ACTA SCIENTIFIC NUTRITIONAL HEALTH (ISSN:2582-1423)

Volume 4 Issue 10 October 2020

Research Article

In Vitro Antioxidant Activity, Spectrophotometric Determination of Caffeine, Total Phenol and Flavonoid Contents in Traditional Omani Qahwa in Comparison with Green and Black Tea

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Received: August 17, 2020

Published: September 30, 2020

Abstract

The aim of the present work was to quantify caffeine and total phenolic content and to evaluate the antioxidant activity in commercially available black tea, green tea, Turkish coffee and traditional Omani qahwa. Aqueous extracts of tea and coffee samples prepared by decoction were subjected to the phytochemical screening test for phenols and caffeine. Caffeine in the samples was quantified with the help of a linear regression equation obtained from the standard plot. Total phenolic and flavonoid contents in the tea and coffee were estimated colorimetrically. Antioxidant activity of the samples was investigated by DPPH (1, 1 diphenyl picrylhydrazyl) free radical assay and phosphomolybdenum methods. All the test extracts showed the presence of phenolic compounds and caffeine. The results indicated a variation in caffeine content, phenolic content, flavonoid content and antioxidant activity between the two extracts and among same extracts with different brand names. In general, Mumtaz black tea was found to contain the highest caffeine (24.44 mg/g) and phenolic content (8.27 mg/g of GAE). It also showed the highest total antioxidant activity (10.46 mg/g of ascorbic acid equivalent) but surprisingly its flavonoid content and antioxidant activity by DPPH method was lower than other samples. Tea was found to be richer in phenols and caffeine but Omani qahwa showed better antioxidant activity. It can be concluded that phenolic content and the antioxidant activities depend on the origin, brands and additives of these beverages that may lead to variation in the content and bioactivity.

Keywords: Turkish and Omani Qahwa; Black Tea; Green Tea; Phenols; Antioxidant; Caffeine

Abbreviations

DPPH: (1, 1 diphenyl picrylhydrazyl); GAE: Gallic Acid Equivalents; SCG: Spent Coffee Grounds; LDL: Low Density Lipoproteins; UV: Ultra Violet; FCR: Folin-Ciocalteu Reagent; TFC: Total Flavonoid Contents

Introduction

Coffee and tea are the most commonly consumed beverages worldwide. These aromatic beverages were used in Middle East, India, Europe, China and USA while Green tea was popular in Japan and East Asia countries. Tea and coffee provides energy during the entire day and have some effect in mood elevation [1]. They have a significant role in reducing physical fatigue and restore mental alertness when unusual weakness or drowsiness occurs, which are primarily due to the phytochemicals found in coffee beans and tea leaves. Caffeine content and other bioactive phytochemicals differ in their concentration among the different brands available in the market. These hot drinks have also significant medical properties,

which primarily come from the antioxidants found in coffee beans and tea leaves. These beverages contains a lot of bioactive compounds which are rich in health benefits such as phenolic compounds and antioxidants including chlorogenic acids in coffee and flavanols in tea, cafestol, caffeine and many more [2].

Hundred different kinds of teas, with their own individual appearance, taste and aroma are available e.g. Black, green, white, yellow, herbal fermented, Oolonge and many other types of tea [3]. Every harvest of tea will vary year to year due to changes in climate, rainfall and other seasonal conditions [4]. The compounds that produce the familiar aroma, flavor and taste in the tea extract include polyphenols, amino acids, methylxanthines and volatile organic compounds. Hundreds of volatile substances in tea leaves make up the flavours and aroma [5].

Polyphenols (catechins) and flavonoids are metabolites produced by the plant as a defense against predators. These make up 39% of the dry weight of fresh tea leaves. Chlorogenic acids make up 8% of the composition of raw coffee [2]. Green tea contains natural polyphenols that are thought to provide anti-inflammatory and anti-carcinogenic effects. It has a great role in the improvement of brain function, fat loss and reducing the aging signs [6]. There are two main species of coffee beans in the world which are *Coffee arabica* and *Coffee robusta*. More than three-quarters of the beans that are sold in the world today are *Coffea arabica*, the majority of the remaining bulk are *Coffea robusta* which is also known as *Coffea canephora* [7,8]. Both tea and coffee contain caffeine, a methylxanthine that delivers a characteristic bitter taste and famous stimulant effect by blocking the action of adenosines [9].

The caffeine content in coffee and tea is a central nervous system stimulant, source and has immediate effects on the mood and energy level. It excites us, accelerates our heart rate and raises blood pressure. Several studies have suggested that the flavonoids and caffeine in green tea can help elevate metabolic rate, increase fat oxidation and even improve insulin activity. Consumption of green tea and caffeine helps to lose an average of 2.9 pounds during a 12-week period, while sticking to regular diet and increase in calorie output was equal to about 100 calories over a 24-hour period [10]. Tea is also known to lower the risk of heart disease, stress relief, fat reduction and weight loss [11]. Regular black tea drinkers have also shown to have a lower risk of developing heart disease. Green tea also shows beneficial effects assisting to lower blood cholesterol, control high blood pressure, lower blood sugar, suppress ageing, prevent and treat skin diseases, stop cavities and fight viruses [6]. Drinking at least three cups of coffee per day has been linked to a lower risk of developing Type 2 Diabetes [12].

Spent coffee grounds (SCG) are rich in dietary fibers, when it is fermented by the human gut flora, it produces short chain fatty acids which has the ability to suppress inflammation mediators [13]. Coffee silver skin which is a by-product during coffee roasting can resist the oxidative damage into skin cells that can be used in anti-aging cosmetics [14]. An isolated compound called Javamide-11 from coffee extracts which could be a potent inhibitor of sirt1/2 used in treating neurodegenerative diseases and act as an anticancer agent [15]. Consumption of black tea raises the antioxidant level *in vivo* due to its composition which contains theaflavins, catechins, thearubigins and also helps in lowering the LDL level significantly [16]. Green tea consumption can prevent bone loss and improving bone mineral density [17].

It is also well known that the method of extraction and processing can affect the phenolic compounds and antioxidant activity [18]. It has been known that phenolic content decreases as the roasting temperature increases, while the antioxidant activity is influenced by other factors [19]. Green coffee beans of Robusta coffee have been reported to exert a 2-fold higher antioxidant activity than Arabica coffee, but after roasting this differences were no longer significant. Among coffee, cocoa and tea, soluble coffee has the highest antioxidants on a cup-serving basis [20]. The question regarding the comparison of caffeine content in traditional Omani qahwa (coffee), Turkish coffee, black and green tea is not disclosed by any researchers. Hence this research focus on the comparison of caffeine content in traditional Omani coffee, Turkish coffee, black and green tea and also to compare total phenolic content and antioxidant activity.

Materials and Methods

Chemicals

Two brands of black tea (Lipton® and Mumtaz®), two brands of green tea (Lipton® and Twinings®), Omani and Turkish coffee were purchased from the local hypermarket in Muscat, Oman. Folin-Ciocalteu reagent, Gallic acid, pure caffeine, DPPH (1,1 diphenyl picrylhydrazyl) were purchased from Sigma Aldrich, Germany. Aluminum chloride, Sodium hydroxide, Methanol, Ethanol, Ferric chloride, Ascorbic acid, Sulfuric acid, Ammonium molybdate and

Sodium phosphate were purchased from the local chemical distributors. All chemicals and solvents used were of analytical grade.

Preparation of extracts

The aqueous extract of the test samples was prepared by boiling 2g of black tea/green tea/Turkish or Omani coffee respectively in 100 mL of water on a magnetic hot plate for 15 - 20 minutes. The obtained tea and coffee extracts were cooled to room temperature and filtered using a Buckner funnel and Whatman No. 1 filter paper.

Phytochemical analysis

Aqueous extracts were phytochemically tested to determine the presence of phenols using standard protocol [21]. Any change of colors or the precipitate formation was used as an indicative of positive response to this test.

Quantitative analysis of caffeine by UV spectrophotometry

In this method, a comparison between different concentrations of pure caffeine samples and the extracted samples of tea and coffee were done. The λmax of pure caffeine solution was determined by UV spectrophotometer and then a standard curve of caffeine (32 - 160 µg/mL) was plotted by measuring the absorbance at 277 nm. The absorbance of prepared extracts after suitable dilution is also measured at λmax 277 nm. The extract samples were prepared by adding 2.5 mL of tea extract into 25 mL of water (sample 1) and 4.5 mL of coffee extract into 25 ml of water (sample 2) in volumetric flasks. Then from each sample (1 and 2), 4 mL was added to 25 mL of water. This step was done to make the volume more dilute to obtain the accurate results [22].

Determination of total phenolic content by Folin-Ciocalteu reagent

The total phenolic content was determined using a Folin-Ciocalteu calorimetric method [23]. For the preparation of Folin-Ciocalteu Reagent (FCR), 1 mL of FCR was added to 9 mL of water (1:10). A stock solution of pure gallic acid was prepared. For the experiment, in each test tube was added, 0.25 mL of each extract/gallic acid, 5 mL of sodium carbonate and 0.25 mL FCR. The test tubes were kept in dark for 45 minutes. This procedure is performed in triplicate. Absorbance was measured at 765 nm using water as blank. Blank was prepared by substituting the extract with distilled water. The amount of phenolic compounds as gallic acid equivalent (GAE) in extract was calculated from the regression line equation obtained for the standard plot of gallic acid.

Total phenolic content in mg/g of dried sample is calculated as follows:

$$\frac{\text{(Concentration from curve) x 100 mL (volume of extract prepared) x dilution}}{\text{2 (Mass of extract taken in g)}}$$

Determination of total flavanoids

Total flavanoid content of tea and coffee aqueous extracts was measured with the aluminum chloride colorimetric assay by measuring the absorbance at 510 nm. Briefly, in each test tube 1 mL of sample, 4 mL distilled water and 0.3 mL of 5% sodium nitrite solution were added. After 5 minutes 0.3 mL of 10% aluminum chloride was added. At the 6th minute, 2 mL of 1M sodium hydroxide was added. Finally, volume was made up to 10 mL with distilled water and mixed well. The absorbance of the orange yellowish color so developed after the reaction was measured. Quercetin was used to construct the standard curve to quantify the total flavonoids in the extract. The experiment was performed in triplicate in addition to 2 blanks which were prepared using distilled water [24].

Determination of anti-oxidant activity by *in-vitro* assay

DPPH (1,1 diphenyl picrylhydrazyl) assay method

The free radical solution of DPPH was prepared by dissolving 4 mg DPPH in 100 mL methanol in a volumetric flask. From each tea and coffee samples, three concentrations were prepared (100%, 50% and 25%) by dilution with water. For the standard, 25 mg of ascorbic acid was dissolved in 25 mL water to yield a solution of 1000 µg/mL. From this solution, a stock solution of 100 µg/mL concentration was prepared by suitable dilution (1:10) [25]. Then from this stock solution, 4 dilutions (10, 20, 40, 100 µg/mL) were prepared. In each labeled test tubes, 1 mL of sample (25%, 50 % and 100% extract)/ascorbic acid was mixed with 2 mL of DPPH solution. The test tubes were kept in dark for 20 minutes and then absorbance was measured at 517 nm. A blank was prepared by using water in place of sample. The experiment was repeated in triplicate and free radical scavenging activity was calculated by using the following standard formula:

$$\% \text{ radical scavenging activity} = [(A_c - A_t)/ A_t] *100$$

Where, A_c = Absorbance of Control and A_t = Absorbance of test/standard.

Total antioxidant activity by phosphomolybdenum method

The phosphomolybdenum reagent was prepared by using 3.33 mL of concentrated sulfuric acid (0.6M) in 100 mL water, 0.335g

sodium phosphate (28 mM) in 100 mL water and 0.494g ammonium molybdate (4 mM) in 100 mL water. Ascorbic acid in 4 different concentrations (10, 20, 40 and 80 µg/mL) was used as a positive control for calculating the total antioxidant activity. From each extract/standard 0.2 mL was taken in a test tube and to this was added 2 mL of phosphomolybdenum reagent and 1.8 mL of water. Then, the test tubes were kept at 95°C in a water bath for 90 minutes. After 90 minutes, the absorption of the extract/standard solution was recorded at 695 nm. The experiment was performed in triplicates [25].

Results and Discussion

Phytochemical analysis confirmed presence of phenols by ferric chloride test. The tested samples of tea and coffee extracts turned into dark green color indicating the presence of phenols. The quantity of caffeine was quantified with the help of a linear regression equation computed from the calibration curve of caffeine $y = 0.0016x + 0.0208$ with correlation r^2 of 0.9973 and its shown in figure 1. Twinings green tea had the lowest absorbance value which indicated the low caffeine content (11.28 mg/g) followed by Lipton green tea (15.88 mg/g). On the other hand, Mumtaz black tea showed the highest absorbance value which reflected the high amount of caffeine level (24.44 mg/g), followed by Lipton black tea (23.78 mg/g). It has been reported that black tea has less caffeine than coffee; however, the amount of caffeine depends on the type of tea as it may vary between different brands available in the market [26]. It was assumed that a standard cup of tea has half the amount of caffeine compared to coffee [7]. According to Center for Science in the Public Interest, caffeine content in a variety of commercial teas is varied. It was reported that that 8-ounce serving of black tea brewed for three minutes contains roughly 30 to 80 milligrams of caffeine. In comparison, an 8-ounce serving of green tea brewed for three minutes contains about 35 to 60 milligrams of caffeine [27]. In the present study, Omani coffee has same caffeine content (21.15 mg/g) compared to Turkish coffee (21.48 mg/g) and the results are shown in figure 2. The caffeine content of the present study samples was observed in the following order: Black tea > Coffee > Green tea.

The estimation of total phenolic content by Folin-Ciocalteu experiment indicated that twinings green tea has the lowest value with total phenolic content of 7.26 mg/g while the Lipton green tea showed higher content of phenols, which is more than Omani and Turkish coffee. Mumtaz black tea contained the highest total phenolic content of 8.27 mg/g followed by Lipton black tea. A study done by using six commercial tea varieties of black and green teas revealed that the maximum phenolic content was found in green tea which was contrary to our study results [28]. In our results, Omani coffee showed higher phenolic content than Turkish coffee and this may be due to the presence of flavors such as cinnamon and saffron in Omani coffee which may affect the results. The phenolic content of the present study samples was found to be in the following order: Mumtaz black tea > Lipton black tea > Lipton green tea > Omani and Turkish coffee > Twinings green tea and the comparative results are shown in table 1.

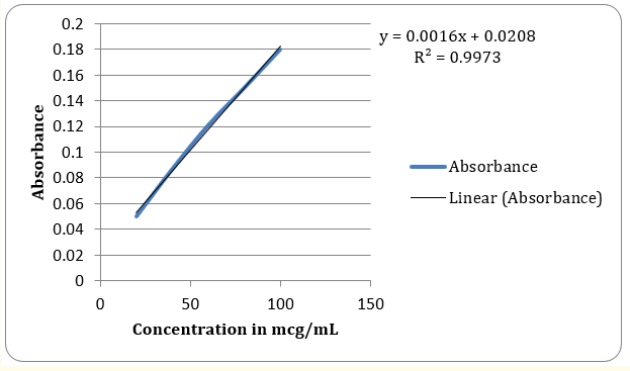

Figure 1: Standard curve of pure caffeine (with different concentrations).

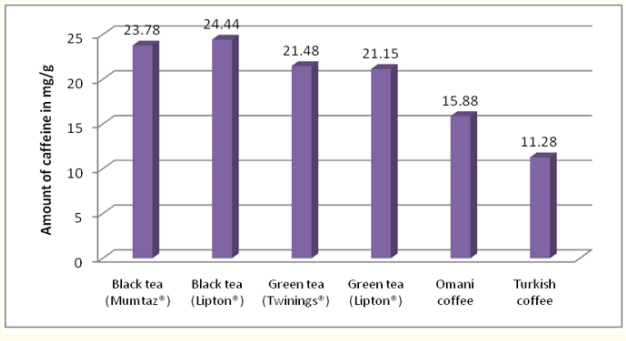

Figure 2: Amount of caffeine in different brands of black tea, green tea and coffee samples.

Test Sample	TPC in mg/g of gallic acid equivalent in dried sample	TFC in mg/g of Quercetin equivalent in dried sample
Black tea (Mumtaz®)	8.27	0.788
Black tea (Lipton®)	8.1	0.788
Green tea (Twinings®)	7.26	0.847
Green tea (Lipton®)	8.13	1.204
Omani qahwa (coffee)	7.58	1.442
Turkish coffee	7.35	1.085

Table 1: Total phenolic content (TPC) and flavonoid content (TFC) in different tea and coffee extracts.

The results of antioxidant activity using DPPH and phosphomolymdenum assay methods revealed that Lipton green tea possessed the highest antioxidant activity in comparison to black tea and it is in the agreement with the previous results [29]. All other brands in this study were low in antioxidant activity. It is important to mention that in DPPH method, black coffee had no absorbance readings due to intense black color. But the Turkish and the Omani coffee had the lowest absorbance which means the lowest antioxidant activity with respect to other samples in phosphomolybdenum method. DPPH scavenging activity increases while roasting the coffee which is conflicting to the present results [8]. The comparative results of antioxidant activity of tea and coffee extracts using *in-vitro* DPPH (1,1 diphenyl picrylhydrazyl) method is illustrated in figure 3.

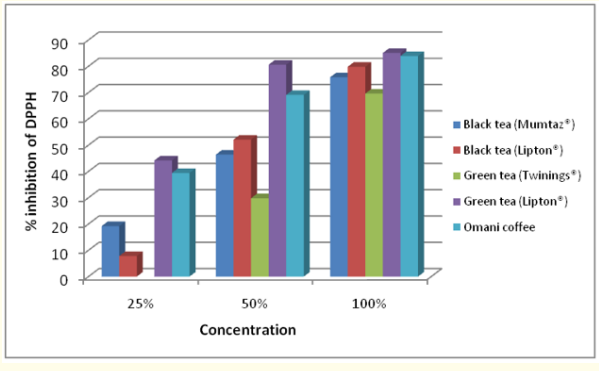

Figure 3: Comparison of antioxidant activity among tea and coffee samples.

The total flavonoid contents (TFC) of the various tea/coffee extracts is expressed in terms of quercetin equivalent (mg/g of the dried sample). The total flavonoid content was calculated using the linear regression equation obtained from the standard plot of quercetin ($y = 0.0084x - 0.0076$ with r^2 value of 0.9973) and the results are shown in table 2. In the results of TFC, Omani coffee showed the highest flavanoid content in comparison to black coffee followed by green tea. The unroasted coffee has a higher TFC comparing to roast one and it can be applicable in case of Omani coffee [19]. In addition, the additives in Omani coffee such as saffron and cardamom can contribute in increasing the TFC. While the black tea either Lipton or Mumtaz had the lowest flavanoid content which is comparable to the results of Kopjar., *et al*. and they reported that the green tea contains much higher flavanoids than the black tea [30]. There was statistically significant difference between the antioxidant activities of black tea and coffee extract, however no relation between phenolic content, flavonoid content and antioxidant activity could be established.

Test Sample	Total antioxidant capacity as mg/g of Ascorbic acid equivalent
Black tea (Mumtaz®)	8.37
Black tea (Lipton®)	10.46
Green tea (Twinings®)	10
Green tea (Lipton®)	10.45
Omani qahwa (coffee)	4.8
Turkish coffee	6.5

Table 2: Total antioxidant activity of tea and coffee samples as mg/g of ascorbic acid equivalent.

Conclusion

Consumption of coffee and tea is a traditional practice and thus it is very important to evaluate the phytochemicals through scientific methods and provide information especially Omani and Turkish coffee that could be used in the future for their beneficial properties. In conclusion, the phytochemical analysis showed that the extract contains major phytochemicals like phenolic compounds and flavonoids. Phenolic content and the antioxidant activities depend on the origin, brands and additives of these beverages that may lead to variation in the data of two commercially available brands of the same extract. Tea appears to be richer in phenols and caffeine but Omani qahwa along with Turkish coffee showed better antioxidant activity. The quantitative DPPH assay indicated that the study samples has potent antioxidant activity which can be an excellent option for biological and chemical analysis. This method of study using Omani and Turkish coffee is an excellent choice and can be further subjected for the isolation of therapeutically active substance with antioxidant and other pharmacologically effective agents. This study can contribute to a better knowledge of the levels of caffeine in commercial tea and traditional coffee for Omani consumers to estimate how many cups of coffee people should have every day.

Conflict of Interest

Authors declare no conflicts of interest and there is no financial support for this research.

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