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Qualitative and quantitative determination of the effective components of the plants in different herbal slimming products in Turkey by HPLC

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Abstract: Obesity is a serious disease that can be due to genetic and environmental reasons and is defined by the World Health Organization as abnormal or excessive fat accumulation that may impair health. There is an increasing trend towards herbal slimming products in obesity treatment. The quality control analysis of these products is important for public health. In this study, new, simple, and sensitive high performance liquid chromatography methods were developed for qualitative and quantitative determination of arbutin, hydroquinone, ursolic acid, chlorogenic acid, epigallocatechin gallate, epigallocatechin, epicatechin gallate, and catechin in eleven herbal slimming products in Turkey containing *Camellia sinensis*, *Ilex paraguariensis*, and *Calluna vulgaris*. The intra- and interday precisions, stated as the relative standard deviation, were less than 2% and accuracy, based on relative error, was less than 10%. The limits of detection and quantification for arbutin and hydroquinone were 0.80 and 2.40 µg/mL and for the other compounds were 0.30 and 0.90 µg/mL, respectively. The methods developed can be used for routine quality control analysis of these effective compounds in different herbal slimming products.

Key words: High performance liquid chromatography, herbal slimming product, *Camellia sinensis*, *Ilex paraguariensis*, *Calluna vulgaris*

1. Introduction

Obesity is a serious disease that can be due to genetic and environmental reasons.[1] It is an important risk factor for a range of diseases like metabolic syndrome, type 2 diabetes, hypertension, hyperlipidemia, and cancer.[2,3] Pharmacological treatment for obesity could be considered after dietary restrictions, physical exercise, and lifestyle modifications.[3,4] Drugs such as phentermine, dexfluramine, orlistat, rimonabant, and sibutramine have been used in pharmacotherapy for obesity.[4,5] The synthetic medicines for obesity treatment have several adverse effects, namely headache, insomnia, nausea, diarrhea, flatulence, abdominal pain, dyspepsia, and increased risk of heart attack and stroke, and so there is an increasing trend towards herbal products in this field.[6,7]

For many years, herbal medicines have been used for the prevention and treatment of diseases. Natural products may be a good alternative approach for the development of effective and safe antiobesity drugs. Crude plant extracts and isolated pure natural compounds could be used in obesity treatment. Natural products with lipase inhibitory, appetite suppressant, energy expenditure stimulant, adipocyte differentiation inhibitory, and

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lipolysis-enhancing effects have an important role in treating obesity.[7] *Camellia sinensis*, *Ilex paraguariensis*, and *Calluna vulgaris* are some of the plants in herbal products for obesity treatment.

Camellia sinensis (L.) Kuntze (green tea) is an evergreen shrub grown in China, India, Sri Lanka, Japan, Indonesia, Kenya, Turkey, Pakistan, and Argentina.[8] It is a member of the family Theaceae. The leaves of *C. sinensis* contain caffeine and various phenolic compounds (e.g., epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, quercetin, and myricetin).[9] The antiobesity effects of *C. sinensis* have been correlated with its catechin content.[10] *C. sinensis* has demonstrated an antiobesity effect by reducing nutrient absorption and modifying appetite. Green tea catechins have increased energy expenditure and promoted fat oxidation. It has been shown that green tea catechins decreased glucose absorption by inhibition of α-amylase and α-glucosidase enzymes.[11]

Ilex paraguariensis A.St.-Hil. (mate) is a South American plant and a member of the family Aquifoliaceae. The medicinal parts of the plant are dried or roasted leaves. *I. paraguariensis* contains chlorogenic acid, caffeine, theobromine, rutin, isoquercitrin, kaempferol glycosides, and triterpene saponins (ursolic acid and mate saponins).[8] *I. paraguariensis* decreases body weight, adiposity, cholesterol levels, and leptin release by adipose tissue.[12] Ursolic acid showed reduced lipid accumulation in the livers of mice and it gave rise to decreases in liver lipid depositions.[13] Furthermore, ursolic acid increased lipolysis in rat fat cells.[14] It has been reported that chlorogenic acid has an inhibitory effect on cell population growth in preadipocytes.[15]

Calluna vulgaris (L.) Hull. (heather) is a perennial shrub of the family Ericaceae distributed in Europe, Russia, Asia Minor, and the Atlantic coast of North America. The medicinal parts of *C. vulgaris* are the complete herb with leaves and flowers. Secondary metabolites of heather are flavonoids, tannins, proanthocyanidins, caffeic acid derivatives, phenols, triterpenes, steroids, and hydroquinone glycosides (arbutin).[4] *C. vulgaris* has been used as a diuretic.[16] Hydroquinone is the metabolite of arbutin that has a diuretic effect.[17,18]

To the best of our knowledge, there is no study about the qualitative and quantitative determination of the effective components of *C. sinensis*, *I. paraguariensis*, or *C. vulgaris* in different herbal slimming products by high performance liquid chromatography (HPLC). In the present study, rapid, precise, and accurate HPLC methods were developed and validated for eight compounds, i.e. epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), catechin, ursolic acid, chlorogenic acid, arbutin, and hydroquinone, in eleven herbal slimming products in Turkey containing *C. sinensis*, *I. paraguariensis*, and *C. vulgaris.*

2. Results and discussion

2.1. Method development and optimization

For the analysis of arbutin and hydroquinone, the composition of the mobile phase was methanol:water (10:90, h/h). The best peak resolution was at 280 nm.

For the analysis of ursolic acid, the composition of the mobile phase was methanol:water (95:5, h/h). Detection was at 215 nm.

For the analysis of chlorogenic acid, the composition of solvents was: (A) water:acetic acid (98.5:1.5) and (B) methanol. The mobile phase composition was 75% of solvent A and 25% of solvent B. Data were obtained at 325 nm.

The composition of solvents for the analysis of (–)-EGCG was: (A) water:formic acid (99.9:0.1) and (B) methanol. The mobile phase composition was 70% of solvent A and 30% of solvent B. The appropriate wavelength for detection was selected as 280 nm.

The composition of solvents for the analysis of (–)-EGC was: (A) water:formic acid (99.9:0.1) and (B) methanol. The mobile phase composition was 80% of solvent A and 20% of solvent B. Detection was at 210 nm.

The composition of solvents for the analysis of (–)-ECG was: (A) water:formic acid (99.9:0.1) and (B) methanol. The mobile phase composition was 65% of solvent A and 35% of solvent B. Data were obtained at 280 nm.

The composition of solvents for the analysis of (+)-catechin was: (A) water:formic acid (99.9:0.1) and (B) methanol. The mobile phase composition was 80% of solvent A and 20% of solvent B. The best peak resolution was at 280 nm.

The column temperature was 30 °C, the flow rate was 1 mL/min, and the injection volume was 10 µL for all analytes.

2.2. Validation of the method

The analytical HPLC methods were validated in terms of linearity, precision, accuracy, limits of detection, and quantification.

The linearity of the calibration curve for arbutin and hydroquinone was demonstrated in the concentration range 2.5–125 µg/mL. The linearity range for chlorogenic acid, (–)-EGCG, (–)-EGC, (–)-ECG, and (+)-catechin was 1–100 µg/mL and for ursolic acid it was 1–150 µg/mL. The calibration curves constructed were evaluated according to their correlation coefficients. The linear regression equations and correlation coefficient values of the compounds are given in Table 1.

Table 1. Features of the calibration curves of arbutin, hydroquinone, ursolic acid, chlorogenic acid, (–)-EGCG, (–)-EGC, (–)-ECG, and (+)-catechin.

Standard compounds	Linearity (µg/mL)	Regression equation[a]	Correlation coefficient (R)	RSD (%)	Relative error (%)	Recovery (%)	LOD (µg/mL)	LOQ (µg/mL)
Arbutin	2.5–125	y = 6.654x – 5.0492	0.9916	0.51	8.25	91.75	0.80	2.40
Hydroquinone	2.5–150	y = 9.120x – 6.6006	0.9998	0.30	0.80	99.20	0.80	2.40
Ursolic acid	1–150	y = 5.973x + 28.774	0.9981	0.38	3.08	96.92	0.30	0.90
Chlorogenic acid	1–100	y = 33.490x + 90.657	0.9956	0.59	1.84	98.16	0.30	0.90
(–)-EGCG	1–100	y = 12.797x + 81.612	0.9806	0.90	2.16	97.84	0.30	0.90
(–)-ECG	1–100	y = 18.953x + 25.783	0.9987	0.51	0.55	99.45	0.30	0.90
(–)-EGC	1–100	y = 90.919x + 210.780	0.9936	2.12	1.73	98.27	0.30	0.90
(+)-Catechin	1–100	y = 7.634x + 9.0115	0.9993	0.33	0.40	99.60	0.30	0.90

[a]Based on calibration curves, y: peak height, x: concentration.

The precision of the HPLC methods was obtained by repeatability (intraday) and intermediate precision (interday) and it was determined by relative standard deviation (RSD). The accuracy of the methods was evaluated as the percentage relative error. For all the concentrations studied, intra- and interday RSD values were $\leq$2% and the relative errors were $\leq$10%. To determine the accuracy of the HPLC methods and to study the interference of herbal slimming product formulation additives, the recovery was checked at three different concentration levels and analytical recovery experiments were performed by adding known amounts of pure standard compounds to preanalyzed samples of herbal slimming product extracts. The percentage recovery obtained for the herbal slimming product extracts was between 91.75% and 99.60%. The results are given in Table 1.

Limits of detection (LOD) and quantification (LOQ) for the studied compounds were detected by the

signal-to-noise ratio. The signal-to-noise ratios were 3 and 10, respectively, by analysis of a range of diluted standard solutions in terms of 10-µL injection. The results are shown in Table 1.

The analytical method for arbutin and hydroquinone was validated as described in U.S. Pharmacopoeia (2008), for ursolic acid the method was validated as described by Xu et al., for chlorogenic acid the method was validated as described by Grujic et al., and for (–)-EGCG, (–)-EGC, (–)-ECG, and (+)-catechin the methods were validated as described by Wang et al.[19–21]

2.3. Application of methods to herbal slimming products

The developed HPLC methods were used for qualitative and quantitative determination of effective components of the plants in different herbal slimming products. The amounts of components were calculated from the calibration curve. The retention times of arbutin, hydroquinone, ursolic acid, chlorogenic acid, (–)-EGCG, (–)-EGC, (–)-ECG, and (+)-catechin were 7.1, 9, 9.1, 8.9, 7.9, 10.8, 9.1, and 11.7, respectively (Figure). The mean amounts and percentages of the compounds found in the different herbal slimming products are given in Tables 2 and 3. The HPLC chromatograms of products 2–4 (10 mg/mL) are shown in the Supplement.

Some of the related methods were evaluated with a literature survey. An HPLC method was reported for separating and determining arbutin and hydroquinone from different plant extracts.[22] In this method, the calibration curve of the HPLC method was linear for arbutin and hydroquinone in the range 7.5–500 µg/mL and 1.4–98 µg/mL, respectively. Intra- and interday precision, expressed as the RSD, were less than 3.23% and 3.27% for arbutin and hydroquinone, respectively. The proposed method indicated high precision and sensitivity when compared with another method.[22] The LOD value of the reported method was 1.01 µg/mL, whereas the present method's LOD was 0.80 µg/mL; the LOQ of the reported method was 3.07 µg/mL, whereas the present method's LOQ was 2.40 µg/mL for arbutin.

In an HPLC method for the analysis of tea catechins in vegetable oils, the calibration curve was linear for catechins in the range 0.05–100 µg/mL.[23] Intra- and interday precision, expressed as the RSD, was less than 5.0% for EGCG, EGC, ECG, and catechin. Furthermore, an HPLC method was reported for the analysis of catechins in tea samples.[24] The LOD and LOQ values of this method were, respectively, 1.3 and 4.6 µg/mL for EGC, 0.4 and 1.3 µg/mL for catechin, and 1.4 and 4.8 µg/mL for EGCG. The present method's LOD and LOQ were 0.30 and 0.90 µg/mL, respectively. The proposed methods indicated high precision and sensitivity when compared with other methods in the literature.[23–27]

Product 11 was a *Camellia sinensis* tea sample. Chlorogenic acid is a component of *C. sinensis* but it was determined that product 11 did not contain any chlorogenic acid (Table 2). Due to these confusing results, in our opinion, the quality and reliability of some commercially available herbal slimming products are controversial.

In an HPLC method for the determination of ursolic acid in different parts of plant material, the calibration curve was linear for ursolic acid in the range 33.24–664.8 µg/mL.[28] Intra- and interday precision, expressed as the RSD, was less than 1.2% for ursolic acid. The LOD value of this method was 0.39 µg/mL and the present method's LOD was 0.30 µg/mL. An HPLC method was reported for quality control of Semen Strychni.[29] Intra- and interday precision, expressed as the RSD, was less than 3.50% and accuracy (relative error) was better than 3.00%. The proposed methods indicated high precision and sensitivity when compared with other methods in the literature.[19,20,26,28–32]

The advertisements and easy accessibility of herbal products even from markets and the Internet lead to

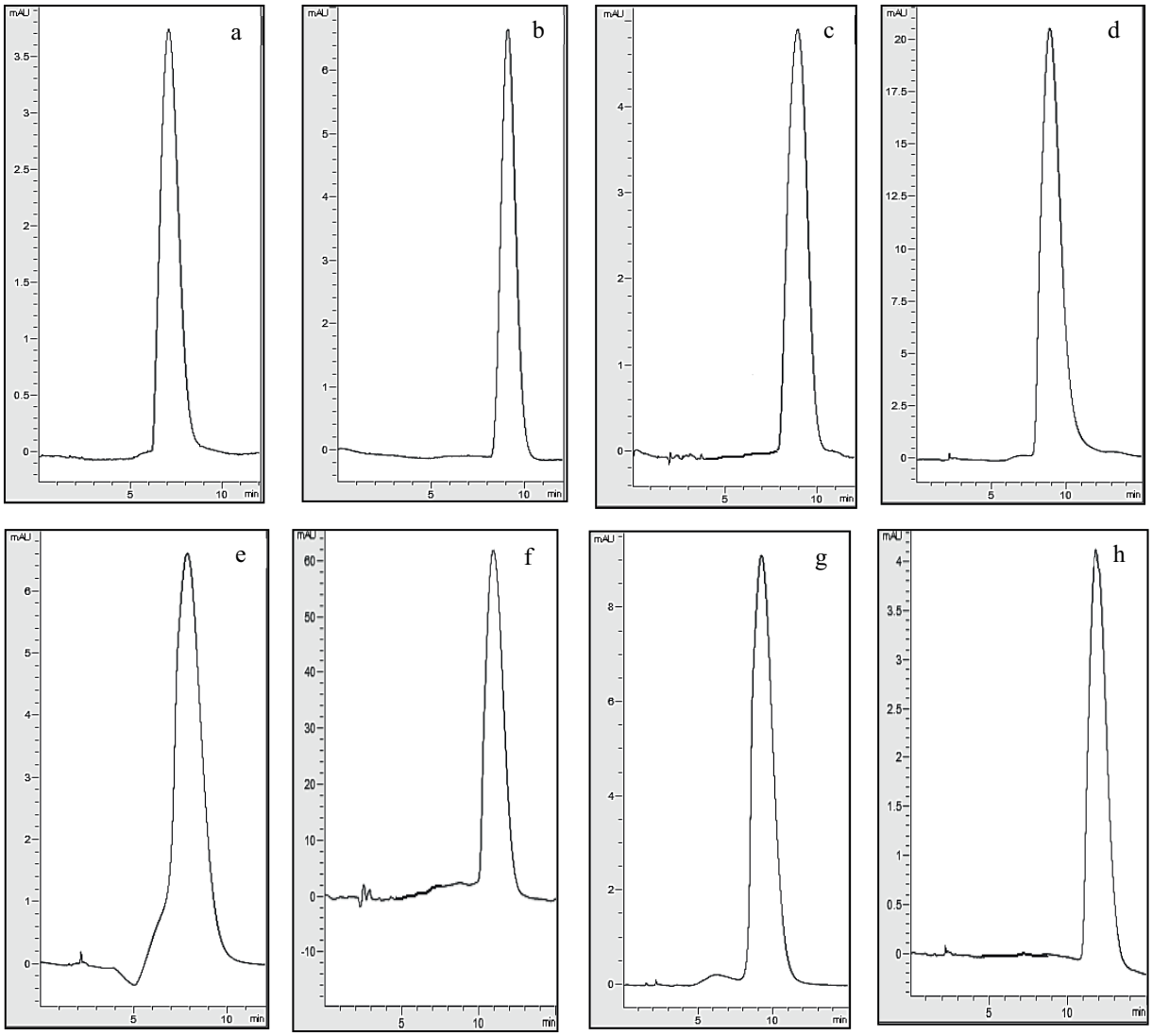

Figure. The HPLC chromatograms of arbutin (a), hydroquinone (b), ursolic acid (c), chlorogenic acid (d), (–)-EGCG (e), (–)-EGC (f), (–)-ECG (g), and (+)-catechin (h).

their widespread usage. The effectiveness of herbal slimming products and their compliance to standards is a matter for discussion. Therefore, the quality control analysis of these products is important for public health. In the present work, new, simple, and sensitive HPLC methods were developed for the qualitative and quantitative determination of the effective components in different herbal slimming products. The methods were validated for linearity, precision, accuracy, and limits of detection and quantification and can be used for routine quality control of herbal raw materials.

3. Experimental

3.1. Chemicals and reagents

HPLC-grade methanol (France) and formic acid (Germany) were purchased from Sigma-Aldrich, while acetic acid (Germany) was obtained from Riedel-de Haen.

Table 2. Amounts of arbutin, hydroquinone, ursolic acid, and chlorogenic acid in different herbal slimming products (10 mg/mL).

Sample name (10 mg/mL)		Arbutin (µg/mL)	% conc.	Hydroquinone (µg/mL)	% conc.	Ursolic acid (µg/mL)	% conc.	Chlorogenic acid (µg/mL)	% conc.
1	Aqueous extract	n.d.	-	+	0.054	n.d.	-	+	0.010
	Methanol extract	+	0.019	n.d.	-	+	5.324	n.d.	-
2	Aqueous extract	+	0.085	+	0.056	n.d.	-	+	0.306
	Methanol extract	n.d.	-	+	0.034	+	5.844	+	0.331
3	Aqueous extract	+	0.098	n.d.	-	n.d.	-	+	0.273
	Methanol extract	n.d.	-	n.d.	-	+	5.797	+	0.470
4	Aqueous extract	+	0.018	+	0.507	n.d.	-	+	0.012
	Methanol extract	+	0.028	+	0.035	+	1.036	+	0.017
5	Aqueous extract	+	0.210	+	0.018	n.d.	-	+	0.357
	Methanol extract	+	0.012	+	0.012	+	4.048	+	0.164
6	Aqueous extract	+	0.013	+	0.011	n.d.	-	+	0.012
	Methanol extract	+	0.050	+	0.011	+	7.269	+	0.023
7	Aqueous extract	+	0.012	+	0.045	-	-	-	-
	Methanol extract	n.d.	-	n.d.	-	-	-	-	-
8	Aqueous extract	+	0.017	+	0.175	n.d.	-	+	0.018
	Methanol extract	+	0.021	n.d.	-	+	1.483	n.d.	-
9	Aqueous extract	+	0.044	+	0.023	-	-	-	-
	Methanol extract	n.d.	-	n.d.	-	-	-	-	-
10	Aqueous extract	-	-	-	-	n.d.	-	+	2.398
	Methanol extract	-	-	-	-	+	5.679	+	1.383
11	Aqueous extract	-	-	-	-	-	-	-	-
	Methanol extract	-	-	-	-	-	-	-	-

conc: concentration, n.d.: not determined.
(+): The analyte is present in sample, (-): The analyte is not present in sample.

(–)-EGCG (Germany), (–)-EGC (India), (–)-ECG (China), (+)-catechin (Germany), ursolic acid (France), chlorogenic acid (Germany), arbutin (UK), and hydroquinone (Switzerland) were purchased from Sigma-Aldrich.

3.2. Plant materials and pharmaceutics

Eleven herbal slimming products containing *Camellia sinensis* (L.) Kuntze, *Ilex paraguariensis* A.St.-Hil., and *Calluna vulgaris* (L.) Hull. were procured from the Internet and different pharmacies in Turkey. The herbal slimming products 1 to 7 were mixed herbal teas and product 8 was a slimming tea capsule. The products 9, 10, and 11 were *C. vulgaris*, *I. paraguariensis*, and *C. sinensis* teas, respectively.

Products 1 to 9 contained *C. vulgaris*, products 1 to 10 (except 7 and 9) contained *I. paraguariensis*, and products 1 to 11 (except 5, 7, 9, and 10) contained *C. sinensis*.

3.3. Extraction of the herbal slimming products

Ten grams of each product was extracted with distilled water and methanol (100 mL × 2) separately at 40 °C for 30 min. The extracts were filtered. Then the aqueous extracts were cooled at –80 °C and lyophilized. The methanol was evaporated to dryness and the methanol extracts were obtained.

Table 3. Amounts of (–)-EGCG, (–)-ECG, (–)-EGC, and (+)-catechin in different herbal slimming products (10 mg/mL).

Sample name (10 mg/mL)		EGCG (µg/mL)	% conc.	EGC (µg/mL)	% conc.	ECG (µg/mL)	% conc.	Catechin (µg/mL)	% conc.
1	Aqueous extract	+	0.013	n.d.	-	n.d.	-	n.d.	-
	Methanol extract	+	0.035	+	0.011	n.d.	-	n.d.	-
2	Aqueous extract	+	0.055	+	0.234	n.d.	-	n.d.	-
	Methanol extract	+	0.215	+	0.779	n.d.	-	n.d.	-
3	Aqueous extract	+	0.069	+	0.617	n.d.	-	n.d.	-
	Methanol extract	+	0.241	+	0.862	n.d.	-	n.d.	-
4	Aqueous extract	n.d.	-	n.d.	-	+	0.090	n.d.	-
	Methanol extract	n.d.	-	n.d.	-	+	0.009	n.d.	-
5	Aqueous extract	+	0.001	n.d.	-	n.d.	-	n.d.	-
	Methanol extract	+	0.045	n.d.	-	n.d.	-	n.d.	-
6	Aqueous extract	+	0.028	n.d.	-	n.d.	-	n.d.	-
	Methanol extract	+	0.054	+	0.176	n.d.	-	n.d.	-
7	Aqueous extract	+	0.037	n.d.	-	n.d.	-	n.d.	-
	Methanol extract	+	0.054	n.d.	-	n.d.	-	n.d.	-
8	Aqueous extract	+	0.015	n.d.	-	n.d.	-	+	0.106
	Methanol extract	n.d.	-	n.d.	-	n.d.	-	n.d.	-
9	Aqueous extract	-	-	-	-	-	-	-	-
	Methanol extract	-	-	-	-	-	-	-	-
10	Aqueous extract	+	0.636	n.d.	-	n.d.	-	n.d.	-
	Methanol extract	+	0.344	n.d.	-	n.d.	-	+	0.520
11	Aqueous extract	+	0.483	+	8.233	n.d.	-	n.d.	-
	Methanol extract	+	0.445	n.d.	-	n.d.	-	+	0.479

conc.: concentration, n.d.: not determined.
(+): The analyte is present in sample, (-): The analyte is not present in sample.

3.4. Apparatus and analytical conditions

HPLC analysis was carried out using an Agilent 1200 Infinity Series HPLC system (Agilent Technologies, Germany) including an autosampler, a quaternary pump, a temperature-controlled column compartment, and a diode array detector. An ACE 5 C_{18} column (250 × 4.6 mm, 5 µm) was used for separation.

3.5. Standard preparation

First 1 mg each of (–)-EGCG, (–)-EGC, (–)-ECG, (+)-catechin, ursolic acid, chlorogenic acid, arbutin, and hydroquinone was dissolved in 1 mL of mobile phase to obtain 1 mg/mL stock solutions. Then the solutions were serially diluted with distilled water to achieve standard working solutions at concentrations of 2.5, 5, 10, 25, 50, 75, 100, and 125 µg/mL for arbutin; 2.5, 5, 10, 25, 50, 75, 100, 125, and 150 µg/mL for hydroquinone; 1, 2.5, 5, 10, 25, 50, 100, and 150 µg/mL for ursolic acid; and 1, 2.5, 5, 10, 25, 50, 75, and 100 µg/mL for chlorogenic acid, EGCG, EGC, ECG, and catechin.

3.6. Sample preparation

First 10 mg of each extract of herbal slimming products was dissolved in 1 mL of mobile phase. Then the samples were filtered through a 0.22-µm filter (Millex GV, Ireland) and transferred to HPLC vials.

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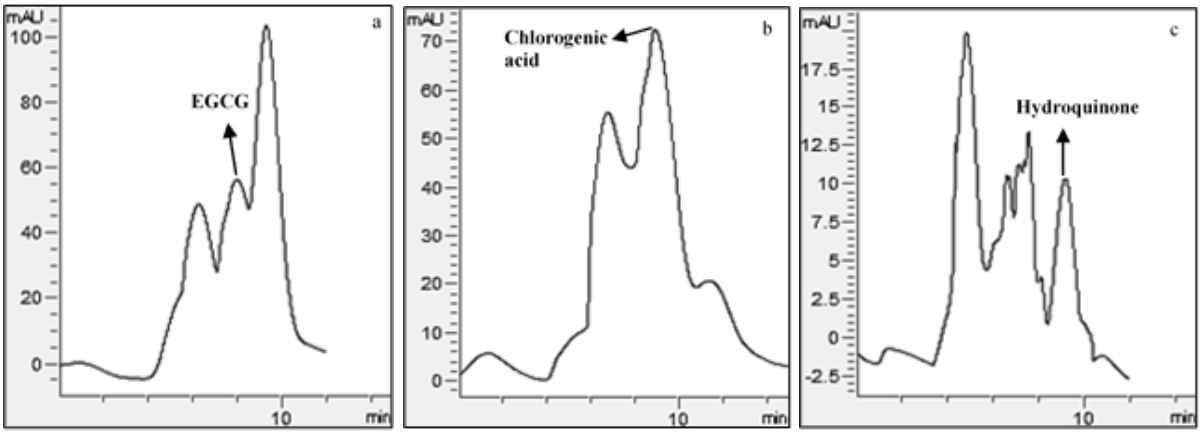

Supplement. The HPLC chromatograms of product 2 (a), product 3 (b), and product 4 (c) (10 mg/mL).