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RESEARCH ARTICLE/**ARAŞTIRMA MAKALESİ**

DETERMINATION OF BASIC POLYPHENOLIC COMPOUNDS IN SOME TURKISH TEA SAMPLES BY HPLC METHOD*

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ABSTRACT

The basic polyphenolic compounds present in several tea samples from Turkish market have been determined using a high performance liquid chromatographic method (HPLC) with diode array detector. Chromatographic separation was performed by delivering the mobile phase, A: acetonitrile: TFA:water (10:0.3:89.7 v/v/v) and B: acetonitrile:water (30:70 v/v) at a flow rate of 1mL/min. A Macherey-Negal C_8 column (150 x 3.00mm, particle size 5µm) was used for separation. Four basic polyphenolic compounds originated from tea were separated in 17 minutes. The detections were carried out at 278 nm. Twenty tea samples present in Turkish market were analysed with this method and results showed that 2% infusions of all samples contain the basic polyphenols at µg/mL level.

Keywords: Tea, Polyphenol, Determination, HPLC

BAZI TÜRK ÇAY ÖRNEKLERINDEKİ ANA POLİFENOLİK BİLEŞIKLERİN YBSK YÖNTEMİ İLE TAYINİ

ÖZ

Türk pazarındaki çeşitli çay örneklerinde bulunan ana polifenolik bileşikler YBSK yöntemi ile diode array dedektör kullanılarak tayin edilmiştir. Kromatografik ayırım, A: asetonitril: TFA : su (10:0.3:89.7 h/h/h) ve B: asetonitril : su çözeltilerinin karışımından (30:70 h/h) oluşan hareketli fazın 1 mL/dakika akış hızı ile sisteme verilerek sağlanmıştır. Ayırım amacı ile Macherey-Negal C_8 kolon (150 x 3.00mm, partikül büyüklüğü 5µm) kullanılmıştır. Çayda bulunan dört ana polifenolik bileşik 17 dakika içinde ayrılmıştır. Dedeksiyon 278 nm dalga boyunda gerçekleştirilmiştir. Türk pazarında bulunan 20 çay örneği bu yöntem ile analiz edilmiş ve elde edilen sonuçlar tüm çay örneklerinin %2'lik infüzyonlarının, ana polifenolik bileşikleri µg/mL düzeyinde içerdiğini göstermiştir.

Anahtar Kelimeler: Çay, Polifenol, Miktar Tayini, YBSK

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1. INTRODUCTION

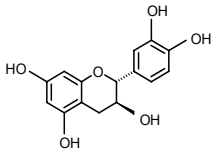
Camellia sinensis (tea plant) is an evergreen tree belonging to the family of Theaceae (Weisburger, 1997). Tea is the most popular beverage consumed by over two thirds of the world's population. After harvesting process, tea leaves are processed differently to give green, black or oolong tea (Kuroda and Hara 1999). Tea leaves are stabilized by dry heating or steaming, rolled, dried rapidly and more or less roasted to give green tea (Bruneton, 1999). It's consumed predominantly in Japan, China, India, a few countries in North Africa and the Middle East (Ahmad and Mukhtar, 1999). Tea leaves are wilted for about 20 hours, rolled, fermented in a humid atmosphere, than dried with hot air for the production of black tea (Bruneton 1999). It's commonly consumed in the western countries (Ahmad and Mukhtar, 1999). If the leaves are partially fermented, the product is oolong tea (Bruneton, 1999).

Tea is pharmacologically effective in clinical treatment of amebic dysentery, bacterial dysentery, gastroenteritis and hepatitis. It has also been reported to antiatherosclerotic effects and Vitamin P activity (Duke 1985). Black tea has beneficial effects on cerebravascular and cardiac accidents. Tea is also used as a diuretic and stimulant (Bruneton 1999) A lot of laboratory and epidemiological studies carried out in recent years have shown that polyphenolic compounds, present in tea leaves, have antioxidant, antimutagenic, anticarcinogenic and chemopreventive effects (Kuroda and Hara 1999, Ahmad and Mukhtar, 1999, Fujiki et al. 1998, 2000, Inoue et al. 2001).

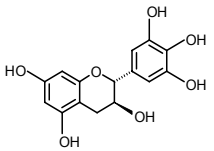
The most important chemical compounds in tea are polyphenols belonging to the catechines family and caffeine. The amounts of polyphenolic compounds depend on the genetic make up and environmental factors such as climate, light, rainfall, temperature, nutrient availability and leaf age (Kuroda and Hara 1999). The polyphenolic compounds account for up to 30% of the dry weight of green tea leaves. While green tea is containing the major polyphenolic compounds (EC, ECG, EGC and EGCG), the black tea also contains oxidative products such as teaflavins and tearubigens (Bruneton, 1999) The major polyphenolic compounds present in green tea and black tea are represented in Table 1. The chemical structures of basic green and black tea catechins are illustrated in Figure 1.

Table 1. Polyphenols in green tea and black tea

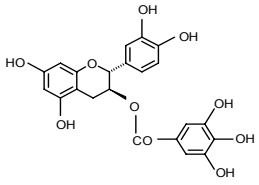
Polyphenol	Green Tea	Black Tea
(-)-Epicatechin (EC)	+	+
(-)-Epicatechin-3-gallate (ECG)	+	+
(-)-Epigallocatechin (EGC)	+	+
(-)-Epigallocatechin-3-gallate (EGCG)	+	+
Teaflavin (TF)	-	+
Teaflavin gallate A (TFA)	-	+
Teaflavin gallate B (TFB)	-	+
Teaflavindigallate (TFDG)	-	+



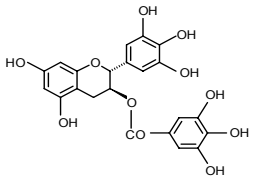
(-)-Epicatechin (EC)



(-)Epigallocatechin(EGC)



(-)- Epicatechin gallate(ECG)



(-)- Epigallocatechin gallate(EGCG

Figure 1. Chemical structures of catechins in green tea and black tea

The tea plant has been growing since the end of 1800's in northeast of Turkey. Today, there are more than 40 plants producing black and green tea in Rize (Baytop 1999). The products are marketed in Turkey and also exported.

Several methods including capillary zone electrophoresis (CZE) (Horie et al. 1997, Arce et al. 1998), micellar electrokinetic capillary chromatography (MEKC) (Nelson et al. 1998, Larger et.al. 1998), gas chromatography (GC) (Luthria et al. 1997), thin layer chromatography (TLC) (Dalluge ve Nelson 2000), paper chromatography (PC) (Dalluge ve Nelson 2000), visible spectrophotometry (Hibasami et.al. 1996), chemiluminescence (Sagesaka-Mitane 1990), nuclear magnetic resonance (NMR) (Ahmad and Mukhtar 1999), liquid chromatography (LC and HPLC) (Dalluge ve Nelson 2000; Tsuchiya et al. 1997, Lee and Ong 2000) have been published for the determination of tea polyphenols.

In present study we aimed to determine the quantities of EC, EGC, EGCG and ECG, the most important polyphenols having health beneficial effects, in 20 different black and green tea samples from Turkish market using an HPLC method established by Tsuchiya et al. (1997).

2. MATERIAL AND METHOD

2.1. Apparatus

The HPLC system consisted of an LC10A liquid chromatograph (Shimadzu), a Macherey-Negal C_8 column (150 x 4.6mm, particle size 5µm), a SPD-M10A diode array detector, a LC10AT gradient pump (Shimadzu), a CBM-10A connector module

(Shimadzu) and a Rheodyne injection port (Cotati) with a 20µL loop.

2.2. Chemicals and Reagents

EGCG was purchased from Phytoplan Co.(Germany) EC, EGC and ECG standards were obtained from Sigma Co. Trifluoro acetic acid and methanol were obtained from Merck Co. Bidistilled water was prepared in our laboratory.

2.3. Standard Solutions

The stock standard solutions were prepared as follows: EGCG; 4.4mg/10mL bidistilled water, EC; 15.9mg/50mL 10% methanol. EGC; 5mg/3mL bidistilled water and ECG; 4.4mg/10mL 10% methanol. Standard solutions were stored at +4°C in the dark.100µL of each solution were mixed and used as standard mixture solution for HPLC analysis.

2.4. Sample Preparation

20 different tea samples were purchased from several markets. 100mL of boiling water was added to 2g of tea and boiled 5 minutes more. This procedure is similar to the preparation of beverage by human. The infusion preparation was carried out just before the analysis and injected to HPLC after filtered from 0.45µm filter (Orange Scientific).

2.5. Mobile Phase and Instrumental Parameters

Chromatographic separation was performed by delivering the mobile phase, A; acetonitrile:TFA:water (10:0.3:89.7 v/v/v) and B; acetonitrile:water (30:70 v/v) at a flow rate of 1mL/min. The gradient was prepared as follows: 100% A at 0 min to 25% A and 75% B at 17 min in a linear gradient elution mode and than 100% A for five minutes. In routine analysis polyphenols were detected at 278 nm.

3. RESULTS AND DISCUSSION

The retention times of EGC, EC, EGCG and ECG were found as 4.8, 7.9, 10.9 and 15.6 minutes respectively, via injecting the standard solutions of each compound alone to the HPLC system. The peak areas were determined analysing the standard mixture solution. Four important polyphenolic compounds separated well with the gradient system used (Figure 2).

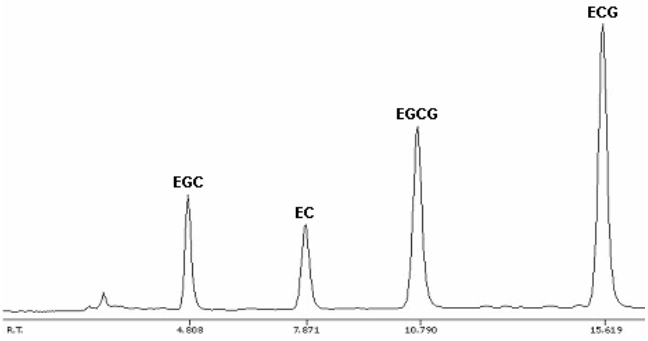


Figure 2. Chromatogram of standard mixture

Stability of the standard solutions stored at +4°C in the dark and at room temperature under light was evaluated comparing the retention times and peak areas every day by HPLC chromatograms. The solutions stored at +4°C in the dark, kept their stabilities at least 4 days, but the others stored at room temperature under light maintained their stabilities just 1 day.

After the repeatability of the method had acquired, 2% infusions of the samples were analysed. The chromatograms of the standard mixture solution and tea infusions are displayed in Figure 3.

Standard solutions of the substances (EGC 3.2 µg/mL- 16 µg/mL, EC 6.36 µg/mL-31.8 µg/mL, EGCG 0.88 µg/mL-4.4 µg/mL and ECG 0.88 µg/mL-4.4 µg/mL concentration rate) were prepared at five different concentrations to investigate the linearity. The calibration equations, found by the evaluation of the improved area rates of the peaks obtained from five concentration levels, are given in Table 2.

Table 2. Concentration-area relationships of the standard compounds.

Standard Compound	Concentration range (µ g/mL)	LOD	LOQ	Retention Time (min)	Equation of regression curve	Correlation coefficient (r)
EGC	3.2 – 16	.06	0.35	4.8616 ± 0.0246	942.2813x + 289.1	0.9968
EC	6.36 – 31.8	2.15	0.64	7.9992 ± 0.0355	494.7956x – 885.3	0.9997
EGCG	0.88 - 4.4	0.30	0.09	10.9265 ± 0.0353	10068.07x – 2176.45	0.9980
ECG	0.88 - 4.4	0.30	0.09	15.6675 ± 0.0359	15753x – 1527.49	0.9969

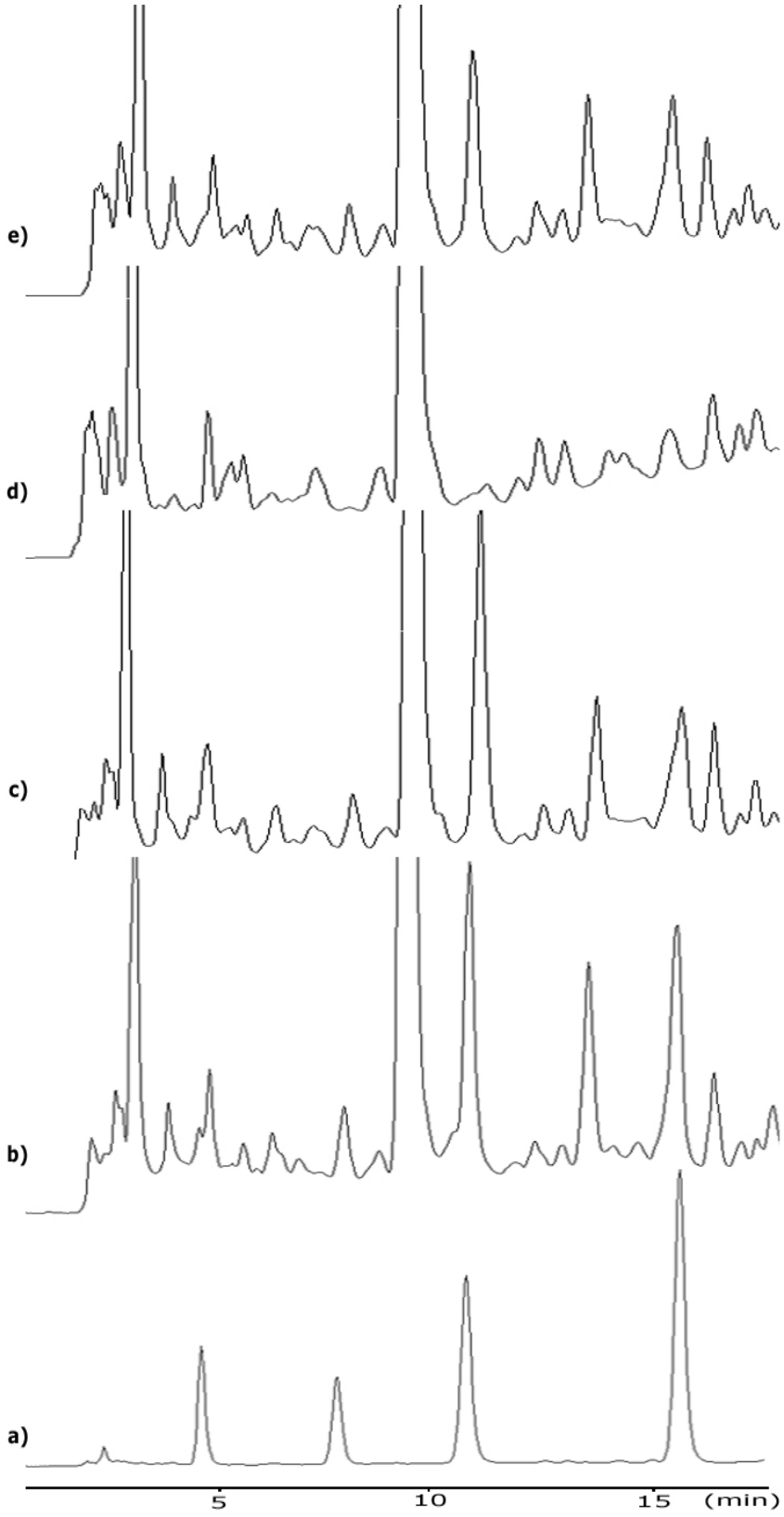


Figure 3. a) Chromatogram of standard mixture solution
b) Chromatogram of sample 17
c) Chromatogram of sample 2
d) Chromatogram of sample 11
e) Chromatogram of sample 3

Recovery and analytical reproducibility were evaluated for the accuracy of the method. Spiked tea samples were prepared with standard polyphenols which almost correspond to be concentrations in tea extract. Good recoveries were obtained in all samples between 96-99 % .

The amounts of the basic polyphenols determined by HPLC in 2% infusions of 20 different tea samples are displayed in Table 3.

Table 3: Quantities of the basic polyphenols in 2% infusions of the samples

Sample No.	EGC (μg/mL)	EC (μg/mL)	EGCG (μg/mL)	ECG (μg/mL)
1 (green)	146.72	164.46	54.87	11.31
2 (green)	325.29	341.11	116.15	37.02
3 (black)	255.31	347.07	80.82	44.51
4 (black)	366.40	242.67	25.87	28.45
5 (black)	164.34	85.82	11.37	17.89
6 (black)	532.84	195.37	8.43	25.21
7 (black)	1162.81	677.72	132.63	77.34
8 (black)	311.98	113.46	13.81	24.11
9 (black)	585.47	108.14	7.05	23.26
10 (black)	198.52	19.36	3.78	12.43
11 (black)	235.44	25.01	4.59	15.28
12(black)	206.70	16.02	3.29	15.41
13 (black)	255.15	55.85	4.61	19.82
14 (black)	405.03	13.46	3.41	14.71
15 (black)	425.46	25.99	3.40	13.06
16 (black)	156.10	12.81	3.77	12.65
17 (green)	92.58	186.66	44.70	24.67
18 (green)	115.21	130.52	46.33	28.13
19 (green)	127.56	153.85	85.34	40.55
20 (black)	334.46	325.17	28.52	17.22

According to the results of this study, although all the polyphenols have been detected in all analysed samples, quantities of them were less than the data in literature (Kuroda and Hara 1999a). This difference may primarily be related to the variety of the plant grown in Turkey and production processes. Moreover, storing conditions, packaging, climate and laboratory conditions may be the secondary factors. This study has also shown that the HPLC method, developed by Tsuchiya et.al. (1997) to determine the tea polyphenols in human saliva, can be used effectively to analyse the polyphenols in tea extract directly for routine analyses without further pretreatment.

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