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In vitro antidiabetic effect, quantitative studies and UPLC-TOF-MS analysis of black tea samples from Turkish market

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ABSTRACT: *In vitro* antidiabetic effects of seven Turkish and five Sri Lankan black tea (*Camelia sinensis*) samples collected from Turkish market were investigated through enzyme inhibition assays. Foreign matter, non-oxidized particles, water soluble matter, loss on drying and total ash values were determined and compared with the Turkish Food Codex-Tea Notification and found to be compliant with the standards. Total phenolic and flavonoid contents, caffeine, gallic acid and rutin amounts of tea samples were measured. Ultra-pressure liquid chromatography with time of flight mass spectroscopy (UPLC-TOF-MS) was used to compare the major compounds of tea samples. All tea samples had potent inhibition against alpha glucosidase (>100-97.86% at 3 mg/ml). Water soluble matters of Sri Lankan teas are higher than them of Turkish samples (except TL1). Caffeine amount of the Sri Lankan tea samples were approximately twice of Turkish black tea samples. Sixteen common compounds were identified in tea samples by UPLC-TOF-MS.

KEYWORDS: Antidiabetic; black tea; *Camellia sinensis*; UPLC-TOF-MS, HPLC, caffeine, rutin, gallic acid.

1. INTRODUCTION

Tea is one of the oldest and the most widely consumed beverages in the world. It is the infusion prepared from the freshly harvested two or three of the top young leaves and bud of *Camellia sinensis* (L.) O. Kuntze (Theaceae) known as tea plant. According to the report of Food and Agriculture Organization, world tea consumption is increasing day by day and China is the largest producer. Sri Lanka and Turkey are two of the important black tea producing countries with 6.8 and 4.5 percent of the world total [1].

Processed commercial tea is classified into three main types according to degree of fermentation as green tea, oolong tea and black tea. Black tea is categorized as fully fermented tea in which the catechins have been extensively oxidized into teaflavins, tearubigins and other oligomers. This oxidation process darkens the green color of tea leaves to the deep brown color almost near to black.

Black tea is divided into two major types as orthodox and CTC (crush, tear and curl) tea according to the manufacturing method. The orthodox term is used to express the traditional process of tea manufacturing which the whole tea leaves are processed manually by hand or rolled using a rolling table after withering stage. Orthodox tea is light, brisk, and generally bright with a flavor that has many layers. CTC machines crush, tear and curl the tea leaves to prepare the granular leaf particles of tea that come in different sizes known as brokens, fannings, and dust. The brokens come with the largest sized grains and the dusts are the finest particles. The smaller sized grains in the CTC tea offer a larger combined surface area thus CTC tea brews quicker. Hence, flavor, color and quality of tea change according to the degree of fermentation during manufacturing [2-4].

Many medicinal properties of black tea have been comprehensively studied such as antioxidant, diuretic, digestive, anti-inflammatory, antimicrobial, anticancer and preventive effect against cardiovascular diseases. Hence, many of its beneficial effects on health are well-known by the consumers [5-11].

Today, Turkey has one of the highest per capita consumption rates of black tea with about 1,000 cups per year. In Turkey, it is a cultural and traditional drink prepared by using double tea pot, drunk in small tulip-shaped glasses. Over 200,000 families are involved in the cultivation of tea either as owners of tea

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plantations, sharecroppers, or employees in the nearly 300 tea producing factories. Thus, tea is much more than a drink and it is one of the most important plants consumed all over the country. By the way, we aimed to compare the enzyme inhibitory and antioxidant activities, chemical profiles and some quantitative properties of Turkish and Sri Lankan tea samples collected from Turkish market in this study.

2. RESULTS AND DISCUSSION

This is the first study on the comparison of Turkish tea samples with Sri Lankan teas, their antidiabetic effects, chemical profiles and quantitative analysis. According to Turkish Food Codex-Tea Notification (TFC) [12], the black tea samples must be free of foreign matter and non-oxidized particles are allowed to be less than 8%. Macroscopic examination of black tea samples has revealed that the samples contained neither foreign matter nor non-oxidized particles. Total ash values of the samples were compatible with the TFC and all of them were between the given limits (4-8% of the dried leaves). Highest total ash value was measured in SL3 with 5.47% and the lowest was in TL2 with 4.27%. Humidity of the dried black tea samples should be less than 7% according to the TFC and loss on drying values of all the tea samples suited to this limit (between 2.97-5.23 %) (Table 1). Water soluble matter of black tea samples were ranging between 21.03-33.65% in leaf samples and 24.26-31.47 in tea bag samples. The highest values were detected in Sri Lankan teas with 33.65 in SL2 and with 31.47 in SB1.

Table 1. Codes, commercial names, water soluble matter (WSM), total ash and loss on drying values (%), total phenolic contents (TPC), total flavonoid contents (TFC) (%), and α-glucosidase inhibitory activities (Inhibition % ± S.D.) of tea samples.

Sample	Commercial Name	WSM %	Total Ash %	Loss on Drying %	TPC %	TFC %	α-Glucosidase Inhibitory Activities			
							3000 µg/ml	1000 µg/ml	300 µg/ml	100 µg/ml
TL1	Caykur Altinbas	23.78	4.74	3.63	21.83 ± 0.33	8.93 ± 1.34	97.86 ± 1.52	96.25 ± 0.22	72.90 ± 4.60	32.69 ± 8.32
TL2	Caykur Tiryaki	22.49	4.27	3.08	24.26 ± 0.55	9.35 ± 0.17	98.49 ± 0.65	90.92 ± 0.82	57.69 ± 1.85	7.73 ± 0.20
TL3	Caykur Turist	22.58	4.55	3.31	18.85 ± 1.13	7.38 ± 0.27	99.22 ± 0.50	94.00 ± 0.23	69.91 ± 0.02	19.43 ± 2.83
TL4	Caykur Organic Hemsin	21.03	5.16	2.97	19.32 ± 0.13	8.47 ± 0.61	98.71 ± 1.29	97.03 ± 0.40	79.82 ± 0.75	30.26 ± 6.00
TL5	Lipton Yellow Label	21.66	4.80	3.10	14.89 ± 0.18	4.36 ± 0.02	98.84 ± 0.26	94.06 ± 0.52	71.01 ± 4.06	19.93 ± 3.89
SL1	Istıkan	22.81	4.64	5.11	12.01 ± 0.32	3.90 ± 0.44	98.73 ± 0.25	97.80 ± 0.04	84.13 ± 1.52	38.19 ± 7.96
SL2	Interleon	33.65	5.34	3.15	18.96 ± 0.22	4.30 ± 0.10	99.46 ± 0.53	94.25 ± 0.12	34.62 ± 5.98	5.05 ± 2.18
SL3	Champion	24.85	5.47	3.83	37.52 ± 1.06	7.96 ± 0.17	99.68 ± 0.56	98.78 ± 0.18	89.80 ± 1.22	66.22 ± 4.74
SL4	Layalina	26.57	5.16	3.55	32.39 ± 0.41	4.05 ± 0.07	> 100.00	98.06 ± 0.57	90.63 ± 1.33	59.10 ± 3.88
TB1	Caykur Organic Hemsin Tea Bag	24.26	4.74	4.57	17.81 ± 0.35	5.74 ± 0.71	99.49 ± 0.12	96.29 ± 0.53	68.59 ± 7.42	15.73 ± 1.03
TB2	Lipton Yellow Label Tea Bag	26.84	5.20	3.42	23.11 ± 0.83	7.69 ± 0.00	> 100.00	96.67 ± 0.48	78.99 ± 0.86	40.57 ± 8.69
SB1	Ahmad Tea Bag	31.47	4.29	5.23	30.38 ± 1.03	7.09 ± 0.30	> 100.00	98.37 ± 0.10	80.00 ± 1.37	32.19 ± 1.26
							100 µg/ml	**30 µg/ml**	**10 µg/ml**	**3 µg/ml**
Ref.	Acarbose	-	-	-	-	-	98.67 ± 0.17	98.02 ± 0.03	96.13 ± 0.62	92.77 ± 1.05

Total phenolic contents of the tea samples vary from 12.01 to 37.52% and the highest phenolic contents were determined in Sri Lankan teas in SL3, SL4 and SB1 with 37.52, 32.39 and 30.38 (mg gallic acid equivalent in 100 g extract) respectively. On the contrary, total flavonoid contents of the Turkish tea samples were higher than the Sri Lankan's. The total flavonoid content of Turkish leaf tea samples was between 9.35-4.36 mg quercetin equivalent in 100 g extract and the highest flavonoid content in tea bag samples were determined in TB2 with 7.69 mg quercetin equivalent in 100 g extract (Table 1).

High performance liquid chromatography was used to determine the caffeine, rutin and gallic acid amounts of selected tea samples and the results are given in Table 2. Gallic acid contents of Sri Lankan tea samples (1.10-0.23%) were found to be much more than them of Turkish samples (0.23-0.05%). Only gallic acid content of SB4 was below the quantification limit, thus it could not be calculated. According to TFC caffeine content of black tea samples should not be less than 1.6% (g/g). In our study, caffeine content of all tea samples

were over the limit and the highest caffeine was measured in SL4 sample (7.24%) that is nearly twice of the Turkish leaf samples. According to the results, rutin amounts in the tea samples were close to each other ranging from 0.11 to 0.34%.

Table 2. Gallic acid, caffeine and rutin percentages of black tea samples.

	Gallic Acid %		Caffeine %		Rutin %	
Sample	**Extract**	**Tea Sample**	**Extract**	**Tea Sample**	**Extract**	**Tea Sample**
TL1	0.94	0.22	16.74	3.98	1.05	0.25
TL2	0.88	0.20	13.45	3.03	0.66	0.15
TL3	0.85	0.19	15.28	3.45	0.74	0.17
TL4	0.25	0.05	9.08	1.91	0.89	0.19
TL5	0.70	0.15	15.08	3.27	0.51	0.11
SL1	2.34	0.53	18.06	4.12	0.52	0.12
SL2	0.67	0.23	11.74	3.95	0.64	0.22
SL3	4.44	1.10	22.15	5.50	0.85	0.21
SL4	-	-	27.25	7.24	0.80	0.21
TB1	0.62	0.15	10.68	2.59	1.04	0.25
TB2	0.843	0.23	16.41	4.40	1.27	0.34
SB1	1.79	0.56	15.50	4.88	0.61	0.19
Retention time	4.1		10.7		24.8	
Standard curve	*y=27107.4x+323999*		*y=145904x+743818*		*y=83235.9x+1185930*	
Linearity	r^2=0.9921		r^2=0.9975		r^2=0.9890	

UPLC-TOF-MS was used to compare chemical profiles of the Turkish and Sri Lankan leaf and tea bag samples, the results are given in Table 2 and Table 3. Caffeine, catechin/epicatechin, catechingallate/epicatechin gallate, gallic acid, gallocatechin, kaempferol hexoside, kaempferol rhamnohexoside, *L*-teanin, *p*-coumaroyl quinic acid, quercetin hexoside, rutin, teaflavin, teaflavin digallate, teaflavin gallate, theagallin, and theabromine were the common compounds detected in all samples. Additionally, chlorogenic acid was determined in only seven samples in TL5, TB2, and in all Sri Lankan tea samples. Epigallocatechingallate/gallocatechingallate, epigallocatechin, *p*-coumaric acid, epigallocatechin-3,5-digallate, epicatechin-3,5-digallate, elagic acid, caffeic acid, quercetin, isochlorogenic acid were not existing in the samples and they were thought to be absent (Figure 1-3).

After chemical analysis, enzyme inhibitory effects of tea samples were investigated. It was surprising to see all the samples were inactive on *a*-amylase enzyme. On the contrary, all of the extracts inhibited *a*-glucosidase enzyme strongly at 3000, 1000, 300 and 100 µg/ml concentrations. The effects were dose dependent and promising compared to the reference drug acarbose.

Tea is the most widely consumed beverage all around the world and it confers many beneficial effects to the health of consumers with its medicinal properties. Taste, color, odor, chemical profile, and biological activities of the black tea vary with species, season, horticultural conditions and particularly with degree of fermentation during the manufacturing process. Numerous studies have reported on the variation of catechins, phenolic acids and caffeine contents of commercial black tea samples hitherto [4, 13, 14].

In Sri Lanka, traditional medical practitioners recommend consumption of 6-10 cups of black tea for prediabetics and mild diabetics [15]. Thus, many studies have been conducted to reveal the hypoglycaemic effect of black tea *in vivo* and *in vitro* [3, 16-20]. These studies exhibited that black tea and its active constituents have hypoglycemic, antihyperglycemic and antidiabetic activities on experimental animals by inhibiting intestinal glucose absorption, impairing *a*-glucosidase and *a*-amylase enzymes, and improving insulin

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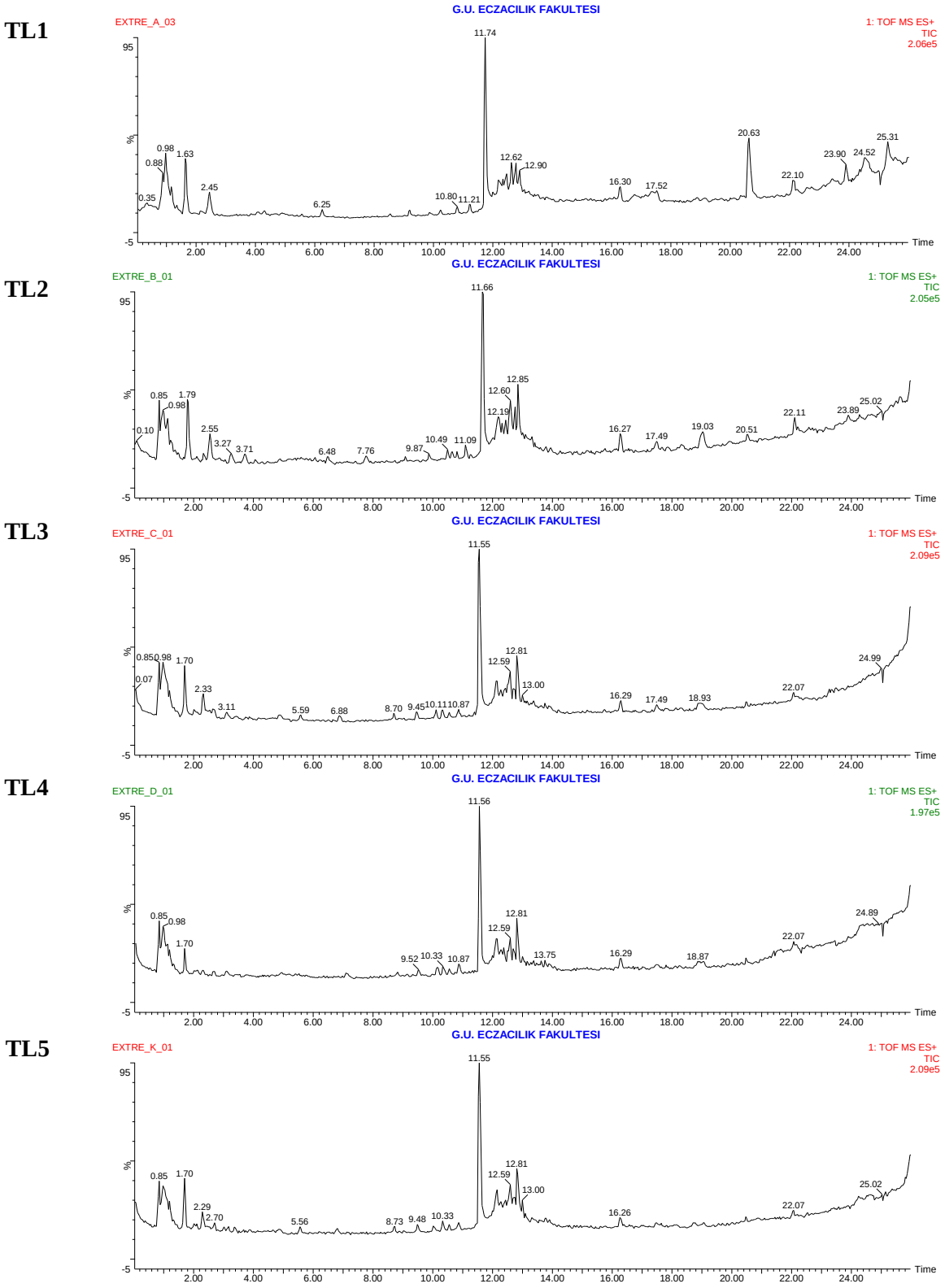

Figure 1. Ultra-pressure liquid chromatography with time of flight mass spectroscopy (UPLC-TOF-MS) total ion chromatograms of Turkish leaf tea samples

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sensitivity. Additionally, Jin et al. [21] proved that teaflavins are one of the functional components which contributed to the antiobesity and lipid lowering effects of black tea, and might reduce the risk of type 2 diabetes and cardiovascular disease in obese patients.

Table 3. Compounds determined by UPLC-TOF-MS in black tea samples and their retention times.

Compound	Molecular Formula	Selected Ion	*m/Z* Experimental	Selected Ion	*m/Z* Experimental	Retention times (Rt)											
						TL1	TL2	TL3	TL4	TL5	SL1	SL2	SL3	SL4	TB1	TB2	SB3
L-Teanin	$C_7H_{14}N_2O_3$	$[M-H]^-$	173.0926	$[M+H]^+$	175.1083	2.08	2.23	2.26	2.26	2.08	2.11	2.24	2.48	2.08	2.14	2.42	2.17
Gallic acid	$C_7H_6O_5$	$[M-H]^-$	169.0137	$[M+H]^+$	171.0293	4.31	3.71	3.20	3.18	3.11	3.18	3.18	3.24	3.17	3.21	3.73	3.17
Theagallin	$C_{14}H_{16}O_{10}$	$[M-H]^-$	343.0665	$[M+H]^+$	345.0822	6.19	5.09	4.90	5.06	4.99	5.06	4.94	4.90	4.99	5.12	6.00	4.96
Theobromine	$C_7H_8N_4O_2$	$[M-H]^-$	179.0569	$[M+H]^+$	181.0726	9.29	9.08	8.73	8.86	8.73	8.89	8.92	8.76	8.89	8.76	9.17	8.76
Gallocatechin	$C_{15}H_{14}O_7$	$[M-H]^-$	305.0661	$[M+H]^+$	307.0818	11.05	10.81	10.55	10.80	10.58	10.58	10.62	10.65	10.74	10.61	10.87	10.58
Caffeine	$C_8H_{10}N_4O_2$	$[M-H]^-$	193.0726	$[M+H]^+$	195.0882	11.75	11.69	11.55	11.56	11.55	11.55	11.56	11.56	11.55	11.56	11.65	11.55
Chlorogenic acid	$C_{16}H_{18}O_9$	$[M-H]^-$	353.0873	$[M+H]^+$	355.1029	-	-	-	-	11.74	11.71	11.75	11.68	11.74	-	11.90	11.74
p-Coumaroyl quinic acid	$C_{18}H_{18}O_8$	$[M-H]^-$	337.0923	$[M+H]^+$	339.1080	12.43	12.12	12.27	12.34	12.43	12.43	12.22	12.18	12.34	12.34	12.31	12.34
Catechin/Epicatechin	$C_{15}H_{14}O_6$	$[M-H]^-$	289.0712	$[M+H]^+$	291.0869	12.28	12.19	12.12	12.19	12.12	12.12	12.12	12.18	12.12	12.15	12.15	12.14
Quercetin hexoside	$C_{21}H_{20}O_{12}$	$[M-H]^-$	463.0877	$[M+H]^+$	465.1033	12.96	12.91	12.84	12.91	12.97	12.97	12.99	12.97	12.93	12.97	12.97	12.84
Rutin	$C_{27}H_{30}O_{16}$	$[M-H]^-$	609.1459	$[M+H]^+$	611.1612	12.84	12.81	12.77	12.69	12.65	12.68	12.69	12.78	12.78	12.78	12.84	12.75
Kaempherol rhamnohexoside	$C_{33}H_{40}O_{19}$	$[M-H]^-$	739.2086	$[M+H]^+$	741.2242	12.94	12.84	12.80	12.91	12.94	12.90	12.78	12.78	12.78	12.84	12.94	12.84
Catechingallate/ Epicatechingallate	$C_{22}H_{18}O_{10}$	$[M-H]^-$	441.0822	$[M+H]^+$	443.0978	12.97	12.91	12.90	12.93	12.94	12.93	12.91	12.97	12.93	12.91	13.06	13.09
Kaempferol hexoside	$C_{21}H_{20}O_{11}$	$[M-H]^-$	447.0927	$[M+H]^+$	449.1084	13.21	13.23	13.24	13.12	13.15	13.21	13.28	13.09	13.28	13.22	13.10	13.15
Teaflavin	$C_{29}H_{24}O_{12}$	$[M-H]^-$	563.1190	$[M+H]^+$	565.1346	13.85	13.79	13.75	13.75	13.72	13.78	13.78	13.78	13.75	13.91	13.88	13.78
Teaflavin gallate	$C_{36}H_{28}O_{16}$	$[M-H]^-$	715.1299	$[M+H]^+$	717.1456	13.91	13.88	13.87	13.88	13.94	13.85	13.94	14.03	13.84	13.94	13.88	13.87
Teaflavin digallate	$C_{43}H_{32}O_{20}$	$[M-H]^-$	867.1409	$[M+H]^+$	869.1565	14.03	13.94	13.91	13.91	13.93	13.94	13.94	13.87	14.03	13.94	13.94	13.90

In Turkey, black tea is consumed in breakfast, just after lunch and dinner every day by the Anatolian people. Our results supported the current literature that all tested tea samples have shown strong *a*-glucosidase enzyme inhibitory activity *in vitro*. Thus, black tea can help to lower postprandial glucose of the consumers.

Our results have also expressed that the black tea samples in the Turkish market are compatible with the standards of Turkish Food Codex Tea Notification. On the other hand, the chemical profiles of the teas from the same country were very much alike (Figure 1 and Figure 2). Although there were some differences in the total content of phenolics and flavonoids, amounts of caffeine, gallic acid and rutin (Figure 4-7), UPLC analysis have shown that the chemical composition of black teas in tea bags are similar (Figure 3).

3. CONCLUSION

Black tea is the most consumed beverage in Turkey. Our quantitative and chemical studies have shown the high content of bioactive compounds and biological studies supported its beneficial effects in daily consumption. It is well-known that taste, color, odor, chemical profile, and biological activities of the black tea vary with species, season, horticultural conditions and with degree of fermentation during the manufacturing process. Thus, some differences in the chemical profiles were detected between Turkish and Sri Lankan tea samples.

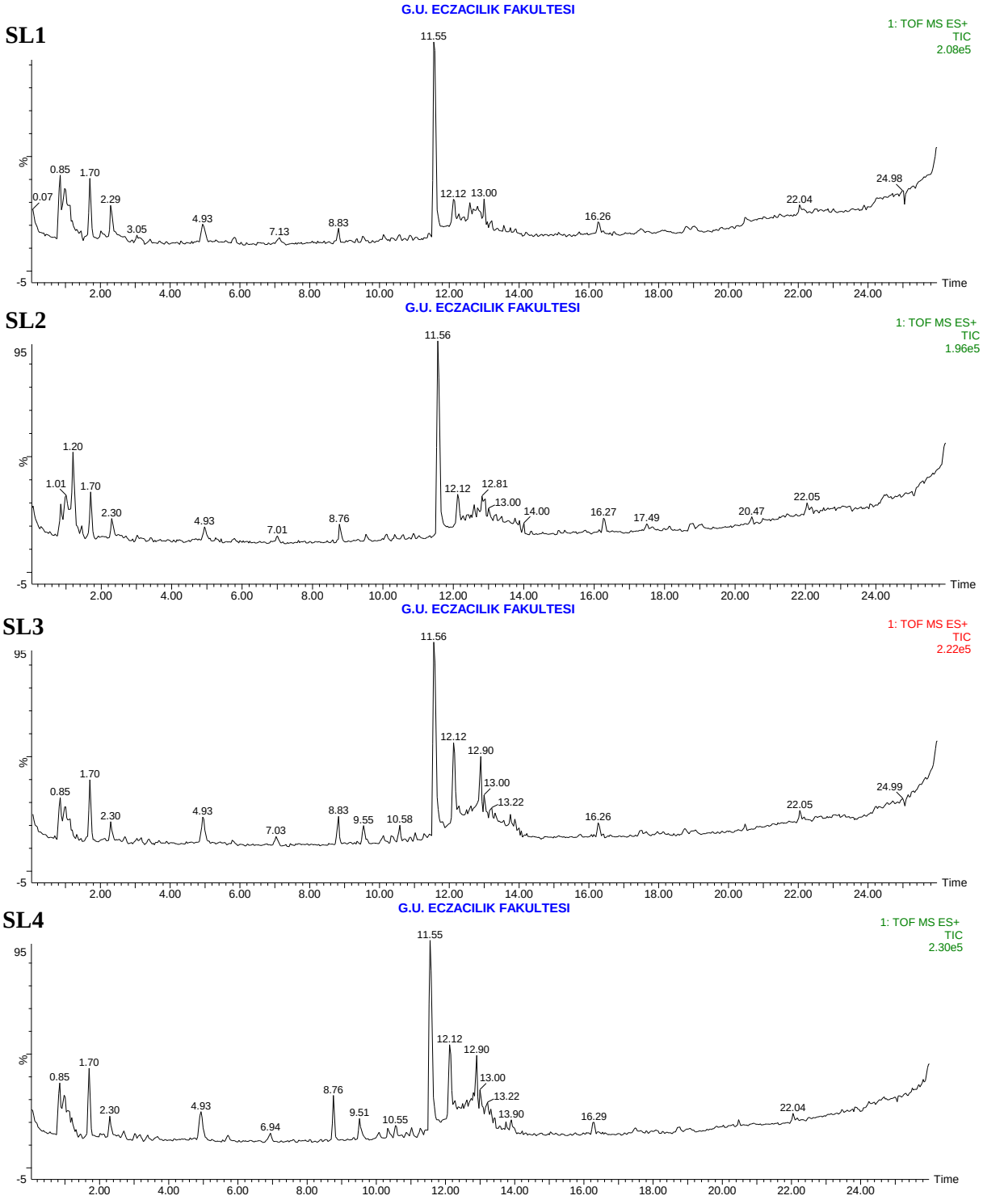

Figure 2. UPLC-TOF-MS total ion chromatograms of Sri Lankan leaf tea samples.

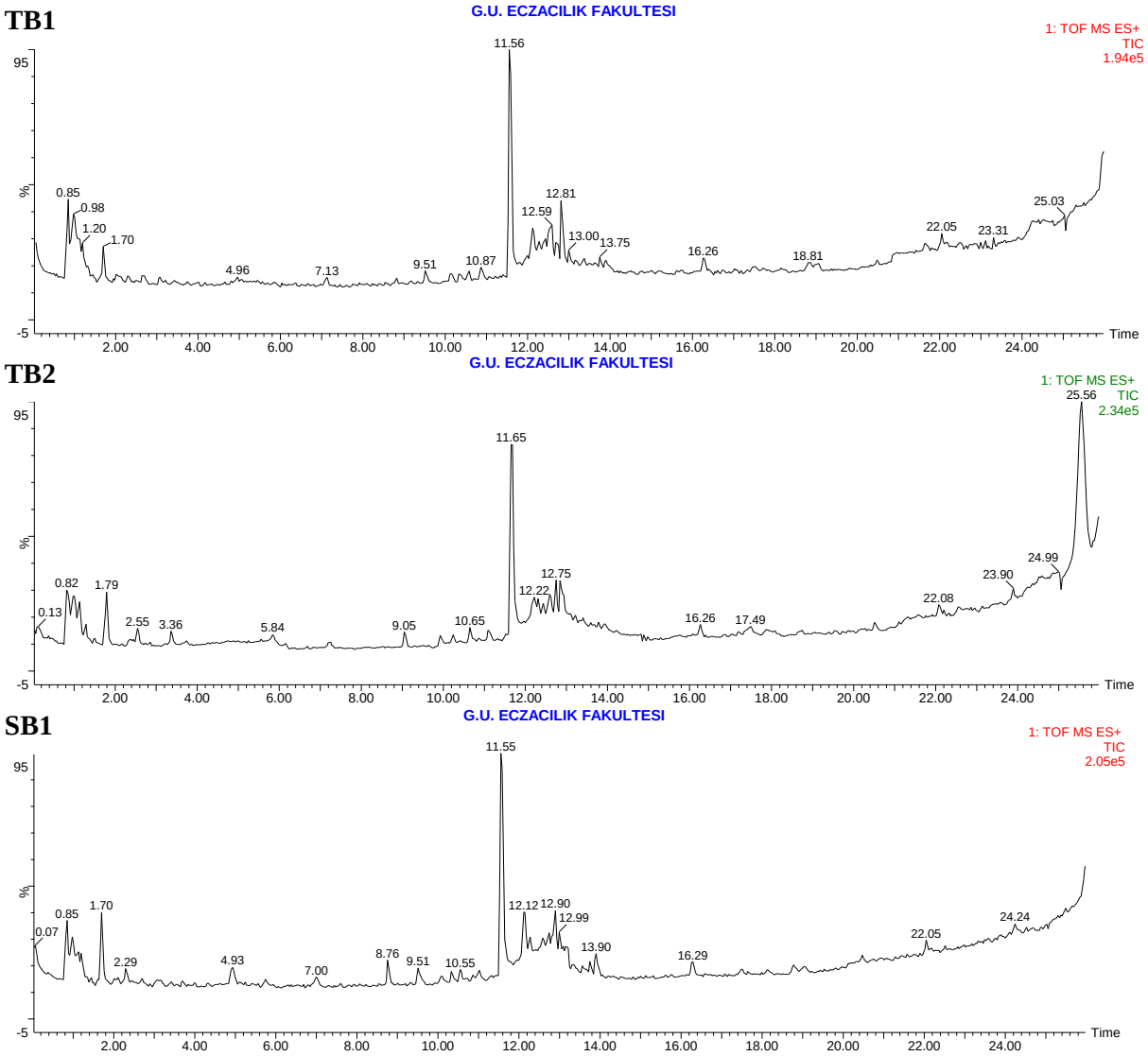

Figure 3. UPLC-TOF-MS total ion chromatograms of tea bag samples.

4. MATERIALS AND METHODS

4.1. Plant materials

Twelve black tea samples were collected from Turkish market. Tea samples grown in Turkey (n=7) are coded with T and Sri Lankan tea samples are coded with S (n=5). Tea samples having whole leaves or big tea particles as brokens and fannings are coded as L (leaf) (n=9) and tea bag samples produced from dusts are coded as B (bag) (n=3).

4.2. Foreign matter and non-oxidized particles

10 g each of black tea samples were spread in thin layers on a white paper. Foreign matters and non-oxidized particles were detected by inspecting with naked eye and separated. According to Turkish Food Codex-Tea Notification (TFC) [12], black tea should not contain any foreign particles such as parts of other plants, animal originated particles, stone, plastic or similar materials and artificial substances. Non-oxidized particles are allowed to be less than 8% (g/g).

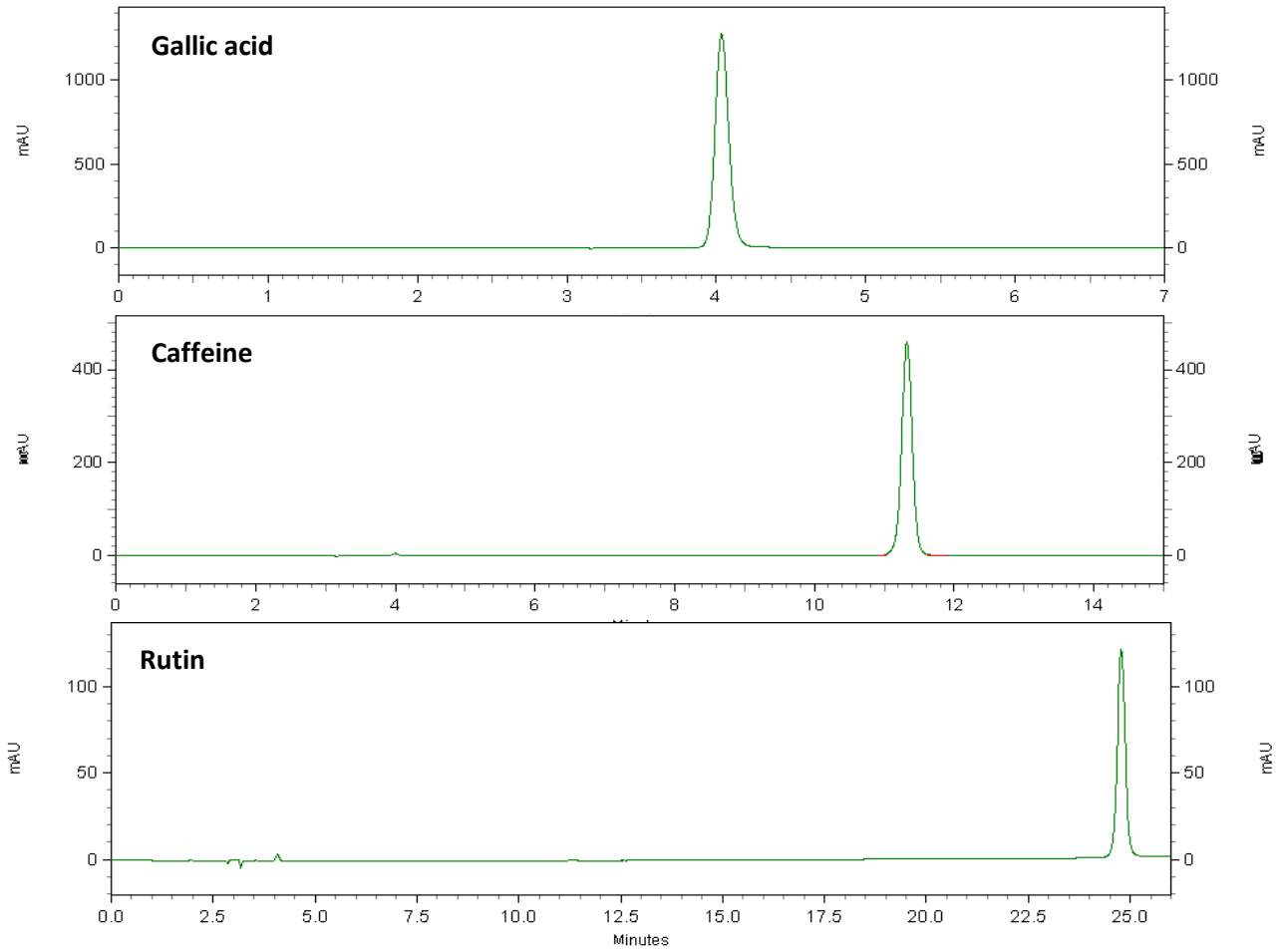

Figure 4. HPLC chromatograms of the standards.

4.3. Total ash

Tea samples were weighted (1.000 g) in silica crucibles previously ignited by a furnace to a constant mass. Samples were incinerated at 600 ˚C for 3 h and weighted after they were allowed to cool in desiccators. Tree replicates were prepared and average total ash % values were calculated. Total ash values should be between 4-8% (g/g) of the dried leaves [12].

4.4. Loss on drying

Tea samples were accurately weighted (1.000 g) in glass weighing bottles previously dried in an oven to a constant mass. The percentages of weight losses were calculated after drying powdered drugs in an oven at 105 ˚C for 2 h. Humidity of the dried black tea samples should be less than 7% according to the TFC [12].

4.5. Water soluble matter

To prepare water extract and to measure water soluble matter, infusion of the samples were prepared. For this aim, 50 ml boiling water was added on 1 g of tea samples. Tea samples were kept on boiling water bath for 12 minutes. Filtered extracts were freezed at -80 ˚C. Freeze-dried extracts were weighted and water soluble matter was calculated as w/w %.

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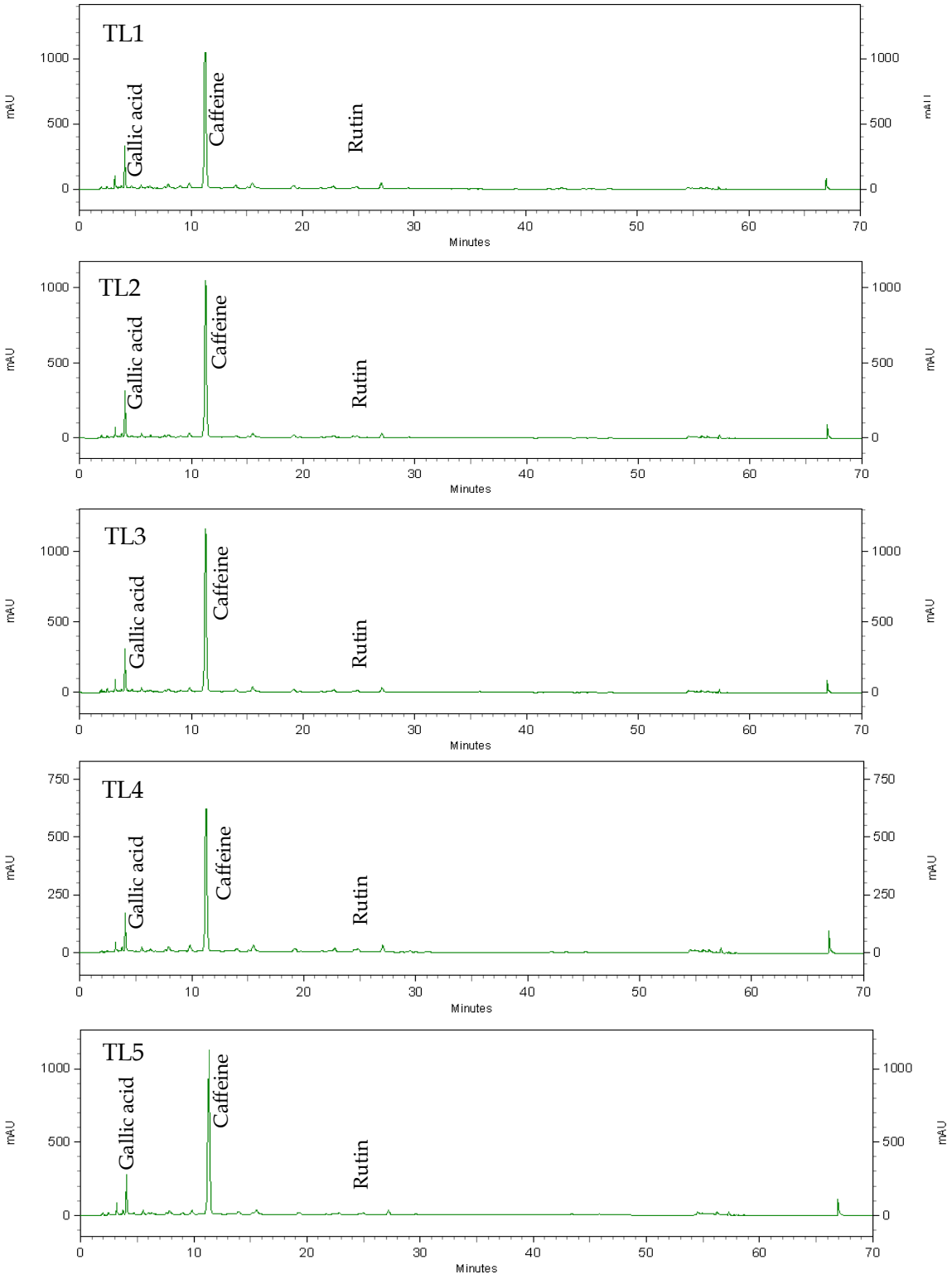

Figure 5. High-pressure liquid chromatography (HPLC) chromatograms of Turkish leaf tea samples.

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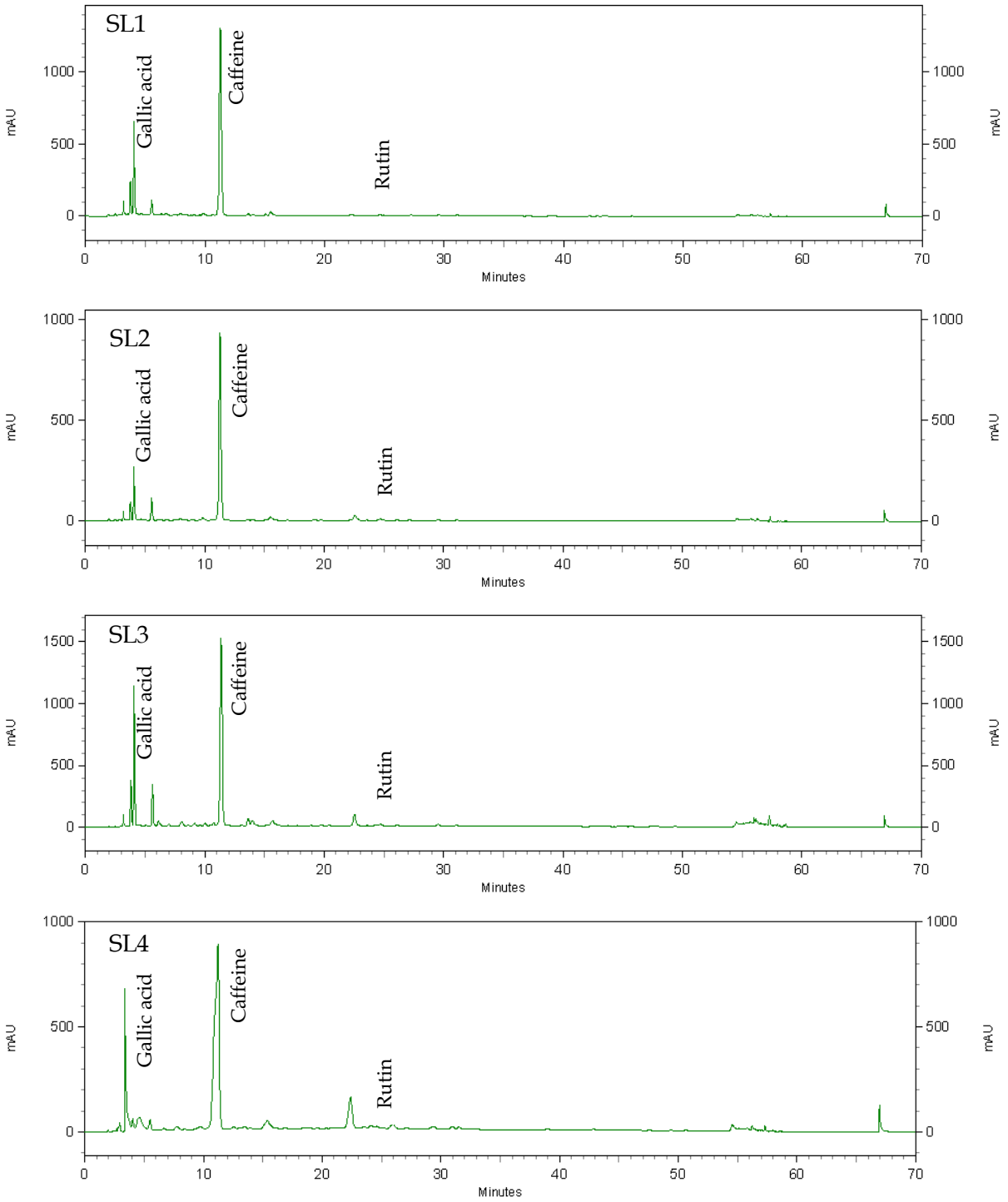

Figure 6. High-pressure liquid chromatography (HPLC) chromatograms of Sri Lankan leaf tea samples.

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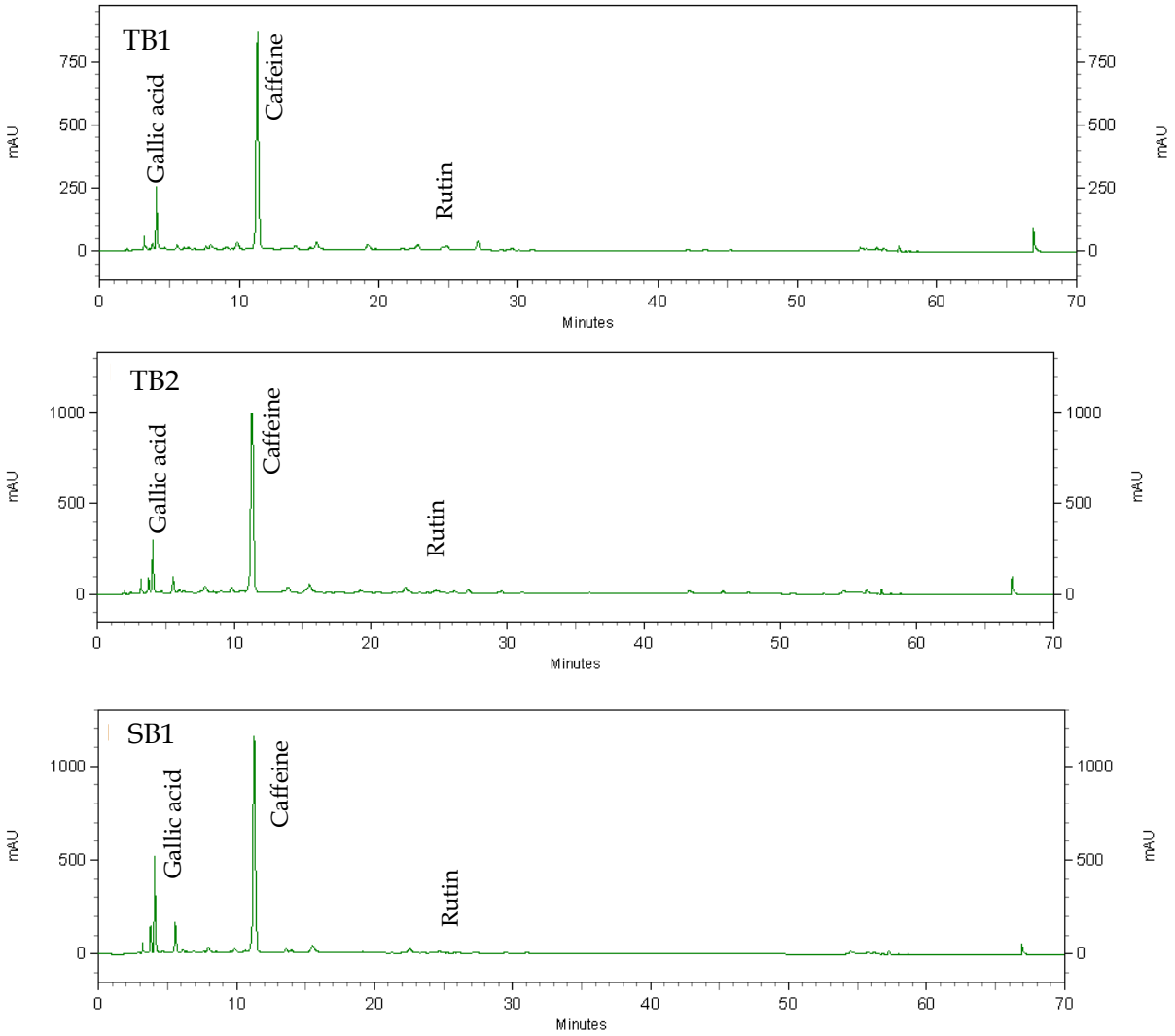

Figure 7. High-pressure liquid chromatography (HPLC) chromatograms of tea bag samples.

4.6. Determination of total phenolic content

The total phenolic contents (TPC) were determined by the Folin-Ciocalteu method following one of our previous literature [23]. Five readings were done and the results were averaged. Percent of TPC was mentioned in mg of gallic acid equivalents (GAE)/100 g extracts.

4.7. Determination of total flavonoid content

The total flavonoid contents (TFC) of the samples were calculated by using a common method described in Orhan et al. [23]. The absorbance was measured at 415 nm. TFC of the extracts were given in mg of quercetin equivalents (QE)/100 g extracts.

4.8. Chromatography

4.8.1. Solvents and chemicals

HPLC grade acetonitrile, chromatographic grade double-distilled water, analytical grade acetic acid and formic acid were used for HPLC and UPLC analysis. All solvents were filtered and degassed before use. Caffeine (C0750), gallic acid (G7384) and rutin (R5143) were purchased from Sigma-Aldrich (Germany).

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4.8.2. High performance liquid chromatography (HPLC) analysis

Amount of caffeine, gallic acid and rutin in the tea extract samples were determined by using HPLC. Thermo Electron Corporation Finnigar Surveyor HPLC system equipped with a LC pump, an auto-sampler, a column oven, and a Thermo Finnigar Surveyor PDA Detector was used. Data analysis was performed using Chromequest. The separation was made on Phenomenex Luna C18, (250x4.6 mm, 5 µ). The mobile phase was a mixture of acetonitrile (solution A), acetic acid 2% in water (solution B). The composition of the gradient was (A:B), 8:92 at 0 min, 31:69 at 50 min, 100:0 at 55 min, 100:0 at 60 min, 8:92 at 70 min. The injection volume was 20 µl. The detector was set at 230, 280, and 360 nm and quantification was performed at 280 nm. The column temperature was 30 °C. Tea extracts, caffeine, gallic acid and rutin were dissolved in ultrapure water-acetonitrile (1:1) mixture to maintain concentration of 1 mg/ml. All the extracts and standards were filtered by a 0.45 µm filter before injection. To obtain calibration curves, 5 different concentrations of standards were prepared from the stock solutions (1 mg/ml) (Figure 4). According to Turkish Food Codex-Tea Notification [12], caffeine content of black tea samples should not be less than 1.6% (g/g).

4.8.3. UPLC-TOF-MS analysis

Reference compounds (caffeine, gallic acid, and rutin) and extracts were dissolved in methanol before the experiments (1 mg/ml) and the extracts were filtered by using a filter membrane. Three microliters of the samples/reference compounds were injected to the chromatographic system and monitored. Acquity Ultra Performance Liquid Chromatography system (Waters Corp, Milford, MA) was used for the determination of major compounds with a 2.1 mm x 100 mm Acquity UPLC BEH and 1.7 µm C18 column (Waters Corp, Milford, MA). The mobile phase, TOF instrumentation data, data processing and the method used was described in detail in our previous study [23]. Chemical constituents of black tea leaves were investigated by a literature search. 29 compounds were selected and their molecular formulas were enrolled. Their M+ and M- values were calculated accurately by using MassLynx V 4.1 software.

4.9. Assay for *a*-amylase inhibitory activity

a-Amylase type VI (EC 3.2.1.1, Sigma) was used to determine the *a*-amylase inhibitory activity. The spectrophotometric method used in this study was described in our previous study in detail [22]. Absorbance was read at 540 nm and Acarbose (Bayer, Turkey) was used as reference. Percent of inhibition was calculated as: Inhibition %=[(Maltose $_{Control}$ -Maltose $_{Sample}$)/Maltose $_{Control}$)]×100

4.10. Assay for *a*-glucosidase inhibitory activity

a-Glucosidase inhibitory activities of the extracts were measured using the already published method [22]. Acarbose (Bayer, Turkey) was used as positive control. The inhibition percentage (%) was calculated by the equation: Inhibition %=[(AControl-ASample)/AControl]×100

4.11. Statistical analysis

All analyses were carried out in triplicates and the results were averaged. All values are expressed as the mean ± standard deviation (S.D.) or standard error of the mean (S.E.M.); linear regression analyses and calculations were done by using Microsoft Excel and GraphPad Instat softwares.

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