Historical textiles dyeing with *Genista tinctoria* L.:

A comprehensive study by UPLC-MS/MS analysis

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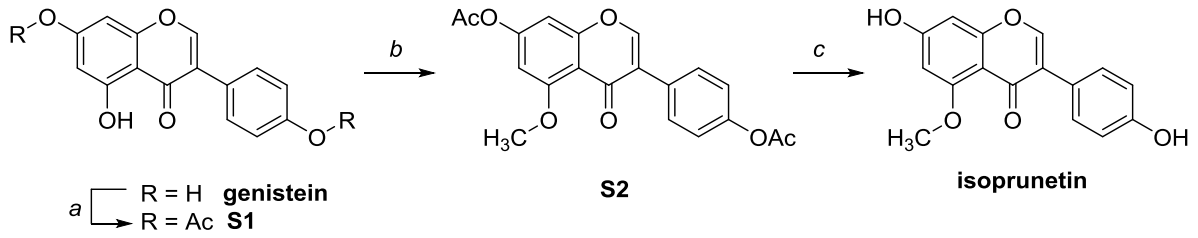
SUPPORTING INFORMATION FILE

SI1: Preparation of an authentic sample of isoprunetin



Scheme S1: Reagents and Conditions: (a) Ac_2O, py, rt, 48 h; (b) $(MeO)_2SO_2$, K_2CO_3, acetone, reflux, 5 h; (c) $NaHCO_3$ aq., MeOH:THF, 60°C, 3 h then rt, 40 h.

7,4'-diacetoxy-5-hydroxyisoflavone (**S1**) was prepared as according to the method of Al-Maharik and Botting[1] from **genistein** (100 mg) to give **S1** (113 mg, 86% yield) as a colourless solid which was used without further purification. **MP** = 197-198°C, **Lit.**[1] **MP** = 200-203°C; δ_H (500 MHz, $CDCl_3$) 12.76 (1H, s, 5-OH), 8.00 (1H, s, H-2), 7.58 (2H, d, *J* = 8.7 Hz, H-2' & H-6'), 7.22 (2H, d, *J* = 8.7 Hz, H-3' & H-5'), 6.80 (1H, ddd, *J* = 2.1 Hz, H-6), 6.63 (1H, dd, *J* = 2.1 Hz, H-8), 2.36 (3H, s, OAc), 2.35 (3H, s, OAc). ^{1}H NMR spectroscopic data in good agreement with the literature.[1]

Isoprunetin was prepared through adaptation of the methods of Compton, Larsen and Weavers,[2] and Al-Maharik and Botting.[1] Thus **S1** (100 mg, 0.282 mmol) and K_2CO_3 (262 mg, 1.89 mmol) were dissolved in anhydrous acetone (30 mL) to which dimethylsulfate (0.80 mL, 8.48 mmol) was added under nitrogen. The reaction mixture was heated under reflux with stirring for 5 h and then cooled to room temperature. Solids were removed by filtration and the solvent removed *in vacuo* to give a pale yellow oil (58.9 mg) which was revealed by ^{1}H NMR to be an ~5:1 mixture of **7,4'-diacetoxy-5-methoxyisoflavone** (**S2**) and unreacted **S1**. δ_H (500 MHz, $CDCl_3$) 7.87 (1H, s, H-2), 7.59 (2H, d, *J* = 8.8 Hz, H-2' & H-6'), 7.16 (2H, d, *J* = 8.8 Hz, H-3' & H-5'), 6.89 (1H, d, *J* = 2.1 Hz, H-6), 6.62 (1H, d, *J* = 2.1 Hz, H-8), 3.99 (3H, s, OCH_3), 2.38 (3H, s, OAc), 2.34 (3H, s, OAc). The crude mixture (58.9 mg) was suspended in MeOH:THF (1.5 mL; 1:1) and $NaHCO_3$ was added (3 mL; 10% aq.). The reaction mixture was heated with stirring at 60°C for 3 h, then cooled to room temperature and stirred for a further 40 h. The volume of the reaction mixture was reduced by half *in vacuo*, and then the solution was cautiously quenched with HCl (2.0 mL; 2% aq.) to near neutral pH, extracted with ethyl acetate (3 x 10 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo* to give a colourless solid (43.1 mg) which was revealed by ^{1}H NMR to be a ~60:40 mixture of **isoprunetin** and **genistein**. These could be separated by flash chromatography (Hexane:EtOAc, 6:4; then Hexane:EtOAc:MeOH, 6:4:1)[3] to yield a sample of **isoprunetin** (9.5 mg) as a colourless solid which was shown to be of >95% purity by UPLC. $\mathbf{R_f}$ (Hexane:EtOAc:MeOH, 6:4:1) = 0.22; δ_H (500 MHz, acetone-d_6) 7.94 (1H, s, H-2), 7.42 (2H, d, *J* = 8.7 Hz, H-2' & H-6'), 6.88 (2H, d, *J* = 8.7 Hz, H-3' & H-5'), 6.47 (2H, s, H-6 & H-8), 3.87 (3H, s, OCH_3); δ_C (125 MHz, acetone-d_6) 173.7 (C), 162.1 (C), 162.0 (C), 149.8 (CH), 130.4 (2CH), 125.5 (C), 123.9 (C), 114.7 (2CH), 96.3 (CH), 94.9 (CH), 55.5 (CH_3) - C8a carbon not seen. ^{1}H NMR spectroscopic data in good agreement with the literature.[3]

[1]: N. Al-Maharik, N. P. Botting, *Tetrahedron*, **2003**, *59*, 4177-4181.
[2]: B. J. Compton, L. Larsen, R. T. Weavers, *Tetrahedron*, **2011**, *67*, 718-726.
[3]: S. A. Adensanya, M. J. O'Neill, M. F. Roberts, *Phytochemistry*, **1985**, *24*, 2699-2702.

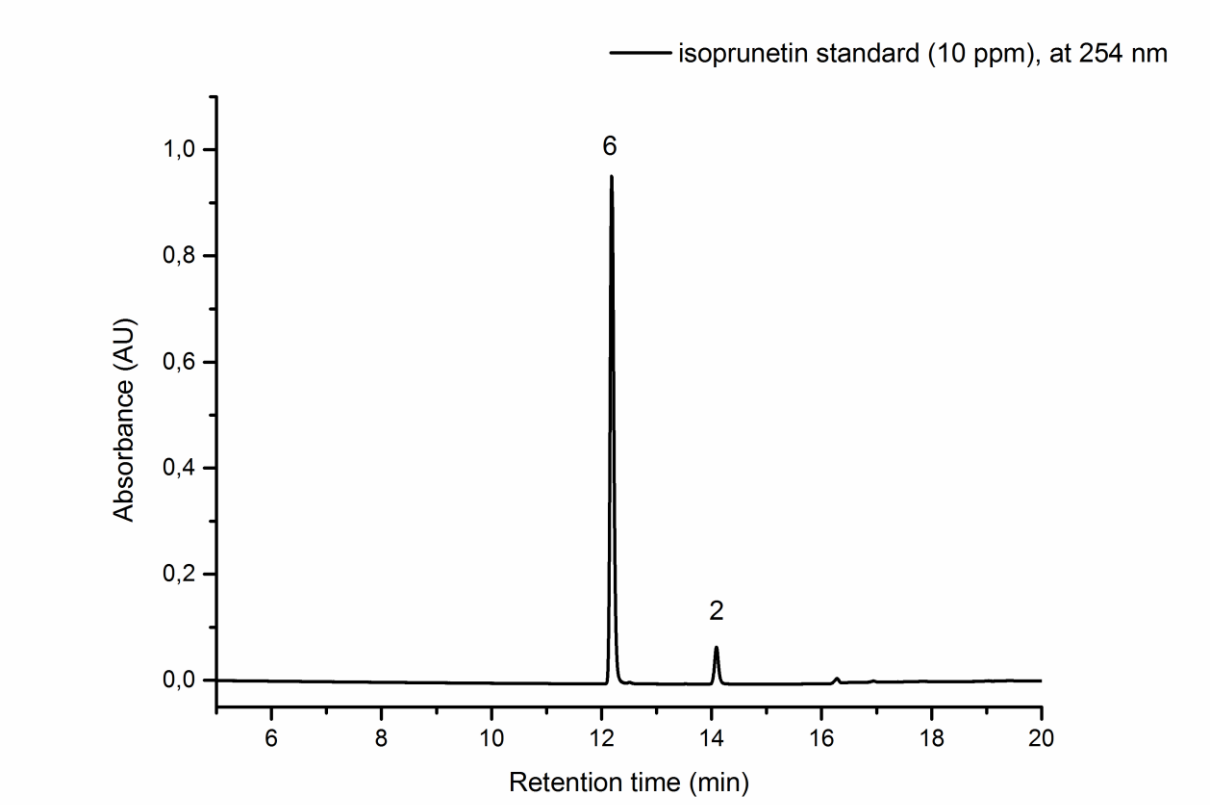
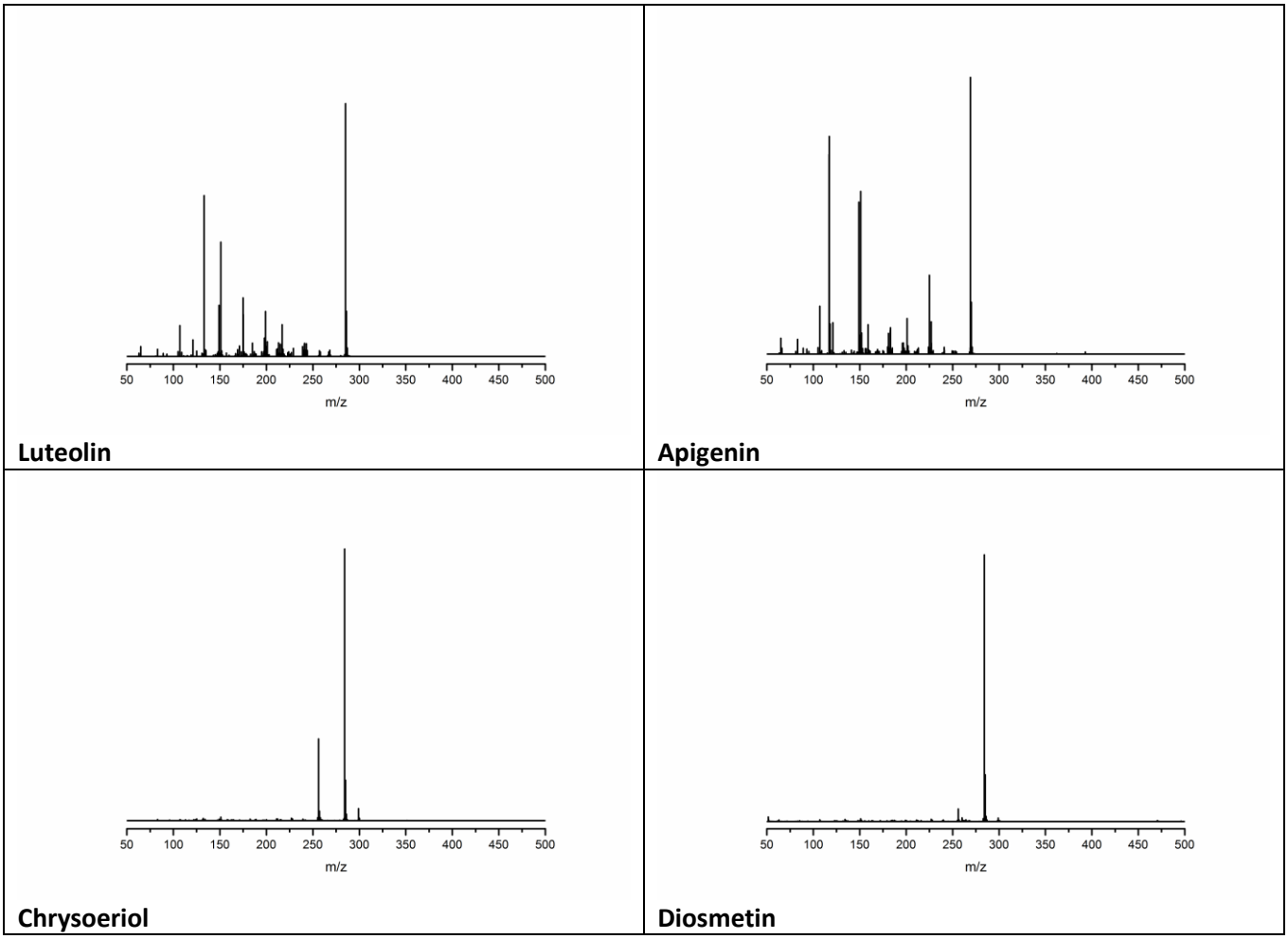


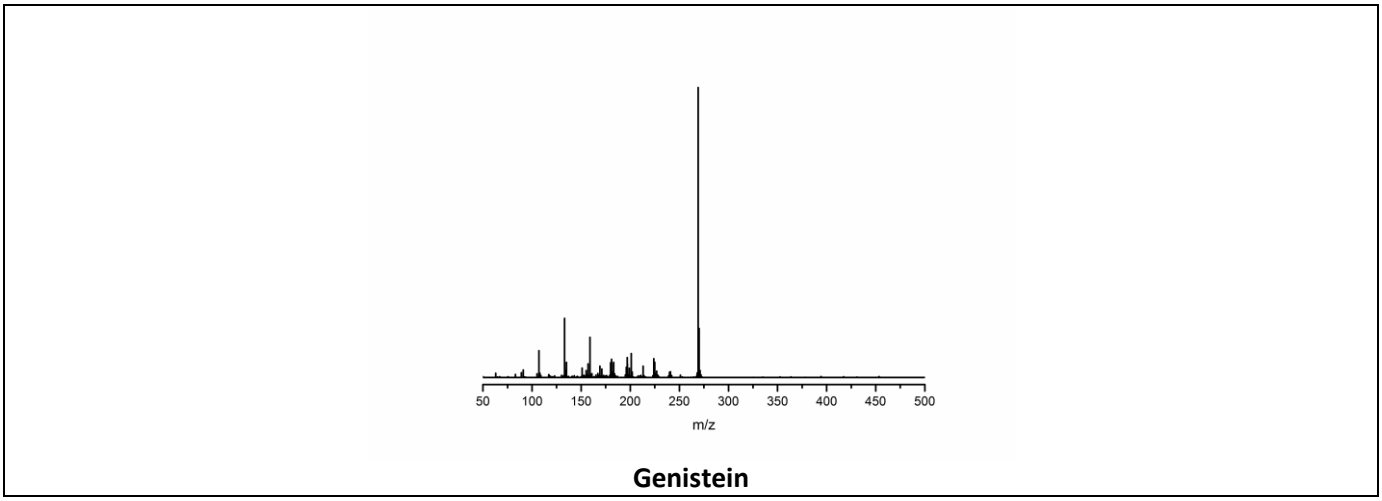
Fig. S1: UPLC chromatogram of isoprunetin (**6**), showing residual genistein (**2**) starting material, monitored at 254 nm.

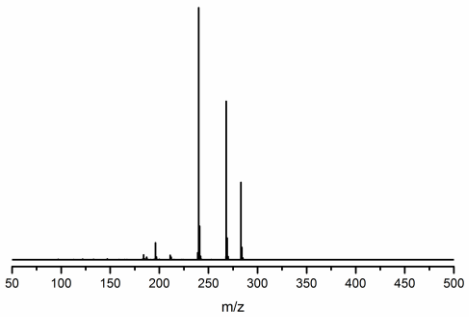
SI 2: (ESI$^-$) MS^2 fragmentation of flavonoid and isoflavonoid standards at CE: 25 eV

Flavones

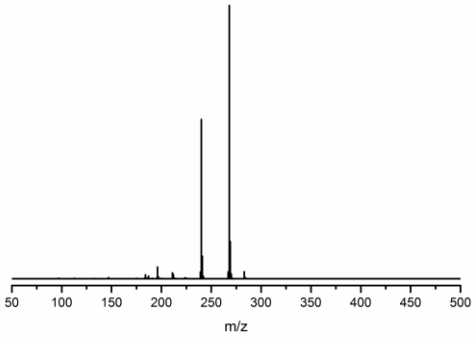


Isoflavones

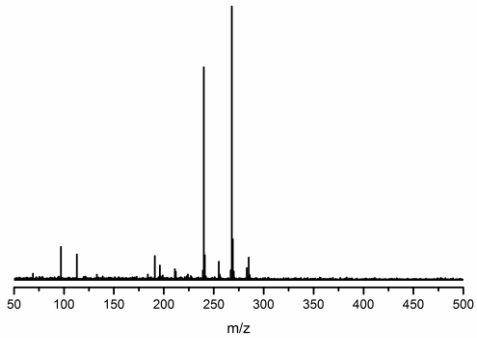




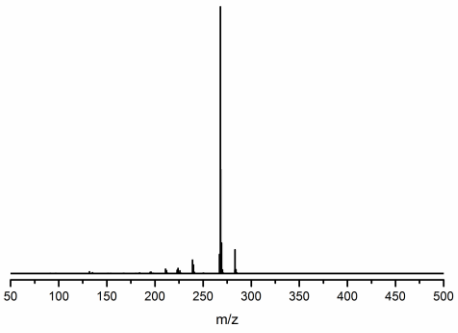
Isoprunetin



Glycitein

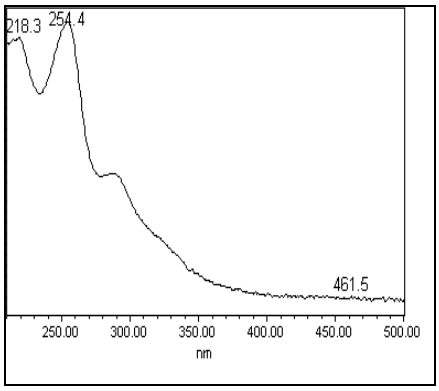


Prunetin

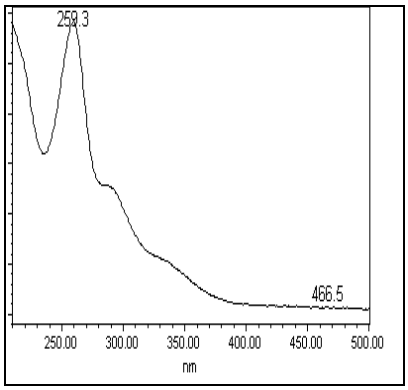


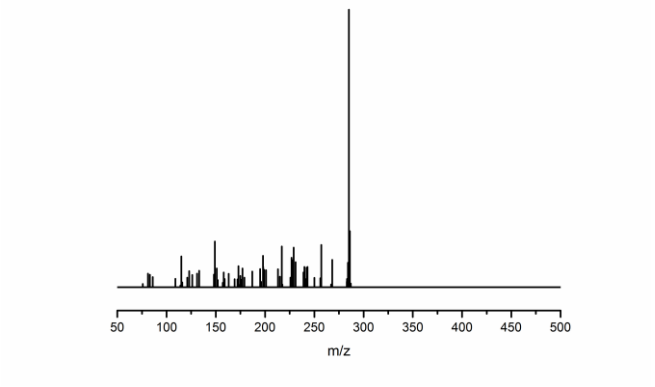
Biochanin A

SI 3: UPLC-PDA and (ESI$^-$) MS^2 fragmentation of Gt compounds at CE: 25 eV

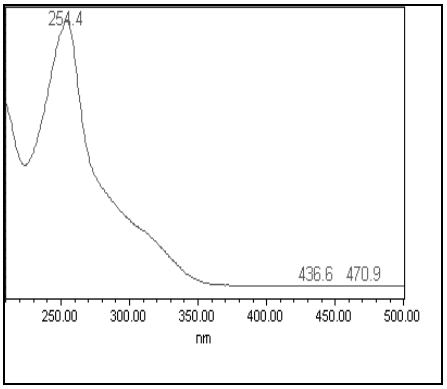


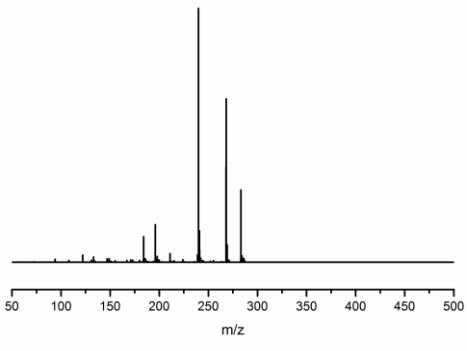
Gt$_1$



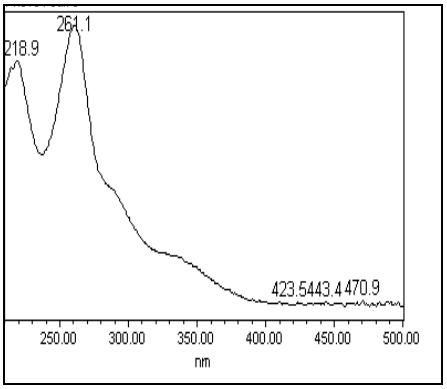


Gt$_2$





Gt$_3$


264.1
218.9
423.5443.4 470.9
250.00
300.00
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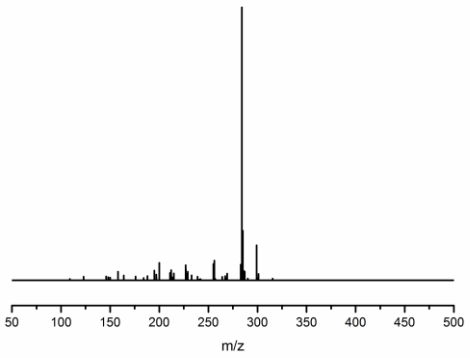
nm


50
100
150
200
250
300
350
400
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500
m/z

Gt_4

SI 4: UPLC-PDA investigation of references and historical yarns

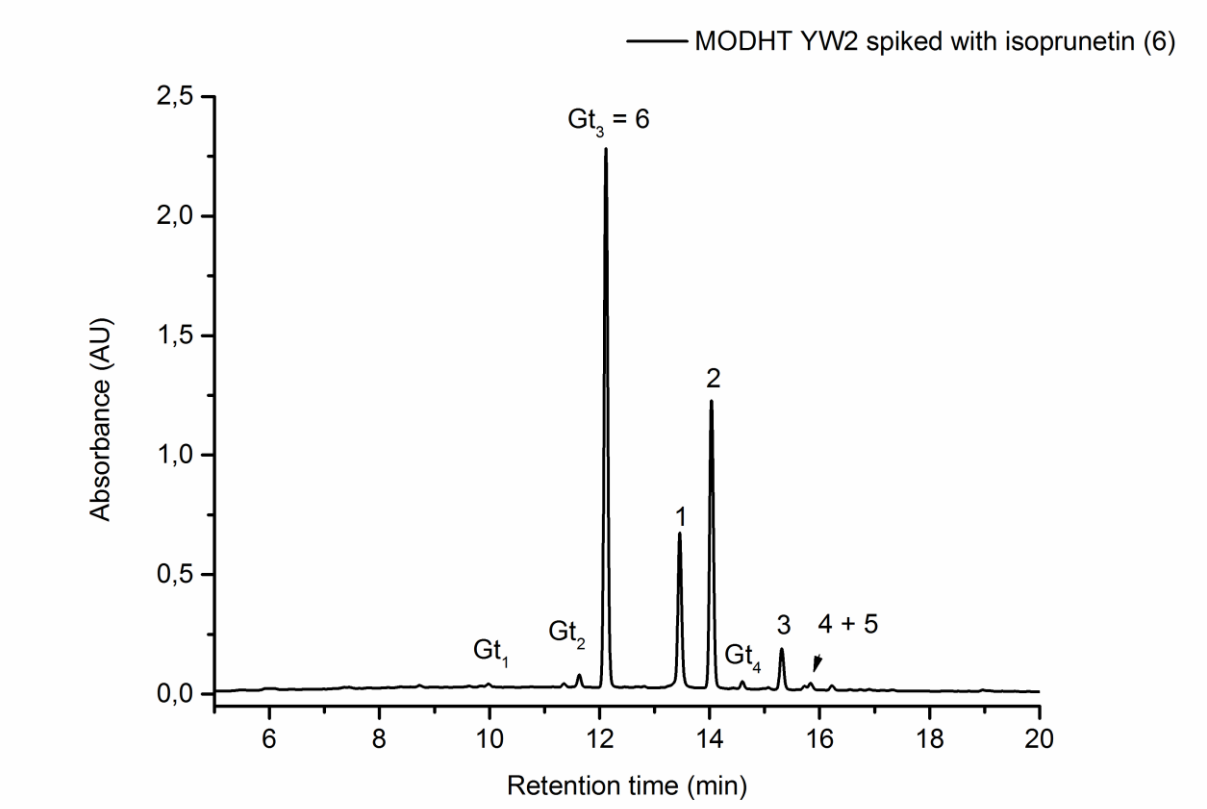

Fig. S2: UPLC chromatogram of the acid hydrolysed extract of reference yarn MODHT YW2 spiked with a solution of isoprunetin (**6**), identifying the unknown Gt_3 compound as isoprunetin (**6**), monitored at 254 nm.

	Relative Amount at 254 nm: average ± s (%), n=4				
	MODHT YW2	MODHT YS3a	MODHT YS3b	MODHT YS3c	MODHT YS3d
Gt_1	--	2.0 ± 0.9	0.7 ± 1.2	--	1.5 ± 1.2
Gt_2	4.6 ± 0.5	3.6 ± 1.3	1.1 ± 1.6	3.5 ± 1.0	4.9 ± 1.1
Gt_3	20.8 ± 1.8	19.2 ± 2.8	14.0 ± 1.5	11.4 ± 1.8	7.0 ± 2.1
luteolin (**1**)	25.5 ± 2.6	30.2 ± 2.7	39.8 ± 1.4	42.9 ± 2.3	58.2 ± 3.7
genistein (**2**)	34.8 ± 1.0	34.3 ± 1.6	33.2 ± 2.7	28.5 ± 2.3	19. 5 ± 1.2
Gt_4	3.9 ± 2.2	2.1 ± 0.2	2.0 ± 0.6	3.1 ± 0.2	1.9 ± 0.3
apigenin (**3**)	6.7 ± 0.3	5.2 ± 0.4	4.6 ± 0.4	5.0 ± 0.7	2.9 ± 0.3
chrysoeriol (**4**)	1.3 ± 0.6	1.2 ± 0.2	1.4 ± 0.1	1.6 ± 0.4	1.3 ± 0.1
diosmetin (**5**)	2.4 ± 0.7	2.3 ± 0.3	3.2 ± 0.3	4.0 ± 0.8	2.8 ± 0.4

Table 1: The relative amount of the flavonoid and isoflavonoid dyes characterised in the acid hydrolysed extract of reference yarns dyed with dyer's greenweed, YW2 and YS3a and others subjected to over dyeing (YS3b to d), monitored at 254 nm.

	Relative Amount at 254 nm					
	MAP2 YS12	47.21 YS21	47.21 YS 28	47.21 YS2	47.21 YS1	47.21 YS4
Gt_2	3.5	--	--	--	--	--
Gt_3	4.9	11.3	16.5	9.2	13.1	14.0
luteolin (**1**)	12.9	25.1	22.0	37.4	45.7	34.1
genistein (**2**)	71.5	40.0	53.9	34.9	23.4	32.1
Gt_4	2.7	5.0	--	--	4.0	7.7
apigenin (**3**)	2.7	12.4	7.6	12.7	9.7	12.1
chrysoeriol (**4**)	1.1	6.1	0.0	3.0	1.1	--
diosmetin (**5**)	0.7	--	--	2.9	3.1	--

Table 2: The relative amount of the flavonoid and isoflavonoid dyes characterised in the acid hydrolysed extract of Historical Yellow Silk (YS) sampled from a 16th century *Sheldon tapestry map of Oxfordshire* from the Bodleian Library in Oxford - UK (MAP2 YS12); and early English tapestry from the Burrell Collection in Glasgow - UK (tapestry 47.21, *coat of arms of Walter Jones and Eleanor Pope*), monitored at 254 nm.

	Relative Amount at 254 nm				
	47.7 GW2	47.10 GW9	47.12 GW3A	47.12 GW3B	47.14 GW3
Gt_2	--	--	--	--	--
Gt_3	17.5	24.9	16.5	15.2	16.1
luteolin (**1**)	33.1	44.7	52.2	52.9	52.4
genistein (**2**)	26.9	20.4	15.4	19.1	19.8
Gt_4	6.5	--	--	--	--
apigenin (**3**)	11.7	9.5	10.7	8.3	6.1
chrysoeriol (**4**)	4.3	0.5	5.2	4.5	3.9
diosmetin (**5**)	--	--	--	--	--

Table 3: The relative amount of the flavonoid and isoflavonoid dyes characterised in the acid hydrolysed extract of Historical Green Wool (GW) sampled from several early English tapestries from the Burrell Collection in Glasgow - UK (tapestries 47.7; 47.10; 47.12 and 47.14, *the Story of Susanna and the Elders*), monitored at 254 nm.