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The effect of the essential oils: tea tree, thyme, and peppermint on *Staphylococcus aureus* growth.

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ABSTRACT

Essential oils are liquids extracted from plants which contain compounds that are easily evaporated. They produce strong fragrances and are often used for healing practices. The purpose of this experiment is to investigate the antibacterial activity of the essential oils: tea tree, thyme, and peppermint on *Staphylococcus aureus* growth. In this experiment, different factors such as the duration of each trial and the dilution percent of each oil will be taken into consideration. Tea tree, thyme, and peppermint oil were all ordered from Plant Therapy Incorporated as a therapeutic grade. Results showed that all the oils proved to be effective against *Staphylococcus aureus.*

Keywords: *antibacterial, antimicrobial, essential oils.*

INTRODUCTION

Recent studies released by the Centers for Disease Control and Prevention (CDC) in 2019 showed that more than 119,000 people suffered from bloodstream *Staphylococcus aureus* infections in the United States in 2017, and about 20,000 people died from it. However, recent research has shown that the rates of serious staph infections in the United States are decreasing. (Helmut, et al., 2019) Treatments for this infection includes, taking antibiotics, cleaning and draining the wound, or surgery to remove the infection (Helmut, et al., 2019.)

Staphylococcus aureus is a gram-positive bacteria. Gram-positive bacteria have a very thick cell wall made of a protein called peptidoglycan, which retains the crystal violet dye used in gram staining. This bacterium is the most dangerous of all the many staphylococcal bacteria and can cause minor skin infections, pneumonia, osteomyelitis and endocarditis. Staphylococcus aureus produces the enzyme, catalase. This allows staph to covert hydrogen peroxide to water and oxygen, thus helping distinguish Staphylococcus aureus from other staphylococcal organisms. (Kalemba, et al., 2003)

Essential oils are hydrophobic liquids extracted from plants which contain compounds that are easily evaporated. (Irshad, et al., 2018) The use of these oils range from cancer treatment, aromatherapy, scents, and food preservations. The use of essential oils date back to the late 1400s. Queen Hatshepsut of Egypt influenced the nation to gather plants and oils to use as resources of medication. (Irshad, et. al., 2018) In India, the use of essential oils are still practiced today. In Indian culture, religion plays a role in the different types of medications used. There, basil is a sacred plant and is believed to grant the energy of love and devotion by opening the heart and mind. In China, religion also plays a role in their use of essential oils. When Shennong, otherwise known as the “God-King of Chinese Medicine and Agriculture,” ruled, he taught his people different practices of agriculture. As years progressed, the use of essential oils continued throughout the world. (Davies, Lorene)

For the past 100 years, Australia has used tea tree oil as an antiseptic. Around the world today, Tea tree oil is used to treat nail fungus, athlete’s foot, and acne. (Carson, et al., 2006) Tea tree oil comes from the plant, Melaleuca alternifolia. Due to the component, Terpinen-4-ol, tea tree oil has been found to have strong antimicrobial and anti-inflammatory properties. (Pazvar, et al., 2012) Studies revealed that tea tree oil shows promising efficacy in treating Staphylococcus aureus. (Halcon, et al., 2004.) Tea tree oil is an antimicrobial, anti-inflammatory that has demonstrated the ability to activate monocytes. Monocytes are a type of white blood cell. This oil strongly inhibited the growth of *Staphylococcus aureus.* A study demonstrated that tea tree oil is effective in treating osteomyelitis, *Staphylococcus aureus*, and other infected chronic wounds and case studies. Tea tree oil currently known as being one of the most popular essential oils used for home remedies. (Halcon, et al., 2004.)

Thyme oil comes from the species, Thymus vulgarism, and is also part of the Lamiaceae family. The antimicrobial activity of thyme oil against multi-drug resistant strains of Staphylococcus showed that thyme oil could be used in the prevention of various human infections. Researchers concluded that the use of the essential oil from thyme in the prevention and treatment of various human infections may be reasonable. Thyme oil was tested against strains of bacteria from patients with infections within the mouth, abdominal cavity, respiratory and genitourinary tracts, and skin. The results provided allowed researchers to conclude that the use of thyme oil may be reasonable in the prevention and treatment of human infections. (Sienkiewicz, et al. 2012)

The mint plant, otherwise known as Mentha, comes from the family of Lamiaceae and has different species. In Greek mythology, Mentha was after by Pluto, the God of the underworld, thus making his wife jealous and forcing Mentha into the ground. Pluto, feeling bad for Mehta, then turned her into a plant so that people could appreciate her in the future. Piperita, peppermint, is a species from the Lamiaceae family. Chemical components that make up peppermint oil includes: menthol, menthone, methyl acetate, methofurn, limonene, and more. The use of peppermint oil includes headaches, aching feet, muscular pains, and painful menstrual cycles. (Kligler, 2007) Recent studies have indicated that peppermint oil possesses antibacterial and anti-fungal activities. A study aimed to evaluate the antimicrobial activity of peppermint oil against Staphylococcus aureus exotic production. (Li, et al. 2011) An exotoxin is a toxin released by a living bacterial cell into its surroundings. Data revealed that peppermint oil contained high contents of menthone, isomethone, neomenthol, menthol, and menthyl acetate which inhibited the growth of this strain of bacteria. Also, A-Toxin is a secreted protein responsible for the hemolytic activity of *Staphylococcus aureus* and in previous studies, results revealed that peppermint oil decreases *S. aureus* growth (Li, et al. 2011).

The purpose of this experiment is to introduce new methods in fighting *Staphylococcus aureus* using the essential oils: tea tree, thyme, and peppermint. In this experiment, different factors such as, the duration of the trials and the amount of each oil will be taken into consideration to evaluate the effects of inhibition.

MATERIALS AND METHODS

Tea tree, thyme, and peppermint oil were ordered from Plant Therapy Incorporated as a therapeutic grade. All the petri dishes and wafer disks used in the experiment were sterilized in an autoclave and prepared under a fume hood to prevent contamination. Under the fume hood, Difco Mueller Hinton Agar was poured into each petri dish. After the petri dishes were prepared with agar, *Staphylococcus aureus* was then spread across the agar, and then tested with different dilutions of essential oils to test resistance after being placed in an incubator at 37 degrees Celsius for 24 hours.

i. Preparing Agar Plates

Mueller Hinton nutrient agar was used and poured onto sterilized petri dishes. To prepare the agar, 38 grams of the Mueller Hinton powder was weighed out and mixed in one liter of distilled water in a two liter flask. The flask was then covered with aluminum foil to avoid spillage onto the hot plate. The solution was then heated at 400 degrees Celsius and spun at 300 rpms on a hot plate. The flask was then removed from the hot plate, then was autoclaved at 120 degree Celsius for 15 minutes. The flask is then removed from the autoclave and placed inside an incubator to cool down to 45 degrees Celsius. Once the agar cooled down to 45 degrees Celsius, it was then poured into petri dishes and placed into the refrigerator until needed.

ii. Reactivation of *Staphylococcus aureus*

The bacteria, *Staphylococcus aureus,* was reactivated by the method below. The culture was removed from the resealable bag that was stored in the refrigerator until use. 1 L of the rehydration medium was removed from the test tube and added into the culture and gently mixed with a sterile pipet. The rehydrated culture was removed from the vial placed into a sterilized test tube and placed in a 37 degree Celsius incubator for 24 hours.

iii. Inoculation

To maintain the purity of the bacteria, the tube was then inoculated. For inoculation, a sterilized wire loop, a Bunsen burner, and five test tubes were used. The wire loop was placed in the flame to be sterilized then dipped into the reactivated *Staphylococcus aureus* medium. After being carefully dipped, the loop containing the bacteria was then placed into a test tube and incubated at 37 degrees. This was repeated for five different tubes. The oils were purchased from Plant Therapy Incorporated and are used for therapeutic purposes.

iv. Study with essential oils

The essential oils: tea tree, thyme, and peppermint were first studied at a 100 percent concentration with the bacteria. 30 microliters of each oil were added to each disk. There were four disks in each petri plate. The control used was DMSO. After looking at the results of the first part of the study. The oils were then diluted to 50 percent with DMSO. 30 micrometers of was then added to the disks and placed into the plate. The oils were then diluted to 10 percent and the results were analyzed. These steps were repeated for each oil. After putting the oils on the petri dishes each oil was incubate d at 45 degrees for 24 hours.

RESULTS

Table 1. Zone of inhibition measured in mm from the essential oils: tea tree, thyme, and peppermint at different concentrations. Test results were collected by measuring the area from the disk that the bacteria was killed. Each oil was diluted with DMSO to create 50% and ten percent oil composition.

Zone of Inhibition Measured in mm for Tea Tree, Thyme, and Peppermint Oils at Different Concentrations			
	Tea Tree	Thyme	Peppermint
Control DMSO	No Bacteria was killed.		
100%	9.25 mm	100% Inhibition	100% Inhibition
50%	5.33 mm	100% Inhibition	100% Inhibition
10%	No bacteria was killed.	100% Inhibition	No bacteria was killed.

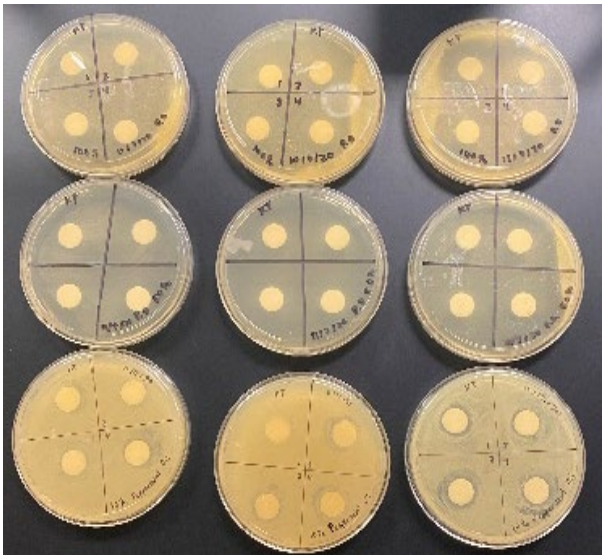

Figure 1. Zones of inhibition caused by essential oils using disk diffusion method.

DISCUSSION

Results showed that all the oils prove to be effective against *Staphylococcus aureus* growth, however their effectiveness varied depending on their composition. At 100% and 50%, both thyme and peppermint oil killed all of the bacteria, however at ten percent composition, only thyme oil showed antibacterial activity by killing all the bacteria present on the plate, while tea tree and thyme didn't kill any.

Thyme oil showed to be the most affective at all compositions by killing all of the bacteria on the plate each trial. The active ingredient in Thyme oil is thymol. Thymol is a phenol that was used as a pesticide in 1964 to repel birds, rats, mice, cats, and dogs (EPA,1993). Discovered and raised in Southern Europe, in ancient times, thyme was used as medicine to clear mucus in the lungs, prevent cough, relieve muscle spasms, and help promote the passing of urine. (Boruga, et al., 2014).

Essential oils that contains a phenol group on their chemical structure have shown to be effective against Gram-positive bacteria (Nazarro et al, 2013). This explains the strong results we've seen from thyme oil. Tea tree oil also contains a phenol group, however there must be a high composition of the oil present to show effective results. This may be due to the proteins found in *Staphylococcus aureus*. The oil composition must be higher in order to denature the proteins present (Nazarro et al, 2013).

Essential oils are capable of inhibiting the growth of bacteria by targeting the membrane and cytoplasm (Nazarro et al, 2013). Gram-positive bacteria are more susceptible to essential oils due to the essential oil's hydrophobic nature. In gram positive bacteria, essential oils can go through the bacteria (Nazarro et al, 2013). The essential oils are able to go through the cell's membrane due to the cell's inability to separate the essential oils from the bacteria. Once in the cell, the essential oil has the ability to denature the proteins inside of the cell (Nazarro et al, 2013).The essential oils: tea tree, thyme, and peppermint shown to be effective against *Staphylococcus aureus* growth. Further studies need to be done to understand the effectiveness of essential oils at different compositions.

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