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Original Article

ANTIBACTERIAL ACTIVITY OF GREEN TEA EXTRACT IN COMBINATION WITH CEFOTAXIME ON DIARRHEA CAUSING ESBL PRODUCING *E. COLI*

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ABSTRACT

Objective: Owing to the continuous evolution of antibiotic resistance, treatment with natural products represents significant adjuncts or alternatives to conventional antibiotic therapy. *Enterobacteriaceae* especially *Escherichia coli* have acquired antibiotic resistance due to production of Extended spectrum Beta-lactamases (ESBL).

Methods: Effect of Aqueous extract of Green tea (AGTE) was studied on 44 ESBL producing, diarrhea causing *E. coli.* ESBL production of the strains was confirmed by phenotypic confirmatory disc diffusion test (PCDDT) and E-test. Antibacterial activity of AGTE was studied by disc diffusion method and the Minimum Inhibitory Concentration (MIC) of AGTE and Cefotaxime were determined by an agar dilution technique. The combined activity of AGTE and Cefotaxime was determined by calculating the fractional inhibitory concentration (FIC index) by the checkerboard method. The active ingredients epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG) present in the extract were quantitated by High Performance liquid Chromatography (HPLC).

Results: AGTE exhibited significant antibacterial activity as the observed zones of inhibition ranged from 10–26 mm against the 44 ESBL producing *E. coli* strains. The MIC of AGTE was found to be in the range of 2.5-20 mg/ml with a mean of 7.23 mg/ml. 8 test strains showed synergism whereas the remaining showed an additive effect between AGTE and Cefotaxime. By HPLC analysis the concentration of EC, ECG, EGC, EGCG were found to be 7.03, 10.08, 9.99 and 43.57 respectively, expressed as percentage w/w.

Conclusion: As green tea extract has shown substantial antibacterial as well as synergistic activity, it can be of potential therapeutic value against ESBL producing *Escherichia coli* gastrointestinal infections.

Keywords: ESBL, Green tea, EGCG, Synergy.

INTRODUCTION

Drug resistance is a natural step in microbial evolution, with many microorganisms, especially of the *Enterobacteriaceae* family being highly adaptable and easily capable of mutating to acquire means of exhibiting drug resistance. Since β-Lactam antibiotics are the most widely used, it is therefore not surprising that resistance to many β-lactams is common and still evolving. Mechanisms of resistance to β-lactams in *Escherichia coli* can be divided into three categories, namely, enzymatic inactivation of the antibiotic, alteration of the target, and reduced penetration of the antibiotic. Enzymatic inactivation to β-lactams is most often caused by the production of enzymes extended spectrum β-lactamases (ESBLs).

Over the last 15 years, numerous outbreaks of infection with organisms producing ESBLs have been observed worldwide [1]. The advent of ESBLs producers has posed a great threat to the use of many classes of antibiotics particularly cephalosporins. There are indications that poor outcome occurs when patients with serious infections due to β-lactamase producing organisms are treated with antibiotics to which the organism is resistant [2].

Extended spectrum Beta-lactamases (ESBL) hydrolyze extended spectrum cephalosporins and are inhibited by Clavulanic acid, Sulbactum and Tazobactum [3]. *E. coli* has been revealed to be one of the most commonly encountered ESBL producing organisms in various parts of India [4-6]. Treatment of such infections with a combination of a β-lactam with a β-lactamase inhibitor is common, but it may not always prevent the emergence of resistance [7].

Several studies in the recent past emphasized the urgent need for new therapeutic strategies, including the use of plant extracts and phytochemicals extracted from them [8,9]. Such an approach can help curb or considerably decrease the occurrence of drug resistance among bacteria as plant extracts usually have multiple active ingredients that act at various target sites in the bacteria.

Green tea (*Camellia sinensis* leaves) has been consumed since ancient times in China and has gained popularity in several other countries including India. Green tea extracts being a popular beverage consumed worldwide, inexpensive, and non-toxic, serves as a very important a source of alternative medicine. It is known to possess preventive activity against cardiovascular diseases and some forms of cancer [10]. Also, it has antioxidant [11], antitumor, anti-inflammatory, anti diabetic, immunomodulatory [12] and antibacterial activities [8,9]. Green tea consists of catechins viz. epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG). Other polyphenols include flavanols and their glycosides [13]. EGCG, a main constituent of green tea polyphenol, has been reported to have great antimicrobial activity and also exhibits synergistic properties with other antibiotics against Gram-positive and Gram-negative bacteria [14-16]. By means of molecular dynamic simulations it was revealed that a number of tea catechins have a strong affinity for the lipid bilayer via hydrogen bonding to the lipid bilayer and among them EGCG showed the strongest interaction as it can form large number of hydrogen bonds.

However the effect of Green tea for diarrhea causing ESBL producing E. coli still needs to be elucidated. The objective of the present study is thus to prepare an aqueous extract of Green tea and validate its antibacterial activity against diarrhea causing ESBL producing E. coli.

MATERIALS AND METHODS

The present study was carried out in the Department of Microbiology, Topiwala National Medical College and BYL Nair Charitable Hospital, Mumbai, India and approved by the local Ethics Committee of our institution.

Bacterial strains

E. coli strains were isolated and identified using standard methods from stool samples obtained from patients suffering with acute diarrhea admitted to our tertiary care hospital.

Determination of antimicrobial susceptibility

Antimicrobial Susceptibility Testing (AST) was performed using the disk diffusion method as described by the Clinical and Laboratory Standard Institute (CLSI) using Kirby-Bauer method [18]. Antibiotic discs were obtained from Himedia Ltd. Antibiotics used were Chloramphenicol (C), Ciprofloxacin (Ci), Nalidixic acid (NA), Amoxyclav (AMC), Ceftriaxone (CTR), Ceftazidime (CAZ), Cefotaxime (CTX), Co-trimoxazole (CO) and Imipenem (I).

Screening for ESBL production

All the isolates showing resistance to 3^{rd}generation cephalosporins, namely Ceftazdime, Ceftriaxone and Cefotaxime, were tested for confirmation of β-lactamase production by phenotypic confirmatory disc diffusion test (PCDDT). The Optical Density (O. D.) of the cultures were adjusted to 0.5 McFarland (~1.5 × 108 CFUmL−1) and swabbed on Mueller-Hinton (MH) Agar plates. The screening was done as per CLSI guidelines [18].

Confirmation of ESBL production by E-test

Confirmation of ESBL production was done using Multi-Ezy MICTM Strips (Hi-Media Laboratories Pvt. Ltd.). These strips differ from the conventional E-strips in that they contain a gradient of 3 antibiotics with and without Clavulanic acid on either side of the strip respectively instead of one antibiotic. The Multi-Ezy MIC™ Strips contain Cefotaxime, Ceftazidime and Cefipime (MIX) on one side in a twofold gradient and the same antibiotics with Clavulanic acid (MIX+) on the other side. A ratio of inhibition zones for MIX and MIX+of ≥ 8 was considered as a positive E -test [19]. *E. coli* ATCC 25922 as negative control and *Klebsiella pneumonia* ATCC 700603 as positive control were used in the test.

Preparation of green tea extract

The dried Green tea powder was provided by Konark Herbals and Health Care, Mumbai. 10 g of dried powder was boiled with 100 ml of distilled water for 10 min. The heated solution was filtered and evaporated at 40°C. The aqueous green tea extract (AGTE) thus prepared was then kept in the refrigerator for further use.

High-performance liquid chromatography analysis

The High-performance liquid chromatography (HPLC) system consisted of a Shimadzu LC-2010CHT model (Shimadzu, Tokyo, Japan), with a column of Phenomenex Luna-C18 (4.6 × 250 mm, 5 µm Merck) and detection at a wavelength of 280 nm. Elution was carried out at a flow rate of 1.6 ml/min under a linear gradient of Buffer made up of 0.136 g of anhydrous potassium dihydrogen phosphate in water and 0.5 ml of orthophosphoric acid (solvent A) and acetonitrile (solvent B), with a run time of 35 min. The AGTE was dissolved in water and 20 µl was injected into the HPLC. The presence of epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG) was confirmed by the same retention time of their standards (Natural Remedies, India). The amount of catechins in the sample was calculated using the formula:

$$\frac{\text{Area of sample}}{\text{Area of standard}} \times \frac{\text{weight of standard(mg)}}{\text{standard dilution (ml)}} \times \frac{\text{Sample dilution (ml)}}{\text{weight of the sample (mg)}} \times \text{Purity of the standard (\%)}$$

Antibacterial activity of AGTE

The antibacterial activity of the extract was tested using the disk diffusion method described by the Clinical and Laboratory Standards Institute. Briefly, sterile paper discs (6 mm; Hi-Media, India) were loaded with 20 µl of extracts at concentration of 500 mg/ml dissolved in sterile distilled water and then left to dry for 18 h at 37 °C. The bacterial suspensions were then diluted to a turbidity of approximately 0.5 McFarland (~1.5 × 108 CFUmL−1). Ceftazidime/Clavulanic acid (30/10 µg) CAC and Imipenem (10 µg) (Hi-Media, India) was used as the positive control. After incubation at 37 °C for 24 h, the diameter of the zone of inhibition around each of the discs was measured and recorded. Each experiment was performed in triplicate.

Determination of MIC

The MIC value of the AGTE was determined using the Agar dilution technique [19]. The suspensions of all 44 ESBL producing *E. coli* cultures were adjusted to a turbidity of 0.5 McFarland. The AGTE was added to Mueller and Hinton agar in order to obtain concentrations from 0.05 % to 2 % (0.5 mg/ml to 20 mg/ml) and the plates were poured. Similarly plates containing Cefotaxime in the range of 5 to 1000µg/ml were prepared. Saline suspensions of the *E. coli* strains were inoculated onto the plates. The lowest concentration capable of inhibiting visible growth after 24 h of incubation at 37 °C was then recorded as the MIC.

Determination of combined activity by Checkerboard assay

Cefotaxime stock (10 mg/ml) and 10 % AGTE was used for the Checkerboard assay. The concentration range of Cefotaxime was from 5 to 1000 µg/ml and AGTE ranged from 0.05% to 2% (0.5 mg/ml to 20 mg/ml). Visible growth after 24 h of incubation at 37 °C was checked and the MIC in combination for Cefotaxime and AGTE were determined for each strain of *E. coli*. The fractional inhibitory concentration was calculated as follows:

FIC of compound a (FICa) = MIC of compound A (Cefotaxime) in combination/MIC of compound A alone

FIC of compound b (FICb) = MIC of compound B (AGTE) in combination/MIC of compound B alone

The sum of fractional inhibitory concentration (FICs) indices of two compounds in the combination was calculated as follows: FICa+FICb = FICs

FIC indices were interpreted as synergic when values were ≤ □0.5 and as antagonistic when values were>4. The results between synergy and antagonistic tendency (>0.5 and<4) were defined as additive or indifferent. [20]

RESULTS

E. coli strains isolated from stool samples obtained from patients suffering from diarrhea were screened for their susceptibility to antibiotics Chloramphenicol (C), Ciprofloxacin (Ci), Nalidixic acid (NA), Amoxyclav (AMC), Ceftriaxone (CTR), Ceftazidime (CAZ), Cefotaxime(CTX), Co-trimoxazole (CO) and Imipenem (I). The susceptibility pattern of the test strains is as shown in table 1. All the strains were resistant to Ampicillin, Ceftriaxone, Ceftazidime, Cefotaxime and Nalidixic acid. 57% of the strains exhibited resistance towards Amoxyclav, 66% each towards Co-trimoxazole and Ciprofloxacin. 61% were resistant to Chloramphenicol whereas all were sensitive to Imipenem as observed in table 1. Resistance to all the third generation Cephalosporins viz. Ceftriaxone, Ceftazidime, Cefotaxime indicates that the stains are ESBL producers, which was confirmed by PCDDT and E-test. The Phenotypic confirmatory disc diffusion test was positive for all the *E. coli* strains as an increase in the zone diameter for Ceftazidime-Clavulanic acid by≥ 5 mm as compared to Ceftazidime alone was observed. E-test using Ezy-MICTM strips confirmed the ESBL production of all the 44 E. coli strains as a ratio of inhibition zones for MIX and MIX+of ≥ 8 was observed. To estimate the amounts of active ingredients EC, ECG, EGC, and EGCG, HPLC analysis was carried out and were found to be 7.03, 10.08, 9.99 and 43.57 respectively, expressed as percentage w/w. EGCG being the main catechin is present in maximum amount in AGTE.

The antimicrobial effect of AGTE was determined using the disc diffusion method, carried out in triplicates. The average zone sizes ranged from 10–26 mm with a mean of 16 mm for the test strains. The individual zone sizes of the test strains are as shown in fig. 1. The MIC of AGTE was determined by the agar dilution technique. Concentrations from 0.05% to 2% (0.5 mg/ml to 20 mg/ml) were used to determine the MIC. MIC of AGTE for the 44 strains ranged from 2.5-20 mg/ml with a mean of 7.23 mg/ml as depicted in table 2. As the organisms are all ESBL producers their MIC values for Cefotaxime were very high. A MIC ≥of 4 µg/ml indicates ESBL production. As seen in table 3 all the 44 strains showed MIC of Cefotaxime ≥ 10 µg/ml hence clearly highlights the ESBL production of the test strains.

Table 1: Pattern of Antibiotic susceptibility of the *E. coli* strains

Antibiotic	A	AMC	C	Co	Ci	NA	CAZ	CTX	CTR	I
No. of strains resistant	44	25	27	29	29	44	44	44	44	0
Percentage of resistant strains	100	57	61	66	66	100	100	100	100	0

Note: n = 44

Table 2: MIC of AGTE against ESBL producing *E. coli.* Table shows number and percentage of strains inhibited by various concentrations of AGTE

Concentration of AGTE (mg/ml)	0.5	1.0	2.5	5.0	10	15	20	25
No. of strains inhibited	-	-	13	8	21	-	2	-
Percentage of strains inhibited	-	-	29.5	18.2	47.7	-	4.5	-

Note: n = 44

Table 3: MIC of Cefotaxime against ESBL producing *E. coli.* Table shows number and percentage of strains inhibited by various concentrations of Cefotaxime

Concentration of Cefotaxime (µg/ml)	10	50	100	150	200	250	300	350	500	1000
No. of strains inhibited	4	4	7	6	3	2	9	3	-	6
Percentage of strains inhibited	9.1	9.1	15.9	13.6	6.8	4.5	20.5	6.8	0	13.6

Note: n = 44

The combined activity of Cefotaxime and AGTE was determined by checkerboard assay. Calculations of FICs were performed as shown below for strain E8 as an example as shown in fig. 2. For strain E8, FIC a = 50/350 = 0.14. FIC b = 2.5/10 =0.25

So FICs = 0.14+0.25 = 0.39 rounded up to 0.4, which indicates a synergistic effect between Cefotaxime and AGTE. The results obtained for all the 44 strains are as mentioned in table 4 where 8 strains are showing synergistic activity with FICs≤ 0.5, whereas all the remaining strains showed an additive effect with FICs between 0.5 and 4. None of the strains exhibited Antagonism between AGTE and Cefotaxime.

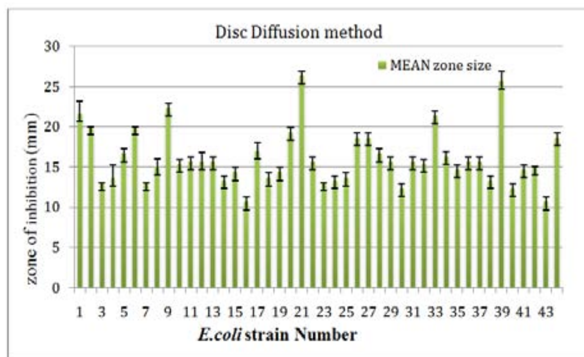

Fig. 1: Bar chart showing mean zone sizes of AGTE by disc diffusion method. Vertical bars indicate standard deviation (n=3)

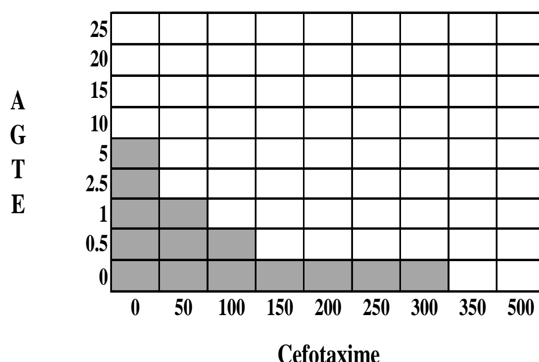

Fig. 2: Interpretation of combined effect of AGTE and Cefotaxime by Checkerboard assay for strain E8

DISCUSSION

For the last several years, frequent occurrence of infection with organisms producing β-lactamases has been observed globally [21]. The advent of β-lactamases producers has established a great hazard to the use of many classes of antibiotics particularly cephalosporins. There are indications that poor outcome arises when patients with severe infections due to β-lactamase producing organisms are treated with antibiotics to which the organism is resistant [2]. The high incidence of gastrointestinal tract infections in the general population, the potential for complications, especially in developing countries like India and the associated costs of treatments calls attention to the importance of alternate therapy. In this regard plant extracts are being comprehensively researched by scientists [15, 22, 23].

E. coli strains were isolated from patients suffering from severe diarrhea. Resistance to antimicrobial agents in these species has become an increasingly relevant problem for health care providers. These isolates were confirmed to be ESBL producers by PCDDT and E test. Due to ESBL production these organisms were resistant to third generation cephalosporins. Their resistance was also extended to other groups of antibiotics like Ciprofloxacin and Co-trimoxazole. Many times ESBL producing organisms posses or have acquired genes that encode co-resistance to two or more groups of antibiotics. Management and treatment of infections caused by such resistance pathogens offer a great difficulty and increase health care cost. Therefore, there is a need to investigate alternate methods of therapy.

Green tea is consumed worldwide now and its beneficial physiological and pharmacological effects are very well known. A study by Nima *et al.* [24] stated the MIC of aqueous green tea for 18 *E. coli* isolates to be in the range of 75 to 150 mg/ml with a mean of 122 mg/ml. In another study by Tiwari *et al.* [16] the MIC of green tea extract against *E. coli* was found to be 88.30 mg/ml. In this study the MIC values ranged from 2.5-20 mg/ml with a mean of 7.23 mg/ml which is much lesser than the previous study suggesting better extraction of tea catechins. There are various studies that have carried out *in vivo* experiments to demonstrate the significance of Green tea. One such study by Toda *et al.* [25] proved that a mixture of tea catechins protected rabbits from an experimental infection caused by *V. cholerae* and suggested that patients with cholera could benefit if tea extracts were added to oral rehydration solutions.

Antibiotic therapy combined with phytochemicals may delay the emergency of bacterial resistance and may also produce desirable synergistic effects in the treatment of infections caused by ESBL producing organisms. This could be due to the fact that crude plant extracts have many different phytochemicals, which inhibit bacteria by different unknown mechanisms. This dual attack of both phytochemicals and antibiotic on different target sites of the bacteria

could lead to either an additive or a synergistic effect. AGTE showed a decrease in the MIC of Cefotaxime for all the test strains. 8 strains showed synergism whereas the remaining test strains showed an additive effect. None of the test strains showed antagonistic effect between AGTE and Cefotaxime. Such synergistic activity was also demonstrated by Cui Y. *et al.* in 2012 [15], Tiwari *et al.* in 2004 [16] and Zhi-Qing Hu *et al.* in 2002 [20] on different test organisms. Hence there is no doubt about Green tea being a boon, antimicrobial benefit of which should be reaped in the form of providing lead molecules in developing better and effective drugs by pharmacists.

Table 4: FIC values and results of combined effect of Cefotaxime and AGTE against ESBL producing *E. coli*

Strain No.	FICs	Effect	Strain no	FICs	Effect
E1	2.9	A	E23	0.6	A
E2	0.1	S	E24	0.6	A
E3	1.3	S	E25	1.5	A
E4	2.0	A	E26	2.3	A
E5	0.8	A	E27	2.1	A
E6	0.8	A	E28	2.8	A
E7	2.5	A	E29	1.6	A
E8	0.4	S	E30	2.5	A
E9	0.9	A	E31	0.2	S
E10	2.0	A	E32	0.3	S
E11	2.1	A	E33	2.0	A
E12	2.5	A	E34	1.7	A
E13	0.3	S	E35	1.3	A
E14	1.5	A	E36	0.8	A
E15	1.5	A	E37	1.7	A
E16	1.8	A	E38	0.8	A
E17	0.4	S	E39	1.7	A
E18	1.0	A	E40	1.0	S
E19	1.3	A	E41	0.6	A
E20	1.5	A	E42	0.8	A
E21	0.6	A	E43	2.5	A
E22	1.7	A	E44	1.6	A

Note: S means Synergistic effect as FICs ≤ 0.5, A means Additive effect with FICs between 0.5 and 4

CONCLUSION

Green tea (*Camellia sinensis*) extract has been proved to be effective against ESBL producing *E. coli* not only alone but also in combination with antibiotic. The tea catechins can be purified and tested further *in vivo* which will help to confirm the therapeutic value of Green tea. Thus, Green tea can be used as an alternate therapy or can be used as an addition to the existing conventional therapy to cure diarrheagenic infections caused by ESBL producing *E. coli.*

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CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Palucha A, Mikiewiez B, Hryniewiez W, Griadkowski M. Concurrent outbreaks of extended spectrum β-lactamase producing organisms of the family Enterobacteriaceae in a Warsaw Hospital. J Antimicrob Chemother 1999;44:489-99.
2. Paterson LD, Wen-Chien Ko, Gottberg AV, Casellas JM, Rice LB, Macormack JG, *et al*. Outcome of cepaholsporin treatment for serious infections due to apparently susceptible organisms producing extended spectrum β-lactamases. Implications for the clinical microbiology laboratory. J Clin Microbiol 2001;39:2206-12.
3. Sarah M Drawz1, Robert A Bonomo. Three decades of β-Lactamase Inhibitors. Clin Microbiol Rev 2010;23(1):160–201.
4. Srikrishna AJ, Rita Swaminathan. Prevalance of extended spectrum beta-lactamases (ESBLs) among enterobacteriaceae. Bombay Hospital J 2013;55:46-51.
5. Maninder Kaur, Aruna Aggarwal. Occurrence of the CTX-M, SHV and the TEM Genes among the extended spectrum β-Lactamase producing isolates of enterobacteriaceae in a tertiary care hospital of North India. J Clin Diagn Res 2013;7(4):642-5.
6. B Sasirekha Prevalence of Esbl, Ampc β-Lactamases and mrsa among uropathogens and its antibiogram. EXCLI 2013;12:81-8.
7. Klibanov OM, Raasch RH, Rublein JC. Single versus combined antibiotic therapy for gram-negative infections. Ann Pharmacother 2004;38:332-7.
8. Cho YS, Schiller NL, Oh KH. Antibacterial effects of green tea polyphenols on clinical isolates of methicillin-resistant Staphylococcus aureus. Curr Microbiol 2008;57:542-6.
9. Shimamura T, Zhao WH, Hu ZQ. Mechanism of action and potential for use of tea catechin as an anti-infective Agent. Anti-Infect Agents Med Chem 2007;6:57-62.
10. Michael D. Brown. Green Tea (Camellia Sinensis) extract and its possible role in the prevention of cancer. Altern Med Rev 1999;4(5):360-8.
11. Henning SM, Niu Y, Lee NH, Thames GD, Minutti RR, Wang H, *et al.* Bioavailability and antioxidant activity of tea flavanols after consumption of greentea, blacktea, or a green tea extract supplement. Am J Clin Nutr 2004;80:1558-64.
12. Arshad H Rahmani, Yousef H Aldebasi, Salah M Aly. Role of green tea and its constituent epigallocatechin-3-gallate in the health management. Int J Pharm Pharm Sci 2015;7(3):6-12.
13. Graham HN. Green tea composition, consumption,and polyphenol chemistry. Prev Med 1992;21:334-50.
14. Hu ZQ, Zhao WH, Asano N, Yoda Y, Hara Y, Shimamura T. Epigallocatechingallate synergistically enhances the activity of carbapenems against methicillin-resistant Staphylococcu saureus. Antimicrob Agents Chemother 2002;46:558-60.
15. Cui Y, Oh YJ, Youn M, Lee I, Pak HK, Park W, *et al.* AFM study of the differential inhibitory effects of the green tea polyphenol (-)-epigallocatechin-3-gallate(EGCG) against gram-positive and gram-negative bacteria. Food Microbiol 2012;29:80-7.
16. RP Tiwari, SK Bharti, HD Kaur, RP Dikshit, GS Hoondal. Synergistic antimicrobial activity of tea & antibiotics. Indian J Med Res 2004;122:80-5.
17. Sirk TW, Brown EF, Sum AK, Friedman M. Moleculardynamics study on the biophysical interactions of seven green tea

catechins with lipid bilayers of cell membranes. J Agric Food Chem 2008;56:7750-8.

18. Clinical and laboratory standards institute. Performance standards for antimicrobial disc susceptibility tests: approved standard-11th ed. CLSI document M02-A11. CLSI, Wayne PA; 2012.
19. Clinical and laboratory standards institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: CLSI document M07-A9, vol. 32, no. 2. CLSI, Wayne, PA; 2012.
20. Zhi-Qing Hu, Wei-Hua Zhao, Yoshiyuki Yoda, Nozomi Asano, Yukihiko Hara, Tadakatsu Shimamura. Additive, indifferent and antagonistic effects in combinations of epigallocatechin gallate with 12 non-β-lactam antibiotics against methicillin-resistant Staphylococcus aureus. J Antimicrob Chemother 2002;50:1051-4.
21. Palucha A, Mikiewiez B, Hryniewiez W, Griadkowski M. Concurrent outbreaks of extended spectrum β-lactamase producing organisms of the family Enterobacteriaceae in a Warsaw Hospital. J Antimicrob Chemother 1999;44:489-99.
22. Silva NCC, Fernandes Júnior A. Biological properties of medicinal plants: a review of their antimicrobial activity. J Venom Anim Toxins 2010;16(3):402-13.
23. Dilek Keskin Sevil. Toroglu Studies on antimicrobial activities of solvent extracts of different spices. J Environ Biol 2011;32:251-6.
24. Nima Hosseini Jazani, Minoo Zartoshti Shaharam Shahabi, Zahra Yekta, Shahin Nategi. Evaluation of the synergetic effect of water soluble extracts of Green tea (Camellia sinensis) on the activity of ciprofloxacin in urinary isolated E. coli. J Biosci 2007;7(8):1500-3.
25. Toda M, S Okubo, H Ikigai, T Suzuki, Y Suzuki, Y Hara, *et al*. The protective activity of tea catechins against experimental infection by Vibrio cholerae O1. Microbiol Immunol 1992;36:999–1001.