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Antibacterial Effects of Water Soluble Green Tea Extracts on Multi-Antibiotic Resistant Isolates of *Pseudomonas aeruginosa*

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Abstract: In this research we evaluated the antibacterial activity of water soluble green tea extracts on 43 hospital isolates of *Pseudomonas aeruginosa*. A total of 43 strains of *Pseudomonas aeruginosa* were collected from clinical specimens at two hospitals in Tehran, Iran. The susceptibilities of isolates to different antibiotics were tested using agar disk diffusion method. Antibacterial activity of water soluble green tea extract was measured by Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs). 35.6% of isolated strains showed resistance to 5 antibiotics or more and 55.8% of all strains were Multi-Drug Resistant (MDR) strains. The average MICs and MBCs of the extract against all strains of *Pseudomonas aeruginosa* were 2.06 ± 1.76 and 2.54 ± 2.22 mg mL⁻¹, respectively. Our study suggests that green tea has significant activity with bactericidal action on multi-drug resistant strains of *Pseudomonas aeruginosa*.

Key words: Green tea, MBC, MIC, *Pseudomonas aeruginosa*

INTRODUCTION

Tea from the leaves of plant *Camellia Sinensis*, next to water, is the most widely consumed beverage in the world. Depending upon the level of fermentation, tea can be categorized into three types: green (unfermented), oolong (partially fermented) and black (highly to fully fermented). The potent biological activities of green tea polyphenols in, for example cancer and cardiovascular disease prevention have in particular been attracting scientists in medical and pharmaceutical fields (Siddiqui *et al.*, 2006; Tiwari *et al.*, 2005).

Various studies have shown significant suppressive effects of green tea polyphenols against many microorganisms, for example *Salmonella typhimurium* (Shetty *et al.*, 1994), *Salmonella typhi*, *Shigella dysenteriae*, *Yersinia enterocolitica*, *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae*, *Campylobacter jejuni*, *Plesiomonas shigelloides* and many other species of bacteria (Toda *et al.*, 1989; Taguri *et al.*, 2004; Yam *et al.*, 1997; Kim *et al.*, 2004; Stapleton *et al.*, 2004; Yam *et al.*, 1998).

Pseudomonas aeruginosa is one of the leading gram-negative organisms associated with nosocomial infections and can cause different kinds of infections in human, especially in immunocompromised hosts. Many strains of *P. aeruginosa* show high rate of resistance to several

antibiotics and other kinds of antimicrobial agents (Brooks *et al.*, 2004; Obritsch *et al.*, 2005), consequently introducing of the new antimicrobial agents against this bacterium is one of the most important goals in treatment of such infections. However limited studies investigated the antibacterial effects of green tea extracts on this microorganism and some of them showed that green tea have significant activity with bactericidal action against *P. aeruginosa* (Lee *et al.*, 2003), but alternatively some reports showed that tea samples had no effect on the growth of *P. aeruginosa* strains (Toda *et al.*, 1989), but all these studies have investigated only a few isolates of *P. aeruginosa*.

In this study we evaluated the antibacterial activity of water soluble green tea extracts on 43 antibiotic resistant strains of *P. aeruginosa*.

MATERIALS AND METHODS

Bacterial strains and culture media: A total of 43 strains were collected from clinical specimens of two hospitals in Tehran, Iran. The isolates were further processed by the standard methods to identify as the strains of *P. aeruginosa*. Strains were maintained for long storage on skimmed milk medium (BBL) by adding 10% glycerol in -70°C, cultures were maintained for daily use on nutrient agar (BBL) slants on 4°C. The Muller Hinton Agar (MHA)

and Muller Hinton Broth (MHB) medium (Pronadisa) were used for antibiogram of strains and measurement of the MIC and MBC of tea leaves extracts against each strain, respectively.

Preparation of tea leaves: Commercial green tea was used for doing experiments. Tea samples were stored in plastic bags at 4°C. Crude tea extracts were prepared by the method described by Tiwari *et al.* (2005).

Determination of antimicrobial activity of green tea extracts: The frozen bacterial strains were thawed and inoculated on nutrient agar medium and then cultured overnight at 36±0.5°C. The bacteria were suspended in 10 mL of sterile buffer saline and used as inoculate within 1 h after adjustment.

Sixty eight milligrams of the resulting green tea powder were dissolved in 2 mL of MHB. The solution was diluted serially in 8 stages.

Bacterial inoculate were added to serial dilutions of green tea, with final bacterial concentrations of (1.5×10⁶ cell mL⁻¹). Antibacterial activity was measured by determining MIC and MBC by investigation of the tubes turbidity and culturing on MHA medium in a sterile Petri dish respectively. After overnight incubation at 36±1°C, the tubes were examined visually for growth (turbidity) and no growth (no turbidity). The highest dilution inhibiting the growth was taken as MIC. Five microliter of the highest dilution streaked on MHA plates which did not show any bacterial growth after overnight incubation was taken as MBC (Tiwari *et al.*, 2005; Sahm and Weissfeld, 2002).

Determination of the strains sensitivity to antibiotics: The susceptibilities of isolates to different antibiotics were tested using agar disk diffusion method (Bauer *et al.*, 1966). To represent the different classes of antimicrobial agents commonly used for the treatment of *P. aeruginosa*, we used amikacin, gentamicin, tobramycin, ceftazidime, ceftriaxone, ceftizoxime, cefoperazone, carbenicillin, piperacillin, ticarcillin, ciprofloxacin, lomefloxacin and imipenem (Hi-media, Mombay, India). *P. aeruginosa* ATCC 27853 was used as control.

RESULTS

A total of 43 *P. aeruginosa* isolates were collected from specimens submitted to the clinical microbiology laboratories of selected hospitals in Tehran. Strains were isolated from hospital environment (6 strains), wound swab (2 strains), urine (16 strains), sputum (12 strains), stool (3 strains), throat swab (3 strains) and pleural fluid (1 strain).

Table 1: The average of minimum inhibitory concentrations for isolated strains of *P. aeruginosa*

No. of strains	MICs for each strain (mg mL ⁻¹)
7	0.53
12	1.06
18	2.12
4	4.25
2	8.5
Total = 43	Average of MICs±SD = was 2.06±1.76 mg mL ⁻¹

Table 2: The average of minimum bactericidal concentrations for isolated strains of *P. aeruginosa*

No. of strains	MBC for each strain (mg mL ⁻¹)
6	0.53
10	1.06
17	2.12
6	4.25
4	8.5
Total = 43	Average of MBCs±SD = 2.54±2.22 mg mL ⁻¹

The type of infection and patient characteristics were not important to the study design and are not presented here.

Sensitivity of Bacterial Species to selected Antibiotics:

The rates of resistance to different antibiotics for 43 studied isolates were as follows: ceftizoxime (97.6%), lomefloxacin (93%), ceftazidime (58.1%), ticarcillin (51.1%), ceftriaxone (44.1%), cefoperazone (37.2%), tobramycin (34.8%), piperacillin and gentamicin (33.4%), carbenicillin (25.6%), amikacin (23.2%), ciprofloxacin (16.3%), imipenem (3.4%). 34.9% of isolated strains showed resistance to 5 antibiotics or more and 55.8% of all strains were Multi-Drug Resistant (MDR) strains.

Sensitivity of bacterial species to green tea water soluble extracts:

The average MICs and MBCs of the water soluble green tea extract against all strains of *P. aeruginosa* was 2.06±1.76 (Table 1) and 2.54±2.22 mg mL⁻¹ (Table 2), respectively.

DISCUSSION

With the emergence of antibiotic resistant bacteria, it is reasonable to explore new sources of natural compounds with antibacterial activity. Edible plants have been proven to be harmless and are economical (Lee *et al.*, 2003).

Green tea has been studied extensively by Japanese investigators. In addition to its anticancer and anti-hypercholesterolic activities, it has anti-bacterial activity that includes inhibition of gram positive cocci, gram negative bacilli and resistant strains such as vancomycin-resistant enterococci and MSRA (Hamilton-Miller, 1995).

Hospital isolated *P. aeruginosa* usually has multi-drug resistance to several antibiotics and other type of antimicrobial agents; however our results showed that water soluble green tea extract has antibacterial effects against antibiotic resistant strains of *P. aeruginosa* (Table 1 and 2).

Although limited studies showed that green tea extracts had no effect on the growth of *P. aeruginosa* strains (Toda *et al.*, 1989), but taken together, our study and the data from Lee *et al.* (2003) suggest that green tea has significant activity with bactericidal action on antibiotic resistant strains of *P. aeruginosa* (Lee *et al.*, 2003). However they investigated the antibacterial effects of green tea on only one strain of *P. aeruginosa*, so our study is the first study that examined the antibacterial effects of green tea on several hospital isolates of MDR *P. aeruginosa*. In conclusion Green tea seems to be a safe agent that has the potential for exploration of broader applications as an anti-*pseudomonas* agent against antibiotic resistant strains of this bacterium.

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