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Inconsistency between declared versus determined *trans*-resveratrol and/or quercetin contents in food supplements

Dicle ÇEVİK[1], Elif Begüm YILDIRIM[2], Etil GÜZELMERİÇ[2*], Erdem YEŞİLADA[2]

1 Department of Pharmacognosy, Faculty of Pharmacy, Trakya University, Balkan Campus, 22030, Edirne, Türkiye
2 Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, Kayışdağı, 34755, İstanbul, Türkiye
* Corresponding Author. E-mail: etil.ariburnu@yeditepe.edu.tr

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ABSTRACT: The utilization of nutraceuticals consisting of (*trans*)-resveratrol or quercetin or combination of these two compounds or food supplements contain extracts rich with (*trans*)-resveratrol or quercetin have become very popular due to their protective and health-beneficial effects especially after the Coronavirus 2019 (COVID-19) pandemic. As the safety and quality control of the food supplements is very important in terms of well-being of consumers, the objective of this study was to qualitatively analyze commercially available (*trans*)-resveratrol and/or quercetin supplements sold in the Turkish market by using High-Performance Thin-Layer Chromatography (HPTLC) and to measure *trans*-resveratrol and quercetin quantitatively by using a newly developed and validated High-Performance Liquid Chromatography (HPLC) method. The HPTLC analysis was performed on silica gel 60 F_{254} glass HPTLC plates using toluene-chloroform-ethyl acetate-formic acid (3:2:10:0.1, *v/v/v/v*) as a developing solvent system. Standard compounds *trans*-resveratrol and quercetin were parallelly analyzed with sample test solutions belong to different brands. The chemical fingerprintings of analyzed samples showed that 8 of the 33 of them did not exhibit the expected zones corresponding to reference compounds. According to quantitative analysis via HPLC, the determined contents of *trans*-resveratrol and quercetin were found to be different from the declared amounts in all of the investigated products. The deviation of determined *trans*-resveratrol content from the declared content was calculated as ± 10.0% in only four food supplements (R4, R5, R8, QR4; percent of declared *trans*-resveratrol contents ranged between 90.3% and 101.2 %), whereas deviation of quercetin levels was found higher in all quercetin containing products. To conclude, the majority of the marketed products containing (*trans*)-resveratrol and/or quercetin in Türkiye had lower levels of the active compounds indicating the inconsistency between the declared and the actual content of these substances. It should be highlighted that quality control of food supplements must be done by authorities before marketing.

KEYWORDS: *Trans*-resveratrol; Quercetin; Nutraceuticals; Food supplements; High-Performance Thin-Layer Chromatography; High-Performance Liquid Chromatography; Quality control.

1. INTRODUCTION

Resveratrol, having 3,5,4′-trihydroxystilbene skeleton is a polyphenolic phytoalexin with two isomeric forms (*cis* and *trans*) and commonly found in the skin of red grapes, peanuts, berries and most notably in red wine [1,2]. Additionally, Japanese knotweed (*Fallopia japonica* syn. *Polygonum cuspidatum*) which is a common source of resveratrol for most of the dietary supplements owing to its high resveratrol content [3]. *Trans*-resveratrol is the isoform that largely responsible for its many biological properties including cardioprotective, anti-inflammatory, antioxidant, antiviral, anti-proliferative, proapoptotic and anticancer effects [4–8].

Quercetin has a 3,3',4',5,7-pentahydroxyflavone nucleus belonging to the flavonol subfamily of flavonoids [9]. It has been shown to possess a wide range of therapeutic properties such as antioxidant, cardioprotective, anticancer, antitumor, anti-ulcer, anti-allergic, antiviral, anti-inflammatory, antidiabetic and gastroprotective effects [9,10]. While quercetin is found especially in onion, asparagus, and berries at high concentrations, small quantities are found in many different fruits and vegetables [11]. Besides, skins of grapes also contain quercetin, hence resveratrol supplements as well as wine products yielded from red grapes might consist of quercetin too [1,12].

Food supplements are defined as concentrates or extracts of nutrients such as vitamins, minerals, proteins, carbohydrates, fibers, fatty acids, amino acids or other substances of plant and animal origin, bioactive compounds that have nutritional or physiological effects taken in variety of dosage forms (capsule, tablet, lozenge, liquid ampoule etc.) with determined daily intake doses in order to supplement the normal

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diet according to Turkish Medicines and Medical Devices Agency [13]. Additionally, nutraceuticals are defined by Zeisel (1999) as "Nutraceuticals are food supplements that deliver a concentrated form of a presumed bioactive agent from a food, presented in a non-food matrix, and used with the purpose of enhancing health in dosages that exceed those that could be obtained from normal foods." [14]. Recently, there is a growing demand for nutraceuticals and food supplements to maintain good health. Especially, products consisting of quercetin or *trans*-resveratrol and sometimes combinations of these two compounds have become very popular nutraceuticals most importantly due to their antioxidant properties that contribute to the immune system [15–17]. The combination of quercetin and *trans*-resveratrol have been shown to exhibit antioxidant synergism through *in vitro* models. However, there are very limited researches on this topic [18,19]. Furthermore, the potential antiviral effects of quercetin and/or trans-resveratrol against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection are thought to contribute to the increased use of these supplements by patients during the Coronavirus Disease 2019 (COVID-19) pandemic [4,20–22]. Supplements containing quercetin recorded a 74.1% sales increase in 2020, according to United States market records [23]. Quercetin was identified as a potential angiotensin converting enzyme-2 (ACE-2) receptor inhibitor by using computer-based *in silico* study [24,25]. A randomized clinical investigation by Shohan et al. (2022) demonstrated that quercetin (1000 mg/day for seven days) reduced the hospitalization period and alleviated the levels of C-reactive protein (CRP), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) when combined with antiviral therapy [26]. Quercetin (1000 mg/day) application with antiviral therapy for 30 days indicated a reduction in frequency and length of hospitalization, non-invasive oxygen therapy need, intensive care progression and number of deaths through a prospective, randomized, controlled, open-label study on 152 COVID-19 outpatients [27]. An *in vivo* study on rats showed that resveratrol 50 mg/kg/day increased ACE-2 protein level which might propose an improved severity in SARS-CoV-2 related illness [28]. According to a clinical study conducted with 230 severe COVID-19 patients, thirty patients who were given *trans*-resveratrol (5.6 mg) and copper (560 ng) orally, once every 6 hours, along with the standard care led to two-fold reduction in mortality [29].

Besides, increases in internet sales as one of the consequences of COVID-19 pandemic also caused an increase in the usage of food supplements [30]. However, there is very limited control by authority in Türkiye for the evaluation of quality and safety of these kinds of products which may lead to adulterated and non-standardized food supplements in the Turkish market. One of the most commonly occurred challenges in this sector is the inconsistency between the declared and the actual content of substances in food supplements [30–33]. Therefore, the safety of food supplements is very important in terms of the well-being of consumers.

In light of these facts, the primary objective of this study was to qualitatively analyze commercially available (*trans*)-resveratrol and/or quercetin supplements sold in the Turkish market by using an high-performance thin-layer chromatography (HPTLC). The secondary objective was to measure *trans*-resveratrol and quercetin quantitatively using rapid, simple, and cost-effective, newly developed and validated high-performance liquid chromatographic (HPLC) method and compare these values with those labeled in each product.

2. RESULTS and DISCUSSION

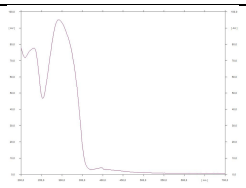
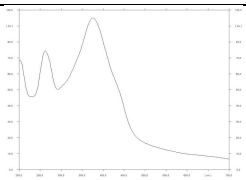
2.1. HPTLC analysis

Totally 33 sample test solutions belonging to (*trans*)-resveratrol (R1-R11), quercetin (Q1-15) and both quercetin and (*trans*)-resveratrol (QR1-7) containing products were first screened by HPTLC. The identity of the *trans*-resveratrol and quercetin reference compounds was qualitatively evaluated in investigated products by using their retention factor (R_F) values, UV spectra, as well as band colors derivatized with Natural Product (NP) and Polyethylene glycol (PEG 400) reagents respectively, as presented in Table 1.

Through HPTLC, simultaneous estimation of quercetin and *trans*-resveratrol in marketed products in a single chromatogram was aimed. Imran at al. (2019) developed HPTLC method for analyzing quercetin and resveratrol in one lipid-based nanoformulation. They used a solvent mixture containing toluene-ethyl acetate-formic acid (6:2.5:1.5, *v*/*v*/*v*) to separate quercetin and resveratrol. However, the separation of the peaks were found to be not good enough, this study was not taken into consideration during the trials [34]. In a recent study by Sethuraman et al. (2021), analysis of quercetin and *trans*-resveratrol in quercetin/resveratrol-loaded nanoformulations as well as *Sesbania grandiflora* leaf extract was achieved by HPTLC using a developing solvent system consisting of toluene-chloroform-ethyl acetate-formic acid (3:2:4.9:0.1%, *v/v*) [35]. After trying this method, it was thought that the mobile phase system should be

changed because the compounds examined were positioned close to the application position and they were detected very close to each other. Therefore, the ethyl acetate composition was modified for future studies. Thus, toluene-chloroform-ethyl acetate-formic acid (3:2:10:0.1, *v/v/v/v*) developing solvent system was used for the better separation of these compounds.

Table 1. R_F values, UV spectra, and band colors of *trans*-resveratrol and quercetin analyzed by HPTLC.

Compounds	R_F	UV Spectrum	Derivatized with NP + PEG 400 reagents (366 nm)
Trans-resveratrol	≈ 0.45		Blue-colored zone
Quercetin	≈ 0.35		Yellowish-colored zone

The results of HPTLC analysis of nutraceuticals and food supplements was evaluated by using HPTLC densitograms (Figure 1) together with pictures of HPTLC plates after derivatization.

Accordingly, not R11 but other analyzed samples claimed to contain (*trans*)-resveratrol found to consist *trans*-resveratrol (blue-colored zone at $R_F \approx 0.45$) (Figure 1A and Figure 2). The other zones apart from reference compounds were also observed in the profiles of R6 and R7 which might be due to metabolites belonging *Euterpe oleracea* and/or *Polygonum cuspidatum* regarding the declared components of these products. As all samples applied at the same concentration *per* band, it can be concluded that the amounts of *trans*-resveratrol in R9 and R10 were lower than the other products.

Additionally, yellowish-colored zone of quercetin at $R_F \approx 0.35$ was detected in the quercetin containing investigated samples coded as Q1-Q4, Q6-Q11, Q13 and Q14, whereas 3 of the investigated samples (Q5, Q12 and Q15) were devoid of quercetin zone in their chemical profiles (Figure 1B and Figure 3).

The examination of HPTLC fingerprinting of the samples claimed to contain both (*trans*)-resveratrol and quercetin indicated that only QR1, QR4, and QR5 contained both of the examined reference compounds (Figure 1C, Figure 1D and Figure 4). In HPTLC profiles of samples QR3 and QR6, there were no zones corresponding to either *trans*-resveratrol or quercetin. Fade zone of *trans*-resveratrol was detected in QR2, indicating that it contained the amount lower than its claimed *trans*-resveratrol content while no trace of quercetin was observed in this sample. Although all samples except QR7 analyzed at 1 ng/band, the amount of concentration at the application position of QR7 was not known because of not indicating the concentrations of the ingredients. Therefore, QR7 was applied at two different concentrations. Although yellowish colored zone appeared close to the R_F of quercetin, it could not belong to quercetin due to the presence of different spectra. For QR4, QR5, and QR7, some zones detected between application position and at $R_F \approx 0.4$ might belong to components of extracts of grape seeds.

As a result, the above-mentioned findings revealed that 8 of 33 investigated products did not exhibit the expected zones in their HPTLC profiles with regard to their labeled constituents. Further analysis was performed through HPLC to quantify components to compare the obtained results with the declared values and discuss the quality of the investigated products more accurately.

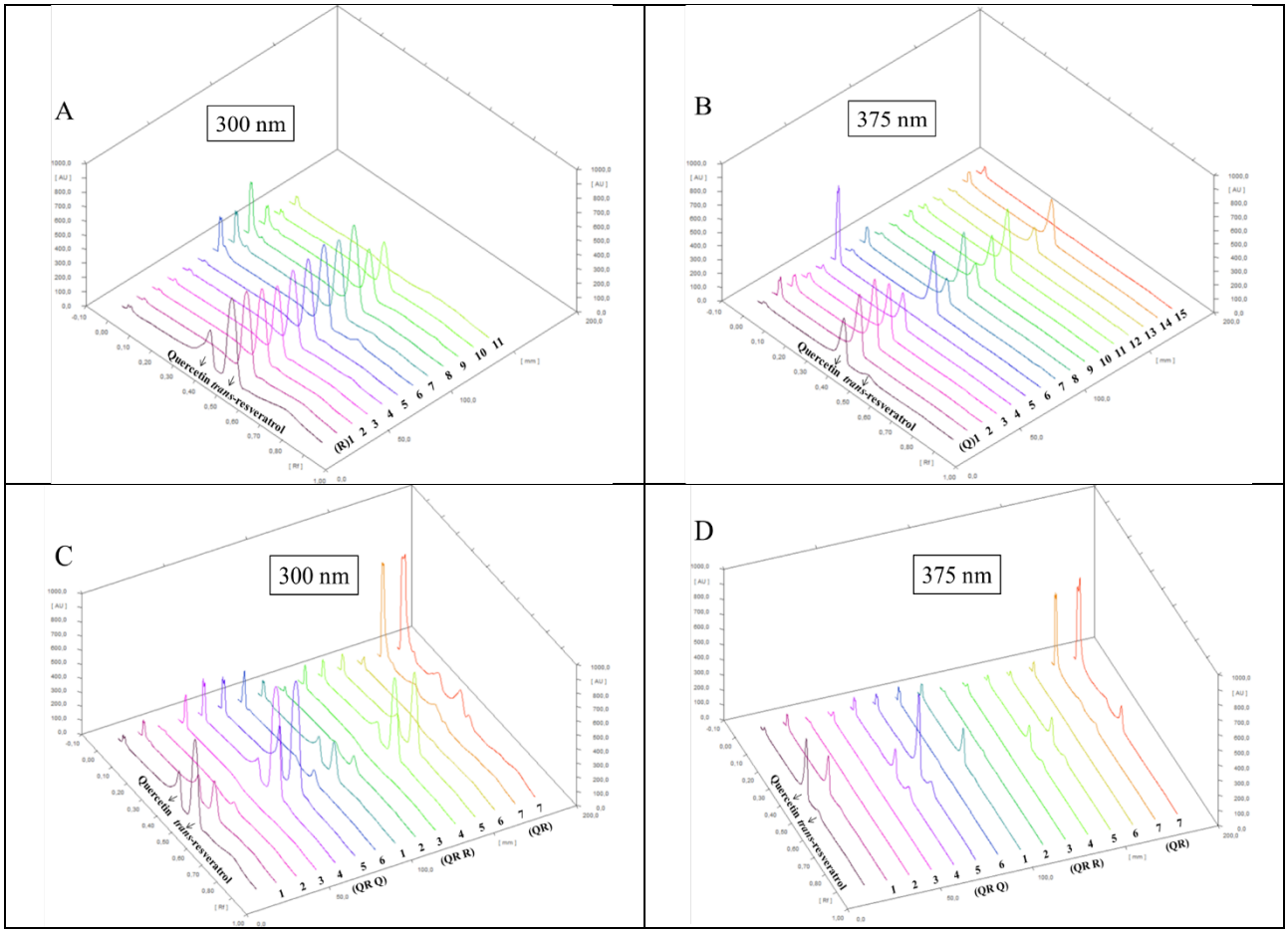


Figure 1. HPTLC densitograms showing standards and samples using toluene-chloroform-ethyl acetate-formic acid (3:2:10:0.1, *v/v/v/v*) mobile phase system. Investigated products containing (A) (*trans*)-resveratrol (R1-11, 1 ng/band) analyzed at 300 nm, (B) quercetin (Q1-11, 1 ng/band) analyzed at 375 nm, (C) and (D) quercetin and (*trans*)-resveratrol (QR R1-6 and QR Q1-6, 1 ng/band). QR7 was applied at different concentrations and evaluated both at 300 and 375 nm.

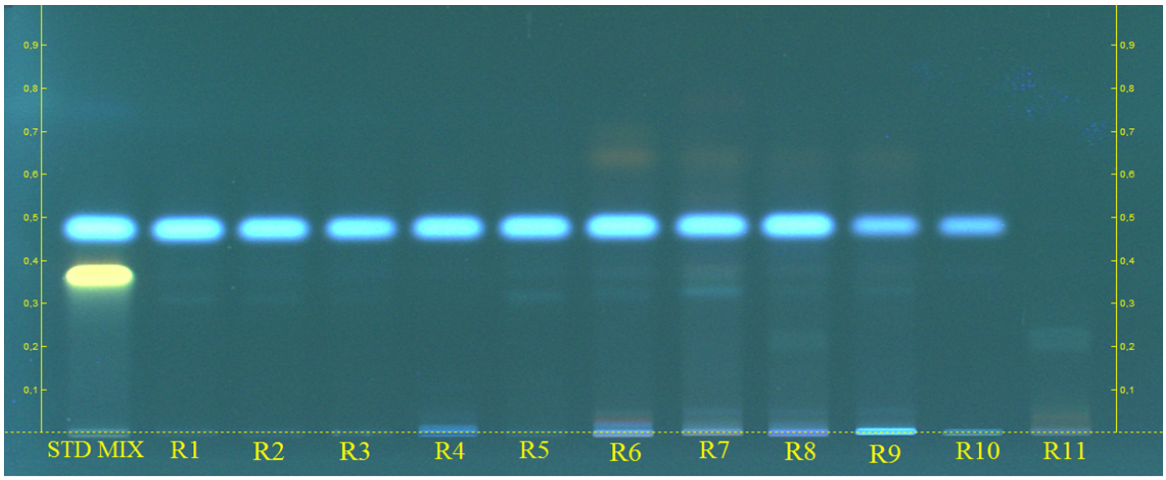


Figure 2. HPTLC chromatogram of (*trans*)-resveratrol containing products (R1-11) sold in the market captured at 366 nm after derivatization with NP and PEG reagents, respectively. Sample amount: 1 ng/band.

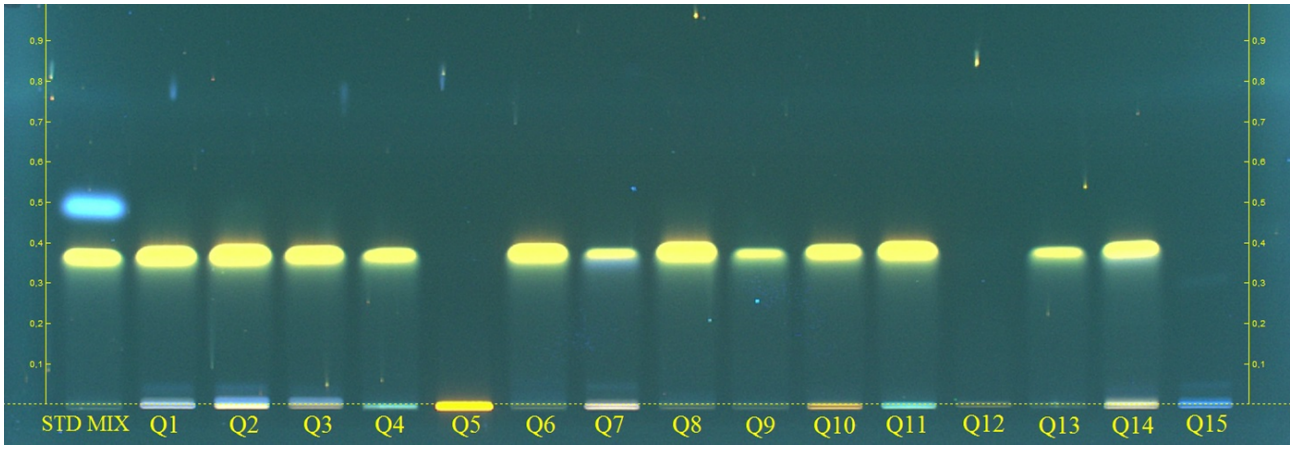


Figure 3. HPTLC chromatogram of quercetin containing products (Q1-15) sold in the market captured at 366 nm after derivatization with NP and PEG reagents, respectively. Sample amount: 1 ng/band.

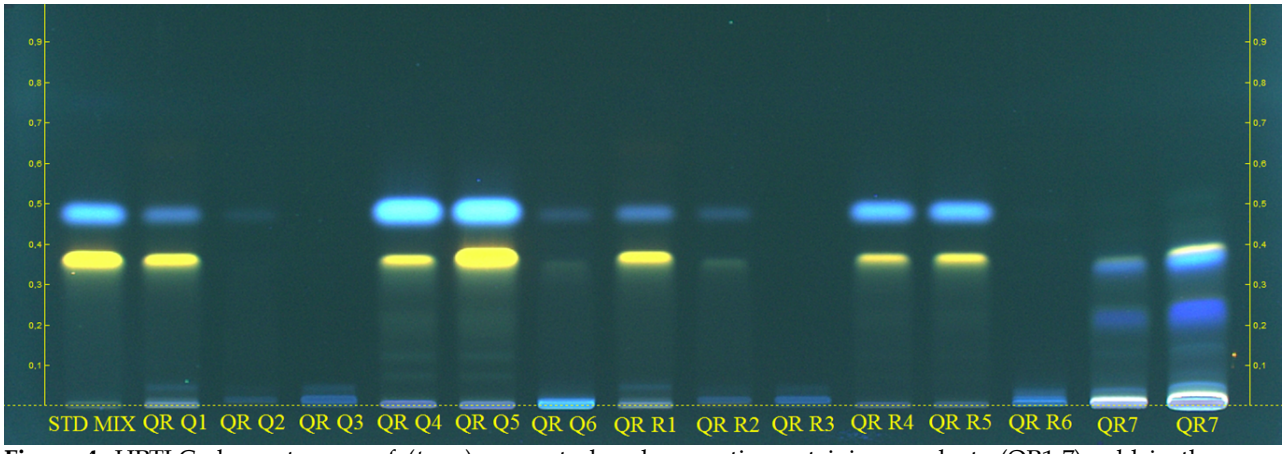


Figure 4. HPTLC chromatogram of (*trans*)-resveratrol and quercetin containing products (QR1-7) sold in the market captured at 366 nm after derivatization with NP and PEG reagents, respectively. Quercetin concentration *per* band of the first six samples encoded as QR Q1-6 and (*trans*)-resveratrol concentration of next six samples encoded as QR R1-6 was 1 ng/band. QR7 was applied 2 µL and 5 µL as no claimed concentration on the food supplement.

2.2. HPLC analysis

2.2.1. HPLC method validation

A newly developed HPLC method was validated to measure *trans*-resveratrol and quercetin contents in the samples with regard to International Conference on Harmonisation (ICH) rules using the specificity, linearity (r^2), detection and determination limits (LOD and LOQ), intraday and interday precision, and accuracy (recovery) parameters [36].

For the determination of the specificity of the HPLC method, the least concentration of the standard solutions belonging *trans*-resveratrol and quercetin were comparatively analyzed with the blank solution. *Trans*-resveratrol t_R = 8.08 ± 0.01 and quercetin t_R = 11.91 ± 0.01 were not detected on the blank chromatogram indicating the specificity of the developed method. To confirm the reference compounds in the samples, t_R values and UV spectrums of *trans*-resveratrol (at 305 nm) and quercetin (at 370 nm) standard solutions were compared with those of the sample test solutions (Figure 5A and Figure 5B).

To examine the linearity, seven different concentration levels (0.5-50 µg/mL) of each freshly prepared standard solution were analyzed in triplicate. The calibration curve area versus concentration (µg/mL) was found to be linear in the range of 0.5-50 µg/mL (0.5, 1.0, 2.5, 5.0, 10.0, 25.0 and 50 µg/mL) with r^2 = 0.999 for *trans*-resveratrol, and r^2 = 0.999 for quercetin (Table 2). LOD and LOQ values were determined from the equation as 3.3 x (SD/S) and 10 x (SD/S), respectively. Accordingly, LOD and LOQ were determined as

0.032 µg/mL and 0.106 µg/mL for *trans*-resveratrol and 0.025 µg/mL and 0.084 µg/mL for quercetin, respectively (Table 2).

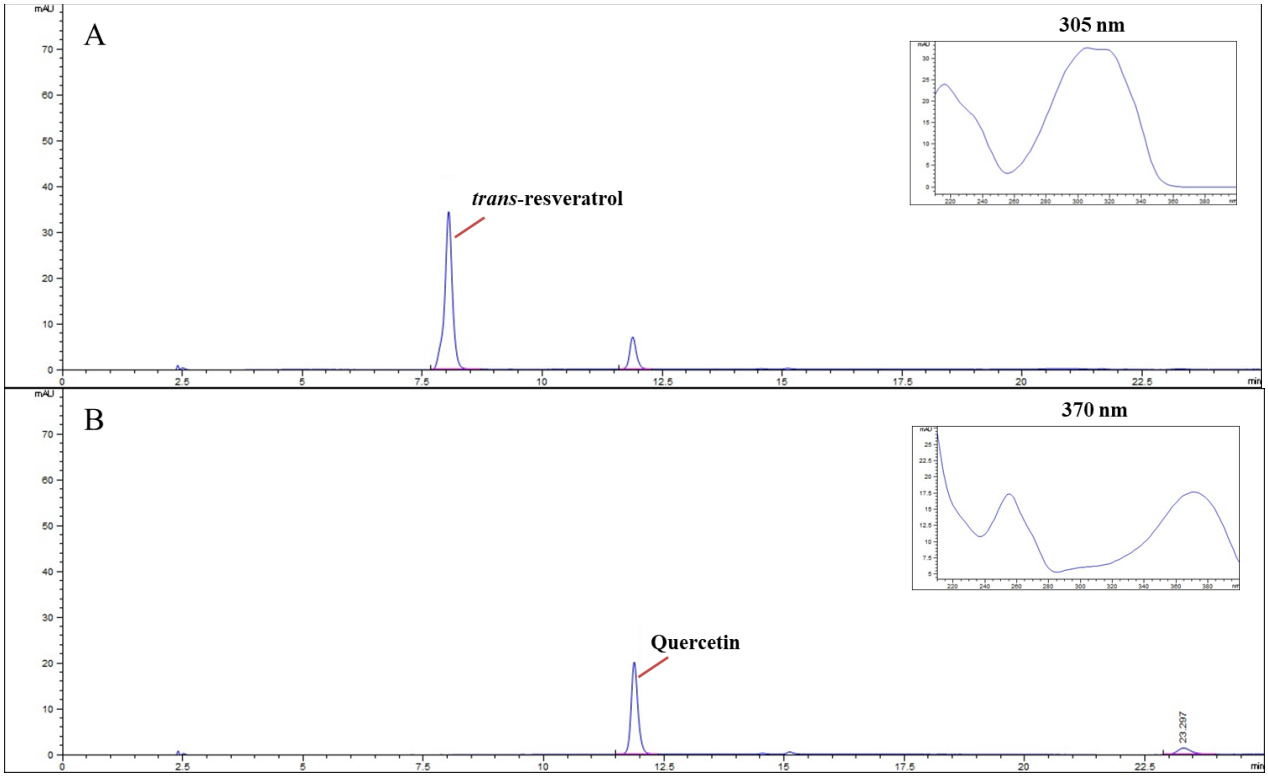


Figure 5. HPLC chromatogram of standard mixture (5 µg/mL) at 370 nm ; (A) *trans*-resveratrol t_R =8.08 ± 0.01 and (B) quercetin t_R = 11.91 ± 0.01.

Table 2. Linearity data of the calibration curves belonging to quercetin and *trans*-resveratrol, and LOD and LOQ values.

Standards	Parameters						
	Linearity range µg/mL	r^2	a	b	SD	LOD µg/mL	LOQ µg/mL
Trans-resveratrol	0.5-50	0.999	73.049	14.635	0.7760	0.032	0.106
Quercetin	0.5-50	0.999	38.074	1.588	0.318	0.025	0.084

The calibration equation was 'y=ax+b'.
SD= Standard deviation

For the determination of the intraday precision of the method, the experiment was replicated in triplicate, whereas interday precision was examined by repeating the analysis three times in three consecutive days. The results are depicted in Table 3.

Table 3. Repeatability and intra-/interday precision data.

Standards	Intraday precision		Interday precision		Interday precision		Interday precision	
	Average value[a]	RSD	Average value[a]	RSD	Average value[a]	RSD	Average value[a]	RSD
***Trans*-resveratrol** (5 µg/mL)	5.219 ± 0.040 5.215 ± 0.012 5.194 ± 0.003	0.758 0.223 0.055	5.214 ± 0.006	0.120	5.239 ± 0.003	0.066	5.281 ± 0.043	0.812
Quercetin (5 µg/mL)	5.075 ± 0.033 5.058 ± 0.014 5.033 ± 0.009	0.657 0.286 0.183	5.040 ± 0.004	0.080	5.032 ± 0.017	0.340	5.044 ± 0.048	0.949

[a] The average values were expressed as µg/mL ± SD; *n*=3.

Recovery was determined by the ratio in percent between the known spiked amount of the compound and the experimentally found result [36]. The three different concentrations of standard compounds (3.0, 6.0 and 15.0 µg/mL) were analyzed. The results showed that the developed method has a good recovery range from 99.134 to 100.473% for *trans*-resveratrol and from 99.994 to 100.544% for quercetin as shown in Table 4.

Table 4. Recovery results.

Standards	Theoretical value[a]	Amount found[b]	Recovery (%)	RSD
***Trans*-resveratrol**	3.0	2.974 ± 0.021	99.134	0.697
	6.0	5.973 ± 0.019	99.556	0.318
	15.0	15.071 ± 0.025	100.473	0.168
Quercetin	3.0	3.016 ± 0.031	100.544	1.032
	6.0	6.028 ± 0.014	100.467	0.226
	15.0	14.999 ± 0.068	99.994	0.452

[a]Theoretical value of the standards were expressed as µg/mL.
[b]The average found amounts were expressed as µg/mL ± SD, *n*=3.
RSD: Relative standard deviation

2.2.2. Quantitative analysis of trans-resveratrol and quercetin and assessment of compliance of declared and determined contents

HPLC quantification of *trans*-resveratrol and quercetin was performed for all marketed products except for QR7 due to not labeling contents of the ingredients (Table 5). Regarding the obtained results based on HPLC, compliance of the declared and labeled (*trans*)-resveratrol and quercetin contents with the determined *trans*-resveratrol and quercetin contents in nutraceuticals and food supplements were evaluated.

The declared quercetin contents in the products encoded as Q1-15 ranged from 100 mg to 500 mg *per* unit (capsule, tablet, effervescent), while the determined quercetin content ranged from 0 to 168.13 mg *per* unit (Table 5). Thus, all of the quercetin containing products (Q1-Q15) were found to contain lower amounts of quercetin than the declared amounts.

The measured *trans*-resveratrol content was found in the range of 0.80 to 236.00 mg/unit (capsule, tablet) in (*trans*)-resveratrol containing supplements (R1-11), while the declared (*trans*)-resveratrol contents were from 40 mg to 250 mg *per* unit. Amongst the investigated products, the content of *trans*-resveratrol was determined as higher than the labeled amounts in R1, R3 and R8. Whilst, in the other eight supplements (samples R2, R4-R7, R9-R11), the levels of *trans*-resveratrol contents were lower than declared.

HPLC analysis of products consisting both (*trans*)-resveratrol and quercetin (QR1-6) indicated that contents of *trans*-resveratrol and quercetin were lower than the labeled quantities in all of the investigated samples.

Using the declared and actually measured contents of *trans*-resveratrol and quercetin, the percent of these declared contents were calculated as seen in Table 5. As a result, the determined contents of *trans*-resveratrol and quercetin were found to be different from the declared contents in all of the tested products, being higher or lower than 100% of declared amounts. The percent of the declared (*trans*)-resveratrol content was found in the range of 0 - 118% while, differences between the declared and determined quercetin contents were ranging from 0% to 88.8%. The deviation of determined *trans*-resveratrol content from declared content were calculated as ± 10% in only four investigated samples (R4, R5, R8, QR4; percent of declared (*trans*)-resveratrol contents ranged between 90.3% and 101.2 %), whereas none of the products consisted quercetin in ± 10% deviation range (Figure 6 A and B).

In a very recent study by Bensa et al. (2023), 95% of the analyzed marketed products amongst 20 products, resveratrol content was calculated as higher or lower than declared amounts by using HPTLC. More interestingly, the determined resveratrol contents were found to be higher than declared in the 60% of the analyzed samples, while %35 of the samples contained lower resveratrol than declared [30].

It is not usually possible to contain exact ingredient levels such as nutrients in food supplements regarding their labels because of the quality of the raw material, variations in extraction procedures and formulation processes (heat application, pH factor etc.), and storage conditions. Acceptable tolerance range for vitamins in food supplements should not be more than 50% and less than 20% from the declared value on the labels according to the regulation of European Parliament and of the Council 924/2006/EC, Reg.

1925/2006/EC [37]. However, to the best our knowledge there is no legislation about the control for compliance of active ingredients or marker components in food supplements.

Table 5. Labeled and measured amounts of quercetin and *trans*-resveratrol contents (mg/unit) in products containing *(trans)*-resveratrol and/or quercetin.

		Declared Amount (mg/unit)		Measured Amount (mg/unit)	
	PRODUCTS	**Quercetin**	**(*Trans*)-resveratrol**	**Quercetin (%*)**	***Trans*-resveratrol (%*)**
Samples containing quercetin	**Q1**	250 mg (1 tablet)	-	114.75 mg (45.9%)	-
	Q2	250 mg (1 tablet)	-	88.31 mg (35.3%)	-
	Q3	200 mg (1 tablet)	-	95.30 mg (47.7%)	-
	Q4	100 mg (1 capsule)	-	88.83 mg (88.8%)	-
	Q5	125 mg (1 capsule)	-	2.97 mg (2.4%)	-
	Q6	250 mg (1 capsule)	-	168.13 mg (67.3%)	-
	Q7	250 mg (1 capsule)	-	54.94 mg (22.0%)	-
	Q8	250 mg (1 capsule)	-	164.50 mg (65.8%)	-
	Q9	500 mg (1 capsule)	-	119.00 mg (23.8%)	-
	Q10	250 mg (1 capsule)	-	95.19 mg (38.1%)	-
	Q11	100 mg (1 capsule)	-	60.58 mg (60.6%)	-
	Q12	500 mg (1 capsule)	-	17.75 mg (3.6%)	-
	Q13	500 mg (1 capsule)	-	148.00 mg (29.6%)	-
	Q14	250 mg (1 capsule)	-	99.13 mg (39.7%)	-
	Q15	250 mg (1 effervescent)	-	n.d. (0.0%)	-
Samples containing (*trans*)-resveratrol	**R1**	-	200 mg (1 capsule)	-	236.00 mg (118.0%)
	R2	-	250 mg (1 capsule)	-	201.75 mg (80.7%)
	R3	-	100 mg (1 capsule)	-	115.50 mg (115.5%)
	R4	-	200 mg (1 capsule)	-	185.00 mg (92.5%)
	R5	-	250 mg (1 capsule)	-	225.75 mg (90.3%)
	R6	-	100 mg (1 capsule)	-	83.90 mg (83.9%)
	R7	-	75 mg (1 capsule)	-	58.20 mg (77.6%)
	R8	-	100 mg (1 capsule)	-	101.20 mg (101.2%)
	R9	-	50 mg (1 tablet)	-	19.65 mg (39.3%)
	R10	-	40 mg (1 capsule)	-	9.36 mg (23.4%)
	R11	-	50 mg (1 capsule)	-	0.80 mg (1.6%)
Samples containing quercetin & (*trans*)-resveratrol	**QR1**	100 mg (1 tablet)	100 mg (1 tablet)	23.68 mg (23.7%)	20.35 mg (20.4%)
	QR2	250 mg (1 capsule)	100 mg (1 capsule)	7.25 mg (2.9%)	7.30 mg (7.3%)
	QR3	40 mg (1 tablet)	50 mg (1 tablet)	n.d. (0.0%)	n.d. (0.0%)
	QR4	25 mg (10 mL)	100 mg (10 mL)	16.66 mg (66.6%)	92.90 mg (92.9%)
	QR5	50 mg (25 mL)	200 mg (25 mL)	26.06 mg (52.1%)	133.70 mg (66.9%)
	QR6	20 mg (10 mL)	100 mg (10 mL)	0.79 mg (4.0%)	1.75 mg (1.8%)

n.d.: Not detected
*The percent of declared contents in nutraceuticals and food supplements

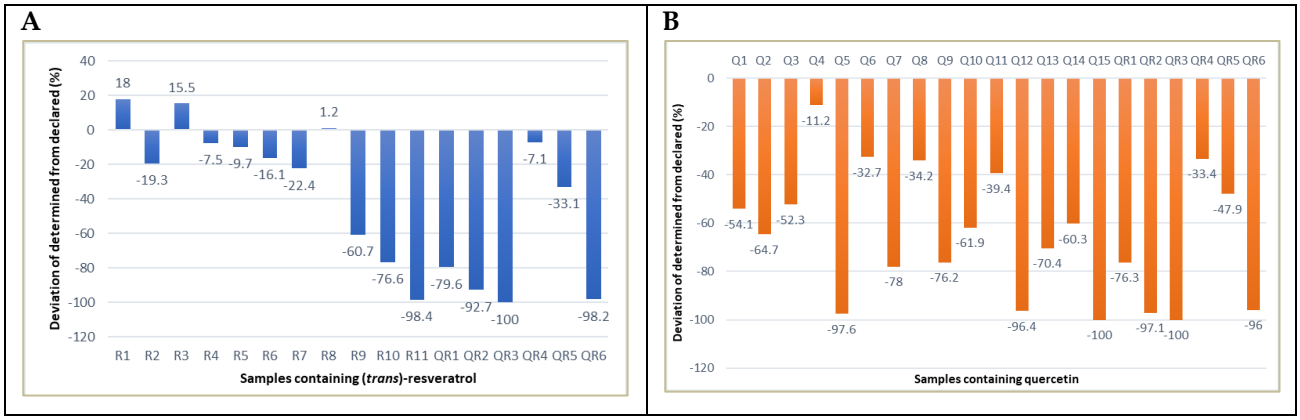


Figure 6. Deviation of determined content from declared content (%) of (A) *trans*-resveratrol in marketed products containing (*trans*)-resveratrol (R1-11 and QR1-6) and (B) quercetin in marketed products containing quercetin (Q1-15 and QR1-6).

Careri et al. conducted a newly developed HPLC analysis of quercetin and *trans*-resveratrol in red wine, grape, and winemaking byproducts by using isocratic elution of mobile phase consisting 1% (*v*/*v*) formic acid aqueous solution-acetonitrile-2-propanol (70:22:8) [12]. The highest *trans*-resveratrol was found in grape skin (27.5 µg/g) whereas the highest quercetin (103.9 µg/g) was measured in grape pomace. Additionally, the highest *trans*-resveratrol and quercetin were calculated as 2.86 mg/mL and 8.84 mg/mL, respectively in red wine samples. In another study by Omar et al., a reverse-phase HPLC-UV method for the simultaneous and quantitative analyses of *trans*-/*cis*-resveratrol, emodin, and quercetin was developed for the quality investigation of 28 resveratrol supplements retrieved from Canada and United States of America [1]. As a result of the comparison of the concentrations of labeled resveratrol with the analyzed values by HPLC, less *trans*-resveratrol (- 11-76%) than labeled amount was detected in six samples (21% of samples), while higher contents of *trans*-resveratrol (+ 18-162%) than that reported were measured in three samples. Authors suggested that the remaining 18 samples were found to be in fairly good agreement with those given on the product labels which contained up to -10% or +%17 content of *trans*-resveratrol [1]. According to these points of view, the stated amounts of *trans*-resveratrol could be accepted as reasonably accurate in five of the tested samples representing 15.63% of the samples including R3 (115.5%), R4 (92.5%), R5 (90.3%), R8 (101.2%) and QR4 (92.9%). However, any of the stated quercetin levels (0.0-88.8%) was not found to be consistent with determined quercetin levels amongst the tested products.

This is the first report comparatively evaluating the fingerprinting and quantitative analysis of the marketed (*trans*)-resveratrol and/or quercetin containing products in Türkiye by using HPTLC and HPLC methods. These results demonstrated that more attention to quality control processes, not only for raw material but also for final product must be given by manufacturers and authorizations to ensure efficacy and safety of food supplements. The newly developed and validated HPLC method might be appropriate for routine analysis of formulations consisting of (*trans*)-resveratrol and/or quercetin.

3. CONCLUSION

According to simultaneous chemical fingerprinting analysis of marketed products containing (*trans*)-resveratrol and/or quercetin (R1-R11, Q1-15 and QR1-7) in variety of formulations through HPTLC method using *trans*-resveratrol and quercetin as reference compounds; 8 of 33 investigated products did not exhibit the expected zones belonging to reference compounds with regard to their labeled constituents. Further quantification analysis via HPLC showed that all of the quercetin containing products (Q1-Q15, QR1-QR6) were found to contain lower amounts of quercetin than the declared amounts possessing from 0.0% to 88.8% of the labeled quercetin content. Amongst the products consisting (*trans*)-resveratrol (R1-R11 and QR1-QR6), the amount of *trans*-resveratrol was determined as higher than the labeled amounts in R1, R3 and R8 (118.0%, 115.5% and 101.2%, respectively), whereas in the remaining (*trans*)-resveratrol containing supplements including R2, R4-R7, R9-R11 and QR1-QR6, the level of *trans*-resveratrol content was calculated as lower than declared, ranging from 0.0 to 92.9%. To conclude, inconsistency was detected between the declared and the actual content of active compounds in most of the marketed products containing (*trans*)-resveratrol and/or quercetin in Türkiye.

4. MATERIALS AND METHODS

4.1. Chemicals and solvents

Toluene, chloroform, ethyl acetate, formic acid, *o*-phosphoric acid, and HPLC grade methanol were purchased from Sigma-Aldrich (Steinheim, Germany). 2-Aminoethyl diphenylborinate was obtained from Fluka (Steinheim, Germany). Polyethylene glycol 400 was acquired from Merck (Darmstadt, Germany). The ultrapure water was obtained from Millipore, Simplicity UV (Darmstadt, Germany). Reference samples of quercetin (≥ 95%) was purchased from Sigma-Aldrich (Steinheim, Germany) and *trans*-resveratrol (phamaceutical grade, 99% pure *trans*-resveratrol) as a raw material were obtained from Mega Resveratrol® (Danbury, USA).

4.2. Nutraceuticals containing (*trans*)-resveratrol and/or quercetin and food supplements

Nutraceuticals containing (*trans*)-resveratrol and food supplements containing extracts such as Japanese knotweed, grape seed, and acai (R1-11) and fifteen supplements containing quercetin (Q1-15) and related plant extracts were provided in either tablets, effervescents or capsules (Table 6).

Additionally, seven samples containing both quercetin and (*trans*)-resveratrol (QR1-7) were acquired in capsule, tablet, shot or syrup forms (Table 7).

These supplements were provided from pharmacies, markets and e-commerce websites in Türkiye acquired via mostly donated or purchased for research. The amount of quercetin or (*trans*)-resveratrol declared on the labels of these commercial formulations ranged from 20 to 500 mg.

4.3. Preparation of standard solutions

4.3.1. Standard solutions for HPTLC analysis

Standard solutions of *trans*-resveratrol and quercetin were prepared in methanol at 0.4 mg/mL concentration. Standard solutions were mixed to prepare a standard mixture solution consisting 0.1 mg/mL *trans*-resveratrol and 0.25 mg/mL quercetin (std mix).

4.3.2. Standard solutions for HPLC analysis

Stock solutions of quercetin and *trans*-resveratrol (1000 µg/mL) were prepared in methanol and further diluted to obtain a calibration curve with seven data points and to perform recovery studies.

4.4. Preparation of sample test solutions

Six capsules or tablets containing either quercetin or (*trans*)-resveratrol were randomly selected, pooled, and mixed in a beaker and weighted to determine the mean weight of each capsule and tablet. Then, different volumes of MeOH were added to the powdered samples according to the claimed quercetin and (*trans*)-resveratrol amounts on the investigated products to adjust the final concentration as 50 mg/mL (stock sample test solution). Then, these samples were sonicated for 30 minutes and filtered through a syringe filter (0.45 µm) for further analysis.

Products containing both quercetin and (*trans*)-resveratrol in capsules or tablet forms (QR1-QR3) were also prepared as described above. However, these samples consisted different amounts of quercetin and (*trans*)-resveratrol except QR1. Therefore, two different sample test solutions were prepared for these products according to their claimed quercetin and (*trans*)-resveratrol contents. The samples prepared according to the quercetin amounts were coded as QR Q1 to QR Q3, while samples were encoded from QR R1 to QR R3 with regard to their (*trans*)-resveratrol content.

On the other hand, samples in the form of liquid forms as syrup (QR4 and QR6) or shot (QR5) were diluted in half with methanol, and after 30 minutes of sonication, they were filtered through a syringe filter to yield test solutions with different concentrations. The last sample containing both of (*trans*)-resveratrol and quercetin in syrup form (QR7) which had no label information about their amounts was also diluted to half with MeOH and filtered after sonication.

Finally, all sample test solutions except QR7 were adjusted to 0.5 mg/mL using MeOH for HPTLC analysis. To analyze *trans*-resveratrol content by HPLC analysis, R1-11 were prepared at the concentration of 10 µg/mL whereas QR1-6 were prepared at the concentration of 20 µg/mL. For the quantification of

quercetin content, samples containing quercetin (Q1-15 and QR1-6) were prepared at the concentration of 40 µg/mL.

Table 6. Detailed information on declared main ingredients and formulations of investigated nutraceuticals and food supplements R1-R11 and Q1-Q15.

Code	Formulation	Main ingredients
R1	Capsule	Japanese knotweed extract (resveratrol)
R2	Capsule	Resveratrol, Black pepper extract (piperine)
R3	Capsule	Resveratrol (*Polygonum cuspidatum*) (root)
R4	Capsule	Resveratrol (*trans*-resveratrol), Vitamin E, Vitamin C, Vitamin A, Selenium
R5	Capsule	Resveratrol
R6	Capsule	Acai (*Euterpe oleracea*), Japanese knotweed (*Polygonum cuspidatum*), Resveratrol, Coenzyme Q10
R7	Capsule	Japanese knotweed (*Polygonum cuspidatum*) root extract (resveratrol), Coenzyme Q10, Astaxanthin, Piperine
R8	Capsule	Japanese knotweed (*Polygonum cuspidatum*) root extract (resveratrol), Grape seed extract (*Vitis vinifera*)
R9	Tablet	Hydrolyzed Collagen Type I, Resveratrol, Alpha Lipoic acid, Hyaluronic acid, Vitamin C
R10	Tablet	Hydrolysed collagen, Hyaluronic acid, Resveratrol, Pomegranate peel extract
R11	Capsule	Collagen Hydrolysate, Lactoferrin, Boswellia Gum Resin Extract *(Boswellia serrata*), Resveratrol, Selenium, Vitamin D, Manganese, Copper, Vitamin K, Folate
Q1	Tablet	Quercetin, Vitamin C, Bromelain, Zinc, Vitamin D
Q2	Tablet	Vitamin C, Quercetin, Zinc, Vitamin D3
Q3	Tablet	Quercetin, Vitamin C, Zinc
Q4	Capsule	Quercetin
Q5	Capsule	Quercetin
Q6	Capsule	Quercetin
Q7	Capsule	Vitamin C, Quercetin, Bromelain, Zinc, Vitamin D3
Q8	Capsule	Quercetin, Boron
Q9	Capsule	Quercetin
Q10	Capsule	Vitamin C, Quercetin, Bromelain, Citrus bioflavonoid complex, Rosehips powder (*Rosa canina*) (fruit), Acerola extract, Rutin
Q11	Capsule	Vitamin C, Quercetin, Bromelain, Zinc, Vitamin B6 (pyridoxine), Vitamin D
Q12	Capsule	Quercetin, Vitamin C, Bromelain, Citrus bioflavonoid, Rosehips (fruit), Acerola (berry), Rutin
Q13	Capsule	Quercetin, Acerola fruit extract (*Malpighia punicifolia*), Bromelain, Zinc, Copper, Vitamin D
Q14	Capsule	Quercetin, Acerola extract, Oleuropein, Vitamin C, Zinc, Vitamin D3
Q15	Effervescent tablet	Bromelain, Vitamin C, Quercetin, Coenzyme Q10, Chrome

Table 7. Detailed information on declared main ingredients and formulations of investigated nutraceuticals and food supplements QR1-QR7.

Code	Formulation	Main ingredients
QR1	Tablet	Vitamin C, Resveratrol, Quercetin
QR2	Capsule	Quercetin, Resveratrol, Lactoferrin, Vitamin D3, Vitamin C, Zinc gluconate
QR3	Tablet	Vitamin C, Bromelain, Citrus bioflavonoids, Rosehip, Acerola, Resveratrol, Quercetin
QR4	Syrup	Resveratrol, Grape seed extract, Quercetin
QR5	Shot	Resveratrol, Grape seed extract, Quercetin
QR6	Syrup	Resveratrol, Quercetin, Vitamin C
QR7	Syrup	Black grape skin and seed extract (resveratrol and catechin derivatives), Quercetin, Citric acid

4.5. HPTLC method

Standard mixture solution containing quercetin and *trans*-resveratrol (5 µL) and sample test solutions coded as R1-11, Q1-15 and QR1-7 (2 µL) were applied as bands by using Linomat V semi-automatic sample applicator (Camag, Muttenz, Switzerland) coupled with 100 µL Hamilton syringe on the silica gel 60 F_{254} glass HPTLC plate (20 x 10 cm). The plates were developed up to 7 cm in a saturated (20 min) twin-trough chamber (Camag) containing toluene-chloroform-ethyl acetate-formic acid (3:2:10:0.1, *v/v/v/v*). After development, plates were dried with cold air. Then, the plates were scanned by using TLC Scanner 3 (Camag) in absorption/reflectance mode at 300 nm for *trans*-resveratrol and at 375 nm for quercetin, using slit dimensions 6 mm × 0.30 mm the scanning speed 20 mm s^{-1} and the data resolution 100 m/step. In addition, HPTLC plates were scanned from 200 nm to 700 nm to obtain UV spectrum of *trans*-resveratrol and quercetin. For visual evaluation of the *trans*-resveratrol and quercetin in the analyzed samples, HPTLC plates were first heated on a TLC heater (Camag) at 100°C for 3 minutes and then dipped into two different solutions containing 2-aminoethyl diphenylborinate called as Natural Product reagent and polyethylene glycol (PEG) 400, respectively by using TLC Immersion III (Camag). The images of the derivatized plates were documented under 366 nm by using a TLC Visualizer (Camag).

4.6. HPLC method

Agilent 1260 Infinity HPLC system (Darmstadt, Germany) consisting of an Agilent ChemStation software, quaternary pump (G1311B), auto-sampler (G1329B), thermostatted column compartment (G1316A), and diode array detector (G4212B) was utilized for HPLC analysis. Agilent® Zorbax Extend-C_{18} Column (4.6 mm x 250 mm, 5-µm particle size) was used at 40 ˚C to analyze the reference compounds in samples with the mobile phases A (*o*-phosphoric acid-water, 0.1:99.9, *v/v*) and B (methanol). The following gradient elution system was applied: 35-70%B (0-16 min.), 70-80% B (16-18 min.), 80% B (18-20 min.), and 80-35% B (20-25 min.). 10 µL of the standard and test solutions were injected into the system. The flow rate was 1 mL/min. Quercetin was monitored at 370 nm, while *trans*-resveratrol was detected at 305 nm. This newly developed method was validated following the International Conference on Harmonisation (ICH) 1995 guidelines [36].

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pREFERENCES

[1] Omar JM, Yang H, Li S, Marquardt RR, Jones PJH. Development of an improved reverse-phase high-performance liquid chromatography method for the simultaneous analyses of *trans* -/ *cis* -resveratrol, quercetin, and emodin in commercial resveratrol supplements. J Agric Food Chem. 2014; 62: 5812–5817. https://doi.org/10.1021/jf5001277
[2] Burns J, Yokota T, Ashihara H, Lean MEJ, Crozier Alan. Plant foods and herbal sources of resveratrol. J Agric Food Chem. 2002; 50: 3337–3340. https://doi.org/10.1021/jf0112973
[3] Ke J, Li M-T, Xu S, Ma, Jianpeng M, Liu M-Y, Han Y. Advances for pharmacological activities of *Polygonum cuspidatum* - A review. Pharm Biol. 2023; 61: 177–188. https://doi.org/10.1080/13880209.2022.2158349
[4] Filardo S, Di Pietro M, Mastromarino P, Sessa R. Therapeutic potential of resveratrol against emerging respiratory viral infections. Pharmacol Ther. 2020; 214: 107613. https://doi.org/10.1016/j.pharmthera.2020.107613
[5] Wiciński M, Socha M, Walczak M, Wódkiewicz E, Malinowski B, Rewerski S, Górski K, Pawlak-Osińska, K. Beneficial effects of resveratrol administration—Focus on potential biochemical mechanisms in cardiovascular conditions. Nutrients. 2018; 10(11): 1813. https://doi.org/10.3390/nu10111813
[6] Ćućuz V, Cvejić J, Gojković-Bukarica L. Clinical trials of resveratrol efficacy and safety. Vojnosanit Pregl. 2022; 79: 613–618. https://doi.org/10.2298/VSP201126006C
[7] Chen L, Musa AE. Boosting immune system against cancer by resveratrol. Phyther Res. 2021; 35: 5514–5526. https://doi.org/10.1002/ptr.7189
[8] Shahcheraghi SH, Salemi F, Small S, Syed S, Salari F, Alam W, Cheang WS, Saso L, Khan H. Resveratrol regulates inflammation and improves oxidative stress via Nrf2 signaling pathway: Therapeutic and biotechnological prospects. Phyther Res. 2023; 1590–1605. https://doi.org/10.1002/ptr.7754
[9] Batiha GES, Beshbishy AM, Ikram M, Mulla ZS, El-Hack MEA, Taha AE, Algammal AM, Elewa YHA. The pharmacological activity, biochemical properties, and pharmacokinetics of the major natural polyphenolic flavonoid: Quercetin. Foods. 2020; 9: 374. https://doi.org/10.3390/foods9030374
[10] David AVA, Arulmoli R, Parasuraman S. Overviews of biological importance of quercetin: A bioactive flavonoid. Pharmacogn Rev. 2016; 10: 84–89. https://doi.org/10.4103/0973-7847.194044
[11] Dabeek WM, Marra MV. Dietary quercetin and kaempferol: Bioavailability and potential cardiovascular-related bioactivity in humans. Nutrients. 2019; 11. https://doi.org/10.3390/nu11102288
[12] Careri M, Corradini C, Elviri L, Nicoletti I, Zagnoni I. Direct HPLC analysis of quercetin and *trans*-resveratrol in red wine, grape, and winemaking byproducts. J Agric Food Chem. 2003; 51: 5226–5231. https://doi.org/10.1021/jf034149g
[13] Directive 2023/32169/Turkish Medicines and Medical Devices Agency of 20 April 2023 on Health Declaration In Food And Supplementary Foods Regulations About The Use. https://www.resmigazete.gov.tr/eskiler/2023/04/20230420-5.htm (accessed on 25 August 2023).
[14] Zeisel SH. Regulation of nutraceuticals. Science. 1999; 285:1853–1855. https://10.1126/science.285.5435.1853
[15] Frémont L, Belguendouz L, Delpal S. Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. Life Sci. 1999; 64: 2511–2521. https://doi.org/10.1016/S0024-3205(99)00209-X
[16] Coballase-Urrutia E, Pedraza-Chaverri J, Cárdenas-Rodríguez N, Huerta-Gertrudis B, García-Cruz ME, Montesinos-Correa H, Sánchez-González DJ, Camacho-Carranza R, Espinosa-Aguirre JJ. Acetonic and methanolic extracts of *Heterotheca inuloides*, and quercetin, decrease CCl_4-oxidative stress in several rat tissues. Evidence-Based Complement Altern Med. 2013; 2013: 659165. https://doi.org/10.1155/2013/659165
[17] Xu D, Hu MJ, Wang YQ, Cui YL. Antioxidant activities of quercetin and its complexes for medicinal application. Molecules. 2019; 24: 1123. https://doi.org/10.3390/molecules24061123
[18] Murugesan N, Damodaran C, Krishnamoorthy S, Raja M. *In-vitro* evaluation of synergism in antioxidant efficiency of quercetin and resveratrol. Chem Biol Lett. 2023; *10*(2): 534. https://pubs.thesciencein.org/journal/index.php/cbl/article/view/534
[19] Murugesan N, Damodaran CP. Antioxidant activity of synergistic quercetin resveratrol. J Mol Chem. 2023; *3*(1): 581. https://www.pubs.thesciencein.org/journal/index.php/jmc/article/view/a581
[20] Quiles J, Rivas-García L, Varela-López A, Llopis J, Battino M, Sánchez-González C. Do nutrients and other bioactive molecules from foods have anything to say in the treatment against COVID-19? Environ Res J. 2020; 191: 110053. https://doi.org/10.1016/j.envres.2020.110053
[21] Hurcan M, Irkin R. A review of natural foods consumed during the COVID-19 pandemic life. Postepy Hig Med Dosw. 2022; 76: 188–198. https://doi.org/10.2478/ahem-2022-0020
[22] Bijelić K, Hitl M, Kladar N. Phytochemicals in the prevention and treatment of SARS-CoV-2—Clinical evidence. Antibiotics. 2022; 11: 1614. https://doi.org/10.3390/antibiotics11111614
[23] Smith T, Majid F, Eckl V, Reynolds CM. Herbal supplement sales in US increase by record-breaking 17.3% in 2020. HerbalGram. 2021; 131: 52-65.
[24] Sargiacomo C, Sotgia F, Lisanti MP. COVID-19 and chronological aging: Senolytics and other anti-aging drugs for the treatment or prevention of corona virus infection? Aging. 2020; 12: 6511–6517. https://doi.org/10.18632/AGING.103001
[25] Derosa G, Maffioli P, D'Angelo A, Di Pierro F. A role for quercetin in coronavirus disease 2019 (COVID-19).

Phyther Res. 2021; 35: 1230–1236. https://doi.org/10.1002/ptr.6887
[26] Shohan M, Nashibi R, Mahmoudian-Sani MR, Abolnezhadian F, Ghafourian M, Alavi SM, Sharhani A, Khodadadi A. The therapeutic efficacy of quercetin in combination with antiviral drugs in hospitalized COVID-19 patients: A randomized controlled trial. Eur J Pharmacol. 2022; 914: 174615. https://doi.org/10.1016/j.ejphar.2021.174615
[27] Di Pierro F, Derosa G, Maffioli P, Bertuccioli A, Togni S, Riva A, Allegrini P, Khan A, Khan S, Khan BA, Altaf N, Zahid M, Ujjan ID, Nigar R, Khushk MI, Phulpoto M, Lail A, Devrajani BR. Ahmed S. Possible therapeutic effects of adjuvant quercetin supplementation against early-stage covid-19 infection: A prospective, randomized, controlled, and open-label study. Int J Gen Med. 2021; 14: 2359–2366. https://doi.org/10.2147/IJGM.S318720
[28] Horne JR, Vohl MC. Biological plausibility for interactions between dietary fat, resveratrol, ACE2, and SARS-CoV illness severity. Am J Physiol - Endocrinol Metab 2020; 318: E830–E833. https://doi.org/10.1152/AJPENDO.00150.2020
[29] Mittra I, Souza R de, Bhadade R, Madke T, Shankpal PD, Joshi M, Qayyumi B, Bhattacharjee A, Gota V, Gupta S, Chaturvedi P, Badwe R. Resveratrol and Copper for treatment of severe COVID-19: an observational study (RESCU 002). MedRxiv. 2020. https://doi.org/10.1101/2020.07.21.20151423
[30] Bensa M, Vovk I, Glavnik V. Resveratrol food supplement products and the challenges of accurate label information to ensure food safety for consumers. Nutrients. 2023; 15: 474. https://doi.org/10.3390/nu15020474
[31] Güzelmeriç E, Çevik D, Yeşilada E. Quality assessment of marketed food supplements and herbal tea products of olive leaf in Turkey. J Res Pharm. 2020; 24: 159–169. https://doi.org/10.35333/jrp.2020.123
[32] Çevik D, Güzelmeriç E, Şen NB, Yeşilada E. Qualitative and quantitative evaluation of the marketing *Passiflora incarnata* L. formulations in Turkey. J Res Pharm. 2023; 27: 609–619. https://doi.org/10.29228/jrp.343
[33] Calahan J, Howard D, Almalki AJ, Gupta MP, Calderón AI. Chemical adulterants in herbal medicinal products: A review. Planta Med. 2016; 82: 505–515. https://doi.org/10.1055/s-0042-103495
[34] Imran M, Kashif Iqubal M, Ahmad S, Ali J, Baboota S. Stability-indicating high-performance thin-layer chromatographic method for the simultaneous determination of quercetin and resveratrol in the lipid-based nanoformulation. JPC-Modern TLC. 32: 393–400. https://doi.org/10.1556/1006.2019.32.5.7
[35] Sethuraman V, Janakiraman K, Krishnaswami V, Natesan S, Kandasamy R. Combinatorial analysis of quercetin and resveratrol by HPTLC in *Sesbania grandiflora*/phyto-based nanoformulations. Nat Prod Res. 2021; 35(13): 2243-2248. https://doi.org/10.1080/14786419.2019.1662012
[36] ICH Harmonised Tripartite Guideline Validation of Analytical Procedures: Text and Methodology Q2 (R1). https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf (accessed on 25 August 2023).
[37] General Guidance Document For Competent Authorities For The Control Of Compliance with EU Legislation. December 2012. European Commission. Health and Consumers Directorate. https://food.ec.europa.eu/system/files/2016-10/labelling_nutrition-vitamins_minerals-guidance_tolerances_1212_en.pdf (accessed on 27 August 2023).