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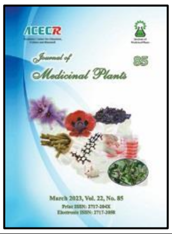

Research Article

Aromatic composition, catechins content, and metal elements profiling of forty-three Iranian black tea (*Camellia sinensis* (L.) Kuntze samples

Fatemeh Direkvand-Moghadam[1], Mohammad Hossein Mirjalili[2], Mohammad Naghi Padasht Dehkai[3], Hassan Rezadoost[1,*]

[1] *Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran*

[2] *Department of Agriculture, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran*

[3] *Horticulture Crops Research Department, Guilan Agricultural and Natural Resources Research and Education Center, AREEO, Rasht, Iran*

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ABSTRACT

Background: Tea (*Camellia sinensis*), belongs to the family Theaceae, is a well-known perennial and evergreen plant that is processed and widely used as a daily drink across the world. **Objective:** The present study was aimed to explore aromatic composition, catechins content, and metal elements (MEs) profiling of forty-three black tea samples collected across Iran. **Method:** Black tea samples were prepared from the Iranian Tea Research Institute. MEs content of the plant dried samples was measured by atomic absorption spectrometer. Aromatic composition and catechins content of the studied samples were analyzed by GC-FID, GC-MS and HPLC, respectively. **Result:** Hexanal (0.3-27.6 %), *cis*-linalool oxide (0.1-44.7 %), *trans*-linalool oxide (0.2-48.3 %), linalool (0.2-39.2 %), benzyl alcohol (0.2-38.9 %), phenylethanol (0.1-37.9 %), and (-)-Myrtenol (4.4-26.8 %) were the major volatile oil compounds of the studied samples. The highest content of hexanal, *cis*-linalool oxide, *trans*-linalool oxide, linalool, benzyl alcohol, phenylethanol and (-)-Myrtenol was measured in IR5 (Zarin), IR13 (Leil 1), IR23 (Shariat), IR31 (Roozmehr 1), IR34 (Noshiran), IR36 (Roozmehr 3) and IR18 (Amard 2), respectively. Catechin content was ranged from 0.589 ± 0.0285 in IR32 (Roozmehr 2) to 0.65 ± 0.0088 in IR29 (Aramgol 1). The level of the copper, Iron and lead were generally within the safe limitation mentioned in the world. **Conclusion:** Sample of Roozmehr was characterized with high aromatic compounds, catechins content, and low level of MEs. This information can be interestingly considered by food industrials to process black tea products.

Abbreviations: WHO, World Health Organization; FDA, Food and Drug Administration; MEs, Metal Elements; GC-FID, Gas Chromatography-Flame Ionization Detector; GC-MS, Gas Chromatography-Mass Spectroscopy; HPLC, High-Performance Liquid Chromatography; AAS, Atomic Absorption Spectroscopy; PCA, Principle Component Analysis

*Corresponding author: h_rezadoost@sbu.ac.ir

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1. Introduction

Herbs, spices and drinks are the most important herbal products that have been used by humans for years and their importance is increasing day by day. According to the WHO and FDA, trade of these plant materials will exceed $ 5 trillion by 2050 [1-2]. The active ingredients of these herbs are currently used in a wide range of medicinal, food and cosmetic products. Many of these herbs, especially those used in herbal tea, are an integral part of the diet of the people across the world [3].

Camellia sinensis (L.) Kuntze (Tea) is a perennial and evergreen plant of the family Theaceae that has many therapeutic properties. An infused processed leaf of the plant (black tea) is one of the most popular drinks throughout the world [4]. Tea has been historically used as a medicinal herb and dates back 4,700 years in China. Drinking tea is currently accepted in the daily diet of the people especially in Asia [3]. Tea is effective on controlling many diseases such as skin cancer, Parkinson's, stroke and other heart diseases [5].

India, Sri Lanka, Kenya, Japan, Indonesia, Turkey and Malawi are the other major suppliers of tea in the world [5]. About 26,000 hectares of tea gardens are currently located in Guilan and Mazandaran Provinces in the north of Iran, of which about 21,000 hectares are exploiting. In total, it is estimated that about 100,000 households or 500,000 people directly and indirectly engage in the tea industry from the cultivation, harvesting and production of green tea leaves to tea factories, packaging, distribution and sales services. Per capita consumption of tea in Iran has been estimated to be about 1.5 kg. Thus, Iran, with 1 % of the total population of the world, accounts for about 4.5 % of the total consumption of tea in the world, indicating the great tendency of Iranians to this drink [6]. Color, texture strength, aroma and taste of tea are the most important quality parameters those affect tea marketing. Phenolic compounds are implicated in the color and taste of tea, while volatile compositions are attributed in its odor. More than 600 volatile compositions have been identified in fully fermented black tea so far, of which 41 compositions have been identified as important factors in black tea aroma [7]. Variation in the metabolites of different types of tea is affecting the taste quality and nutritional value of this well-known drink [8]. *trans*-2-Hexenal, benzaldehyde, methyl-5-hepten-2-one, methyl salicylate and indole have been reported as the most important compositions of fermented and non-fermented types of tea that are varied among different types of tea including green, oolong and black tea. *trans*-2-Hexenal and methyl salicylate may help classify semi- and fully fermented tea [3]. Catechins have been widely used in medicine, chemistry, environment and other fields. Catechin has strong antioxidant properties due to its polyphenolic structure. This is an important indicator for evaluating the quality of tea [9]. In addition, catechins also have health care functions in reducing blood lipids and blood sugar and eliminating free radicals [10].

The other composition of tea leaves such as metal elements (MEs) is also the subjected to broad toxicological studies. So, the accurate determination of MEs content is thus very important in assessing the standard and quality of tea as well as any potential implications to health [11] Although the chemical composition of the tea has been reported from the other parts of the world [10], but the volatile oil composition, catechin content and MEs profiling of the tea have not been studied from Iran yet. Due to (i) the importance of the tea as a commercial drink throughout the world, (ii) a wide diversity and distribution of the plant in Iran, and (iii) the assessment necessity of the nutrient composition specially the essential, non-essential and toxic minerals in tea plant materials, the present study was aimed to analyze the essential oils composition, catechin determination, and MEs profiling of Iranian black tea samples. This information can be interestingly considered by the plant producers and the black tea processing industries for further commercial purposes.

2. Materials and methods

2.1. Plant materials

Forty-three samples of Iranian tea were collected from the Iranian Tea Research Institute (Sheikh Zahed St., Lahijan, Guilan Province, 50° 0 '12.943" longitude, 37° 12' 23.563" latitude and 1 m altitude) (Table 1). The samples were stored under appropriate conditions at room temperature of 23-25 °C until the test.

2.2. Essential oil isolation and analysis procedure

Powdered samples of Iranian black tea (80 g) were hydro-distilled (3 h) by using Clevenger-type apparatus in accordance with the British Pharmacopeia (1993). The essential oils were then analyzed by gas chromatography-flame ionization detection (GC-FID) and GC-mass spectrometry (GC-MS) as described previously. The device was set according to the standard methods of the manufacturer (automatic tuning operation).

The device was set according to the standard methods of the manufacturer (automatic tuning operation). The calibration and parts of the device were investigated, the signal to noise of the device was checked, the metabolites were isolated based on the gas chromatography temperature program and removing the signal related to the solvents by 70 electron volts by the device filament and scanning 40-500 daltons. First, the normal standard of alkanes is injected into the device from C_8-C_{20} and then 1 microliter of each sample was injected into the device with 3 replications. The column of the device was HP5-MS with a length of 30 m and an inner diameter of 25 microns. The oven temperature program started at 60 °C and was maintained for 5 minutes at this time. It was then increased by 5 °C to 280 °C and kept at this temperature for 10 minutes. After isolating the sample compositions, data identification and processing were performed by the device library and other standard methods.

2.3. Extraction and catechins determination by HPLC

Catechin and epigallocatechin was extracted from the studied studied samples according to the method mentioned in the National Standard of Iran (Anonymous YEAR). No. 8986-2 entitled "Green and black tea - Measurement of specific materials - Part 2: Determining the total content of catechin in green tea by high performance liquid chromatography" [12]. HPLC device (Kanvar, Germany) and the necessary solvents with high purity and HPLC grade prepared from Merck Co. (Germany) simultaneously with the standard solutions of catechin and epigallocatechin at the wavelength of 278 nm with a dedicated test column. The values were determined according to the National Standard with three replications.

Table 1. Localities and geographical data of the studied Iranian black tea samples

No.	Sample name	Code	Collection site	Latitude	Longitude	Altitude (m)
1	Heshmat 1	IR1	Fooman	37° 13' 56.513"	49° 19' 8.597"	27
2	Heshmat 2	IR2	Fooman	37° 13' 56.513"	49° 19' 8.597"	27
3	Zarineh 1	IR3	Fooman	37° 13' 56.513"	49° 19' 8.597"	27
4	Zarineh 1	IR4	Fooman	37° 13' 56.513"	49° 19' 8.597"	27
5	Zarin	IR5	Zideh village	37° 13' 15.122"	49° 12' 33.211"	105
6	Foumanat	IR6	Fooman	37° 13' 56.513"	49° 19' 8.597"	27
7	Sohreh 1	IR7	Leila Kooh	37° 8' 51.047"	50° 9' 43.942"	21
8	Sohreh 2	IR8	Leila Kooh	37° 8' 51.047"	50° 9' 43.942"	21
9	Neginbaharan 1	IR9	Rasht	37° 16' 50.873"	49° 35' 33.000"	3
10	Neginbaharan 2	IR10	Rasht	37° 16' 50.873"	49° 35' 33.000"	3
11	Jam 1	IR11	Lahijan	37° 12' 23.563"	50° 0' 12.943"	1
12	Jam 2	IR12	Lahijan	37° 12' 23.563"	50° 0' 12.943"	1
13	Leil 1	IR13	Leil village	37° 6' 32.402"	50° 7' 0.922"	46
14	Leil 2	IR14	Leil village	37° 6' 32.402"	50° 7' 0.922"	46
15	Moein	IR15	Lashkajan	37° 5' 34.379"	50° 17' 15.097"	4
16	Saei	IR16	Moridan	37° 7' 14.382"	50° 9' 41.082"	12
17	Amard 1	IR17	Sowme'eh Sara	37° 18' 0.877"	49° 18' 50.284"	6
18	Amard 2	IR18	Sowme'eh Sara	37° 18' 0.877"	49° 18' 50.284"	6
19	Kosar 1	IR19	Amlash	37° 5' 49.418"	50° 11' 3.832"	43
20	Kosar 2	IR20	Amlash	37° 5' 49.418"	50° 11' 3.832"	43
21	Sykooh 1	IR21	Otaghvar	37° 6' 32.402"	50° 7' 0.922"	46
22	Sykooh 2	IR22	Otaghvar	37° 6' 32.402"	50° 7' 0.922"	46
23	Shariat	IR23	Peltan	36° 48' 21.600"	50° 49' 22.364"	23
24	Famil	IR24	Lahijan	37° 12' 23.563"	50° 0' 12.943"	1
25	Chaiiran	IR25	Lahijan	37° 12' 23.563"	50° 0' 12.943"	1
26	B.U.T	IR26	Lahijan	37° 12' 23.563"	50° 0' 12.943"	1
27	Evimgolden	IR27	Sowme'eh Sara	37° 18' 0.877"	49° 18' 50.284"	6
28	Naderi 1	IR28	Komeleh	37° 9' 7.662"	50° 10' 32.600"	-9
29	Aramgol 1	IR29	Emlesh	37° 9' 29.299"	50° 13' 49.835"	-22
30	Aramgol 2	IR30	Emlesh	37° 9' 29.299"	50° 13' 49.835"	-22
31	Roozmehr 1	IR31	Lahijan	37° 12' 23.563"	50° 0' 12.943"	1
32	Roozmehr 2	IR32	Lahijan	37° 12' 23.563"	50° 0' 12.943"	1
33	Tima	IR33	Lahijan	37° 12' 23.563"	50° 0' 12.943"	1
34	Noshiran	IR34	komeleh	37° 9' 7.662"	50° 10' 32.600"	-9
35	Bineh	IR35	Lahijan	37° 12' 23.563"	50° 0' 12.943"	1
36	Roozmehr 3	IR36	Lahijan	37° 12' 23.563"	50° 0' 12.943"	1
37	Mehmandoost	IR37	Lahijan	37° 12' 23.563"	50° 0' 12.943"	1
38	Golkis	IR38	Lahijan	37° 12' 23.563"	50° 0' 12.943"	1
39	Behbooteh	IR39	Rankooh	37° 2' 55.778"	50° 14' 7.906"	62
40	Naderi 2	IR40	Komeleh	37° 9' 7.662"	50° 10' 32.600"	-9
41	Morgheshgh	IR41	Lahijan	37° 12' 23.563"	50° 0' 12.943"	1
42	Zarkhal	IR42	Roodsar	37° 8' 19.212"	50° 16' 53.648"	-19
43	Barangarkrod	IR43	Rankooh	37° 2' 55.778"	50° 14' 7.906"	62

2.4. Determination of metal elements concentration

Calibration curves were prepared to determine the MEs extracted in tea samples by FAAS using standard one-element solutions of Fe, Zn, Cu, Pb, Cd, Ni, Mn, Cr and Al at 5 ml of 2 normal HCl, reached 100 ml by doubled distilled water, and then injected into the flame atomic absorption spectrometer (Varian Spectra AA-880) for analysis. The adsorption obtained from standard solutions was used to plot the calibration curves of each analyte and the resulting equations were used to assess the metals in the samples.

2.5. Statistical analysis

SAS 9.4 software was used to perform descriptive statistics and mean comparison of data based on Duncan's multiple range test at the probability level of 5 %.

3. Results

3.1. Essential oil content and composition

The major essential oil content (w/w %) of the Iranian tea samples was ranged from 2.6 in IR42 to 92.7 in IR31. Hexanal (0.3-27.6 %), *cis*-linalool oxide (0.1 - 44.7%), *trans*-linalool oxide (0.2 - 48.3 %), linalool (0.2-39.2 %), benzyl alcohol (0.2 - 38.9 %), phenylethanol (0.1 - 37.9 %), and (-)-Myrtenol (4.4 - 26.8 %) were the major volatile oil compounds of the studied samples. The highest percentage of hexanal was observed in IR5 (Zarin). The highest content of *cis*-linalool oxide, *trans*-linalool oxide, linalool, benzyl alcohol, and phenylethanol was measured in IR13 (Leil 1), IR23 (Shariat), IR31 (Roozmehr 1), IR34 (Noshiran), and IR36 (Roozmehr 3), respectively (Table 2).

3.2. Content of catechin and epigallocatechin

The catechin and epigallocatechin was reported of forty-three Iranian black tea (Table 2). Due to phytochemical analysis of the results showed that this concentration is different in samples. The level of Catechin showed that the highest of composition was identified in IR32 (4.633 ± 0), the lowest level this compound was IR29 (0.65 ± 0.0088). According to Duncan's mean comparison test, the highest level of epigallocatechin in IR16 was 17.68 ± 0.1878, while the lowest level in Iranian tea IR22 was 1.76 ± 0.1703.

3.3. Metal elements (MEs) concentration

Five elements (Fe, Cu, Zn, Mn and Pb) were analyzed using Atomic Absorption Spectroscopy (Table 3). The concentration of Fe showed that the highest of composition was identified in IR40 (0.589 ± 0.0285), the lowest concentration this compound was IR39 (0.125 ± 0.0095).

Due to Metal elements analysis of the results showed concentration of Cu is different in samples the highest concentration of Cu in IR26 was 0.089 ± 0.0015, while the lowest concentration in IR19 were 0.015 ± 0.0035 and IR32 0.015 ± 0.0009. Zn had the highest concentration in IR19 (0.061 ± 0.0015) and the lowest concentration in IR5 (0.003 ± 0.0019) and IR21 (0.003 ± 0.0005). For Mn element, the highest concentration was 4.404 ± 0.1178 in IR2, while the lowest concentration was related to IR19 0.911 ± 0.0048. The toxic element of Pb was not found in most of the samples except sample IR1 (0.011 ± 0.0009).

Table 2. Essential oil composition of the studied Iranian black tea samples

Sample code	Content (%)							Total identified
	Hexanal	*cis*-Linalool oxide	*trans*-Linalool oxide	Linalool	Benzyl alcohol	Phenylethanol	(-)-Myrtenol	
IR1	0.3	-	13.3	8.5	6.6	37.9	10.7	77.3
IR2	7.4	2.8	3.0	3.8	13.9	28.3	10.6	69.8
IR3	8.6	4.3	6.5	2.5	9.8	19.9	7.4	59
IR4	7.6	7.5	13.9	1.1	5.1	11.4	4.4	51
IR5	27.6	3.0	7.3	2.9	12.1	22.8	9.2	84.9
IR6	5.2	3.7	7.5	2.3	5.9	18.5	8.5	51.6
IR7	21.6	-	4.3	-	9.1	19.8	6.2	61
IR8	2.2	0.7	4.5	-	2.9	15.5	5.5	31.3
IR9	4.5	3.4	4.6	2.6	11.0	24.3	11.0	61.4
IR10	5.4	4.3	8.1	2.7	11.0	25.4	11.0	67.9
IR11	2.6	2.4	4.7	2.6	11.3	26.9	11.8	62.3
IR12	1.0	-	2.0	4.4	20.2	41.5	17.6	86.7
IR13	3.0	16.3	10.5	2.6	9.2	30.9	15.2	87.7
IR14	1.8	3.4	8.9	3.4	13.7	35.0	14.5	80.7
IR15	3.2	5.2	12.4	2.5	10.6	22.7	9.9	66.5
IR16	0.9	2.1	1.4	2.6	3.0	11.3	6.3	27.6
IR17	3.3	1.4	1.9	4.0	16.0	35.5	17.2	79.3
IR18	3.8	3.6	1.5	3.8	17.0	37.5	20.8	88
IR19	-	3.7	0.2	0.2	0.2	0.2	16.7	21.2
IR20	0.6	0.4	0.7	3.3	4.6	1.8	-	11.4
IR21	2.6	0.7	0.8	0.7	7.9	11.1	-	23.8
IR22	1.2	0.9	3.0	1.9	9.1	17.3	6.2	39.6
IR23	3.7	9.9	26.1	8.1	10.3	4.4	-	62.5
IR24	4.9	7.6	17.2	10.5	20.9	8.9	-	70
IR25	4.7	5.7	11.9	13.1	18.5	9.3	-	63.2
IR26	2.5	4.9	11.0	13.3	29.8	11.7	-	73.2
IR27	1.7	7.7	17.0	7.3	22.0	9.6	-	65.3
IR28	-	8.3	2.5	18.6	30.2	14.4	-	74
IR29	4.3	1.9	2.5	11.4	27.1	12.1	-	59.3
IR30	7.5	4.5	8.2	10.0	19.6	8.3	-	58.1
IR31	-	5.1	13.7	55.8	18.1	-	-	92.7
IR32	-	3.0	1.7	14.5	38.9	-	18.4	76.5
IR33	0.6	0.8	1.2	6.9	13.1	6.2	-	28.8
IR34	1.1	-	1.9	21.5	40.4	21.0	-	85.9
IR35	0.4	1.6	2.2	16.8	0.4	0.1	16.7	38.2
IR36	3.7	4.7	9.7	13.3	20.9	7.2	-	59.5
IR37	1.7	6.0	7.8	7.5	15.5	7.2	-	45.7
IR38	5.0	2.5	3.9	4.3	18.4	28.5	-	62.6
IR39	0.8	3.3	6.0	5.8	11.6	5.4	-	32.9
IR40	1.6	2.1	2.7	28.6	22.7	12.2	-	69.9
IR41	1.1	2.1	2.7	23.6	21.7	11.2	-	62.4
IR42	-	0.1	0.5	1.2	0.7	0.1	-	2.6
IR43	-	4.7	17.2	23.1	23.1	6.6	-	74.7

Table 3. The level of metal elements (MEs) and catechins in the Iranian black tea samples

Sample code	MEs (ppm)					Catechins (ppm)	
	Fe	**Cu**	**Zn**	**Mn**	**Pb**	**Catechin**	**Epigallo catechin**
IR1	$0.571 \pm 0.0046^{e-h}$	$0.043 \pm 0.0007^{l-q}$	$0.021 \pm 0.0021^{i-s}$	$3.389 \pm 0.1579^{e-h}$	0.011 ± 0.0009^{a}	0.91 ± 0^{wx}	4.32 ± 0.1304^{vwx}
IR2	$0.398 \pm 0.0075^{n-s}$	0.025 ± 0.0015^{CDE}	$0.011 \pm 0.0008^{w-E}$	4.404 ± 0.1178^{a}	0 ± 0.0003^{b}	2.197 ± 0.0208^{b}	$3.853 \pm 0.3516^{v-y}$
IR3	0.628 ± 0.018^{de}	$0.03 \pm 0.0017^{w-E}$	$0.022 \pm 0.0017^{h-r}$	$2.787 \pm 0.1388^{k-n}$	0 ± 0^{b}	0.85 ± 0.0088^{y}	12.48 ± 0.2544^{g}
IR4	$0.36 \pm 0.0147^{p-t}$	0.017 ± 0.0013^{F}	$0.016 \pm 0.0009^{n-z}$	$2.903 \pm 0.0513^{i-l}$	0 ± 0.0009^{b}	1.617 ± 0.024^{c}	15.097 ± 0.1637^{d}
IR5	$0.369 \pm 0.006^{o-t}$	$0.033 \pm 0.0014^{s-C}$	0.003 ± 0.0019^{F}	$2.32 \pm 0.0371^{p-t}$	0 ± 0^{b}	$1.177 \pm 0.0186^{j-m}$	9.397 ± 0.0897^{i}
IR6	$0.401 \pm 0.0305^{n-s}$	$0.029 \pm 0.001^{w-E}$	0.03 ± 0.0027^{fgh}	3.194 ± 0.0344^{ghi}	0 ± 0.0012^{b}	$1.083 \pm 0.0231^{o-r}$	11.48 ± 0.1397^{h}
IR7	$0.553 \pm 0.0296^{e-i}$	$0.041 \pm 0.0028^{m-s}$	$0.007 \pm 0.0047^{A-F}$	$2.558 \pm 0.1194^{m-q}$	0 ± 0.0026^{b}	1.42 ± 0.024^{e}	8.557 ± 0.2004^{jk}
IR8	$0.328 \pm 0.0462^{r-u}$	$0.026 \pm 0.0023^{B-E}$	$0.019 \pm 0.0006^{j-v}$	$2.191 \pm 0.0699^{r-v}$	0 ± 0.0004^{b}	$1.21 \pm 0.0033^{i-l}$	$8.03 \pm 0.1462^{k-n}$
IR9	$0.509 \pm 0.0256^{g-l}$	$0.035 \pm 0.0026^{q-y}$	$0.023 \pm 0.0024^{h-o}$	3.787 ± 0.1363^{cd}	0 ± 0^{b}	1.27 ± 0.0318^{fgh}	4.763 ± 0.19^{uv}
IR10	$0.389 \pm 0.0146^{o-s}$	0.024 ± 0.0003^{DE}	$0.02 \pm 0.0012^{j-u}$	3.185 ± 0.0834^{ghi}	0 ± 0.0001^{b}	$0.937 \pm 0.0115^{u-x}$	$6.04 \pm 0.3762^{q-t}$
IR11	$0.505 \pm 0.0378^{g-m}$	0.053 ± 0.0016^{hij}	$0.012 \pm 0.0012^{v-E}$	$3.302 \pm 0.2026^{e-h}$	0 ± 0.0012^{b}	1.247 ± 0.0321^{ghi}	4.47 ± 0.2948^{vwx}
IR12	$0.385 \pm 0.0145^{o-s}$	$0.042 \pm 0.0001^{m-r}$	$0.025 \pm 0.0003^{g-m}$	$3.161 \pm 0.0399^{g-j}$	0 ± 0^{b}	1.61 ± 0.0033^{c}	6.373 ± 0.3003^{pqr}
IR13	$0.541 \pm 0.037^{f-i}$	0.067 ± 0.0003^{ef}	$0.01 \pm 0.0016^{y-F}$	3.627 ± 0.1865^{de}	0 ± 0.0006^{b}	$1.057 \pm 0.0208^{p-s}$	3.677 ± 0.8854^{xyz}
IR14	$0.393 \pm 0.0194^{o-s}$	$0.039 \pm 0.0015^{n-v}$	$0.029 \pm 0.0015^{f-i}$	3.29 ± 0.0175^{fgh}	0 ± 0.0002^{b}	$0.94 \pm 0.0601^{u-x}$	5.507 ± 0.5445^{stu}
IR15	$0.482 \pm 0.0628^{i-n}$	$0.028 \pm 0.0012^{x-E}$	0.005 ± 0.0025^{DEF}	$3.254 \pm 0.0808f^{gh}$	0 ± 0.0012^{b}	0.97 ± 0.0451^{u}	15.95 ± 0.34^{b}
IR16	$0.446 \pm 0.0244^{k-p}$	$0.033 \pm 0.0049^{s-C}$	$0.006 \pm 0.0048^{C-F}$	$2.624 \pm 0.2401^{l-p}$	0 ± 0.002^{b}	0.913 ± 0.0757^{vwx}	17.68 ± 0.1878^{a}
IR17	$0.545 \pm 0.0548^{e-i}$	$0.046 \pm 0.0044^{j-n}$	0.004 ± 0.0026^{EF}	$3.393 \pm 0.0743^{e-h}$	0 ± 0.0006^{b}	$0.78 \pm 0.0384^{z B}$	$3.823 \pm 0.4161^{v-y}$
IR18	$0.407 \pm 0.0656^{n-r}$	$0.035 \pm 0.0021^{p-y}$	$0.021 \pm 0.003^{i-s}$	$3.054 \pm 0.133^{h-k}$	0 ± 0.0003^{b}	$1.103 \pm 0.0088^{n-q}$	2.12 ± 0.1184^{CD}
IR19	0.096 ± 0.0116^{v}	0.015 ± 0.0035^{F}	0.061 ± 0.0015^{a}	0.911 ± 0.0048^{G}	0 ± 0.0004^{b}	0.833 ± 0.0058^{yz}	6.98 ± 0.1284^{op}
IR20	$0.569 \pm 0.0339^{e-h}$	$0.027 \pm 0.^{0091y-E}$	$0.025 \pm 0.0027^{g-l}$	$2.74 \pm 0.0283^{k-n}$	0 ± 0^{b}	0.85 ± 0.0033^{y}	13.25 ± 0.1671^{f}
IR21	$0.432 \pm 0.05l^{-p}$	$0.031 \pm 0.0031^{v-E}$	0.003 ± 0.0005^{F}	$2.878 \pm 0.0969^{i-m}$	0 ± 0.0009^{b}	1.107 ± 0.0033^{nop}	2.65 ± 0.1386^{ABC}
IR22	$0.519 \pm 0.0164^{g-k}$	0.066 ± 0.0058^{efg}	$0.012 \pm 0.0062^{u-E}$	$2.309 \pm 0.2299^{p-t}$	0 ± 0.0015^{b}	$1.07 \pm 0.0088^{p-s}$	1.76 ± 0.1703^{D}
IR23	0.165 ± 0.0077^{v}	$0.044 \pm 0.0015^{k-o}$	0.042 ± 0.0031^{cd}	0.285 ± 0.1134^{H}	0 ± 0^{b}	1.147 ± 0^{mn}	8.487 ± 0.0964^{jkl}
IR24	$0.362 \pm 0.0053^{p-t}$	0.08 ± 0.0027^{c}	$0.028 \pm 0.0015^{f-i}$	$2.037 \pm 0.0507^{s-x}$	0 ± 0.0023^{b}	$1.21 \pm 0^{i-l}$	3.73 ± 0.0742^{xyz}
IR25	$0.432 \pm 0.0115^{l-p}$	$0.044 \pm 0.0007^{l-p}$	0.044 ± 0.0023^{bcd}	$1.838 \pm 0.1691^{w-B}$	0 ± 0.0013^{b}	1.387 ± 0^{e}	2.313 ± 0.2254^{CD}
IR26	$0.456 \pm 0.0165^{j-o}$	0.089 ± 0.0015^{ab}	0.032 ± 0.0025^{efg}	$1.644 \pm 0.0942^{z-E}$	0 ± 0^{b}	1.413 ± 0.0033^{e}	4.303 ± 0.0982^{vwx}
IR27	$0.512 \pm 0.0068^{g-l}$	0.076 ± 0.0019^{cd}	$0.014 \pm 0.0003^{s-C}$	$1.544 \pm 0.1279^{B-E}$	0 ± 0^{b}	1.213 ± 0^{ijk}	2.477 ± 0.1411^{BCD}
IR28	0.318 ± 0.0121^{stu}	$0.033 \pm 0.001^{t-D}$	$0.02 \pm 0.0002^{j-u}$	$2.189 \pm 0.0314^{r-v}$	0 ± 0^{b}	0.69 ± 0.0033^{CDE}	$6.3 \pm 0.0433^{p-s}$
IR29	$0.559 \pm 0.0281^{e-i}$	$0.039 \pm 0.0026^{n-u}$	$0.008 \pm 0.0007^{z-F}$	1.206 ± 0.0366^{FG}	0 ± 0^{b}	0.65 ± 0.0088^{E}	5.477 ± 0.0467^{tu}
IR30	$0.383 \pm 0.0308^{o-s}$	$0.026 \pm 0.0005^{A-E}$	0.05 ± 0.0018^{b}	$1.652 \pm 0.0279^{y-E}$	0 ± 0^{b}	1.23 ± 0.0067^{hij}	4.673 ± 0.105^{v}
IR31	$0.52 \pm 0.0173^{g-k}$	$0.026 \pm 0.0014^{z-E}$	$0.007 \pm 0.0024^{B-F}$	1.358 ± 0.0768^{EF}	0 ± 0^{b}	$1.18 \pm 0.0058^{j-m}$	6.337 ± 0.1885^{pqr}
IR32	$0.359 \pm 0.0052^{p-t}$	0.015 ± 0.0009^{F}	$0.014 \pm 0.0004^{s-C}$	$1.651 \pm 0.073^{y-E}$	0 ± 0^{b}	4.633 ± 0^{a}	3.687 ± 0.2252^{xyz}
IR33	$0.537 \pm 0.0222^{f-j}$	$0.03 \pm 0.0017^{w-E}$	$0.028 \pm 0.0018^{f-i}$	3.568 ± 0.0452^{def}	0 ± 0^{b}	1.133 ± 0^{mno}	$6.047 \pm 0.2987^{q-t}$
IR34	$0.404 \pm 0.0095^{n-s}$	$0.029 \pm 0.0003^{w-E}$	$0.023 \pm 0.001^{h-p}$	4.256 ± 0.0525^{ab}	0 ± 0^{b}	1.29 ± 0.0033^{fg}	4.577 ± 0.1677^{vw}
IR35	$0.539 \pm 0.022^{f-j}$	$0.039 \pm 0.0017^{n-v}$	$0.023 \pm 0.0011^{h-p}$	$2.846 \pm 0.15^{j-m}$	0 ± 0^{b}	$1.153 \pm 0.0067l^{mn}$	2.663 ± 0.1804^{ABC}
IR36	$0.435 \pm 0.0225^{l-p}$	0.025 ± 0.0048^{DE}	$0.012 \pm 0.003^{u-E}$	$2.839 \pm 0.0255^{j-m}$	0 ± 0^{b}	0.987 ± 0^{tu}	1.83 ± 0.2636^{D}
IR37	$0.575 \pm 0.043^{e-h}$	$0.041 \pm 0.0015^{m-t}$	$0.026 \pm 0.0014^{f-k}$	$2.374 \pm 0.1863^{o-s}$	0 ± 0^{b}	1.317 ± 0.0088^{f}	$4.127 \pm 0.283^{v-y}$
IR38	$0.383 \pm 0.0224^{o-s}$	0.024 ± 0.002^{EF}	$0.013 \pm 0.0009^{t-D}$	3.214 ± 0.0274^{ghi}	0 ± 0^{b}	$1.19 \pm 0^{j-m}$	3.387 ± 0.382^{yzA}
IR39	0.125 ± 0.0095^{v}	$0.052 \pm 0.0006^{h-k}$	$0.009 \pm 0.0002^{z-F}$	$2.545 \pm 0.0536^{m-q}$	0 ± 0^{b}	1.533 ± 0^{d}	2.32 ± 0.151^{CD}
IR40	0.589 ± 0.0285^{efg}	$0.041 \pm 0.0014^{m-s}$	$0.023 \pm 0.0009^{h-n}$	$2.304 \pm 0.0105^{p-u}$	0 ± 0^{b}	1.627 ± 0.0033^{c}	2.657 ± 0.0529^{ABC}
IR41	$0.372 \pm 0.0081^{o-t}$	0.067 ± 0.0013^{ef}	$0.015 \pm 0.004^{o-A}$	4.051 ± 0.0078^{bc}	0 ± 0^{b}	1.633 ± 0^{c}	$5.833 \pm 0.0935^{q-t}$
IR42	$0.584 \pm 0.0082^{e-h}$	$0.037 \pm 0.0013^{o-w}$	$0.018 \pm 0.0019^{k-x}$	$3.132 \pm 0.1077^{g-j}$	0 ± 0^{b}	$1.067 \pm 0.0033^{p-s}$	3.383 ± 0.1735^{yzA}
IR43	$0.4 \pm 0.014^{n-s}$	$0.027 \pm 0.0006^{y-E}$	$0.025 \pm 0.0003^{g-l}$	3.187 ± 0.0234^{ghi}	0 ± 0^{b}	0.74 ± 0.0033^{BC}	5.463 ± 0.1159^{tu}

3.4. Correlation coefficient

The results of the correlation between the studied traits are presented in Fig. 1. The results showed a negative correlation between catechin as the most important composition of tea, epigallocatechin (r = -026*), and lead (r = -0.20*) while had a positive and significant correlation with benzyl alcohol (r = 0.37*). *Cis*-linalool oxide had the highest positive and significant correlation (r = 0.52**) with lead content. Phenylethanol also had a positive and significant correlation with manganese (r = 0.63**) and hexanal (r = 0.36*) and a significant negative correlation (r = -0.35*) with linalool.

3.5. Principal component analysis

Principal component analysis was performed based on eigenvalues higher than 0.5 regardless of the relevant sign, as significant coefficients after Varimax rotation. Under the above conditions, 6 factors were identified and all of them explained 67.34 % of the diversity in the traits (Table 4). Under the above conditions, the first factor, which accounted for 19.254 % of the total changes, had positive factor coefficients for phenylethanol and manganese and a negative factor coefficient for linalool. The selection based on the second factor, which accounted for 12.65 % of the data changes, including the traits of benzyl alcohol, catechin and epigallocatechin, will lead to the herbs with high catechin. The third factor had large and positive coefficients for *cis*-linalool oxide and lead. This factor justified 10.48 % of the data changes (Table 4).

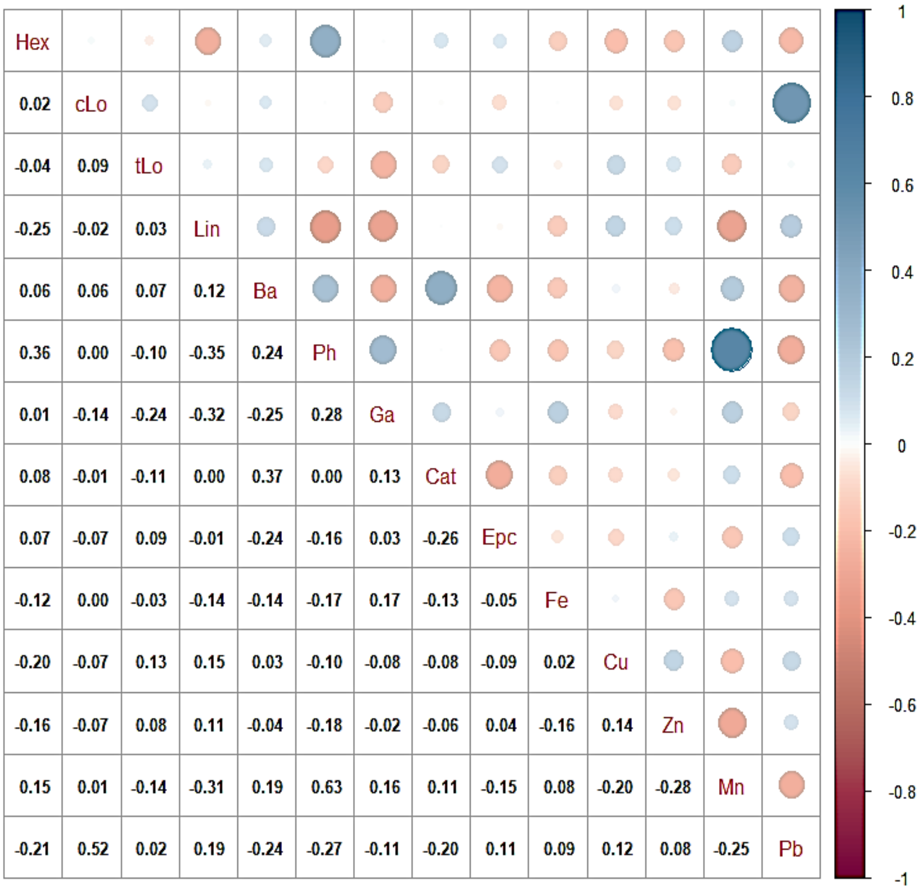

Fig. 1. Pearson correlation between studied traits of Iranian tea

Table 4. Principal component analysis by Varimax rotation for Iranian tea based on all traits

variable	Factor					
	1	**2**	**3**	**4**	**5**	**6**
Hex	0.420	-0.060	-0.080	0.128	**-0.537**	0.221
cLo	0.087	0.097	**0.897**	0.115	-0.097	-0.001
tLo	0.024	-0.174	-0.008	**0.708**	0.201	0.023
Lin	**-0.659**	0.228	0.047	0.202	0.056	0.104
Ba	0.115	**0.725**	-0.083	0.362	-0.040	0.112
Ph	**0.870**	0.172	-0.041	-0.016	-0.067	0.138
Ga	0.352	-0.158	-0.125	**-0.725**	0.116	-0.053
Cat	-0.085	0.701	-0.104	-0.279	-0.180	0.115
Epc	-0.141	**-0.661**	-0.084	0.096	-0.319	0.151
Fe	-0.015	-0.144	0.009	-0.128	0.177	**-0.820**
Cu	-0.051	0.018	-0.024	0.207	**0.759**	0.049
Zn	-0.188	-0.141	-0.013	-0.123	0.454	**0.615**
Mn	**0.713**	0.262	-0.042	-0.041	-0.133	-0.238
Pb	-0.263	-0.222	**0.819**	-0.045	0.141	-0.021
Variance (%)	19.254	12.650	10.480	9.475	7.990	7.496
Cumulative %	19.254	31.904	42.384	51.859	59.849	67.345
Eigen value	2.696	1.771	1.467	1.326	1.119	1.049

4. Discussion

One of the most important environmental factors that have a significant effect on the quantity and quality of active ingredients of medicinal herbs are temperature and altitude as well as physical and chemical properties of the soil [11]. In addition, phytochemical composition of the plants such as tea can be influenced by the plant varieties, origin of collection as well as pre- and post-harvest treatments [13]. The major volatile compositions are the biosynthetic pathways of terpenoids and shikimates or the oxidation of fatty acids and carotenoids [14] in semi-fermented and fermented tea of linalool and diastromers of linalool oxides with different taste characteristics at concentrations up to 50 % of volatile compositions [15]. Aromatic compositions derived from carotenoids contribute to the aroma quality of tea. Some of these compositions are among the most effective flavoring elements and play a significant role in the quality of tea. In particular, *β*-ionone and damascenone, although do not have much effect on human sense of smell, are significantly effective on the taste of black tea [7].

According to the results obtained from the analysis of essential oils of Iranian and non-Iranian tea, seven constituents including hexanal, *cis*-linalool oxide, *trans*-linalool oxide, linalool, benzyl alcohol, phenylethanol and (-)-myrtenol, which had the highest percentage were identified using GC-MS method. In line in our result, phenylethanol (14.7 %), linalool (7.9 %), (E)-linalool oxide, were identified as the main compositions of essential oils of tea [16]. In general, linalool and hexanal content played a key role in tea quality [17]. In the present study, it was found that the highest and lowest hexanal composition was in IR5 (27.6) and IR1 (0.3), respectively. Hexanal was not detected in IR19, IR28, IR31, IR32 and IR 43 samples. The results showed, the maximum Linalool IR31 (55.8) and minimum were IR19 (0.2), Linalool composition not detected in IR7 and IR8 samples. [18] reported the volatile compositions of black tea Hexanal, benzyl alcohols and linalool which are consistent with the results of the above test.

Another study confirmed the result of present study, Linalool (threshold value: 6 ppb in water), benzyl alcohol, and 2-phenylethanol are mainly volatile compounds in black tea [19] .In another study, the compositions hexenal, linalool and linalool oxides were identified as the most important compositions of black tea [18]. The difference in compositions between the studied tea can be due to geographical profiles, especially altitude. In this regard, [19]. Studied the key essential oil compositions of black tea. They pointed out that altitude levels affect the growth properties and essential oil compositions of black tea samples [20].

Catechin can effectively prevent the occurrence of cancers due to its strong antioxidant capacity and Catechins are one of the most abundant flavonoids found in the tea, and the daily intake per person is about 120 mL [21]. There are many reports that catechins in human diet can play an important role in preventing degenerative diseases, cardiovascular diseases, visceral diseases and some cancers. Therefore, it can be concluded that determining the content of catechin in different teas is very important [22]. Due to hplc analysis of Catechin and Epigallocatechin in different black tea, the results showed, The Catechin was highest and lowest in the IR 32 (4.633 ± 0) and IR29 (0.65 ± 0.0088), and it was found in the current study that highest and lowest Epigallocatechin IR16 (17.68 ± 0.1878) and IR22 (1.76 ± 0.1703) respectively.

Examination of the catechin contents of different black tea samples showed differences, which may be attributed to the high level of tea and abundant organic compounds in the soil. In addition, it may be caused by the difference in weather [9]. The toxic element of Pb was not found in most of the samples except sample IR1 (0.011 ± 0.0009). The increase in the concentration of lead in the tea consumed by the people of the world can be a worrying factor for consumers and producers, so each country determines its own limit for the concentration of lead in tea leaves. In Europe it is 5 mg/kg, in Japan it is 20 mg/kg, while in China the limit is 2 mg/kg [23]. In Iran, the permissible limit of lead is 1 mg/kg [24]. According to reports, tea leaves contain 350 to 900 L/g of manganese, which is an essential element for plants, microorganisms, and higher animals, including humans. Daily consumption of 2 to 5 mg of manganese per day is recommended, and tea can be a good source of this amount of manganese [25]. Based on the results, the IR2 (4.404 ± 0.1178) samples and IR19 (0.911 ± 0.0048) have maximum and minimum levels of Mn element, respectively. The research results of manganese concentration in tea leaves, including herbal extracts with several flavoring additives, are in a wide range with the lowest (79 L/g) and the highest (768 L/g). On the other hand, Mn content in different Indian tea brands ranged from 371 to 758 Lg/g with a mean value of 575 ± 96 Lg/g [26]. In this study, Cu content ranged between IR26 (0.089 ± 0.0015) and in IR19 (0.015 ± 0.0035) and IR32 (0.015 ± 0.0009). In a study, it was reported that the copper content was in the range of 9.6-20.9 Lg/g in three brands of Chinese tea [27].

According to the test result Cu contents in investigated tea samples ranged from 9.61 to 30.00 µg/g with the mean of 16.18 µg/g [22]. The obtained results showed those values (13.2 ± 0.96) reported by [28] (2005) and the Cu contents (27.7 ± 0.7), (24.8 ± 1.4), (18.1 ± 6.9) µg/g reported by [29]. In Iran, the permissible limit of lead is 150 mg/kg [30].

The maximum levels of Fe in the studied Samples IR40 (0.589 ± 0.0285) and the minimum level of IR39 (0.125 ± 0.0095). In another study reported, the levels of Fe, ranging between 97.9 and 488.49 µg/g with the mean of 193.82 µg/g [30]. [31] reported amount of Fe estimated in thirty samples, was 9.38 ppm.

5. Conclusion

Tea is a favorite drink of people in the Middle East. The results of this study show that the studied copper, iron and lead levels are generally within the safe range and compare well with the levels of similar teas in other parts of the world. Routine inspection and frequent analysis of food is required to avoid the risk of excessive consumption beyond the limit of tolerance standards. In this test, Iranian tea was difference of phytochemical compositions and metal elements profiling, indicating different effects of geographical. Roozmehr was characterized with high aromatic compounds, catechin content, and low level of MEs. This information can be interestingly considered by food industrials to process black tea products.

Author contributions

F.DM.: Contributed to the conception of the study, doing laboratory work, data collection and analysis, interpretation of data, drafting the manuscript. MH.MJ.: Interpretation of data reviewing and editing the manuscript. M.NPD.: Interpretation of data, reviewing and editing the manuscript. H.R.: Supervised the study, assist in the analysis and interpretation of results, drafting the manuscript.

Conflict of interests

The authors declare that there is no conflict of interest.

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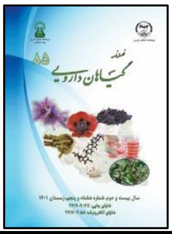

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ترکیبات معطر، محتوای کاتچین‌ها و پروفایل عناصر فلزی ۴۳ نمونه چای سیاه ایرانی

فاطمه دیرکوند مقدم[۱]، محمدحسین میرجلیلی[۲]، محمدنقی پاداشت دهکائی[۳]، حسن رضادوست[۱،*]

[۱] گروه فیتوشیمی، پژوهشکده گیاهان دارویی و مواد اولیه دارویی، دانشگاه شهید بهشتی، تهران، ایران

[۲] گروه کشاورزی، پژوهشکده گیاهان دارویی و مواد اولیه دارویی، دانشگاه شهید بهشتی، تهران، ایران

[۳] گروه تحقیقات محصولات باغی، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی گیلان، AREEO، رشت، ایران

اطلاعات مقاله

گل‌واژگان:
مس
اپی گالوکاتچین
اسانس
کروماتوگرافی گازی
فیتوشیمیایی
تئاسه

چکیده

مقدمه: چای (*Camellia sinensis*)، متعلق به خانواده چای (Theaceae)، گیاهی شناخته شده چند ساله و همیشه سبز است که به عنوان یک نوشیدنی روزانه در سراسر جهان فرآوری و استفاده می‌شود. **هدف:** مطالعه حاضر با هدف بررسی ترکیبات معطر و مشخصات عناصر فلزی (MEs) چهل و سه نمونه چای سیاه جمع‌آوری شده در سراسر ایران انجام شد. **روش بررسی:** نمونه‌های چای سیاه از موسسه تحقیقات چای ایران تهیه شد. محتوای MEs نمونه‌های خشک شده گیاهی با استفاده از طیف سنج جذب اتمی اندازه‌گیری شد. ترکیب آروماتیک و محتوای کاتچین نمونه‌های مورد مطالعه به ترتیب با GC-FID و GC-MS و HPLC مورد تجزیه و تحلیل قرار گرفت. **نتایج:** هگزانال (۰/۳-۲۷/۶ درصد)، سیس- لینالول اکسید (۰/۱-۴۴/۷ درصد)، ترانس- لینالول اکسید (۰/۲-۴۸/۳ درصد)، لینالول (۰/۲-۳۹/۲ درصد)، بنزیل الکل (۰/۲-۳۸/۹ درصد)، فنیل اتانول (۰/۱-۳۷/۹ درصد) و (-)- میرتنول (۴/۴-۲۶/۸ درصد) عمده‌ترین ترکیبات اسانس نمونه‌های مورد مطالعه بودند. بیشترین میزان هگزانال، سیس- لینالول اکسید، ترانس- لینالول اکسید، لینالول، بنزیل الکل، فنیل اتانول و (-)- میرتنول به ترتیب در IR5 (زرین)، IR13 (لیل ۱)، IR23 (شریعت)، IR31 (روزمهر ۱)، IR34 (نوشیران)، IR36 (روزمهر ۳) و IR18 (آمارد ۲) اندازه‌گیری شد. محتوای کاتچین از ۰/۰۲۸۵ ± ۰/۵۸۹ در IR32 (روزمهر ۲) تا ۰/۰۰۸۸ ± ۰/۶۵ در IR29 (آرامگل ۱) متغیر بود. سطح مس، آهن و سرب به طور کلی در محدوده ایمن ذکر شده در جهان بود. **نتیجه‌گیری:** نمونه روزمهر با ترکیبات معطر بالا، محتوای کاتچین و سطح پایین ME مشخص گردید. این اطلاعات می‌تواند مورد توجه صنایع غذایی برای فرآوری محصولات چای سیاه قرار گیرد.

مخفف‌ها: WHO، سازمان جهانی بهداشت؛ FDA، سازمان غذا و داروی آمریکا؛ MEs، عناصر فلزی؛ GC-FID، کروماتوگرافی گازی-دتکتور یونیزاسیون شعله‌ای؛ GC-MS، کروماتوگرافی گازی متصل به طیف سنج جرمی؛ HPLC، کروماتوگرافی مایع با کارآیی بالا؛ AAS، طیف‌سنج جذب اتمی؛ PCA، تجزیه و تحلیل اجزای اصلی

* نویسنده مسؤول: h_rezadoost@sbu.ac.ir

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