Interactions between lemongrass and lavender essential oils in combination with ampicillin influencing antibacterial activity on Sporosarcina ureae and Serratia liquefaciens

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ABSTRACT: The purpose of this study is to determine the effects of various combinations of essential oils (EOs) with antibiotics on bacterial growth. The molecular mechanisms behind the effects of individual phytochemicals in EOs and antibiotics is well understood, unlike the mechanisms behind the interactions between multiple phytochemicals and antibiotics in a mixture. Serratia liquefaciens and Sporosarcina ureae were exposed to various treatments of different combinations of Lavandula officinalis (lavender oil), Cymbopogon citratus (lemongrass oil) with ampicillin. For each treatment group, mean zones of inhibition (ZOI) were measured after exposure for 48 hours. Controls for both species did not yield any ZOI whereas all other treatments resulted in the inhibition of bacterial growth in both Serratia liquefaciens and Sporosarcina ureae. Statistical analyses showed that the combination of lemongrass oil and ampicillin was significantly more effective than all other treatments for Serratia liquefaciens. The lemongrass oil and ampicillin treatment was the only treatment that displayed additive effects. All treatments for Sporosarcina ureae, with the exception of the control and lavender oil treatments, showed a significantly higher mean ZOI when compared to control and lavender oil treatments. It was concluded that lemongrass oil was a better candidate to be included in antibacterial cocktails than lavender oil. However, further investigation is required to elucidate EOs that interact synergistically with ampicillin when acting on Serratia liquefaciens and Sporosarcina ureae. Additionally, further investigation into the molecular mechanisms behind the interactions of the components found in these EOs with ampicillin is required.

KEYWORDS: Antibacterial cocktail, synergy, essential oil, bacteria

Introduction

Disease-causing microorganisms have become increasingly resistant to single-target antibiotics, rendering conventional antibiotics less effective. This increase in resistance has resulted in the search for new antimicrobial agents to develop a cocktail of multi-target drugs (1).

Current research focuses on developing multi-target drugs using antimicrobial compounds found in essential oils extracted from herbs and spices because multi-drugs have fewer associated side-effects and are relatively cheaper than antibiotics (1, 2). Essential oils (EOs) are natural oils of plants extracted via distillation. EOs contain varying concentrations of many different constituents known as phytochemicals (3). Major components in EOs such as polyphenols and terpenoids exhibit antimicrobial properties due to their aromatic and phenolic content. These characteristics allow the molecules to associate with bacterial membranes to increase their permeability, which disrupts cellular processes within bacteria (4). The high lipophilicity of terpenoids due to their hydrocarbon tails allows these phytochemicals to permeate through bacterial cell walls (1). Plants have evolved to protect their most vulnerable parts, such as their leaves, by concentrating phytochemicals in these areas to target proteins and glycoproteins in the cell membranes of potentially harmful microorganisms (3). Plants that contain EOs, like Lavandula officinalis (lavender) and Cymbopogon citratus (lemongrass), have been used as natural medicines in a variety of cultures because of their antimicrobial and...
antioxidant properties (5, 6). Lavender (lav) is a fragrant shrub that has violet flowers. Lemongrass (lem) is a grass that smells like lemon.

Recent studies have shown that various combinations of antibiotics with EOs could produce synergistic, additive, or antagonistic effects with regards to their antibacterial properties (4). Synergy occurs when the combined effects are greater than the sum of their constituent effects in a mixture; an additive effect is when the total effect is equal to the sum of each constituent effect in a mixture; and an antagonistic effect is less than the sum of each constituent effect in a mixture (1). Synergy occurs in multi-target drugs that have multiple constituents cooperating together by binding to several targets (1). This type of synergy is observed in EOs where multiple phytochemicals can act upon different components in a bacterial cell (1). Other factors that contribute to synergy are the interactions between major and minor constituents within EOs (1). Minor constituents do not possess antibacterial properties, however they may increase solubility and resorption rates of major constituents that do possess antibacterial properties, increasing their potency (1). Studies have shown that EOs significantly reduce bacterial growth compared to their individual major components (4). In contrast, additive effects sometimes occur due to a shared target between multiple compounds (1). In antimicrobial cocktails, synergistic interactions between antibiotics with EOs or between different EOs show great promise in being an effective way to treat disease with respect to bacteria that develop antibiotic resistance.

Nevertheless, more research is needed to determine the specific molecular mechanisms behind the synergistic interactions between phytochemicals in EOs and antibiotics in terms of their antibacterial activity. A deeper understanding of these interactions could help with the development of drugs that target resistant defenses in bacteria. Since bacteria differ in their physiology, they also differ in their susceptibility to antimicrobial cocktails (4). For instance, one study researched antibiotic resistant bacteria that contained a gene encoding for beta-lactamase, which is an enzyme that degrades beta-lactam-containing antibiotics such as ampicillin (amp) and amoxicillin (7). Adding lavender oil to the initially ineffectve antibiotics effectively inhibited bacterial growth (7). It is believed that phytochemicals can bind to the active site of beta-lactamase and cease its activity, allowing for antibiotics like ampicillin to inhibit enzymes that build the cell walls in bacteria, leading to lysis (1). Researchers can use this knowledge to make new drugs that target multiple pathways in pathogenic bacteria (2).

This study tests the susceptibility of *Serratia liquefaciens* (Grimes and Hennerty) and *Sporosarcina ureae* (Beijerinck) Kluyver and van Niel to antimicrobial cocktails containing lavender and lemongrass oils with ampicillin. *Serratia liquefaciens* is a rare pathogenic gram-negative bacteria part of the Enterobacteriaceae bacterial family. Enterobacteriaceae are known to develop antibiotic resistance by overexpressing efflux pumps in their membranes such that antibiotics are removed from the cell preventing their accumulation (8, 9). Some EOs contain compounds known as efflux pump inhibitors which prevent the removal of antibiotics in these bacteria lowering the chances of infection occurring (8). *Serratia liquefaciens* can cause infection in the bloodstream and urinary tract of dialysis patients as a result of contamination due to breaches in health protocols and improper handling of dialysis equipment (9). Bloodstream infections in chronic dialysis patients lead to mortality 12-38% of the time (10). Dialysis patients must be given antibiotics, usually ampicillin, to reduce risk of infection because gram-negative bacteria have an extra membrane composed of lipopolysaccharides (LPS) that evade eukaryotic immune responses (11). Due to reduced kidney function in these patients, which also reduces their ability to eliminate drugs, ampicillin must be administered in intervals of low doses (11). The concerted use of EOs, such as lavender and lemongrass oils, with ampicillin may reduce the dosage of the latter, alongside its negative consequences. They can also be useful for reverting possible antibiotic resistance in *Serratia liquefaciens*. Additionally, lavender oil is a diuretic and can aid patients by compensating for their reduced kidney function. *Sporosarcina ureae* is a non-pathogenic gram-positive bacteria that is unique because it contains an enzyme known as urease, which degrades urea (12). Patients with reduced kidney function often experience overgrowth of natural bacteria in the gut, which leads to an excess of ammonia production (12). The liver converts this ammonia into urea, which is toxic to the body in high concentrations and cannot be removed because the kidneys of these patients cannot properly filter such toxins (12). *Sporosarcina ureae* is encapsulated and used as a probiotic to degrade urea, reducing the burden on the ailing kidney by preventing bacterial overgrowth in the gut and accumulation of harmful urea (12). EOs have therapeutic uses such as reducing emotional stress, boosting mood and promoting restful sleep, which patients can highly
benefit from. There are no known studies exploring the effects that antibacterial cocktails containing EOs, such as lavender and lemongrass oils, and ampicillin have on *Serratia liquefaciens* and *Sporosarcina ureae*. This information would be important when treating patients on dialysis with reduced kidney function.

The objective of this experiment was to determine if the combination of lavender and lemongrass oils with ampicillin will inhibit *Serratia liquefaciens* and *Sporosarcina ureae* synergistically, additively or antagonistically. It was hypothesized that treatments of different combinations of lavender and lemongrass oils and ampicillin exposed to *Serratia liquefaciens* and *Sporosarcina ureae* would yield dissimilar mean zones of inhibition (ZOI). It was also hypothesized that the treatment groups would differ in their mean ZOI compared to the control groups.

Methods

The ZOI of the bacterial strains *Sporosarcina ureae* and *Serratia liquefaciens* were measured in this study. The ZOI is a circular region of a bacterial culture that has been killed off, in this case through the application of an assay disk with a given antibacterial treatment. Agar plates were divided into four quadrants and separated into groups of two, one group for each bacterium to be spread on. Prior to plating the bacteria, their respective flasks were swirled to ensure randomization in the sampling of the bacteria. To create seven treatments, assay disks were soaked in individual stock solutions of lavender, lemongrass, ampicillin, solutions that combined each EO with ampicillin (100 mg/mL), both EOs together and a mixture of all three solutions. The concentration of all EOs used was 100%. Three agar plates were assigned to each treatment. As a control, *Sporosarcina ureae* and *Serratia liquefaciens* were grown on agar plates and exposed to assay disks soaked in distilled water.

Assay disks were randomly placed into seven empty petri dishes and each petri dish assigned to a treatment. Assay disks were administered 30 µL of treatment each and left to soak for ten minutes. After soaking, assay disks of the same treatment were placed onto each of the four quadrants of the same agar plate, equidistant from each other. There were three agar plates per treatment, giving a total of 12 replicates per treatment. *Sporosarcina ureae* and *Serratia liquefaciens* were grown at 25 and 30 °C respectively for 48 hours in the absence of light. After this growth period, the ZOIs (mm) of the bacteria were measured using a calibrated electronic caliper. Perpendicular diameters were used to measure the ZOI. The two diameter measurements were then averaged and recorded.

Statistical analysis

The statistical program IBM SPSS Statistics 24 (Version 24.0., IBM Corp.) was used to conduct an one-way analysis of variance and a post-hoc Tukey test to analyze the differences among the mean ZOI of each treatment for both *Sporosarcina ureae* and *Serratia liquefaciens* (alpha = 0.05).

Results

In *Serratia liquefaciens*, there were no significant changes (F=127.121, P<0.001) in mean zone of inhibition (mm ± SD) of *Serratia liquefaciens* growth after incubation at 30°C for 48 hours. Means labelled with the same letter within each treatment are not significantly different (P<0.05) according to Tukey's test.

In *Sporosarcina ureae*, there was a significant increase (F=899.240, P<0.001) in mean zone of inhibition (mm) between Control and Lav. All treatments, with the exception of Control and Lav, killed all bacteria present on the agar plate giving a maximum mean zone of inhibition (mm) (Fig. 1).

In *Sporosarcina ureae*, there was a significant increase (F=899.240, P<0.001) in mean zone of inhibition (mm) between Control and Lav. All treatments, with the exception of Control and Lav, killed all bacteria present on the agar plate giving a maximum mean zone of inhibition (mm) (Fig. 2).
Discussion

The results of this present study supported the initial hypothesis that each treatment group including the control group would yield different mean ZOI when used on Serratia liquefaciens and Sporosarcina ureae. Due to the varying compositions of these cocktails, different interactions between their constituents occur— influencing antimicrobial activity. The data supported that both Serratia liquefaciens and Sporosarcina ureae are susceptible to all antimicrobial cocktails of lavender and lemongrass oils with ampicillin. However, Sporosarcina ureae was significantly much more susceptible to all of the treatments compared to Serratia liquefaciens (Fig.2., Fig.1.). The combination of lemongrass oil and ampicillin showed a significantly higher mean ZOI than any other combinational treatment for Serratia liquefaciens (Fig.1.), showing additive effects and potentially being beneficial for their use with antibiotics. All other combinational treatments do not show any additive or synergistic effects when used on Serratia liquefaciens. The same conclusions cannot be made for Sporosarcina ureae because every treatment other than the control and the lavender oil treatment showed no bacterial growth.

One study concluded that lavender showed synergistic effects with piperacillin when exposed to gram-negative bacteria that was resistant against beta-lactam containing antibiotics; however, there was no interaction between lavender oil and ampicillin (7). This supported the results of the present study, which showed no interaction between lavender and ampicillin in Serratia liquefaciens (Fig. 1.). In previous studies, both lavender and lemongrass oils had stronger inhibitory effects on gram-positive bacteria than gram-negative bacteria (5, 6). This was observed when the lavender treatment was three times more effective in preventing bacterial growth for Sporosarcina ureae in comparison to Serratia liquefaciens (Fig.1., Fig.2.). The reason behind this trend is because gram-negative bacteria have an extra membrane layer containing LPS that serves as an extra barrier against macromolecules and hydrophobic molecules such as EOs and antibiotics (5). Another study observed that lemongrass oil showed consistent synergy with antibiotics when exposed to multi-drug resistant gram-positive and gram-negative bacteria (6). These results did not agree with the results of the present study since only additive effects were observed when treating Serratia liquefaciens with lemongrass oil and ampicillin (Fig. 1.). This is explained by the fact that different strains and types of bacteria vary in their susceptibility to the same antibacterial cocktails (4). Additionally, factors such as the plant chemotype, method of plant harvest, and storage conditions of the plant affect the concentration of certain constituents which affect antibacterial properties within the EOs (13).

The reason behind lemongrass oil being a much more effective additive to ampicillin can be explained by comparing its composition of phytochemicals to that of lavender oil. Both lavender and lemongrass oils contain a phytochemical known as limonene that is shown to decrease membrane permeability by dissociating unsaturated fatty acids from membranes of bacteria allowing for better passage of antibiotics into bacteria (2). This compound causes leakage of cytoplasmic material that causes a disruption in the bacteria’s regulation of water and can possibly follow a cascade causing lysis in bacteria (2). In the EOs used in this study, limonene was a major constituent in lemongrass oil and was a minor constituent in lavender oil. In lavender oil, on the other hand, linalool is a major constituent that binds to LPS, weakening gram-negative bacterial defenses against antibiotics; however, it shows weaker inhibitory effects than other phytochemicals like limonene (2, 4). This explains why lemongrass oil was much more effective than lavender oil in inhibiting bacterial growth in both Serratia liquefaciens and Sporosarcina ureae (Fig. 1., Fig. 2.).

Both lavender and lemongrass oils contain compounds that have been shown to disrupt...
quorum-sensing mechanisms in bacteria. Quorum-sensing (QS) mechanisms allow for motility in response to environmental changes and conjugal plasmid transfer between bacteria to spread resistance against a foreign threat (14). EOs have been shown to be effective to treat infection very efficiently possibly due to their effects on QS in bacteria (14). This explains why both lavender and lemongrass oils were effective in inhibiting growth in both Serratia liquefaciens and Sporosarcina ureae (Fig. 1., Fig. 2.).

Future experiments may investigate the interactions between a wider variety of EOs with ampicillin at different concentrations to explore combinations that display synergistic interactions and reduce antibiotic doses. Since Serratia liquefaciens and Sporosarcina ureae are quite different, different antibacterial cocktails can be selectively inhibitory to each bacterium, which is important when treating bacterial infections in patients with reduced kidney function who take probiotics. It is also important to conduct in vivo experiments in mice to determine if such synergistic interactions between EOs and antibiotics extend to such an environment and to make sure that these antibacterial cocktails are not toxic. Due to the possibility of antibiotic resistance in Serratia liquefaciens, future experiments should explore the susceptibility of antibiotic resistant strains of this bacteria to antibacterial cocktails. Lastly, due to the complexity of EOs, specific mechanisms for the interactions of their compounds are poorly understood and may be investigated in future experiments.

Conclusion

In conclusion, synergism between the combinational treatments of lavender and lemongrass oils with ampicillin was not established when exposed to Serratia liquefaciens and Sporosarcina ureae. All combinational treatments inhibited Serratia liquefaciens and Sporosarcina ureae growth compared to the control, where Sporosarcina ureae was more susceptible to all treatments compared to Serratia liquefaciens. All other combinations of EOs and antibiotics did not interact, showing no significant differences in their mean ZOI in Serratia liquefaciens (Fig.1.). However, the combination of lemongrass oil and ampicillin showed a significant additive effect to the mean ZOI of Serratia liquefaciens (Fig.1.). Thus, lemongrass oil is a stronger candidate to be used as an additive in antibiotic cocktails to treat bacterial infection compared to lavender oil. More EOs need to be tested in future experiments to elucidate synergistic interactions and their mechanisms when used to treat ampicillin-resistant bacteria so that new drugs can be developed to specifically target pathogenic bacteria.

References