

Epigallocatechin Gallate-Stearate Enhances the Efficacy of Antibiotics

Ayuni Yussof^{1,2}, Umme Habiba², Deborah Liaw², Tinchun Chu^{1*}, Lee H. Lee^{2*}

¹Department of Biological Sciences, Seton Hall University, South Orange, NJ, USA

²Department of Biology, Montclair State University, Montclair, NJ, USA

Email: *Tin-Chun.Chu@shu.edu, *Lee.Lee@montclair.edu

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Abstract

Introduction: The rise in antibiotic resistant cases has caused a global concern. Researchers around the world are trying to find a novel alternative to combat this issue. Green tea with its many health benefits, including antibacterial and antiviral activity, has shown to be one of the most promising candidates to be used as an agent to solve this problem. **Objective:** This study focuses on evaluating the synergistic effects of antibiotics and two green tea polyphenols: epigallocatechin gallate (EGCG), and its modified lipophilic form epigallocatechin gallate stearate (EGCG-S). **Methods:** In this study, twelve antibiotics and eight bacteria: Gram-positive *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*) and *Bacillus megaterium* (*B. megaterium*); Gram-negative *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Serratia marcescens* (*S. marcescens*), and *Enterobacter aerogenes* (*E. aerogenes*); and acid-fast *Mycobacterium smegmatis* (*M. smegmatis*) were used. Antibacterial synergism profiling of EGCG, EGCG-S and antibiotics has been established using a disk diffusion assay. **Results:** The results revealed that both 1% of EGCG and 1% EGCG-S enhanced the antimicrobial activities on antibiotics in various bacteria. Antimicrobial susceptibility study indicated that EGCG-S was able to enhance some antibiotics from the resistant category to intermediate or susceptible and/or from intermediate category to susceptible. Both EGCG and EGCG-S worked comparably on Gram-positive bacteria; in *S. aureus*, both compounds enhanced 5 antibiotics (AM10, CF30, C30, S10 and TE30) activities while EGCG-S had higher efficiency. *B. megaterium* were susceptible to most of the antibiotic treatment, thus the impact of EGCG and EGCG-S was insignificant. EGCG-S worked better than EGCG on Gram-negative bacteria; converted 9 antibiotics susceptibility in *E. coli* and *P. aeruginosa*, and 8 antibiotics in *E. aerogenes*. EGCG and EGCG-S also showed synergism on acid-fast bacteria *M. smegmatis* with EGCG-S has much higher efficiency than EGCG.

Conclusion: The results suggested that EGCG-S might be a promising anti-bacterial synergistic agent with antibiotics to combat antibiotic-resistant bacteria.

Keywords

EGCG, EGCG-Stearate, Antibiotic Resistance, Disk Diffusion Methods

1. Introduction

Antibiotics have been a key element of modern medicine, leading to their extended use in healthcare and agriculture [1]. The extensive global use of antibiotics, lack of new and effective antibiotics and increased spread of multi-drug resistant bacteria have led to the looming threat to global health [2]. This rapid accumulation of antibiotic resistance suggests a reservoir of transferable antibiotic resistance gene determinants in response to antibiotics that were found in hospitals, environmental isolates, and even in permafrost DNA [3]. Evidence of antibiotic resistance in hospitals suggests they may contribute to increasing rates of nosocomial infections or hospital-associated infections. A group of pathogens named “ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter species*)” has been widely studied because they are the leading cause of nosocomial infections and are generally characterized by multi-drug resistance [4] [5]. With multi-drug resistance representing one of the top threats to public health globally, novel natural alternatives are tested to help combat the rise of antibiotic resistant bacteria.

Green tea extracted from *Camellia sinensis* has been shown to have a wide range of health benefits including anticarcinogenic, anti-atherogenic, antimicrobial and antiviral properties [6]. Green tea extract contains a wide range of tea polyphenols including epigallocatechin-gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC) [2]. EGCG is the most abundant polyphenol with the greatest antibacterial activities among the tea polyphenols [7]. One of the advantages of EGCG is that it is non-toxic and can be applied or consumed without adverse effects, which the Federal Drug Administration (FDA) classifies as a safe compound [8] [9]. Under normal conditions, EGCG is a water-soluble polyphenol oxidized and metabolized rapidly, which leads to the loss of potent antimicrobial abilities quickly [10]. To overcome the stability issue, the modified lipophilic polyphenols could be an effective agent as green tea polyphenols (GTPs) [11]. EGCG-stearate (EGCG-S), lipid soluble tea polyphenols (LTPs) that were prepared chemically and enzymatically, can significantly improve the bioavailability of GTPs [12] [13].

Gram-negative bacteria like *Escherichia coli* (*E. coli*), *Enterobacter aerogenes* (*E. aerogenes*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Serratia marces-*

cens (*S. marcescens*) antibiotic resistance reached critical level with limited treatment options [14]. Some of the commonly isolated gram-positive bacteria like *Bacillus megaterium* (*B. megaterium*), *Staphylococcus aureus* (*S. aureus*), and *Staphylococcus epidermidis* (*S. epidermidis*) have been shown to have an increase number of resistant cases [15]. EGCG has been shown to have an inhibitory effect on both Gram-positive and Gram-negative bacteria, but the exact mechanism has not been determined [16] [17] [18].

This study uses both EGCG and EGCG-S along with twelve different antibiotic disks to evaluate the effect of these polyphenols on antibiotics in eight different Gram+, Gram– and acid-fast bacteria. The combination of antibiotics with either EGCG or EGCG-S provides insight into whether the addition of either compound can produce a synergistic effect and enhance the antimicrobial activity by these antibiotics.

2. Material and Methods

2.1. Culture Preparation and Maintenance

Eight microorganisms were included in this study: Gram-positive, Gram-negative, and acid-fast. Gram-positive bacteria: *Bacillus megaterium* (*B. megaterium*), *Staphylococcus aureus* (*S. aureus*), and *Staphylococcus epidermidis* (*S. epidermidis*); Gram-negative bacteria: *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Serratia marcescens* (*S. marcescens*), and *Enterobacter aerogenes* (*E. aerogenes*); and acid-Fast bacteria: *Mycobacterium smegmatis* (*M. smegmatis*) (Carolina Biological Supply Co.). These bacteria were maintained in nutrient broth (NB) and nutrient agar (NA) plate (BD Difco). The media was prepared according to manufacturer directions.

An overnight culture of each bacterium was prepared for each experiment. The overnight culture was grown at 37°C with a constant shaking at 250 rpm except for *Serratia marcescens*, which was grown at room temperature without any shaking. The overnight cultures were checked for purity before used in each experiment.

The stock culture was prepared and stored at 4°C and an original stock for each bacterium was stored at 30% glycerol at –80°C.

2.2. EGCG and EGCG-S Solution Preparation

Pure compound of EGCG and EGCG-S were purchased from Camellix, LLC (Augusta, GA). The 1% stock solutions were prepared by dissolving 1 gram of EGCG or EGCG-S in 100 ml of 100% ethyl alcohol (200 proof), followed by a 0.45 µm membrane filter sterilization.

2.3. Kirby-Bauer Disk Diffusion Assay

Twelve antibiotics with or without 1% EGCG or EGCG-S were evaluated by Kirby-Bauer assay: Ampicillin (AM10), Bacitracin (B10), Cephalothin (CF30), Chloramphenicol (C30), Doxycycline (D30), Erythromycin (E15), Gentamicin

(GM10), Penicillin (P10), Polymyxin B (PB300), Rifampin (RA5), Streptomycin (S10), and Tetracycline (TE30). The plates were incubated at 37°C for 24 and 48 hours. The zone of inhibition (ZOI) was recorded and the categories, susceptible (S), intermediate (I) or resistant (R), were determined based on the criteria published by the Clinical and Laboratory Standards Institute (CLSI) [19]. All experiments were carried out in triplicates and the mean and standard deviation were calculated. The percentage of inhibition was calculated based on the Equation (1) listed below:

$$\text{Percentage of Inhibition (\%)} = \left[\frac{(A - B)}{B} \right] \times 100 \quad (1)$$

where A is the ZOI of the combined treatment and B is the ZOI of the antibiotic alone.

3. Results

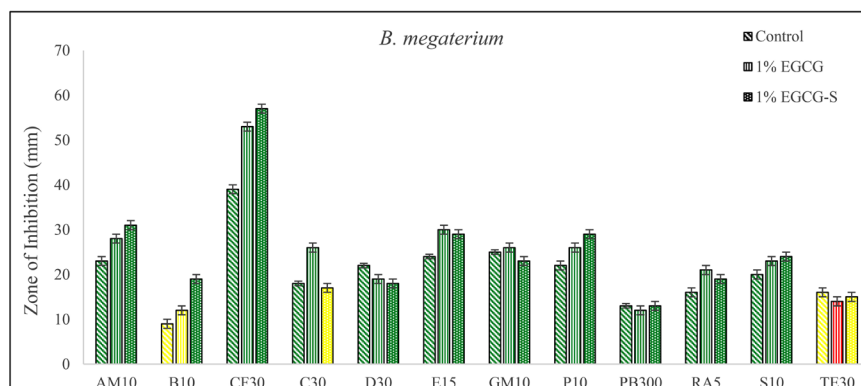
3.1. EGCG and EGCG-S Showed Synergy on Antibiotics in Three Gram-Positive Bacteria

Three Gram-positive bacteria: *B. megaterium*, *S. aureus* and *S. epidermidis*, were used in this study. *B. megaterium* is a Gram-positive bacillus, and an endospore former. The % of increase and % of decrease were calculated using ZOI and shown in **Figure 1(a)**. From the ZOI study, *B. megaterium* was generally susceptible to antibiotic treatments; 10 of 12 antibiotics work on this organism. *B. megaterium* was only intermediate to B10 and TE30. However, addition of EGCG-S converted *B. megaterium* from being intermediate to susceptible to B10 treatment. These results indicate that EGCG-S may be a synergistic agent in enhancing the antimicrobial activity of antibiotics. EGCG and EGCG-S increased the antibiotic efficacy ranging from 4.00% to 44.54% and 18.80% to 112.04% respectively (**Figure 1(b)**). C30 (44.54%) and B10 (112.04%) had the most significant increase for EGCG and EGCG-S respectively. EGCG had a positive impact on 9 antibiotics (AM10, B10, CF30, C30, E15, GM10, P10, RA5 and S10) and EGCG-S had a positive impact on 7 (AM10, B10, CF30, E15, P10, RA5 and S10) with antibiotics.

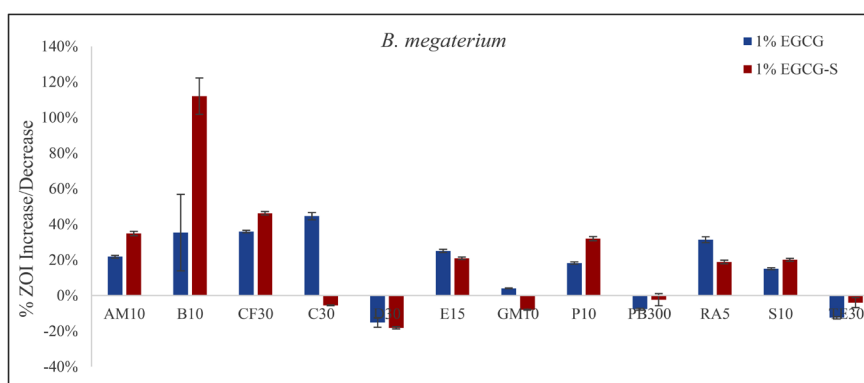
The results from disk diffusion test for *S. aureus* (**Figure 2(a)**) suggest that this bacterium is susceptible to 6 (B10, D30, E15, GM10, PB300, RA5) of 12 antibiotics; intermediate to 4 antibiotics (CF30, C30, S10 and TE30) and resistant to 2 antibiotics (AM10 and P10). Addition of EGCG and EGCG-S changed the category from intermediate to susceptible for all 4 antibiotics (CF30, C30, S10 and TE30). EGCG converted AM10 from resistant to intermediate and EGCG-S converted it to susceptible.

EGCG and EGCG-S increased the antibiotic efficacy ranging from 6.71% to 34.21% and 16.02% to 57.94% respectively (**Figure 2(b)**). S10 (34.21%) and P10 (57.94%) had the most significant increases for EGCG and EGCG-S respectively. Although EGCG-S had the largest positive impact on P10, it still could not convert P10 into a susceptible treatment for *S. aureus*.

The results from disk diffusion test for *S. epidermidis* suggest that this bacterium was susceptible to only three antibiotics (D30, GM10, and S10). It was intermediate to 7 antibiotics (AM10, B10, CF30, C30, PB300, RA5 and TE30) and

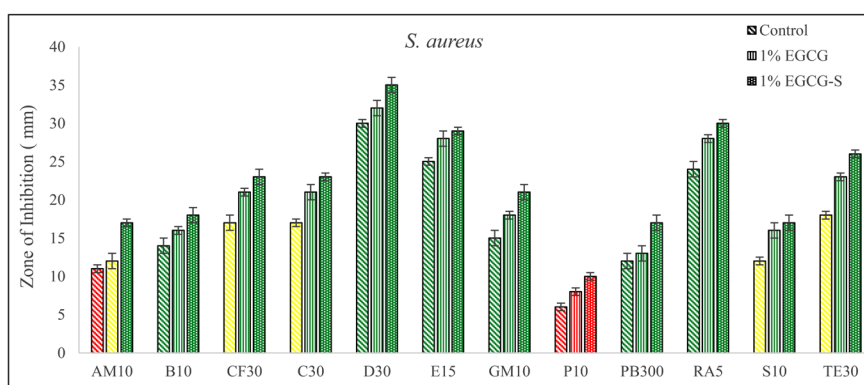


(a)



(b)

Figure 1. (a) The ZOI of *B. megaterium* when treated with 1% EGCG and 1% EGCG-S in combination with various antibiotics. The green color indicates susceptible, yellow indicates intermediate and red indicates resistant. The slanted stripe bar graph indicates antibiotic alone, the vertical stripe bar graph indicates antibiotic plus EGCG and the polka dot bar graph indicate antibiotic plus EGCG-S ($n = 3$); (b) The percentage of ZOI increase/decrease of *B. megaterium* when treated with 1% EGCG and 1% EGCG-S in combination with various antibiotics. The blue bar represent antibiotic plus EGCG, and the red bar represent antibiotic plus EGCG-S ($n = 3$).



(a)

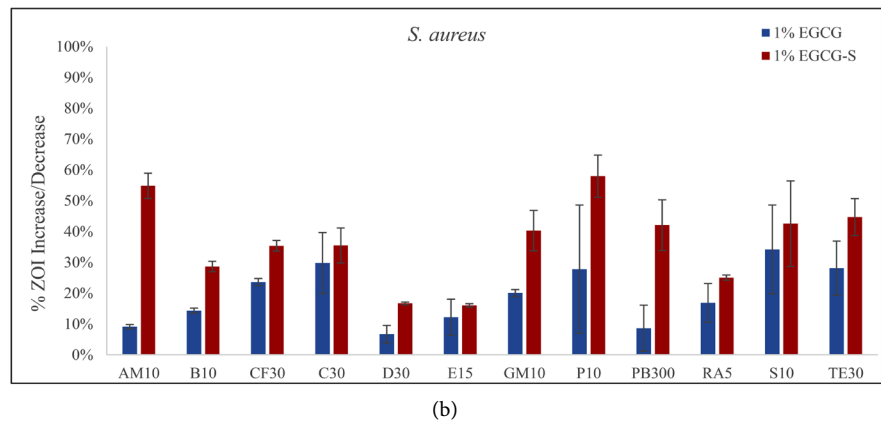


Figure 2. (a) The ZOI of *S. aureus* when treated with 1% EGCG and 1% EGCG-S in combination with antibiotics. The green color indicates susceptible, yellow indicates intermediate and red indicates resistant. The slanted stripe bar graph indicates antibiotic alone, the vertical stripe bar graph indicates antibiotic plus EGCG and the polka dot bar graph indicate antibiotic plus EGCG-S (n = 3); (b) The percentage of ZOI increase/decrease of *S. aureus* when treated with 1% EGCG and 1% EGCG-S in combination with antibiotics. The blue bar represents antibiotic plus EGCG, and the red bar represents antibiotic plus EGCG-S (n = 3).

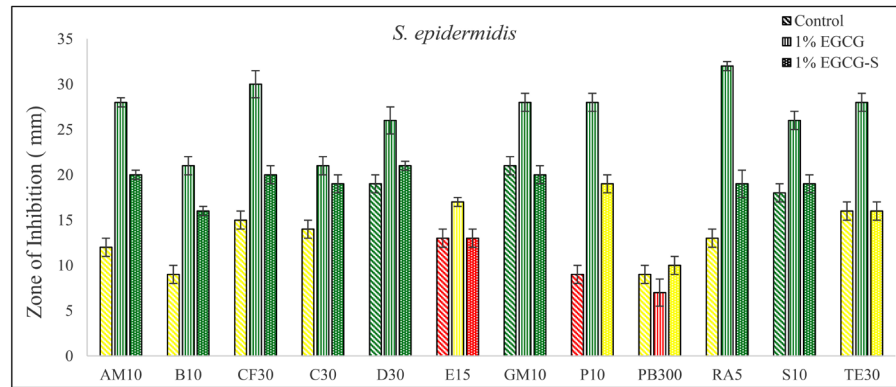
was resistant to E15 and P10. With EGCG, the antibiotic profiles changed and *S. epidermidis* became susceptible to all of the antibiotics except E15 and PB300. EGCG-S changed most antibiotic profiles to susceptible with the exception of E15, PB300, and TE30. EGCG-S changed P10 to the intermediate category (**Figure 3(a)**). EGCG and EGCG-S increased the antibiotic efficacy ranging from 30.89% to 216.20% and 2.78% to 112.04% respectively. P10 had the most significant increase for both EGCG and EGCG-S (**Figure 3(b)**).

3.2. EGCG and EGCG-S Showed Synergy with Antibiotics in Four GRAM-NEGATIVE BACTERIA

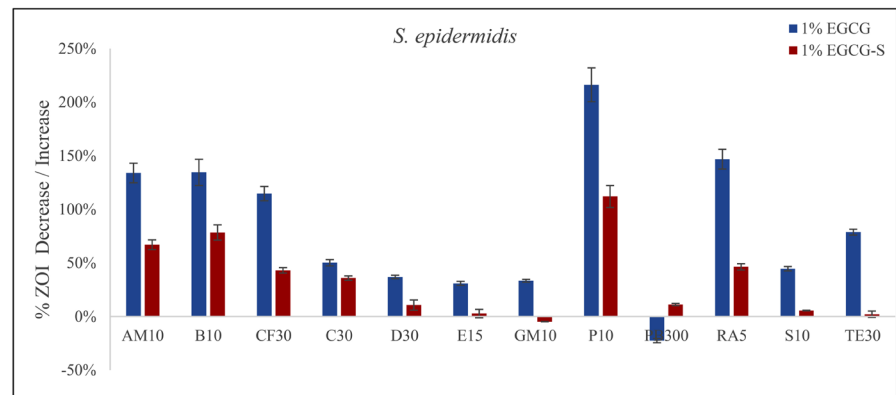
Four Gram-negative bacteria, *E. aerogenes*, *E. coli*, *P. aeruginosa* and *S. marcescens* were used in this study. From the ZOI study, *E. aerogenes* was very resistant to most of the antibiotic treatments, with resistance to 9 (AM10, B10, CF30, D30, E15, P10, PB300, RA5, and TE30) antibiotics; intermediate to S10; and susceptible to only C30 and GM10. In the presence of EGCG, 4 (AM10, B10, PB300, and RA5) out of 9 antibiotics changed categories from resistant to susceptible, and 2 (E15 and P10) antibiotics changed from resistant to intermediate. It also changed S10 from intermediate to susceptible. EGCG was not able to convert CF30, D30 and TE30, thus *E. aerogenes* is still resistant to these three antibiotics. In the presence of EGCG-S, it converted PB300 from resistant to susceptible, 6 (AM10, B10, D30, P10, RA5 and TE30) antibiotics from resistant to intermediate, and S10 from intermediate to susceptible. Although the ZOI increased, EGCG-S did not convert susceptibility category on CF30 or E15 (**Figure 4(a)**).

EGCG and EGCG-S increased the antibiotic efficacy ranging from 19.32% to

144.44% and 12.66% to 164.29% respectively (Figure 4(b)). AM10 (144.44%) had the most significant increase with EGCG converting the antibiotic profile

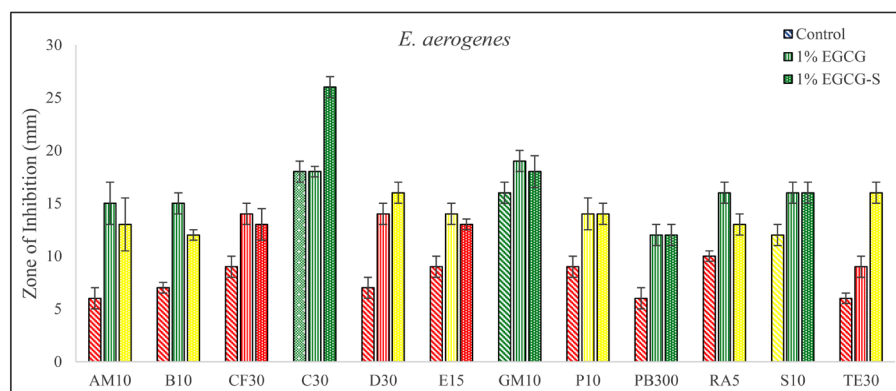


(a)



(b)

Figure 3. (a) The ZOI of *S. epidermidis* when treated with 1% EGCG and 1% EGCG-S in combination with antibiotics. The green color indicates susceptible, yellow indicates intermediate and red indicates resistant. The slanted stripe bar graph indicates antibiotic alone, the vertical stripe bar graph indicates antibiotic plus EGCG and the polka dot bar graph indicate antibiotic plus EGCG-S (n = 3); (b) The percentage of ZOI increase/decrease of *S. epidermidis* when treated with 1% EGCG and 1% EGCG-S in combination with antibiotics. The blue bar represent antibiotic plus EGCG, and the red bar represent antibiotic plus EGCG-S (n = 3).



(a)

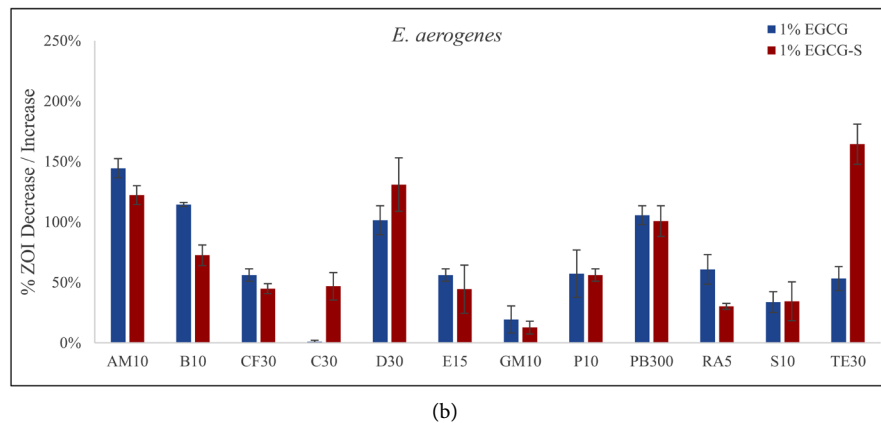


Figure 4. (a) The ZOI of *E. aerogenes* when treated with 1% EGCG and 1% EGCG-S in combination with various antibiotics. The green color indicates susceptible, yellow indicates intermediate and red indicates resistant. The slanted stripe bar graph indicates antibiotic alone, the vertical stripe bar graph indicates antibiotic plus EGCG and the polka dot bar graph indicate antibiotic plus EGCG-S (n = 3); (b) The percentage of ZOI increase/decrease of *E. aerogenes* when treated with 1% EGCG and 1% EGCG-S in combination with antibiotics. The blue bar represents antibiotic plus EGCG, and the red bar represents antibiotic plus EGCG-S (n = 3).

from resistant to susceptible while TE30 (164.29%) had the most significant increase with EGCG-S converting the antibiotic profile from resistant to intermediate. The t-test statistical analysis was carried out and the p-value for both EGCG and EGCG-S were less than 0.01. Thus, these polyphenols have a synergistic effect on these antibiotics.

The ZOI study of *E. coli* indicates resistance to 5 (AM10, B10, D30, P10 and S10) of 12 antibiotics, intermediate to 4 (CF30, E15, RA5 and TE30) of 12 antibiotics, and susceptible to 3 (C30, GM10, and PB300) of 12 antibiotics. EGCG converted 4 (AM10, B10, D30 and S10) antibiotics from resistant to susceptible, 1 (P10) antibiotic from resistant to intermediate, and 2 (RA5 and TE30) antibiotics from intermediate to susceptible. EGCG-S was more effective than EGCG with a conversion of all initially resistant antibiotics to susceptible (AM10, B10, D30, and S10) except for P10 which was only converted to intermediate and all initially intermediate antibiotics to susceptible (CF30, E15, RA5, and TE30) (Figure 5(a)). The results suggested that EGCG-S is a much stronger synergistic anti-*E. coli* agent than EGCG.

EGCG and EGCG-S increased the antibiotic efficacy ranging from 10.93% to 212.04% and 2.10% to 183.33% respectively. EGCG had the highest efficacy for S10 (212.04%) with a conversion from intermediate to susceptible, while EGCG-S had the highest efficacy for P10 (183.33%) with a conversion from resistant to intermediate (Figure 5(b)). The t-test analysis resulted in a p-value of less than 0.05 for both EGCG and EGCG-S indicating a synergistic effect of both compounds to many antibiotics.

The results from the study of *P. aeruginosa* indicates that it is resistant to 6 (AM10, D30, E15, P10, RA5 and TE30) of 12 antibiotics. It is intermediate to 3

(CF30, GM10 and S10) antibiotics and susceptible to 3 (B10, C30, PB300) antibiotics. Addition of EGCG did not convert any of the antibiotics from resistant to susceptible, but four of them were converted to intermediate susceptibility (AM10, D30, E15, and P10). EGCG also converted all the intermediate susceptibility to susceptible (CF30, GM10, and S10). Addition of EGCG-S has a more positive impact on the antibiotics; it converted E15 from resistant to susceptible and 5 (AM10, D30, P10, RA5 and TE30) antibiotics from resistant to intermediate. It also converted all the intermediate to susceptible. This result also illustrated that EGCG-S is more potent synergistic agent with antibiotics than EGCG (**Figure 6(a)**).

EGCG and EGCG-S increased the antibiotic efficacy from 7.17% to 115.87% and 7.17% to 176.19% respectively. EGCG had the most significant increase for E15 (144.4%) but only converted the antibiotic resistant to intermediate. EGCG-S had the most% of increase for E15 (176.19%) and converted it from

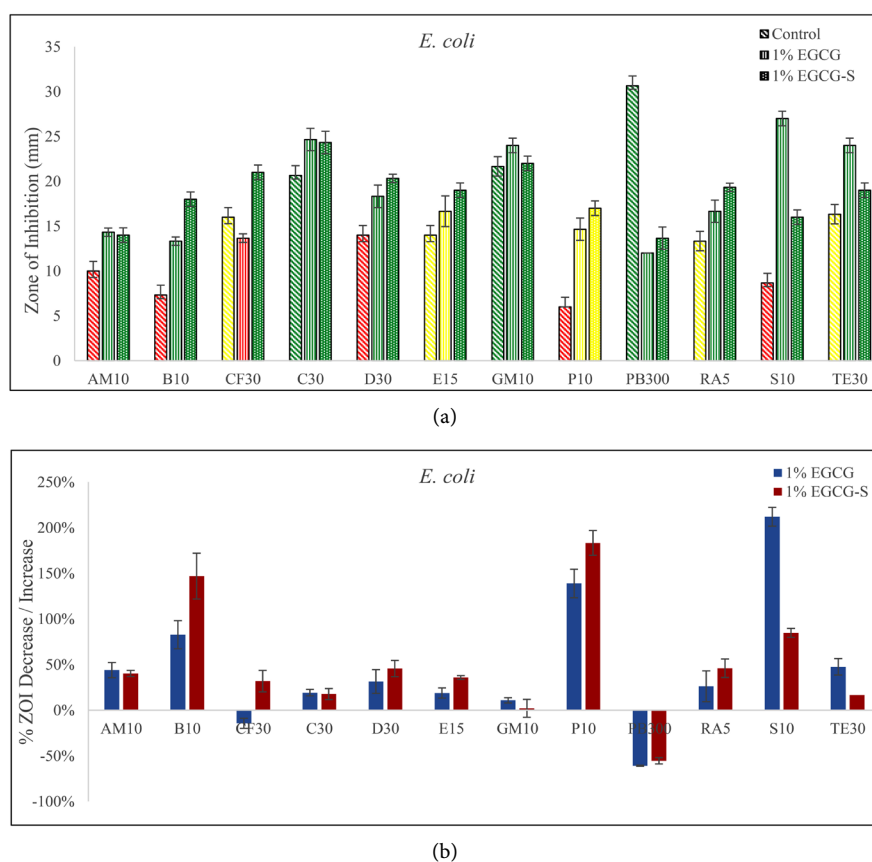
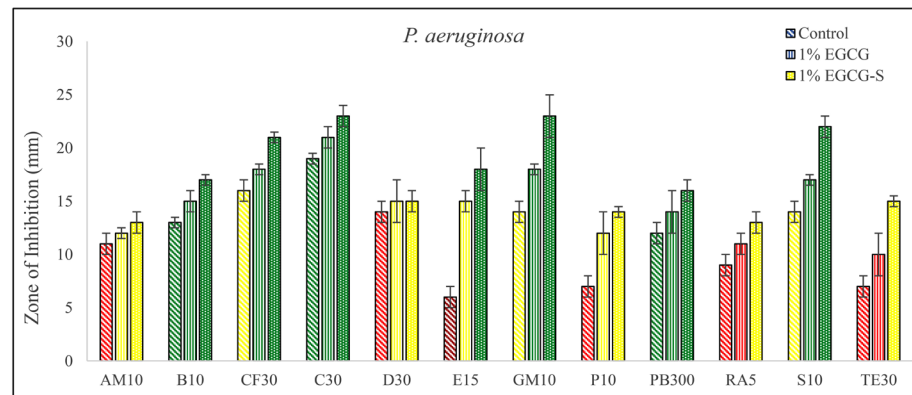
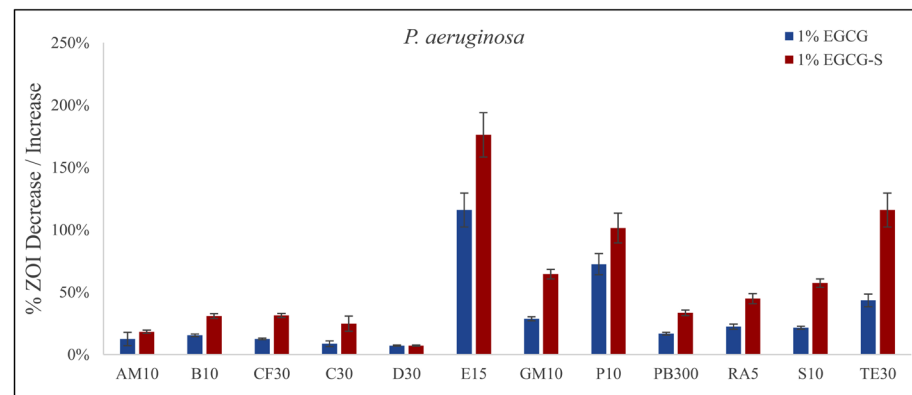


Figure 5. (a) The ZOI of *E. coli* when treated with 1% EGCG and 1% EGCG-S in combination with antibiotics. The green color indicates susceptible, yellow indicates intermediate and red indicates resistant. The slanted stripe bar graph indicates antibiotic alone, the vertical stripe bar graph indicates antibiotic plus EGCG and the polka dot bar graph indicate antibiotic plus EGCG-S; (b) The percentage of ZOI increase/decrease of *E. coli* when treated with 1% EGCG and 1% EGCG-S in combination with antibiotics. The blue bar represents antibiotic plus EGCG, and the red bar represents antibiotic plus EGCG-S (n = 3).



(a)

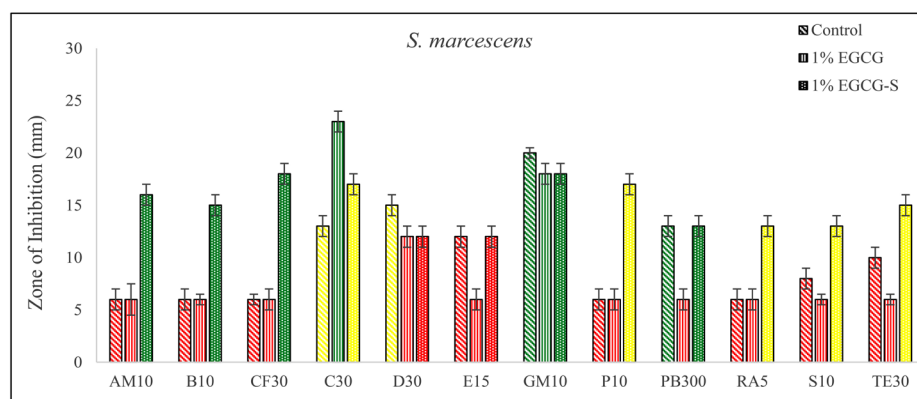


(b)

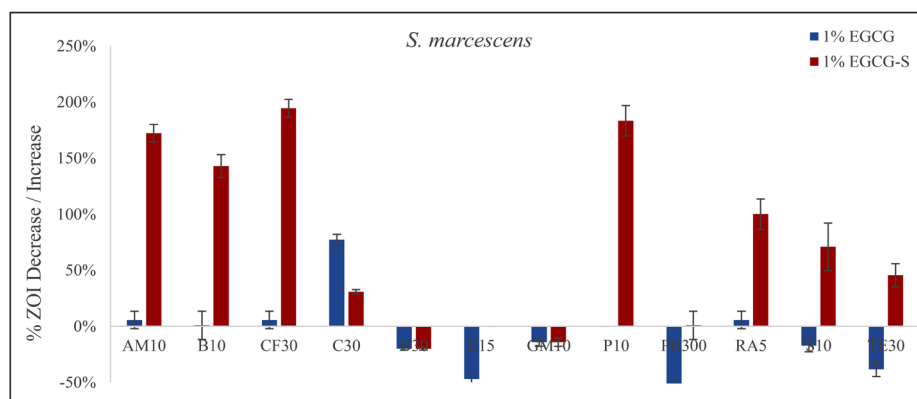
Figure 6. (a) The ZOI of *P. aeruginosa* when treated with 1% EGCG and 1% EGCG-S in combination with various antibiotics. The green color indicates susceptible, yellow indicates intermediate and red indicates resistant. The slanted stripe bar graph indicates antibiotic alone, the vertical stripe bar graph indicates antibiotic plus EGCG and the polka dot bar graph indicate antibiotic plus EGCG-S ($n = 3$); (b) The percentage of ZOI increase/decrease of *P. aeruginosa* when treated with 1% EGCG and 1% EGCG-S in combination with antibiotics. The blue bar represents antibiotic plus EGCG, and the red bar represents antibiotic plus EGCG-S ($n = 3$).

resistant to susceptible (**Figure 6(b)**). The t-test indicated a p-value of less than 0.05 for EGCG and less than 0.01 for EGCG-S demonstrating that the synergistic effect of both compounds are statistically significant.

The ZOI study for *S. marcescens* indicates higher tolerance for antibiotics, with resistance to 8 (AM10, B10, CF30, E15, P10, RA5, S10, TE30) of 12 antibiotics, intermediate resistance to C30 and D30, and sensitivity to only GM10 and PB300. In the presence of EGCG, C30 converted from intermediate to susceptible. EGCG-S was more effective than EGCG with the conversion of 3 (AM10, B10, and CF30) antibiotics from resistant to susceptible and 4 (P10, RA5, S10, and TE30) antibiotics from resistant to intermediate (**Figure 7(a)**). In addition, EGCG-S had greater ZOI percentage increases over a larger array of antibiotics ranging from 30.89% to 194.44%, whereas EGCG-S had a ZOI percentage increase of 77.23% for C30 (**Figure 7(b)**).



(a)



(b)

Figure 7. (a) The ZOI of *S. marcescens* when treated with 1% EGCG and 1% EGCG-S in combination with antibiotics. The green color indicates susceptible, yellow indicates intermediate and red indicates resistant. The slanted stripe bar graph indicates antibiotic alone, the vertical stripe bar graph indicates antibiotic plus EGCG and the polka dot bar graph indicate antibiotic plus EGCG-S ($n = 3$); (b) The percentage of ZOI increase/decrease of *S. marcescens* when treated with 1% EGCG and 1% EGCG-S in combination with antibiotics. The blue bar represents antibiotic plus EGCG, and the red bar represents antibiotic plus EGCG-S ($n = 3$).

While EGCG had the greatest efficacy for C30 (77.23%), this polyphenol only converted C30 from intermediate to susceptible, whereas EGCG-S had the greatest efficacy for CF30 (194.4%) and was able to convert it from resistant to susceptible. The t-test statistical analysis demonstrated a p-value for EGCG-S of less than 0.01 indicating overall synergistic effects with antibiotics.

3.3. EGCG and EGCG-S Showed Synergy with Antibiotics in Acid-Fast Bacteria

The only acid-fast bacterium used for this study was *M. smegmatis*. The ZOI result of *M. smegmatis* indicated that the bacteria is resistant to 5 antibiotics (CF30, D30, PB300, S10, and TE30), intermediate to three antibiotics (AM10, E15, and P10), and susceptible to 4 antibiotics (B10, C30, GM10, and RA5). EGCG converted 3 antibiotics (D30, S10, and TE30) from resistant to suscepti-

ble; PB300 from resistant to intermediate, and E15 from intermediate to susceptible. EGCG-S was more effective than EGCG in synergism with a conversion of 4 antibiotics (D30, PB300, S10, and TE30) from resistant to susceptible and one antibiotic (AM10) from intermediate to susceptible (**Figure 8(a)**).

EGCG and EGCG-S increased antibiotic efficacy ranging from 7% to 185.71% and 7% to 328.57% respectively. EGCG had the highest percentage increase of ZOI for S10 (185.71%) with conversion from antibiotic resistance to susceptible. EGCG-S had the highest percentage increase for TE30 (328.57%) with similar conversions of resistant to susceptible (**Figure 8(b)**).

The effect of crude green tea polyphenols (GTP) and crude lipophilic green tea polyphenols (LTP) on antibiotics has been reported [10]. The comparison of the synergistic effect of the pure EGCG and EGCG-S has been summarized in **Table 1**. The results indicated that the green tea polyphenols have shifted the antimicrobial susceptibility category (R, I, and S) of the antibiotic in all the microorganisms. The EGCG and EGCG-S worked better than the crude extract (GTP and LTP). Both EGCG and EGCG-S worked comparably for Gram-positive. EGCG-S worked better than EGCG on Gram-negative bacteria and acid-fast bacteria. The 1% EGCG-S enhanced seven antibiotics while EGCG only enhanced one antibiotic on in *S. marcescens*.

4. Discussion

We have previously reported that green tea polyphenols, both GTP and LTP, have synergistic antibacterial effects on some antibiotics against Gram+, Gram- and acid-fast bacteria [10]. This study suggested that pure compounds of GTP and LTP, EGCG and EGCG-S, are the active ingredients for the synergy. Green tea, especially EGCG and its derivatives are widely popular for their several beneficial properties such as anti-inflammatory, anti-oxidant, anti-carcinogenic, anti-viral, anti-bacterial, anti-amyloidogenic, anti-biofilm and anticariogenic [20]-[25].

Despite the broad-spectrum effects, the antibacterial mechanisms of EGCG are still elusive. It has been reported that EGCG interacts with bacterial transcription potentially increasing cell permeability and thus facilitates the entry of the antibiotics [23] [26] [27]. Some reported antibacterial mechanisms of EGCG include: 1) Induction of H₂O₂ resulting in disruption/lysis of outer membrane of Gram-negative bacteria [28] [29] [30]; 2) interference with glycocalyx and cell wall/cell membrane interactions [31] [32]; 3) inhibition of peptidoglycan synthesis [30] [33] [34] [35]; and 4) Bind to the fimbriae inhibiting bacterial adherence [36]. EGCG-S is a more stable derivative that can inhibit the production of bacteria secondary metabolites and thus induces endogenous oxidative stress and decreases biofilm formation [26]. Previous report also suggested that EGCG-S could damage the integrity of endospore coat [11]. The mechanisms of EGCG and EGCG-S may work synergistically with antibiotics to increase the susceptibility of antibiotic on antibiotic resistant bacteria.

Table 1. Comparative analysis the synergistic effect of 1% EGCG and 1% EGCG-S.

Bacteria	Synergistic % ZOI Increased	
	1% EGCG	1% EGCG-S
<i>B. megaterium</i>	Not significant	B10 (I→S) 112.04%
	AM10 (R→I) 9.14%	AM10 (R→S) 54.85%
	CF30 (I→S) 23.58%	CF30 (I→S) 35.38%
<i>S. aureus</i>	C30 (I→S) 29.83%	C30 (I→S) 35.48%
	S10 (I→S) 34.21%	S10 (I→S) 42.54%
	TE30 (I→S) 28.14%	TE30 (I→S) 44.65%
	AM10 (I→S) 133.95%	
	B10 (I→S) 134.44%	AM10 (I→S) 66.98%
<i>S. epidermidis</i>	CF30 (I→S) 114.68%	B10 (I→S) 78.43%
	C30 (I→S) 50.17%	CF30 (I→S) 43%
	E15 (R→I) 30.89%	C30 (I→S) 35.84%
	P10 (R→S) 216.20%	P10 (R→I) 112.04%
	RA5 (I→S) 146.73%	RA5 (I→S) 46.34%
	TE30 (I→S) 78.75%	
	AM10 (R→S) 144.44%	AM10 (R→I) 122.22%
<i>E. aerogenes</i>	B10 (R→S) 114.48%	B10 (R→I) 72.42%
	E15 (R→I) 18.78%	D30 (R→I) 45.73%
	P10 (R→I) 57.22%	P10 (R→I) 56.02%
	PB300 (R→S) 105.56%	PB300 (R→S) 100.79%
	RA5 (R→S) 60.77%	RA5 (R→I) 30.20%
	S10 (I→S) 33.74%	S10 (I→S) 34.42%
		TE30 (R→I) 164.29%
		AM10 (R→S) 40.27%
	AM10 (R→S) 43.97%	B10 (R→S) 147.02%
	B10 (R→S) 82.74%	CF30 (I→S) 31.85%
<i>E. coli</i>	D30 (R→S) 22.03%	D30 (R→S) 45.73%
	P10 (R→I) 138.89%	E15 (I→S) 35.84%
	RA5 (I→S) 26.24%	P10 (R→I) 183.33%
	S10 (R→S) 212.04%	RA5 (I→S) 45.94%
	TE30 (I→S) 47.55%	S10 (R→S) 84.72%
		TE30 (I→S) 16.76%
		AM10 (R→I) 18.28%
<i>P. aeruginosa</i>	AM10 (R→I) 12.47%	CF30 (I→S) 31.33%
	CF30 (I→S) 12.53%	D30 (R→I) 7.17%
	D30 (R→I) 7.17%	E15 (R→S) 176.19%
	E15 (R→I) 115.87%	GM10 (I→S) 64.51%
	GM10 (I→S) 28.67%	P10 (R→I) 101.39%
	P10 (R→I) 72.42%	RA5 (R→I) 44.81%
	S10 (I→S) 21.50%	S10 (I→S) 57.34%
		TE30 (R→I) 115.87%
<i>S. marcescens</i>		AM10 (R→S) 172.22%
		B10 (R→S) 142.86%
		CF30 (R→S) 194.44%
	C30 (I→S) 77.23%	P10 (R→I) 183.33%
		RA5 (R→I) 100%
<i>M. smegmatis</i>		S10 (R→I) 70.83%
		TE30 (R→I) 45.45%
	D30 (R→S) 110%	AM10 (I→S) 41.67%
	E15 (I→S) 33.33%	D30 (R→S) 250%
	PB300 (R→I) 12.50%	PB300 (R→S) 137.50%
	S10 (R→S) 285.71%	
	TE30 (R→S) 328.57%	

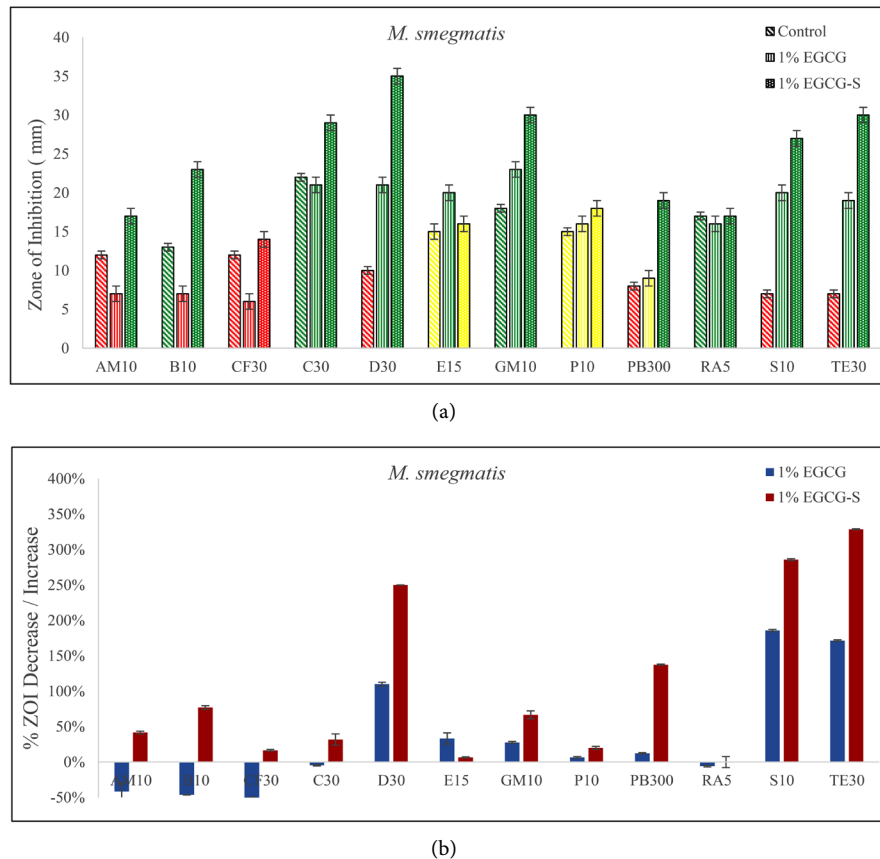


Figure 8. (a) The ZOI of *M. smegmatis* when treated with 1% EGCG and 1% EGCG-S in combination with antibiotics. The green color indicates susceptible, yellow indicates intermediate and red indicates resistant. The slanted stripe bar graph indicates antibiotic alone, the vertical stripe bar graph indicates antibiotic plus EGCG and the polka dot bar graph indicate antibiotic plus EGCG-S (n = 3); (b) The percentage of ZOI increase/decrease of *S. marcescens* when treated with 1% EGCG and 1% EGCG-S in combination with antibiotics. The blue bar represents antibiotic plus EGCG, and the red bar represents antibiotic plus EGCG-S (n = 3).

This study indicated that both EGCG and EGCG-S work synergistically with some antibiotics while EGCG-S possesses higher efficacy than EGCG. When combined with antibiotics, EGCG-S has particularly high potency on Gram-negative bacteria including *E. aerogenes*, *E. coli*, *P. aeruginosa* and *S. marcescens*. Usually the antibiotics are less effective on Gram-negative bacteria due to their outer membranes. Furthermore, both EGCG and EGCG-S enhance anti-mycobacterial activity significantly when combined with antibiotics. Overall, this study highlights that EGCG-S may serve as a promising synergistic agent with antibiotic.

5. Conclusions

This study looked into the potential use of green tea polyphenols EGCG and EGCG-S in combating the antibiotics resistant problem. This study used the Kirby-Bauer Assay to evaluate the effect of both EGCG and EGCG-S in combination with 12 antibiotics.

For Gram-positive bacteria, the antibacterial effect of EGCG was comparable to EGCG-S as seen in **Table 1**. The result showed that EGCG was able to convert categories for 13 antibiotics while EGCG-S was able to convert categories for 12 antibiotics in *S. aureus* and *S. epidermidis*. For Gram-negative and acid-fast bacteria, EGCG is able to convert 27 antibiotics categories while EGCG-S is able to convert 38 antibiotics categories. This overall shows that EGCG-S works better in Gram-negative and Acid-Fast bacteria. Similar to Gram-positive bacteria, EGCG and EGCG-S worked better when compared to LTP and GTP [10]. *S. marcescens* for example, GTP and LTP were able to convert categories for 4 antibiotics [10] while EGCG and EGCG-S were able to convert categories for 8 antibiotics. It was important to note that EGCG-S increased the antibacterial activity up to 328.57% and converted the antibiotic effectiveness from resistance to susceptible category when combined with TE30. In summary, this study demonstrated that EGCG-S could serve as a synergistic antibacterial agent with antibiotics to combat the antibiotic problem.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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