Journal of
Emerging Investigators

Talinum paniculatum root exhibits synergistic antimicrobial activity with Tetracycline, Erythromycin, and Streptomycin against *S. aureus* but has no observed effect on antibiotic efficacy against *E. coli*

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Summary

Bacteria are quickly becoming resistant to antibiotics, and as such, alternative medicines are being explored as treatments for infectious diseases. Plant extracts such as *Panax ginseng* (ginseng) have shown promise in increasing the effectiveness of current therapeutic agents; however, the high cost of ginseng makes it less attainable than other plant species. A comparable plant, *Talinum paniculatum*, has been shown to suppress the growth of *Staphylococcus aureus* and *Serratia marcescens* on its own, yet studies are lacking in testing its effectiveness alongside antibiotic treatment. This study aimed to test the effects of *T. paniculatum* in combination with Tetracycline, Erythromycin, and Streptomycin against *S. aureus* and *E. coli*. The results of the disk diffusion tests indicated that *T. paniculatum* helped increase the efficacy of the antibiotics against *S. aureus*, but had no noticeable effect on *E. coli* when tested with the same antibiotics. This is important because *T. paniculatum* is more cost accessible than ginseng, therefore, it can be more easily acquired and used in combination with antibiotic therapies for treating *S. aureus* infections. Future studies will involve isolating and testing for specific bioactive components of *T. paniculatum*.

Received: January 20, 2017; **Accepted:** November 9, 2017; **Published:** January 9, 2018

Introduction

Antibiotic resistant bacteria pose a public health threat due to resistance accumulating faster than new antibiotics can be produced (1). As such, there is a call for exploration of alternative medicinal therapies for the treatment of infectious diseases (2). Explorative therapies include bark, honey, and plant roots, such as *Panax ginseng* (*P. ginseng*), yet *P. ginseng* can range in cost from $500 to $650 per pound., making it cost prohibitive for some (3). *Talinum paniculatum* (*T. paniculatum*), commonly known as Jewels of Opar or fake ginseng (4), is a popular herb used in many cultures as a substitute for ginseng and as a treatment for gastrointestinal problems and skin wounds (5). *Talinum paniculatum* contains tannins, steroids, and triterpenes (6), which are thought to have antimicrobial activities due to inhibition of cell protein synthesis (7). Experiments using leaf extracts from *T. paniculatum* suggests that metabolites from the plant are inhibitory against a wide spectrum of microorganisms, including *Staphylococcus aureus* (*S. aureus*), *Serratia marcescens, Candida albicans, Mycobacterium bovis,* and *M. tuberculosis* (5).

While *T. paniculatum* on its own has been shown to have antimicrobial activity, we have yet to find a study done to determine whether this plant's roots, in combination with antibiotic therapy, may enhance the efficacy of the antibiotic. To explore this question, we tested the antibacterial efficacy of Tetracycline, Erythromycin, and Streptomycin against *Escherichia coli* (*E. coli*) and *S. aureus* growing on Mueller Hinton and 0.005% *T. paniculatum* root extract Mueller Hinton agars. 0.005% concentration of *T. paniculatum* was used due to a low supply of the plant root. Our results showed that *T. paniculatum* had no observable ramifications on the effectiveness of the antibiotics against *E. coli*, however, there was a statistically significant increase in the zones of inhibition for all antibiotics tested against *S. aureus*. Furthermore, *S. aureus* was sensitive to Streptomycin on 0.005% *T. paniculatum* root extract Mueller Hinton agar, where *S. aureus* was resistant to Streptomycin on Mueller Hinton agar lacking the plant root extract. These results demonstrate that, while not effective at enhancing antibiotic effectiveness against *E. coli*, *T. paniculatum* may increase the sensitivity of *S. aureus* to antibiotics. This could lead to potential treatments for infections caused by antibiotic resistant *S. aureus* species, such as MRSA.

Results

For this study, 0.5 McFarland standards of *S. aureus* and *E. coli* were created. These cultures were then grown on both control Mueller Hinton agar plates and 0.005% *T. paniculatum* root extract Mueller Hinton agar plates, along with antibiotics or a blank (negative control) paper disc, for 24 hours at 35°C. After 24 hours, zone of inhibition (ZOI) measurements were taken by using a standard metric ruler to measure the diameter of the area with no growth. (Figure 1). These values were then compared with antimicrobial sensitivity values obtained from the National Committee for Clinical Laboratory Standards (NCCLS), to determine whether each species of bacteria was sensitive, intermediate, or resistant to the antibiotic in question (8). For Erythromycin, resistance is indicated by ZOIs of less than or equal to 13 mm, intermediate activity by ZOIs of 14-22 mm, and sensitivity to the antibiotic by ZOIs greater than or equal to 23 mm. For Streptomycin, resistance is indicated by ZOIs of less than or equal to 11 mm, intermediate activity by ZOIs of 12-14 mm, and sensitivity to the antibiotic by ZOIs of greater than or equal to 15 mm. Finally, Tetracycline interpretations show that resistance is indicated by ZOIs of ≤ 14 mm, intermediate activity indicated by ZOIs of 15-18 mm, and sensitivity to the antibiotic by ZOIs of greater than or equal to 19 mm.

Table 1 shows the ZOI interpretation of *S. aureus* for each antibiotic. Comparing *S. aureus* growth on just Mueller Hinton agar to the NCCLS standards, it was determined that *S. aureus* was sensitive to both Erythromycin and Tetracycline, but was resistant to Streptomycin. 0.005% *T. paniculatum* root extract Mueller Hinton increased the ZOI for each antibiotic (Table 1). T-tests were performed to determine whether the increased ZOI that was present on the 0.005% *T. paniculatum* root extract Mueller Hinton agars was significant. Using a one-tailed t-test ($\alpha = 0.05$), the p-value was calculated to be $p \leq 0.04$ for Streptomycin alone compared to Streptomycin with 0.005% *T. paniculatum* root extract, $p \leq 0.03$ for Erythromycin alone compared to Erythromycin with 0.005% *T. paniculatum* root extract, and $p \leq 0.02$ for Tetracycline alone compared to Tetracycline with 0.005% *T. paniculatum* root extract (Table 2). These data suggest that *T. paniculatum* enhanced the efficacy of all three antibiotics against *S. aureus*.

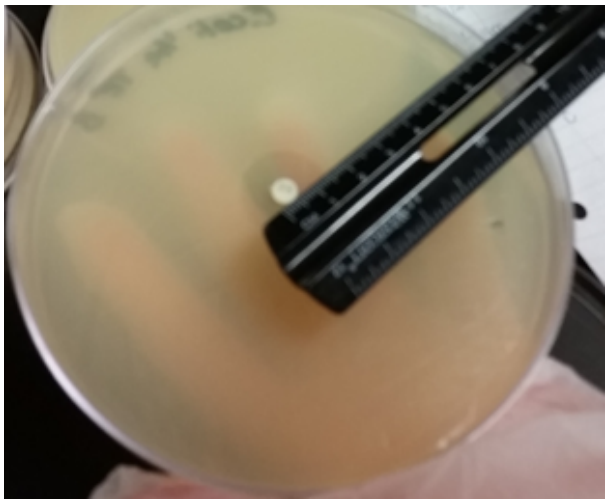

Figure 1. Disk-diffusion to assess antibiotic resistance. In order to determine the zone of inhibition (ZOI), a ruler is used to measure the diameter of the area where the bacteria has not grown.

Table 3 shows the ZOI interpretations of *E. coli* for each antibiotic. Comparing *E. coli* growth on just Mueller Hinton agar to the NCCLS standards, it was determined that *E. coli* is resistant to Erythromycin grown on Mueller Hinton agar alone and in the presence of 0.005% *T. paniculatum* root extract. *E. coli* was sensitive to Tetracycline and Streptomycin, both when grown on Mueller Hinton agar alone and in the presence of

Erythromycin	Tetracycline	Streptomycin
$p \leq 0.03$	$p \leq 0.02$	$p \leq 0.04$

Table 2. T-test results for *S. aureus* antibiotic testing on different agars (Mueller Hinton versus 0.005% *T. paniculatum* Mueller Hinton agar).

	Erythro. 15µg, MH	Erythro. 15µg, MHG	Tetracycline 30µg, MH	Tetracycline 30µg, MHG	Strepto. 10µg, MH	Strepto. 10µg, MHG	Blank MH	Blank MHG
Trial 1	28	30	30	33	14	15	6	6
Trial 2	25	28	28	32	10	14	6	6
Trial 3	25	29	25	32	10	15	6	6
Average ZOI	26	29	28	32	11	15	6	6
Interpretation	Sensitive ≥23	Sensitive ≥23	Sensitive ≥19	Sensitive ≥19	Resistant ≤11	Sensitive ≥15	Resistant	Resistant

Table 1. *S. aureus* zones of inhibition (ZOI). ZOI measurements were taken in millimeters and compared to the NCCLS antibiotic susceptibility guidelines. Erythromycin (E) and Tetracycline (Te) were sensitive on both agar plates, whereas Streptomycin (S) was resistant on Mueller Hinton (MH) Agar, but was sensitive on T. paniculatum – Mueller Hinton (MHG) agar.

	Erythro. 15µg, MH	Erythro. 15µg, MHG	Tetracycline 30µg, MH	Tetracycline 30µg, MHG	Strepto. 10µg, MH	Strepto. 10µg, MHG	Blank MH	Blank MHG
Trial 1	10	10	20	21	25	24	6	6
Trial 2	13	11	22	23	25	24	6	6
Trial 3	15	13	27	26	26	19	6	6
Average ZOI	13	11	23	23	25	22	6	6
Interpretation	Resistant ≤13	Resistant ≤13	Sensitive ≥19	Sensitive ≥19	Sensitive ≥15	Sensitive ≥15	Resistant	Resistant

Table 3. *E. coli* zones of inhibition (ZOI). ZOI measurements were taken in millimeters and compared to the NCCLS antibiotic susceptibility guidelines. Erythromycin (E) was resistant on both types of agar plates, whereas Tetracycline (Te) and Streptomycin (S) were sensitive on Mueller Hinton (MH) and T. paniculatum – Mueller Hinton (MHG) agar.

0.005% *T. paniculatum* root extract. In order to determine whether the presence of 0.005% *T. paniculatum* root extract produced a statistically significant change in antibiotic activity, t-tests were performed. Using a one-tailed t-test (α= 0.05), the p-value produced was $p \leq 0.08$ for Streptomycin compared to Streptomycin with 0.005% *T. paniculatum* root extract, $p \leq 0.24$ for Erythromycin compared to Erythromycin with 0.005% *T. paniculatum* root extract, and $p \leq 0.45$ for Tetracycline compared to Tetracycline with 0.005% *T. paniculatum* root extract (Table 4). With $p \geq 0.05$ for all tests, these data suggest that *T. paniculatum* did not affect antibiotic activity against *E. coli*.

Erythromycin	Tetracycline	Streptomycin
$p \leq 0.24$	$p \leq 0.45$	$p \leq 0.08$

Table 4. T-test results for *E. coli* antibiotic testing on different agars (Mueller Hinton versus 0.005% *T. paniculatum* Mueller Hinton agar).

Discussion

In this study, we examined whether *T. paniculatum* root enhanced the antimicrobial activity of Tetracycline, Streptomycin, and Erythromycin against *S. aureus* and *E. coli*. This was accomplished by growing the bacteria along with antibiotics on Mueller Hinton and 0.005% *T. paniculatum* -Mueller Hinton agars. The results of the experiment showed that the Mueller Hinton agar containing 0.005% *T. paniculatum* root extract statistically enhanced the activity of all the antibiotics against *S. aureus*, as shown by the increased ZOI. Interestingly, 0.005% *T. paniculatum* had a dramatic effect on Streptomycin activity by making *S. aureus* sensitive to the antibiotic, whereas it was resistant when *T. paniculatum* was not present. However, there was no statistically significant effect of *T. paniculatum* on antibiotic activity against *E. coli*. To our knowledge, this is the first study to test combinatorial effects of *T. paniculatum* with antibiotics. Previous research using traditional ginseng (*P. ginseng*) showed similar results in efficacy against *S. aureus* (9). The difference in effects of *T. paniculatum* on antibiotic activity between *S. aureus* and *E. coli* may be attributed to the fact that the ginseng metabolites act differently on each bacteria. (10). Previous studies on ginseng suggest that intestinal bacteria deglycosylate ginsenosides, a class of naturally occuring steroids, create bacterial metabolites which then undergo fatty acid esterification and becoming metabolically active (11). Intestinal bacteria known to activate ginsenosides via this pathway include Prevotella oris, Eubacterium A-44, Bifidobacterium K506, Bacteroides JY6, and Fusobacterium. *S. aureus* may activate tannins, steroids, triterpenes and/or other bioactive molecules in *T. paniculatum* extract via a similar mechanism, thereby explaining the difference in antimicrobial synergy when compared to the *E. coli* plates. This hypothesis will need to be tested in future studies. Another difference in effect may be attributed to the low concentration of *T. paniculatum* in the agar. Using *P. ginseng* studies for comparison, low concentrations of ginseng (1.25%) appear to have a stimulatory effect on the growth of pathogenic Pseudomonas aeruginosa strains (12), with enhancement being attributed to upregulation in synthesis of extracellular proteins. While this phenomenon was not tested here, it is worth exploring in future experiments, as *E. coli*, also a Gram-negative bacterium, may exhibit enhanced growth via a similar mechanism. This may explain why there appeared to be a qualitative reduction in the *E. coli* zones of inhibition for each antibiotic when it was supplemented with *T. paniculatum* extract.

While our results suggest that *T. paniculatum* enhances antibiotic activity against *S. aureus* but not against *E. coli*, there are inherent limitations in this study. The major limitation stems from the fact that whole root extract was used in testing, and molecules from the root extract were not able to be tested individually. Future studies should isolate each specific molecule (for example, tannins, steroids, and triterpenes) thought to attribute to antimicrobial activity (6). It may be possible that the activities of these molecules affect Gram-

positive bacteria differently than Gram-negative bacteria. Another limitation is the lack of bacterial species tested in this experiment. While *T. paniculatum* showed a broad spectrum of activity against a variant of pathogens on its own (10), effectiveness of *T. paniculatum* may be altered for each of these pathogens with respect to antibiotic presence. This warrants further investigation.

Our data suggests that *T. paniculatum* enhances antibiotic activity against *S. aureus*, but has not against *E. coli*. The use of *T. paniculatum* alongside modern antibiotic treatment may offer additional tools for medical professionals to use when treating infections, particularly those infections caused by antibiotic resistant bacteria.

Materials and Methods

T. paniculatum root from a home garden was dehydrated for 2 weeks at room temperature by placing the root in a tray with paper towels. The dehydrated root was then ground up into a powder. Aqueous extraction of the dehydrated root was performed by soaking 2.5g of *T. paniculatum* root powder in 50 mL of autoclaved, distilled water and boiling three times for three minutes, with two minute waiting intervals in between each boiling cycle (5%). This solution was then filtered using a 20 µm filters. The 50 mL filtered solution was then added to 450 mL distilled water and 38g Mueller Hinton agar (autoclaved), creating 0.005% concentration *T. paniculatum* root extract Mueller Hinton agar. Mueller Hinton agar plates without *T. paniculatum* were also made following manufacturer specifications (Remel).

Next, *E. coli* and *S. aureus* were inoculated into separate sterile saline tubes to a 0.5 McFarland standard. A swab was dipped into the bacterial solution and inoculated onto control and 0.005% *T. paniculatum* root extract Mueller Hinton agar using an X, Y, Z quadrant system, creating a confluent lawn of growth. Erythromycin, Tetracycline, and Streptomycin antibiotic discs, as well as a blank disc (BD BBL) were placed onto the inoculated plates. The plates were incubated at 35° for 24 hours. The following day, the zones of inhibition were measured for each antibiotic on both the control Mueller Hinton and 0.005% *T. paniculatum* root extract Mueller Hinton agar plates.

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