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Original Article

COMBATING THE ANTIBIOTIC RESISTANCE THREAT

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ABSTRACT

Objective: Bacteria have developed ability to resist antibiotics that previously served as effective treatment. There is an increasing concern by health care providers to address this problem in healthcare settings especially in underdeveloped countries where access to the latest antibiotics is limited. These antibiotic resistant pathogens, both Gram-positive and Gram-negative bacteria, usually found in health care facilities, can cause severe to fatal infections. Our research focused on five of the most problematic bacteria: Methicillin-resistant *Staphylococcus aureus* (MRSA), Methicillin-sensitive *Staphylococcus aureus* (MSSA), *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

Methods: For centuries home treatments have relied on the use of essential oils to treat ailments. We tested four commonly found essential oils (wintergreen, cinnamon, tea tree, and spearmint) against the five bacteria as well as methylglyoxal, which is an ingredient in Manuka honey. The Kirby-Bauer disk diffusion method was used and diameter of the zone of inhibition for each bacterium was measured to compare with standard antibiotics used for each strain of bacteria. In addition to studying the antibacterial activity of these compounds, we also investigated a way to deliver these compounds to patients, as topical applications, to inhibit the transmission of these multidrug resistant bacteria in healthcare settings.

Results: Wintergreen and cinnamon essential oils as well as methylglyoxal showed high inhibitory effect on the tested bacteria. We also tested and found that Aloe Vera oil, Aloe Vera gel and natural Aloe Vera served as effective carriers with the essential oils and methylglyoxal.

Conclusion: The antibacterial activity found in wintergreen and cinnamon essential oils and in methylglyoxal may offer a cost-effective alternative to commercial antibiotics because these compounds are readily available and relatively inexpensive and would be a benefit to people globally.

Keywords: Methicillin Resistant *Staphylococcus aureus* (MRSA), Methicillin Sensitive *Staphylococcus aureus* (MSSA), *Acinetobacter baumanni*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa*, Manuka, Methylglyoxal, Wintergreen, Cinnamon, Aloe Vera, Antibiotic resistance.

INTRODUCTION

Before the discovery of microorganisms, mankind was successful in using certain plants that were known to have healing potential [1]. Various plants and honeys were known to be an effective treatment against fighting infections and were commonly used in treating wounds. In particular, various types of honey show broad-spectrum antimicrobial activity and show effectiveness against antibiotic resistant bacterial pathogens [2-4]. The capability of essential oils to act as antiseptics is thought to come from the need of plants to be able to withstand harsh elements of nature, including a large degree of deadly microorganisms [5]. Various studies have tested and confirmed that a large number of essential oils have proven to be effective antimicrobial agents [6-8]. Various species of plants have been used for centuries to aid in fighting bacterial infections and many still continue to play a vital role in medicine. Today, the use of commercial antibiotic stands to serve as the main source of treatment of infectious diseases.

In modern times the anti-infectious procedure developed by humans including hygienic measures, epidemiological controls, vaccines, and antibiotics [9], have limited the evolution of pathogenic organisms. Many strains of bacteria, however, have developed mechanisms through which they become resistant to antibiotics and consequently lead to difficulty in treating infected patients [10]. Antibiotic resistance by certain pathogens remains to be a growing problem in the world and has been adversely affecting the lives of hundreds and thousands of people every year. The *Staphylococcus aureus* is a Gram-positive bacterium. *Staph aureus* can be defined into two strains according to their sensitivity to oxacillin, a beta lactam marker. Oxacillin susceptibility is a measure of *Staphylococcus* activity towards beta lactam antibiotics. Beta lactam works by impairing the cell's ability to synthesize the peptidoglycan layer, by binding to the penicillin binding protein (enzymes) that is essential for peptidoglycan synthesis. Strains of *Staphylococcus aureus* that are sensitive to these antibiotics are known as Methicillin Sensitive *Staphylococcus aureus* (MSSA). Strains of *Staphylococcus aureus* that are resistant to oxicillin are known as Methicillin Resistant *Staphylococcus aureus* (MRSA). MRSA has become a 'superbug' that has emerged in both community and hospital environments resulting in many patients developing infections. Vancomycin is the current standard treatment used against MRSA; however, treatment failures have been documented more frequently in the recent past [23]. The judicious use of antibiotics is essential if multi-resistant strains of MRSA are not to become more prevalent [11]. The ways that *Staphylococcus aureus* infections are defined include hospital-acquired MRSA (HA-MRSA) and community acquired MRSA (CA-MRSA). The Centers for Disease Control and Prevention (CDC) has estimated that there are 1.7 million healthcare-associated infections (HAIs) annually in the United States, which results in 99,000 deaths and $4.5 billion in excess healthcare costs [12]. A persistent problem that the world currently faces is the escalation of resistance mechanisms in bacteria, which accounts for substantial increase in related illnesses and deaths every year. Antibacterial agents such as those tested in this study may reduce these infections and prove to be cost-effective by reducing the duration of hospital stay.

In addition to MRSA and MSSA, there are Gram-negative multidrug resistant bacteria found in the hospital settings. Three of the more problematic Gram-negative bacteria are *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [13]. Of these, *P. aeruginosa* ranks second among the Gram-negative pathogens in causing nosocomial infections, with a 58.8% mortality rate [14]. *K. pneumoniae* is a common cause of pneumonia and bacteremia in critically ill patients. An increasing number of strains of *K. pneumoniae* are growing resistant to virtually all antibiotics, with a mortality rate of 50% [15], therefore making it crucial to control its spread. *Acinetobacter baumannii* is also one of the most problematic causative agents of nosocomial infections because of its ability to survive on hospital surfaces and acquire antibiotic resistance, resulting in the global emergence of strains with resistance to multiple antibiotic classes [16].

Infections caused by MRSA, MSSA, *A. baumannii, K. pneumoniae, and P. aeruginosa* can cause life-threatening complications. Patient's cellulitis, post surgical site infections or respiratory pneumonia, may result in bloodstream infections, which can complicate and lead to death. For example, in 2005 it was recorded that approximately 94,000 people developed their first invasive MRSA infection, of which about 19,000 died in the United States [17].

The five bacteria tested in this study are commonly found in hospitals and play a major role in the health of patients that are admitted due to various other types of diseases. In order to reduce the chances of patients acquiring these infections, it is important to find alternatives that are successful in inhibiting the growth of these bacteria and reduce the number of deaths. With each small battle that humans have fought against bacteria with finding new antibiotics and treatments, bacterial infections are still not fully under control because of the escalation of drug resistance mechanisms. The present research studied antibacterial activity of wintergreen and cinnamon essential oils and methylglyoxal. We also tested these compounds as topical applicants with carriers such as Aloe Vera gel, Aloe Vera oil, natural Aloe Vera and lanolin for easy application on soft tissue infected patients.

The published work reveals that Manuka honey obtained from the Manuka tree (*Leptospermum scoparium*) in New Zealand provided greater antibacterial activity than any other honey [18]. Manuka honey has been reported to be a promising functional food, which can also be used for the treatment of wounds and/or stomach ulcers because of its ability to exhibit antimicrobial activity against pathogenic bacteria such as *Staphylococcus aureus*[19]. Antimicrobial activity in most honeys is due to the enzymatic production of hydrogen peroxide [20]. However, methylglyoxal has been shown to be the active ingredient in Manuka honey and Manuka oil [21]. The methylglyoxal also provides suitable properties such as being resistant to heat, body fluids, light and enzymatic activity [22]. The use of such a compound may prove to be highly suitable for patients in hospitals, for the prevention and cure of these harmful infections. Finding an alternative treatment is crucial. Commonly used antibiotics to treat patients with *A. baumannii, K. pneumoniae* and *P. aeruginosa* infections are ampicillin/sulbactam, piperacillin/ tazobactam and or amikacin respectively. And recommended antibiotics to treat MSSA and MRSA are nafcillin and vancomycin respectively. Using specific essential oils and methylglyoxal for treating MRSA, MSSA, *A. baumannii, K. pneumoniae, and P. aeruginosa* may prove to be more effective than the common antibiotics.

MATERIALS AND METHODS

Essential oils

The following essential oils were obtained from West Village Oil Company of Glendale, NY, USA: Wintergreen, cinnamon, tea tree, and spearmint in order to be used in this research. According to the supplier, all of the essential oils were obtained through steam distillation. Manuka honey was obtained from ApiHealth New Zealand Limited (LTD) and the Manuka essential oil was obtained from Barrier Gold- GBI, New Zealand in order to be used for this research. Methylglyoxal (40% in H_2O) was purchased from Sigma-Aldrich®, Missouri, USA.

The standard antibiotics

The antibiotics used as standards for this research were obtained from the Becton, Dickinson and Company (BD), NJ, USA.

1. Vancomycin used for MRSA was used in 5 µg disc form (BD-231352).

2. Streptomycin used for MSSA was used in 10 µg disc form (BD-231328).

3. Amikacin used for *P. aeruginosa* was used in 30 µg disc form (BD-231597).

4. Piperacillin/ Tazobactam used for *K. pneumoniae* was used in 100 µg or 10 µg disc forms, respectively (BD- 2094208).

5. Ampicillin used for *A. baumannii* was used in 10 µg disc form (BD-231660).

Bacterial drug resistant organisms used to test antibacterial activity

Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA) samples were obtained from Jersey Shore University Medical Center (JSUMC) anonymous positive blood culture isolates placed on blood agar plates. *A. baumannii* (ATCC-19606), *K. pneumoniae* (ATCC-13883), and *P. aeruginosa* (ATCC-27853) samples were obtained from American Type Culture Collection (ATCC®, Virginia, USA) and grown under aerobic condition on Mueller Hinton II medium for 24 hours under an incubation period.

Supply of emollients

Among the emollients used, Aloe Vera oil was obtained from House of Nubian, Jersey City, NJ, Aloe Vera 100% Gel was obtained from Fruit of the Earth, Fort Worth, TX, USA and natural Aloe Vera was the product of Squidbat, AZ, USA.

Preparation of muellar-hinton II agar medium

BD Difco™ Mueller Hinton II agar medium was used to provide the important nutrients to support the growth of MRSA and MSSA. This medium proved to be a suitable medium to perform susceptibility testing as well as for growing the microorganisms. It was prepared from a commercially available dehydrated powder as per the manufacturer's instructions. Once prepared, the agar was autoclaved and then poured into sterilized Petri dishes.

Disk diffusion (Kirby Bauer) method

The bacteria were cultured separately in nutrient broths and the disk diffusion test was performed to see the zones of inhibition by the compounds tested. The bacterial cultures were matched to a 0.5 McFarland standard 1 x 10^8 Colony Forming Units per milliliter (CFU/ml) test tube. The Mueller Hinton II agar was poured into petri dishes and then allowed to solidify. After solidification, the bacterial cultures were grown on the Mueller Hinton II and then tested with specific compounds such as Manuka honey, Manuka oil, methyl glyoxal and essential oils. The plates were then incubated for approximately 24 hours. The zones of inhibition by the compounds were with the zone of inhibition by the standard antibiotics-vancomycin, streptomycin, ampicillin/sulbactam, pipercillin/ tazobactam, and amikacin.

The experiments were carried out in triplicate and were performed under aseptic conditions. Sterile blank discs (6 mm) were saturated with 5 µL of one of the compounds being tested. Each of the antibiotic standards was placed on a similar blank disc and positioned in the middle of the petri dish. The zones of inhibition by the different antibiotics for the different strains of bacteria were tested in this manner. The diameters of zones of inhibition were measured after 24 hours of incubation at 37°C.

RESULTS AND DISCUSSION

Wintergreen and cinnamon essential oils as well as methylglyoxal obtained from Manuka honey were tested for antibacterial activity against multidrug resistant bacteria: MRSA, MSSA, *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa,* and found to be more effective than the respective standard antibiotics for each of the five tested bacteria (Table 1 and fig. 1). Spearmint and tea tree essential oils were also tested in this study, however, the results obtained were not better than the respective standard antibiotics (Table 1 and 2). Wintergreen, cinnamon and methylglyoxal were highly effective in inhibiting the growth of MRSA, MSSA and *A. baumannii.* Wintergreen essential oil was also effective in inhibiting the growth of *K. pneumoniae,* better than the standard antibiotic, piperacillin/tazobactam. This was not the case with cinnamon essential oil and methylglyoxal. Similarly, these three compounds did not show better results with *P. aeruginosa* than the standard antibiotic, amikacin (Table 1). However, the zones of inhibition created by these compounds with *P. aeruginosa* were still significant.

Table 1: Mean diameter of zone of inhibition of standard antibiotics used to treat multidrug resistant bacteria

Bacteria	Standard antibiotic	Mean diameter of zone of inhibition (mm)
MRSA	Vancomycin	15
MSSA	Streptomycin	15
A. baumannii	Ampicillin/ Sulbactam	29
K. pneumoniae	Piperacillin/ Tazobactam	31
P. aeruginosa	Amikacin	24

Table 2: mean diameters of zone of inhibition of methylglyoxal, and wintergreen, cinnamon, tea tree and spearmint essential oils at various dilutions

	K. pneumoniae Avg., mm	*A. baumannii* Avg., mm	*P. aeruginosa* Avg., mm	MRSA Avg., mm	MSSA Avg., mm
Methylglyoxal 40% (aq.)	29.6	33.6	21.4	42.0	41.2
Methylglyoxal 20% (aq.)	24	28	18	35	37
Methylglyoxal 10% (aq.)	20	21	14	32	32.5
Wintergreen	36	35	9.3	28.5	34.5
Wintergreen 50% (aq.)	45	41	0	23	37
Cinnamon	27.8	36.2	20.8	33.7	37.7
Cinnamon 50% (aq.)	30	31	20	33	37
Cinnamon 25% (aq.)	30	29	18	35	39
Tea Tree	13.8	12.8	0	0	0
Spearmint	14.5	12	0	0	9.5

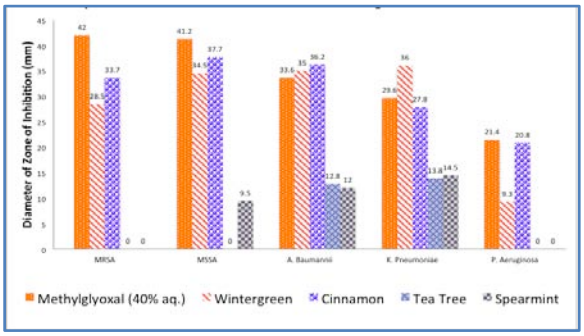

Fig. 1: Efficacy of Methylglyoxal, and Wintergreen, Cinnamon, Tea Tree and Spearmint Essential Oils on Common Multi-Drug Resistant Bacteria

This study appears to be the first to test methylglyoxal, wintergreen and cinnamon essential oils on hospital derived patient samples of MRSA and MSSA, as well as on three Gram-negative multidrug resistant organisms: *K. pneumoniae, A. baumannii* and *P. aeruginosa*.

Dilute aqueous solutions of wintergreen, cinnamon essential oil as well as methylglyoxal, prepared by using sterilized water, were also tested against the five drug resistant bacteria. Methylglyoxal (40%) was diluted to 20% and 10% aqueous solutions, whereas wintergreen was diluted to 50%, and cinnamon essential oil was diluted to 50% and 25% aqueous solutions (Table 2). The results of the tests showed that these compounds, even as diluted aqueous solutions are more effective in inhibiting the growth of MRSA, MSSA and *A. baumannii*, and almost as effective with *K. pneumoniae* and *P. aeruginosa* compared to the respective standard antibiotics. This study demonstrates that wintergreen and cinnamon essential oils and methylglyoxal could be beneficial in treating infections caused by these bacteria.

Natural Aloe Vera, Aloe Vera oil, and commercial Aloe Vera gel were used as carriers for the essential oils and methylglyoxal. The gel from the natural Aloe Vera leaf was aseptically removed and used with the essential oils and methylglyoxal in equal quantities. The results of this procedure (fig. 2) indicated that natural Aloe Vera gel when combined with methylglyoxal and wintergreen decreased the zones of inhibition with the five bacterial species tested. However, when natural Aloe Vera gel was combined only with cinnamon essential oil, the activity was enhanced. The mean of zones of inhibition obtained for MRSA, *K. pneumoniae* and *P. aeruginosa* with the cinnamon essential oil alone were 33.7 mm, 27.8 mm and 20.8 mm, respectively (fig. 1). These zones of inhibition increased with the use of natural Aloe Vera gel to 35.0 mm, 40.0 mm and 31.0 mm, respectively (fig. 2). The mean zones of inhibition obtained for MSSA and *A. baumannii* with the cinnamon essential oil alone were 37.7 mm and 36.0 mm, respectively (fig. 1). These zones of inhibition decreased with the use of natural Aloe Vera gel to 36.0 mm and 18.0 mm, respectively (fig. 2).

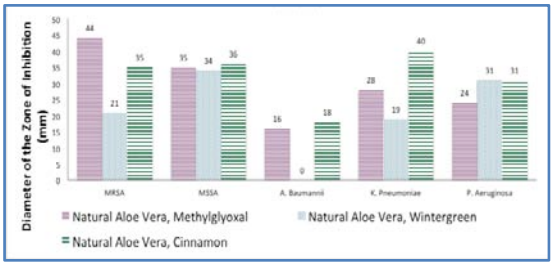

Fig. 2: Carrier Natural Aloe Vera with Methylglyoxal, Wintergreen and Cinnamon

Aloe Vera oil was also tested as another carrier for the essential oils and methylglyoxal on the five different bacterial species. The Aloe Vera oil was used in equal quantities with the wintergreen and cinnamon essential oils, as well as methylglyoxal. The results showed that the activity of the Aloe Vera oil in conjunction with methylglyoxal, wintergreen and cinnamon essential oils showed high mean zones of inhibition for *P. aeruginosa*. The mean zones of inhibition obtained for *P. aeruginosa* with the methylglyoxal, wintergreen and cinnamon essential oils (without a carrier) were 21.4 mm, 9.30 mm, and 20.8 mm, respectively (fig. 1). These zones of inhibition increased with the use of Aloe Vera oil to 33.0 mm, 28.0 mm and 27.0 mm, respectively (fig. 3). Similarly, an increase in the mean zones of inhibition was evident for MRSA and MSSA when used with cinnamon essential oil and Aloe Vera oil, and a combination of methylglyoxal and Aloe Vera oil showed an increase when tested with *K. pneumoniae*.

Commercial Aloe Vera gel was also tested as another source of emollient and the results showed that when combined with the essential oils and methylglyoxal, the commercial gel yielded higher mean zones of inhibition for MRSA, MSSA, *K. pneumoniae* and *P. aeruginosa*. For *A. baumannii*, the combination showed lower zones of inhibition when used in conjunction with the Aloe Vera gel. The

results show that for MRSA treated with only wintergreen, the mean zone of inhibition was observed to be 28.5 mm (fig. 1), whereas the zone of inhibition with the Aloe Vera gel increased to 46.0 mm (fig. 4). Similar results were observed for MSSA, *K. pneumoniae* and *P. aeruginosa* (fig. 4).

Commercial Aloe Vera gel by itself was tested and showed a mean zone of inhibition of 19.8 mm, indicating some antibacterial activity of the Aloe Vera gel (fig. 4). Commercial Aloe Vera is known to contain other ingredients such as triethanolamine, and tocopheryl Acetate (Vitamin E) added in the gel.

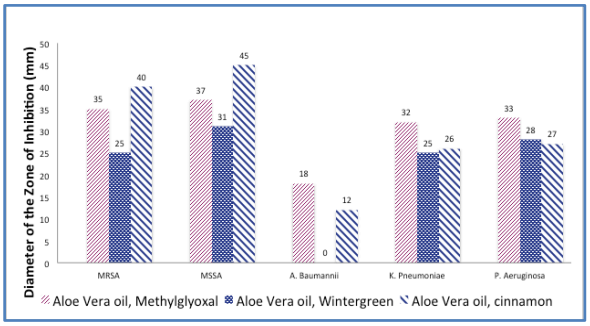

Fig. 3: Carrier Aloe Vera Oil with Methylglyoxal, Wintergreen and Cinnamon

The activity of the commercial Aloe Vera gel may be due to any or a combination of these ingredients. The results of using emollients such as naturally obtained Aloe Vera, Aloe Vera oil, and commercial Aloe Vera gel in conjunction with methylglyoxal and wintergreen, and cinnamon essential oils showed that these compounds could be used in combination with emollients at lower concentrations as a form of topical treatment for treating infections caused by these multidrug-resistant bacteria.

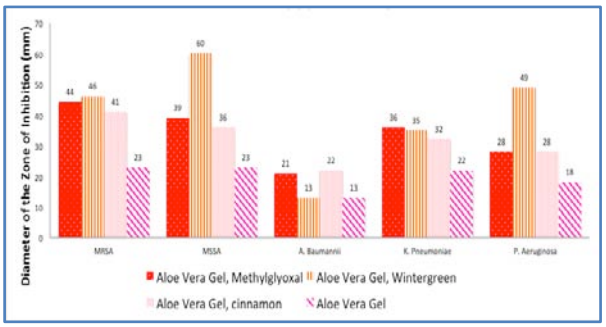

Fig. 4: Carrier Commercial Aloe Vera Gel with Methylglyoxal, Wintergreen and Cinnamon

CONCLUSION

In conclusion, this study revealed strong antibacterial properties of wintergreen and cinnamon essential oils, as well as methylglyoxal (active ingredient in Manuka Honey). These compounds showed high effectiveness against MRSA, MSSA, *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa* than the respective standards antibiotics used to treat infections caused by these bacteria. Test results (fig. 1) with diluted aqueous solutions of the compounds (wintergreen, cinnamon and methylglyoxal) showed the possibility of using these compounds in conjunction with emollients such as Aloe Vera. Results of the tests conducted with carrier oils (fig. 2, 3, 4) demonstrated potential for using such ointments for dermatological use. In order to develop commercial ointments for treatment of bacterial infections, clinical trials using topical emollients will be necessary. The next phase would be to study the biochemical mechanism responsible for inhibiting these bacteria. Understanding this mechanism may lead to the development of a more effective and safer alternative treatment of bacterial infections.

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CONFLICT OF INTERESTS

Declared None

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