



PRIMARY RESEARCH

The antimicrobial activity of green tea extract against the major food born bacteria

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Abstract. Food poisoning and infectious diseases caused by food borne bacteria are worldwide concern and can also be fatal. Green tea exhibits antibacterial effects against a wide range of bacteria. The objective of this study was to determine the antibacterial effect of different concentrations of green tea extract against some of the major food borne bacteria. Antibacterial activities of different concentrations of alcoholic and aqueous green tea extract were evaluated against *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas* spp. by the disk diffusion method and the Minimum Inhibitory Concentration (MIC) assay. The inhibition zones of aqueous extract (concentration 20 μ l) was 14, 15, 15, 17 mm for *Pseudomonas*, *E. coli*, *K. pneumoniae* and *S. aureus* respectively and inhibition zones of alcoholic extract (concentration 20, 28 μ l) was 16, 21, 15, 19 mm for *Pseudomonas*, *E. coli*, *Klebsiella* and *S. aureus* respectively. The most zone of inhibition was observed against *S. aureus* and *Pseudomonas* has a least inhibition zone. Ethanolic extract of green tea was more effective as compared to aqueous extract. Green tea extract showed good activity against food borne bacteria and Our results suggest that this plant has major antibacterial activity against these bacteria comparable with standard antibiotic. This characteristic of green tea may be related to its polyphenolic components.

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INTRODUCTION

Food-borne bacteria are the major cause of disease in many countries [1]. Food poisoning is a food industry concern and can also be fatal. Diseases have varied symptoms include watery or bloody diarrhea, meningitis, chronic renal disorders, immunologic and cardiovascular complications [2]. Considerable amount of food is spoiled due to the non availability of effective preservatives and due to increasing antibiotic resistance of some food borne pathogens [3].

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At 2005, World Health Organization has estimated 1.8 million people died of diarrheal diseases and infections related to consumption of contaminated food and water and in 2006 food borne illness outbreaks have been reported in our country [2-4]. *Escherichia coli* and *Pseudomonas* spp are the most common causes of diarrhea in developing countries and *Staphylococcus aureus* and *Kelebsiella pneumoniae* are another bacterial agents in the incidence of food poisoning and infections [2]. Despite the range of artificial preservation techniques available these days, The increasing demands for 'natural' foods have evoked the search for alternative antimicrobial elements to kill or at least to inhibit growth of bacteria to help control the wholesomeness of food [3-5]. Now day many antibacterial agents used against infectious diseases have been derived from medicinal plants. In different world area several plants are directly or indirectly used to treat various diseases [6]. Their extracts importance in recent years for varied pharmaceutical and food processing applications [7] for several years the green tea has been subjected to numerous medical and antimicrobial studies. The Green tea has been originated from China, but it has become associated with many cultures throughout Asia such as Iran. [8] Green tea contains a variety of enzymes, amino acids, carbohydrates, lipids, sterols and phytochemical compounds, such as alkaloids, saponins, tannins, catechin and polyphenols (Sofowara, 1984; Opara, 1992) and dietary minerals so It is rich in various components [10-12]. Moreover, green tea polyphenols have been applied to functional drug and foods for beneficial to human health for its antibacterial, antiviral, and antifungal activities [13]. In the present study we evaluated the antimicrobial effects of green tea extract as a new antibacterial material [11]. We hypothesized that green tea extract has selective growth-inhibitory activity against various strains of *Escherichia coli*, *Pseudomonas* spp, *Staphylococcus aureus* and *Kelebsiella pneumoniae* strains [9].

MATERIALS AND METHOD

Collection of Plant Samples

The plant Samples were collected from different places at Tehran main market in August –September 2014. The collected samples were put in plastic bags and immediately transferred to laboratory.

Bacterial Strains

This study involves some clinical strains of food borne bacteria as *Escherichia coli* ATCC 8739 *Pseudomonas* spp ATCC 15442, *Staphylococcus aureus* ATCC 9144 and *Kelebsiella pneumoniae* ATCC 1290. Pure cultures were refreshed and maintained on nutrient agar slants and kept in incubator overnight at 37°C and then stored at 4 °C. Aqueous and ethanolic green tea extracts Preparation The plant leaves were properly washed by distilled water and 100 g of each green tea material was grinded with grinder, filtered and then lyophilized under reduced pressure and kept in the dark. For this work, putting dried material in boiling water and was boiled for 45 min. Whatman no. 1 filter paper was used to filtering. The filtrate was heated at 40-50°C using water baths, until thick paste is formed. The obtained extract was then lyophilized. The lyophilized extract stored at 4°C in refrigerator. The

Same stages were used to ethanolic extract preparation by 95% ethanolic solvent instead of sterilized distilled water.

Agar-Well Diffusion Assay of Crude Extracts

The anti-bacterial assay was done on Mueller Hinton agar (Difco) plates by agar well diffusion method. The bacterial inoculum was prepared from overnight-grown cultures (24 h) in nutrient broth (Difco) with turbidity equivalent to 0.5 McFarland units (approximately 1.2×10^8 CFU ml⁻¹). Aliquots (100 µl) of inoculums were cultured on agar plates. Sterile discs (diameter of 6 mm), were placed at the center of 102 each plate and then the green tea extracts were added on the discs. The plates incubated for 24 h at 37°C, 103 and after time duration the zone of bacterial growth inhibition around the disc was measured. . If the organism was more or less sensitive to the extract, clear zone of the growth inhibition was appeared around the disc.

Determination of Minimum Inhibitory Concentration

The lowest concentration of green tea extract that completely inhibits the growth of the organism was defined as MIC. Bacterial cultures were enriched in nutrient broth for 10 hours at 35 °C. Overnight bacteria were seeded into the tubes containing nutrient broth and the green tea extracts were tested at concentration 15 and 20 µl /mL and then incubated for 24 h at 35 °C. Serial broth dilutions method in sterile tubes, was done according to Clinical and Laboratory Standards Institute [14].

RESULTS

Antibacterial Activity of Green Tea Extracts

The selected standard antibiotic inhibition zone diameter against considered strains is shown in Table 1.

TABLE 1. Antibiotic inhibition zone diameter against food borne bacteria

Zone diameter (mm)				Antibiotic
Staphylococcus aureus ATCC 9144	Klebsiella PTCC 1290	Pseudomonas aeruginosa ATCC 15442	E.coli ATCC 8739	
25	22	18	18	Gentamycin
29	R	R	R	Clindamycin
21	R	R	R	Penicillin
R	R	R	R	Vancomycin
24	R	R	20	Chloramphenicol

All of the strains were sensitive to gentamycin because of inhibitory nature of this antibiotic for aminoglycoside synthesis. Although, the inhibition zone

diameter of green tea extracts against selected bacterial at a concentration of 20 µl and 15 µl of both aqueous and alcoholic extract is shown in Table 2. Antibacterial activity of ethanolic extracts of green tea showed that these bacteria were sensitive to the concentrations of 96% ethanolic extracts as a control solution which were 12 mm, 13mm, 13mm, 14mm, 125 and 15 mm for E. coli, Pseudomonas, K.pneumoniae and S. areas respectively.

Calculation of MIC

Antibacterial activity of green tea leaf extract against selected is demonstrated in Table 3. Results showed that MIC of aqueous green tea extract for Pseudomonas and E.coli was 1:128 (512 mg/ml) and their MBC178 was equal to 1:64 (512 mg/ml). MIC and MBC results of alcoholic green tea extract for E.coli was 1:32 (512mg/ml) and MIC for S.aureus equal to 1:16 (512mg/ml). E.coli and pseudomonas were most sensitive strains to aqueous extract and K.pneumoniae was the most resistant strain in 512µg/ml concentration. Among prepared extracts, ethanolic extract was shown the most effective on inhibition of all bacteria strains than aqueous extract. Table 4 shown different dilution of standard antibiotic MIC and MBC against selected food born bacteria Antibacterial activity of green tea leaf extract against selected is demonstrated in Table 3. Results showed that MIC of aqueous green tea extract for Pseudomonas and E.coli was 1:128 (512 mg/ml) and their MBC178 was equal to 1:64 (512 mg/ml). MIC and MBC results of alcoholic green tea extract (512mg/ml) and MIC for S.aureus equal to 1:16 (512mg/ml). E.coli and pseudomonas were most sensitive strains to aqueous extract and K.pneumoniae was the most resistant strain in 512µg/ml concentration. Among prepared extracts, ethanolic extract was shown the most effective on inhibition of all bacteria strains than aqueous extract. Table 4 shown different dilution of standard antibiotic MIC.

TABLE 2. Antimicrobial activity of aqueous green tea extracts comparable with alcoholic green tea extract

Inhibition Zone Diameter				Extracts
S.aureus ATCC 9144	K.pneumonia e PTCC 1290	Pseudomonas ATCC 15442	E.coli ATCC 8739	
17	15	14	15	Aqueous Extract µl 20
19	15	16	21	Alcoholic extract µl 20
20	18	14	16	Aqueous extract µl 15
24	20	17	24	Alcoholic extract µl 15

TABLE 3. MIC and MBC of aqueous and alcoholic green tea extracts on selected bacterial strains

											Extract	Bacteria	
1													
+	+	+	-	-	-	-	-	-	-	-	MIC	Aqueous	E.coli
+	+	+	+	-	-	-	-	-	-	-	MBC	Extract	
+	+	+	+	+	-	-	-	-	-	-	MIC	Alcoholic	
+	+	+	+	+	-	-	-	-	-	-	MBC	Extract	
+	+	+	-	-	-	-	-	-	-	-	MIC	Aqueous	Pseudomonas
+	+	+	+	-	-	-	-	-	-	-	MBC	Extract	
+	+	+	+	-	-	-	-	-	-	-	MIC	Alcoholic	
+	+	+	+	+	-	-	-	-	-	-	MBC	Extract	
+	+	+	+	+	+	-	-	-	-	-	MIC	Aqueous	K.pneumonia
+	+	+	+	+	+	+	+	-	-	-	MBC	Extract	
+	+	+	+	+	+	-	-	-	-	-	MIC	Alcoholic	
+	+	+	+	+	+	+	+	+	-	-	MBC	Extract	
+	+	+	+	+	+	-	-	-	-	-	MIC	Aqueous	S.aureus
+	+	+	+	+	+	+	+	-	-	-	MBC	Extract	
+	+	+	+	+	+	+	-	-	-	-	MIC	Alcoholic	
+	+	+	+	+	+	+	-	-	-	-	MBC	Extract	

TABLE 4. MIC and MBC of aqueous and alcoholic green tea extracts on selected bacterial strains

0/5	1	½	1/	1/	1/1	1/32	1/6	1/1				
			4	8	6		4	28				
-	-	-	-	-	-	-	-	-	MIC	GM	E.coli	
-	-	-	-	-	-	-	-	-	MBC			
-	-	-	-	-	-	-	-	-	MIC	GM	Pseudomonas	
-	-	-	-	-	-	-	-	-	MBC			
-	-	-	-	-	-	-	-	-	MIC	GM	K.pneumonia	
-	-	-	-	-	-	-	-	-	MBC			
-	-	-	-	-	-	-	-	-	MIC	GM	S.aureus	
-	-	-	-	-	-	-	-	-	MBC			
+	+	+	+	+	-	-	-	-	MIC	Clin	E.coli	
+	+	+	+	+	+	-	-	-	MBC			
+	+	+	+	+	+	+	-	-	MIC	Clin	Pseudomonas	
+	+	+	+	+	+	+	+	-	MBC			
+	+	+	+	-	-	-	-	-	MIC	Clin	K.pneumonia	
+	+	+	+	+	+	-	-	-	MBC			
-	-	-	-	-	-	-	-	-	MIC	Clin	S.aureus	
-	-	-	-	-	-	-	-	-	MBC			

Sign (-) indicate inhibitory growth. All of strains sensitive to gentamycin and clindamycin in 1/28 concentration. K.pneumoniae and Pseudomonas were most resistance and most sensitive strains to clindamycin respectively.

DISCUSSION AND CONCLUSION

Multiple drug resistant bacteria a major cause of failure of the public health. Evaluation of plants for antimicrobial property is the first step towards achieving the goal for developing eco-friendly management of infectious diseases of humans by the search for new bio-molecules of plant origin. Plants extract as a drug are effective, available, and cheap without side effects. Green tea was screened for antibacterial susceptibility against five human pathogenic food borne bacteria. A zone of inhibition and the antibacterial assays were evaluated medical effects of the extract against 218 these bacteria and results showed that green tea has major antibacterial activity. The results of this study indicate that green tea extract as a natural antimicrobial agent, has the potential power for preservation of the food from major foodborne bacteria and can be used for preserving various foodstuffs and antifungal or antibacterial therapy in medical. Both the aqueous and ethanolic extracts were effective against bacteria, but there is a considerable difference in the antimicrobial activity of the extract of green tea when the ethanol is the solvent and the extract when water is the solvent. Ethanolic extract of green tea was more effective as compared to aqueous extract. The 100% activity of green tea extract against bacteria was more than imipenem effective. We can note that the anti-bacterial activity of green tea extract is comparable to standard antibiotic. Our results indicate that the activity of aqueous and alcoholic extracts was comparable with gentamicin. This can be because of green tea phytochemicals compounds. Moreover the properties of green tea, which inhibit bacterial Growth maybe related to their polyphenolic Components such as epicatechin, epicatechingallate, epigallocatechin, and epigallocatechingallate. All test organisms were inhibited by extract. The maximum zone of inhibition was observed against *S. aureus* (A Gram ositive organism) and the minimum was against *pseudomonas* (a Gram-negative organism). So that the Gram positive strain was more sensitive to green tea extract. We can see the variation in the size of the inhibition zone between different groups of bacteria. MICs varied widely (512 μ g /ml) between different species. All strains were resistant to vancomycin while sensitive to green tea extract and inhibition zone showed approximately equal to that of gentamycin. Find new sources of natural compounds with antibacterial activity as our study result, useful for Control of infections acquired in hospitals and Communities caused by multi-drug resistant food borne bacteria in developing countries

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— This article does not have any appendix. —