Antibacterial Effect of Iranian Green Tea and Black Tea against *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis* and *Prevotella intermedia*

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ABSTRACT

Background and Objective: Anaerobic bacteria are the main cause of periodontitis. It has been shown that green tea and black tea have antibacterial effect. The aim of this study was to determine he antibacterial effect of Iranian green tea and black tea against *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*.

Methods: Aqueous and methanolic extracts of Iranian green tea and black tea at concentrations ranging from 10 to 500 mg/ml were tested against standard strains of *A. actinomycetemcomitans* (ATCC 33384), *P. gingivalis* (ATCC 33227) and *P. intermedia* (ATCC 25671) using agar disk diffusion, broth microdilution and determination of minimum inhibitory concentration.

Results: *P. gingivalis*, *A. actinomycetemcomitans* and *P. intermedia* were sensitive to the methanolic extract of Iranian green tea at concentrations of 100-500 mg/ml, 10-500 mg/ml and 50-500mg/ml, respectively. *P. gingivalis*, *A. actinomycetemcomitans* and *P. intermedia* were sensitive to the methanolic extract of Iranian black tea at concentrations of 200-500 mg/ml, 20-500 mg/ml and 200-500 mg/ml, respectively. In addition, *P. gingivalis*, *A. actinomycetemcomitans* and *P. intermedia* were sensitive to the aquatic extract of Iranian green tea at concentrations of 200-500mg/ml, 100-500 mg/ml and 200-500 mg/ml, respectively.

Conclusion: The aquatic and alcoholic extracts of Iranian green tea and black tea have antibacterial activity against *A. actinomycetemcomitans*, *P. intermedia* and *P. gingivalis*. Therefore, incorporation of Iranian black tea as an effective native herb could be beneficial for prevention of oral cavity diseases.

Keywords: Tea, Green Tea, Antibacterial Agents, Anaerobic Bacteria.

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INTRODUCTION

Periodontitis is an inflammatory disease that could lead to destruction of supporting structures of teeth. Anaerobic bacteria are the main cause of initiation and progression of periodontitis (1-3). *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis* and *Prevotella intermedia* are among the main anaerobic pathogens involved in periodontitis development (4). Prevention and treatment of periodontitis has been a challenging issue in modern dentistry. While mouthwashes are useful tools for controlling the oral microflora in periodontitis, they have some disadvantages including tooth discoloration and unpleasant taste. Natural plants with therapeutic properties could be considered as alternatives for such products. These plants are easily available and cost effective.

Tea (*Camellia sinensis)* is the most popular drink in Iran. In general, black tea is fully fermented, oolong is partially fermented, and green tea is not fermented or only minimally fermented (5-6).

It has been shown that all types of tea have antibacterial, antifungal and antioxidant properties (7-9). Tea-derived catechins and epigallocatechin gallate (EGCG) can inhibit the activity of periodontal pathogens. In addition, tea polyphenols have a positive effect on the inflammatory response in periodontal structures (10-12). It has been shown that growth conditions including soil composition, temperature and cultivating methods can affect the chemical properties of tea (13). Some studies have previously demonstrated the antibacterial effect of aquatic and alcoholic extracts of Iranian green tea on periodontopathic bacteria (14-15). However, there is limited information about the antibacterial effect of Iranian black tea. Black tea is a popular beverage in Iran, especially among the older population. The aim of this study was to determine the antibacterial effect of Iranian green tea and black tea on a number of periodontitis-causing anaerobic bacteria.

MATERIAL AND METHODS

First, tea leaves were collected from Baz-Kia-Gurab region in Gillan Province, Iran. The samples were verified by the Department of Pharmacognosy (School of Pharmacy) at Shahid Beheshti University of Medical Sciences, Iran.

For alcoholic extraction of green tea and black tea, 500 ml of 70% methanol (Merck Co. Germany) were added to a sterile flask containing 100 g of chopped, powdered green tea and black tea samples, separately. The mixture was place at room temperature for two days. After filtering the mixture through No.1 filter papers (Whatman Co. Germany), the extract was dried in water bath (GFL Co., Germany) at 70 ^{o}C. The dried powder obtained was kept at 4 °C in sealed vials for future analysis. The following concentrations of the extracts were prepared from the stock solution: 1% (10mg/ml), 2% (20mg/ml), 5% (50mg/ml), 10% (100mg/ml), 20% (200mg/ml), 30% (300mg/ml), 40% (400mg/ml) and 50% (500mg/ml) (16).

Aqueous extraction of green tea and black tea was done separately. First, 1L of sterilized boiling water was added to 100 g of tea. After 4 hours, the mixture was filtered by No.1 filter paper (Whatman Co., Germany), and then dried in water bath (GFL Co., Germany) at 70 ^{o}C. A range of different concentrations was prepared from the stock solution similar to the previous step.

Standard strains of *A. actinomycetemcomitans (ATCC 33384), P. gingivalis (ATCC 33227)* and *P. intermedia (ATCC 25671)* were obtained from bacterial collection of School of Medicine, Shahed University, Iran. The bacteria were inoculated into solid and aqueous media containing 41 g/L Brucella agar, 52g/L BHA, 44 g/L anaerobic blood agar, 30 g/L thioglycollate fluid, 29 g/L thioglycollate broth and 30 g/L trypticase soy broth (Merck Co. Germany). The samples were inoculated into 5 mg/ml hemin-containing yeast extract (Sigma Co. Germany), and kept at 37°C in anaerobic conditions by Gas-pak (Merck Co. Germany). Then, 10 µg of vitamin K and 100 ml defibrinated sheep blood (Bahar afshan Co. Iran) were added to the anaerobic blood agar (Merck Co. Germany).

All media were kept under anaerobic condition at 4 °C for future testing. The antimicrobial activity of the alcoholic and aqueous extracts of green tea and black tea was evaluated using disk diffusion, well diffusion and minimum inhibitory concentrations (MICs). The antibacterial effect of green tea and black tea was evaluated against standard strains of *A. actinomycetemcomitans* (ATCC 33384), *P.*

gingivalis (ATCC 33227) and *P. intermedia* (ATCC 25671). Sterile blank paper discs were soaked in the extracts. Then, 20 µl of 0.5 McFarland bacterial suspension was spread on the surface of sterile Muller Hinton agar plates (Merck Co. Germany). Paper disks (Padtan Teb Co. Iran) containing different concentrations of the extracts were placed on the surface of each plate. The plates were incubated at 37 ^{o}C for 72 hours in anaerobic condition. The antibacterial activity of the extracts against the bacteria was assessed by measuring the growth inhibition zone around the disks. MIC of crude extracts was determined by broth dilution method. Briefly, 1ml of sterile trypticase soy broth (Merck Co. Germany) was transferred to a sterile test tube. Then, 10 µl of 0.5 McFarland bacterial suspension was inoculated into a test tube containing 1ml of sterile trypticase soy broth (Merck Co. Germany). Different concentrations of the extracts were added to each test tube. The content of the tubes was mixed thoroughly by gentle shaking. The test tubes were incubated at 37 ^{o}C for 3-7 days in anaerobic condition. A tube without bacteria was also prepared as negative control. Bacterial cultures were swabbed on Mueller Hinton agar plates (Merck Co. Germany) by disk diffusion method. Moreover, the antibacterial activity of the extracts was compared with that of vancomycin, clindamycin, ciprofloxacin, ampicillin, tetracycline, amoxicillin, gentamycin, kanamycin and penicillin paper disks (Mast Co., UK).

RESULTS

As shown in Table 1, *P. gingivalis, A. actinomycetemcomitans* and *P. intermedia* were sensitive to the methanolic extract of green tea at concentrations of 100-500 mg/ml, 10-500 mg/ml and 50- 500 mg/ml, respectively. The MIC of methanolic extract of green tea for *P. gingivalis, A. actinomycetemcomitans* and *P. intermedia* was 50 mg/ml, 20 mg/ml and 10 mg/ml, respectively (Table 2). *P. gingivalis, A. actinomycetemcomitans* and *P. intermedia* were sensitive to the aquatic extract of Iranian green tea at concentration of 200-500 mg/ml, 100-500 mg/ml and 200-500 mg/ml, respectively (Table 1). The MIC of the aquatic extract of green tea for *P. gingivalis, A. actinomycetemcomitans* and *P. intermedia* was 50 mg/ml, 50 mg/ml and 20 mg/ml, respectively (Table 2).

P. gingivalis, A. actinomycetemcomitans and *P. intermedia* were sensitive to the methanolic extract of Iranian black tea at concentrations of 200-500 mg/ml, 20-500 mg/ml and 50-500 mg/ml, respectively (Table 1). The MIC of the methanolic extract of black tea for *P. gingivalis, A. actinomycetemcomitans* and *P. intermedia* was 100 mg/ml, 50 mg/ml and 20 mg/ml, respectively (Table 2). As shown in Table 1, *P. gingivalis, A. actinomycetemcomitans* and *P. intermedia* were sensitive to the aquatic extract of black tea at concentrations of 200-500 mg/ml, 100-500 mg/ml and 200-500 mg/ml, respectively. The MIC of the aquatic extract of black tea for *P. gingivalis, A. actinomycetemcomitans* and *P. intermedia* was 300 mg/ml, 20 mg/ml and 100 mg/ml, respectively (Table 2).

In addition, the methanolic and aquatic extracts of green tea had more antibacterial effect at lower concentrations compared with black tea (Table 2). Moreover, comparison of the antibiotic activity of green tea and black tea with antibiotics is shown in Table 3.

Table 1- Antimicrobial activity of methanolic and aquatic extracts of Iranian green tea and black tea by well diffusion method

		10 mg/ml	20 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	300 mg/ml	400 mg/ml	500 mg/ml
Methanolic extract of *g*reen tea	**Pg**	**-**	**-**	**-**	**7mm**	**9mm**	**12mm**	**14mm**	**15mm**
	Aa	**9mm**	**10mm**	**12mm**	**13mm**	**13mm**	**14mm**	**14mm**	**15mm**
	Pi	**-**	**-**	**8mm**	**10mm**	**10mm**	**12mm**	**13mm**	**14mm**
Aqueous extract of green tea	**Pg**	**-**	**-**	**-**	**-**	**8mm**	**8mm**	**10mm**	**12mm**
	Aa	**-**	**-**	**-**	**8mm**	**9mm**	**10mm**	**11mm**	**14mm**
	Pi	**-**	**-**	**-**	**-**	**8mm**	**8mm**	**12mm**	**12mm**
Methanolic extract of black tea	**Pg**	**-**	**-**	**-**	**-**	**8mm**	**10mm**	**12mm**	**15mm**
	Aa	**-**	**10mm**	**12mm**	**12mm**	**11mm**	**12mm**	**12mm**	**16mm**
	Pi	**-**	**-**	**-**	**-**	**10mm**	**11mm**	**12mm**	**14mm**
Aqueous extract of black tea	**Pg**	**-**	**-**	**-**	**-**	**7mm**	**7mm**	**8mm**	**10mm**
	Aa	**-**	**-**	**-**	**7mm**	**7mm**	**8mm**	**9mm**	**10mm**
	Pi	-	-	-	-	8mm	9mm	10mm	12mm

Pg: *P. gingivalis*
Aa: *A. actinomycetemcomitan*
Pi: *P. intermedia*

Table 2- MIC of methanolic and aqueous extracts of Iranian green tea and black tea against the bacteria tested

		10 mg/ml	20 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	300 mg/ml	400 mg/ml	500 mg/ml
Methanolic extract of green tea	**Pg**	+	+	-	-	-	-	-	-
	Aa	-	-	-	-	-	-	-	-
	Pi	-	-	-	-	-	-	-	-
Aqueous extract of green tea	**Pg**	+	+	-	-	-	-	-	-
	Aa	+	+	-	-	-	-	-	-
	Pi	+	-	-	-	-	-	-	-
Methanolic extract of black tea	**Pg**	+	+	+	-	-	-	-	-
	Aa	+	+	-	-	-	-	-	-
	Pi	+	+	-	-	-	-	-	-
Aqueous extract of black tea	**Pg**	+	+	+	+	+	-	-	-
	Aa	+	-	-	-	-	-	-	-
	Pi	+	+	+	-	-	-	-	-

Pg: *P. gingivalis*
Aa: *A. actinomycetemcomitan*
Pi: *P. intermedia*

Table 3- Comparison of antimicrobial activity of antibiotic discs and the extracts against the bacteria tested by disk diffusion method

	AMX 25mg	AM 10mg	V30 mg	TE 30mg	CP 5mg	K30 mg	GM 10mg	CC 2mg	P 10mg
P. gingivalis	20mm	16mm	16mm	14mm	17mm	-	-	-	-
A. actinomycetem comitans	28mm	16mm	21mm	18mm	30mm	10mm	10mm	-	-
P. intermedia	18mm	15mm	15mm	14mm	23mm	10mm	20mm	12mm	-

AMX: Amoxicillin, AM: Ampicillin, V: Vancomycin, TE : Tetracycline, CP: Ciprofloxacin, K: Kanamycin, GM: Gentamycin, CC: Clindamycin, P: Penicillin

DISCUSSION

In present study, we examined the antibacterial activity of the methanolic and aquatic extracts of Iranian green tea and black tea against a number of anaerobic periodontal pathogens. The results showed that *P. gingivalis, A. actinomycetemcomitans* and *P. intermedia are* sensitive to the extracts, with more sensitivity observed against the extracts of Iranian green tea. These findings are in agreement with previous reports on the aquatic extract of green tea (14-17). Highest antibacterial activity by both extracts of Iranian green tea and black tea was observed at concentration of 200 mg/ml, which is in agreement with results of Sakanaka et al. (18). The MIC of aquatic extracts of Iranian green tea and black tea was 50 mg/ml and 100 mg/ml, respectively. However, Araghizadeh et al. found that *P. gingivalis, A. actinomycetemcomitans* and *P. intermedia* are most sensitive to the aquatic extract of green tea at concentration of 12.5-50 mg/ml (14). The MIC of aquatic extract of green tea for *P. gingivalis, A. actinomycetemcomitans* and *P. intermedia* was 50 mg/ml, 50 mg/ml and 20 mg/ml, respectively. These findings are inconsistent with findings of Araghizadeh et al. (14). This could be due to the differences in the methods used. For instance, we have used bacterial stocks, while Araghizadeh et al. examined the aquatic extract of green tea on oral-derived bacteria.

According to previous studies, consumption of green tea reduces the periodontal destruction and significantly improves periodontal indices (15 -19). The antibacterial activity of tea is mainly attributed to its chemical components such as polyphenolic compounds and catechin (9, 20-22). It has been shown that the bactericidal activity of green tea could also be due to the impact of EGCG on bacterial membrane (23).

P. gingivalis is a key microorganism involved in initiation of periodontitis. The bacterium produces proteolytic enzymes such as collagenases and dipeptidyl aminopeptidase IV. Tea-derived polyphenols inhibit proteases production in *P. gingivalis* (24). Sakanaka et al. demonstrated the effect of green tea-derived polyphenols, especially EGCG on the growth and adherence of *P. gingivalis* onto the oral buccal cells (18). In addition, green tea-derived catechin has bactericidal effects on *Prevotella spp*. with MIC of 1.0 mg/ml (25).

Okamoto et al. suggested that the inhibitory effect of green tea on the activity of *P. intermedia* might be due to the impact of EGCG on tyrosine phosphatase (26). The catechin content of green tea is more than that of black tea and oolong tea. This difference

originates from oxidation of tea leaves during fermentation. The fermentation process leads to conversion of flavanoids into aflevine and arubigin (6).

Various methods and therapeutic agents have been used for eliminating periodontopathic bacteria in mouth. However, they all have some disadvantages such as tooth discoloration. Herbal-based mouthwashes could have some beneficial effects for eradication of the periodontopathic microflora. Such products are safer and more cost-effective compared with currently available chemical counterparts. Based on the results of our study and its antibacterial properties, tea could be incorporated into mouthwashes for prevention or even treatment of oral diseases. However, in vivo studies should be performed on the effect of different concentrations of green tea and black tea extracts on *P. gingivalis, A. actinomycetemcomitans* and *P. intermedia* to verify the results of the present study.

CONCLUSION

The methanolic and aquatic extracts of Iranian green tea and black tea have antibacterial effect on *P. gingivalis, A. actinomycetemcomitans* and *P. intermedia* in a dose-dependent manner. Iranian black tea can be used as an effective herbal mouthwash to prevent oral cavity diseases.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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