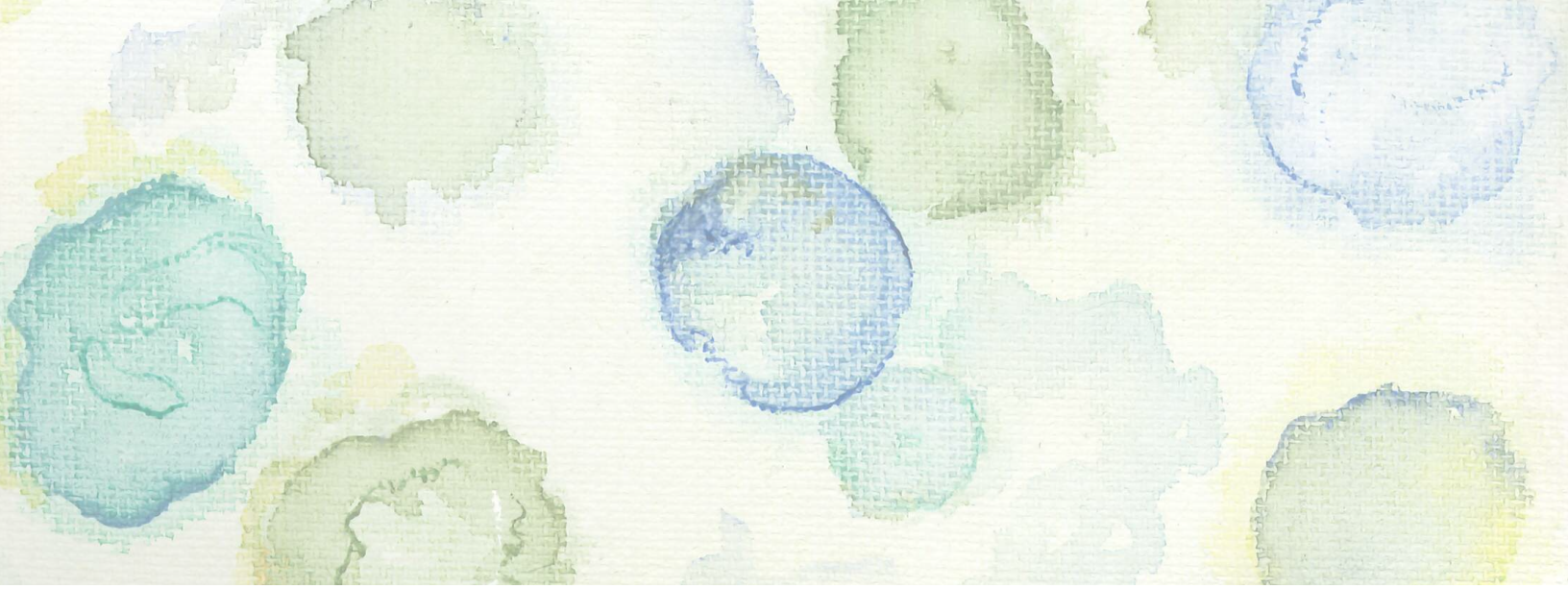

Antibacterial Effects of Bitter Melon Extract in Combination With Commonly Prescribed Antibiotics

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Abstract:

Antibiotics are commonly overprescribed or taken incorrectly, which has resulted in an alarming increase of antibiotic-resistant bacteria. One potential solution to combat this problem is administering multiple antibiotics together to achieve antibiotic synergy; when two or more antibiotics work together to increase antibacterial efficacy. When considering potential synergistic combinations of antibiotics, one possibility is to utilize antibacterial plant extracts in addition to common antibiotics. The goal of our research was to compare the antibacterial properties of the Chinese medicinal plant bitter melon (*Momordica charantia*) and four common antibiotics alone or in combination with bitter melon against *Micrococcus luteus*, *Pseudomonas putida*, and *Escherichia coli*. We hypothesized that combining the antibiotics with bitter melon extract would result in increased antibacterial effects against one or more bacterial strains. Oil from dried bitter melon was prepared using the Soxhlet extraction method. Antibacterial properties of bitter melon extract and carbenicillin, streptomycin, colistin, and tetracycline alone or in combination with the extract were determined by performing disk diffusion assays. Diameters of the resulting zones of inhibition for the two treatments were measured and analyzed for statistical significance by performing a two-tailed, paired sample t-test using *Rguroo*. We found that bitter melon extract individually had little to no antibacterial effect against any of the organisms tested. Interestingly however, combining bitter melon extract with common antibiotics resulted in synergistic effects in some cases, as well as one example of antibiotic antagonism. These results demonstrate that plant-derived extracts can enhance the antibacterial effects of commonly prescribed antibiotics if paired correctly.

Introduction

The injudicious prescription and use of antibiotics creates selective pressure for bacteria to evolve resistance. According to the Centers for Disease Control and Prevention, each year over 2.8 million people are infected with antibiotic-resistant bacteria (4). In the United States alone, a person acquires an antibiotic- resistant infection every 11 seconds, while every 15 minutes someone dies from such an infection (4). This startling statistic can be attributed in part to the fact that nearly one-third of the antibiotics prescribed are not appropriate for the conditions being treated. The continuous misuse of readily available conventional antibiotics is proving to be a catalyst for the persistence, evolution and spread of antibiotic resistance. This severely limits the efficacy of antibiotics that were once used to treat life threatening bacterial infections and is now resulting in increased hospitalization rates and even deaths due to the rise in resistance. In turn, this leads to the requirement for heavier dosages and new antibiotic drugs to treat infections that were once readily treatable (4). Health care professionals, scientists and the public must do their part in combating this growing problem as antibiotic resistance is evolving into a serious threat to the human healthcare field globally.

One potential solution to combat the rising number of antibiotic-resistant strains is administering multiple antibiotics together to achieve antibacterial synergy. If an antibiotic demonstrates stronger antibacterial effects in combination with another antibiotic rather than when used alone, the combination treatment can be deemed synergistic. Alternatively, antibiotic antagonism occurs when the overall antibiotic efficacy of two or more antibiotics administered together is decreased relative to the effects of each when used alone (1). Combining extracts from plants with commonly prescribed antibiotics is one possible way to achieve antibacterial synergy. Plant-derived compounds can exhibit a direct antibacterial activity as well as indirect activity as antibiotic resistance modifying compounds that, when combined with antibiotics, can potentially increase their efficacy (13). The organic compounds in plants are referred to as biologically active substances and include phenolic compounds, terpenes, and alkaloids. These substances can be isolated into crude extracts, some of which have prominent antibacterial activity (13).

In this study, we tested the antibacterial properties of an organic extract prepared from the Chinese medicinal plant bitter melon (*Momordica charantia*) alone and in combination with commonly prescribed antibiotics. While this is not the first investigation of the antibacterial properties of bitter melon, to our knowledge this is the first study in which antibiotic synergy was tested between bitter melon and antibiotics. We hypothesized that bitter melon would itself have antibacterial properties against one or more organisms, as well as a synergistic effect with at least some of the common antibiotics tested.

Materials and Methods

Bacterial Strains and Growth Conditions

Escherichia coli, Micrococcus luteus, and *Pseudomonas putida* were tested in order to compare antibiotic efficacy against a variety of organisms. All of these organisms meet the current biosafety guidelines for Curry College in that all are designated BSL-1, in addition to being clinically relevant organisms. Bacterial cells were suspended in Luria-Bertani (LB, Miller) or Mueller-Hinton broth and incubated with continuous shaking at 225 rpm, or grown on solid agar plates of the same media. *E. coli* and *M. luteus* were incubated at 37 ºC, while *P. putida* was grown at a temperature of 30 ºC.

Media preparation

LB (Miller) broth and agar (Fisher) used for routine growth of bacterial strains was prepared according to the manufacturer's instructions. Mueller-Hinton broth and agar (Fisher) used for the antibacterial susceptibility assays was prepared according to the manufacturer's instructions. Broth was stored at room temperature (68-72 ºF), while plates were stored at 4 ºC.

Preparation of Antibiotic Solutions

The following common antibiotics were tested against each organism: carbenicillin, streptomycin, colistin, and tetracycline. Antibiotics were obtained from Midwest

Scientific (MidSci) and prepared by dissolving the antibiotic powder in molecular grade water (Invitrogen) or 70% ethanol (Fisher) for tetracycline, and then aseptically passing the sample through a 0.22 μm filter (Whatman) using a 10 mL syringe (Fisher). Antibiotic solutions were prepared in the following stock concentrations: carbenicillin (100 μg/μL), streptomycin (10 μg/μL), colistin (30 μg/μL), and tetracycline (30 μg/μL).

Preparation of Bitter Melon Extract

Oil from bitter melon *(Momordica charantia*) was extracted using the Soxhlet method, following a previously developed protocol (19). The bitter melon was purchased at Kam Man Supermarket (Quincy, MA), cut up, then dried in the oven at 200 ºF overnight. 12.45 g of bitter melon was extracted in 250 mL of 80% ethanol and subsequently concentrated by boiling off the ethanol to a final volume of 9 mL.

Disk Diffusion Assays

Disk diffusion assays were performed according to a previously published protocol (7). Briefly, bacteria were grown in an overnight culture in the appropriate conditions for the bacteria as described. The next morning, cultures were diluted to an OD_{600} of 0.05 and swabbed as a lawn on a Mueller-Hinton agar plate, swabbing the entire surface area of each plate three times to ensure complete coverage. Then each antibiotic, bitter melon extract, or combination was tested by gently placing 6mm paper disks (ThermoScientific Oxoid blank disks) containing the appropriate antibiotic solution in the center of each plate using sterilized forceps.

To prepare the disks, 20 μL of antibiotic stock solution, extract, or control (molecular grade water, 70% ethanol, or 80% ethanol) was aseptically pipetted onto each disk tested, and then allowed to dry in sterile conditions for approximately one hour. For disks with both common antibiotic and bitter melon extract, 20 μL of the common antibiotic solution (or water/70% ethanol as control) was first pipetted onto the disk, and then allowed to dry for ~1 hour. Then, 20 μL of bitter melon extract (or 80% ethanol as control) was pipetted onto the disk, and the disk was dried for an additional ~1 hour. Plates were wrapped individually in parafilm, and incubated overnight (~16-24 hours) at the preferred growth temperatures for each organism as described. Four to six replicates were performed for each control, antibiotic, bitter melon extract, or combination tested against all three organisms.

Data Analysis

After overnight growth, the diameter of the zone of inhibition (ZoI), or area resulting in no observable bacterial growth, was measured in millimeters using a ruler and recorded. Average (mean) ZoI diameters were calculated for all replicates along with standard error of the mean to analyze variance in the data.

Data were then categorized by each different organism and further by each common antibiotic tested. The ZoI measurements for each replicate of the common antibiotic alone was included, along with the measurements for the same common antibiotic tested in combination with bitter melon extract. Each spreadsheet was uploaded into the statistical analysis program *Rguroo* (22). A mean inference analysis between two populations (with population 1 as the common antibiotic ZoI diameters and population 2 as the same common antibiotic tested with bitter melon extract) was selected in order to perform a two-tailed, paired sample t-test evaluating the difference between each population mean and generate p-values. Unequal variance was assumed when performing all t-tests. Tests for normality of each dataset were also performed.

Results:

Bitter melon extract alone has little to no effect at preventing bacterial growth

Since our research aimed to test whether or not bitter melon extract enhances the antibacterial properties of commonly prescribed antibiotics, we first tested the efficacy of four different common antibiotics (carbenicillin, streptomycin, colistin, and tetracycline) at preventing growth of *E. coli, P. putida*, and *M. luteus* by setting up disk diffusion assays. For each organism tested, four to six replicates were performed. Controls were also tested for all three organisms by soaking disks in either molecular grade water or 70% ethanol. None of the control disks produced a zone of inhibition. Table 1 shows the average ZoI diameter

measurements for all antibiotics tested for *E. coli, P. putida*, and *M. luteus*, respectively. Unsurprisingly, while different antibiotics demonstrated varying degrees of efficacy against each organism, all were at least somewhat effective at preventing growth and resulted in a ZoI surrounding the antibiotic-soaked paper disk.

Next, we performed disk diffusion assays on the same three organisms using disks soaked with bitter melon extract or control disks soaked in 80% ethanol, the solvent used to prepare the bitter melon extract. Bitter melon extract had no effect at preventing growth of either *E. coli* or *P. putida*, since no ZoI was found around the paper disks (Reported as "0 mm" in Table 1). Bitter melon had only a small effect at preventing the growth of *M. luteus,* resulting in an average ZoI diameter of 5 mm (Table 1). All commonly prescribed antibiotics tested against *M. luteus* were found to produce a substantially greater average ZoI diameter than bitter melon, ranging from 22.1 mm for colistin to 47.25 mm for tetracycline (Table 1). As before, none of the control disks soaked in 80% ethanol as the control for bitter melon produced a ZoI.

Antibiotics:	***E. coli***		***P. putida***		***M. luteus***	
	Average Zol diameter (mm):	Standard Error of the Mean:	Average Zol diameter (mm):	Standard Error of the Mean:	Average Zol diameter (mm):	Standard Error of the Mean:
Carbenicillin	31.25	1.4930	25.2	1.5979	41.45	1.3525
Streptomycin	18.77	1.2925	18.15	0.2872	33.45	1.8715
Colistin	21.4	1.7709	19.6	0.9055	22.1	0.1291
Tetracycline	40	2.1909	23.35	1.0874	47.25	2.2867
Bitter melon	0	0	0	0	5	2.8868

Table 1. **Zone of inhibition measurements for antibiotics and bitter melon extract tested alone against *E. coli, P. putida,* and *M. luteus*. The zone of inhibition (ZoI) diameter measurements (in mm) for each common antibiotic tested (carbenicillin, streptomycin, colistin, and tetracycline, respectively) or for bitter melon extract are reported here. For each antimicrobial substance tested, a total of four to six replicates were performed. The average zone of inhibition diameter was calculated from all replicates performed, and the standard error of the mean was calculated as a relative measure of how consistent the measurements were.**

Bitter melon extract used in combination with commonly prescribed antibiotics influences their antibacterial efficacy

Although bitter melon extract itself had minimal to no effect against the three bacterial strains tested, we decided to test whether bitter melon extract could enhance the antibacterial properties of any of the four commonly prescribed antibiotics when used in combination. Disks for the combination disk diffusion assays were prepared by first soaking with 20 µL of the common antibiotic solution (carbenicillin, streptomycin, colistin, and tetracycline). Once dry, the same disk was then soaked in an additional 20 µL of bitter melon extract and set out to dry completely in a sterile environment. Control disks were also prepared and consistently resulted in no ZoI.

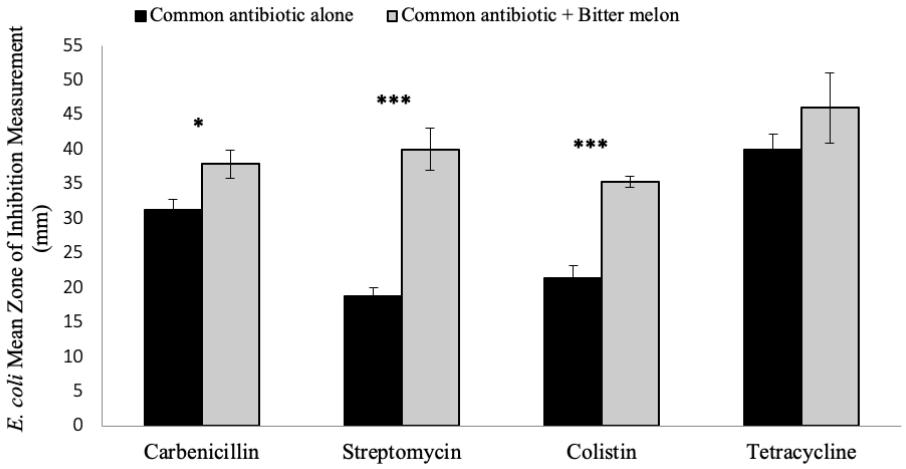

Figure 1. E. coli **average zone of inhibition statistical analysis between common antibiotics alone and in combination with bitter melon extract. The average diameter (in mm) of the zones of inhibition produced in the disk diffusion assays by each common antibiotic or common antibiotic combined with bitter melon extract are represented by the black and grey columns, respectively. Average diameters were calculated from the four to six replicates performed for each assay. Error bars represent the + and - values for standard error of the mean. A t-test was performed (with an assumption of unequal variance) to calculate the difference between the mean values for common antibiotic tested alone or in combination with bitter melon extract; those resulting in statistically significant p-values are indicated with asterisks (* indicates a p-value of <0.05, and *** indicates a p-value of <0.001). The t-test for carbenicillin resulted in a p-value of 0.0295; for streptomycin, 0.0004; for colistin, 0.0002; and for tetracycline, 0.3106.**

For *E. coli*, all four commonly prescribed antibiotics tested in combination with bitter melon extract showed enhanced ability to prevent bacterial growth. The most striking difference was for streptomycin, as the ZoI diameter more than doubled with the combination resulting in an average ZoI of 40 mm relative to the 18.76 mm observed for streptomycin alone (Figure 1).

Interestingly, bitter melon extract used in combination with commonly prescribed antibiotics did not always result in enhanced antibacterial properties. For *P. putida*, bitter melon extract actually decreased the average ZoI diameter from 25.2 mm to 19.55 mm when tested in combination with carbenicillin (Figure 2). The antibiotic effects of the other three antibiotics against *P. putida,* however, were enhanced when combined with bitter melon extract. The most striking enhancement was for colistin, which increased from an average ZoI diameter of 19.6 mm for colistin alone compared to an average of 33.65 mm for the combination (Figure 2). When tested for efficacy at inhibiting *M. luteus* growth, bitter melon extract combined with carbenicillin showed only a modest increase in the average ZoI diameter from 41.45 mm to 49 mm (Figure 3). All other combinations resulted in either the same effect against *M. luteus* growth or a decreased effect against *M. luteus* growth (Figure 3).

Antibacterial synergy between bitter melon extract and some commonly prescribed antibiotics is statistically significant

Since our data suggested that bitter melon extract can influence the antibacterial properties of the commonly prescribed antibiotics tested, our final goal was to determine whether the differences observed between the average ZoI for the common antibiotics alone compared to those for the combinations with bitter melon extract were statistically significant. To determine this, the raw data derived from the disk diffusion assay ZoI measurements was compiled in an excel spreadsheet and categorized by each bacterial species and common antibiotic. The data for each replicate was included, and each spreadsheet was uploaded into the

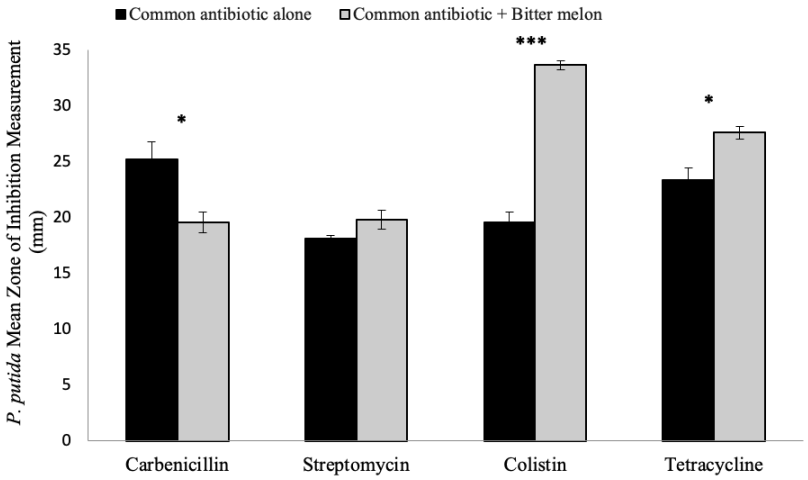

Figure 2. P. putida average zone of inhibition statistical analysis between common antibiotics alone and in combination with bitter melon extract. | The average diameter (in mm) of the zones of inhibition produced in the disk diffusion assays by each common antibiotic or common antibiotic combined with bitter melon extract are represented by the black and grey columns, respectively. Average diameters were calculated from the four to six replicates performed for each assay. Error bars represent the + and - values for standard error of the mean. A t-test was performed (with an assumption of unequal variance) to calculate the difference between the mean values for common antibiotic tested alone or in combination with bitter melon extract; those resulting in statistically significant p-values are indicated with asterisks (* indicates a p-value of <0.05, and *** indicates a p-value of <0.001). The t-test for carbenicillin resulted in a p-value of 0.0296; for streptomycin, 0.1386; for colistin, 0.0001; and for tetracycline, 0.0212.

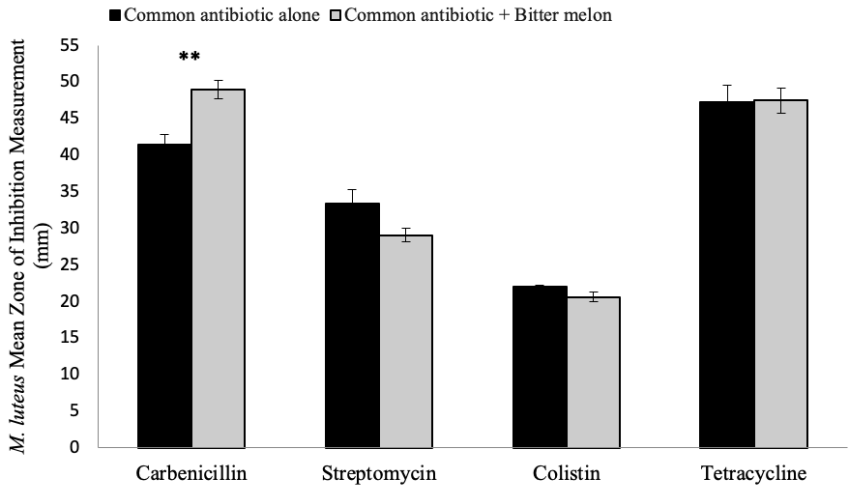

Figure 3. M. luteus average zone of inhibition statistical analysis between common antibiotics alone and in combination with bitter melon extract. The average diameter (in mm) of the zones of inhibition produced in the disk diffusion assays by each common antibiotic or common antibiotic combined with bitter melon extract are represented by the black and grey columns, respectively. Average diameters were calculated from the four to six replicates performed for each assay. Error bars represent the + and - values for standard error of the mean. A t-test was performed (with an assumption of unequal variance) to calculate the difference between the mean values for common antibiotic tested alone or in combination with bitter melon extract; those resulting in statistically significant p-values are indicated with asterisks (** indicates a p-value of <0.01). The t-test for carbenicillin resulted in a p-value of 0.0068; for streptomycin, 0.1002; for colistin, 0.1284; and for tetracycline, 0.9333.

statistical analysis program *Rguroo* (22). A mean inference analysis between two populations (average common antibiotic ZoI diameter relative to the average diameter for each tested with bitter melon extract) was used to perform a t-test evaluating the difference between each population mean and generate p-values (Figures 1, 2, and 3).

For all organisms and antibiotics tested, data was highly repeatable with relatively low variance as measured by standard error of the mean (Table 1, Figures 1, 2, and 3). Despite the low observed variance, t-tests were performed with an assumption of unequal variance, which allowed for a more rigorous comparison. A test for normality of each dataset was also performed in *Rguroo,* with each set of replicates found to be normally distributed (22).

The data for *E. coli* show that there is a statistically significant difference between the mean ZoI diameter for the common antibiotics carbenicillin, streptomycin, and colistin alone relative to the mean ZoI diameter for each of these combined with bitter melon extract, with p-values of 0.0295, 0.0004, and 0.0001, respectively (Figure 1). For *P. putida,* bitter melon extract was found to significantly increase the mean ZoI diameter of colistin and tetracycline relative to the common antibiotics alone, with p-values of 0.0001 and 0.0212, respectively (Figure 2). On the other hand, bitter melon extract combined with carbenicillin when tested against *P. putida* resulted in a significantly smaller average ZoI diameter compared to carbenicillin alone, with a p-value of 0.0296 (Figure 2). For *M. luteus*, bitter melon extract only significantly increased the mean ZoI diameter when combined carbenicillin, with a p-value of 0.0068 (Figure 3).

Discussion:

The extensive use of antibiotics in healthcare has led to a dramatic rise in antibiotic resistance and requires immediate action. The development and discovery of new drugs to treat such resistant infections has risen to be the top priority of the World Health Organization (WHO) (23, 25). It is critical to test new antibiotic combinations in order to treat a wider range of pathogens. The goal of our research was to test the antibacterial properties of bitter melon extract alone and in combination with four commonly prescribed antibiotics (carbenicillin, streptomycin, colistin, and tetracycline) against three different bacterial species: *E. coli, P. putida,* and *M. luteus.* The disk diffusion data show that bitter melon alone had little to no antibacterial properties when tested against the bacteria individually (Table 1). However, when tested in combination with the common antibiotics, there were in some cases significant effects on the efficacy of the antibacterial properties (Figures 1, 2, and 3). The data primarily showed a statistically significant enhancement in the antibacterial activity of the common antibiotics (i.e. antibacterial synergy), but also one example of antibacterial antagonism.

In this study, we aimed to test a broad range of organisms to explore differences in the potential synergistic or antagonistic effects of bitter melon extract. Although the three organisms selected are all Biosafety Level 1 organisms, all have important clinical relevance. *E. coli* is a Gram-negative bacterium that is resistant to many prescribed antibiotics (18). One study found that the rate of adaptive mutations in *E. coli* is about 10^{-5} per genome per generation, which is 1000 times higher than previous estimates (18). These antibiotic-resistant *E. coli* strains are passed on, often carrying multiple drug resistant plasmids that can easily transfer to other organisms (20). *M. luteus* is a Gram-positive organism that is detected as a commensal organism in the mucous membrane as well as in soil and water (27). Although *M. luteus* is described as low virulence, the bacterium can become pathogenic under certain conditions (5). *P. putida* is found in soil and water and has been reported as an opportunistic pathogen that can occasionally cause hospital-acquired infections (11, 12). Strains of this species are known to exhibit resistance to many antibiotics through the presence of plasmids that encode antibiotic resistance factors (12).

Carbenicillin, streptomycin, colistin, and tetracycline were the common antibiotics chosen to test in our study. Carbenicillin is an antibiotic among the semisynthetic penicillin group and is used to treat illnesses such as bladder infections. It has Gram-negative coverage but limited Gram-positive coverage (17). Streptomycin is an antibiotic that is commonly used to treat aerobic Gram-negative bacterial infections such as tuberculosis. The original wide spectrum of activity exhibited by streptomycin against both Gram-negative and Gram-positive bacteria has severely decreased due to the rise of antibiotic resistance; commonly

to Enterobacteriaceae such as *E. coli* (24). Colistin is a polymyxin "last line" antibiotic used to treat infections caused by multi-drug resistant Gram-negative bacteria such as *P. aeruginosa*. Being a "last line" antibiotic, this means there are no other novel antibiotics to treat such infections and research for an alternative treatment is crucial (16). Tetracycline is a protein synthesis inhibitor antibiotic that is used to manage and treat bacterial infections. Originally tetracycline showed antibacterial activity against most medically relevant aerobic and anaerobic bacteria, both Gram-positive and Gram-negative (21).

Many plants used in Chinese traditional medicine have been shown to have antibacterial properties. In one study, essential oils prepared from 21 different plants were all found to have at least some degree of antibacterial activity against 10 *Pseudomonas* species tested, with oil from *Cinnamomum zeylanicum* displaying the highest inhibitory effect (9). In another study, berberine, an alkaloid derived from goldenseal (*Hydrastis canadensis)* was itself found to have antibacterial activity against *Staphylococcus aureus.* Flavonoids isolated from a different part of the plant were found to have a synergistic effect on berberine efficacy despite having no inherent antibacterial effect when used individually (8). Previous studies have explored the various biological activities of bitter melon such as antiviral, antioxidant, and antibacterial components (3, 6, 15, 26). For example, one study determined that essential oils of bitter melon have significant inhibitory effects on *S. aureus* growth, as well as significant antibacterial activity against several other bacteria including *Pseudomonas multocida, Salmonella typhi,* and *Staphylococcus epidermidis*(6). Bitter melon pulp extract also was proven to have a broader spectrum of antibacterial activity against *E. coli, Staphylococcus*, and *Pseudomonas* (6). In addition to plant-derived compounds themselves exhibiting antibacterial properties, many plant extracts have been shown to have synergistic effects with antibiotics, thus warranting our research (8, 13).

As we expected, all commonly prescribed antibiotics tested had at least some effect at inhibiting the growth of each bacterial species tested (Table 1). It was also unsurprising that the efficacy of each antibiotic tested varied depending on the organism. For example, ZoIs for *P. putid*a were typically smaller for all antibiotics tested. *Pseudomonas* species are known for their high degree of intrinsic antibacterial resistance, which can account for this observation (14). Bitter melon extract tested alone had no effect against either *E. coli* or *P. putida,* and only a very minimal effect against *M. luteus* (Table 1). We found this result surprising given that previous research has shown antibacterial activity of bitter melon against some microorganisms, including *E. coli* and *Pseudomonas* species (6). Differences in bitter melon's antibacterial activity however, have been observed depending on which part of the plant was tested (i.e. seeds, leaves, fruit), and whether mature or immature plants were tested (15, 26). It is thus possible that by using the entire plant, antibacterial properties were diluted out and not detected in our assays. It is also possible that differences in the methodology and solvent used for extraction of oils as well as the amount of plant used and extent to which the oils were concentrated account for the lack of antibacterial activity we observed.

Although bitter melon extract had little to no antibacterial activity against the strains tested when applied individually to paper disks, we nonetheless decided to test whether bitter melon could enhance the antibacterial activity of any of the four commonly prescribed antibiotics tested (Figures 1, 2, and 3). While most studies that assay for synergistic combinations of antibacterials test two or more compounds that have antibacterial activity individually, a study by Junio *et al*. (2011) found that some compounds can have synergistic effects on other antibacterials without themselves having any direct antibacterial properties (8). Interestingly, our results do suggest that bitter melon extract can have indirect effects on some of the antibiotics tested, when used against specific organisms. For *E. coli,* bitter melon extract significantly increased the ZoI size when used in combination with carbenicillin, streptomycin, and colistin, compared to each of these antibiotics administered alone (Figure 1). For *P. putida*, statistically significant enhancement in ZoI size for antibiotics used in combination with bitter melon extract was seen for colistin and tetracycline (Figure 2). For *M. luteus,* significant enhancement of growth inhibition was seen when bitter melon extract was used in combination with carbenicillin, compared to carbenicillin alone (Figure 3). This suggests that bitter melon extract has bioactive components that work synergistically in combination with

commonly prescribed antibiotics, when used against certain microorganisms. Of note, we did find one potentially antagonistic combination, as carbenicillin combined with bitter melon extract resulted in less inhibition of *P. putida* growth than carbenicillin alone (Figure 2).

Our finding of significant antibiotic synergy between colistin and bitter melon extract when used against *P. putida* is of particular interest. Colistin is commonly used as a "last line" antibiotic to treat multidrug- resistant *Pseudomonas aeruginosa* infections, but resistance to colistin has been steadily increasing over the years (10). If our results are found to be repeatable when tested against *P. aeruginosa,* this synergistic combination could provide another potential avenue for treatment of these deadly infections. Testing for synergistic antibiotic combinations to treat *P. aeruginosa* is already underway. For example, the combination of streptomycin and cefadroxil were reported to be synergistic against *Pseudomonas aeruginosa* isolates in vitro when compared to the effects of each of these antibiotics alone (2). Further testing will be the key to determining which combinations work best in vivo, when treating patients.

On the other hand, our result of an antagonistic effect of bitter melon extract when used with carbenicillin relative to carbenicillin used alone against *P. putida* is also noteworthy. Since carbenicillin is a commonly prescribed antibiotic for Gram-negative pathogens such as *Pseudomonas* species, it is important to be aware of the limitations of combination treatments and have a thorough understanding of both synergistic and antagonistic combinations in order to optimize treatment outcomes (1, 17). Antibiotic antagonism is not unexpected when combining antibiotics. For example, Murray and colleagues found that treating *P. aeruginosa* first with low concentrations of polymyxin B resulted in its decreased antibiotic susceptibility when later exposed to gentamicin, neomycin, tobramycin, or ciprofloxacin (14). It is therefore critical that combinations of antibiotics are tested prior to use either combinatorially or subsequently to avoid the possibility of antagonistic effects such as this.

Our research presents a possible solution to combat the growing problem of antibiotic resistance. Although the results of our study are preliminary, they contribute to the foundational understanding necessary for future advancements in furthering the discovery of synergistic antibiotic combinations. Future directions of this work include repeating assays using isolated and purified components of bitter melon. Bitter melon has many phytochemicals with antioxidant, anti-inflammatory, and antibacterial properties (3, 15). Further research on the efficacy of bitter melon's antibacterial properties when used either alone or combinatorially should thus be done through fractionation and chromatography assays, as well as minimum inhibitory concentration analysis (MIC). Ultimately, our findings will need to be tested using in vivo models and with physiologically relevant concentrations of antibiotics and purified bitter melon components. In conclusion, the findings of this research give one potential solution to help combat the drastic increase in antibiotic-resistant pathogens that are threatening our global healthcare.

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