Short Communication

THE SYNERGY OF GREEN TEA AND PENICILLIN G AGAINST *BACILLUS SUBTILIS*

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ABSTRACT

Tea is extracted from the leaves of *Camellia sinenesis*. Methanoic extract of black tea samples diluted in ethanol has been shown to enhance antibacterial activity against *Streptococcus mutans* in combination with Chloramphenicol, Levofloxacin and Gentamycin. This study investigated the influence of boiled water green tea extract on the effectiveness of Penicillin G against the *Bacillus subtilis* bacterium. Using a range of dilutions of green tea solution, a positive relationship ($p<0.001$) was found between the relative green tea concentration and the area of inhibition.

Key words: Antibacterial activity, green tea, *Bacillus subtilis*, synergistic activity

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Running Title: Green tea synergy

INTRODUCTION

The beverage tea is produced from the plant *Camellia sinenesis*. A recent investigation reported that the antibacterial activity obtained using methanoic extract of black tea diluted in ethanol is enhanced in combination with Chloramphenicol, Levofloxacin and Gentamycin when applied against *S. mutans* (Mughal et al., 2010). However, information regarding possible synergy between green tea extracts and antibiotics is scant. This study investigated the modification of the antibacterial activity of aqueous green tea extract in conjunction with Penicillin G against the gram positive bacterium *Bacillis subtilis.*

MATERIAL AND METHODS

Dried green tea leaves of mass 10g were packed into small metal thimbles and immersed in boiling distilled water ($100cm^3$) for 10 minutes. The contents were drained into a sterilised beaker to form the stock solution. Samples of green tea extract with relative concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 were prepared; the sample of relative

concentration of 1 was obtained by diluting the stock solution with distilled water in the ratio 1:4. In addition, distilled water was used as a control solution.

Bacterial samples of *Bacillus subtilis* were cultured on nutrient agar using Petri dishes, with three antibiotic discs (Penicillin G, 6μg) on each surface. Agar samples had been prepared by agar dilution (Wiegand et al., 2008) using the green tea extract samples of the respective concentrations (agar: tea extract 3:1); there were three Petri dishes per concentration. The dishes were sealed and incubated at 30°C for 24 hours.

Following incubation, the area of each inhibition zone was estimated. Inhibition area was plotted against green tea concentration. Strength of association was measured using Pearson's correlation. A linear regression line was fitted to the data, and a statistical test based on the *t*-distribution (Bland, 1995) was applied to determine whether the gradient observed was statistically significant.

RESULTS

A positive association (Pearson's correlation $r = 0.755$) was found between the inhibition area and the relative concentration of green tea extract. The regression line, shown in the Figure, indicates a relationship of strong statistical significance ($p<0.001$).

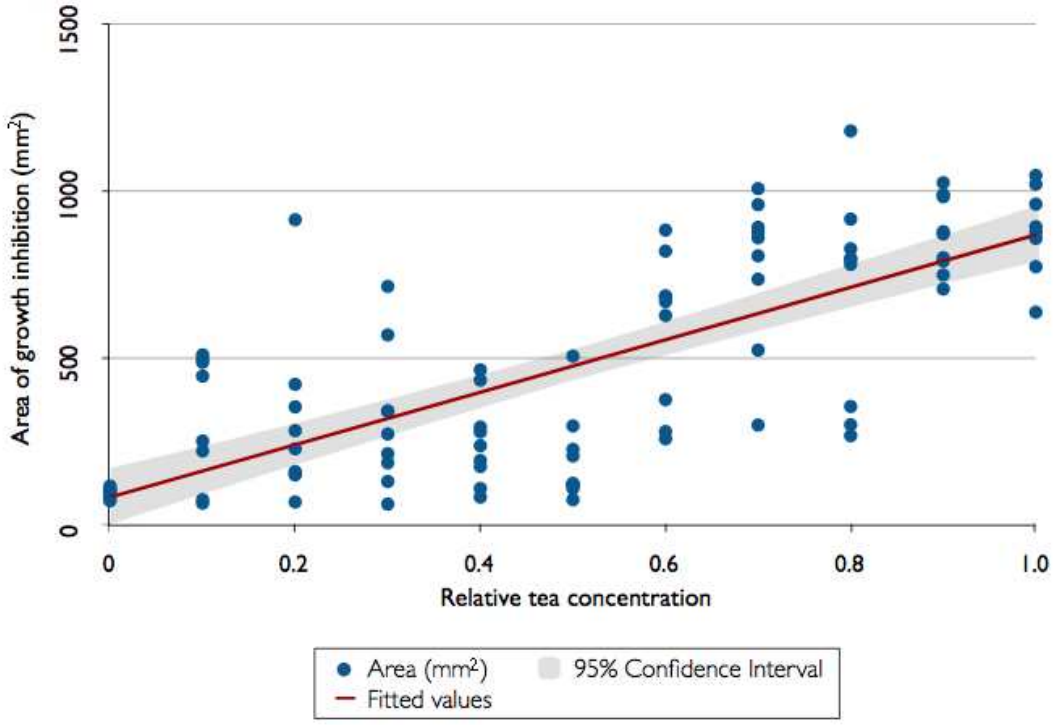

Figure 1. Relationship of growth area inhibition and relative tea concentration; statistical significance ($p<0.001$).

These findings suggest synergism between green tea extract and antibiotics, in like manner to that reported by Mughal et al. (2010) for black tea extract. Mughal et al. investigated synergism for Chloramphenicol, Tetracycline, Levofloxacin and Gentamycin using black tea extract concentrations between 0.1 mg/ml and 1.0 mg/ml. The minimum inhibitory concentration (MIC) values found were 0.1 mg/ml for Chloramphenicol, Levofloxacin,and Gentamycin. Tetracycline, with an MIC value of 0.5 mg/ml, was found not to enhance antibacterial activity. It was not possible to estimate the MIC value for Penicillin G with green tea extract as synergy was observed even at the lowest extract concentration.

It is difficult to make a direct comparison between the green tea extract and black tea extract studies. Firstly, different ranges of tea extract concentrations were used; based on the method of preparation for the stock solution the concentrations of green tea extract in the agar were much greater, being in the range 0.5 mg/ml to 5.0 mg/ml. Secondly, this study had an aqueous solvent whereas the black tea extract study used methanol, and methanolic extract diluted in ethanol. Jonathan and Fasidi (2003) reported that compared to water, methanol is a more effective solvent for extracting antimicrobial substances. Thirdly, this investigation involved agar dilution whereas the well diffusion method was used by Mughal et al. Finally, different antibiotics were tested in the two studies. In the light of these differences in study design, a comparison of green tea extract and black tea extract under the same experimental conditions is required.

CONCLUSION

This investigation has shown that aqueous green tea extract solution significantly enhances the antibacterial effect of Penicillin G against the *Bacillus subtilis* bacterium. These findings highlight the potential that green tea extract may offer in addressing the growing problem of bacterial resistance to antibiotics. However, more research is merited, to determine whether similar effects occur with other types of solvent, antibiotic and bacteria.

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