Inhibitory effects of combined *Ocimum basilicum* or *Rosmarinus officinalis* essential oil and Vancomycin therapy on *Staphylococcus aureus* growth

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INTRODUCTION

In nature, plants possess secondary compounds that serve ecological functions. Oftentimes they attract certain insects to pollinate or feed on the plant, but also serve as repellents for many potential predators (Fraenkel 1959). It has also been suggested that these compounds may serve a function in the initial stages of plant growth. For example, the herb rosemary contains a polyphenol, rosmarinic acid. The acid remains at highly concentrated and fairly consistent levels in all of the plant's organs and throughout the plant's growth, indicating an active role in early plant development (José del Bano 2003).

The secondary compounds of plants have also been discovered to be adaptable to medical uses. They have served as model compounds for drug synthesis, and acted as chemical models for the design of new drugs (Balandrin et al 1985). One drug, Etoposide, is derived from *Podophyllum peltatum* (the mayapple) and is used in the treatment of small cell lung carcinomas, refractory testicular carcinomas, nonlymphocytic leukemias, and non-Hodgkin's lymphomas (Balandrin et al 1985).

The secondary compounds of spices in particular have shown potential for medical uses, especially antibacterial applications. In cinnamon, cinnamaldehyde can act against multiple strains of bacteria that are detrimental to human health (Chang et al. 2001). Ginger has demonstrated antibacterial effects against *Escherichia coli*, *Salmonella enteriditis*, *Clostridium perfringens*, *Staphylococcus aureus*, *Campylobacter jejuni*, and *Bacillus cereus* (Sunilson et al. 2007).

The possible uses of spice secondary compounds in combating bacterial growth are especially significant with the increasing number of antibiotic resistant bacteria. The first instances of antibiotic resistant bacteria occurred in military hospitals in the 1930s. By 2004, the list of bacteria resistant to a variety of antibiotics included (but was certainly not limited to) *Streptoccoccus pyogenes Escherichia coli*, *Shigella* and *Salmonella Mycobacterium tuberculosis*, *Enterococcus faecium*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Acinetobacter baumanii* and *Pseudomonas aeruginosa* (Levy & Marshall 2004).

One of these bacteria, *S. aureus*, is of special interest because of its potential for serious infection, abscess formation, sepsis development and mortality (Lowy 1998). *S. aureus* is a gram positive bacterium that is described as a superbug, resistant to multiple drugs (Walsh 1999).

Vancomycin, considered to be an important antibiotic in combating gram-positive bacteria, is often used as a last resort against particularly resilient infections (Walsh 1999). In spite of the effectiveness of vancomycin, some bacteria have developed resistance to it. This includes five strains of enterococci bacteria (Murray 2000) and strains of *S. aureus* (Levy & Marshall 2004).

The effectiveness of two particular spices, rosemary and basil, against bacterial growth has been investigated as a potential solution for treating antibacterial resistant bacteria. Growth of *S. aureus* is inhibited by the essential oil of lemon basil (*Ocimum citriodorum*) and African basil (*O. amerincanum*) (Carovic-Stanko et al 2009). The essential oils in rosemary also demonstrate significant antimicrobial activity against *S. aureus* (Fu 2007).

The following study investigated if a combination of vancomycin and the essential oils of basil or rosemary increased the effectiveness of inhibiting the growth of *S. aureus* more than either treatment alone. The essential oils tested included that of basil and rosemary because these two spices have demonstrated effectiveness against *S. aureus* in previous studies.

Materials and Methods

Twenty Fisher Scientific plates containing agar and Muller Hinton nutrients (Hardy Diagnostics) were streaked with *S. aureus* bacteria provided by the Washington State University Microbiology Prep Laboratory. The plates were streaked to create a lawn pattern of bacterial growth. Each plate was divided into four sections, one for each treatment of vancomycin alone, basil OR rosemary alone, vancomycin and basil OR rosemary, and a control of no treatment.

One 30 mg vancomycin disc (Hardy Diagnostics) was placed on each plate. Ten vancomycin discs were impregnated with 20 µL of 100% essential oil of *O. Basilicum* (organic basil, Oshadi Professional Aromatherapy). Ten vancomycin discs were impregnated with 20 µL of 100% essential oil of *Rosmarinus officinalis* (rosemary, Aura Cacia Pure Aromatherapy). Ten filter paper discs were impregnated with 20 µL rosemary essential oil and ten filter paper discs were impregnated with 20 µL basil essential oil. One disc of each treatment was placed on the respective section of the agar surface. Control sections were left empty. All 20 plates were incubated for seven days at 37° C.

After removal from incubation, the zones of inhibition for each treatment were measured in terms of diameter around the treatment disc (in mm). In instances where the zone of inhibition was irregular in shape, the longest and shortest visible diameters were averaged. All experiments were performed in duplicate. The data were analyzed using two-tailed t-tests comparing the average zone of inhibition of each treatment (p-value=.05).

Results

Basil alone, vancomycin alone, and a combination of the two significantly inhibited the growth of *S. aureus.* Vancomycin alone was the most effective with an average zone of inhibition of 14.05mm. A combination of basil and vancomycin resulted in an average zone of inhibition of 13.6mm, and basil alone was least effective with an average zone of inhibition of 9.4mm (Figure 1). Both treatments containing vancomycin inhibited bacterial growth significantly more than basil alone, but a combination of basil and vancomycin did not inhibit bacterial growth significantly more than vancomycin alone.

Rosemary alone, vancomycin alone, and a combination of the two significantly inhibited the growth of *S. aureus*. A combination of vancomycin and rosemary was the most effective with an average zone of inhibition of 15.7mm. Vancomycin alone resulted in an average zone of inhibition of 15.15mm, and rosemary alone was least effective with an average zone of inhibition of 9.55mm (Figure 2). Both treatments containing vancomycin inhibited bacterial growth significantly more than rosemary alone, but a combination of rosemary and vancomycin did not inhibit bacterial growth significantly more than vancomycin alone.

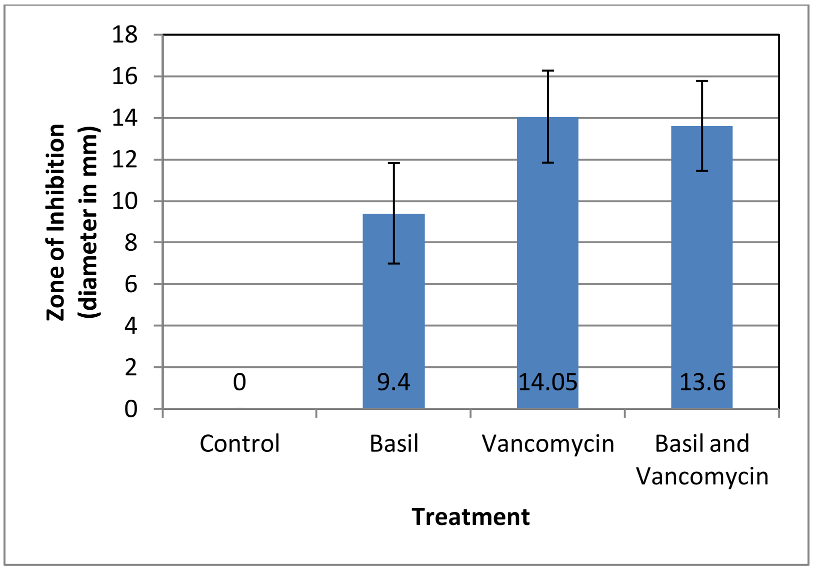

Figure 1. The zones of inhibition of basil, vancomycin, and basil/vancomycin combination treatments on *S. aureus* growth. Error bars indicate standard deviation about the mean for 20 replicates.

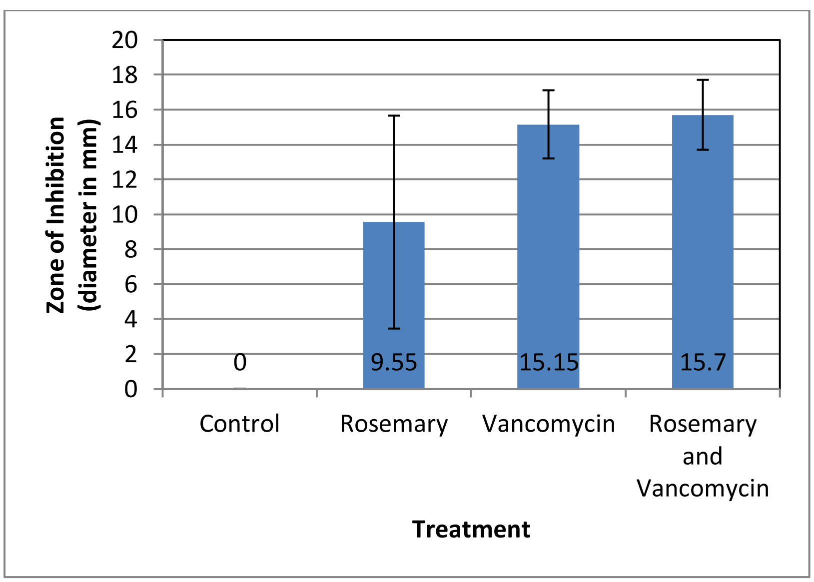

Figure 2. The zones of inhibition of rosemary, vancomycin, and rosemary/vancomycin combination treatments on *S. aureus* growth. Error bars indicate standard deviation about the mean for 20 replicates.

Discussion

The results indicate that vancomycin alone is the most effective treatment for inhibiting the growth of the *S. aureus.* Although both basil and rosemary show potential for inhibiting bacterial growth, combining either of these treatments with the already effective vancomycin did not significantly increase the treatment's inhibitory effect.

These findings on basil are largely concurrent with those of Corovic-Stanko et al. who demonstrated that the essential oil of the basil variety *O. basilicum* significantly inhibits the growth of *E. coli* bacteria but not the growth of *S. aureus*. Two different varieties of basil, *O. americanum* and *O. citriodorum* effectively inhibited the growth of *S. aureus*. Our study used the *O. basilicum* variety, which can explain its limited effectiveness against *S. aureus*. This can also explain why combining this particular oil with vancomycin did not increase the effectiveness at inhibiting bacterial growth.

These findings on rosemary differ from those of Fu et al. who found that rosemary essential oil significantly inhibited the growth of *S. aureus* with an average inhibitory zone diameter of 18.5±1.3mm. The average in the present study was a much lower 9.55±6.1mm. The same variety of rosemary, *Rosmarinus officinalis*, was used in each study, indicating that variety did not contribute to the difference in findings. Each study also employed a similar method of testing through the impregnation of filter paper discs and the placement of these discs on agar streaked with *S. aureus*. However, Fu et al. impregnated each disc with only 5 µL of essential oil while the present study used approximately 20 µL. It is possible that the level of saturation of the filter paper discs affected the inhibitory capabilities of the rosemary essential oil.

Bacterial resistance to antibiotics is still a growing problem. Even vancomycin, a powerful antibiotic against gram-positive bacteria, is ineffective against certain strains of enterococci bacteria (Murray 2000) and strains of *S. aureus* (Levy & Marshall 2004). The essential oils of spices alone show inhibitory effects against bacteria including *S. aureus*, *E. coli*, *E. faecalis*, *P. vulgaris*, *S. epidermidis* and are considered a potentially viable alternative treatment for antibiotic-resistant bacteria (Carovic-Stanko et al. 2009 and Fu et al. 2007). Further research is necessary to determine if a combination of the most effective varieties of spices, such as *O. americanum*, and antibiotic treatment increase the inhibition of bacterial growth.

LITERATURE CITED

Balandrin, M., J. Klocke, E. Wurtele, and W. Bollinger (1985) Natural plant chemicals: sources of industrial and medicinal materials. *Science* 228: 1154-1160.

This article details the multitudes of societal uses of secondary plant metabolites, the potential for the discovery and implementation of future uses, and the technological advances that will aid in making plants a renewable resource for these products. The authors describe the uses of secondary plant metabolites in industrial oils, resins, waxes, dyes, flavors, pharmaceuticals, rubbers, pesticides, and many other products. They also state that although there are opportunities to find even more uses, the window for this chance rapidly closes with the continued destruction of tropical forests.

Carovic-Stanko, K., S. Orlic, O. Politeo, F. Strikic, I. Kolak, M. Milos, and Z. Satovic (2009) Composition and antibacterial activities of essential oils of seven *Ocimum* taxa. *Food Chemistry* 119: 196-201.

The essential oils of seven *Ocimum* varieties were identified and screened for antibacterial activity against common pathogens. This included preliminary screening using the filter paper disc agar diffusion technique, while modification of the disc diffusion method was used for further analysis. The essential oil of *O. basilicum* essential oil inhibited *E. coli* while *Enterococcus faecalis*, *Enterococcus faecium*, *P. vulgaris*, *S. aureus*, and *S. epidermis* were inhibited by the essential oils of *O. americanum* and *O. citriodorum*.

Chang, S., P. Chen, and S. Chang (2001) Antibacterial activity of leaf essential oils and their constituents from cinnamomum osmophloeum. *Journal of Ethnopharmacology* 77: 123-127.

The essential oils of two *Cinnamomum osmophloeum* clones (A and B) were tested for antibacterial activity against nine strains of bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). The B leaf showed the greatest inhibitory effect against all strains of bacteria. Of the essential oil constituents, cinnamaldehyde showed the most effectiveness at varying minimum inhibitory concentrations.

Fraenkel, G. (1959) The Raison d'Etre of secondary plant substances. *Science* 129: 1466-1470.

This article describes the ecological role of secondary plant compounds as either attractants or repellents for insects, and how these chemicals developed through both plant and insect evolution. Certain insects are known to eat exclusively from certain plant families, and this behavior is attributed to those plants containing a chemical attractant. Others plants, those they do not feed on, are believed to contain a chemical repellent, which can also be toxic. Fraenkel details some of the primary insect-plant relationships among six plant families, and explains the roles that secondary plant compounds play in those relations.

Fu,Y., Y. Zu, L. Chen, X. Shi, Z. Wang, S. Sun, and T. Efferth (2007) Antimicrobial activity of clove and rosemary essential oils alone and in combination. *Phytotherapy Research* 21: 989-994.

The essential oils of rosemary and cloves were tested for effectiveness at inhibiting the growth of a variety of bacteria and fungi alone and in combination. The essential oils of each spice alone were significantly effective against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Proteus vulgaris*. Combined, the essential oils demonstrated effectiveness against *Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa.*

José del Bano, M., J. Lorente, J. Castillo, O. Benavente-García, J. Antonio del Río, A. Ortuño, K. Quirin, and D. Gerard (2003) Phenolic diterpenes, flavones, and rosmarinic acid distribution during the development of leaves, flowers, stems, and roots of *Rosmarinus officinalis*. *Journal of Agricultural and Food Chemistry* 51: 4247-4253.

The six polyphenols present in rosemary were studied and determined to all be present in only the leaves of the plant. One of these, rosmarinic acid, has the highest concentration in all of the plant's organs, suggesting a role in both biosynthesis and transport phenomena in different stages of plant development. Rosemary extracts were also found to be very effective antioxidants in both lipid and aqueous systems.

Levy, SB. and B. Marshall (2004) Antibacterial resistance worldwide: causes, challenges, and responses. *Nature Medicine* 10: S122-129.

The emergence of numerous strains of antibacterial resistant bacteria is described. The problem of resistance in hospitals and communities is examined, as well as the process by which bacterial populations become resistant. The authors go on to posit possible solutions for adapting to and preventing the development of antibacterial resistance.

Lowy, F.D. (1998) *Staphylococcus aureus* Infections. *The New England Journal of Medicine* 339: 520-532.

This article describes the development of physician understanding of *S. aureus* infections. This includes the pathogenesis, epidemiology, and management of the infection. The structure of the bacterial cell is examined, as are the modes of transmission and the types of diseases that can result from *S. aureus* infections.

Murray, B.E. (2000) Vancomycin-Resistant Enterococcal Infections. *The New England Journal of Medicine* 342: 710-721.

In enterococci, five types of resistance to vancomycin have been identified as VanA, VanB, VanC, VanD, and VanE. Some enterococci bacteria have an intrinsic resistance to killing by penicillin and vancomycin. The best characterized mode of resistance is found in the VanA group, in which a cluster of seven genes that are transcribed for vancomycin resistance are found on the transposable genetic element.

Sunilson, J., R. Suraj, G. Rejitha and K. Anandarajagopa (2009) In vitro antibacterial evaluation of *Zingiber officinale*, *Curcuma longa* and *Alpinia galanga* extracts as natural food preservatives. *American Journal of Food Technology* 4: 192–200.

Extracts of *Zingiber ofjicinale*, *Curcuma longa* and *Alpinia galangal* varieties of ginger were tested for antibacterial activity against fungi and common food-borne bacteria. While methanol extracts showed the greatest zone of inhibition, all extracts showed effectiveness against bacterial and fungal growth. The authors also determined the potential for selective rhizomes to be used as natural food preservatives.

Walsh, C. (1999) Deconstructing Vancomycin. *Science* 284: 442.

Vancomycin is examined as the prime example of a powerful antibiotic to which particularly resilient bacterial infections have become resistant. The development of these “superbugs” is cited as a developing problem in need of medical research. Possible solutions for antibiotic resistance, including modification of the sugar groups in the peptide backbone of vancomycin, are discussed and recommended for further research.