

Supplementary Material

Comprehensive HPTLC fingerprinting as a tool for a simplified analysis of purity of ginkgo products

Débora Arruda Frommenwiler^{a,b}, Anthony Booker^{c,d}, Roser Vila^a, Michael Heinrich^c, Eike Reich^b and Salvador Cañigüeral^a

^a Unitat de Farmacologia, Farmacognòsia i Terapèutica, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Av. Joan XXIII, 27-31, ES-08028 Barcelona - Spain

^b CAMAG AG, Sonnenmattstrasse 11, 4132 Muttenz - Switzerland

^c Centre for Pharmacognosy and Phytotherapy, Biodiversity and Medicines Research Cluster, Department of Pharmaceutical and Biological Chemistry, UCL School of Pharmacy, 29-39 Brunswick Square, WC1N 1AX London – UK

^d East Medicine Research Group, Department of Life Sciences, University of Westminster, Regent Street 309, W1B 2HW London – UK

Table S1.

Description of the products analysed in this work, their dosage form and country of origin. GP: ginkgo product; GBE: refined ginkgo dry extract; GE: other types of ginkgo dry extract. DER: drug-extract ratio.

Sample N°	Description of products per dosage unit	Dosage form	Country of origin
GP1	GBE 120 mg equivalent to 6000 mg of leaf. Standardised to contain 24% of flavone glycosides (28.8 mg) and 6% of terpene lactones (7.2 mg)	Tablet	UK
GP2	GBE 120 mg equivalent to 6000 mg of leaf, 24% of flavone glycosides and 6% of terpene lactones	Tablet	UK
GP3	GBE 5 mg (DER 50:1) and 250 mg of Ginkgo biloba leaf	Tablet	UK
GP4	GBE 120 mg. Standardised to contain 24% of flavone glycosides (28.8 mg) and 6% of ginkgolides plus bilobalide (7.2 mg)	Tablet	UK
GP5	GBE, equivalent to 6000 mg of dried leaf. Contain 24% of flavone glycosides	Tablet	UK
GP6	GBE 100 mg. Contain 24 mg of flavone glycosides and 6 mg of terpene lactones	Tablet	Denmark
GP7	GBE 600 mg, equivalent to 30.000 mg of leaf. Contain 24% of flavone glycosides (114 mg) and 6% of terpene lactones (36 mg).	Capsule	UK
GP8	GBE 60 mg from leaf. Standardised to contain 24% of flavone glycosides (14.4 mg)	Tablet	USA
GP9	GBE 100 mg. Contain 23 mg of flavone glycosides, 6 mg of terpene lactones, 300 mg of green buckwheat and 10 mg of Vitamin E	Tablet	UK
GP10	GBE 120 mg (DER 50:1), providing 6000 mg	Tablet	UK
GP11	GBE 120 mg (DER 50:1) equivalent to 6000 mg of herb powder, providing 28.8 mg of flavone glycosides and 7.2 mg of terpene lactones	Tablet	UK
GP12	GBE 60 mg. Standardized to contain 14.3 mg of flavone glycosides and 3.6 mg of terpene lactones	Capsule	UK
GP13	GBE 120 mg (DER 50:1), providing 6000 mg	Tablet	UK
GP14	GBE 60 mg (DER 50:1), equivalent to 3000 mg of leaf. Contain 24% of flavone glycosides and 6% of terpene lactones	Tablet	UK
GP15	GBE 120 mg (DER 50:1), providing 6000 mg	Tablet	UK
GP16	GBE 120 mg (DER 50:1), providing 6000 mg	Tablet	UK
GP17	GBE 100 mg, equivalent to 5000 mg Ginkgo biloba. Containing 24 mg of flavone glycosides and 6 mg of terpene lactones	Tablet	UK
GP18	GBE 120 mg (DER 50:1), equivalent to 6000 mg	Tablet	UK
GP19	GBE 120 mg (DER 50:1) equivalent to 6000 mg of whole dried leaf	Tablet	UK
GP20	GBE 120 mg (LI 1370) from cultivated leaf. Contain 25% of flavone glycosides	Tablet	Germany

Table S1 (continued).

Sample N°	Description of products per dosage unit	Dosage form	Country of origin
GP21	GBE 120 mg (DER 50:1), equivalent to 6000 mg of leaf. Contain 28.8 mg of flavone glycosides and 7.2 mg of terpene lactones	Tablet	UK
GP22	GBE 120 mg (DER 50:1), equivalent to 6000 mg of leaf. Contain 28.8 mg of flavone glycosides and 7.2 mg of ginkgolides A B & C and bilobalide	Tablet	UK
GP23	GBE 120 mg from leaf. Standardized to contain 24% of flavone glycosides (28.8 mg) and 6% of terpene lactones (7.2 mg)	Capsule	UK
GP24	GBE 60 mg from leaf. Standardized to contain 24% of flavone glycosides and 6% of terpene lactones and NMT 5% of ginkgolic acids	Capsule	USA
GP25	GBE 8 mg, equivalent to 400 mg of herb powder. Contain 24% of flavone glycosides and 6% of terpene lactones	Capsule	New Zealand
GP26	GBE 120 mg (DER 50:1) equivalent to 6000 mg of whole herb	Capsule	UK
GP27	GBE 60 mg from leaf. Contain 24% of flavone glycosides (14.4 mg) and 30 mg of Ginkgo biloba leaf powder	Capsule	USA
GP28	Ginkgo biloba leaf powder 130 mg	Capsule	UK
GP29	GBE 120 mg, equivalent to 6000 mg of herb powder. Contain 24% of flavone glycosides and 6-7% of ginkgolides A B & C and bilobalide and 50 mg of rutin	Capsule	UK
GP30	GBE 200 mg (DER 50:1), equivalent to 10.000 mg of herb powder.	Capsule	UK
GP31	GBE 100 mg (DER 50:1), equivalent to 5000 mg of Ginkgo biloba powder. Contain 24% of flavone glycosides and 6% of terpene lactones	Soft gel capsule	UK
GP32	GBE 120 mg, equivalent to 6000 mg of whole Ginkgo biloba.	Tablet	Netherlands
GP33	GE 90 mg (DER 3-5:1) prepared from fresh leaf	Tablet	Switzerland
GP34	Ginkgo biloba leaf powder 180 mg	Capsule	UK
GP35	GBE 120 mg. Contain 24% of flavone glycosides (28.8 mg) and 6% of terpene lactones (7.2 mg)	Capsule	USA
GP36	GBE 80 mg. Contain 17.6-21.6 mg of flavone glycosides and 4.0- 5.6 mg of terpene lactones.	Tablet	Switzerland
GP37	Quantified and refined GBE 120 mg from leaf (DER 35-67:1). Contain 26.4-32.4 mg of flavonoid glycosides, 3.12-3.84 mg of bilobalide, 3.36-4.08 mg of ginkgolides A, B & C	Tablet	Switzerland
GP38	Quantified and refined GBE 120 mg (DER 35-67:1). Contain 26.4-32.4 mg of flavonoid glycosides, 3.12-3.84 mg of bilobalide, 3.36-4.08 mg of ginkgolides A, B, C and NMT 5 ppm of ginkgolic acid	Tablet	Switzerland
GP39	Quantified and refined GBE 120 mg (DER 35-67:1). Contain 26.4-32.4 mg of flavonoid glycosides and 6.48-7.92 mg terpene lactones	Tablet	Switzerland
GP40	GBE 120 mg from leaf (DER 35-67:1). Contain 26.4-32.4 mg of flavonoid glycosides, 6.0-8.4 mg of terpene lactones, of which 3.12-3.84 mg of bilobalide, 3.36-4.08 mg of ginkgolides A, B & C	Tablet	Germany
GP41	GBE 120 mg from leaf (DER 35-67:1). Contain 26.4-32.4 mg of flavonoid glycosides, 6.0-8.4 mg of terpene lactones, of which 3.12-3.84 mg of bilobalide, 3.36-4.08 mg of ginkgolides A, B & C	Tablet	Germany
GP42	GBE 120 mg from leaf (DER 35-67:1; EGb 761). Contain 26.4-32.4 mg of flavonoid glycosides, 6.0-8.4 mg of terpene lactones, of which 3.12-3.84 mg of bilobalide, 3.36-4.08 mg of ginkgolides A, B and C	Tablet	Germany
GP43	GBE 120 mg from leaf (DER 35-67:1). Contain 22-27% of flavonoid glycosides, 2.8-3.4% of ginkgolides A, B & C, 2.6-3.2% of bilobalide and NMT 5ppm of ginkgolic acids	Capsule	Germany
GP44	GBE 100 mg (50:1). Contain 24.5 mg flavone glycosides, 6.5 mg ginkgolide terpene lactones	Capsule	Germany
GP45	Standardized GBE (120 mg). Contain NLT 24% of flavonol glycosides and 6% of terpene lactones.	Capsule	Serbian

Table S1 (continued).

Sample N°	Description of products per dosage unit	Dosage form	Country of origin
GP46	Ginkgo biloba leaf 330 mg	Tablet	Spain
GP47	Ginkgo biloba leaf 180 mg	Tablet	Spain
GP48	GBE 40 mg (40-50:1). Contain 8.8-10.8 mg of flavone glycosides, 1.12-1.36 mg of Ginkgolide A, B & C, and 1.04-1.28 mg of bilobalide	Tablet	Croatia
GP49	GBE 120 mg. Contain 24% of flavone glycosides (28.8 mg) and 6% terpene lactone (7.2 mg).	Capsule	Netherlands
GP50	GBE 410 mg and leaf (amount not informed). Contain 24% of flavone glycosides.	Capsule	Italy
GP51	GBE 60 mg. Contain 24% of flavone glycosides	Soft gel capsule	Colombia
GP52	GBE 80 mg. Contain 24% of flavone glycosides and 6% of terpene lactones	Soft gel capsule	Colombia
GP53	GBE 40 mg. Contain 24% of flavone glycosides (9.6 mg)	Soft gel capsule	Colombia
GP54	GBE 80 mg. Contain 19.2 mg of flavone glycosides	Capsule	Colombia
GP55	GE 50 mg, equivalent to 500 mg of GBL	Tablet	Spain
GP56	Ginkgo biloba leaf, equivalent to 500 mg (200 mg of leaf and 40 mg of extract 7.5:1)	Tablet	Spain
GP57	GBE 120 mg. Contain 24% of flavone glycosides (28 mg)	Capsule	USA
GP58	GBE 60 mg. Contain 24% of flavone glycosides (14.4 mg)	Tablet	USA

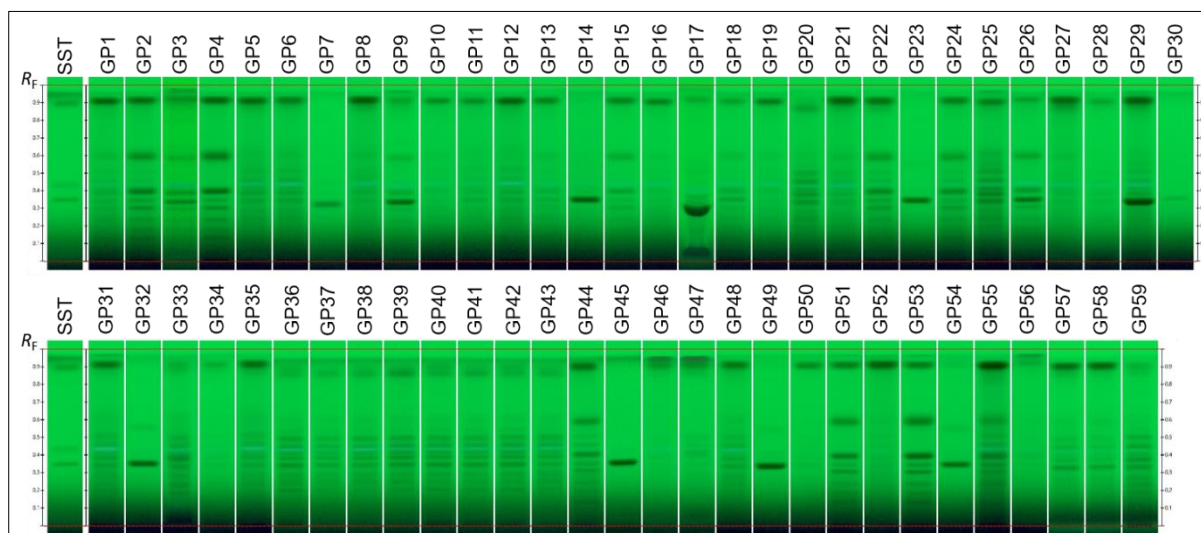


Figure S1. HPTLC fingerprints of all ginkgo products under UV 254 nm prior to derivatization. SST: system suitability test (rutin, chlorogenic acid, and quercetin, increasing R_F values).

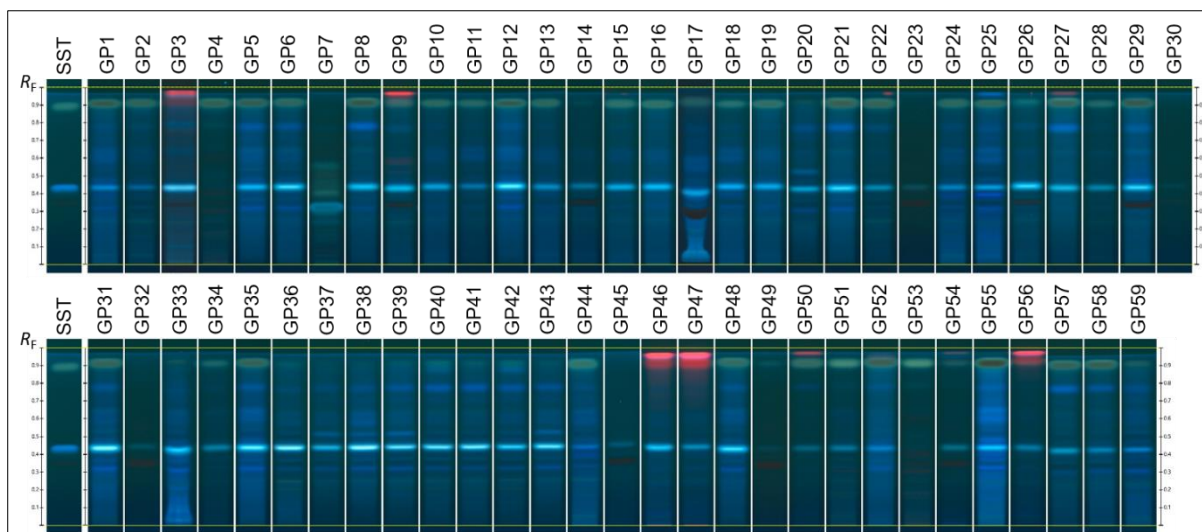


Figure S2. HPTLC fingerprints of all ginkgo products under UV 366 nm prior to derivatization. SST: system suitability test (rutin, chlorogenic acid, and quercetin, increasing R_F values).

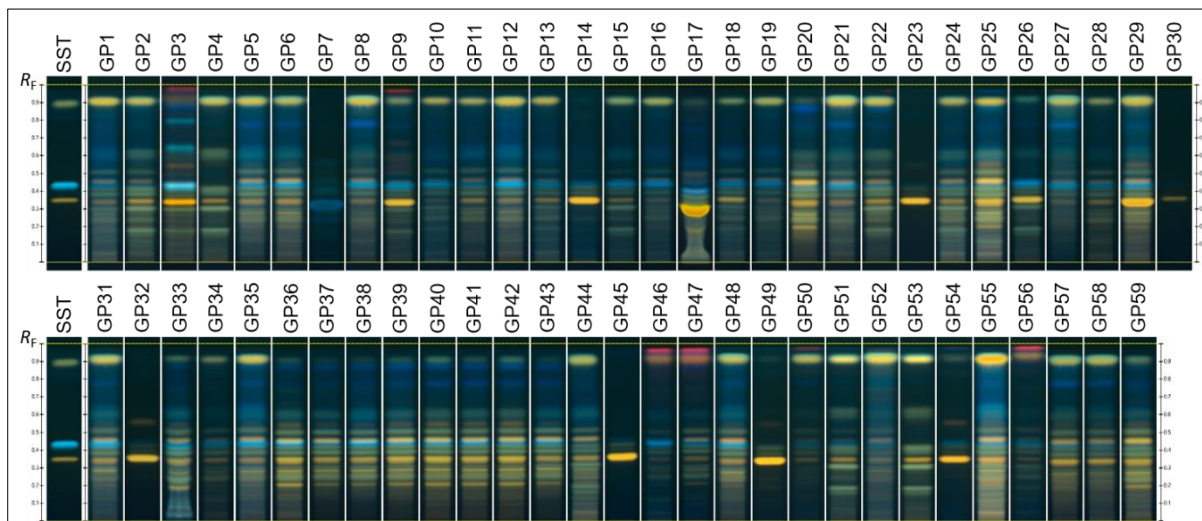


Figure S3. HPTLC fingerprints of all ginkgo products under UV 366 nm after derivatisation 2 (NP reagent). SST: system suitability test (rutin, chlorogenic acid, and quercetin, increasing R_F values).

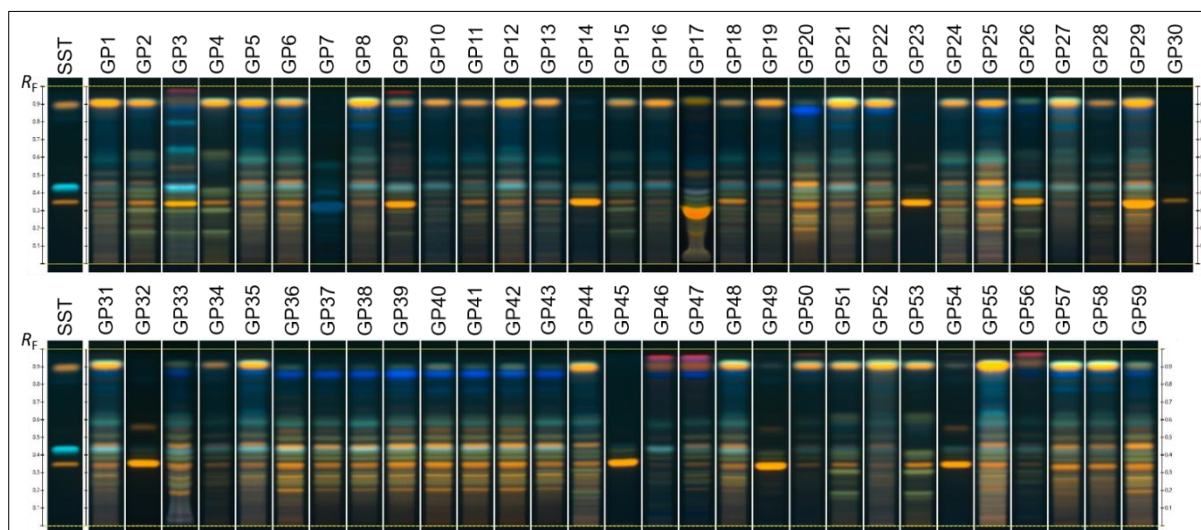


Figure S4. HPTLC fingerprints of all ginkgo products under UV 366 nm after derivatisation 1. SST: system suitability test (rutin, chlorogenic acid, and quercetin, increasing R_f values).

Table S2.

Table of results obtained by HPTLC regarding identity, purity, and percentages of rutin and quercetin, for all products. Products similar to that of reference ginkgo leaf or extract are indicated with green squares, and not similar with red circles. GBE: Refined ginkgo extract; GL: Ginkgo leaf (GBL); SJfr: Sophora fruit; SJfl: Sophora flower bud; BWH: Buckwheat herb; 5-HTP: 5-hydroxytryptophan; n.a.: not applicable.

Sample N°	Similar to GBE or GBL	Contain SJfr	Contain SJfl	Contain BWH	5-HTP only	Intense zone at the position of rutin or only rutin	Rutin (%), by HPTLC ID method	Intense zone at the position of quercetin	Quercetin (%), by HPTLC reversed phase method
GP1	●	x					0.69	x	3.74
GP2	●	x					1.92	x	3.11
GP3 ^a	●	x					n.a.		n.a.
GP4	●	x					1.95	x	3.80
GP5	●						1.35	x	3.16
GP6	●						1.72	x	2.24
GP7	●				x		n.a.		n.a.
GP8	●						0.94	x	2.88
GP9 ^b	●	x		x			n.a.	x	n.a.
GP10	●	x					0.42	x	2.50
GP11	●						1.49	x	1.99
GP12	●						1.46	x	3.52
GP13	●	x					1.31	x	2.53
GP14	●					x	6.61		0.24
GP15	●	x					1.10	x	2.89
GP16	●						0.45	x	2.55
GP17	●		x			x	6.62	x	1.07
GP18	●	x					2.54	x	1.68
GP19	●						0.43	x	2.89
GP20	■						2.20		0.16

Table S2 (continued).

Sample N°	Similar to GBE or GBL	Contain SJfr	Contain SJfl	Contain BWH	5-HTP only	Intense zone at the position of rutin or only rutin	Rutin (%), by HPTLC ID method	Intense zone at the position of quercetin	Quercetin, by HPTLC reversed phase method
GP21	●						1.18	x	2.85
GP22	●	x					1.73	x	2.93
GP23	●					x	6.45		0.29
GP24	●	x					1.22	x	2.14
GP25	●						2.86	x	1.80
GP26	●	x					5.47		0.35
GP27 ^a	●						n.a.	x	n.a.
GP28 ^c	●						n.a.	x	n.a.
GP29 ^d	●		x			x	n.a.	x	n.a.
GP30	●					Only rutin	1.75		0.00
GP31	●						1.74	x	2.79
GP32	●					x	6.51		0.27
GP33 ^e	■						1.71		0.20
GP34 ^c	●						n.a.	x	n.a.
GP35	●						1.41	x	2.79
GP36	■						2.84		0.09
GP37	■						1.96		0.05
GP38	■						2.11		0.04
GP39	■						2.63		0.12
GP40	■						2.79		0.31
GP41	■						2.73		0.16
GP42	■						2.89		0.29
GP43	■						1.93		0.09
GP44	●	x					1.37	x	2.60
GP45	●					x	6.91		0.01
GP46 ^c	●						n.a.		n.a.
GP47 ^c	●						n.a.		n.a.
GP48	●						1.85	x	2.04
GP49	●					x	6.95		0.50
GP50 ^a	●						n.a.	x	n.a.
GP51	●	x					1.78	x	2.10
GP52	●						0.49	x	2.32
GP53	●	x					2.73	x	2.05
GP54	●					x	6.23		0.66
GP55 ^e	●	x					1.66	x	4.44
GP56 ^c	●						n.a.		n.a.
GP57	●						2.82	x	2.15
GP58	●						2.47	x	2.35
GP59	■						2.38		0.39
Total	11	16	2	1	1	9	---	35	---

^a Products that declare to contain mixture of refined ginkgo extract and ginkgo leaf; ^b Products that declare to contain mixture of refined ginkgo extract and green buckwheat; ^c Products that declare to contain ginkgo leaf; ^d Products that declare to contain mixture of refined ginkgo extract and rutin; ^e Products that declare to contain ginkgo extract other than GBE.

Table S3.

HPLC results of the assay of total flavonoids, the peak ratios kaempferol/quercetin (K/Q) and isorhamnetin/quercetin (I/Q), and the limit tests for rutin and quercetin. Samples marked in red show one or more results not compliant with the USP monograph. Samples marked in green are compliant with the USP monograph for the analyses performed. Samples in which conformity to the monograph is not applicable are marked in black. n.a.: not applicable.

Products that declare to contain ginkgo refined extract (22-27% of flavone glycosides)					
Sample No	Total flavonoids^a (22.0-27.0%)^b	Peak area ratio K/Q (≥ 0.7)^b	Peak area ratio I/Q (≥ 0.1)^b	Rutin (%)^a (NMT 4%)^b	Quercetin (%)^a (NMT 0.5%)^b
GP1	24.6	0.3	0.1	0.6	8.13
GP2	22.3	0.9	0.2	1.6	3.18
GP4	25.9	1.1	0.1	1.3	4.08
GP5	24.9	0.6	0.2	1.0	5.16
GP6	16.0	0.9	0.2	1.2	2.60
GP7	Not analysed	Not analysed	Not analysed	Not analysed	Not analysed
GP8	22.3	0.8	0.1	0.4	5.75
GP11	17.8	0.4	0.1	0.8	2.05
GP12	24.8	0.3	0.1	0.4	3.33
GP14	Not analysed	Not analysed	Not analysed	Not analysed	Not analysed
GP17	Not analysed	Not analysed	Not analysed	Not analysed	Not analysed
GP20	23.2	0.9	0.1	2.5	0.12
GP21	25.6	0.9	0.1	0.6	4.70
GP22	25.2	0.9	0.1	1.4	4.15
GP23	10.0	0.04	0.02	23.8	0.33
GP24	22.0	1.0	0.2	1.1	2.72
GP25	Not analysed	Not analysed	Not analysed	Not analysed	Not analysed
GP31	21.2	0.8	0.2	0.7	2.24
GP35	25.9	0.8	0.2	0.9	4.37
GP 36	23.1	1.0	0.3	2.3	0.18
GP 37	23.5	1.2	0.4	2.0	0.18
GP 38	22.2	1.5	0.4	1.8	0.18
GP 39	22.2	1.3	0.5	2.4	0.20
GP 40	23.1	1.0	0.3	2.3	0.34
GP 41	23.0	1.0	0.3	2.3	0.24
GP 42	24.4	1.0	0.3	2.5	0.34
GP 43	23.0	1.6	0.3	1.4	0.22
GP 44	28.8	0.9	0.1	1.1	3.62
GP 45	27.0	0.0	0.0	23.2	0.02
GP 48	26.3	0.8	0.2	1.2	3.13
GP 49	26.9	0.0	0.0	20.6	0.47
GP 51	17.3	1.4	0.1	0.8	1.69
GP 52	19.3	0.8	0.1	0.1	1.53
GP 53	27.0	1.1	0.1	2.1	2.36
GP 54	15.4	0.0	0.0	14.5	0.73
GP 57	25.6	1.0	0.1	2.5	3.11
GP 58	26.4	1.0	0.1	2.0	3.61
GP 59	26.9	1.1	0.4	2.5	0.38

Table S3 (continued).

Products that declare to contain ginkgo dry extract with a DER of 50:1 ^c					
Sample No	Total flavonoids ^a (22.0-27.0%) ^b	Peak area ratio K/Q (≥ 0.7) ^b	Peak area ratio I/Q (≥ 0.1) ^b	Rutin (%) ^a (NMT 4%) ^b	Quercetin (%) ^a (NMT 0.5%) ^b
GP10	12.4	0.3	0.1	0.2	3.01
GP13	13.3	0.3	0.1	0.8	3.05
GP15	11.4	0.8	0.1	0.5	1.93
GP16	10.0	0.3	0.1	0.2	3.24
GP18	Not analysed	Not analysed	Not analysed	Not analysed	Not analysed
GP19	14.1	0.3	0.1	0.2	3.92
GP26	16.3	0.5	0.1	13.0	0.39
GP30	0.1	0.8	0.1	0.7	0.01
GP32	19.3	0.02	0.01	21.0	0.39

Products that declare to contain other types of ginkgo dry extract					
Sample No	Total flavonoids ^a (requirement n.a.)	Peak area ratio K/Q (requirement n.a.)	Peak area ratio I/Q (requirement n.a.)	Rutin (%) ^a (requirement n.a.)	Quercetin (%) ^a (requirement n.a.)
GP33	2.9	1.4	0.4	0.2	0.00
GP55	16.3	0.4	0.1	0.5	3.44

Products that declare to contain ginkgo refined extract and additional rutin					
Sample No	Total flavonoids ^d (requirement n.a.)	Peak area ratio K/Q (requirement n.a.)	Peak area ratio I/Q (requirement n.a.)	Rutin (%) (requirement n.a.)	Quercetin (%) (requirement n.a.)
GP29	37.3	0.2	0.1	n.a.	n.a.

Products that declare to contain ginkgo refined extract and additional green buckwheat					
Sample No	Total flavonoids ^e (requirement n.a.)	Peak area ratio K/Q (requirement n.a.)	Peak area ratio I/Q (requirement n.a.)	Rutin (%) (requirement n.a.)	Quercetin (%) (requirement n.a.)
GP9	8.2	0.2	0.04	n.a.	n.a.

Products that declare to contain mixture of ginkgo refined extract (declaring 22-27% of flavone glycosides or DER 50:1) and ginkgo leaf					
Sample No	Total flavonoids ^f (requirement n.a.)	Peak area ratio K/Q (requirement n.a.)	Peak area ratio I/Q (requirement n.a.)	Rutin (%) (requirement n.a.)	Quercetin (%) (requirement n.a.)
GP3	15.1	0.8	0.1	n.a.	n.a.
GP27	22.3	1.0	0.1	n.a.	n.a.
GP50	Not analysed	Not analysed	Not analysed	Not analysed	Not analysed

Products that declare to contain ginkgo leaf					
Sample No	Total flavonoids ^g (NLT 0.5%) ^h	Peak area ratio K/Q (requirement n.a.)	Peak area ratio I/Q (requirement n.a.)	Rutin (%) (requirement n.a.)	Quercetin (%) (requirement n.a.)
GP28	0.8	n.a.	n.a.	n.a.	n.a.
GP34	0.6	n.a.	n.a.	n.a.	n.a.
GP46	0.5	n.a.	n.a.	n.a.	n.a.
GP47	0.9	n.a.	n.a.	n.a.	n.a.
GP56	0.5	n.a.	n.a.	n.a.	n.a.

^a Results expressed in relation to the extract contained in the product. ^b Requirements of the USP monograph on Powdered Ginkgo Extract. ^c These extracts did not declare any content of flavonoids but a DER of 50:1, typical of the GBE. For this reason, were considered as GBE and thus, compliance to the requirements described in the USP monograph on Powdered Ginkgo Extract was assessed. ^d Results expressed in relation to the sum of the declared amounts of GBE and rutin. ^e Results expressed in relation to the sum of the declared amounts of GBE and green buckwheat. ^f Results expressed in relation to GBE equivalent, sum of the amounts of GBE and GBE equivalent to ginkgo leaf (calculated from a DER 50:1). ^g Results expressed in relation to the ginkgo leaf contained in the product. ^h Requirements of the USP monograph on Ginkgo (ginkgo leaf).

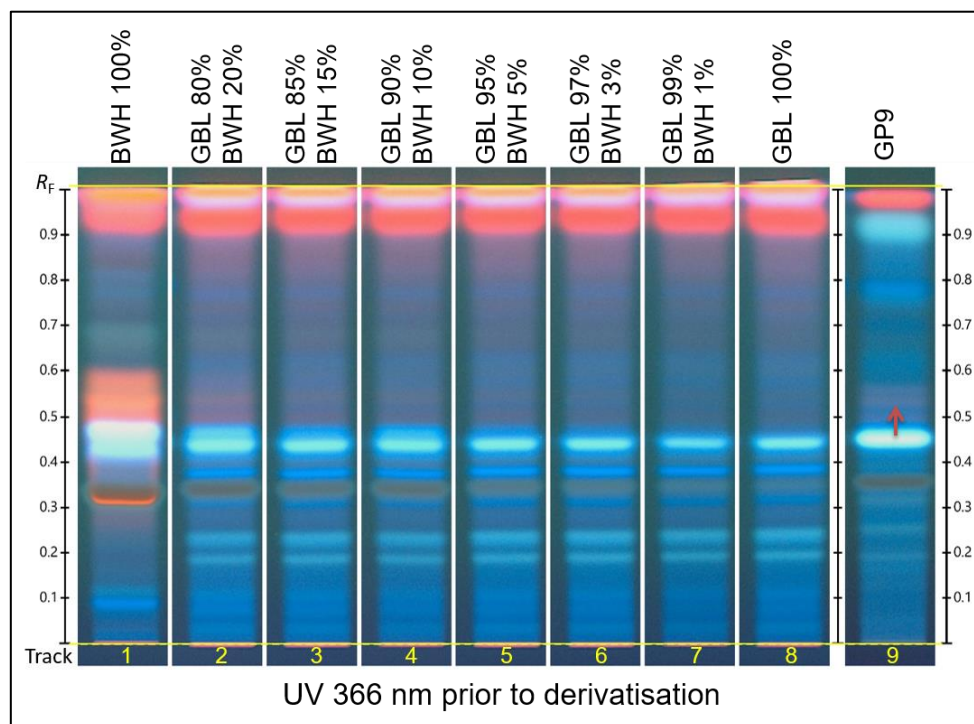


Figure S5. HPLTC fingerprints of the physical mixtures of ginkgo leaf and buckwheat herb, and examples of adulterated product (GP9). BWH: buckwheat herb, GBL: ginkgo leaf. Track 1: 100% BWH, track 8: 100% GBL, tracks 2-8: mixtures of BWH and GBL, track 9: product containing BWH.

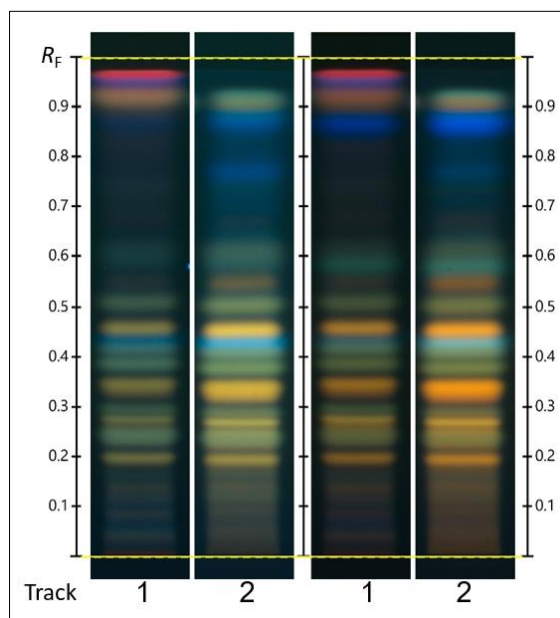


Figure S6. Fingerprints of GBL (track 1) and GBE (track 2) under UV 366 nm after derivatization with NP (left) and NP + PEG (right).

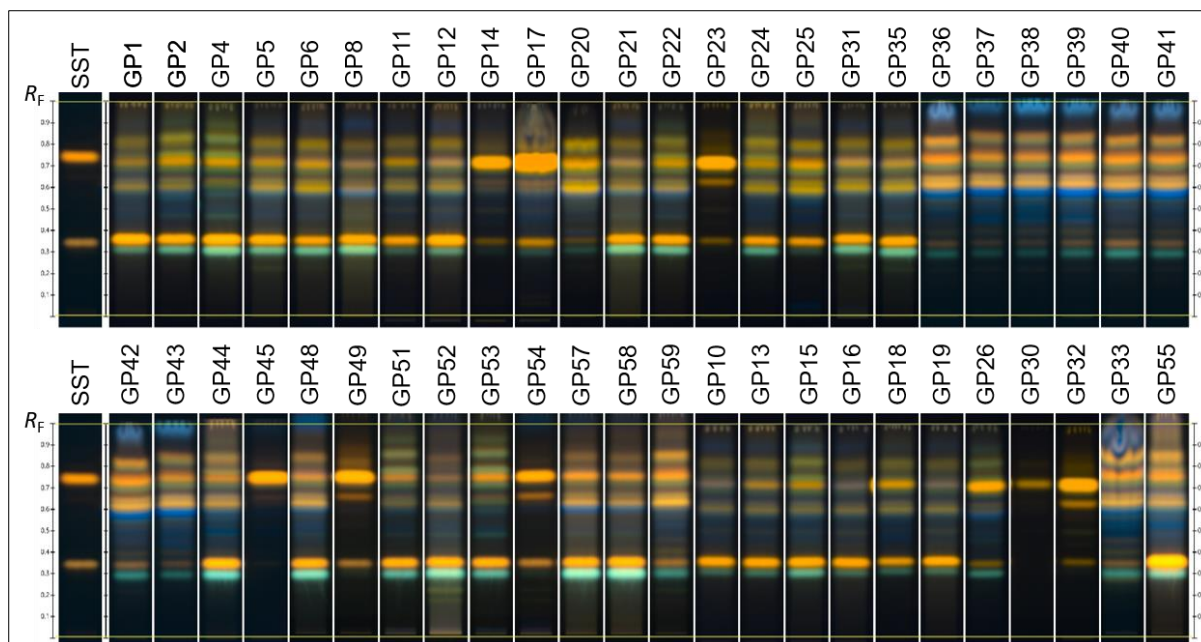


Figure S7. HPTLC fingerprints of forty-eight ginkgo products analysed with the reverse-phase HPTLC method under UV 366 nm after derivatisation 1. SST: system suitability test (quercetin and rutin, increasing R_F values).