AIM OF THE WORK

Quality control of herbal products is of a great concern nowadays. Herbal products are perceived by the public as safe products and their popularity is increasing. Being classified as dietary supplements they are generally exempted from the quality control tests done on allopathic medicines, in spite the fact that they are prone to variation in composition, adulteration, and contamination. Most compendial monographs are inadequate to ensure the quality of botanical products. *Ginkgo biloba* L. is one of the top selling botanicals in the world.

The aims of the present study are:

1. To critically assess current USP monographs for the assay of *Ginkgo biloba* L. extracts and *Ginkgo biloba* L. dietary supplements.
2. To help specify simple method to detect inferior, hydrolysed or counterfeit products containing *Ginkgo biloba* L. extracts.
3. To assess the quality of *Ginkgo biloba* L. containing dietary supplements marketed in Egypt.
4. To draw attention to the potential hazards of botanical dietary supplements used as remedies without undergoing strict regulations mandated for conventional medicines.

MATERIALS, METHODS, & EQUIPMENT

1. Materials

1.1. Reference materials and standards

USP quercetin reference standard, rutin Nutritional biochemicals, quercetrin (97%) Sigma Aldrich, kaempferol (97%) Sigma Aldrich, USP standardized *Ginkgo biloba* L. extract. bilobalide standard (Sigma, Batch: 050M1444, assay = 97.8%),ginkgolide A standard (Fluka, Batch: 1375407V, assay = 99.0%), ginkgolide B standard (Sigma, assay = 97.0%) ,ginkgolide C standard (Fluka, assay = 99.2%), ginkgolide J standard (Fluka assay = 90.1%) , chrysin standard (Aldrich, assay = 99.4%), anacardic acid (C15:0) standard (Sigma, assay = 99.0%) ginkgolic acid (C15:1) standard (Fluka, assay = 99.4%) ginkgolic acid (C17:1) standard (Fluka, assay = 97.2%) 12-hydroxystearic acid standard Acros Organics.

1.2. Solvents and TLC plates

Solvents and chemicals used in this study were all analytical and HPLC grade. methanol HPLC grade Fisher chemical UK, hydrochloric acid analytical grade (36.5-38%) Sigma Aldrich, phosphoric acid analytical grade ADWIC, filtered double distilled water (Millipore system), ethanol HPLC grade Sigma Aldrich, ethyl acetate HPLC grade Sigma Aldrich, anhydrous formic acid analytical grade ADWIC, glacial acetic acid analytical grade (Sd Fine- Chem Limited), 0.25-mm layer of chromatographic pre-coated silica gel TLC and HPTLC fluorescent plates, (silica gel GF- 254), Merck, Darmstadt was used.

1.3. Reagents

Ferric chloride (10%) analytical grade Chemajet Chemical Company. [106]

1.4. *Ginkgo biloba* L. extracts

Two *Ginkgo biloba* L. extracts were obtained from different manufacturers of *Ginkgo biloba* L. dietary supplements in Egypt.

1.5. *Ginkgo biloba* L. products

Ten commercial products of *Gingko biloba* supplements were purchased (Table 3). All products were obtained from the local market (A-I), except product (J) was from United Kingdom. Product A, B, E, and H are suggested to be registered as medicines, while the other products are declared to be registered as dietary supplements. All samples were analyzed during the shelf life.

Table 3. *Ginkgo biloba* L. containing products used for the quality assessments.

Name	Declared amount of *Ginkgo biloba* L. extracts	Batch number	Shelf lifes
A	Each tablet contains 40 mg *Ginkgo biloba* L. Extract (EGb 761)	717127	4 years
B	One film coated tablet contains dried extract from *Ginkgo biloba* L. leaves 40 mg, standardized to a content of 9.6mg Ginkgo flavone glycosides and 2.4 mg terpene lactons (ginkgolides,bilobalides) *Ginkgo biloba* L. extract 761	1208902	5 years
C	Each capsule *contains Ginkgo biloba* L. (*Ginkgo* flavone glycosides 24%) 50mg and ginseng dry extract (10%) 100mg.	030912	3 years
D	Each capsule contains 260 mg of *Ginkgo biloba* L. leaf powder extract standardized as *Ginkgo* flavone glycosides not less than 24%, Total ginkgolides not less than 6%	9032013	3 years
E	Each capsule contains glucosamine sulphate 500 mg, *Ginkgo biloba* L. leaf extract 50 mg	12400	2 years
F	Each capsule contains *Ginkgo biloba* L. 60 mg extract + ginseng 100mg +R. jelly 600 mg	2063006	3 years
1G	Each capsule contains *Ginkgo biloba* L. extract 50 mg, grape seed extract 50 mg	132601	2 years
2G	Each capsule contains *Ginkgo biloba* L. extract 50 mg, grape seed extract 50 mg	332601	3 years
H	Each capsule contains standardized *Ginkgo biloba* L. extract containing 24% Ginkgo glycosides and 6% (ginkgolide-bilobalide) 0.014g , heptaminol hydrochloride 0.3g, troxerutin 0.3g, excipients 0.625g	170028	3 years
I	Each capsule contains *Ginkgo biloba* L. extract 60 mg	009	3 years
J	Each tablet contains *Ginkgo biloba* L. extract 120mg equivalent to *Ginkgo biloba* L. whole leaf providing flavonol glycosides 28.8mg and terpene trilactones 7.2mg	159038	

Table 4. Products containing *Ginkgo biloba* L. extracts and used for the USP microbial enumeration tests.

Name	Batch number	Shelf life
A	917106	4 years
B	1305616	5 years
C	490613	3 years
D	9032013	3 years
E	12400	2 years
G	132601	2 years
I	009	3 years

2. Equipment

2.1. Flavonol glycosides analysis

2.1.1. HPLC

Apparatus: Preliminary work was done using Perkin-Elmer (USA) Series 200, system equipped with an auto sampler (Perkin-Elmer Series 200) and a variable-wavelength UV-Vis detector (Perkin-Elmer Series 200). The chromatograms were recorded with a series 600 chromatography interface supported with "Total chrom Navigator " software , version 6.3.2. Columns: A stainless steel column (250 x 4.6 mm I.D.) Packed with sphere 5 RP- 18 (5 µm) was used (Kromasil columns), and waters xbridge column (150 x 4.6 mm I.D.) Packed with sphere 5 RP- 18 (5 µm).Final work was done using Agilent 1200 infinity series system.

2.1.2. HPTLC

Ultraviolet lamp: (Vilber Lourmat, France), model VL-6.LC, was used to visualize the plates at 254 and 366 nm. Applicator: Samples were applied on plates using CAMAG Linomat IV sample applicator (CAMAG, Switzerland).

2.2. Terpene trilactones and ginkgolic acid analysis

Agilent 1200 Series HPLC instrument, Agilent Technologies. Column used was Poroshell 120 EC-C18, 4.6 x 50mm, 2.7 µm.

2.3. Stability Cabinet

Stability studies of the samples were conducted in a Climacell cabinet made in USA maintained at 40°C ± 0.1 and 75 % Relative Humidity ± 2

3. Methods

3.1. Assay of flavonol glycosides

3.1.1. Method of assay

Method for assay of flavonol glycosides in dry *Ginkgo biloba* extract and products containing such extract is HPLC and HPTLC method.

3.1.2. Sample and extract preparation for HPLC analysis

3.1.2.1. Tablets and capsules extraction for the unhydrolyzed study

The capsules were opened, emptied, weighed and the contents were mixed. Tablets were weighed and ground into a fine powder using a mortar and pestle. An accurately weighed amount equivalent to about 50 mg of flavonol qlycosides was transferred to a 50-ml volumetric flask. 20 ml of methanol was added, and all samples were sonicated for 3 minutes 20 ml of water was added and sonication was done again for 10 minutes. Samples were allowed to cool to room temperature and diluted with methanol to volume. All samples were centrifuged and a portion of each supernatant was transferred to a rubber-capped glass vial and filtered through 0.45 µm PolyVinyliDene Fluoride (PVDF) filters. 10-20µl was injected in duplicate.

3.1.2.2. Preparation of *Ginkgo biloba* L. extract for the unhydrolyzed study

0.2 gm of each *Ginkgo biloba* L. extracts was weighed and transferred to a 50-ml volumetric flask. 20 ml of methanol was added and the samples were sonicated for 3 minutes. 20 ml of water was added and sonication was done again for 10 minutes. The samples were allowed to cool to room temperature and diluted with methanol to volume. All samples were centrifuged and a portion of each supernatant was transferred to a rubber-capped glass vial and filtered through 0.45 µm PVDF filters. 10-20µl was injected in duplicate.

3.1.2.3. Tablets and capsules extraction for the hydrolyzed study[107]

The capsules were opened, emptied, weighed and the contents were mixed. Tablets were weighed and ground into a fine powder using a mortar and pestle. An accurately weighed amount equivalent to about 50 mg of flavonol qlycosides was transferred to a 50-ml volumetric flask. 20 ml of methanol was added, and all samples were sonicated for 3

minutes. 20 ml of 1.5N hydrochloric acid was added and sonication was done again for 10 minutes. Samples were allowed to cool to room temperature and diluted with methanol to volume. All samples were centrifuged and a portion of each supernatant was transferred to a rubber-capped glass vial. All samples were heated in a steam bath for 25 minutes, cooled in an ice bath, and filtered through 0.45 µm PVDF filters. 10-20 µl was injected in duplicate.

3.1.2.4. Preparation of *Ginkgo biloba* L. extract for the hydrolyzed study[107]

0.3 gm of each *Ginkgo biloba* L. extract was transferred to a 250 ml flask fitted with a reflux condenser. 78ml of extraction solvent (50 ml alcohol, 20 ml water, 8 ml HCL) was added and refluxed in a hot water bath for 135 minutes. This was allowed to cool at room temperature, transferred to 100 ml volumetric, completed with water, mixed, and filtered through 0.45 µm PVDF filters. 10-20 µl was injected in duplicate.

3.1.2.5. HPLC conditions

Mobile phase used was methanol: water: phosphoric acid (100:100:1). The flow was isocratic at a rate of 1.5 ml/min. Detection wavelength was at 360 and 370 nm.

3.1.3. Sample and extract preparation for HPTLC analysis

The equivalent of 20mg of *Ginkgo biloba* L. extract was weighed and dissolved in a mixture of methanol and water (4:1). Samples were applied to HPTLC fluorescent plates, rutin standard solution of 0.4mg/ml was used. The developing solvent system was: Ethyl acetate: anhydrous formic acid: glacial acetic acid: water (100:11:11:26 v/v/v/v). The application volume was 5 µl and conditions of application were 1.5 cm from edges, 6 cm band width, 4 cm between bands, and 1 cm from below. Visualization was done at 254 nm. The plate were Sprayed with ferric chloride reagent.

3.2. Assay of terpene trilactones

3.2.1. Method of assay

Method for assay of bilobalide and ginkgolide A, B, C and J in dry *Ginkgo biloba* extract and products containing such extract is HPLC-MS method. This method uses internal standard calibration based on area.

3.2.2. Sample preparation

1 tablet, containing about 40 mg of *Ginkgo biloba* extract was weighed in 25 ml volumetric flask and filled with 20 ml of methanol. The content in flask was sonicated for 15 minutes and after cooling to room temperature filled to mark with methanol. Aliquot amount of dispersion was centrifuged 5 minutes at 6000 rpm at room temperature. Until this point the procedure is equal to procedure for ginkgolic acids. 2 ml of clear supernatant was transferred to 25 ml volumetric flask, spiked with 2.5 ml of chrysin solution (concentration 40.1775 µg/ml in methanol) as IS and filled to mark with methanol. 3 µl was injected to HPLC-MS instrument. This procedure is useful for preparation of sample solution from dry extract of *Ginkgo biloba* or another product containing different amount

of extract by choosing the right weight of the sample or volume of flask to obtain concentration of *Ginkgo biloba* L. extract about 0.05 mg/ml in methanol.

3.2.3. HPLC conditions

Mobile phase: methanol with 0.2% of HCOOH, water with 0.2% of HCOOH. Flow rate: 0.5 ml/min, Injection: 3 µl, Column temperature: 30 ºC, Run time: 13 minutes.
Gradient elution:

Time (min)	MeOH (vol %)	Water (vol%)
0	60	40
4	80	20
4.5	100	0
8	100	0
8.1	60	40
13	60	40

3.3. Assay of ginkgolic acids

3.3.1. Method of assay

Method for assay of ginkgolic acids (anacardic acid, ginkgolic acid 15:1 and ginkgolic acid 17:1) in dry *Ginkgo biloba* extract and products containing such extract is HPLC-MS method. This method uses internal standard calibration based on area. Validation was performed with reference substances and chemicals.

3.3.2. Sample preparation

One tablet, containing about 40 mg of *Ginkgo biloba* L. extract was weighed in 25 ml volumetric flask and filled with 20 ml of methanol. The content in flask was sonicated for 15 minutes and after cooling to room temperature filled to mark with methanol. Aliquot amount of dispersion was centrifuged 5 minutes at 6000 rpm at room temperature. 900 µl of clear supernatant was pipette to auto sampler vial and spiked with 100 µl of internal standard (2-OH stearic acid, conc. about 22.5 µg/ml in methanol). 5µl was injected to HPLC-MS instrument. This procedure is useful for preparation of sample solution from dry extract of Ginkgo biloba or another product containing different amount of extract by choosing the right weight of the sample or volume of flask to obtain concentration of *Ginkgo biloba* extract about 1 mg/ml in methanol.

3.3.3. HPLC conditions

Mobile phase: Filtered mehanol with 0.2% of HCOOH, Flow rate: 0.5 ml/min, Injection: 5 µl, Column temperature: 30 ºC, Isocratic run time: 5 minutes.

RESULTS

The results of analyses of the flavonol glycosides content of examined extracts and products containing *Ginkgo biloba* L. are shown in Table 5. As a requirement of USP the ratio of the flavonol aglycones quercetin/kaempferol/isorhamnetin (Q/K/I) was also determined.

Table 5. Results of HPLC analyses of flavonol glycosides content and the ratio of (Q/K/I) of two commercial extracts (1&2) and 10 products (A-J) containing *Ginkgo biloba* according to USP[(107)].

	Percentage of flavonol glycosides	Ratio of (Q/K/I)
Extract 1	27.4%	1/0.26/0.05
Extract 2	23.7%	1/0.36/0.04
Product A	23.6%	1/0.90/0.20
Product B	26.8%	1/0.84/0.19
Product C	44.4%	1/0.17/0.01
Product D	23.7%	1/0.08/0.02
Product E	27.6%	1/0.11/0.02
Product F	19.3%	1/0.19/0.04
Product G (Batch 1)	11.0%	1/0.54/0.14
Product G*(Batch 2)	14.2%	1/0.34/0.06
Product H	53.3%	Not determined
Product I	6.3%	1/6.13/0.31
Product J	23.7%	1/1.02/0.17

Table 5 shows that only three products (A, B, and J) complied with the USP requirements for flavonol glycosides content (22-27%) as well as the (Q/K/I) ratio (1/0.8-1.2/Not Less Than (NLT) 0.1). Products D and E complied with USP requirements for flavonol glycosides percentage but failed the (Q/K/I) ratio. Products F- I failed both the USP requirements for the flavonol glycosides content and the (Q/K /I) ratio. The two *Ginkgo biloba* L. extracts (1 and 2) though fulfilled the USP requirements for the total

flavonol glycosides, however failed the specified ratio of (Q/K/I). It is interesting to note although most of manufacturers of *Ginkgo* containing products declared fulfillment of their extracts to specifications, however the actual analyses revealed a wide range of flavonol glycosides ranging from 6.3% (product I) to 53.3% (product H)

Table 6. Results of HPLC analyses of 10 products containing *Ginkgo biloba* L. extracts for the quercetin content before and after hydrolysis.

	Percentage of quercetin before hydrolysis (according to the suggested method)	Percentage of quercetin after hydrolysis (according to USP)
Product A	0.09%	5.80%
Product B	0.09%	5.20%
Product C	0.30%	14.90%
Product D	0.50%	8.90%
Product E	0.60%	9.60%
Product F	0.42%	6.07%
Product G (Batch 1)	1.09%	2.60%
Product G*(Batch 2)	0.20%	4.04%
Product H	Not determined	21.25%
Product I	0.04%	0.30%
Product J	2.98%	4.31%

Products A and B which complied with the USP requirements for flavonol glycosides content and (Q/K/I) ratio (Table 5) showed a low percentage of quercetin (0.09%) before hydrolysis and increased percentage of quercetin after hydrolysis (5.2-5.8%. The quercetin percentage of four products (C-F) ranged from (0.3-0.6%) before hydrolysis compared to the (0.09%) of complied products (A and B). However, after hydrolysis quercetin percentage ranged from (6.07 - 14.9%) compared to the (5.2- 5.8 %) of complied products (A & B). For Product G there was no much increase in the percentage of quercetin after hydrolysis compared to the percentage of quercetin before hydrolysis. This indicates an inferior quality product adulterated with quercetin aglycone or hydrolysis. Product I showed low percentage of quercetin before and after hydrolysis. Prodcut J was found to have high percentage of quercetin before hydrolysis (2.98%) compared to quercetin after hydrolysis (4.31%) suggesting adulteration with quercetin or

hydrolysis. Product H contained high percentage of quercetin after hydrolysis (21.25 %) due to the high percentage of troxerutin which upon hydrolysis gives quercetin (Table 6).

Table 7. Results of HPLC analyses of 10 products containing *Ginkgo biloba* L. extracts for the rutin and quercetrin glycosides content before hydrolysis.

	Percentage of rutin	Percentage of quercetrin
Product A	5.00%	6.50%
Product B	4.37%	5.80%
Product C	25.00%	1.54%
Product D	17.40%	0.99%
Product E	17.50%	1.20%
Product F	19.32%	Not determined
Product G (Batch 1)	1.16%	1.40%
Product G* (Batch 2)	8.66%	Not determined
Product H	Supersaturated	supersaturated
Product I	1.20%	1.85%
Product J	1.10%	Not determined

Table 7 shows the results of analyses of rutin and quercetrin in examined products containing *Ginkgo biloba* L. extracts. Products A and B, complied with USP requirements, contained from 4.4 to 5% rutin and from 5.8 to 6.5 % of quercetrin. However products C, D, E, F and G * were found to have varied amount of rutin ranged from 8.66-25%and low percentage of quercetrin ranged from 0.99-1.20%%.Products G, I, and J were found to have low percentage of rutin (1.10-1.20%) and quercetrin.(1.4-1.85%).

Table 8. Ratio of the free aglycones (quercetin and kaempferol) to the intact glycosides quercetrin in the examined *Ginkgo biloba* L. containing products and its indication.

	Ratio of aglycones to glycosides obtained before hydrolysis (quercetin+kaempferol/quercetrin)	Indication
Product A	0.04	No hydrolysis
Product B	0.05	No hydrolysis
Product C	0.81	Hydrolysis
Product D	3.48	Hydrolysis
Product E	1.44	Hydrolysis
Product G	1.48	Hydrolysis
Product H	Not detected	Not detected
Product I	0.06	No hydrolysis
Product J	8.94	Hydrolysis

Table 8 shows the ratio of aglycones to glycosides in extracts of products containing *Ginkgo biloba* L. extracts before hydrolysis and its indication. Products A and B were found to have the lowest ratio (0.04-0.05) suggesting good quality products, while product J were found to have the highest ratio (8.94) suggesting hydrolysis. Also product C, D, E, and G were found to be hydrolyzed having high ratio of aglycones to glycosides (0.81-3.48).

Table 9. Percentage of bilobalide, total ginkgolides and total terpene trilactones in the *Ginkgo biloba* L. containing products assayed by HPLC-MS.

	% Bilobalide	% Sum of ginkgolides A,B, and C	%Terpene trilactones
Product A	3.132	3.351	6.5
Product B	2.115	3.298	5.4
Product C	0.007	0.249	0.3
Product D	0.701	3.392	4.1
Product E	0.336	0.677	1.0
Product G	0.00	2.687	2.7
Product H	0.433	3.665	4.0
Product I	2.076	3.832	5.9

Table 9 shows the results of analyses of eight *Ginkgo biloba* L. containing products for the content of terpene trilactones. Products A, B, and I were found to comply with the USP requirements for the terpene trilactones content. All the other Products Failed the USP requirements.

Table 10. Limits of ginkgolic acids (GAs) found in the examined products assayed by HPLC-MS.

	GA 15:0 (ppm)	GA 15:1 (ppm)	GA 17:1(ppm)	Total Gas (ppm)
Product A	0.00	0.144	0.160	0.304
Product B	0.00	0.100	0.004	0.104
Product C	0.798	14.065	14.497	29.361
Product D	1.570	21.667	23.232	46.468
Product E	23.524	458.163	436.220	917.906
Product G	15.067	479.617	157.829	652.512
Product H	0.00	0.00	0.00	0.00
Product I	63.161	803.236	662.054	1528.452

Content of ginkgolic acids of products A, B, and H was found to be less than 5 ppm, whereas the content of ginkgolic acids in the other products ranged from 29.4 to 1528.5 ppm (Table 10).

Preliminary HPTLC analyses of different products and extracts using equivalent amount of *Ginkgo biloba* L. extract is shown in Figure12 and 13. On the HPTLC plate, the extracts of analysed preparations (1, 2) which are products A and B demonstrated all zones typical for USP standardized extract of *Ginkgo biloba* L. leaves (9).

In the extracts of preparations (1, 2) and in the standard extract (9) the quenching of the characteristic zones was markedly stronger, relative to the zones of all other dietary supplements extracts. Furthermore, extracts of dietary supplements (3, 4, 5, 6, 8) which are products (D, E, C, F, and G*) respectively and *Ginkgo biloba* L. extract (10,11) showed a much stronger quenching in the rutin zone than preparations (1, 2) and *Ginkgo biloba* L. extract (9). Preparation 7 which is product I is an inferior quality product. It has faint bands.

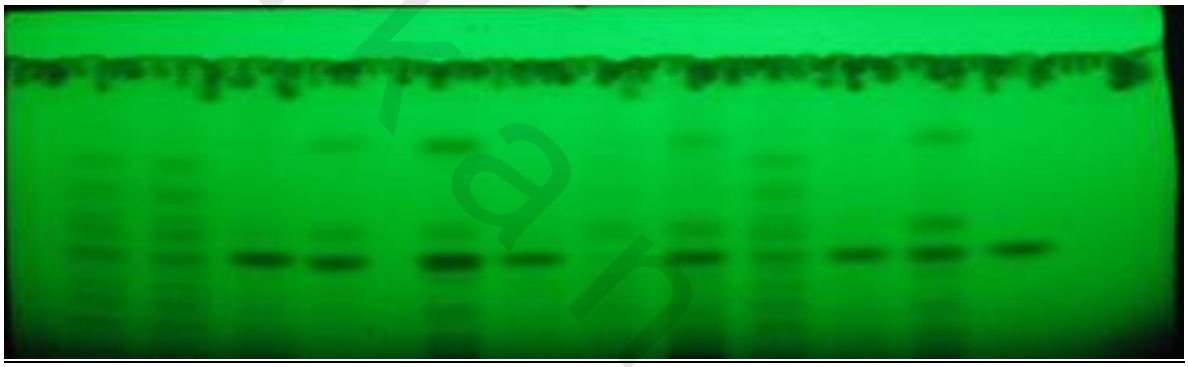


Figure 12.HPTLC chromatograms of unhydrolyzed extracts from preparations (1-8), *Ginkgo biloba* L. extracts (9-11) and standard rutin (12) in UV 254 nm.

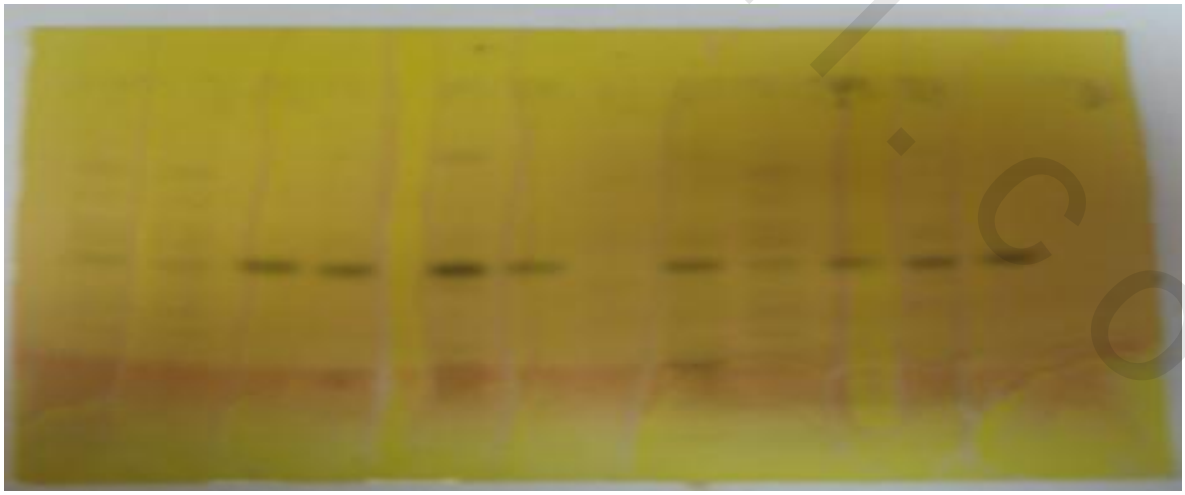


Figure 13. HPTLC chromatograms of unhydrolyzed extracts from preparations (1-8), *Ginkgo biloba* L. extracts (9-11) and standard rutin (12) using ferric chloride reagent.

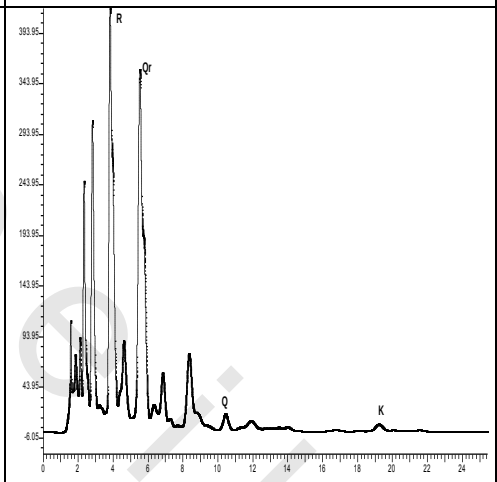
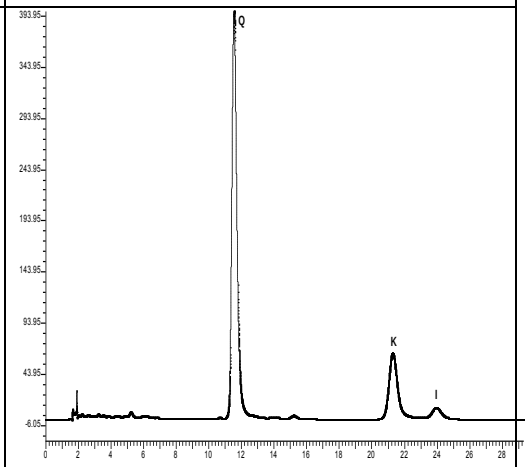
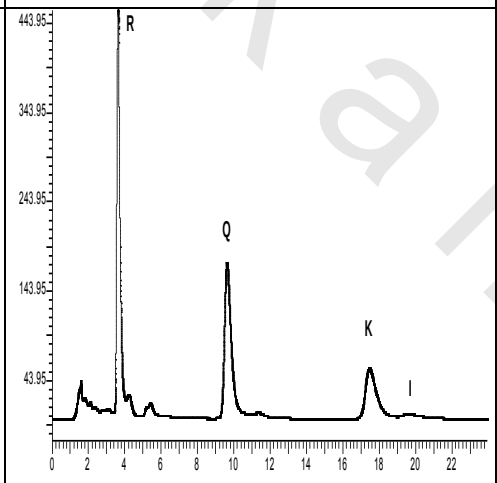
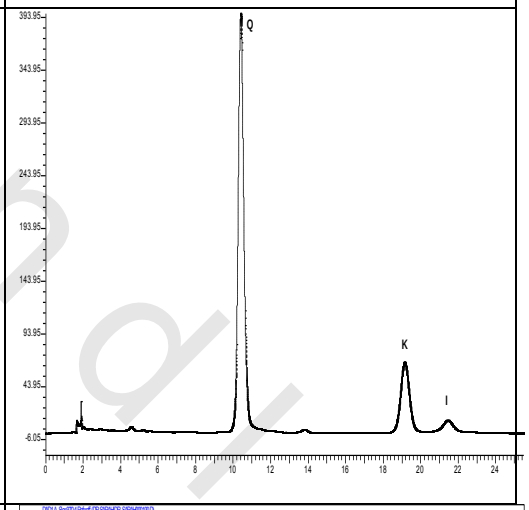
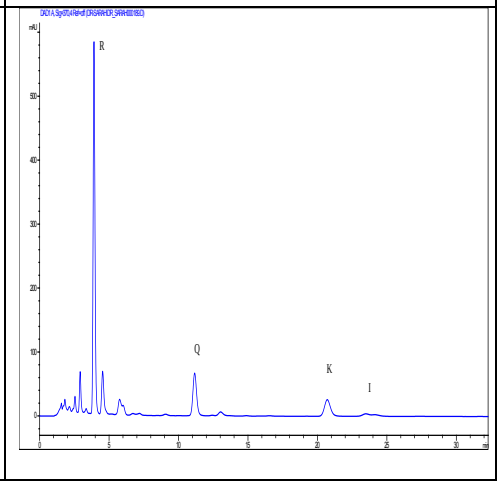
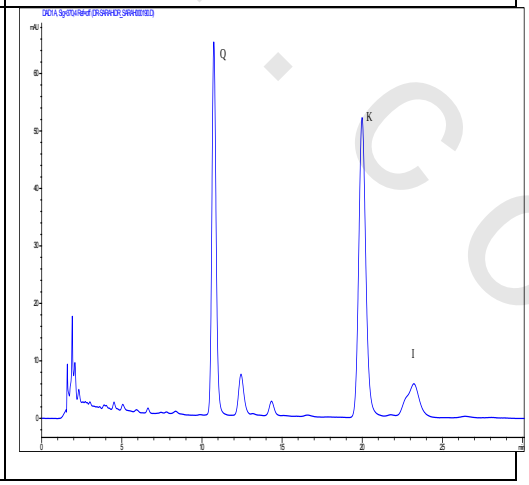
	HPLC fingerprints before hydrolysis	HPLC fingerprints after hydrolysis
USP *Ginkgo* extract		
1		
2		

Figure 15. HPLC fingerprints of USP standardized *Ginkgo biloba* L. extract and two *Ginkgo biloba* L. extracts obtained from different manufacturers of dietary supplements in Egypt (1and 2) before and after hydrolysis.

In Figure 15 the HPLC fingerprints of USP standardized extracts showed in the pre-hydrolysis step a number of prominent peaks mainly due to glycosides of which rutin and quercetrin are prominent. However, the fingerprint revealed the presence of minute quantities of the aglycones quercetin, kaempferol, and isorhamnetin . HPLC fingerprints of extracts 1 and 2 revealed three prominent peaks corresponding to the glycosides rutin and the aglycones quercetin and kaempferol. Unlike the USP standardized *Ginkgo biloba* L. extract, extracts 1and 2 showed no other peaks in the region of the primary glycosides of *Ginkgo biloba* L..

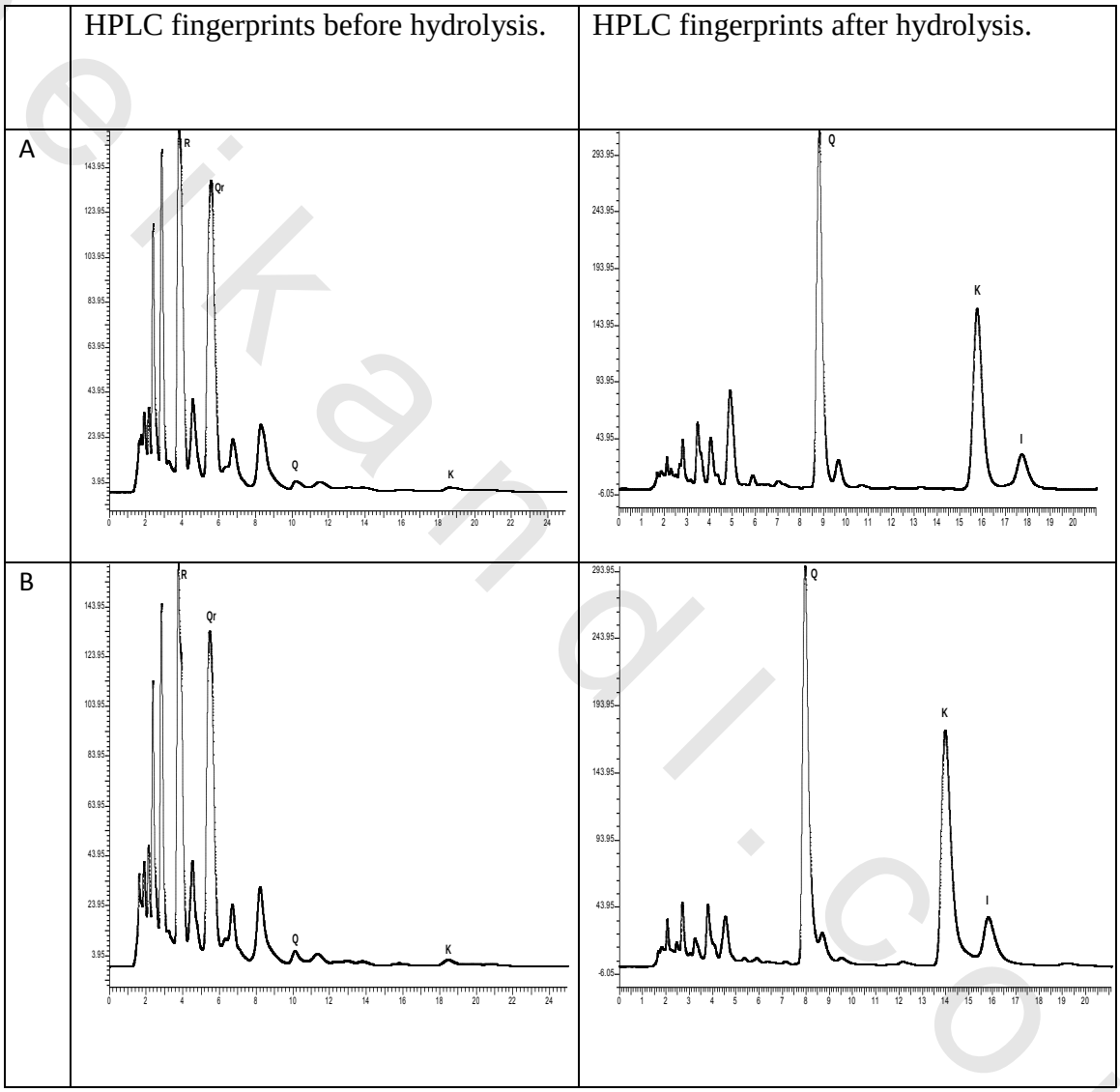


Figure 16. HPLC fingerprints of products A and B before and after hydrolysis.

Figure 16 shows the HPLC fingerprints of products A and B. Products A and B were found to have the same fingerprint of the USP standardized extract with the same prominent peaks of primary glycoside.

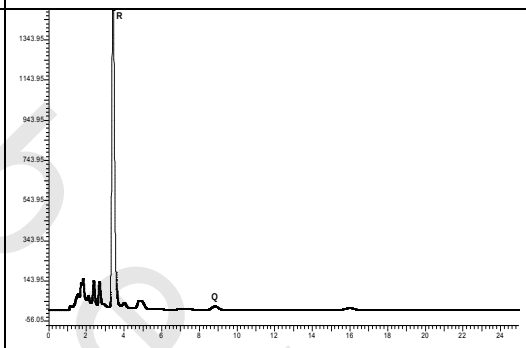
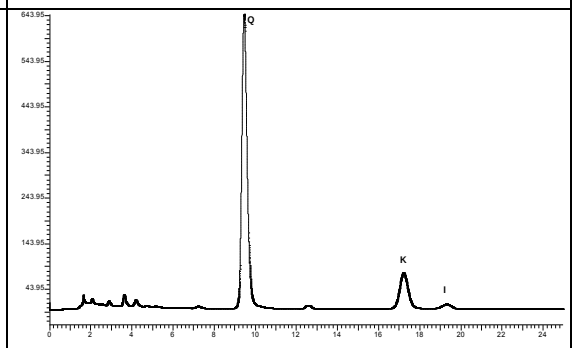
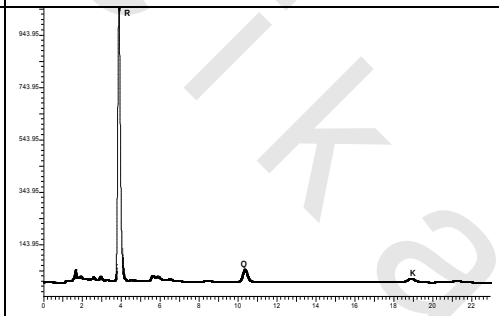
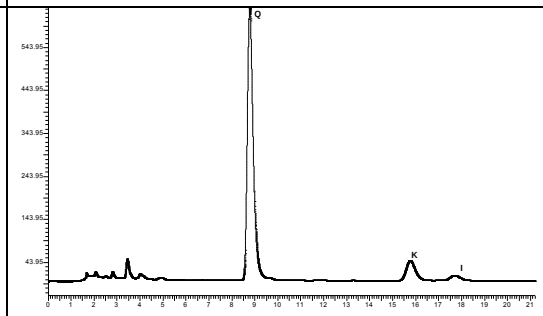
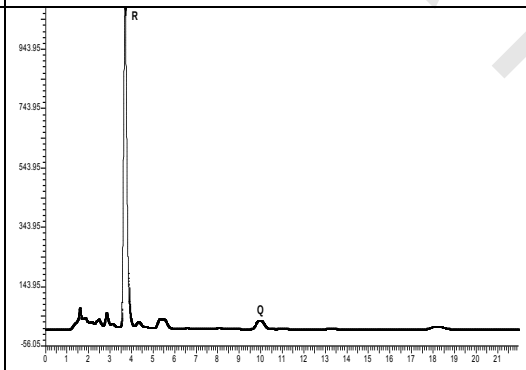
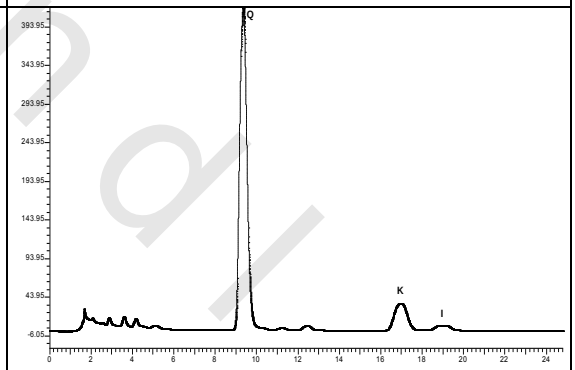
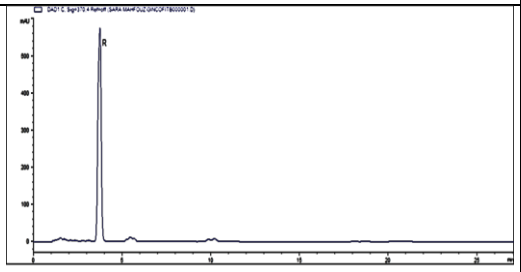
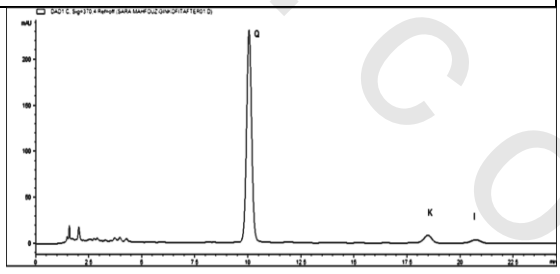
	HPLC fingerprints before hydrolysis.	HPLC fingerprints after hydrolysis.
C		
D		
E		
F		

Figure 17. HPLC fingerprints of products C, D, E, and F before and after hydrolysis.

HPLC fingerprints of products C, D, E, and F are shown in Figure 17. The four products showed only one prominent peak due to rutin, unlike the USP standardized *Ginkgo biloba* L. extract.

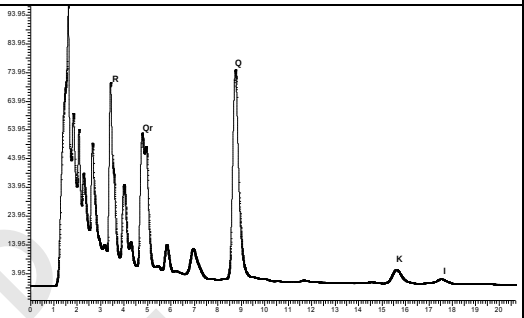
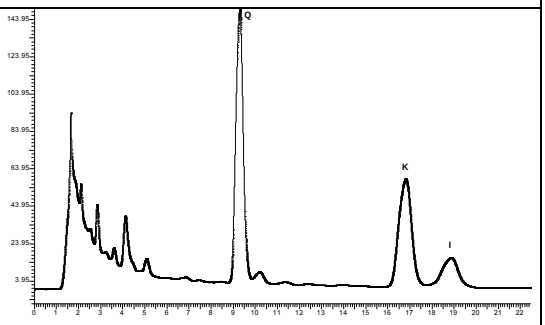
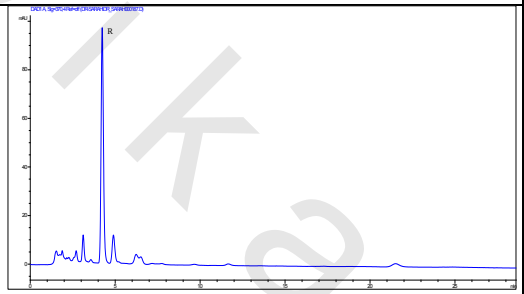
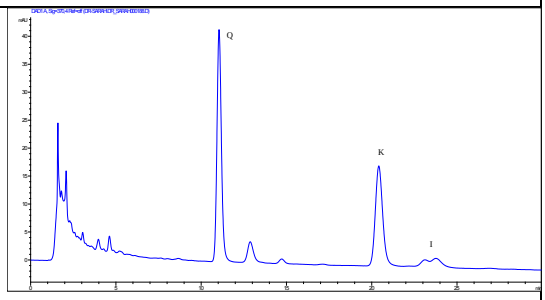
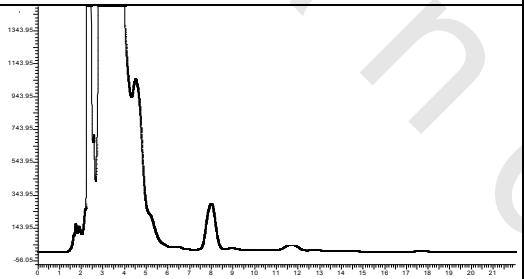
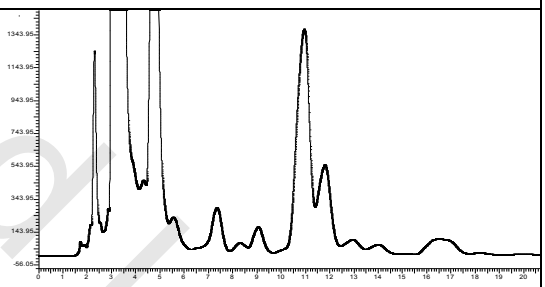
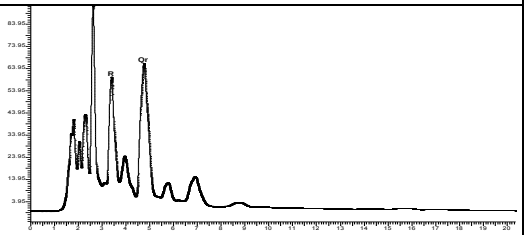
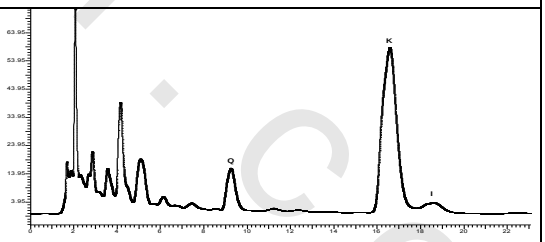
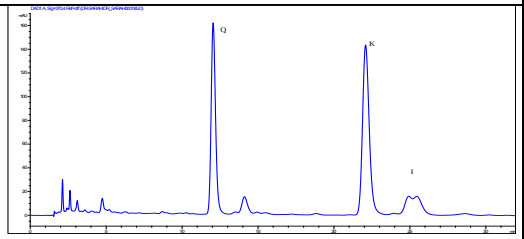
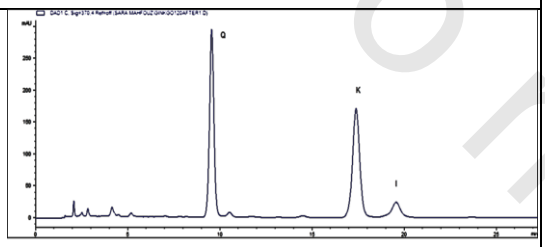
	HPLC fingerprints before hydrolysis.	HPLC fingerprints after hydrolysis
G		
G*		
H		
I		
J		

Figure 18. HPLC fingerprints of products G, G*, H, I, and J before and after hydrolysis.

Although products G and G* are two different batches of the same product, Product G showed prominent peak due to quercetin and G* showed one due to rutin. HPLC fingerprint of product H was of no value due to the interference of other constituents in the product namely trihydroxyethyl rutin which masked the peaks due to the primary glycosides. Product I showed prominent peak of unkown flavonol glycosides. It is reflected in the 6 times increase in the ratio of kaempferol to quercetin (Table 5). HPLC fingerprint of product J before hydrolysis showed prominent peaks due to quercetin, kaempferol, and isorhamnetin (Figure 18).

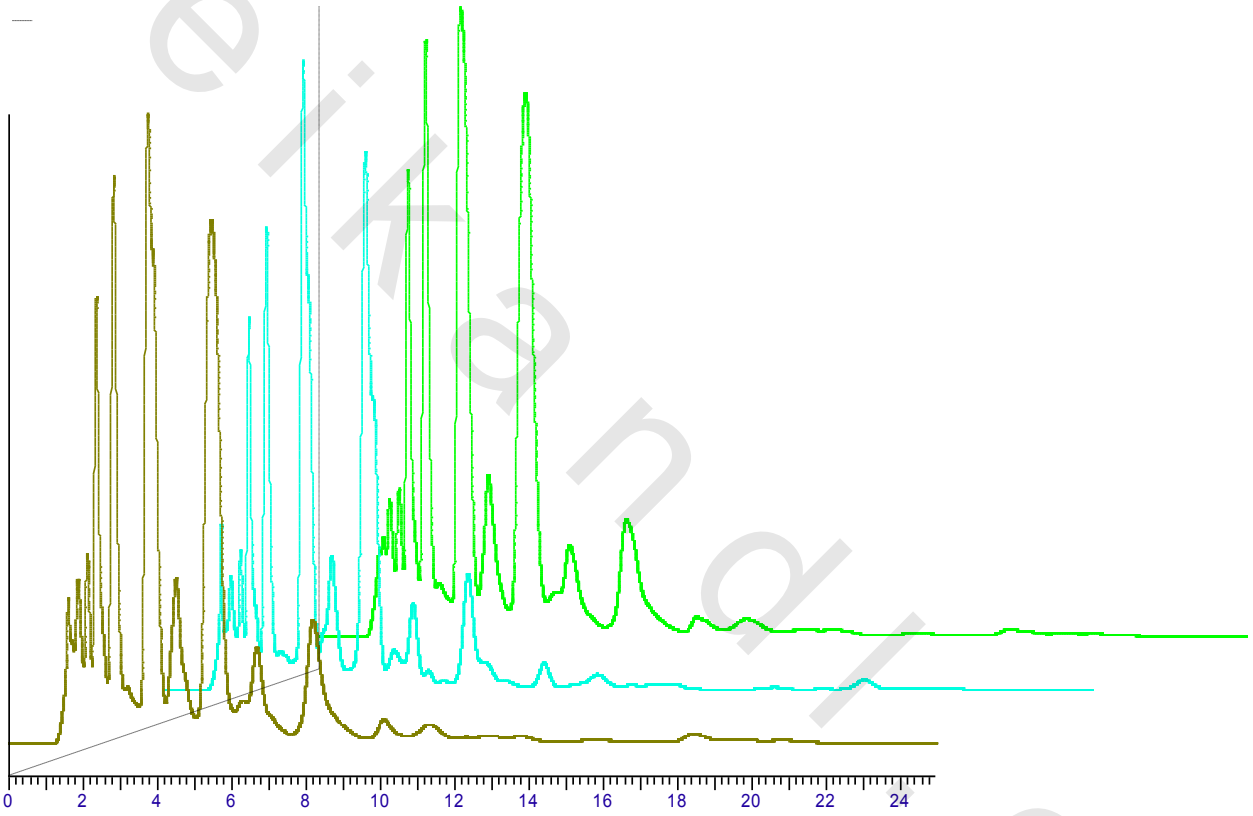


Figure 19. Overlay HPLC fingerprints of product A (green), product B (olive green), and *Ginkgo biloba* L. standardized extract (blue).

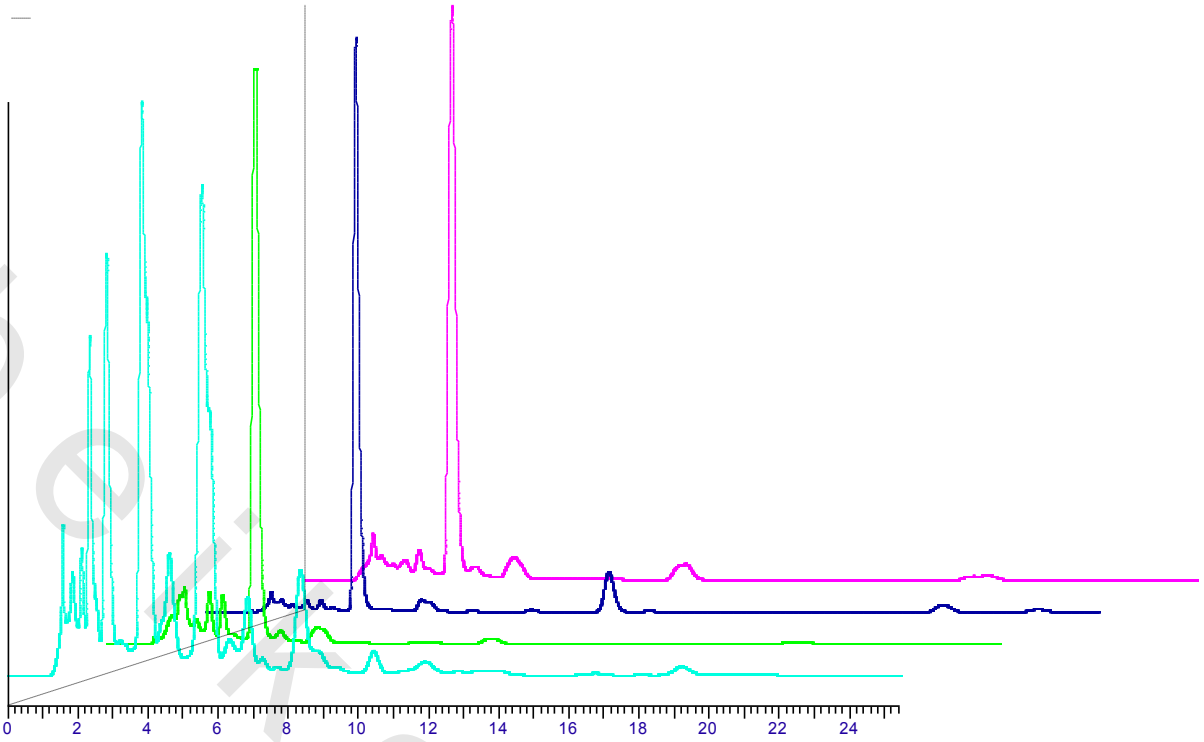


Figure 20. Overlay HPLC fingerprints of product C (Navy), product D (purple), product E (green), and *Ginkgo biloba* L. extract (blue).

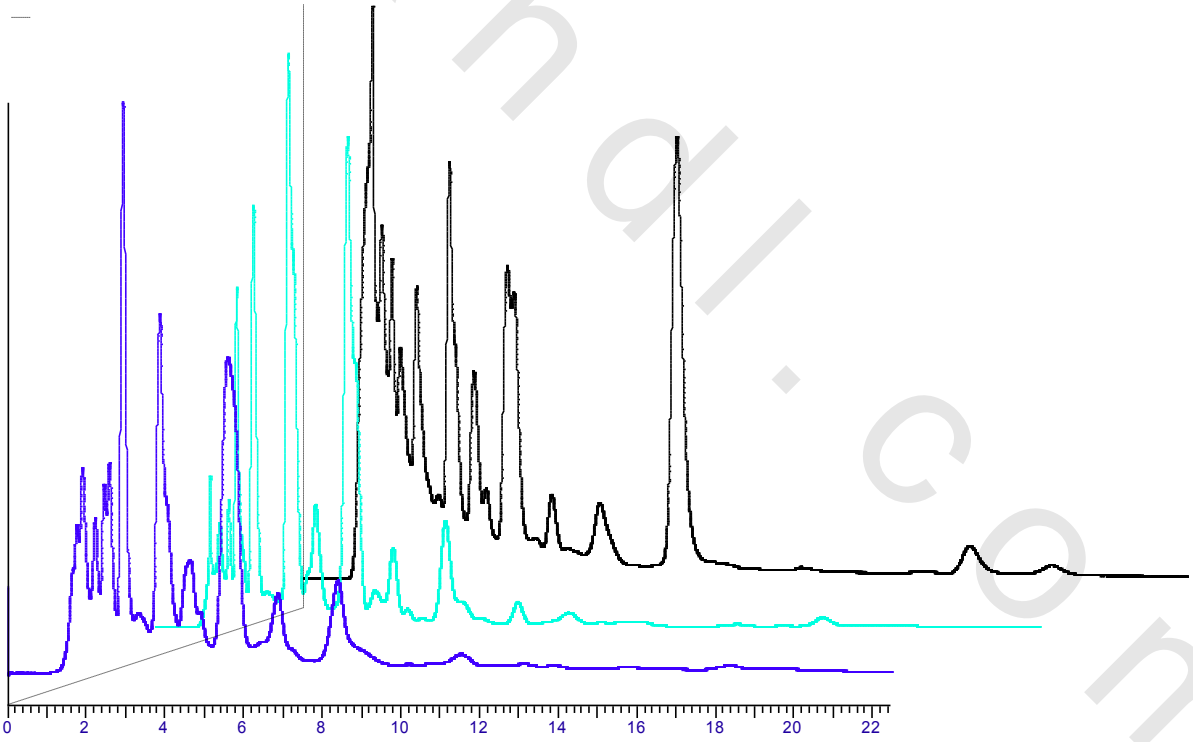
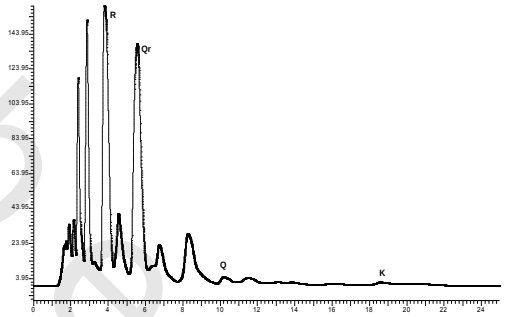
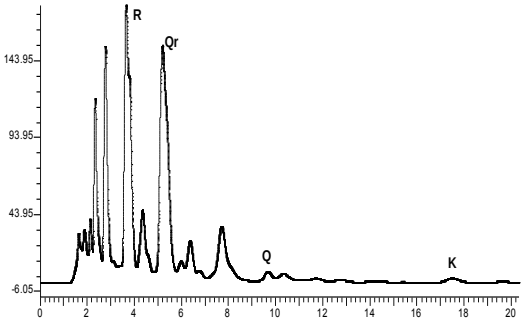
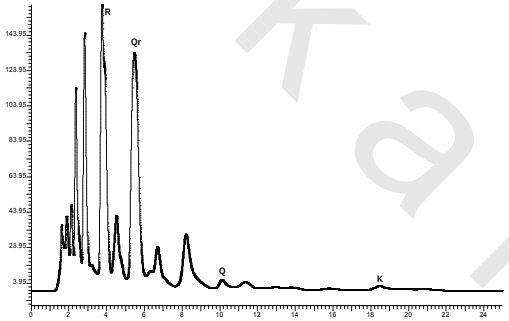
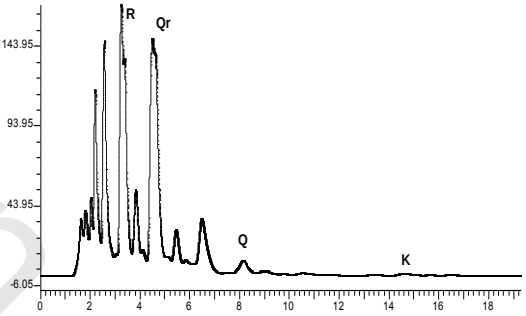
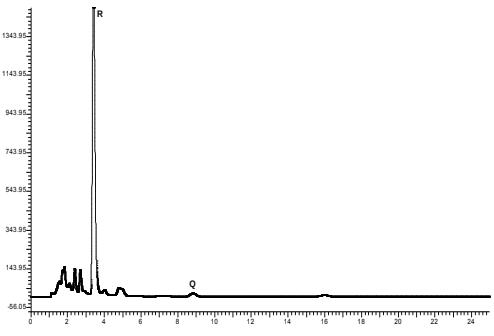
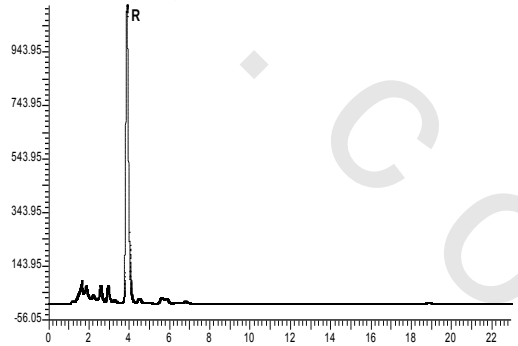


Figure 21. Overlay HPLC fingerprints of product G (black), product I (Navy), and *Ginkgo biloba* L. extract (blue).

	Zero time	After 6 months
A		
B		
C		

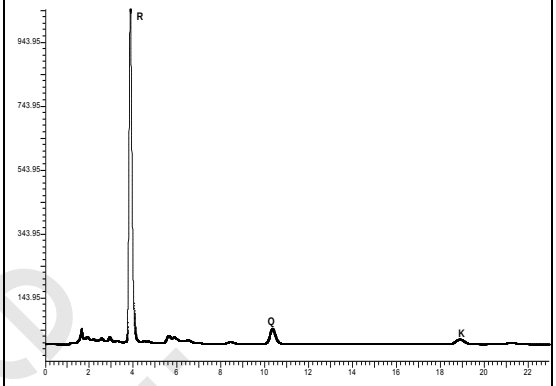
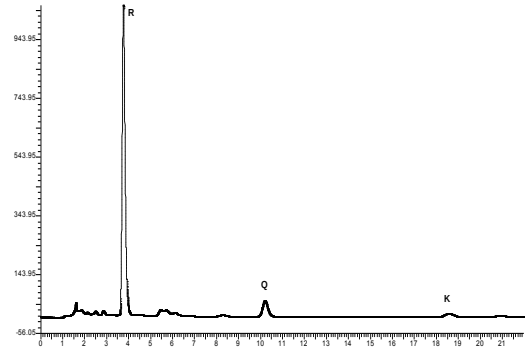
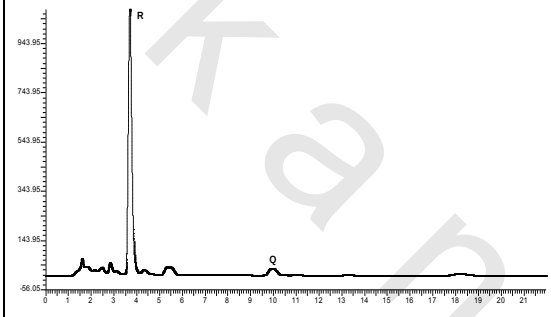
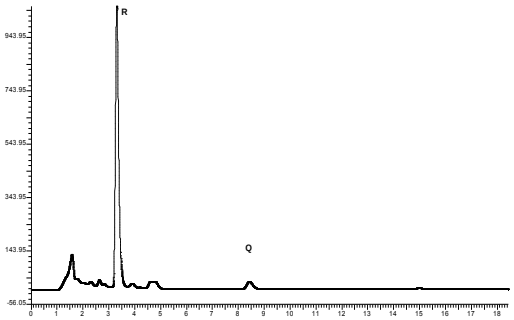
	Zero time	After 6 months
D		
E		

Figure 22. Shows HPLC fingerprints results of five products containing *Ginkgo biloba* L. at zero time and after 6 months.

Table 11. Results of analyses of five products for the rutin and quercetrin contents at zero time and after 6 months.

	Percentage of rutin			Percentage of quercetrin		
	% at zero time	% after 6 months	% difference	% at zero time	% after 6 months	% difference
Product A	5.00%	3.80%	24.00%	6.50%	3.8%	41.53%
Product B	4.37%	3.07%	29.74%	5.80%	3.9%	32.75%
Product C	25%	7.1%	71.60%	1.54%	0.54%	64.93%
Product D	17.4%	6.03%	63.39%	0.99%	0.42%	57.57%
Product E	17.5%	12.6%	28.00%	1.2%	0.7%	41.66%

Stability studies carried on the examined products (during the self-life) at 40 °C and 75 % RH revealed that the accelerated stability study has an effect on the quality of the G. biloba containing products. However extensive stability studies are further requested. Products C, D, and E were found to be adulterated with rutin and had reduced amounts of *Ginkgo* extracts. Thus the accelerated stability study did not reflect the stability of the extracts.