

In vitro antibacterial activity of green tea (*Camellia sinensis*) extract against *Staphylococcus aureus* and MRSA

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Abstract

Context: There is a worldwide emergence of multi drug resistant bacteria. Recently no new antibiotics have been approved for use, although some are in the pipeline, undergoing phase 2 or 3 clinical trials. There is need for alternate compounds for treatment; one such agent is green tea. Green tea has antimicrobial effects against a variety of Gram positive and Gram negative bacteria (e.g., *Staphylococcus aureus*, *Enterococcus* sp, *Escherichia coli*, *Salmonella* sp.)

Aims: To determine the antibacterial activity of green tea extract against Methicillin resistant and sensitive *Staphylococcus aureus* (MRSA & MSSA)

Settings and Design: Department of Microbiology, Cross sectional study

Materials and Methods: A total of 15 consecutive laboratory isolates of *S. aureus* (MSSA) and MRSA each, isolated between August to September 2017 were inoculated in media containing Green tea extract [epigallocatechin gallate (EGCG)] in concentrations of 2.5mg/ml, 5mg/ml, 10mg/ml. After overnight incubation the bacterial broth was subcultured using standard calibrated loop (0.01ml) onto Mueller Hinton agar. The lowest concentration of EGCG in which no growth occurred was considered as the minimum bactericidal concentration (MBC).

Results: EGCG had antimicrobial activity against *S. aureus* ATCC-25923, *P. aeruginosa* ATCC-27853, *K.pneumoniae* ATCC-BAA 1705 and *Esch.coli* ATCC-25922- MBC 99.9% was 5mg/ml. But for *E.faecalis* ATCC 29212 MBC 99.9% was 10mg/ml. EGCG was effective against clinical isolates of MSSA and MRSA with MBC of 5mg/ml except 2 MRSA isolates for which MBC was 10mg/ml.

Conclusions: Green tea extract- EGCG has antibacterial activity not only against standard bacterial strains but also against clinical isolates of *S.aureus* and MRSA.

Keywords: *Camellia sinensis*, *S.aureus*, Minimum bactericidal concentration.

Introduction

Antimicrobial resistance has been increasing over the years due to overuse of antibiotics.¹ Hence trivial infections are progressing to become life threatening diseases. *S. aureus* are highly pathogenic and rate of infection caused by these microorganisms have increased in recent years. With increase in multi drug resistance there is a need for alternate compounds for treatment. One such agent is green tea extract.²

Camellia sinensis (green tea) has a wide range of antioxidant, anti inflammatory, anti-carcinogenic and antibacterial activity against many pathogens.² Green tea is used in the treatment of cardiovascular diseases and obesity. It is safe, non toxic and has no side effects.³ Green tea has antimicrobial effects against a variety of Gram positive and Gram negative bacteria(e.g., *Staphylococcus aureus*, *Enterococcus* sp, *Escherichia coli*, *Salmonella* sp), some fungi(e.g., *Candida albicans*), and a variety of viruses(e.g., HIV, *Herpes simplex virus*, *Influenza virus*).^{4,5}

The chemical composition of green tea is complex and incompletely defined. The most abundant components in green tea are polyphenols, mostly flavonoids such as catechins, catechingallates and proanthocyanidins.⁶

The biological properties of green tea are due to the catechin fraction, which constitutes up to 30% of the

dry leaf weight. There are four main catechins in tea. Epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3- gallate (EGCG). EGCG is the most abundant, representing approximately 59% of the total catechins.⁷

The EGCG-induces damage to the bacterial cell wall with possible interference of its biosynthesis through direct binding with peptidoglycan. This may be one of the major reasons for its antimicrobial activity against Methicillin sensitive and resistant *S. aureus* (MSSA & MRSA).⁸

The present study was designed to assess the in vitro antibacterial activity of green tea extract against MSSA and MRSA isolated from clinical specimens with an objective to design possible therapeutic approach to infections caused by them, by determining Minimum Bactericidal Concentration (MBC) of epigallocatechin gallate (EGCG), extracted from green tea.

Materials and Methods

The present study (IEC-RC/17/02) was reviewed by the Institute Ethics committee and a waiver of consent was obtained as it did not involve human participants. The cross sectional study was conducted in the Department of Microbiology, Pondicherry Institute

of Medical Sciences, Puducherry between August to September 2017. During this period 15 consecutive isolates each of MSSA and MRSA were included.

All *S. aureus* were identified as per standard microbiological protocols.⁹ MRSA was identified based on resistance to antibiotic disc Cefoxitin (30 µg) with inhibition zone diameter <22 mm by Standard Disc diffusion method as per CLSI guidelines.¹⁰ A second screening test for MRSA was performed with 6 mg of oxacillin per ml on Mueller Hinton (MH) agar supplemented with 4% NaCl.¹⁰

Commercially available green tea extract with active ingredient being (-)-epigallocatechin gallate (EGCG) manufactured by Sigma Aldrich (Lot No SLBP0484V) was used in this study. Minimum Bactericidal Concentration (MBC) of Green Tea extract against MSSA and MRSA was done by Broth Dilution method. Different concentrations of EGCG (2.5mg/ml, 5mg/ml, 10mg/ml) was prepared using Mueller hinton broth. These concentrations were based on the pilot study. *S. aureus* was inoculated into peptone water and the turbidity was matched to 0.5 Mc Farland standard (Himedia, India) corresponding to bacterial density of 1.5 X 10⁸ cfu/ml. 100µl of bacterial broth and 100 µl of EGCG (different concentrations were tested) in microtitre wells was incubated at 37°C overnight (Fig 1). The bacterial broth was subcultured using standard calibrated loop (0.01ml) onto Mueller Hinton agar. The lowest concentration of EGCG in which no growth occurred was defined as the MBC. Appropriate controls of uninoculated Mueller Hinton broth without any supplements, uninoculated Mueller Hinton broth (MHB) containing Green tea extract at a concentration of 2.5mg/ml, 5mg/ml and 10mg/ml as EGCG control and 200µl of bacterial isolate in MHB without Green tea extract were used.

Staphylococcus aureus ATCC 25923, *Esherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC BAA 1705 were also tested for assessing efficacy of EGCG.

Results and Discussion

The results of the study showed that the green tea extract epigallocatechin gallate (EGCG) exhibited a potent antibacterial activity as detected by minimum bactericidal activity. The antimicrobial activity of

commercial EGCG against Standard bacterial strains and Clinical isolates of MSSA and MRSA is depicted in Tables 1 and 2 respectively.

Control strains, MSSA and MRSA showed growth in the absence of EGCG.

Amongst the standard bacterial strains EGCG had highest antimicrobial activity with 99.9% MBC at a concentration of 5mg/ml against *S. aureus* ATCC-25923, *P. aeruginosa* ATCC-27853, *K.pneumoniae* ATCC-BAA 1705 and *Esch.coli* ATCC-25922. Against *E.faecalis* ATCC 29212, 99.9% MBC was 10mg/ml concentration (Fig. 2 & 3).

Amongst the 15 isolates of MRSA, EGCG exhibited antimicrobial activity. MBC was 5mg/ml except two isolates 5 and 10. For isolate number 5 and 10 MBC was 10mg/ml (Fig. 3 & 4). Amongst the 15 isolates of MSSA, MBC was 5 mg/ml (Fig. 3). At 2.5mg/ml of EGCG all standard bacterial strains, MSSA and MRSA there was growth (Fig. 4).

The active ingredient of Green tea, *Camellia sinensis* has been reported to have antibacterial activity against various pathogenic bacteria including MRSA.¹¹ The present study showed that commercially available Epigallocatechin gallate (EGCG) from green tea extract has antimicrobial activity not only against standard ATCC strains but also against clinical isolates of MSSA and MRSA. Our observations are in accordance with other studies which have shown that green tea extract exhibit antimicrobial activity against highly resistant bacterial strains such as MRSA.¹¹⁻¹⁶

Green tea catechins interfere with bacterial DNA replication by inhibiting the function of DNA gyrase. The polyphenols of green tea can inhibit the enzyme dihydrofolate reductase in bacteria and yeast effectively blocking the ability of microbes to synthesize purines and pyrimidine.⁷ Studies have suggested that main polyphenolic component of green tea is Epigallocatechin gallate which can lead to reversal of methicillin resistance in MRSA by inhibiting synthesis of PBP2.^{7,12,17}

Further studies need to be carried out to determine the optimal concentration of green tea extract required for its inhibitory activity. Moreover, it needs to be determined whether these concentrations can be achieved and sustained in vivo. The EGCG plasma concentration after intake of Green tea needs to be evaluated.

Table 1: MBC of EGCG against Standard bacterial strains

ATCC strains	MBC
<i>Staphylococcus aureus</i> ATCC 25923	5mg/ml
<i>Escherichia coli</i> ATCC 25922	5mg/ml
<i>Pseudomonas aeruginosa</i> ATCC 27853	5mg/ml
<i>Enterococcus faecalis</i> ATCC 29212	10 mg/ml
<i>Klebsiella pneumoniae</i> ATCC BAA 1705	5mg/ml

Table 2: MBC of EGCG against *Staphylococcus aureus* and MRSA isolates

MRSA Isolate Number	MBC	MSSA Isolate Number	MBC
1	5mg/ml	16	5mg/ml
2	5mg/ml	17	5mg/ml
3	5mg/ml	18	5mg/ml
4	5mg/ml	19	5mg/ml
5	10mg/ml	20	5mg/ml
6	5mg/ml	21	5mg/ml
7	5mg/ml	22	5mg/ml
8	5mg/ml	23	5mg/ml
9	5mg/ml	24	5mg/ml
10	10mg/ml	25	5mg/ml
11	5mg/ml	26	5mg/ml
12	5mg/ml	27	5mg/ml
13	5mg/ml	28	5mg/ml
14	5mg/ml	29	5mg/ml
15	5mg/ml	30	5mg/ml

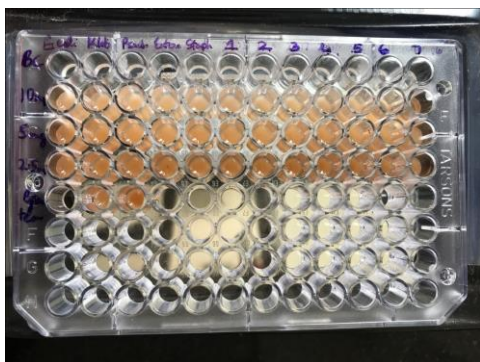


Fig. 1: Microtitre wells with EGCG and Bacterial broth

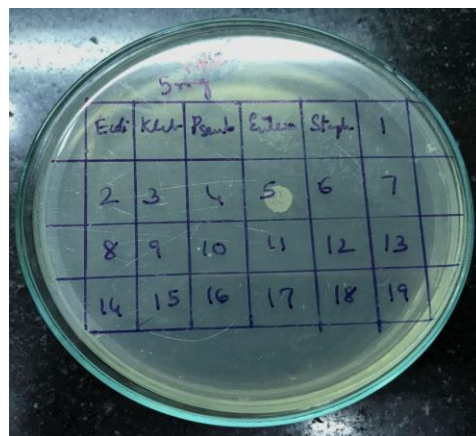


Fig. 3: EGCG of 5 mg/ml with bacterial broth of ATCC strains, *S.aureus* and MRSA isolates subcultured onto MHA

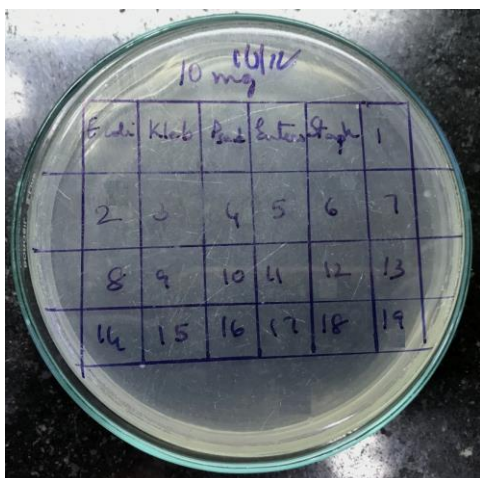


Fig. 2: EGCG of 10mg/ml with bacterial broth of ATCC strains, *S.aureus* and MRSA isolates subcultured onto MHA

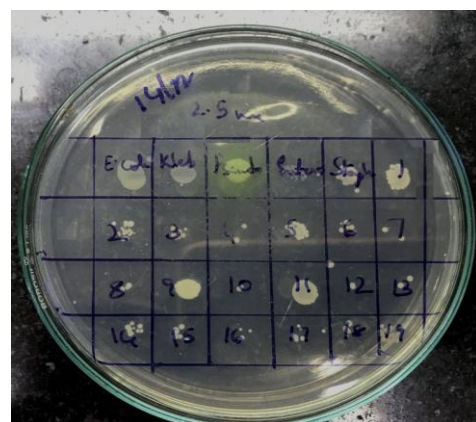


Fig. 4: EGCG of 2.5 mg/ml with bacterial broth of ATCC strains, *S.aureus* and MRSA isolates subcultured onto MHA

Limitations

Only few clinical isolates were tested in the present study. The antibacterial activity of green tea extract EGCG against multi drug resistant bacteria like MDR *Acinetobacter*, *Pseudomonas* and other nosocomial pathogens were not evaluated.

Conclusion

Green tea (*Camellia sinensis*) extract EGCG which is commercially available acts as a potent antibacterial agent. It is effective against *S. aureus* ATCC-25923, *P. aeruginosa* ATCC-27853, *K.pneumoniae* ATCC-BAA 1705 and *Esch.coli* ATCC-25922, *E.faecalis* ATCC 29212 and against clinical isolates of *S.aureus* and MRSA. These green tea extract (polyphenolic compounds) can be used as singly or as adjuvant therapeutic agent in the treatment of multidrug resistant infections.

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