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Original Article

Comparison of Antimicrobial and Antioxidant Activities of Four Different Tea Extracts

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Introduction: Increasing of food-related diseases has led to the perception of diet importance. Plant-derived products (especially tea) as important sources of antioxidant and antimicrobial compounds play a major role in reducing food pathogens. In this study, total phenolic content, antioxidant and antimicrobial activity of four tea extracts including green tea, white, black and red teas were evaluated. **Methods:** The total phenolic amount was determined using Folin–Ciocalteu method and 1-diphenyl-2-picryl hydrazyl radical (DPPH) method was used for antioxidant activity measurement. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of tea extracts against eight species of tested bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumonia*, *Saprophyticus Staphylococcus*, *Enterococcus faecali*, *Acinetobacter baumannii*, *Proteus mirabilis* and *Serratia marcescens*) were evaluated by microdilution technique. **Results:** The results of this study showed that green tea and white tea extracts had the highest total phenolic content and antioxidant scavenging activity. Also, a strong positive correlation was observed between phenolic content and antioxidant activity in green tea and red tea. **Conclusion:** All four tea extracts showed inhibitions of several microorganisms. However, gram-negative bacteria were more resistant to inhibitory effects of tea extracts. As a result, non-fermented tea extracts showed more antioxidant activity and inhibition effect against tested bacteria. *J Med Microbiol Infec Dis, 2015, 3 (3-4): 57-61*

Keywords: Antioxidants, Phenols, Plant extracts, Tea.

INTRODUCTION

Nowadays, the number of food-related illnesses caused by pathogens is increasing [1]. The pathogenic species, *Bacillus cereus*, *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica* and *Staphylococcus aureus*, are related to foodborne diseases [2]. These diseases have been intensified due to increased use of antibiotics and subsequently, increased resistance to these compounds [1]. Herbal products are source of antimicrobial compounds that lead to reduction of food pathogens [2]. So, high interest in the development of antimicrobial compounds derived from plants, such as tea leaves compounds has risen [1].

Plant species of *Camellia sinesis* has leaves and buds that are used for the production of popular tea beverage. *Camellia* is a genus of flowering plants in the family of Theaceae. The white, green, oolong and black teas are all obtained from *Camellia sinesis* species, but they process differently to make various levels of antioxidant [3]. Tea is known as a famous drink. Also, it is used in a wide range of foods, beverages and cosmetic products all over the world [4]. The compounds found in tea leaves are affected by the fermentation process. According to this process, there are three types of tea: unfermented tea (green and white teas), partially fermented tea (red and oolong tea) and fermented tea (black tea) [5]. Tea leaves produce organic compounds that are involved in plant defense against pathogens including insects, bacteria, fungi and viruses. These compounds include metabolites of polyphenol, catechins, methylxanthine alkaloids caffeine, theobromine and theophylline [6]. Many reported physiological activities of tea extracts are due to their polyphenol compounds [7]. Tea polyphenols, particularly catechins, are important antioxidants with a positive effect on human health [8]. In addition to phenolic compounds, proanthocyanidins and tannins of tea have shown antimicrobial activity [9]. The most important mechanism of antioxidant and antibacterial activity in tea plant is free radicals scavenging and preventing of oxidation in cells and cell membranes [10]. Many studies have shown health-related properties of tea such as antioxidant and antimicrobial activity [6, 11, 12]. So, the importance of diet has known to prevent disease [13]. However, many studies have not been conducted on comparison the antibacterial and antioxidant effects of different types of tea extracts, yet. The aim of this study was to compare the total phenolic content and antioxidant activity of black, white, green and red teas. Also, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of four mentioned tea types on eight strains of pathogenic bacteria investigated.

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MATERIAL AND METHODS

Plant material. Plants of green, white, black and red teas were collected from Zabol city (61 longitudes and 31 latitudes), Sistan and Baluchestan province of Iran, during August 2015.

Preparation of samples for phenolic compounds and antioxidant measurement. For sample preparation and extraction, 10 g of dried leaves from each type of tea including green tea, white tea, black tea and red tea were weighted and powdered by the mill. Amounts of 100 ml of methanol 80% were added to the powdered samples and incubated for 48 h. Then, the samples were filtered by the vacuum pump; the solvent was removed from them by the rotary device, and the condensates were stored at -4°C until analysis.

Determination of total phenolic compounds and antioxidant activity. Total phenolic tea compounds were determined using the method described by others [14]. Briefly, 300 µL of methanol extracts of samples were combined with 2.1 ml of sodium carbonate and 5.1 ml of Folin-Ciocalteu reagent. Samples absorption was read at the wavelength of 765 nm, by a spectrophotometer. Antioxidant activity was determined using the DPPH method [15]. In this approach, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used to determine the free radical scavenging activity of extracts. For this purpose, different concentrations of extracts were added to a methanol solution of DPPH (0.15 mM). After 15 min of incubation at room temperature, the absorption was measured at the wavelength 517 nm. Inhibition percent of 50 or IC50 was determined.

Preparation of samples for antimicrobial activity. Dried leaves of four tea plants were powder by a mortar. Amounts of 10 g powder of each plant were soaked in 100 ml of 95% ethanol and shaken for 48 h at room temperature. The solvent was evaporated by rotary device at the temperature of 370°C. Extract was stored at 4°C.

Measurement of MIC and MBC. The *in vitro* antimicrobial activity of ethanol extracts of green, white, black and red teas was conducted against eight various microbes (*S. aureus, S. pyogenes, S. pneumonia, S. Staphylococcus, E. faecali, A. baumannii, P. mirabilis, S. marcescens*). The dilution was done using 96-well micro-plates and serial concentrations. Concentration of extracts ranged from 0.625 mg/ml to 320 mg/ml. Amounts of 100 µL of each extract and 95 µL of Mueller Hinton Broth medium and finally 5 µL of bacterial suspension were added to the wells. The micro-plate wells were incubated at 37°C for 18 to 24 h [16]. MIC was determined as the least concentration of extracts that inhibit growth of 90% of microbial colonies. MBC was defined as the lowest concentration of extracts which inhibited 99.9% growth of microbial cells [17].

Microbial species. (Standard forms) Reference strains of microbes were prepared from Center of Agricultural Biotechnology, University of Zabol, Iran. All microbe strains were cultured in 10 ml of tryptic broth medium (TSB, Merck, Germany). The bacterial concentration was determined by using McFarland standard (1.5x10^8 cfu). The bacteria species included gram-positive and gram-negative bacteria. The data of the bacteria is reflected in table 1.

Statistical analysis. The means of antioxidant activity (DPPH) and total phenolic content were calculated by SAS software and one-way analysis of variance (ANOVA) with Duncan test.

RESULTS

Total phenolic content. The Mean and standard deviations of the total phenolic compounds of green, white, black and red teas is shown in figure 1. The green and white tea had higher amount of total phenolic compounds compared to black and red teas. However, there was no significant difference in phenolic content of green tea and white tea. The results also showed that the red tea contains the least amount of phenolic compounds compared to other three types of tea.

Antioxidant activity. Figure 2 shows the results of antioxidant activity of four tested tea types. According to these results, there were significant differences between antioxidant activity of green, white, black and red teas. Antioxidant activity in green tea was significantly higher than white, black and red teas. Also, the results showed that red tea contains the least antioxidant activity compared to other types of studied teas.

Antimicrobial effect of tea extracts. Effect of four tea extracts against human pathogens and their MIC and MBC are illustrated in tables 2 and 3. According to these results, extracts of four teas in different concentrations inhibited the growth of the tested microbes (Figure 3). White tea and green tea, similarly, had the lowest mount of MIC and MBC against studied bacteria's. The least MIC and MBC in these two teas were 0.62 mg/ml and 1.25 mg/ml, respectively. The highest inhibitory concentration of white tea was 2.5 mg/ml and observed on *S. saprophyticus*, *A. baumannii*, *P. mirabilis* and *S. marcescens*. Black tea extract showed the highest amount of MIC and MBC against tested microbes. However, the lowest MIC and MBC of black tea were 2.5 mg/ml and 5 mg/ml, respectively. Also, red tea extract was found to be effective on studied bacteria's. Red tea had the least rate of MIC and MBC against *S. saprophyticus* and *E. faecalis*.

Table 1. List of bacterial strains used in this study

Bacterial strain	Standard number	Gram reaction
S. aureus	*ATCC® 25923*	positive
S. pyogenes	*ATCC® 19615™*	positive
S. pneumonia	*ATCC 49619*	positive
S. Staphylococcus	*ATCC®15305*	positive
E. faecali	*ATCC 29212*	positive
A. baumannii	*ATCC 19606*	negative
P. mirabilis	*ATCC 35659*	negative
S. marcescens	*ATCC 274*	negative

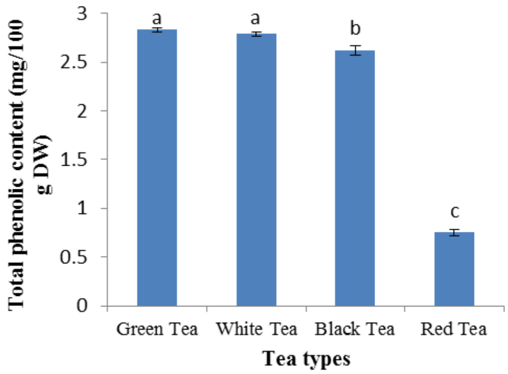

Fig. 1. Total phenolic compounds (mg/100 g DW) of four tea extracts. Different superscript letters, in each column, express significant (*P<0.01*) differences among results

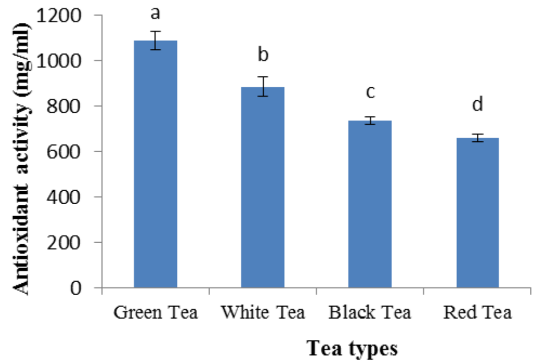

Fig. 2. Antioxidant activity (mg/ml) of four tea extracts. Different superscript letters, in each column, express significant (*P<0.01*) differences among results

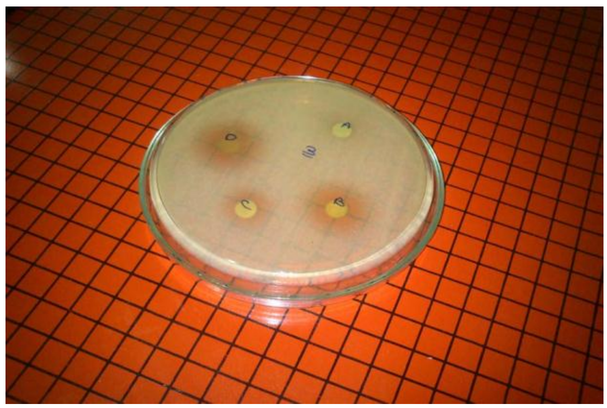

Fig. 3. Inhibitory effect of four tea extracts (A: red tea, B: green tea, C: black tea and D: white tea) on one of the tested microbes (*S. pneumonia*)

Table 2. Values of MIC (mg/ml) for different tea extracts obtained by micro dilution method

Microbes	Minimum inhibitory concentration (mg/ml)			
	GREEN TEA	WHITE TEA	BLACK TEA	RED TEA
S.aureus	1.25	1.25	2.5	2.5
S.pyogenes	1.25	0.62	2.5	2.5
S.pneumoniae	2.5	1.25	5	2.5
S. saprophyticus	0.62	2.5	2.5	1.25
A. baumannii	2.5	2.5	2.5	2.5
E. faecalis	1.25	1.25	2.5	1.25
P. mirabilis	2.5	2.5	5	5
S. marcescens	2.5	2.5	5	2.5

Each assay was done in duplication

Table 3. Values of MBC (mg/ml) for different tea extracts obtained by micro dilution method

Microbes	Minimum bactericidal concentration (mg/ml)			
	GREEN TEA	WHITE TEA	BLACK TEA	RED TEA
S.aureus	2.5	2.5	5	5
S.pyogenes	2.5	1.25	5	5
S.pneumoniae	5	2.5	10	5
S. saprophyticus	1.25	5	5	2.5
A. baumannii	5	5	5	5
E. faecalis	2.5	2.5	5	2.5
P. mirabilis	5	5	10	10
S. marcescens	5	5	10	5

Each assay was done in duplication

DISCUSSION

Plant polyphenols are aromatic hydroxyl compounds. They are useful bioactive and therapeutic materials [18]. Also, they are the most effective antioxidant constituent in plant food [19]. Genotype and species of plants are the effective factors on their secondary metabolites and phenolic content [20, 21]. The content and composition of polyphenols in tea plants are affected by several factors; including variation in tea type, harvest season, climate and processing method [22]. In this regard, Oh *et al*. evaluated total phenolic content (TPC) and total flavonoid content (TFC) of rooibos tea, green, black and rosemary teas. Green tea ethanol extract showed the highest TPC and TFC in comparison to other extracts. They suggested this result was related to the high flavonoid of catechins in green tea [23]. Tshivhandekano *et al*. showed that there were significant differences in the total polyphenol content of bush tea, special tea and synergy (combination of bush tea and special tea) [24]. Turkmen Erol *et al*. showed the lower content of polyphenol in green tea compared to fresh tea leaves was due to polyphenol destruction during heat treatment [11]. Also, Pilar Almajano *et al*. reported that significant differences were found between the polyphenol content of black, red, green and white teas. In this way, green tea and white tea had the highest amount of polyphenol, but the lowest content of polyphenol was observed in red tea. They suggested that this difference was caused by damage and change of tea leave compositions during the fermentation process, packaging and preparation [8]. According to the results of this study (Table 1), higher content of phenolic compounds in green tea and white tea may be due to less fermentation and processing. Also, red tea had the lowest content of total phenolic. So, in addition to fermentation and treatment amount of tea leaves, plant species is one of the effective factors in phenolic content.

Antioxidants have an important role in preventing free radicals damage to blood, cells, and tissues [25]. The present study showed that (Table 1) green tea had the highest antioxidant activity compared to white, black and red teas. Also, a strong positive correlation was found between total phenolic content and antioxidant activity in green tea (R^2= 0.97) and red tea (R^2= 0.82). In other words, there was a significant relationship between the content of phenolic compounds and antioxidant activity. So, polyphenols contribute to the antioxidant activity of plant extracts [26]. Similar observation was reported by Tshivhandekano *et al*. who suggested that higher antioxidant activity of special tea compared to bush tea and synergy can be associated with higher polyphenol content (as a primary source of antioxidant activity) [24]. In the study carried out by Anissi *et al*. the lower amount of antioxidant activity of black tea compared to green tea attributed to compounds change during the fermentation and processing [27]. Also, Stewart *et al*. declared the highest antioxidant activity in green tea is attributed to its high flavonoid content (flavan-3-ols) [28]. According to a study by Erkan *et al.*, there is a close relationship between antioxidant activity and total phenolic compounds of natural sources extracts [29]. Similar to these reports, the different antioxidant activity of the tea samples in this study may be due to the difference in fermentation amount. Also, different amount of phenolic compounds as an antioxidant source in tea samples is probably an effective factor on antioxidant activity. So, green tea with the highest phenolic content and red tea with the lowest phenolic amount showed the highest and the lowest antioxidant activity, respectively.

Active compounds of the plants and type of solvent for extraction may be effective on antimicrobial activity of plant extracts [30, 31]. The current study has shown that green, white, black and red teas had antimicrobial activity on tested bacteria strains. However, green and white teas inhibited of tested microorganisms in lower concentrations. In this regard, Kaur *et al*. found that green tea extract possesses stronger effect on gram-positive and gram-negative bacteria compared to black tea and divya peya tea [32]. Similarly, Pilar Almajano *et al*. demonstrated that antimicrobial activity of non-fermented tea is higher than semi-fermented and fermented tea, confirming our results. Oh *et al*. declared among 11 tea extracts, only green tea extract inhibits of tested pathogens. They suggested that different antimicrobial activity of green tea and black tea was due to composition changes in manufacturing process [23]. Antioxidant activity amount and polyphenol content may be substantial factors in antimicrobial property of tea extracts [8, 33]. So, the high antimicrobial activity of non-fermented extracts of green tea and white tea in this study can be attributed to high antioxidant and total phenolic amount. Also, the highest inhibitory and bactericidal concentrations of four tea types extract were found against gram-negative bacteria, *P. mirabilis,* and *S. marcescens*. In support of this study, Wu *et al*. showed that different tea extracts had antimicrobial activity against *S. aureus* and *B. subtilis*, but *E. coli* (one gram-negative bacteria) was resistance to the extracts [34]. In general, gram-negative bacteria are more resistant to polyphenols because of their different composition of the cell wall [35]. So, more resistance of *P. mirabilis* and *S. marcescens* against applied tea extracts in our study can be related to their different structure and composition of the cell wall. In conclusions, the present study showed that fermentation and processing amount might be effective factors on phenolic content and antioxidant activity of tea plants. Green tea and white tea (non-fermented teas) had the highest total phenolic and free radicals scavenging amount. The highest antimicrobial activity has been observed in samples with high phenolic content and antimicrobial effect. Green tea and white tea extracts can be useful sources that inhibit of pathogenic microorganisms. So, the use of these teas as safe plant-sources with other antimicrobial additives represents a practical strategy to reduce the food-related diseases.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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