

Synergistic antimicrobial activity of tea & antibiotics

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Tea leaves are known for its antibacterial activity against many microorganisms. In this study we attempted to describe the synergistic antimicrobial activity of tea and antibiotics against enteropathogens. Antimicrobial activity of boiled water tea extract and organic solvent extract were studied against *Salmonella typhimurium* 1402/84, *S. typhi*, *S. typhi* Ty2a, *Shigella dysenteriae*, *Yersinia enterocolitica* C₇₇₀, and *Escherichia coli* (EPEC P₂ 1265) determining minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and death rate kinetics at MBC of tea extract in presence of subinhibitory concentration of antibiotic. Both green tea or black tea extracts effectively inhibited the growth of *S. typhimurium* 1402/84, *S. typhi*, *S. typhi* Ty2a, *S. dysenteriae*, *Y. enterocolitica* C₇₇₀, and *E. coli* (EPEC P₂ 1265). However, the growth inhibitory concentration of tea extract was lower for green tea as compared to black tea extract. Antimicrobial activity of green tea tea methanol: water extract tea was better as compared to boiled water tea extract of green tea. Based on death rate kinetics results, *S. typhi* Ty2a appeared to be highly sensitive and *Y. enterocolitica* C₇₇₀ the most resistant. Chloramphenicol and tea extract in combination inhibited the growth of *S. dysenteriae* at 2.5 µg/ml chloramphenicol (MIC 5 µg/ml) and 5.094 mg/ml black tea extract (MIC 9.089 mg/ml). Tea extract showed synergistic activity with chloramphenicol and other antibiotics like gentamycin, methicillin and nalidixic acid against test strains.

Key words Antibiotic - enteropathogens - synergistic activity - tea extract

Tea from the leaves of plant *Camellia sinensis* has been shown to have wide range of antioxidant, anti-inflammatory, anti-carcinogenic and antibacterial activity against many pathogens¹⁻⁵. Acidic, basic and neutral methanol extract fractions of *Camellia japonica* inhibited the growth of food borne pathogens in microbiological media and food⁶. *Staphylococcus aureus*, *Vibrio parahemolyticus*, *Clostridium perfringens*, *Bacillus cereus*, *Pleisomonas shigelloides* and *Aeromonas sobria* failed to grow in tea normally consumed by Japanese people⁷. Tea

components also inhibit the growth of *Vibrio cholerae* O1⁸, *Streptococcus mutans*⁹, *Shigella dysenteriae*¹⁰ and other bacteria grown *in vitro*. Differences in antimicrobial activities of tea have been found to be related with the kind and degrees of fermentation of tea¹¹. Green tea contains high concentrations of catechins such as (0)-epicatechin (EC), (0)-epigallocatechin (EGC), (0)-epicatechingallate (ECG) and (0)- epigallocatechin gallate (EGCg). Isogai *et al*¹² reported synergy between green tea extract and levofloxacin against enterohaemorrhagic

Table. Death rates kinetics of test bacterial strains cultured in nutrient broth supplemented with black tea extract equivalent to MIC of respective pathogen

Test organism	Log ₁₀ cfu/ml									Tea extract MIC (mg/ml)
	Incubation time (h)									
	0	2	4	8	10	12	16	20	24	
<i>Shigella dysenteriae</i>	6.30	3.00	3.34	2.78	3.00	0	0	0	0	9.09
<i>Yersinia enterocolitica</i>	4.29	3.87	3.70	3.60	3.00	2	0	0	0	47.30
<i>E. coli</i>	3.48	3.08	2.89	2.21	1.51	0	0	0	0	88.30
<i>S. typhi</i>	4.60	3.80	3.40	3.10	2.50	0	0	0	0	79.56
<i>S. typhi</i> Ty2a	4.60	4.00	3.30	2.00	0.00	0	0	0	0	91.98
<i>S.typhimurium</i> 1402/84	7.74	5.04	4.90	3.46	4.00	0	0	0	0	94.61
MIC, minimum inhibitory concentration; cfu, colony forming units										

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Escherichia coli. Susceptibility of bacterial strains to the tea extract has been shown to be related to differences in cell wall components¹³. Catechins partitioning in the lipid bilayer membrane result in loss of cell structure and function and finally the cell death¹³⁻¹⁶. We studied the antimicrobial activity of tea in combination with other antibiotics on enteropathogens.

Clinical isolates *Salmonella typhimurium* 1402/84, *S. typhi*, *S. typhi* Ty2a, *S. dysenteriae*, *Yersinia enterocolitica* C₇₇₀, and *Escherichia coli* (EPEC P₂ 1265) were procured from Departmental culture collection. Human faecal isolates, *Salmonella typhimurium* 98 (procured from Institute of Microbial Technology, Chandigarh) and *S. typhi* 6 (provided by Dr Shobha Ram, Department of Microbiology, Daya Nand Medical College, Ludhiana) were also obtained. Strains were maintained for long storage on Le Minor medium stabs (meat extract 5g, peptone 10g, sodium chloride 3g, disodium hydrogen orthophosphate 2g, agar 10 g and distilled water to make 1000 ml, pH 7.4) at 4°C in screw-capped tubes. Cultures were maintained for daily use on nutrient agar slants at 4°C.

Lipton brand black tea was purchased from American Embassy, New Delhi, India. It contained 190 mg of flavonoids per 2g black tea containing Orange Pekoe and Pekoe ingredients (as per manufacturer's information). Medium grade green tea (Kangra Asha, Kangra Jwala, TV-23 and TV-3) was from Kangra and Jwala, Himachal Pradesh, India

(kindly donated by Dr P.D. Sharma, University Institute of Pharmacy, Panjab University, Chandigarh, India). Tea samples were stored in plastic bags at 4°C. Crude tea extract was prepared following the method described by Yam *et al*¹⁷. Boiling water (100 ml) was added to 2 g of tea leaves and mixture was filtered after standing for 10 min. The resulting 2 per cent tea extract was labeled as boiled tea extract and was stored at 4°C. Known volume of tea extract was dried in preweighed crucibles in oven at 100°C for finding concentration of tea in mg/ml. Organic solvent tea extract was prepared by the method of Hertog *et al*¹⁸. Tea was extracted with methanol: water mixture (62.5:37.5 v/v) for two hour. The extract was concentrated to one-fifth volume, filter sterilized and stored at 4°C.

Antibiotic disc impregnated with chloramphenicol, kanamycin, tetracycline, gentamycin, methicillin, nalidixic acid and chloramphenicol in powder form was purchased from Hi-Media Laboratories Limited Mumbai, India.

Test bacterial strain was grown in nutrient broth for 16-18 h at 37°C on rotary shaker. Cells were harvested by centrifugation (8,000 g, 15 min, 4°C). Bacterial pellet thus obtained was given three washings with sterile phosphate buffer saline (0.1 M, pH 7.2) and finally suspended in the same buffer. Two-fold serial dilutions of black tea-extract or antibiotic were made in sterile nutrient broth. Each dilution was inoculated with 10 µl of 1:100 diluted overnight

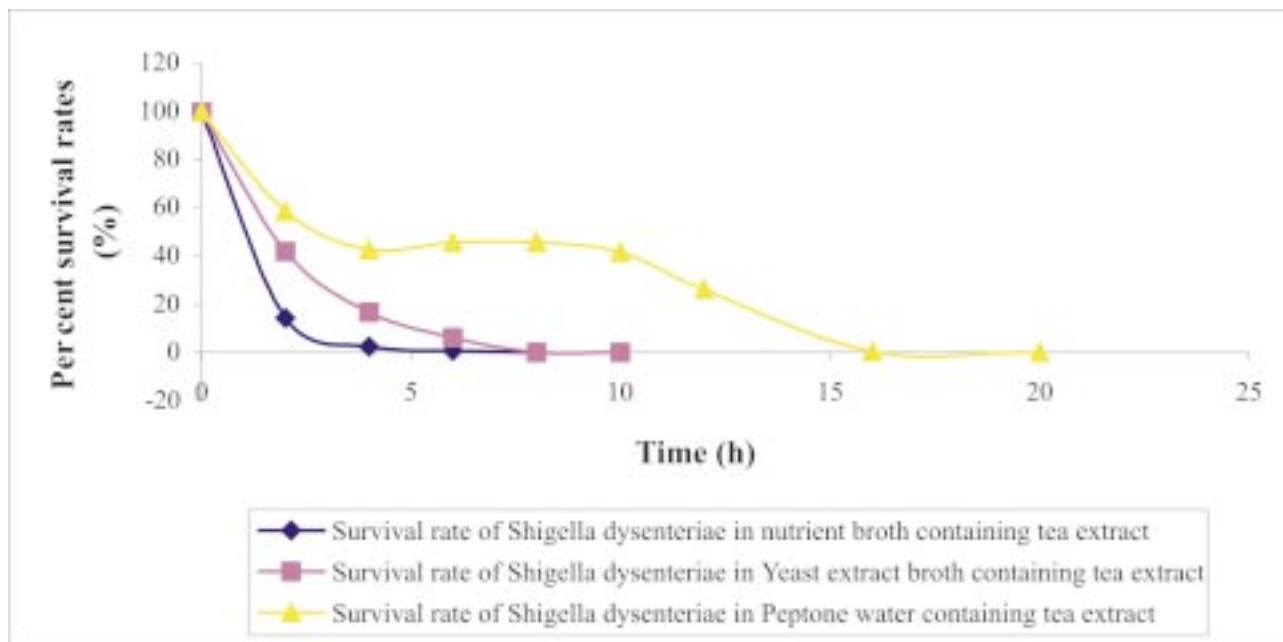


Fig. Survival rates of *Shigella dysenteriae* in different growth media (nutrient broth, yeast extract broth and peptone water) containing black tea extract (9.09 mg/ml).

grown test bacterial cultures and incubated at 37°C. Next day, the tubes were examined visually for growth (turbidity) and no growth (no turbidity). The highest dilution inhibiting the growth was taken as minimum inhibitory concentration (MIC). A loopful from the highest dilution streaked on nutrient agar plates which did not show any bacterial growth after overnight incubation was taken as minimum bactericidal concentration (MBC).

Nutrient medium (20 ml/ 100 ml flask) with and without boiled black tea extract (concentration equivalent to MIC for the test-strains) in duplicates was inoculated with 20 µl of 1:100 diluted overnight grown test bacterial cultures. Samples withdrawn at intervals were used for determining bacterial viable counts by spread plate on nutrient agar plate.

Standard method of Bauer *et al*¹⁹ was used for determining antibiotic sensitivity of *S. dysenteriae*. Based on the antibiotic sensitivity pattern, chloramphenicol was selected for confirmation of synergistic effect with tea extract. Chloramphenicol (MIC 5 µg/ml) was added at sub-inhibitory concentration (2.5 µg/ml) to the growth medium containing boiled tea extract (2.01-9.089 mg/ml) in separate tubes (2 ml/ tube). All these tubes were

inoculated with 10 µl actively growing young culture. The tubes were examined for growth inhibition after incubating at 37°C for 24 h.

Our results showed wide differences in the MIC (9.089-94.61 mg/ml) of tea extract against different bacterial strains *e.g.*, *S. typhimurium* 1402/84 (94.61 mg/ml) > *S. typhi* Ty2a (91.98mg/ml) > *S. typhi* (79.56 mg/ml) > *E. coli* (88.30 mg/ml) > *Y. enterocolitica* (47.30 mg/ml) > *S. dysenteriae* (9.09 mg/ml) (Table). Differences were also seen in the MICs of different tea extracts against *S. dysenteriae* which were in this order: organic solvent green tea extract 3.3 mg/ml < boiled green tea extract 6.27 mg/ml < black tea extract 9.09 mg/ml. MIC of green tea organic solvent extracts (Kangra Jwala) was lowest (3.3 mg/ml) followed by boiled water green tea extract (6.27 mg/ml) and green tea infusion (6.94 mg/ml). Enhanced antibacterial activity with organic solvent extracts may be due to higher content of catechin (30-40% w/w) and some oil fractions besides water-soluble fractions¹⁵. Differences in antibacterial activity of tea were seen with respect to test bacterial strain, type of tea, and method of extraction; all the green tea extracts showed better antimicrobial activity as compared to the black tea.

Based on death rate kinetics of enteropathogens used in this study, bacterial viable count was less than 1/ml after 10 h for *S. typhi* Ty2a, 12 h for *S. dysenteriae*, *E. coli*, *S. typhi*, *S. typhimurium* and >16 h for *Y. enterocolitica*. Differences in MIC values of bacteria may be related to differential susceptibility of bacterial cell wall, which is the functional barrier and minor differences present in outer membrane in the cell wall composition²⁰.

S. dysenteriae was used for comparing antibacterial activity of different tea extract and studying synergistic activity with antibiotics. Differences were observed in the survival rates of *S. dysenteriae* in boiled black tea extract or tea extract equivalent to MIC added to different growth media (Fig). *S. dysenteriae* was found to be more susceptible to growth inhibition by chloramphenicol, gentamycin, methicillin and nalidixic acid as the zones of inhibition were wider ranging from 1 to 4 mm on nutrient agar plates supplemented with 4.415 mg/ml black tea extract as compared to the zones of inhibition on nutrient agar plates without tea extract. Growth inhibition of *S. dysenteriae* at low concentration of chloramphenicol (2.5 µg/ml) and tea extract (5.09 mg/ml) as compared the MIC of individual agent (chloramphenicol 5 µg/ml or black tea extract 9.09 mg/ml) further confirm the synergistic activity. Synergistic microbial growth inhibition by black tea extract and antibiotics could be attributed to the presence of dual binding sites on the bacterial surface for antibiotic and tea extract. The results are in agreement to the marked reduction in MIC of oxacillin and β lactams reported in presence of epicatechin gallate in methicillin resistant *S. aureus* and enhanced effect of Japanese tea on inhibitory activities of antibiotics against MRSA strains²⁰⁻²². The combined use of tea and antibiotics could be useful in fighting emerging drug-resistance problem especially among enteropathogens²³. Further, *in vivo* experiments are needed to confirm these findings.

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