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Review article

Effects of green tea on *Escherichia coli* as a uropathogen

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ABSTRACT

Escherichia coli is the most common cause of urinary tract infections. The development of antibiotic resistance in *E. coli* is an important problem. Finding alternative antimicrobial agents from plant extracts has received growing interest. *Camellia sinensis* is a safe, nontoxic, cheap beverage that has been reported to have antimicrobial effects against various pathogenic bacteria including *E. coli.* Polyphenolic components of green tea (綠茶 lǜ chá) have antibacterial activity. Catechins also have synergistic effect with antibiotics such as chloramphenicol, amoxicillin, sulfamethoxazole, azithromycin, levofloxacin, gentamycin, methicillin, naldixic acid, and, especially ciprofloxacin. In this review, all experimental studies that evaluated the effect of green tea on *E. coli* were collected. Data from *in vitro* studies on the antimicrobial effects of green tea are promising, but human data are currently lacking. *In vivo* studies on antibacterial effects of green tea and evaluating the efficacy of its catechins in the treatment of urinary tract infection are needed.

1. Introduction

Urinary tract infections (UTIs) are the most common type of nosocomial infection in females and males, and have resulted in billions of dollars in medical care costs.[1,2] The most important cause of 80–90% of all UTIs is *Escherichia coli.*[3] Nonpathogenic strains of *E. coli* are important facultative aerobes in the normal intestinal flora of human and animals. However, pathogenic strains of these bacteria are the most common cause of urinary tract infections.[4] Uropathogenic *E. coli* infects the urinary tract by producing special surface proteins (adhesins), which make them to attach to and attack the epithelial cells that line the urinary bladder.[5] If pathogenic *E. coli* is in the bladder (uncomplicated UTI), and is not eliminated, it may travel up the ureters to the kidneys and cause complicated UTIs which can be accompanied by renal damage and renal failure.[3,4,6] The development of antibiotic resistance in bacteria is a growing problem worldwide. A number of *E. coli* isolates have been collected from urine specimens of patients with UTI that are resistant to antimicrobial agents commonly used to treat UTIs (β-lactams, trimethoprim–sulfamethoxazole, fluroquinolones, nitroforantoin, etc.).[1,7,8] Therefore, treatment options are replaced with a second or third choice of antibiotics, which are much more expensive.[9] These challenges have been receiving growing interest to find alternative antimicrobial agents from plant extracts that need to be developed and used to control multidrug-resistant bacteria.[3,10,11] *Camellia sinensis* is one of the most popular beverages in the world, and has been reported to have antimicrobial effects against various pathogenic bacteria.[6,10,12–24] Tea can be cultivated in many regions from sea level to high mountains. It is generally safe, nontoxic, cheap, and available and is a popular drink, traditionally in Asian countries.[3,4] These properties make it a very good alternative antimicrobial agent. For green tea (綠茶 lǜ chá) production, freshly harvested tea leaves of *C. sinensis* must be processed with the least amount of oxidation, while oolong and black tea are made from fermented leaves of the same plant. Studies on the antibacterial activity have shown that green tea inhibits the growth of *E. coli* by its polyphenolic components (also known as catechins). The most important catechins in green tea are (−)-epicatechin (EC), (−)-epigallocatechin-3-gallate (EGCg), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG). EGC and EGCg have been shown to have the greatest antimicrobial effects, but only EGC has been shown to be excreted in urine.[25] EGC and EGCg have the highest amounts in green tea and are excreted in bile.[3,4,6,14,26,27]

There are different mechanisms for antimicrobial effects of green tea such as:

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1. Polyphenols are anti-inflammatory agents that inhibit clinical symptoms of UTIs.[7,26]
2. Catechins induce production of cytokines such as IL-12 and IL-10.[7]
3. Green tea polyphenols decrease tumor necrosis factor-α gene expression, which is important in pathogenesis of *E. coli* infection.[7]
4. Catechins, by blocking the connection of conjugated R plasmid in *E. coli*, have bactericidal and antitoxin effects.[7]
5. Catechin-copper (II) complexes damage the cytoplasmic membrane of *E. coli*.[28–30]
6. EGC can bind to the ATP site of the DNA gyrase β subunit of bacteria and inhibit the activity of the gyrase enzyme.[7,28]
7. The bactericidal action of catechin is due to its hydrogen peroxide generation.[29]
8. The highest antimicrobial activity of tea is due to presence of catechins and polyphenols which damage the bacterial cell membrane.[30]
9. Catechins interfere with the expression of β-lactamases in staphylococci and inhibit the extracellular release of verotoxin from enterohemorrhagic *E. coli* (EHEC) 0157.[27,31]

Several research studies have focused on the effects of green tea on microorganisms. In the present review, the antimicrobial effect of green tea on *E. coli* (the major pathogen of UTI) is discussed in experimental studies.

2. Method

A literature review was conducted using PubMed, Scopus, Medline, Cochrane central register of controlled trials, Cochrane database systematic reviews and Google scholar. Search Keywords used were 'green tea', 'catechin', '*E. coli*', 'UTI', 'EGC', 'synergistic', 'antimicrobial', and 'mechanism'. No time limit was considered when organizing this review. All English language studies that evaluated the effect of green tea on *E. coli* as a main surrogate endpoint were included.

3. Results

3.1. Experimental studies on the antimicrobial effects of green tea against E. coli

Antimicrobial effects of green tea on *E. coli* have been suggested in different experimental studies.[3,12,32,33] In this part, all the experimental studies that were found are reviewed. A summary of these studies is shown in Table 1.

Ikigai et al[34] reported the results of their research on EGCg and EC, two of the strongly antimicrobial catechins found in green tea. They used *E. coli* K-12 strain G6 and *Staphylococcus aureus* ATCC25932 as Gram-negative and Gram-positive bacteria, respectively. EC and EGCg were extracted from water-soluble extract of green tea. The minimal growth inhibitory concentration (MIC) was determined by the agar dilution method. The MIC of EGCg for *E. coli* and *S. aureus* were 573 μg/mL and 73 μg/mL respectively. The MIC of EC for *E. coli* and *S. aureus* were >1145 μg/mL and 183 μg/mL, respectively. Catechins had greater activity against Gram-positive than Gram-negative bacteria. Liposomes were used as a model of bacterial membranes. EC showed little absorption through liposome membranes at 0.6 mM. They used EGCg to examine the effects of catechin on bacterial membranes. EGCg inhibited cytoplasmic membrane function by inducing leakage of small molecules from the intraliposomal space. Therefore, catechins damaged bacterial membranes and impaired membrane function.[34]

Hoshino et al[28] studied the effect of catechins (EGC and EC)-copper (II) complexes on the cytoplasmic membrane of *E. coli*. *E. coli* were incubated with EGC in the presence of Cu^{2+} at 37°C and, after 60 minutes, the supernatant was separated by centrifuging and the copper concentration of the supernatant (using atomic absorption with a Shimadzu spectrophotometer, Kyoto, Japan AA-660) and also the amount of copper ions binding to *E. coli* cells were determined. They concluded that EGC and EC (100 μM each) and Cu^{2+} (1 μM) separately have no effect on the viability of *E. coli*, while the combination of Cu^{2+} (1 μM) with EGC (1 μM, 10 μM, and 100 μM) or EC (100 μM) killed *E. coli* cells. To determine ATP levels in *E. coli* cells, they incubated *E. coli* with EGC or EC in the presence of Cu^{2+} for 60 minutes at 37°C and ATP (using an ATP bioluminescence assay kit based on the method of Stanley) and cellular and unbinding potassium levels (using atomic absorption) were measured. To analyze DNA of *E. coli* cells, first they isolated DNA from *E. coli* cells and then incubated it with EGC or EC in the presence of Cu^{2+} for 60 minutes at 37°C. They found that the DNA double strands did not break in the killing process, while depletion in both the ATP and potassium pools of the had an important role in killing of *E. coli*. Therefore, bactericidal activity of catechins in the presence of Cu^{2+} is derived from damage to the cytoplasmic membrane of *E. coli*.[28]

Sugita-Konishi et al[27] investigated the effects of six catechin derivatives of green tea (catechin, EGC, EC, ECG, EGCg, and gallocatechin gallate) on the production and extracellular release of verotoxins (VTs) from EHEC. Different concentrations of mentioned catechins were added to culture medium of EHEC (10^7 cells/L) and incubate at 37°C for 24 hours. They used the reversed passive latex agglutination assay to determine the amounts of VT in the EHEC culture supernatant fluid. Among the six catechins examined, EGCg

Table 1
A summary of experimental studies on antimicrobial effect of green tea extract.

Reference	Pathogen	Result
Ikigai et al[34]	*Escherichia coli* [1] K-12 strain G6	Catechins acted on and damaged bacterial membranes.
Hoshino et al[28]	*E. coli* ATCC 11775	Bactericidal activity of catechins in the presence of Cu^{2+} is derived from damage to the cytoplasmic membrane of *E. coli*.
Sugita-Konishi et al[27]	Enterohemorrhagic *E. coli* O157:H7	Epigallocatechin gallate and gallocatechin gallate in green tea inhibited extracellular release of Vero toxin from *E. coli*.
Arakawa et al[29]	*E. coli* ATCC 25922	Hydrogen peroxide, which is generated by EGCg, appears to be involved in the bactericidal action of EGCg.
Shahidi et al[35]	Two strains of *E. coli* (PTCC No. 1330 and PTCC No. 1338)	Green tea has antibacterial effect against only one strain of *E. coli* (PTCC No. 1338) with 10 mm inhibition zone diameter.
Cho et al[30]	*E. coli* ATCC 25922	Tea polyphenols have a dose-dependent bactericidal effect on *E. coli* and a unique change in saturated and unsaturated fatty acids was seen in cell membrane of *E. coli* cultures treated with tea polyphenols.
Kumar et al[32]	*Staphylococcus*, *Streptococcus*, *Pseudomonas*, *E. coli*, *Proteus*, *Bacillus*	Aqueous extract showed little antimicrobial activity against six bacteria isolated; methanolic extract showed maximum antibacterial activity.
Reygaert et al[3]	*E. coli* isolated from UTI cultures during 2007–2008.	All of the strains tested, except one, had minimum inhibitory concentrations of ≤4.0 mg/mL (99%). Green tea had antimicrobial effect on *E. coli* causing UTI.

EGCg = epigallocatechin-3-gallate; UTI = urinary tract infection.

and gallocatechin gallate had greatest effects on suppressing VT release from EHEC cells into the culture supernatant fluid at concentrations of 0.05 mg/mL or higher. They also found that catechins suppress release of other periplasm proteins such as maltose binding protein. They concluded that green tea can be used to prevent food poisoning caused by EHEC.[27]

Arakawa et al[29] showed the role of hydrogen peroxide in bactericidal action of catechin. *Escherichia coli* ATCC 25922, containing 10^6 colony forming units (CFU)/mL was used as bacterial strain and green tea extracts were measured with the peroxalate chemiluminescence detection system. Standard solutions of ECG and EGCg (1 mM) in H_2O were prepared and serially diluted with H_2O. A stoke solution of hydrogen peroxide (0.1 M) in H_2O was prepared and stored at 4°C until use. They used chemiluminescent methods and Erythrocyte Sedimentation Rate (ESR) measurement to confirm that EGCg efficiently generated hydrogen peroxide and it is dependent on the pH of solution, which indicates that hydrogen peroxide is generated from catechin by one electron reduction to dissolved oxygen. The investigation also confirmed that bactericidal action of catechin is due to hydrogen peroxide generated from catechin and the intensity of action appears to be dependent on the sensitivity of bacterium for reactive oxygen and ability of bacterium to adsorb catechin.[29]

Shahidi Bonjar et al[35] evaluated the antibacterial effect of some botanical plants that were grown in the southern region of Iran against two strains of *E. coli* (PTCC No. 1330 and PTCC No. 1338). One of these plants was *C. sinensis*. Methanolic extract of *C. sinensis* (20 mg/mL) was used. Green tea had antibacterial effect against only one strain of *E. coli* (PTCC No. 1338) with 10 mm inhibition zone diameter (IZD). They suggested that green tea can be used against *E. coli* that was resistant to antibiotics such as trimethoprim and sulfamethoxazole.[35]

Cho et al[30] reported that concentration of 500 μg of tea polyphenols could inhibit the growth of *E. coli* ATCC 25922 and that concentrations of ≥5000 μg/mL were considered bactericidal. The mechanism of these effects was that tea polyphenols downregulate the production of protein such as EF-2 (elongation factor for protein translation); protein involved in energy metabolism and in phospholipid. They determined the cellular response and proteomic analysis of *E. coli* that exposed to tea polyphenols extracted from *C. sinensis*. Tea polyphenols had a dose-dependent bactericidal effect on *E. coli* and unique changes in saturated and unsaturated fatty acids were seen in cell membrane of *E. coli* cultures treated with tea polyphenols.[30]

Kumar et al[32] studied the antimicrobial activity of green tea extracts against various bacteria isolated from environmental sources. Different bacteria were isolated from sewage samples collected from different places at Solan Himachal Pradesh. Isolated bacteria were identified by Gram staining and biochemical tests. A total of six different bacteria were identified (*Staphylococcus*, *Streptococcus*, *pseudomonas*, *E. coli*, *Proteus* and *Bacillus*). Aqueous, ethanolic, and air-dried and powdered extracts of green tea were prepared using standardized protocols. The disc diffusion method was used to test antimicrobial activity of all extracts, and antimicrobial assays were performed at concentrations of 10 μL, 20 μL, and 30 μL. For all extracts, significant antimicrobial activity was reported. Aqueous extract showed little antimicrobial activity against the six bacteria isolates; however, methanolic extract has shown maximum antibacterial activity.[32]

Reygaert and Jusifi[3] evaluated an antimicrobial effect of green tea on urinary tract infections caused by *E. coli*. In this study, they used bacterial strains that were part of a research collection of *E. coli* isolated from UTI cultures during 2007–2008. Eighty isolates, which represent a wide spectrum of antimicrobial susceptibility patterns were selected from this collection; in addition, two control strains that were susceptible to all the clinically tested antimicrobials were selected. A standardized green tea (*C. sinensis*) extract (standardized to 7.0% polyphenols) was used. Luria–Bertani (LB) broth and dehydrated Müller–Hinton agar were used as media. Various concentrations of green tea extract (0 mg/mL, 2.5 mg/mL, 3 mg/mL, 3.5 mg/mL, and 4.0 mg/mL) were prepared and the MICs were determined by the agar dilution method. The results were as follows: 99% of strains were susceptible to the green tea extract at a concentration of ≤4.0 mg/mL (one strain was not susceptible at even 4.0 mg/mL); 94% of strains were susceptible at ≤3.5 mg/mL; 76% of strains were susceptible at ≤3.0 mg/mL; 40% of strains were susceptible at ≤2.5 mg/mL; and the control strains varied, one being susceptible at ≤2.5 mg/mL and the other susceptible at ≤3.5 mg/mL. Therefore, all of the strains tested, except one, had MICs of ≤4.0 mg/mL (99%). The results of this study show that green tea can have an antimicrobial effect on *E. coli* bacteria that causes UTIs.[3]

All these studies show that green tea has antimicrobial effect on *E. coli* through different mechanisms. This effect is due to its catechins. Based on these studies, we conclude that green tea can be used as antimicrobial agent against *E. coli*.

3.2. Experimental studies on synergy between green tea and antibiotics against E. coli

In this part, all experimental studies that evaluated the synergistic effects between green tea and antibiotics against *E. coli* are reviewed. A summary of these studies is presented in Table 2.

Table 2
A summary of experimental studies on synergistic effect of green tea extract.

Reference	Pathogen	Result
Isogai et al[36]	*Escherichia coli* 0157	Extracts of *Camellia sinensis* leaves in combination with levofloxacin were protected gnobiotic mice against oral challenge with enterohemorrhagic *E. coli* 0157.
Tiwari et al[37]	*E. coli*	Green tea extract showed synergistic activity with the antibiotics chloramphenicol, amoxicillin, cotrimoxazol, azithromycin, levofloxacin, gentamycin, methicillin, nalldixic acid and ciprofloxacin.
Lee et al[7]	*E. coli* Z17 O2:K1:H, uropathogen	Combination treatment of catechin and ciprofloxacin has synergistic effects.
Esimone et al[38]	*Staphylococcus aureus* ATCC 12600 *E. coli* ATCC 11775	Gentamycin, tetracycline, cefotaxime, and ceftazidime have additive effects against *E. coli.* Streptomycin, ceftriazone, ciprofloxacin, ofloxacin, and norfloxacin have antagonistic effects against *E. coli*.
Jazani et al[39]	*E. coli* isolates collected from urine specimens submitted to a clinical diagnostic laboratory in Urmia, Iran	Combination of water soluble green tea extracts and ciprofloxacin had *in vitro* synergistic effect on urinary tract isolated *E. coli*.
Neyestani et al[34]	*E. coli* ATCC 25920	Green tea extract increased the antibacterial effects of gentamicin and amikacin, at the amount of 1.25 mg had an inhibitory effect on norfloxacin and sulfamethoxazole.
Passat[40]	*E. coli* isolates were collected from urine specimens submitted to a diagnostic microbiology laboratory of selected hospital during October and November 2009	Green tea had synergistic effect with: chloramphenicol, amoxicillin, azithromycin, ciprofloxacin and cefodizim and antagonistic effect with amikacin, streptomycin, amikacin, gentamicin, tobramycin, streptomycin, cefepim, azithromycin, piperacillin, and kanamycin.

Isogai et al[36] investigated the synergistic effects between green tea extract and levofloxacin. They used female (19–22 g) and male (22–26 g) mice at age 4–5 weeks. They divided mice in to four groups: Group 1, Japanese green tea ethanolic extract (JGTE) diet plus levofloxacin (LVFX); Group 2, JGTE diet alone; Group 3, normal diet alone; and Group 4, normal diet plus LVFX. On the basis of the MIC result and concentration of JGTE in a cup for drinking, a special diet with JGTE (1 mg/g catechins) was prepared by Funabashi Farm Co. The EHEC strain was deposited intragastrically through a catheter to germ-free IQI mice. LVFX, 20 mg/kg was administered to the mice once a day for 6 days. The antibiotic therapy was started on Day 1 of the infection (normal diet group), or Day 7 of the infection (JGTE diet group). When EHEC was fed to IQI mice, about 10^9–10^{10} CFU/g *E. coli* was colonized in feces, while in the JGTE diet, the number of EHEC cells dropped to 10^5–10^6 CFU/g. The bacteria were eliminated completely by the LVFX diet. No mice had organ damage in the JGTE diet and conversely in LVFX diet. LVFX and JGTE diet eliminated the EHEC cells completely and organ damage was not seen in mice. This study showed that although green tea could not eliminate EHEC completely, it clearly had antibacterial effects. They concluded *C. sinensis* has protective effects due to inhibiting inflammation and ulceration of intestine mucosa and can be used to increase the safety of antibiotic such as LVFX.[36]

The synergistic effect between catechin and ciprofloxacin on chronic bacterial prostatitis (CBP) rat model was published by Lee et al in 2005.[7] They prepared an experimental CBP model by instilling 0.2 mL of bacterial suspension (*E. coli*, containing 1×10^8 CFU/mL) into the prostatic urethra of 70 male Wistar rats. After 4 weeks of bacterial instillation, 58.6% of rats (41 of 70) were demonstrated to model CBP by microbiology and histology tests. These CBP rat models were randomly divided into four groups: control group ($n = 10$): 2 mL of phosphate-buffered saline (pH = 7.2) administered through an oral gavage in two divided doses daily for 2 weeks; catechin group ($n = 10$): 300 mg/kg body weight of catechin concentrate dissolved in 2 mL of distilled water and administered as for the control group for 2 weeks; ciprofloxacin group ($n = 11$): 5 mg/kg body weight of ciprofloxacin dissolved in 2 mL of distilled water and administered as before; and catechin with ciprofloxacin group ($n = 10$): 5 mg/kg ciprofloxacin and 300 mg/kg catechin dissolved in 2 mL distilled water and administered like the other groups. After 2 weeks of drug treatment, the results of microbiological cultures and histological findings of the prostate and urine samples were analyzed. In the prostate tissue culture, CFU count in the ciprofloxacin and catechin with ciprofloxacin groups significantly decreased when compared with the control group ($p < 0.05$). The catechin with ciprofloxacin group demonstrated significantly decreased CFU count in prostate tissue culture compared with ciprofloxacin group ($p < 0.05$). The catechin group also decreased CFU count in prostate tissue culture compared with the control rats, but did not reach a statistically significance ($p > 0.05$). Three parameters of chronic inflammatory cell infiltration, acinar changes and intestinal fibrosis were evaluated as histological data after 2 weeks of treatment. In the catechin group, there was no significant change compared with the control group. All three parameters improved significantly in the ciprofloxacin and catechin with ciprofloxacin group compared with the control group ($p < 0.05$). The severity scores of chronic inflammatory cell infiltrations were 1.91 ± 0.70 in the ciprofloxacin group ($p < 0.05$), and 1.00 ± 0.71 in the catechin with ciprofloxacin group ($p < 0.05$). Microbiological cultures and histological findings of the prostate and urine samples showed that combination treatment of catechin and ciprofloxacin has synergistic effect and may be effective in treating CBP.[7]

Antimicrobial activity of boiled water tea extract and organic solvent extract were studied by Tiwari et al.[37] In this study, they tried to describe the synergistic antimicrobial activity of tea and antibiotics against enteropathogens such as *E. coli*. Black tea and green tea were purchased from India and crude tea extract (2% tea extract) was prepared following the method described by Yam et al.[31] Antibiotic disc impregnated with chloramphenicol, kanamycin, tetracycline, methicillin, naldixic acid, and gentamycin. A dilution assay was used for determining MIC in which the tubes were examined visually for growth (turbidity) and no growth (no turbidity). A loopful from the highest dilution streaked on nutrient agar plates, that did not show any bacterial growth after overnight incubation, was taken as minimum bactericidal concentration (MBC). The MIC of green tea organic solvent extracts was the lowest (3.3 mg/mL) compared with boiled water green tea extract (6.27 mg/mL) and green tea infusion (6.94 mg/mL). These results showed that organic solvent extracts have a better antimicrobial activity and this effect may be due to higher content of catechin (30–40% w/w). Both green tea and black tea extracts inhibited the growth of *E. coli* but the growth inhibiting concentration of green tea extract was lower than black tea extract and both showed synergistic activity with chloramphenicol, gentamycin, methicillin, and naldixic acid.[37]

Esimone et al[38] studied the interaction of tea (*C. sinensis*) with antimicrobial agents *in vitro*. In this study, they used crude extract of *C. sinensis* and concentrations of 1.5 mg/mL, 2.0 mg/mL, 2.5 mg/mL, 3.0 mg/mL, and 3.5 mg/mL were prepared by diluting the extract with distilled water. Antibiotic discs used contained ampicillin (10 μg), cloxacillin (5 μg), gentamicin (10 μg), streptomycin (10 μg), tetracycline (25 mg), ceftriaxone (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), ciprofloxacin (10 μg), ofloxacin (10 μg), and norfloxacin (10 μg). Bacterial strains used in this study were *S. aureus* ATCC 12600 and *E. coli* ATCC 11775 with concentrations of 1×10^7 CFU/mL for each organism. They performed susceptibility tests with different concentration of green tea extract to determine sub-bacteriostatic concentration (1.5 mg/mL). They used the agar well diffusion method to determine the susceptibility of the microorganisms to tea extract. At the end, IZD were measured with a rule. To determine the interaction of the tea extract with antibiotics, they used overlay the inoculum susceptibility disc method. A plate that had 1.5 mg/mL tea extract in nutrient agar was used as the test agar plate and the control agar plate, which contained nutrient agar, had no tea extract. Finally, four kinds of IZD were determined. Synergism (when IZD increment of 19% or more), additive (<19% increase in IZD), indifference (when there was no variation in IZD), and antagonism (when IZD of control > IZD of test). Ampicillin and cloxacillin were inactive against *E. coli*. Gentamycin, tetracycline, cefotaxime, and ceftazidime had additive effects against *E. coli.* Streptomycin, ceftriazone, ciprofloxacin, ofloxacin, and norfloxacin had antagonistic effects against *E. coli*.[38]

Jazani et al[39] evaluated the synergistic effect of water-soluble green tea extract on the activity of ciprofloxacin against isolated *E. coli*. During a 2-month period, they collected 18 isolates from urine specimens submitted to a clinical diagnostic laboratory in Urmia, Iran. They used water soluble green tea extract (2% tea extract was prepared following the method described by Tiwari et al[37]) and determined MIC and MBC of bacterial isolates for measuring antimicrobial activity of green tea extracts and ciprofloxacin. The mean of MBC and MIC for all 18 isolates were 122.9 ± 40.3 mg/mL. To determine the synergistic activity of green tea water extract with ciprofloxacin, they used a sub-MIC concentration of ciprofloxacin. Each dilution was inoculated with 3×10^6 CFU/mL of bacteria. After overnight incubation, they measured MIC and MBC of green tea extract in the presence of ciprofloxacin. There was a reduction in MIC of green tea extracts in the presence of sub-MIC doses of ciprofloxacin, for 93.7% (15 of 16 tested) of bacterial isolates. Therefore, they confirmed that

combination of water soluble green tea extracts and ciprofloxacin had *in vitro* synergistic effect on urinary tract isolated *E. coli*.[39]

Neyestani et al[40] investigated microbiologic effects of tea extract on certain antibiotics against *E. coli in vitro*. They used bacterial strain ATCC 25920 and crude tea extracts. Different concentrations of black or green tea extracts (6.25 mg/mL, 12.5 mg/mL, 25 mg/mL, 50 mg/mL, and 100 mg/mL) were used for this study. They used the method of disc diffusion for bacterial sensitivity tests. Green tea at 20 mg/mL concentration inhibited *E. coli* growth completely. The antibiotics used were norfloxacin (10 μg/disc), amikacin (30 μg/disc), sulfametoxazole (10 μg/disc), and gentamycin (10 μg/disc). They used the mean diameter of growth inhibition for further statistical analyses. The results showed that green tea extract increased the antibacterial effects of gentamicin and amikacin. Green tea at the amount of 1.25 mg had an inhibitory effect on norfloxacin and sulfamethoxazole but, when increasing its amount to 2.5 mg, antibacterial effect of sulfamethoxazole and norfloxacin were restored and increased respectively. Therefore, the microbiologic effects of green tea extracts on certain antibiotics against *E. coli* may vary depending on the amount of the extract and the antibiotic being used.[40]

Passat[41] studied the interactions of black and green tea water extracts with antibiotics activity in local urinary isolated *E. coli*. Crude boiling water extracts of black and green tea were prepared by the method described by Araghizadeh et al.[15] A total of 17 *E. coli* isolates were collected from urine specimens of patients with UTI. Two bacterial isolates were selected (ED_1, ED_2), grown on brain heart infusion and incubated at 37°C for 24 hours. For antimicrobial sensitivity test, they used the Kirby–Bauer method. Twenty five antibiotic discs containing: amikacin (10 mg), gentamicin (50 mg), streptomycin (25 mg), tobramycin (30 mg), kanamycin (30 μg), cefaclor (30 mg), cefepime (30 mg), cefodizime (30 mg), cefradin (30 mg), chloramphenicol (10 μg), vancomycin (30 μg), lincomycin (2 mg), azithromycin (15 mg), clarithromycin (15 mg), erythromycin (10 μg), amoxicillin (25 mg), ampicillin (10 μg), penicillin G (10 U), piperacillin (100 μg), ciprofloxacin (10 mg), naldixic acid (30 mg), tetracycline (30 μg), rifampicin (30 μg), colistin (10 μg), and bacitracin (10 U), provided by Bioanalyse, Ankara, Turkey, were used in this test. To determine the MICs, they used the tube test method. First, the concentrations 150 mg/mL, 125 mg/mL, 100 mg/mL, and 75 mg/mL were prepared from the stock solution of black tea (200 mg/mL) and the concentrations 250 mg/mL, 225 mg/mL, 200 mg/mL from a stock solution of green tea (300 mg/mL). Then 0.1 mL of microbial suspensions, which were serially diluted to 10^{-3} (containing 10^5 CFU/mL) were inoculated in the plant extracts concentrations and tubes incubated at 37°C for 24 hours. After that, 0.1 mL of each concentration inoculated on nutrient agar plates and incubated at 37°C for 24 hours. The lowest antimicrobial concentration that inhibited visible growth of bacteria was recorded as MIC. They investigated the interaction between antibiotics and sub-MIC dose by taking (0.1 mL) from the sub-MIC dose and spreading the inoculums on nutrient agar; antibiotic discs were placed, and the plates were incubated at 37°C for 24 hours. After that, they measured the diameter of inhibition zones around the discs. The results showed that MIC of green tea water extract was 275 mg/mL (ED_1), 250 mg/mL (ED_2) and the MIC of black tea water extract was 150 mg/mL (ED_1) and 100 mg/mL (ED_2). Green tea extract has synergistic effect with chloramphenicol, amoxicillin, azithromycin, and ciprofloxacin for ED_1, and cefodizim for ED_2. Green tea extract showed antagonistic effect with amikacin and streptomycin for ED_1 and amikacin, gentamicin, tobramycin, streptomycin, cefepim, azithromycin, piperacillin, and kanamycin for ED_2. The extract has no effect on cefradin, vancomycin, lincomycin, erythromycin, ampicillin, penicillin G, or bacitracin. They found that soluble green tea extract has synergistic activity with ciprofloxacin among 93.7% of urinary tract *E. coli* isolates. They showed that using tea was reasonable for treatment of UTI because of high levels of green tea polyphenols, which were found in urine after drinking tea in humans and experimental animals. Catechin affected antibiotic resistance by perturbing the function of key processes associated with the bacterial cytoplasmic membrane. Catechin intercalated into phospholipid bilayers and made the microorganisms more susceptible to the antibacterial agents.[41]

The reviewed studies show that green tea potentiates the effects of some antibiotics and also can antagonize the effect of some other antibiotics. Although the results of these studies are conflicting for some antibiotics, we can conclude that green tea can increase the antimicrobial effect of common antibiotics used in UTI.

4. Conclusion

In this review, antimicrobial and synergistic effects of green tea for treatment of UTIs have been evaluated. UTIs are the most common nosocomial infections, and result in billions of dollars in medical care costs.[1,3] Green tea is a safe, nontoxic, cheap, and widely available drink in Asian countries.[1] Green tea catechins have antimicrobial effects against different bacteria and synergistic effect with antibiotics like chloramphenicol, amoxicillin, sulfamethoxazole, azithromycin, levofloxacin, gentamycin, methicillin, naldixic acid, and, especially, ciprofloxacin.[7,36,40,42–45] Therefore, it may improve the treatment of UTI and decrease its costs. Different studies have reported the antimicrobial effect of green tea against *E. coli*, which is the most important cause of 80–90% of all UTIs. EGC and EGCg have been shown to have the greatest antimicrobial effects but only EGC has been shown to be excreted in urine. Several studies showed that a cup of Japanese green tea (approximately contains 7.5 g of dried green tea leaves) is equivalent to approximately 150 mg of EGC. Urinary excretion of EGC peaked 8 hours after a single ingested dose and ECG levels in the urine reached 3–5 mg, which is a high enough concentration to potentially be effective as an antimicrobial agent.[3,46] Data from *in vitro* studies on the antimicrobial effects of green tea are promising, but human data are currently lacking. Therefore, it is essential to have *in vivo* studies on antibacterial effects of green tea and evaluated the efficacy of its catechins in the treatment of UTIs in the future. Human clinical trials also need to evaluate the synergistic effect between green tea and antibiotics used in UTIs.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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