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Research Article:

Determination of Caffeine, Total Phenol, and Heavy Metals Content in Green and Black Tea Collected From Gilan Province, Iran by Spectroscopic Method

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ABSTRACT

Background: Because of its stimulating and valuable effects, tea is the most commonly used beverage in the world after water. These effects are due to caffeine and phenolic compounds in tea. Also, most of the tea in Iran is planted in Gilan Province. However, food pollution from heavy metals is one of the most important problems.

Objectives: In this research, we measured the total phenolic content, caffeine, lead, cadmium, and chromium in green and black tea cultivated in Gilan Province.

Methods: Ten samples of green and black tea were purchased from different regions of Gilan Province. Methanol and dichloromethane extracts were prepared. Total phenol and caffeine were detected by spectroscopy. Quantification of heavy metals was done by digestion methods and examined by atomic absorption spectrophotometer.

Results: The Mesn±SD amounts of total phenolic content, caffeine, lead, cadmium, and chromium in green tea from all regions comprised 27.13±1.54%, 3.20±0.01%, 0.81±0.66 ppm, 1.54±1.39 ppm, and 0.28±0.20 ppm, respectively, and these amounts in black tea were 14.90±1.53%, 3.20±0.05%, 1.00±0.79 ppm, 1.65±0.93 ppm, and 0.24±0.15 ppm, respectively.

Conclusion: The content of total phenol and lead in tea was different in various regions of Gilan Province. Green tea contains more phenolic compounds than black tea, but the caffeine in green and black tea is almost equal. The amounts of cadmium and chromium in some Gilan Province areas are higher than the standard level, which needs to be investigated.

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Introduction

Tea is a typical drink in the world due to its taste and health properties [1]. Over the past decade, the tea production in the world grew at 1.81% a year, while global tea consumption grew at a rate of 2.05%. Iran produces 60000 tons of tea in the Caspian Sea region of Gilan and Mazandaran provinces annually [2].

Caffeine is the most consumed drug in the world found in various herbs, including coffee, cocoa, cola, and tea [3]. It is a psychoactive drug and affects the central nervous system. In addition to the central nervous system, it can affect other organs, including the heart, peripheral blood vessels, kidneys, gastrointestinal tract, and respiratory system. Excessive caffeine consumption can increase gastric acid and digestive problems, increase the risk of breast cancer, premenstrual problems, blood glucose levels, and even abortions [4, 5].

Phenols are essential components in medicinal plants, and they are used as colorants, flavors, perfumes, and antioxidants in the food industry [6]. Green and black tea are used as excellent sources of polyphenols. Green tea has five times more antioxidants than black tea because of its higher concentration of polyphenols [7]. More than three-quarters of polyphenols are found in the tea leaf are flavanol, known as catechin [8]. This compound can protect the inner lining of the artery from free radicals and thus prevent heart disease [9]. It also inhibits the pancreatic lipase enzyme activity, preventing a sudden rise in blood triglycerides after eating [10]. Flavonoids in tea increase the metabolic rate of lipid peroxidation and improve insulin activity [11]. They can prevent tooth decay by oral bacteria. Fluoride in tea protects enamel [12].

Heavy metals are among the most important environmental pollutants. Human is exposed to these compounds through water and food. The contamination can cause acute, chronic, and dangerous poisoning [13]. These metals are neither quickly metabolized nor excreted after entering the body. They are accumulated in tissues and cause many diseases and complications, including the spread of viral, bacterial, and fungal infections in the body [14].

Poisoning with heavy lead metal can induce complications such as blood disorders, mental retardation, and the prevention of intellectual development and neurological complications in children [15]. Excessive cadmium accumulation can cause prostate cancer, high blood pressure, demyelinating testicular tissues, red blood cells, cramps, and coagulation of specific proteins [16]. Also, chromium poisoning can cause allergic and eczematous skin reactions, dermatitis, bronchial carcinomas, mucous membrane ulcerations, hepatocellular deficiency, gastroenteritis, and renal oligoanuric deficiency [17].

Although tea is the most popular and consumable drink in Iran, studies on tea leaf production in Iran's factories are few. So research on beneficial tea compositions such as total phenol and caffeine and heavy metal contamination is critical.

Materials and Methods

Chemicals

The materials used in this study were caffeine ($C_8H_{10}N_4O_2$, Sigma-Aldrich Co., Inc., USA), dichloromethane (CH_2Cl_2, Dr. Mojallali Industrial Chemical Complex Co., Inc., Iran), Folin–Ciocalteu reagent (Sigma-Aldrich Co., Inc., USA), gallic acid ($C_7H_6O_5$, Sigma-Aldrich Co., Inc., USA), methanol (CH_3OH, Dr. Mojallali Industrial Chemical Complex Co., Inc., Iran), and potassium bicarbonate ($KHCO_3$, Merck & Co., Inc., USA).

Plant materials

About 200 g of black and green tea were provided from ten factories in different Gilan Province regions based on the geographical location (north, south, east, and west) and transferred to the laboratory.

Liquid-liquid extraction of caffeine

Fifty grams of tea leaves were stirred with 150 mL of hot water for 15 min. After cooling, the solution was filtered, and lead acetate was added to remove tannic compounds. Sediments were removed with filter paper. Then, 20 mL of dichloromethane was added and stirred. Dichloromethane was separated with a separatory funnel. Dichloromethane extraction was repeated three times to obtain the caffeinated extract. Also, different concentrations of standard caffeine were prepared at 10, 20, 30, 40, and 50 µg/mL, and the standard curve of absorbance concentration at 274 nm was plotted. By comparing the absorbance of samples with the standard curve, the caffeine concentration of the samples was calculated [5].

Measurement of total phenolic content

The total phenolic content was measured using a Folin-Ciocalteu reagent spectrophotometric method and a standard curve [18]. Fifty grams of each tea sample was weighed, and methanolic extracts were prepared by repeated maceration three times with 80% methanol and

Table 1. Ten regions of Gilan Province

Region Name	Sample Code	Region Name	Sample Code
Astaneh-ye Ashrafiyeh	1	Rudsar	6
Gurab	2	Lat Leyl	7
Soostan	3	Amlash	8
Langarud	4	Kakroud	9
Kumeleh & Otaqvar	5	Siah Lat	10

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concentrated by a rotary evaporator. Then, the extract was dried in a freeze dryer. Concentrations of 25, 50, 100, 200, and 400 µg/mL of gallic acid were prepared. Then, 5 mL of Folin-Ciocalteu reagent (diluted 1:10) was added to 1 mL of each sample. After 10 minutes, 4 mL of sodium carbonate solution was added at a concentration of 75 mg/mL. The solution was incubated for 60 minutes in the dark. Absorbance was read by a spectrophotometer at 760 nm, and a standard curve of gallic acid was drawn. Finally, methanolic extracts of green and black tea were prepared at 1 mg/mL (in distilled water). Similar to the above method, the Folin-Ciocalteu reagent and sodium carbonate were added, and the results were compared with the standard curve. Total phenolic content was reported as g gallic acid equivalent/100 g dried extract powder.

Determination of heavy metal concentration

The amount of heavy metals is determined based on the reported methods [19]. According to this method, 2 g of each sample was dried in an oven at 105oC for 48 hours. Then, 10 mL of 70% perchloric acid, 5 mL of sulfuric acid, and 30 mL of 70% nitric acid were added to each sample. The samples were placed on the heater for half an hour until their solvent evaporated slowly and the solution became clear. Finally, the resulting solution was diluted to 25 mL with distilled water, and then the amount of heavy metals were measured using an atomic absorption spectrometer.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8 software. The normality of data was checked by the Kolmogorov-Smirnov test. To compare samples in terms of concentration of lead, cadmium, and chromium, 1-way ANOVA and t test were used. $P<0.05$ was considered statistically significant.

Results

The present study showed that the amount of phenolic compounds in the tea of 10 regions (according to Table 1) was significantly different (except between regions 2 and 4) ($P<0.05$). It was higher in regions 1 and 7 and lower in region 5. Also, the amount of phenolic compounds in green tea is significantly higher than that in black tea ($P<0.05$) (Figure 1). The Mean±SD total phenol content of all regions in green tea was 27.13±1.54 g gallic acid equivalent/100 g powder and in black tea was 14.90±1.53 g gallic acid equivalent/100 g powder.

However, the amount of caffeine in green and black tea was not significantly different ($P<0.05$) (Figure 2). The Mean±SD amount of caffeine in 100 g of green tea is 3.20±0.01 g and in black tea is 3.20±0.05 g. Also, there is no significant difference between caffeine levels in different regions ($P<0.05$).

The Mean±SD amounts of lead in green and black tea are 0.81±0.66 ppm and 1.00±0.79 ppm, respectively. There is no significant difference between the mean lead levels in green and black tea ($P<0.05$), but there is a significant difference between the lead levels of different regions of Gilan Province ($P<0.05$) (Figure 3). The highest amount of lead belonged to green and black tea in region 2. The lowest amount of lead belonged to green and black tea in region 6. In region 9, the amount of lead in black tea was significantly higher than in green tea ($P<0.05$). Also, in all regions (except for region 2), the lead content is lower than the standard level (2 ppm).

The amount of cadmium in green tea in regions 3, 5, 9, 10, and black tea in regions 5, 8, and 9 were higher than the standard level (2 ppm) (Figure 4). The Mean±SD amount of cadmium in black tea is 1.65±0.93 ppm and in green tea is 1.54±1.39 ppm. Also, in regions 1 and 8, the amount of cadmium in black tea was significantly higher than in green tea ($P<0.05$).

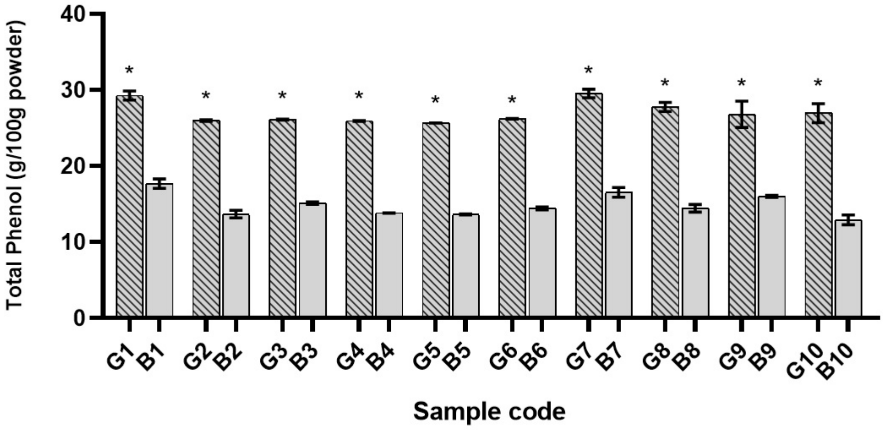

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Figure 1. Comparing total phenolic content in green and black tea of 10 regions

G1: Green Tea Region 1, B1: Black Tea Region 1). *Total phenolic content of green tea in this region are significantly higher than black tea with P<0.05.

The Mean±SD amount of chromium in green tea is 0.28±0.20 ppm and in black tea is 0.24±0.15 ppm. There is no significant difference between the chromium content of green and black tea (P>0.05). According to Figure 5, the amount of chromium in green tea in regions 1, 2, 5, 6, 7, 9, and black tea in regions 1, 5, 6, 7, 9, and 10 are higher than the standard level (0.25 ppm). There is no significant difference between the amount of chromium in green and black tea and its allowable level (P>0.05).

Discussion

Tea has been used in Iran and many East Asian countries since ancient times. Today, due to the beneficial effects of tea, especially green tea, its consumption has increased. The beneficial effects of tea on the body are related to its phenolic compounds and stimulant effects of caffeine. In this study, the amount of these two compounds and heavy metals, which are the environmental pollutants in tea, are measured.

Anesini et al. conducted a study on the samples of green and black tea from Argentina. The results showed that the phenolic content of green tea is higher than black tea [20], which confirms our findings. In the Huagae region of South Korea, Park Chan-sung measured various components of green tea, including protein, fat, ascorbic acid, minerals, and polyphenols. The amount of polyphenols in green tea in this region was 7.8%-9.3% [21]. Our research showed that the amount of polyphenolic compounds in green tea in Gilan Province is higher than in Huagae. Also, the amount of phenolic compounds is different in various geographical areas, which explains the difference in the phenolic compounds of Gilan (Iran)

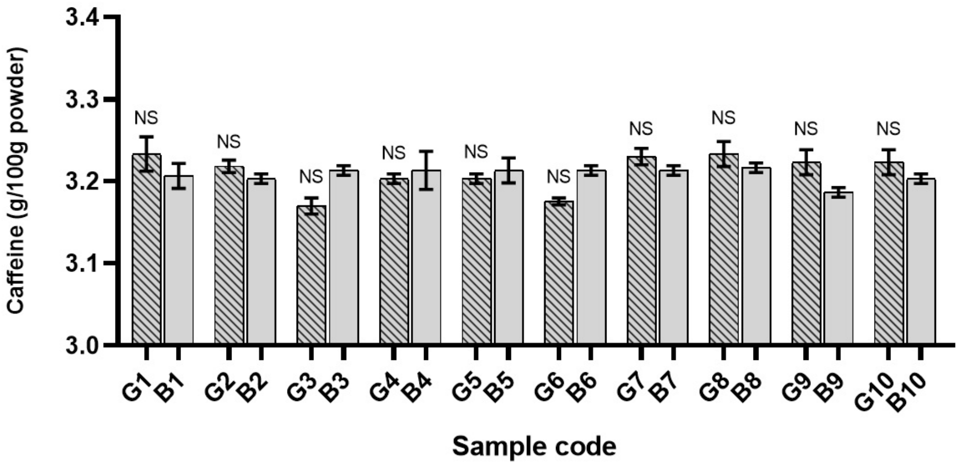

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Figure 2. Comparing caffeine content in green and black tea of 10 regions

G1: Green tea region 1, B1: Black tea region 1.NS: The amount of caffeine in green and black tea of this region was not significantly different.

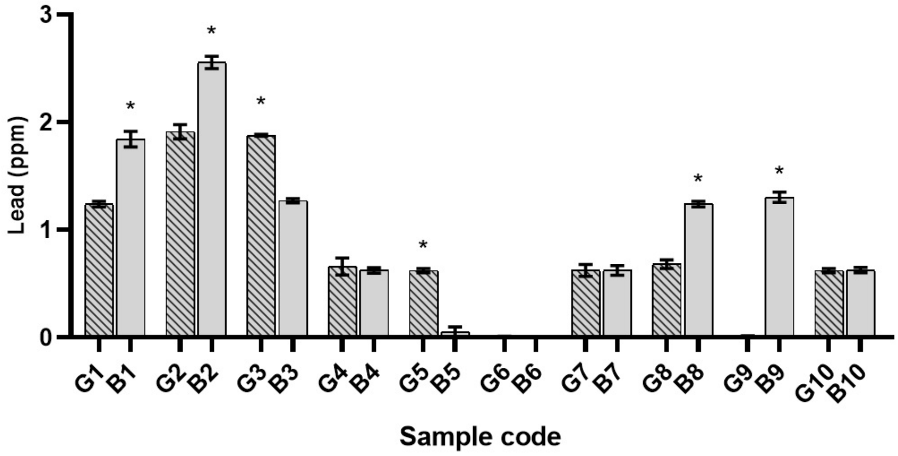

Figure 3. Comparing lead content in green and black tea of 10 regions

G1: Green Tea Region 1, B1: Black Tea Region 1.* Lead amount of green and black tea are significantly different in this region with P<0.05.

and Huagae (South Korea) tea. The total phenolic content of Brazilian green coffee was reported as 14% [22]. Our studies showed that the amount of total phenolic content in green tea (27.13±1.54%) is higher than green coffee; therefore, the higher antioxidant effects of green tea are due to the higher total phenolic content. In our study, the higher phenolic content of green tea proves the beneficial effects of this tea compared to black tea. Also, it indicates the oxidation and degradation of phenolic compounds during the fermentation process and conversion to black tea.

Maidon et al. evaluated the effect of different solvents (water, ethyl acetate, and chloroform) on caffeine extraction from tea. They showed that chloroform solvent has the highest efficiency in caffeine extraction [23]. This finding indicates the need to consider using different solvents in comparing caffeine in different regions. It should be noted that the solvent used in our research is dichloromethane. Determining the amount of caffeine in different tea types showed that white tea has the highest amount of caffeine. The caffeine content in tea is found in the leaves, and fresh leaves have higher caffeine levels. Nonetheless, the fermentation process does not affect the amount of caffeine [4]. In the present study, the tested green and black tea were also prepared from the first spring harvest, and there was no significant difference between the caffeine content of green and black tea. A study on the amount of caffeine in some famous tea brands in different countries (China, India, Namibia, Kenya, and Sri Lanka) showed that these teas have different amounts of caffeine from about 1.4% to 2.8% [24]. While our research indicates that Gilan tea has higher amounts of caffeine

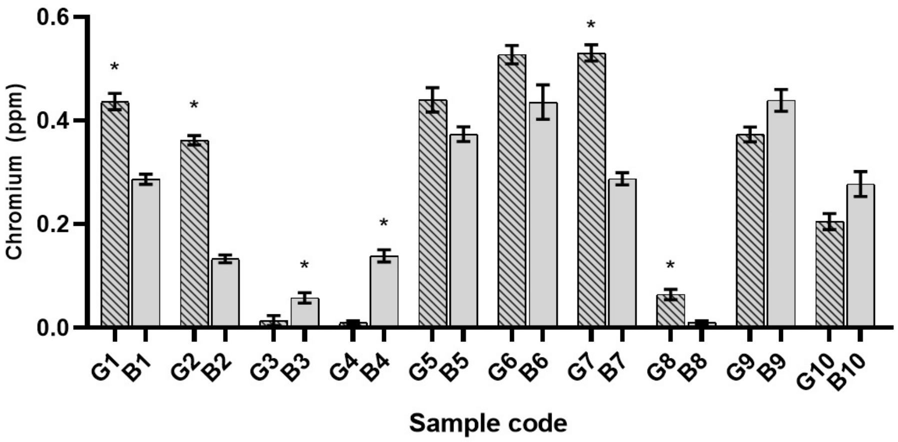

Figure 4. Comparing cadmium content in green and black tea of 10 regions

G1: Green Tea Region 1, B1: Black Tea Region 1. * Cadmium amount of green and black tea are significantly different in this region with P<0.05. p<0.05)

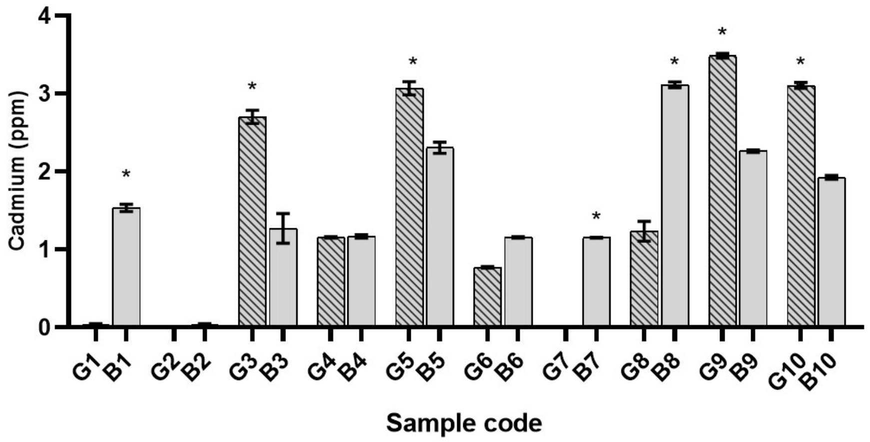

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Figure 5. Comparing chromium content in green and black tea of 10 regions

G1: Green Tea Region 1, B1: Black Tea Region 1. * Chromium amount of green and black tea are significantly different in this region with P<0.05.

(3.20±0.01%). The amount of caffeine in green tea extract and tea bags (green tea) was compared by the HPLC (high-performance liquid chromatography or high-pressure liquid chromatography) method. In this study, it was shown that the amount of caffeine in green tea leaf extract (1.96%-3.54%) is more than in green tea bags (1.39%-3.57%) [25]. The caffeine measured in this study in green tea leaf extract (extraction by dichloromethane (is similar to the amount we reported.

In a study in China on tea produced in 19 orchards in six tea-producing cities, the range of lead concentration in tea leaves was 0.11-4.55 mg/kg (mean 2.33 mg/kg) [26]. In 2006, the concentration of lead in tea samples collected from China during 1999-2001 was investigated, and the concentration was reported as 0.9-2.97 mg/kg. In this study, the lead concentration in 32% of the samples was higher than the national maximum acceptable concentration (2 mg/kg) [27]. In contrast, our studies showed that the lead concentration in Gilan tea leaves was 0.81-1.81 mg/kg, which is lower than the average concentration of lead in Chinese tea leaves. This limit was allowed by the Ministry of Agriculture of Iran (2 ppm, P<0.05), and packaged foreign tea was available in the market. In a study on Mazandaran Province tea, the lead concentration was 0.02-0.02 mg/kg (with an average of 2.82±1.25 mg/kg). This amount was significantly higher than Iran standards [2]. In 2009, Malakootian et al. evaluated 11 samples of foreign tea consumed in Tehran City, by atomic absorption spectrometer. The Mean±SD concentration of lead in tea samples was 6.97±4.78 mg/kg, which is higher than the national standard. The maximum concentration of lead was observed in the Ahmad tea bag [28]. These findings require more attention to the tea production and packaging industry in Iran because, despite the higher amount of lead in foreign tea than the national standard, this tea is used even more than native tea. Karimi et al. evaluated five types of Iranian and non-Iranian teas collected from Mashhad City, Iran, by atomic absorption spectrophotometry method. The average lead of the teas was reported as 2.31 mg/kg [29], which is higher than the national standard.

Although cadmium is known to be a human carcinogen, few studies have examined the level of cadmium in tea. In 2005, Ebadi et al. studied the concentrations of cadmium, chromium, and zinc in green tea leaves cultivated in Gilan Province (Lahijan and Fooman regions). The amounts of cadmium, chromium, and zinc were lower than the standard [30]. Also, Lahijan was one of the study areas in our research, and the amount of cadmium and chromium in this region was lower than the standard level. Chen Yu et al. reported a range of 0.03-0.08 mg/kg for cadmium concentrations in Chinese tea leaves [31]. Seenivasan et al. evaluated 100 tea samples grown in different parts of India. The amount of cadmium varied between 0.05 and 0.38 mg/kg [32].

In previous studies, the amount of cadmium was reported 0.07-2.7 mg/kg in Mazandaran tea [2], while our studies showed that the cadmium content of Gilan tea is 0.01-3.46 mg/kg, which is higher than other studies. Lin et al. reported that heavy metals accumulate in the soil in an accessible form then are transported through the soil to plants and agricultural products. Also, a relationship between the amount of these metals in soil and their concentration in plants was reported [33]. Our studies showed that the amount of lead in the Bazigorab area was the highest. This area is located in the shortest distance from industrial towns in Gilan Province compared to other regions, indicating the entry of pollutants into the plant through water and soil.

Conclusion

The amounts of total phenol and lead in tea were different in various regions of Gilan Province. Astaneh Ashrafieh tea has the highest amount of total phenol, and Gurab tea has the highest amount of lead. Green tea contained more phenolic compounds than black tea, but the amount of caffeine in green and black tea was almost equal. The amount of cadmium and chromium in some Gilan Province areas was higher than the standard level, which needs to be investigated. It is suggested that further studies be conducted on the water supply sources of tea plantations and factories that produce environmental pollutants.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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