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Original Article

DETERMINATION OF PHENOLIC COMPONENTS AND ANTIOXIDANT ACTIVITY OF SOME EGYPTIAN TEA SAMPLES

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ABSTRACT

Objective: Tea is the most widely consumed beverage in the world. Green tea (*Camellia sinensis*) is a good source of bioactive compounds and it is gaining interest due to its health benefits. The present study was conducted to determine the total polyphenols and total flavonoid content as well as the antioxidant activity of ethanolic extracts from different Egyptian tea samples.

Methods: The determination process carried out using standard spectrophotometric methods and quantified various phenolic and flavonoid compounds by using high performance liquid chromatography (HPLC) method. The comparison was also made between Egyptian tea samples and commonly tea samples in the markets.

Results: Results from this study showed that Egyptian green tea showed a significant higher values of total phenol and flavonoid, contents. The same trend was also observed in antioxidant activity as assessed using DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. It revealed that the total antioxidant activity could be due to its total phenol content.

Conclusion: The study concludes that Egyptian green tea offers higher amount of antioxidant properties.

Keywords: Phenolic compounds, Flavonoids, Antioxidant activity, Egyptian tea.

INTRODUCTION

Tea is one of the most widely consumed beverages in the world. Black and green teas are the two main types [1]. A recent awareness of health benefits has increased consumers' interest in this beverage especially green tea. Green tea is derived from drying and steaming the fresh tea leaves and thus no oxidation occurs, resulting in high levels of catechins [2]. Most commercially prepared tea is obtained from the leaf of the plant *Camellia sinensis*. There are two varieties of tea plant. *Camellia sinensis* var *sinensis* (China tea) is grown extensively used in China and Japan, while *C. sinensis* var *assamica* (Assam tea) predominates in South and South-East Asia.

Traditionally, Green tea was used to improve blood flow, eliminate alcohol and toxins, improve resistance to disease, relieve joint pain and to clear urine and improve its flow [3]. Tea contains large amounts of polyphenolic compounds with antioxidant properties, and these may prevent oxidative damage of DNA [4]. Tea is also rich in flavonoids and other polyphenol compounds which have different beneficial activity such as anticarcinogenic or prevent tumor cell growth, cholesterol lowering, antiviral, antibacterial, reduce cardiovascular disease, reduce cholesterol and induce body weight loss [5, 6]. Green tea catechins have the potential to alleviate symptoms of the metabolic syndrome [2].

One of the advantages of tea is that it has high antioxidant activities due to the presence of polyphenols that enable it to scavenge free radicals. Green tea extract has strong antioxidant due to the presence of (+) catechin, (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG) and (-)-epigallocatechin-3-gallate (EGCG). Catechin is a compound which does not evaporate and it contained about 8-15% of the dry weight of plant [7]. Catechin solution is colorless, however, it tastes bitter [8]. Moreover, production of black tea leaves involved extensive enzymatic oxidation of the leaf polyphenols to dark products such as theaflavins and the arubigens. The major theaflavins in black tea are theaflavin (TF1), theaflavin monogallate A (TF2A), theaflavin monogallate B (TF2B) and theaflavin digallate (TF3).

From previous studies, green tea was found to contain higher antioxidant activity than black tea [9, 10].

A number of studies showed that catechins in tea function as anticancer, antibacterial, antiviral, antitoxin and antifungal. Besides, drinking black tea showed similar benefits as in green tea from the perspective of antioxidant capacity. This could be explained by the presence of theaflavin in black tea having the similar amount of catechins as present in green tea. Every tea differs from the perspective of composition and concentration of an antioxidant compound. Black tea has low amount of theaflavin (2.0-6.0%) and high thearubigin (20%), while green tea has higher catechins (30-42%), especially EGCG which has the highest amount of catechins [11-13].

Recently, the antioxidant activity of tea leaves has been studied intensively. However, there is no literature in our knowledge that shows the determination of the antioxidant properties and the polyphenol composition of different samples of Egyptian tea. Therefore, the objective of this study is to evaluate and compare the antioxidant activity as well as total phenol and total flavonoid content of Egyptian tea samples, namely Egyptian fresh tea, Egyptian black tea, and Egyptian green tea compared with Black yellow label tea and Green yellow label tea.

MATERIALS AND METHODS

Apparatus

Spectrophotometric measurements were performed on a UV/Vis spectrometer (Varian Cary 100) equipped with 10 mm quartz cuvettes.

The liquid chromatography system used was Hewllet Packared (series 1050) HPLC system equipped with auto-sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quantity HP pump (series 1100). System control and data processing were carried out by a ChemStation 32 Software. The analytes were eluted on a Chromasil C18 analytical column (250×4.6 mmID，5 μm) (Elite, Dalian, China) protected by a Chromasil-C18 precolumn (20×4.6 mm ID，10 μm)（Elite, Dalian, China) maintained at room temperature using gradient mobile phase composed of methanol+acetonitrile running at a flow rate of 1.0 mL·min^{-1}.

Materials

Acetonitrile (CH_3CN), methanol (MeOH), all of HPLC grade, were purchased from Tedia Company (USA) and all other chemicals used

were of analytical grade. The double–distilled water was used. Folin–Ciocalteau phenol reagent, gallic acid and 2, 2-azinobis [3-ethylbenzothiazoline-6-sulphonic acid] diammonium salt (ABTS) was purchased from Fluka (Buchs, Switzerland). Trolox ([±]-6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was purchased from Sigma–Aldrich (St. Louis, MO, USA). All standard solutions were prepared in methanol.

Sample collection

The Egyptian fresh, green and black tea leaves used in this experiment were cultivated in Agriculture Research center, Egypt and The three tea types were air-dried, cut into pieces and ground into powder. Commercial black, yellow label and green, yellow label tea (Lipton tea) were obtained from local markets in Giza, Egypt.

Sample extraction and preparation

Samples of Egyptian fresh, green and black tea (2.0 g each) were extracted with 50 ml of 70% ethanol for 1.0 h on an orbital shaker to produce crude extracts containing a wide range of active compounds. The mixture was centrifuged at 4500 rpm for 10 min. The samples were re-extracted at identical conditions. The respective extracts were filtered using Whatman No.1 filter paper and dried under reduced pressure at a temperature below 45°C in rotavapour to yield a dense residue. The supernatants was combined and stored at-18°C until further analysis [14]

Green tea: Freshly picked leaves are steam-blanched for 10 min to deactivate enzymes in the leaves. Then, the leaves were dried and ground into smaller particles [15].

Black tea: The leaves were dried in oven for 10 min at 100 °C, followed by 10 min at 90 °C, 10 min at 60 °C and finally 10 min at 40 °C until the moisture content was reduced to approximately 5.0 % [16].

Determination of total phenolic and total flavonoid content

Spectrometric method of total phenolic content

Total phenolics were determined using Folin-Ciocalteu's reagent as adapted from [13]. 100 µl of the test extract solution was mixed with 750 µl of Folin-Ciocalteu reagent and allowed to stand at 22 °C for 5.0 min; 750 µl of (60 g/l) sodium bicarbonate solution. The mixture was shaken thoroughly and the volume was made up to 2.0 ml.

The mixture was allowed to stand for 1.0 h in the dark. Then the absorbance at 725 nm was determined. These data were used to estimate the total phenolic content using the standard curve which was obtained using various concentrations of Gallic acid (mg GAE/g) [17].

Spectrometric method of total flavonoid content

One mL aliquot of the extract and appropriately diluted standard solution of quercetin (20, 40, 60, 80 and 100 mg/l) was added into a 10 ml volumetric flask containing 4.0 mL deionized water. At zero time, 0.3 ml of 10% $AlCl_3$ was added. At 6.0 minutes, 2.0 ml of 1.0M NaOH was added to the mixture. Immediately, the reaction flask was diluted to the volume with the addition of 2.4 ml of deionized water and thoroughly mixed. Absorbance of the mixture, pink in color was determined at 510 nm versus prepared water blank. Total flavonoid of the samples was expressed on a dried weight as quercetin equivalent (mg QE/g) [18].

HPLC method for determination of total phenolic and total flavonoid content

Phenolic and Flavonoid compounds were determined by HPLC Hewllet Packared (series 1050) equipped with auto-sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quantity HP pump (series 1100) [19] as follow:

A 5.0 g of sample were mixed with ethanol and centrifuged at 10000 rpm for 10 min.: and the supernatant was filtered through a 0.2 µm Millipore membrane filter then 1.0-3.0 ml was collected in a vial for injection. Chromatographic separation conditions were described as follows: Gradient separation was carried out with methanol and acetonitrile as a mobile phase at a flow rate of 1.0 ml/min and injection volume was 10 µl. The column temperature was maintained at 35 ºC. An XBridge C18 (4.6 × 250 mm, 3.5 µm) column from Waters (Ireland) was used for chromatographic separation. Phenolic and flavonoid acids are standard from sigma co. were dissolved in a mobile phase and injected into the HPLC. Retention time and peak area were used for calculation of phenolic and flavonoid compound concentration in the data analysis of Hewllet packaged software.

Total antioxidant activity assay

1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity was measured by the spectrophotometric method [20]. A solution of DPPH (0.3 mM) in methanol was prepared freshly. 3.0 ml aliquots of this solution were mixed with 0.1 ml of the samples. The solutions in the test tubes were shaken well and incubated in the dark for 15 min at room temperature. The absorbance was measured at 517 nm against methanol as blank. Control tube containing 1.0 ml of methanol and 3.0 ml of DPPH reagent was also noticed for absorbance. Gallic acid was used as a standard and total antioxidant capacity was expressed as Gallic acid Equivalent.

The antioxidant activity (AA) was calculated as below:

$AA\% = 100 - [(Abs_{sample} - Abs_{empty\ sample})/Abs_{control} \times 100$

Where Abs; is absorbance

Empty sample= 3.0 ml methanol+0.1 ml extract

Control sample= 3.0 ml 0.3 mM DPPH+0.1 ml methanol

Statistical analysis

All of the experiments were carried out in triplicate. Total phenolic content, flavonoid content and antioxidant activity are reported as the mean±standard deviation (SD).

RESULTS

Total phenolic content

In this study, 50% aqueous ethanol was used as solvent to extract tea. From the results (table 1), the ranking of the total phenolic content, total flavonoid content and antioxidant capacity were recorded in table 1: Egyptian green tea>black, yellow label tea>green, yellow label tea>Egyptian Black tea>Egyptian fresh tea which means they are highly correlated between the total phenolic content and total flavonoid content and their antioxidant activity. Moreover, Egyptian green tea showed the highest value for total phenolic content and total flavonoid content and antioxidant activity compared to other samples. Also, the content of phenolic contents using HPLC method was evaluated and recorded in table 2 and fig 1.

Table 1: Total phenolic and flavonoid content and antioxidant activity for the studied tea samples

Samples	Total phenolic content (mg GAE/ g dry weight)	Total flavonoid content (mg QE/ g dry weight)	Antioxidant activity (% A. A.)
Egyptian fresh tea	59.72±0.48	22.61±0.42	49.0±1.06
Egyptian black tea	60.34±0.52	23.87±0.82	52.0±1.13
Egyptian green tea	74.51±0.90	35.09±0.56	75.0±1.41
Black yellow label tea	70.39±1.08	30.44±1.24	78.0±1.60
Green yellow label tea	68.50±0.97	28.76±0.95	54.0±1.21

Data are expressed in mean±SD (n=3).

Table 2: Polyphenols compounds presence of the studied tea samples (Conc. %) using HPLC method.

Samples compounds	Egyptian fresh tea	Egyptian black tea	Egyptian green tea	Black yellow label tea	Green yellow label tea
Catechein	-	0.010707	0.02712	0.25425	0.95306
Protocatachein	0.00174	0.00289	0.00284	0.07011	0.15779
Gallic	0.00056	0.00245	-	0.03829	0.07544
Caffeic	0.00056	-	0.00306	-	0.02293
Vanillic	0.00293	0.000156	0.10682	0.19949	0.31509
Chlorogonic	0.00295	-	-	0.06784	0.74407
Catecol	0.00584	-	-	0.019785	0.24783
Chrysinool	-	-	-	-	0.00435
Syringic	-	0.00144	0.00129	-	0.00111
Ellagic	0.00993	0.00406	-	-	-
Cinnanic	0.00138	-	-	0.00222	-
Chrysin	0.00099	0.00062	-	0.00192	-
Coumarin	0.00137	0.00132	0.00064	0.00323	-
Ferulic	0.00070	-	-	-	-

(-) Not detected,

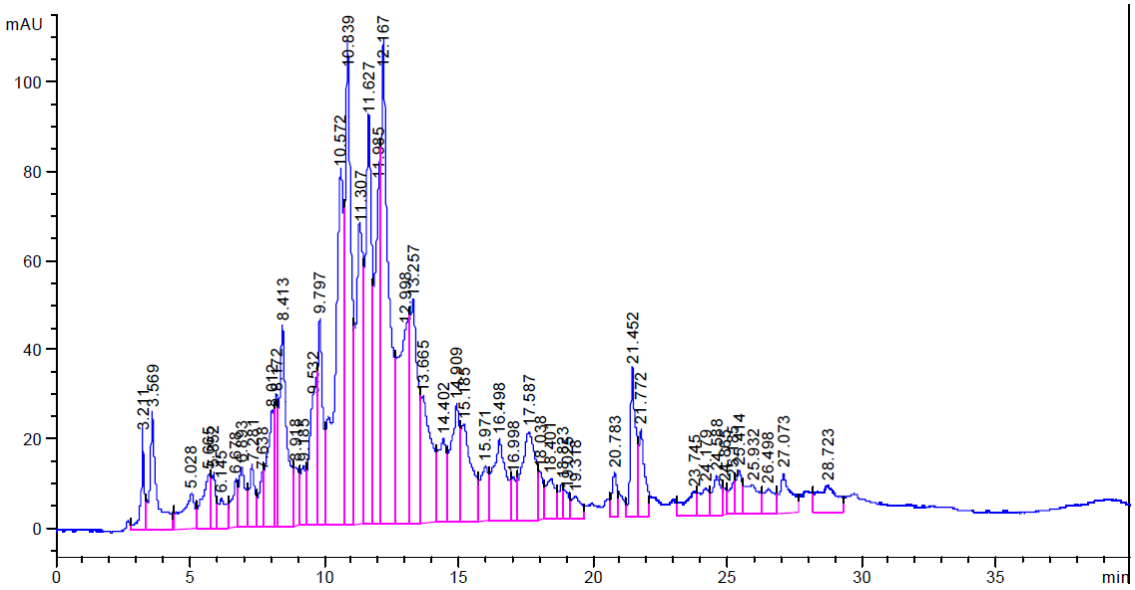

Fig. 1: HPLC chromatogram of phenols compounds in Egyptian green tea

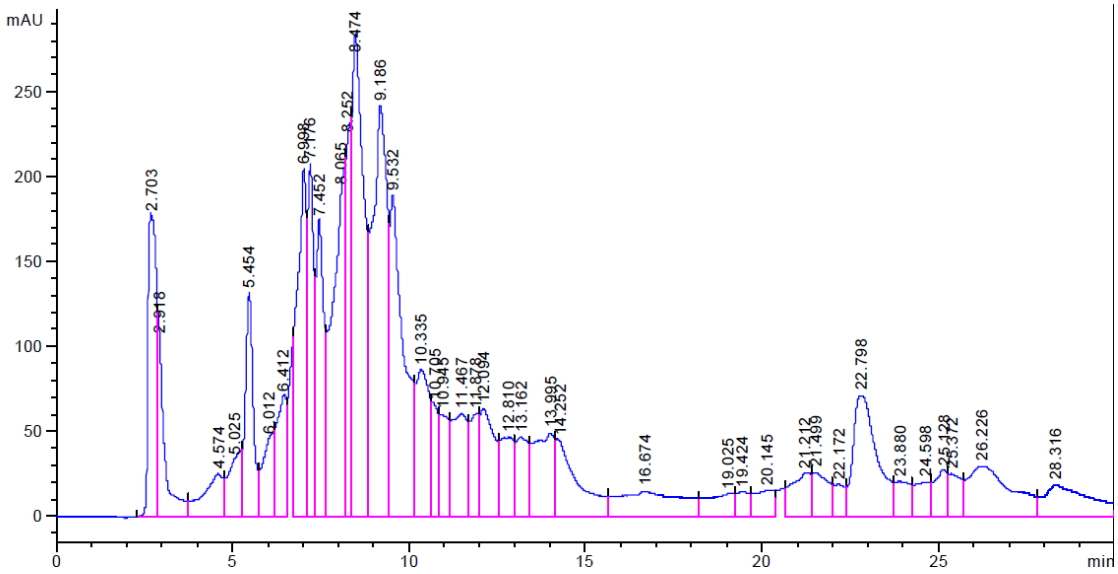

Fig. 2: HPLC Chromatogram of flavonoids compounds in Egyptian green tea

Table 3: Fractionation of flavonoids in the studied tea samples (Conc. %)

Samples Compounds	Egyptian fresh tea	Egyptian black tea	Egyptian green tea	Black yellow label tea	Green yellow label tea
Querctin	-	0.00335	-	0.02757	0.01478
Nargein	-	0.00194	-	0.00847	0.02386
Rosmarinic	0.00319	-	-	0.01480	0.03471
Rutin	0.03149	-	-	0.08864	-
Hispertien	0.02540	-	0.00376	0.09336	-
Kampferol	-	0.00679	0.00462	-	-

(-) Not detected

Total flavonoid content

In this study, Egyptian green tea exhibited significantly better results in green, yellow labeled tea, especially antioxidant activities and polyphenols compound (phenols and flavonoid). This showed that Egyptian green tea has a great antioxidant potential. Moreover, the total phenol content of Egyptian green tea was much higher than Egyptian black tea and this current study was the agreement of the findings of [13, 22, 23]. Also, the content of flavonoid contents using HPLC method was evaluated and recorded in table 3 and fig 2.

Antioxidant activity

In this study, the antioxidant capacity of ethanol extracts of different tea samples was systematically evaluated. The DPPH inhibition of different tea samples is summarized in table 1. Ethanolic extracts of leaves of Egyptian green tea possessed the highest DPPH scavenging activity (75.0±1.41% inhibition of the DPPH radical), followed by Egyptian black tea and Egyptian fresh tea extracts (52.0±1.13% and 49.0±1.06%, respectively), compared to the commercial green label yellow tea and black label yellow tea antioxidant (54.0±1.21% and 78.0±1.60%). The antioxidant activity of the leaves of *C. sinensis* could be due to the presence of a wide variety of bioactive compounds, such as phenolics, flavonoids, carotenoids, and tannins in this plant.

DISCUSSION

The literature [13, 14] reported that the extraction with aqueous-methanol contributed to higher antioxidant activity if compared to methanol and hot water extraction. So, a solvent that has higher polarity is more efficient to scavenge free radicals than less polar solvent. Different solvents with different polarities will definitely contribute to the efficiency of determining antioxidant activities. According to that ethanol is an efficient solvent to extract polyphenols.

Phenolics are well established to show antioxidant activity and contribute to human health. Phenolic is a kind of polyphenols that can be divided into tannin, propanoid and flavonoid. Phenolic compounds are known as powerful chain breaking antioxidants, which may contribute directly to antioxidative action [21]. These compounds are very important constituents of plants and their radical scavenging ability is due to their hydroxyl groups. Phenolic compound has been reported to protect plants against microorganisms and herbivores. This might explain the importance of high phenolic compound in the leaves. Moreover, high correlations were observed in antioxidant capacities as well as total phenolic and flavonoid content of *C. sinensis*. This finding was in agreement and compared with the references [13, 22] in which high correlations were observed between antioxidant activities and polyphenol phytochemicals content. Thus antioxidantnt activities most probably might be contributed by polyphenols contents in the plant extracts.

In this study, the total phenolic content was determined using two methods spectrophotometric method (Folin–Ciocalteu method) and HPLC method. The content of phenolics using was evaluated and expressed in GAE as milligrams per gram of extract (mg GAE/g extract) (table 1). The total phenolic content of the ethanol extracts of Egyptian tea samples, namely Egyptian fresh tea, Egyptian black tea, and Egyptian green tea compared with Black yellow label tea and Green yellow label tea, showed large variations.

Flavonoid was believed to be responsible for antioxidant activity, anticarcinogenic and anti-arteriosclerosis. Flavonoid in tea involved catechins, quercetin, kaempferol and myricetin. Flavonoid in tea has high antioxidant activities and radical scavenging. the result showed that Egyptian green tea has higher phenolic content (table 1).

Antioxidant tests could be based on the evaluation of lipid peroxidation or on the measurement of free radical scavenging potency (hydrogen-donating ability). The radical scavengers donate hydrogen to free radicals, leading to non toxic species and therefore to inhibition of the propagation phase of lipid oxidation. The use of DPPH radical provides an easy, rapid and convenient method to evaluate the antioxidants and radical scavengers. The antioxidant activity of medicinal plants is mainly related to their bioactive compounds, such as phenolics and flavonoids.

CONCLUSION

Polyphenols in Egyptian tea samples contributed significantly to the antioxidant activities of tea extracts. Egyptian green tea showed the most promising result as an antioxidant agent. The potential medicinal uses of these teas from Egyptian tea Plantation are supported by the presence of above mentioned antioxidants and polyphenolic compounds. This study indicates that Egyptian tea is one of the most effective plant in terms of antioxidant properties and can serve as natural sources to the free radical scavengers and antioxidant agents. Hence, the need to exploit the potentials of *C. sinensis* especially in pharmaceutical industries arises.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests with the company name used in the paper.

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