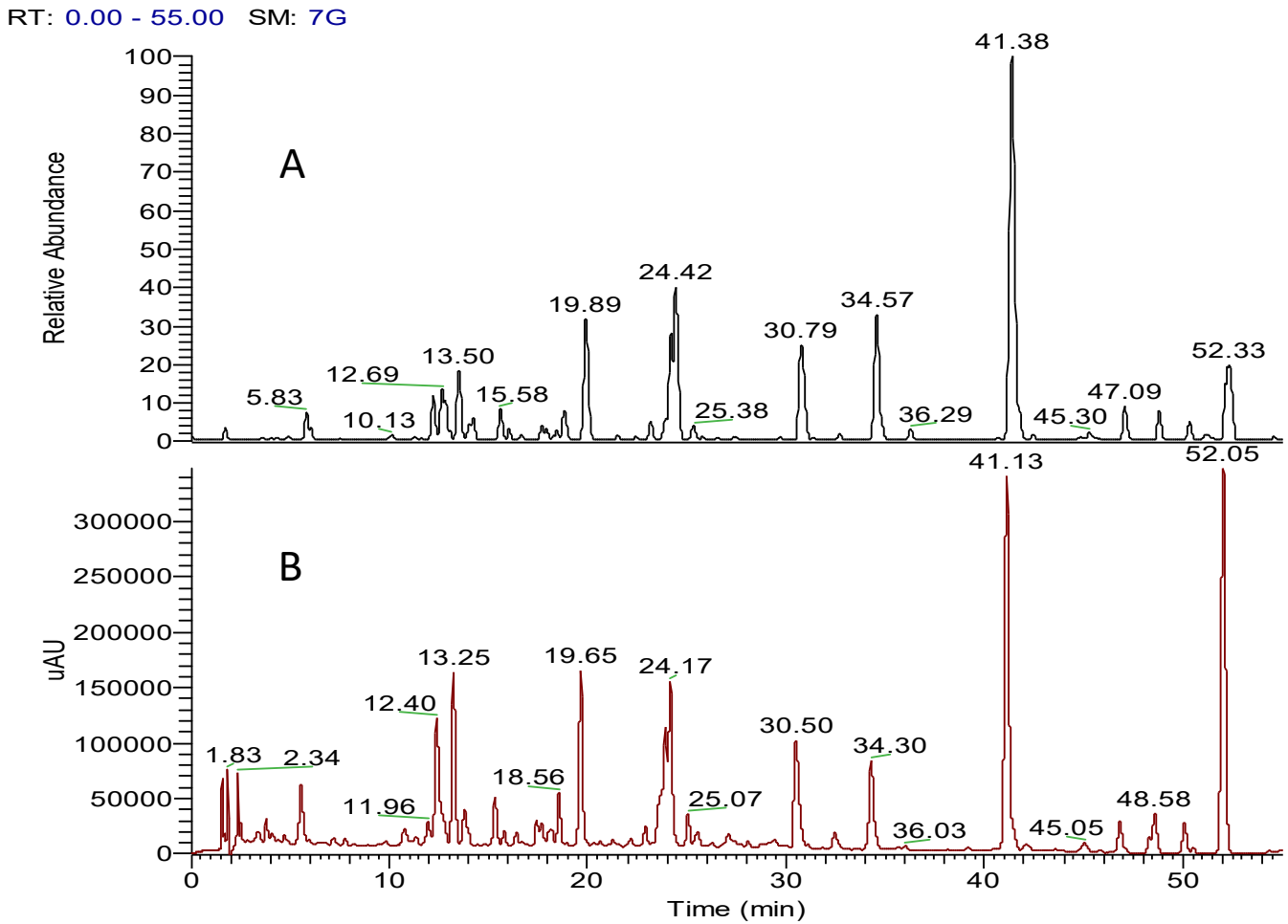


Figure S14. HPLC-PDA-MS chromatogram of the Butanol extract of *Matricaria pubescens* flowers. A: MS chromatogram; B: UV chromatogram at 330 nm.

Table T5. MS fragmentation and UV-vis absorption data of the compounds detected in the butanol extract of *Matricaria pubescens* flowers.

Rt	UV(nm)	MS-ESI(-)	Identification
5.60	296, 326	191 [quinic acid-H]$^-$, 353 [M-H]$^-$	Chlorogenic acid
11.96	248	447	Not identified
12.40	259, 274, 356	317 [A-H]$^-$, 447, 479 [M-H]$^-$	quercetagenin-3-O-glucoside
13.25	255, 269, 349	327 [M-120-H]$^-$, 357 [M-90-H]$^-$, 447 [M-H]$^-$	Isoorientin (luteolin-6C-glucopyranoside)
14.00	254,268, 344	327 [M-120-H]$^-$, 357 [M-90-H]$^-$, 447 [M-H]$^-$	Orientin (luteolin-8C-glucopyranoside)
15.34	281, 344	301 [A-H]$^-$, 463 [M-H]$^-$	6-hydroxyluteolin-7-O-glucoside
15.78	254, 306, 354	313.8 [A-H]$^-$, 638.9 [M-H]$^-$	isorhamnetin-3-O-dihexoside
18.56	259, 277, 356	331 [A-H]$^-$, 493 [M-H]$^-$	patuletin-3-O-glucoside
19.65	255, 267sh, 345	285 [A-H]$^-$, 447 [M-H]$^-$	luteolin-7-O-glucoside
22.96	253, 266, 348	299, 314, 477 [M-H]$^-$	isorhamnetin-3-O-hexoside
24.00	268, 338	285 [A-H]$^-$, 447 [M-H]$^-$	luteolin-4'-O-glucoside

24.17	266, 336	268 $[M-2H]^-$, 431 $[M-H]^-$	apigenin-7-O-glucoside
25.07	266, 341	269 $[A-H]^-$, 461 $[M-H]^-$	apigenin-7-O-glucuronide
30.50	298	519, 639	Tri-p-coumaroyl spermine derivative (cis and trans isomer)
34.30	253, 266, 348	285 $[M-H]^-$	luteolin
41.13	267, 291, 335	269 $[M-H]^-$	apigenin
46.84	275	545, 665, 785 $[M-H]^-$	N1(Z)-N5(Z)-N10(Z)-N14(Z)-tetra-p-coumaroyl spermine/thermospermine (cis-isomers)
48.58	290	545, 665, 785 $[M-H]^-$	N1(Z)-N5(Z)-N9(Z)-N14(Z)- tetra-p-coumaroyl spermine/thermospermine (cis and trans-isomers)
50.10	292, 308	785 $[M-H]^-$	N1(E)-N5(E)-N10(E)-N14(E)- tetra-p-coumaroyl spermine (trans-isomer)
52.05	297, 308	665, 785 $[M-H]^-$	N1(E)-N5(E)-N9(E)-N14(E)- tetra-p-coumaroyl thermospermine (trans-isomer)

A: aglycon

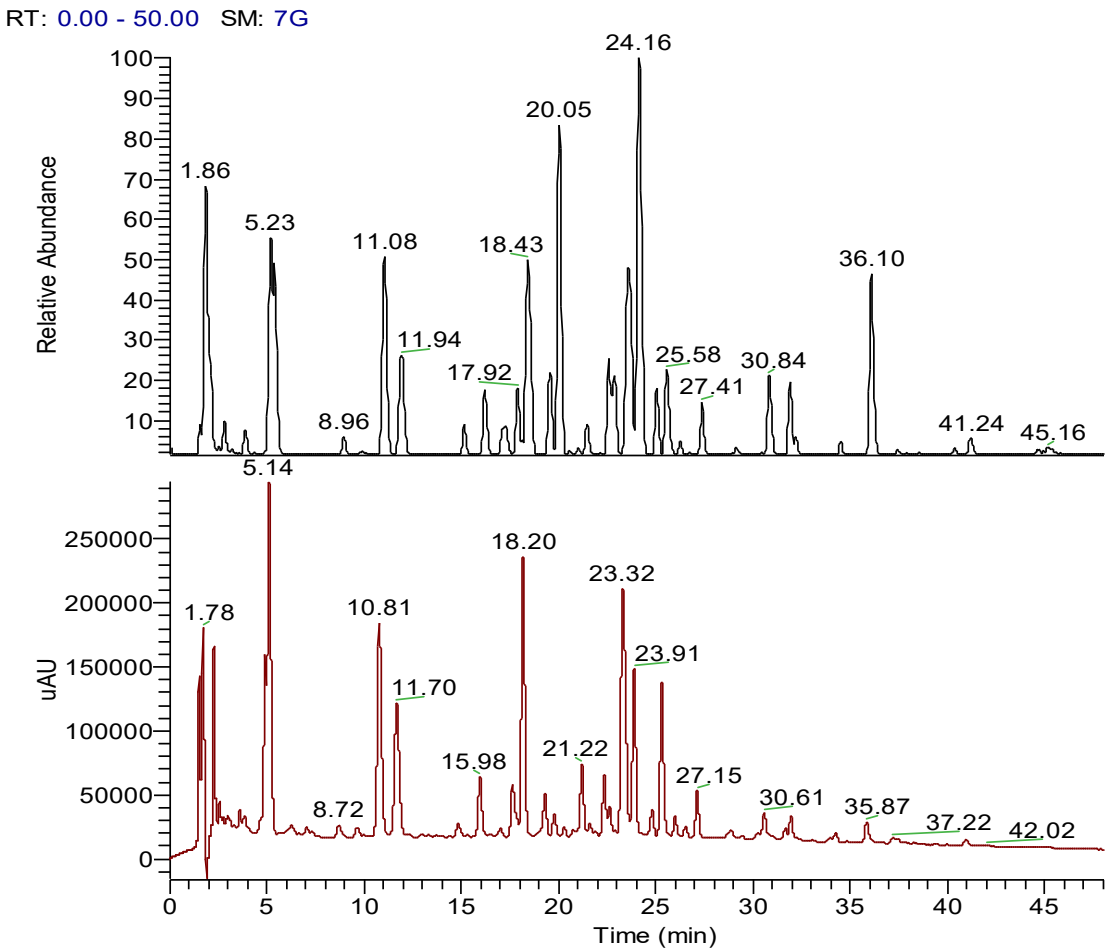


Figure S15. HPLC-PDA-MS chromatogram of the MeOH50% extract of *Matricaria recutita* flowers. A: MS chromatogram; B: UV chromatogram at 330 nm.

Table T6. MS fragmentation and UV-vis absorption data of the compounds detected in the MeOH:H_2O extract of *Matricaria recutita* flowers.

Rt	UV	MS-ESI(-)	Identification
4.94	297, 326	191 [quinic acid-H]$^-$, 353 [M-H]$^-$	Chlorogenic acid
5.14	281, 301	134, 149, 193, 355 [M-H]$^-$, 711 [2M-H]$^-$	cis-2-hydroxy-4-methoxycinnamic-oxo-2-O-β-D-glucopyranoside
10.81	295, 318	135, 149, 193, 355 [M-H]$^-$, 711 [2M-H]$^-$	trans-2-hydroxy-4-methoxycinnamic-oxo-2-O-β-D-glucopyranoside
11.70	258, 273, 345	317 [A-H]$^-$, 479 [M-H]$^-$	quercetagenin-3-O-glucoside
15.98	255, 370	301 [A-H]$^-$, 463 [M-H]$^-$	quercetin-7-O-hexoside
17.68	258, 369	331 [A-H]$^-$, 493 [M-H]$^-$	patuletin-7-O-glucoside
18.20	259, 356, sh382	331 [A-H]$^-$, 493 [M-H]$^-$	patuletin-3-O-glucoside
19.35	256, 267, 346	285 [A-H]$^-$, 447 [M-H]$^-$	luteolin-7-O-glucoside
19.80	-	711	Not identified
21.22	299, 325	173, 179 [caffeic acid-H]$^-$, 191 [quinic acid-H]$^-$, 335, 353 [M-caffeoyl group-H]$^-$, 515 [M-H]$^-$	3,4-O-dicaffeoylquinic acid
22.61	255, 370	315 [A-H]$^-$, 477 [M-H]$^-$	isorhamnetin-7-O-hexoside
22.87	254, 348	315 [A-H]$^-$, 477 [M-H]$^-$	isorhamnetin-3-O-hexoside
23.32	245, 300, 327	179 [caffeic acid-H]$^-$, 191 [quinic acid-H]$^-$, 353 [M-caffeoyl-H]$^-$, 515 [M-H]$^-$	3,5-O-dicaffeoylquinic acid

23.91	267, 336	269 $[A-H]^-$, 431 $[M-H]^-$	apigenin-7-O-glucoside
24.81	253, 268, 345	461 $[M-H]^-$	chrysoeriol-7-O-glucoside
25.31	245, 300, 327	173, 179 [caffeic acid-H]$^-$, 191 [quinic acid-H]$^-$, 353 [M-caffeoyl-H]$^-$, 515 $[M-H]^-$	4,5-O-dicaffeoylquinic acid
27.15	296, 321	149, 161, 193, 323, 517, 539 $[M-H]^-$	Dicaffeoylquinic acid derivative
30.61	267, 329	269 $[A-H]^-$, 473 $[M-H]^-$	apigenin-4′-acetyl-hexoside (tentatively identified)
31.70	267, 336	269 $[A-H]^-$, 473 $[M-H]^-$	apigenin-7-acetyl-hexoside
35.87	267, 335	269 $[A-H]^-$, 473 $[M-H]^-$	apigenin-7-acetyl hexoside isomer

A: aglycon

In vitro protective activity on BALbC 3T3 mouse skin fibroblasts

The culture medium was consisted of Fetal Bovine Serum (10%) and antibiotic-antimycotic (1%) and DMEM with 4mM L-glutamine. The incorporation of chamomile extracts was performed in all phases of the experiment, both in the culture medium (DMEM) and in the irradiation medium or buffer solution (PBS). Six concentrations of the chamomile extracts were tested, covering a large range, e.g. 0.1 μg/mL, 1μg/mL, 10μg/mL, 50μg/mL, 100μg/mL, 200μg/mL. Irradiation Conditions: Doses of 6 J/cm^2 and 7 J/cm^2 (as measured in the UVA range) were determined to be cytotoxic to Balb/c 3T3 cells and sufficiently potent to reveal anti-inflammatory and cytoprotective properties of the extracts. The irradiance was adjusted to achieve 6 J/cm^2 within a time period of 60min and to achieve 7 J/cm^2 within a time period of 70min.

Test Procedure

1st day

A cell suspension (BALbC 3T3 mouse skin fibroblasts, ATCC) was prepared in culture medium. Using a multichannel pipette, an appropriate volume of cell suspension was dispensed into the appropriate number of wells in two 96 well plate. The number of cells per well was equal to 10^4. The cells are incubated for 24 h (5% CO_2, 37 °C, > 90% humidity) so that cells form a half-confluent monolayer. This incubation period ensured cell recovery, adherence and progression to exponential growth phase. The wells were examined under a phase contrast microscope to ensure the condition of exponential cell growth into all plate wells.

2nd day

After 24 h incubation, the culture medium was aspirated from the cells. 100 μL of treatment medium (DMEM, 5% FBS) was added per well. The six concentrations of each extract (*Matricaria pubescens* methanolic and hydromethanolic extracts & *Matricaria recutita* methanolic and hydromethanolic extracts) or control were added in three different wells for each concentration (3 replicates). Cells were again incubated for 24h (5 % CO_2, 37 °C, > 90 % humidity).

3rd day

After 24 h incubation, the culture medium was aspirated from the cells. The six concentrations of each extract (*Matricaria pubescens* methanolic and hydromethanolic extracts & *Matricaria recutita* methanolic and hydromethanolic extracts) were prepared by diluting the stock solution into phosphate buffered salt solutions (pH= 7.4), free from protein components and light absorbing components (e.g., pH-indicator colors and vitamins) to avoid interference during irradiation. 100

µL of the buffer containing the appropriate concentration of the extracts were added per well, as well as the solvent (blank). Cells were incubated in the dark for 60 minutes. To perform the +UV exposure, the cells were irradiated at room temperature for approximately 60 minutes through the lid of the 96-well plate to achieve the selected radiation doses. The test solution was decanted, and the cells carefully washed twice with 150 µL of the buffered solution used for incubation, but not containing the test material. The buffer was replaced with culture medium and incubated overnight (18-22 h). The Neutral Red-(NR) solution was prepared and incubated at 37^{o}C overnight.

4th day

After 24 h treatment, plates were examined under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Changes in morphology of the cells due to cytotoxic effects of the test extracts were recorded. The cells were washed with 150 µL PBS. The washing solution was then removed and 100 µL of ~~and~~ NR solution, after being centrifuged at 600g for 10 min (to remove NR crystals), was ~~ere~~ added. Cells were incubated at 37 °C in a humidified atmosphere of 5 % CO2 for 3h. After incubation, the NR solution was removed, and cells were washed with 150 µL PBS. 150 µL NR desorb solution was added to all wells, including blanks. The microtitre plate was shaken at 300rpm on a minishaker for 10 min until NR was extracted from the cells and formed a homogeneous solution. The absorption of this solution was measured at 540 nm.