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Estimation of total phenolic contents and antioxidant capacities in some green and black tea of Saudi Arabia markets and evaluation of their antibacterial activity

Abstract: Tea is the most commonly consumed beverage in the world after water and considered a good antioxidant nutrient against free radical reactions. Teas are classified into more than one type depending on the manufacturing process. Green and black tea leaves are rich in phenolic compounds that are known for their antioxidant activity, having a beneficial effect on human health. The objective of this study was to determine phenolic content, antioxidant capacities as well as the antibacterial activity of green and black tea for the most popular brands in Saudi Arabia, Lipton and Rabea. The results showed that the content of polyphenols was higher for green brands 524.10 ± 11.8 and 553.75 ± 14.0 mg/g than black brands 468.70 ± 10.5 and 466.25 ± 6.8 mg/g for Lipton and Rabea types respectively. Moreover, it was found that all tea types exhibited high antioxidant activity, but green tea brands were higher 93.94 and 94.08 % than black brands 93.64 and 93.32 % for Lipton and Rabea brands respectively. Test of antibacterial activity showed that green tea was more effective in inhibiting the growth of the test organisms than black tea, where it showed antibacterial activity with a mean diameter of inhibition zone ranged from 9.0 ± 0.22 to13.0 ± 0.24 mm in comparison with black tea 9.0 ± 0.20 to 11.0 ± 0.10 mm. The present study indicated that no significant differences were noted in total phenolic contents and antioxidant activities for black and green tea. but Rabea green tea exhibited stronger antibacterial activity than black tea brands. To our knowledge, no studies have so far been done to compare these properties of two commercial black and green teas available in the markets of Saudi Arabia.

Key words: green, black tea, total phenolic content, radical scavenging activity, Saudi Arabia.

Introduction

In the last decades, lots of epidemiological studies were focused on bioactive phytochemicals such as phenolic compounds due to their beneficial effect on human health. It has been suggested that an initial cause of most chronic diseases is a free radical attack on biomolecules; thus, consumption of foods rich in phenolic compounds that are capable of the scavenging of reactive species may be a mechanism of protection recommending those foods for maximum health benefits [1; 2]. Historically, tea has important roles not only as ancient health therapy but also as a subject of the visual and literary arts. Tea was painted, drawn and figured on textiles and ceramics. Its shape, color, perfection and social interactions were described in many Odes [3; 4]. Tea is the most widely consumed drink in the world after water. It has been cultivated and consumed for more than 2000 years. Tea comes from the leaves of two classes of *Camellia sinensis* plant: assamica and sinensis, which originate in China and Southeast Asia [5; 6]. Teas are classified into three major types depending on the manufacturing process. Non-fermented green tea is produced by drying and steaming the fresh leaves to inactivate the polyphenol oxidase and thus, oxidation does not occur. Semi-fermented oolong tea is produced when the fresh leaves are subjected to a partial fermentation stage before drying. Fermented black and red (Pu-Erh) teas undergo a post-harvest fermentation stage before drying and steaming. However, the manufacturing process of black and red teas is not identical, since the fermentation of black tea is due to oxidation catalyzed by polyphenol oxidase, while the fermentation of red tea is attained by using microorganisms [7; 8]. The percentage of each type of tea produced and consumed in the world is 78% black, 20% green and 2% oolong tea [9]. Among the different types of tea, green tea is now preferred as it has much more positive health benefits in comparison to other natural products [10]. Tea has a complex structure, which contains polyphenols, amino acids, proteins, alkaloids, minerals, carbohydrates, volatile compounds and trace elements [11]. Due to the presence of polyphenols, many scientific studies have proved the health benefits of tea such as anti-carcinogenic, anti-oxidant, anti-aging, anti-mutagenic, anti-viral, anti-bacterial, and anti-inflammatory properties [12; 13]. The antimicrobial effect of tea was first demonstrated almost a century ago, in 1906, in the laboratory by McNaught, who showed that brewed black tea killed *Salmonella typhi* and *Brucella melitensis*. Thus, he recommended that the water bottles of troops should be filled with tea in order to prevent outbreaks of infections due to these agents. The precise antimicrobial spectrum of tea is difficult to be defined due to variation in definitions of 'susceptible' and 'resistant' as well as due to variation in the methods of testing, that have been used [14]. Tea polyphenols are also known for their antibacterial activity. In general, antibacterial activity decreases when the extent of tea fermentation is increased, implying stronger activity in green tea than black tea [15; 16]. Green tea catechins, particularly EGCG and ECG, have antibacterial activity against both Gram-positive and Gram-negative bacteria [17-19]. There are many types of tea (local and exotic) commonly consumed in Saudi Arabia. Most of these teas are traded and consumed without knowing their positive health benefits. So, comparing these teas with its chemical and biological activities is greatly required. Thus, the purpose of our research was to compare the total phenolic content, antioxidant capacities and antibacterial activity of two popular black and green tea brands from local Saudi markets by using several different methods. According to our knowledge, this is the first study to compare these characteristics of the most common green tea brands available in the Saudi markets.

Materials and methods

Tea samples. Camellia sinensis green teas (Lipton and Rabea), and black teas (Lipton and Rabea) were purchased from some supermarkets of Rafha governorate at the Northern Border region in Kingdom of Saudi Arabia. The tea samples were stored in a cool dry place before analysis.

Chemicals. 1,1-diphenyl-2-picrylhydrazyl (DPPH) used as the source of free radicals and Folin-Ciocalteu's phenol reagent used for estimation of total phenolic content were purchased from Sigma-Aldrich Chemical Co. (Pool, UK).

Samples and extract preparation. Two different brands of commercial black and green tea, purchased from the local markets (Lipton classic and Rabea tea), were used in this study. Tea samples were extracted using the hot water method [20]. Teas (25 g) were extracted with 250 ml of hot water three times, with continuous swirling at 120 rpm in an orbital shaker, for 1 h each time. The boiling water was allowed to cool throughout the extraction process to mimic tea brewing. After filtration under suction through Whatman No. 1 filter paper, the residues were re-extracted again with 250 ml of hot water. The water in the extracts was removed using a freeze dryer. Dried extracts were kept at -20°C in a freezer for further analysis.

Folin-ciocalteu assay. Total phenolic content (TPC) of extracts was determined using the Folin-Ciocalteu method [21; 22]. Samples (300 µl, in triplicate) were introduced test tubes wrapped in aluminum foil followed by addition of 1.5 ml of FC reagent (10 times dilution) and 1.2 ml of sodium carbonate solution (7.5% w/v). The tubes were allowed to stand in the dark for 30 min before absorbance was measured at 765 nm. TPC was expressed as gallic acid equivalent (GAE) in mg/g of sample. The calibration equation for gallic acid was $y = 0.0111x + 0.0148$ ($R2 = 0.9998$).

DPPH radical scavenging assay. Antioxidant activity was measured using the DPPH radical scavenging assay [22; 23]. Different dilutions of the extracts (1 ml) were added to 2 ml of DPPH (5.9 mg/100 ml methanol) in test tubes wrapped in aluminum foil. Absorbance (A) was measured at 517 nm after 30 min incubation in the dark. All measurements were made with distilled water as a blank. The scavenging ability (%) of the samples was calculated as ($A_{control} - A_{sample})/A_{control} \times 100$) and calculated as IC_{50}, the concentration of sample needed scavenge DPPH free radicals by 50%. IC50 was expressed as ascorbic acid equivalent antioxidant capacity (AEAC) using the equation: AEAC (mg AA/g sample) = $IC_{50}(AA)/IC_{50}(sample) \times 10^5$. The IC_{50} of AA used for calculation of AEAC was 0.00387 mg/ml.

Disc-diffusion method. Antibacterial activity of extracts and fractions of green and black teas were tested against Gram-positive *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538, and against Gram-negative *Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 7839. Antibacterial activity was measured using the disc-diffusion method [24]. Inoculums (100 µl) were spread evenly onto 20 ml Mueller-Hinton agar set in 90-mm Petri dishes using a sterile cotton swab. Sterilized paper discs (6-mm diameter) were impregnated with plant samples (2 mg per disc) using a micropipette and firmly placed onto the inoculated agar ensuring even distribution to avoid overlapping of zones. Streptomycin susceptibility discs (10 µg) were used as positive controls. After incubation overnight at 37ºC, the minimum inhibitory dose (MID) or lowest concentration of extract or fraction in mg/disc required to show a zone of inhibition was recorded [25; 26].

Statistical analysis. The data were statistically analyzed using Microsoft Excel 2019. Results with $p < 0.05$ were considered statistically significant. All experiments were performed in triplicate and the values were expressed as mean ± SD. The differences between the samples were assessed using single factor analysis of variance (ANOVA).

Results and discussion

Tea is consumed more than any other beverage worldwide [27]. It is a hot water infusion of the dried, young leaves and/or buds of the evergreen *Camellia sinensis* plant [28; 29]. In 2012, 4.884 million tons of tea was produced and consumed globally [30]. The chemical composition of tea is complex and not completely understood. The detailed investigations which have been made were done mainly to understand how tea gets its characteristic flavor and appearance. In the present study, hot water extract of two popular black and green tea brands from local Saudi markets (Lipton and Rabea tea) were prepared for estimation of total phenolic content, antioxidant capacities and antibacterial activity. Hot water was used for extraction since it is the traditional way of brewing tea and previous studies have shown it to be an efficient way of extracting tea [31; 32]. Studies have shown that water temperature is an important factor when extracting tea. Significantly higher yields of hot water than cold water extraction of green tea and stronger radical scavenging activity of oolong tea extracted with hot water of increasing temperature have been reported [33; 34]. For green, oolong, and black teas, extraction with water at 100ºC for 3 min yielded higher total flavanol content than extraction with water at 60 and 80ºC [35].

Total phenolic content (TPC). Polyphenols are a class of chemical constituents with one or more hydroxyl groups associated to the aromatic arene (phenyl) ring. Consequently, they are considered as one of the most potent antioxidative compounds that contribute to the antioxidant activity [36]. Hence, it is important to quantify polyphenol content and to estimate its contribution to antioxidant activity. Total phenolic contents of black teas (Lipton and Rabea) and green teas (Lipton and Rabea) brands were expressed as gallic acid equivalent. The results showed that the content of polyphenols was higher for green brands 524.10 ± 11.8 and 553.75 ± 14.0 mg/g than black brands 468.70 ± 10.5 and 466.25 ± 6.8 mg/g for Lipton and Rabea brands respectively (Table 1).

Table 1 – Total phenolic content (TPC) of extracts of *Camellia sinensis* teas

No	Type	Brand (County)	TPC[a] (mg GAE/g)
1	Black	Lipton (United Arab Emirates)	468.70 ± 10.5
		Rabea (Saudi Arabia)	466.25 ± 6.8
2	Green	Lipton (United Arab Emirates)	524.10 ± 11.8
		Rabea (Saudi Arabia)	553.75 ± 14.0
Note: [a]TPC (total phenolic content) represented by means ± SD (n = 3).			

Our results showed that the concentration of total phenol content was high in both brands especially in the green teas, this result may be referred to the high antioxidant activity of tea catechins, which are mainly found in green tea. The concentration of TPC obtained in this study is slightly lower than TPC obtained in the previous study concerned with the comparison of TPC activity of two popular green tea beverages available in the local market of Saudi Arabia recorded high phenolic content for the two green brands [37].

DPPH radical scavenging activity. DPPH assay is used to evaluate the free radical scavenging activity of hydrogen donating antioxidants in many plant extracts. DPPH is a stable free radical with a dark violet color. This method is based on the principle that DPPH accepts a hydrogen atom from the antioxidant, resulting in the reduction of DPPH to DPPH2, the violet color changes to yellow with a consequent decrease in absorbance at 517 nm. The efficiency of the antioxidant compound is measured by its ability to change color. The percentage of inhibition caused by green tea brands was higher 93.94 and 94.08 % than black brands 93.64 and 93.32 % for Lipton and Rabea brands respectively (Figure 1).

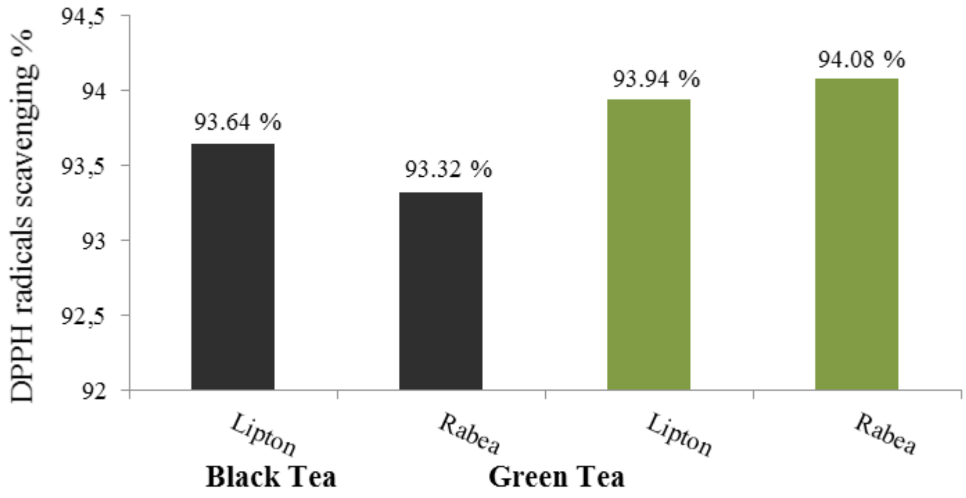


Figure 1 – Radical scavenging activity by black and green (Lipton and Rabea) teas
The value expressed as means ± SD (n = 3).
Compression of means was made using unpaired t-test (P > 0.05)

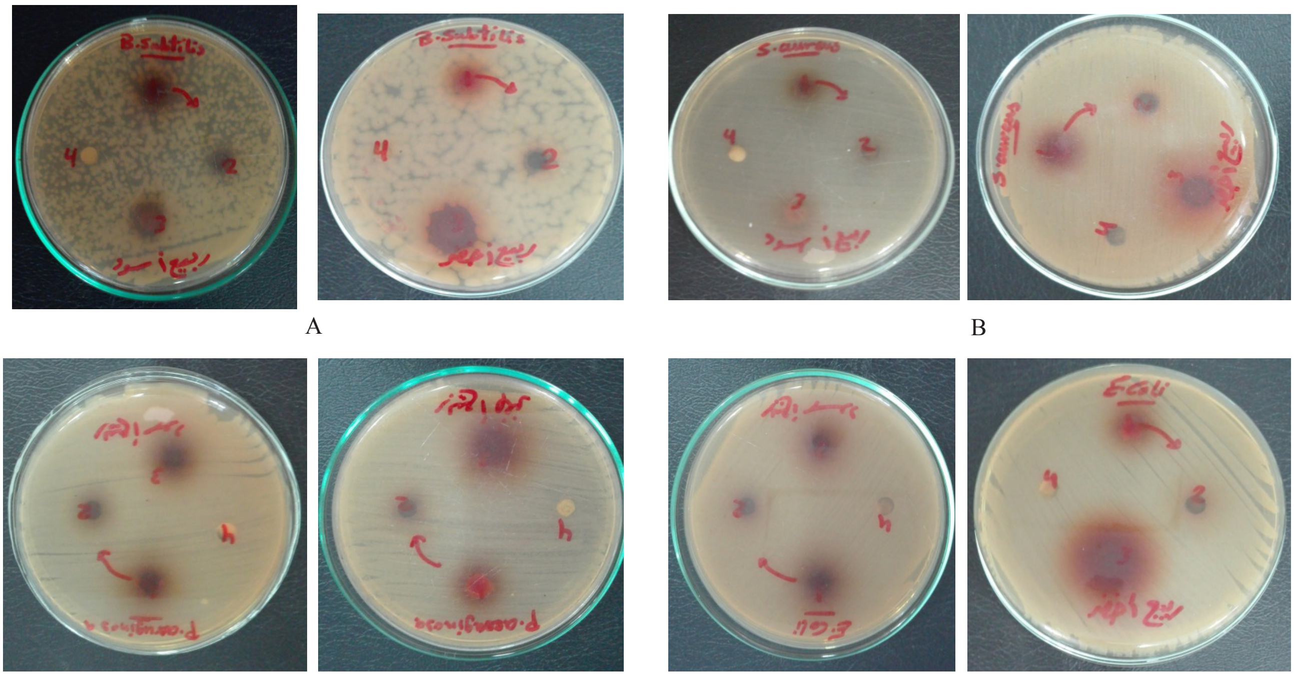
Results demonstrate that black and green of the two brands have the ability to scavenge free radicals. Therefore, both have the same high antioxidant activity. The presence of chlorophyll and pheophitin in green tea extract could explain the higher DPPH scavenging activity in these samples (Figure 1). The main green tea catechins are Epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) [38]. In general, Epigallocatechin gallate is the most powerful catechins in tea and it is responsible for the majority of the biological activity of green tea. It is well known that green tea has greater total catechins than black tea due to the fermentation process; catechins in the black tea are reduced as they are converted to the flavins and thearubigins [39]. Moreover, our results showed that the two brands have the ability to scavenge free radicals. Studies conducted by Omar et al. [37] and Gramza et al. [40], indicated that the analysis of DPPH free radicals scavenging ability by green tea showed higher radicals scavenging efficiency than black tea extract.

Antibacterial properties. Antibacterial activity of hot water extracts of black and green teas are recorded in Table 2 and represented in Figure 2.

The results obtained showed that green tea was more effective in inhibiting the growth of the test organisms than black tea, where it was showed antibacterial activity with mean diameter of inhibition zone ranged from 9.0 ± 0.22 to 13.0 ± 0.24 mm in comparison with black tea 9.0 ± 0.20 to 11.0 ± 0.10 mm. Furthermore, it was found that green Rabea brand was the most active one where it was active against all of the bacterial test organisms, while the weakest one was in case of black Rabea brand where it exhibited activity against *B. subtilis* ATCC 6633 with inhibition zone diameter 9.0 ± 0.20 mm and non-active against the other test organisms. The inhibition of tea extracts against *P. aeruginosa* and *E. coli* has been reported [16; 18] although an earlier study has explicitly reported that tea extracts are not effective against *P. aeruginosa* and *E. coli* [19]. The disparity in findings could be due to different strains of bacteria used, and to the different concentrations and types of extracts investigated. Gram negative bacteria are less susceptible to antibiotics as their outer membrane of lipoproteins and lipopolysaccharides is able to regulate the access of antibacterial agents into the underlying structures [41]. Several studies have shown that catechins from green and black teas, particularly EGCG and ECG, inhibited the growth of many bacterial species [17].

Table 2 – Antibacterial activity of extracts of *Camellia sinensis* teas using the disc-diffusion method

Type	Brand (County)	Inhibition zone diameter (mm)			
		Gram positive		Gram negative	
		B. subtilis ATCC 6633	*S. aureus* ATCC 6538	*P. aeruginosa* ATCC 9027	*E. coli* ATCC 7839
Black	Lipton (United Arab Emirates)	11.0 ± 0.10	0.0	0.0	0.0
	Rabea (Saudi Arabia)	9.0 ± 0.20	0.0	0.0	0.0
Green	Lipton (United Arab Emirates)	13.0 ± 0.24	0.0	0.0	12.0 ± 0.15
	Rabea (Saudi Arabia)	13.0 ± 0.15	9.0 ± 0.22	11.0 ± 0.10	9.0 ± 0.25



A B C D

Figure 2 – Antibacterial activity of black and green teas (Lipton and Rabea) against (A): *B. subtilis,* (B): *S. aureus*, (C) *P. aeruginosa* and (D): *E. coli*.

Contrary to findings from this study, earlier studies have reported that black teas inhibited the growth of Gram positive bacteria [18; 42]. Extracts of green tea have been reported to be more effective in inhibiting bacterial growth than black tea [15]. In general, antibacterial activity decreased when the extent of tea fermentation increased [16; 19].

Conclusion

Our research work showed that green and black tea of the most popular brands in Saudi Arabia, Lipton and Rabea showed similar activity in their total phenolic contents (524.10 ± 11.8 to 553.75 ± 14.0 and 466.25 ± 6.8 to 468.70 ± 10.5 mg GAE/g) and antioxidant activity (93.64 to 93.32% and 93.94 to 94.08%). However, green tea exhibited stronger antibacterial activity against all tested strains (0.0 to 13.0 mm) than black tea which was active against only one strain (0.0 to 11.0 mm). Continued researches are needed to further the current knowledge on the health-promoting effects of this popular beverage using different supplements by different mechanisms.

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