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Infusion of Essentail Oils in Agarose Gels to Create Antimicrobial Surfaces

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INFUSION OF ESSENTIAL OILS IN AGAROSE GELS

TO CREATE ANTIMICROBIAL SURFACES

by

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Abstract

The current use of antibiotics is very excessive in multiple settings, from treating patients to being present in cleaning products. This overuse of antibiotics is resulting in multiple consequences, ranging from having a negative impact on human health to breeding antibiotic-resistant strains of microorganisms. Antimicrobial surfaces are useful today to aid in sterilization, especially in the medical setting. This would help prevent the spread of infection of microorganisms in these settings. In our research, agarose acts as the antimicrobial surface and essential oils would act as the solution incorporated into the surface that has the antimicrobial properties. Agarose is a polysaccharide that derives from red algae. It forms long chains and has charged groups linked to it, like sulfate. These residues are responsible for many of agarose's properties, such as electroendosmosis, which allows it to undergo gel electrophoresis. Thirty-three essential oils tested against bacteria to see if they had any antimicrobial properties. Many of them have historical uses that include as a medicine to treat infections. To create the agarose plates, agarose powder and the solvent heat up to dissolve. The solution went into the plates and multiple essential oils underwent testing for antimicrobial properties by the dispersion of the oils in the plates. Bacteria inoculated the plates and they grew. The results show that of the thirty-three oils tested, oregano, clove, ginger, lemongrass, and frankincense have antimicrobial properties. Of these oils, oregano, clove, and lemongrass have similar results to others in the same field. Ginger and frankincense oils did not have as much research conducted on their antimicrobial properties to determine, so these experiments helped bring new information about these oils to light. Essential oils can help bring natural products to medicine where natural products are rarely used. Future research on this topic can help us determine more essential oils that can be used and more ways these oils can work in a medical setting.

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I. Introduction:

A. Antibiotics, Bacterial Resistance, and the Need for Antimicrobial Surfaces

Some everyday sanitary items, such as hand soaps and disinfectant wipes, now contain antibiotics, which is not necessary and can become harmful. This is because of the new culture emerging in the Western world where there is a need for over-cleanliness. Everything needs to be disinfected and clean, but this kind of cleanliness is beginning to have a negative impact on human health. These disinfecting products usually have alcohol or antibiotics. This can lead to people not having enough bacterial exposure for their immune systems to develop properly, especially in children. This may also explain why there are a larger number of children with asthma and allergies in the Western world compared to that of developing nations. This unnecessary use of antibiotics also results in the increase in pathogens that are resistant to the antibiotic. An example of this is MRSA, which is methicillin-resistant *Staphylococcus aureus*, which is a strain of *Staphylococcus aureus* that is resistant to the antibiotic methicillin. This can result in people dying from infections that once had a cure. With MRSA, this is already occurring as the treatment for MRSA has led to the development of a deadlier strain of *S. aureus*, VRSA, which is vancomycin-resistant *Staphylococcus aureus*. This is a deadlier version of *S. aureus* because of its high mortality rate.

Another reason for increased bacterial resistance is that when a doctor prescribes an antibiotic, some people do not finish the full term of the medicine. They assume that since they feel better, they do not need to continue with treatment. The antibiotic cannot eradicate the entirety of the bacterial infection, which allows some bacteria to generate resistance to that antibiotic. This allows these bacteria to reproduce and cause disease in the host again. When they get sick again, they find out that the original antibiotic is no longer effective. The patient

will need stronger antibiotics or, if the infection is untreatable with antibiotics and becomes serious enough, it may require hospitalization.

Hospitals and other medical facilities also have the problem where there is high bacterial contamination on surfaces. This is because of the high volume of sick people in a certain area allows for high concentrations of bacteria on different surfaces. Any surface or person, when exposed directly or indirectly to a pathogen, can result in contamination of that surface or person with the pathogen. Instead of looking to antibiotics for a way to keep surfaces clean in places where contamination can be dangerous, like in medical settings, agarose infused with natural oils may provide a better alternative.

B. Agarose and Its Properties

Agarose is a polysaccharide, which means it is composed of multiple sugars. The sugars that make up agarose are 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactopyranose (see Appendix I). Charged groups left behind from agaropectin, like sulfate, link to the structure, which allows it to have special properties (see Appendix I). One of these special properties is the ability to undergo gel electrophoresis, which is the most common use of agarose and the name of this property is electroendosmosis. In gel electrophoresis, the gel is negatively charged. It acts as a track for negatively charged particles to travel to the positively charged end of the box. D.A. Rees was the person who described the mechanism of how agarose becomes a gel and S. Arnott and colleagues were the ones who demonstrated it. The agarose in solution has a random coil, but then becomes a double helix when it starts to gel and then becomes a bundle of double helices towards the end of gelation. Agarose also has high gel strength even when it is at low concentration, which is due to its hydrogen bonds. This strength,

however, decreases over time because of the hydrolysis of the agarose polysaccharide chains that occurs spontaneously (see Appendix II).

Agarose comes from different species of seaweed that can differ genetically where the genera of the seaweed differ. The genera that agarose can be derived from are *Gelidium*, *Gracilaria*, *Acanthopeltis*, *Ceramium*, *Pterocladia*, and *Campylaephoru*. Agarose has many different applications. Five different categories help organize these applications. One category is for the use in immunodiffusion and diffusion techniques. Another, which is the most common use, is for electrophoresis of charged particles. These particles help in the understanding of proteins, nucleic acids, and polysaccharides. Also included in this category are immunoelectrophoresis, reverse electrophoresis, and electrofocusing. Another category is for chromatographic techniques, like gel chromatography, ion exchange chromatography, affinity chromatography, and chromatofocusing. Another category is the use of agarose in bioengineering where it exists as a raw material in bead form where the beads are in chromatographic columns to allow for the separation of proteins. Agarose also exists as cross-linked beads. These beads help attach active molecules and have them recovered. Another category is its use in microbiology where agarose allows for the growth of special cultures. These multiple uses of agarose make it a very important compound across multiple disciplines.

There is a very large difference between the gelling temperature and melting temperature. Gelling temperature is the temperature at which agarose becomes a gel. Melting temperature is the temperature at which the gel melts and becomes a liquid. This is called gelation hysteresis, it occurs because of the greater number of hydrogen bonds, and a lack of sulfate groups compared to other compounds that have similar uses, such as agaropectin (see Appendix I). This difference

in hydrogen bonds and sulfate groups results in a gel that has short helix pitches and has no cation reactivity.

There are multiple ways to make agarose. Acetylation separates agarose in terms of its solubility in chloroform. Another technique is selective solution where the agarose separates in terms of its solubility in aqueous media. Quaternary ammonium precipitation produces agarose by observing the insolubility of the products that result from the reaction of agaropectin with quaternary ammonium salts and discovered by S. Hjerten in 1962. The solubility of agarose in a polyethylene glycol containing media as a method to separate agarose is another technique, discovered by B. Russell, T.H. Mead, and A. Polson in 1964. Another technique is observing the solubility of agarose in dimethyl sulfoxide discovered by S. Tagawa in 1966. Ammonium sulfate precipitates agaropectin, which is a technique discovered by G.Y. Azhitskii and G.V. Kobozev in 1967. Ion exchange using citrate or acetate forms is another technique to make agarose, discovered by B.A. Zabin in 1969. In 1969, S.J. Barteling discovered insoluble support absorption, which is where agaropectin is absorbed on a non-reactive support, like aluminum hydroxide gel, resulting in the formation of agarose. In 1970, K. Izumi discovered the technique of chromatographic separation of agarose and agaropectin. T. Fuse and F. Goto discovered acrinol precipitation in 1971 as another technique to separate agarose. In 1971, S. Hjerten also discovered that electrophoresis could separate agar gels and over powdered agar that are granulated or non-granulated. Agaropectin precipitating with rivanol is another technique and discovered in 1971 by S.M. Sviridov, V.A. Berdnikov, and V.N. Ivanov. Chitin and chitosan have absorbent properties, which can help to remove agaropectin, discovered by G.G. Allan, P.G. Johnson, P.G. Lai, Y.Z., and K.V. Sarkanen in 1971. Lastly, in 1973, N.B. Patil and N.R.

Kale discovered that agarose is able to precipitate with ethanol or 2-methoxyethanol in a urea buffer.

C. Natural Oils

Ginger oil comes from the steam distillation of the root of the ginger plant, *Zingiber officinale*. It is mostly composed of the compounds cineole and limonene, which is an allergen (see Appendix III). It originates from China and its uses include in aromatherapy, cosmetics, and food. It is flammable, can cause irritation to the skin and lungs, and is toxic to aquatic life. It may also treat DNA damaged by hydrogen peroxide. It is unknown if ginger oil has antimicrobial properties because not enough research has been conducted.

Chamomile oil is an oil that comes from the steam distillation of the *Anthemis nobilis* plant. Chamomile oil is a flammable liquid that can irritate the eyes, skin, and lungs. Its traditional use is for reducing inflammation, digestive problems, and for disinfecting wounds. The chemicals present in the oil help decrease gas, relax muscles, and sedate pain. Today, it acts as a flavor in foods and as a fragrance in cosmetics. It may have antimicrobial properties due to its historic use as a wound disinfectant.

Orange blossom oil is another name for neroli oil and it comes from the flower of *Citrus sinensis*. Aromatherapy is its main use and it is flammable. The three compounds that make up most of this oil are linalool, limonene, and beta pinene (see Appendix III). It is toxic to marine life and can cause irritation if exposed to skin, eyes, lungs, or digestive tract. It is unknown if it has antimicrobial properties.

Palmarosa oil is flammable and comes from the steam distillation of the *Cymbopogon martini* plant that originates in Nepal and India. It can cause irritation if inhaled, ingested, or if it

has contact with the skin or eyes. It is composed of mostly geraniol and geranyl acetate, but does contain limonene, citral, and farnesol (see Appendix III). It has multiple uses, like as a treatment of skin, nervous, and digestive systems, and even as an herbal medication for cold sores. It is unknown if it has antimicrobial properties.

Propolis oil comes from a resin called propolis, oftentimes called beeglue, which bees produce by extraction from plant parts and from bee secretions. It is made of organic matter, like waxes, resins, aromatics, and pollen. The resin also contains different vitamins and metals, like vitamins C and E, silver, mercury, and copper. It is effective to use against cold sores, genital herpes, and pain after mouth surgery. It also slows down blood clotting. Propolis oil may have antimicrobial characteristics.

Yarrow oil is an oil extracted from the *Achillea millefolium* plant, which originates in India, Hungary, and China. It is made of multiple organic compounds, such as bornyl acetate, eugenol, and myrcene (see Appendix III). Yarrow oil is also a flammable substance that is toxic to aquatic organisms. It is unknown if yarrow oil has antimicrobial characteristics.

Black elderberry oil comes from the fruit of the *Sambucus nigra* plant, whose traditional uses have been to treat flus, colds, and other conditions. The flower itself is toxic, so the oil must be prepared carefully. Even though it has been used to treat the symptoms of many conditions, it is unknown if it has any antimicrobial properties.

Tamanu oil comes from the seeds of a large hardwood tree called *Calophyllum inophyllum* or, more commonly, the Tamanu tree. It is flammable and acts as a medicine by some Southeast Asian cultures to treat skin infections, wounds, ulcers, and rheumatism. It is

made of many organic compounds, like calophyllic acid, calophyllolide, and canophyllol (see Appendix III). Tamanu oil has antiseptic and disinfectant properties.

Key-lime oil comes from the *Citrus aurantifolia* plant. It is flammable and can cause irritation of the skin, lungs, and digestive tract. It is very toxic to aquatic life and is harmful for the environment. It is unknown if it has any antimicrobial properties.

Thyme oil comes from the *Thymus vulgaris* plant. It can cause serious damage to the eyes and digestive tract and irritates the skin and lungs. It is flammable and toxic to marine life. It has been extensively studied and found to have antibiotic and antifungal properties.

Grapeseed oil comes from the seeds of grapes. It can be an irritant if it made contact with the skin or eyes, if ingested, or if inhaled. It is also flammable. It can slow blood clotting, treat leg swelling after six weeks, and treat eye stress from glare. It is unknown if it has antimicrobial properties.

Sage oil comes from the extraction of the sage plant, *Salvia officinalis*. Sage oil is a flammable liquid that is yellow in color and is sensitive to light. It can cause irritation of the skin, lungs, and digestive tract. It is unknown if sage oil has antimicrobial characteristics.

Rosehip seed oil comes from the rose hips of the rose flower. It is susceptible to oxidation if exposed to light. It has a high vitamin C concentration and may treat digestive tract problems, kidney and urinary tract disorders, high cholesterol, high blood pressure, and more. It may be effective to treat osteoarthritis. It is inconclusive if it has antimicrobial properties.

Moroccans have used argan oil for many centuries to treat skin infections and for its cardiovascular benefits. It is a flammable compound and is made of mostly mono-unsaturated fatty acids, some saturated fatty acids, and alcohols as its minor components. These alcohols and

the mono-unsaturated fatty acids may be the components that result in the medicinal properties. Not enough research proves that argan oil has antimicrobial properties.

Clove oil comes from the steam distillation of the dried flower buds of *Syzygium aromaticum* plant. It can be irritating if it enters the body, eyes, or skin. It contains high concentrations of the compound eugenol. Clove oil's original use was for dental pain, diarrhea, hernia, bad breath, and digestive problems. Clove oil may also slow blood clotting. Research shows that clove oil can be antimicrobial because it inhibits Gram-negative bacteria, Gram-positive bacteria, and yeast.

Peppermint oil comes from the steam distillation of the leaves of the *Mentha x piperita* plant. It can treat respiratory illnesses and digestive illnesses, especially irritable bowel syndrome (IBS). It has been used to treat the cold and topically for headaches, muscle aches, and itching. Another use is for its aromatic properties and flavor. It is mostly made of menthols and menthone, but it does include small percentages of limonene and pinenes (see Appendix III). Research on peppermint oil cannot determine if it has antimicrobial properties.

Myrrh oil is a flammable oil extracted as an oily resin from *Commiphora* trees. It is an irritant, toxic to marine life, and contains the compound para cymene (see Appendix III). Research shows that it may have antibacterial and antifungal properties.

Spearmint oil comes from the flower of the *Mentha spicata* plant. The oil may be able to treat digestive illnesses, for pain, for colds, and to reduce inflammation. It is a flavoring agent for foods and in oral hygiene products, like mouthwash and toothpaste. It may cause damage to the liver and cause drowsiness. There is not enough evidence that shows that spearmint oil has antimicrobial properties.

Eucalyptus oil comes from the leaves of different species of the *Eucalyptus* plants. It can irritate the skin, eyes, lungs, or digestive tract and is flammable. It consists of mostly cineole, but it also contains limonene, alpha pinene, and beta pinene and is toxic to marine life (see Appendix III). It can reduce pain and inflammation and it can help alleviate symptoms of respiratory illness. It is in oral hygiene products, in cosmetics as a fragrance for its aromatic properties, and in foods as flavor. It also helps control blood sugar. There is not enough information from research to prove it has antimicrobial properties.

Geranium oil is a volatile oil that comes from the leaves and flowers of the *Pelargonium graveolens* plant. It is composed of mostly citronellol and geraniol, but it also contains menthones and pinenes. It is a natural mosquito repellant, even though the effectiveness has not been determined. Geranium oil is fragrant, so it is included in aromatherapy. It can be an irritant if it gets in the skin, eyes, lungs, or digestive tract. It is toxic to aquatic life and it originates from Northern Africa. It is unknown if it has antimicrobial properties.

Rosemary oil is a volatile oil that comes from the *Rosmarinus officinalis* plant. Its properties make it useful in aromatherapy and in skincare. Rosemary oil is toxic to marine life and to the environment. Research shows that it is effective at killing spider mites and is a good alternative to growth promoter antibiotics in broiler chickens. More research on rosemary oil will determine if it is antimicrobial.

Lemongrass oil is an oil derived from the *Cymbopogon citratus* plant. It can cause skin irritation and sever problems if it enters the eye. It is also a danger to the environment. Its uses include as an ingredient in cosmetics. Research indicates that lemongrass oil is antifungal and antibacterial by having multiple targets in the bacterial cell. It may also affect the formation of biofilms for bacteria.

Vitamin E oil is an oil that is a known antioxidant to fight free radicals. It can also oxidize readily. Vitamin E works by aiding the immune system and by aiding in metabolic processes. The main ingredient is tocopherol (see Appendix III). It cannot be stored near heat, cold, or light and it cannot be stored in certain types of plastic because it can degrade certain plastics. It is unknown if vitamin E oil is antimicrobial.

Guava seed oil comes from the seeds of the *Psidium guajava* plant and may be able to improve the appearance of skin. It also has antioxidants to fight free radicals and can improve the appearance and health of hair. It is made of mostly linoleic acid, but contains traces of palmitic, stearic, and oleic acids (see Appendix III). Some research shows that guava plant roots may be antimicrobial, but no research shows the antimicrobial properties of guava seed oil.

Almond oil comes from the squeezing of almond kernels from the plant *Prunus amygdalus*. It is made mostly of the fatty acid oleic acid and some linolenic acid, but also contains trace amounts of palmitic acid, stearic acid, and alpha linolenic acid (see Appendix III). Almond oil is also a substance that is prone to oxidation when exposed. Its uses include softening the skin and massage. It is unknown if it has antimicrobial properties.

Bergamot oil comes from the cold pressing of the peels of the unripe fruits of the plant *Citrus aurantium bergamia*. The oil undergoes distillation and rectification after the cold pressing. Its uses include aromatherapy, in cosmetics, as a flavor, and in skin care, but it may cause irritation of the skin, lungs, and eyes. It can treat psoriasis, mycosis fungoides, and vitiligo. It is unknown if it has antimicrobial properties.

Hemp seed oil comes from the cold pressing of the seeds of the plant *Cannabis sativa*. It is composed mainly of the fatty acids linoleic acid and alpha linolenic acid, but traces of palmitic

acid, stearic acid, oleic acid, and gamma linolenic acid are also present (see Appendix III). It can help in maintaining the health of skin. It is unknown if it has any antimicrobial properties.

Carrot seed oil comes from the extraction of the seeds from the plant *Daucus carota*. It contains mostly alpha pinene and limonene, but there are traces of beta pinene, geraniol, alpha terpineol, and linalool (see Appendix III). Its primary use is in skin treatment, but it can be irritating to the skin, eyes, and lungs. It can cause an allergic reaction and it is toxic to marine life. It is unknown if it has any antimicrobial properties.

Frankincense oil comes from the *Boswellia sacra* plant from the steam distillation of its resin. Its uses date back to the traditional use of it throughout Africa, the Middle East, and South Asia as a fragrance in incense and perfumes. One study shows that it may be able to suppress cancer cells. Frankincense oil is a flammable liquid that can cause irritation to the skin, eyes, and lungs and can be lethal if ingested. It is unknown if it has antimicrobial properties.

Wintergreen oil is an oil that comes from the wintergreen plant. It contains methyl salicylates, which is a compound that acts similar to aspirin (see Appendix III). As a result, it treats muscular aches and pains. The oil's original concentration is too high for use on the skin, so dilution helps reduce its danger for the skin. Even after dilution, it can still cause skin irritation and ingestion is not safe. It is unknown if it has antimicrobial properties.

Jojoba oil comes from the extraction of the oil from the seeds of the *Simmondsia chinensis* plant. It is not toxic, but it is sensitive to light and heat. There is also no known toxicity to the environment and it biodegradable. Jojoba oil is in cosmetics, like in facial masks, cosmetics for hair, and for general treatment of skin ailments like acne and lesions. It is unknown if it has antimicrobial properties.

Oregano oil comes from the steam distillation of the plant *Origanum vulgare*. It can cause irritation and inflammation of the eyes, skin, and lungs. The current uses of oregano oil are to treat multiple conditions, such as acne, the flu, dandruff, and more, but there is insufficient evidence to support these claims. It can reduce blood sugar, slow blood clotting, and decrease the absorption of copper, iron, and zinc. It has antibacterial and antifungal properties, which may be due to the compound carvacrol (see Appendix III), and current research shows its potential antiviral properties.

Sunflower oil comes from the pressing of sunflower seeds, which come from the plant *Helianthus annus*. Today, sunflower oil's use is in cooking, but its original use was as medicine and in cosmetics. It has a high concentration of different compounds, such as vitamin E (tocopherol) and vitamin F (linolenic acid) (see Appendix III). It is biodegradable, so it has very little impact on the environment. It is unknown if sunflower oil has any antimicrobial properties.

D. Bacterial Species Tested

1. *Staphylococcus aureus*

Staphylococcus aureus is a strain of bacteria that occurs naturally in the noses of humans and in other animals. It transmits in a multitude of ways. One way is through the ingestion of enterotoxin containing food. Another way is through contact with the bacteria through the nose, draining lesions, and discharges. It can also spread through contact between people, fomites, and infected animals. It grows in tryptic soy broth. The virulence of the bacterial species differs for each strain. The incubation goes from four to ten days.

It does not usually cause harm in its host because it acts as an opportunistic infection, which means that it takes advantage of a weakened immune system to start an infection. As a

result, infections of *S. aureus* are commonplace in hospitals. This is because patients in hospitals are at risk of developing this infection because their immune systems are not as strong as a healthy person's immune system. This makes is much easier for a severely sick person to contract an infection from a bacterial species that does not cause disease in the healthy. Even though anyone can develop a staph infection, certain people are more at risk than others are. People who have certain conditions, like diabetes, cancer, heart disease, eczema, lung disease, and HIV are at a much higher risk for developing a staph infection. In a healthcare facility, like a hospital, those who have undergone surgeries or have catheters are also at risk of infection because of the opening provided by the catheter.

Several different infections can occur in the body due to *S. aureus* infection. One type of infection is bacteremia, also known as sepsis, which is the spread of bacteria through the blood stream. Pneumonia can also occur, which is the inflammation of the lungs due to infection that fills the lungs with fluid. Pneumonia mainly affects those with lung disease or those who are on ventilators. Endocarditis can also result from an *S. aureus* infection, which is an infection of the heart valve, which can lead to heart failure or stroke. Another complication of *S. aureus* infection is osteomyelitis, which is a bone infection that results from *S. aureus* traveling to the bone through the blood stream or through direct transmission through trauma. Other less serious complications of infection are skin infections, such as abscesses and boils, fever, impetigo, nausea, cramps, vomiting, and diarrhea.

There are also antibiotic resistant strains of *S. aureus*, such as Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-intermediate *Staphylococcus aureus* (VISA), and Vancomycin-resistant *Staphylococcus aureus* (VRSA). These infections care very dangerous and

can be deadly because they are resistant to certain antibiotics, which make treatment near impossible. As a result, people are dying from bacterial infections that once had a cure.

S. aureus is a Gram-positive, cocci shaped bacterial species. These spheres also tend to cluster together. It does not form spores and is non-motile. It is coagulase positive and they are anaerobes circumstantially, not due to environmental conditions. Gram-positive means that when it undergoes a Gram stain, it shows purple spheres. Since it is Gram-positive, it means that it has several layers of peptidoglycan above its plasma membrane. This is easier to kill compared to a Gram-negative bacterial species.

2. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a strain of bacteria that occurs in humans, animals, plants, water sources, and hospitals, which can result in nosocomial infections. It grows in Brian Heart Infusion broth. It can transmit in a multitude of ways. One way is through the direct contact with aerosols and contaminated water. Transmission can also occur through the exposure of wounds to contaminated sources. Also through the indirect contact with secretions of the respiratory system and mucous membranes. There is no vaccine available to prevent contraction of the bacterial infection. If infected, the treatment includes topical antibiotic ointment or an aggressive antibiotic regime for severe infections. It can cause infections in the eyes, lungs, urinary tract, and in any open wounds.

It mostly causes infections in hospitals because it is an opportunistic infection, which means that it takes advantage of a weakened immune system to start an infection. This means that exposure to *P. aeruginosa* to those with a strong immune system are able to fight off infection easily. For those with immune systems that are weak or already facing another medical

problem, *P. aeruginosa* can easily infect, which is why it mostly infects those who are in the ICU because of their compromised state of health. It can also spread through water as its medium, especially where the water is not properly chlorinated.

P. aeruginosa is a Gram-negative bacillus bacterial species, which means that it has an outer membrane over its peptidoglycan layer, which makes it significantly more difficult to kill. Staining the bacteria via the Gram test and observing it under a microscope would show red rods. *P. aeruginosa* is also a species of bacteria that is hard to treat because it has increased antibiotic resistance. Not only does the extra membrane of the Gram-negative bacteria help with resistance, but also the way that *P. aeruginosa* stays in the body as a biofilm contributes to the resistance. It is also aerobic and does not form spores.

P. aeruginosa was originally treated with different types of antibiotics, but in the past few decades, these antibiotics have stopped being effective. This has led to a dangerous strain called multidrug-resistant *Pseudomonas aeruginosa*, which is a serious threat to people in hospitals, especially those in critical care. Since modern antibiotics are becoming less and less effective, it has led to the use of more traditional antibiotics, which are notorious for their ill side effects. As a result, there is currently an epidemic where diseases that were once curable with antibiotics are now fatal because there is no longer any effective treatment.

II. Materials and Methods:

A. Preparation of Agarose Solution

To make a plate of the agarose, the materials needed are agarose powder, ethylene glycol (see Appendix IV), a beaker, a hot plate, and a plate. To prepare the agarose gel, obtain a 100 mL beaker. 1.00 g of agarose corresponds to one plate. The agarose combines with 8 mL of ethylene

glycol for each gram of agarose in a beaker. The tested essential oil is also included into the solution. Heat the beaker on a hot plate at a low heat for 5-10 minutes. The solution should have a consistency that is in between liquid and slimy.

Another technique introduced to make the plates helped use less agarose powder because the technique described above has an excessive use of the powder. The other technique used to make the plates involves the same materials, except 1X TAE buffer (see Appendix IV), which is made of tris base, acetic acid, and EDTA, replaces the ethylene glycol. 1.00 g of agarose corresponds to 100 mL of 1X TAE buffer, which makes 10-20 plates of agarose gel. They go into a 200 mL beaker and the solution heats up in the microwave for one minute, with the swirling of the beaker after 30 seconds. The solution goes into the plates and the oils mix into the gels before they solidify.

B. Infusion of Oils and Testing

Label a plate with what its contents will be. Once the solution has a slimy consistency, it goes into the plate. The solution then cools and solidifies at room temperature. Once the gel is solidified and dry, parafilm seals the plates from any contamination. Once the results come from the lab, record and analyze them.

With the new technique, the infusion of the oils also changed. The infusion of the gels comes after the heating of the agarose and 1X TAE solution and after the solution goes into the plate. The drops of the essential oil go into the plate and diffuse into the solution. Parafilm seals the plates and they go to the lab. The recording and the analysis of the results of the experiment occur once the results come from the lab.

III. Results:

A. Trial 1

In the first trial, the tested essential oils were black elderberry, clove, propolis, yarrow, and ginger dissolved in ethylene glycol (see Appendix V). A blank was also prepared to ensure the bacteria were able to grow by inoculating the blank plate with bacteria and if the bacteria grew, the results of the plates using the same bacterial solution would be accurate. The hypothesized results were that clove and propolis would be able to inhibit growth because of their previously research abilities to do so. In this trial, the oils that were able to inhibit the growth of bacteria were ginger and clove. The other oils showed to have growth on them. All the plates tested against *S. aureus*, which is a bacterial species that is easier to kill because it is Gram-positive. As a result, if the oils were unable to inhibit the growth of this Gram-positive bacterial species, it would be unable to inhibit the growth of a Gram-negative species. Ginger and clove were both able to inhibit the growth of *S. aureus*, but they differed in the way they reacted in the presence of *P. aeruginosa*. Since ginger and clove were able to inhibit the growth of the *S. aureus*, they were the only two plates that tested against *P. aeruginosa*. Ginger was unable to prevent its growth. Clove was mostly able to prevent its growth, other than a film that formed in its presence, which may have resulted due to bacterial growth.

B. Trial 2

In the second trial, the tested essential oils were clove in ethylene glycol, ginger in ethylene glycol, and the rest of the oils in 1X TAE buffer (see Appendix VI). The twelve oils tested in the 1X TAE buffer were clove, ginger, sage, oregano, spearmint, myrrh, rosemary, thyme, bergamot, eucalyptus, carrot seed, and neroli. A blank of the agarose gel with the 1X TAE buffer was also prepared to ensure the bacteria were able to grow by inoculating the blank plate with bacteria and if the bacteria grew, the results of the plates using the same bacterial

solution would be accurate. In this trial, *P. aeruginosa* did not test against the antimicrobial properties of the oils. The hypothesized results were that the oils clove, ginger, oregano, myrrh, and thyme would be able to inhibit growth because of research previously done by others and results from the first trial. The only oil that was able to inhibit the growth of the *S. aureus* was oregano.

C. Trial 3

In the third trial, all of the oils dissolved in 1X TAE buffer. Twenty-one oils were tested against the bacteria and these oils were clove, ginger, oregano, peppermint, geranium, palmarosa, frankincense, chamomile, wintergreen, lemongrass, rosehip, tamanu, key-lime, hemp seed, almond, sunflower, grapeseed, argan, guava seed, vitamin E, and jojoba oil (see Appendix VII). A blank of the agarose gel with the 1X TAE buffer was also prepared to ensure the bacteria were able to grow by inoculating the blank plate with bacteria and if the bacteria grew, the results of the plates using the same bacterial solution would be accurate. In this trial, *P. aeruginosa* also did not test against the antimicrobial properties of the oils. The hypothesized results were that oregano, clove, ginger, lemongrass, peppermint, rosehip, tamanu, guava seed, chamomile, and argan oils would be able to inhibit growth because of previous research and results from the first and second trials. The oils that were able to inhibit the growth of the *S. aureus* were oregano, frankincense, and lemongrass.

IV. Discussion:

This experiment shows some success, but there were troubles early on in the experimentation, which prevented the formation of many more plates and more trials. When the experimentation process began, I used ethylene glycol to dissolve the agarose. With the prior

knowledge that 1.00 gram of agarose can easily dissolve in 1X TAE buffer, I assumed that the solubility of the ethylene glycol would be similar to this. When the ethylene glycol-agarose solution originally did not solidify, I let it set overnight. When I went back to see the results, I found that they never solidified because I had used too much ethylene glycol. I slowly started to use less ethylene glycol, which resulted in a solution with chunks of agarose gel in ethylene glycol. As a result, I went through multiple trials just to find out the formulation of the ethylene glycol-agarose solution. Once I did find it, it was shocking because the ratio of agarose to ethylene glycol was significantly larger than the ratio of agarose to 1X TAE buffer. I continued with the experimentation with ethylene glycol until I realized that I could make a significantly larger number of plates with the same amount of agarose if I use 1X TAE buffer as the solvent instead of ethylene glycol. As a result, I have both ethylene glycol and 1X TAE buffer solutions in the second trial.

A. Trial 1

In this trial, clove was able to prevent the growth of both *S. aureus* and *P. aeruginosa*, which is something that previous research supports. Clove can inhibit the growth of Gram-negative bacteria and Gram-positive bacteria. Since *S. aureus* is Gram-positive, clove was able to prevent its growth. This is because of the high concentration of the compound eugenol in clove. Eugenol is a compound that, with the phenolic compounds in clove essential oil, reacts with the cell membrane of bacteria. It does this by denaturing proteins and reacting with the phospholipids in the cell membrane. This makes the permeability of the cell membrane change, which is what inhibits the growth of bacteria. Since this effect is present in previous studies, the ability of clove oil to inhibit the growth of several different types of microorganisms is not surprising.

In this trial, ginger was also able to prevent the growth of S. *aureus*, but not of *P. aeruginosa*, which is new information. The ability for ginger oil to inhibit the growth of bacteria is unknown because there is not enough previous research that shows this ability. This means that the ability for ginger to inhibit the growth of S. *aureus* helps us understand the properties of ginger better than before. It also helped better understand how different chemicals present in ginger oil might be able to interact with each other to produce the observed antimicrobial properties. As a result, this experiment helped us find out that ginger has antimicrobial properties.

The other oils in this trial were unable to inhibit the growth of *S. aureus*. These oils, which were black elderberry oil, yarrow oil, and propolis, mostly did not have any known antimicrobial characteristics. Black elderberry oil and yarrow oil did not have any previous studies done on them that suggest that they would be antimicrobial. Propolis, however, did have previous research that stated that it might be antimicrobial. This study found that most bacterial species except *S. aureus* was very sensitive to propolis, with *S. aureus* showing minimal inhibition. If propolis tested against another bacterial species, the results we could get may be different.

B. Trial 2

In this trial, oregano was able to inhibit the growth of *S. aureus*. Analysis of this result shows that this result makes sense because of the known properties of oregano. Prior to this experiment, other researchers found that oregano has antibacterial and antifungal properties. The antiviral properties of oregano were also under study. Oregano can inhibit the growth of *S. aureus*, but previous research shows that oregano is strong enough to interact with the Gram-negative structure of bacteria. If oregano tested against *P. aeruginosa*, it would most likely be

able to inhibit its growth too. This is because the primary component of oregano is a compound called carvacrol (see Appendix III), which might be the chemical compound that gives it its antimicrobial properties.

The other oils in this trial were unable to inhibit the growth of S. aureus. These oils were clove, ginger, sage, spearmint, myrrh, rosemary, thyme, bergamot, eucalyptus, carrot seed, and neroli. These results are surprising because some of these oils have previously researched antimicrobial properties. Clove and ginger both showed results in the previous trial to be able to inhibit growth. These oils tested in both ethylene glycol and 1X TAE buffer and growth formed in both solvents. The only explanation for this is human error either in this trial or in the first trial. This also applies to thyme oil, an oil observed to exhibit antimicrobial properties. Myrrh oil is another essential oil that might have antimicrobial properties, but did not produce results. Spearmint oil, rosemary oil, and eucalyptus oil are oils that have only a few studies that observe antimicrobial properties, so their results are not surprising. The oils of sage, bergamot, carrot seed, and neroli lack sufficient background studies, so the presence of antimicrobial properties in these oils is unknown. The growth of bacteria on these plates counters what previous research states.

This trial's 1X TAE buffer plates also had problems because of the strange way the plates came out. While creating the plates, they started to gel, but they were still wet, so they dried overnight under a hood in the lab. The next morning, the gels looked like they had all but disappeared. Considering this did not happen with the previously made ethylene glycol plates and that there were significant amounts of gel in the plates before they were left to dry, this occurrence can be the reason that the results did not line up with what was expected.

C. Trial 3

In this trial, oregano, frankincense, and lemongrass were able to inhibit the growth of S. aureus. Analysis of this result shows that this result mostly makes sense because of the known properties of oregano and lemongrass. Oregano previously exhibited antimicrobial properties in trial 2, which may be a result of the compound carvacrol (see Appendix III). Lemongrass underwent testing for the first time in this trial. Prior to this experiment, other researchers found that lemongrass is antibacterial, antifungal, and can affect the formation of biofilms. Lemongrass oil attacks bacteria by having multiple targets in the bacterial cell. Frankincense, however, did not have any previous research conducted on it to prove that it had any antimicrobial properties, which is a surprise because it did inhibit bacterial growth. The research on frankincense oil is very limited in terms of its antimicrobial properties because most of the research on it focuses on the ability of this oil to fight cancer cells. This trait, however, might be useful in identifying why frankincense oil inhibits bacterial growth if we understood the pathway of how it kills cancer cells. Something that bacteria and cancer cells have in common is their ability to divide rapidly. A way that frankincense oil may react with cancer cells is that it can affect the division of cancer cells. If this is true, the oil can also halt the way that bacterial cells reproduce. This would explain the results of the experiment.

The other oils in this trial were unable to inhibit the growth of S. aureus. These oils were clove, ginger, peppermint, geranium, palmarosa, chamomile, wintergreen, rosehip, tamanu, key-lime, hemp seed, almond, sunflower, grapeseed, argan, guava seed, vitamin E, and jojoba oil. These results are surprising because some of these oils have previously researched antimicrobial properties. Clove and ginger both showed results in the first trial to be able to inhibit growth. This could be because of the use of 1X TAE buffer instead of ethylene glycol as a solvent or due to human error. Another oil known for its antimicrobial properties that did not produce results is

peppermint. This may be because the concentration of the oil was not high enough for it to exhibit these properties. Rosehip oil is another essential oil that might have antimicrobial properties, but did not produce results. Tamanu oil also has observed antimicrobial properties, but it did not inhibit bacterial growth. Other oils that did not inhibit bacterial growth, despite previous research deeming it otherwise are guava seed oil, chamomile oil, and argan oil. Geranium, palmarosa, wintergreen, key-lime, hemp seed, almond, sunflower, grapeseed, vitamin E, and jojoba oil are oils that have not been studied enough to observe antimicrobial properties, so their results are not surprising. The growth of bacteria on these plates counters what previous research states.

V. Conclusions:

These experiments helped find that multiple oils in the study prove to be natural sources of antimicrobial properties. By testing essential oils against two different bacterial species, we were able to determine which oils were able to deter the growth of even a Gram-negative bacterium. Some of the oils did share certain compounds, like limonene and linalool (see Appendix III), which may play a role in their ability to inhibit bacterial growth. The essential oils also helped make the experiment more natural because there are many chemical substances that humans have exposure to every day, some that have the potential of being carcinogenic. These chemicals do not have enough research done on them for them to be completely safe to use. As a result, essential oils help us avoid these unseen consequences because their use date back hundreds, or even thousands, of years throughout the world. Some of these oils acted as medicine in healing practices before the time of modern medicine. Other oils had different uses, like for taste and for improving health. The safety of these oils are not nearly as questionable as the safety of the chemicals in our everyday materials. Using these oils with agarose gels as a surface

can be very useful in places where there are high rates of infection, like in a hospital or medical facility. They can inhibit growth on commonly used surfaces, like doorknobs, bedsides, medical technology, and more. The inclusion of this surface can help make the medical setting more biologically safe. Doctors would be at a lower risk of infection of a disease and patients would be less likely to contract nosocomial infections. There would be less of a need for antibiotics because less people would be getting sick, so the consequences of overuse of antibiotics would also decrease. This research helps bring us closer to a safer medical world.

This research, however, is not enough to determine if those particular oils are antimicrobial. Many more plates with oils must test against bacteria to reach such a conclusion. This is because the sample size of this experiment was too small. Even though there were many plates, some of the oils tested against bacteria only once. For results with fewer variations, there must be a higher sample number. The consequence of having a small sample size is evident in the results for clove and ginger oils. They were able to inhibit the growth of bacteria in the first trial, but unable to do so in the second and third trials. If there were more samples, there would have been results that are more consistent. Further research in this field would be more successful by making more samples.

Despite the problems with the sample sizes, this research has helped pave a way for future research. One aspect of the research that can result in more research is the difference in results with different solutions. Ethylene glycol was the solvent that was first used in the experimentation, but it was switched to 1X TAE buffer because it used less agarose when dissolving. With the ethylene glycol solvent, the clove and ginger oils were able to produce negative results for growth of bacteria. In the second trial, the results came positive for growth for the ethylene glycol plates for clove and ginger. For the 1X TAE buffer plates, however, the

results came back positive for growth both times. This can be because the interaction of the oil with the solvent can affect the way the oil can interact with bacteria. In the case of the clove and ginger oils, the 1X TAE buffer might prevent the expression of antimicrobial properties of the oils compared to the ethylene glycol.

Another topic in the research that can result in important information is the concentration of effectiveness of those oils that can inhibit bacterial growth. The essential oils tested against bacteria at a similar concentration, unless the oil had the ability to inhibit growth. If this were the case, the concentration of the oils was increased. Further research can find at what concentrations the oils are the most effective. If this research were to be done on the other oils that were determined to be ineffective, the results might show that those oils might also be able to inhibit bacterial growth, but at a higher concentration. This would also explain why some oils that researchers found had antimicrobial properties were unable to inhibit the growth of *S. aureus*. If the concentrations were not high enough, the bacteria would be able to grow.

Another way this research can lead to further research is finding if mixing oils makes the bacterial growth lower than the oils separately. Finding out if mixing oils would result in a surface that is less prone to bacterial growth would be very crucial because this would introduce more questions and answers. A question that can arise from this is if the oils can counteract with each other, causing the bacteria to grow in the presence of a mixture of both oils. Another possible outcome would be that the growth is the same, which would also be useful information because the inhibition of bacterial growth could be because of the mixed oils interacting with the bacteria in the same way, resulting in a similar growth pattern.

This research can help bring natural substances back into the medical setting. It can help prevent the spread of disease and help understand the way these oils work. The particular

structure of agarose alongside the properties of the different oils make different surfaces for each of the oils. Learning more about the ways the different oils act can also help better understand the ability of these oils to be able to prevent the growth of bacteria. As a result, the research not only can lead to the betterment of medicine, but to the understanding of medicinal history.

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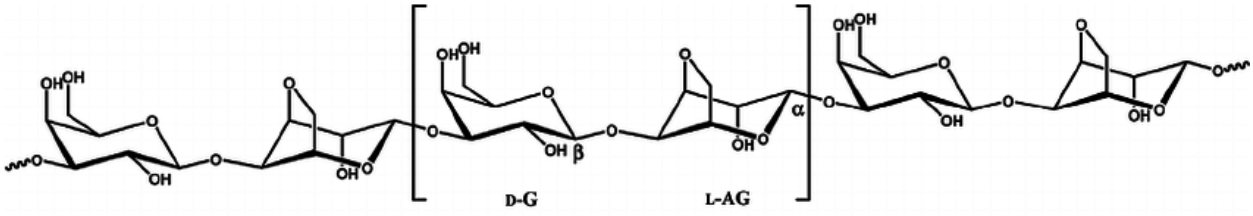
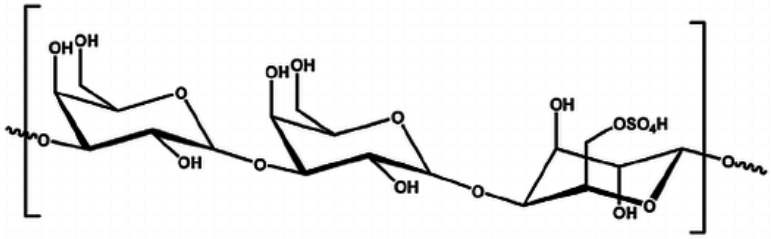
Appendix I: Structures of Agarose and Agaropectin

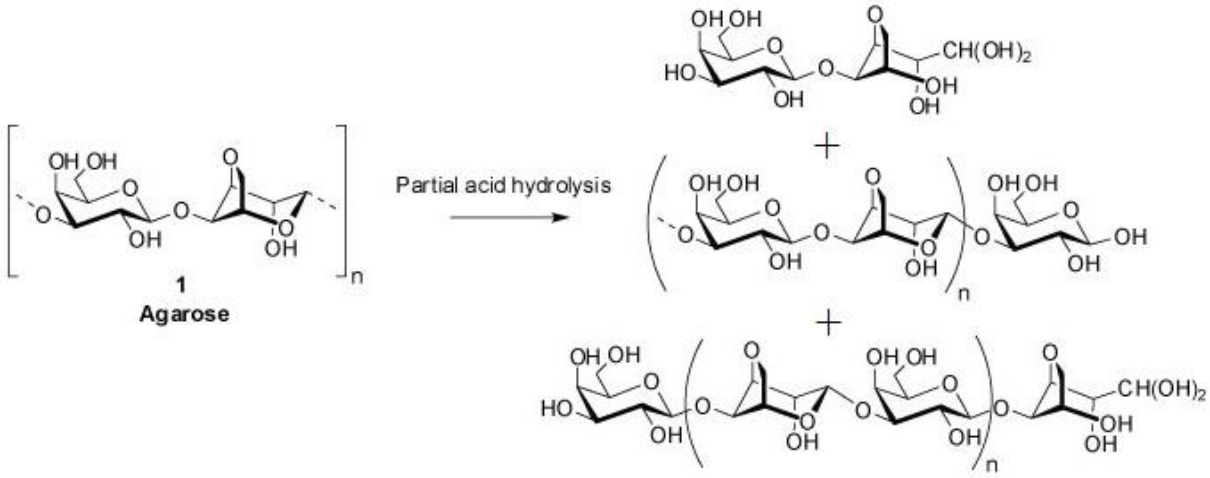


Agarose

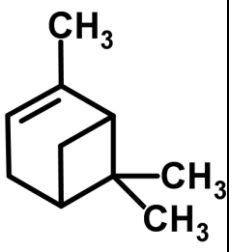
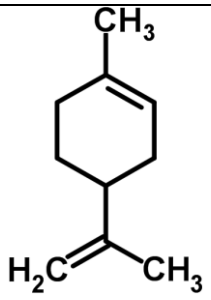
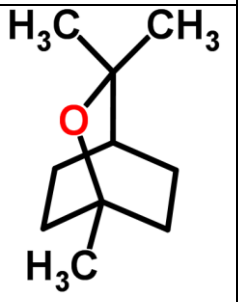
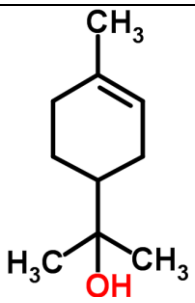
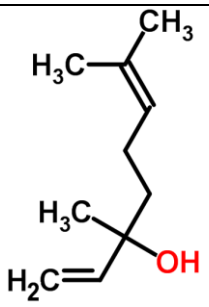
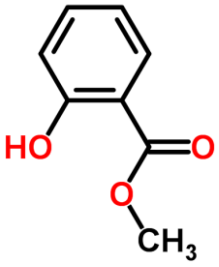
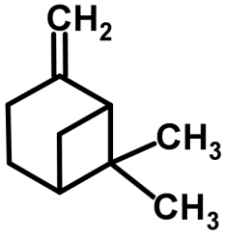
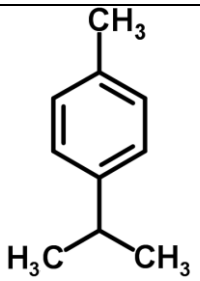
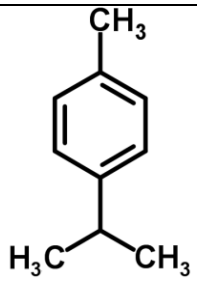
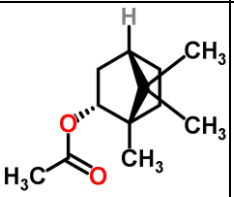
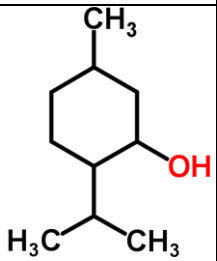
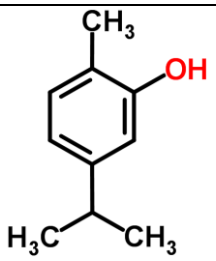
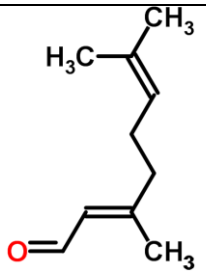
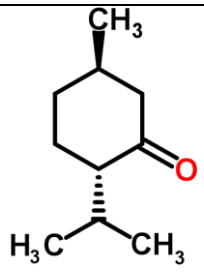
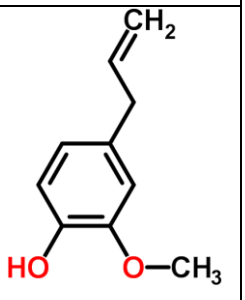


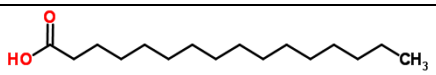
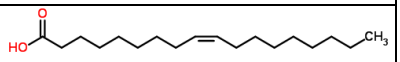
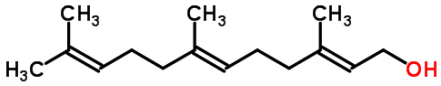
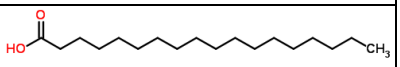
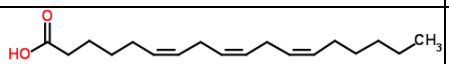
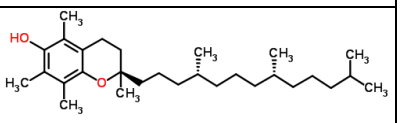
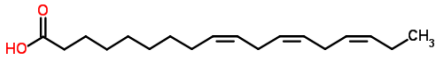
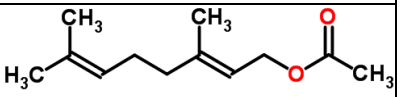
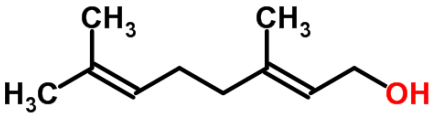
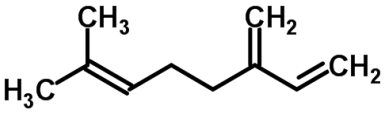
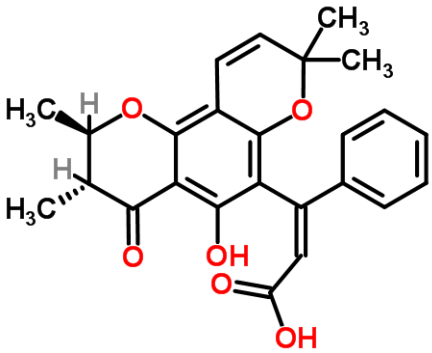
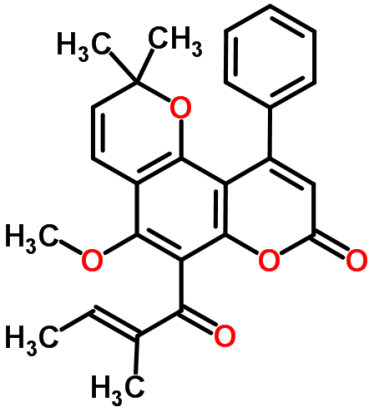
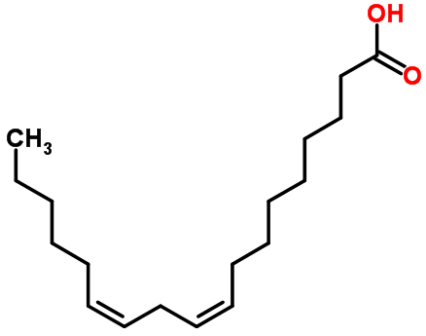
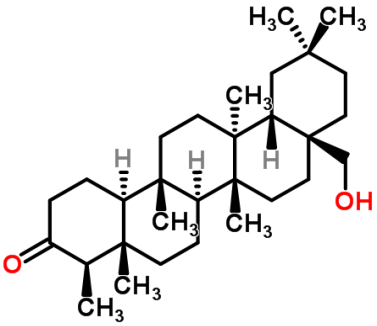
Agaropectin

Appendix II: Hydrolysis of Agarose

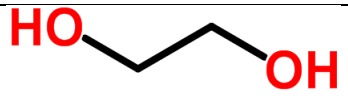
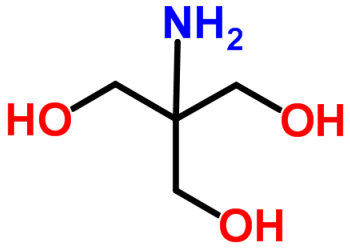
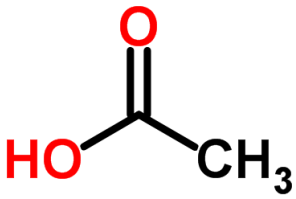
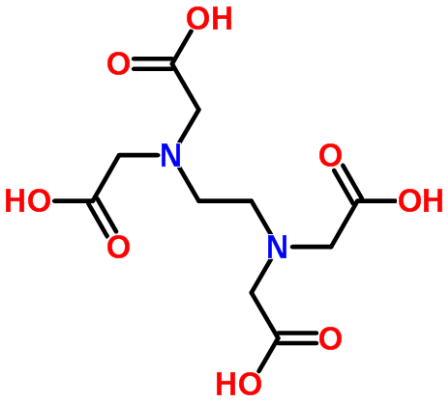


Appendix III: Structures of Compounds in the Essential Oils

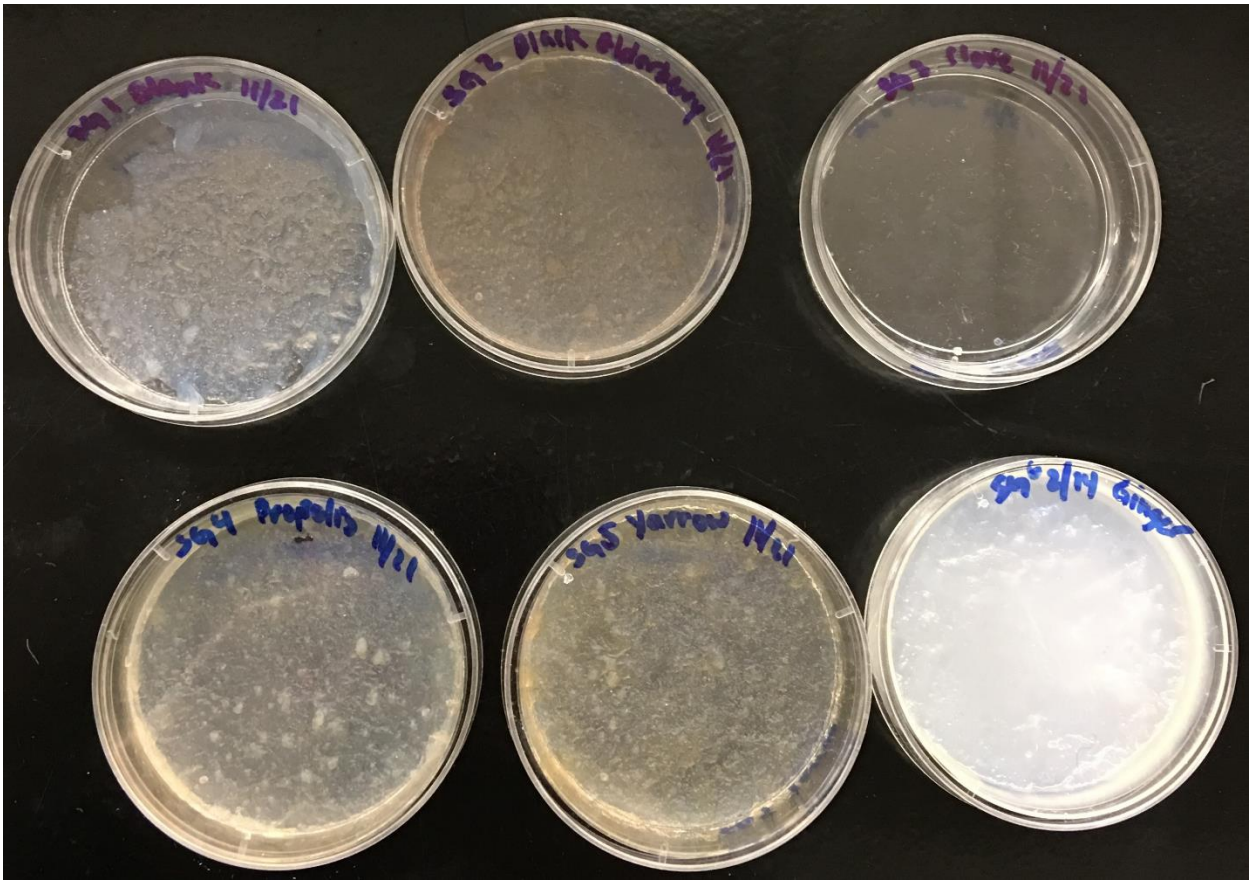
Compound	Structure	Compound	Structure	Compound	Structure
Alpha pinene		Limonene		Cineole	
Alpha terpineol		Linalool		Methyl salicylate	
Beta pinene		Para cymene		Para cymene	
Bornyl acetate		Menthol		Carvacrol	
Citral		Menthone		Eugenol	

Compound	Structure	Compound	Structure
Palmitic acid		Oleic acid	
Farnesol		Stearic acid	
Gamma linoleic acid		Tocoperol	
Alpha linoleic acid		Geranyl acetate	
Geraniol		Myrcene	
Calophyllic acid		Calophyllolide	
Linoleic acid		Canophyllol	

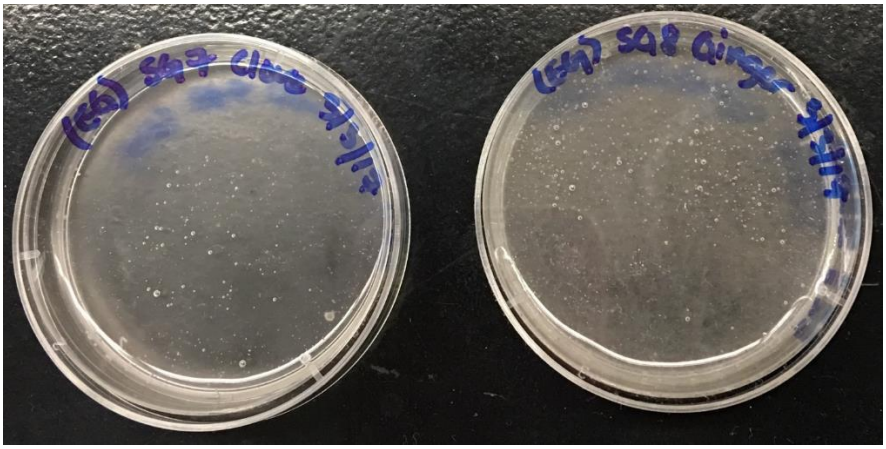
Appendix IV: Composition of TAE Buffer and Ethylene Glycol

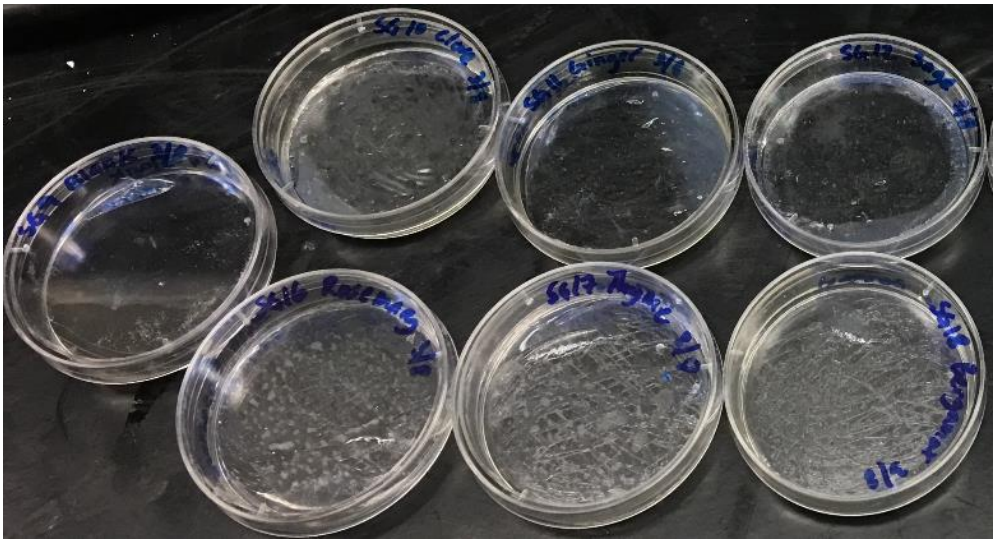
Compound	Structure
Ethylene glycol	
Tris base [Tris(hydroxymethyl)aminomethane]	
Acetic acid	
EDTA (Ethylenediaminetetraacetic acid)	

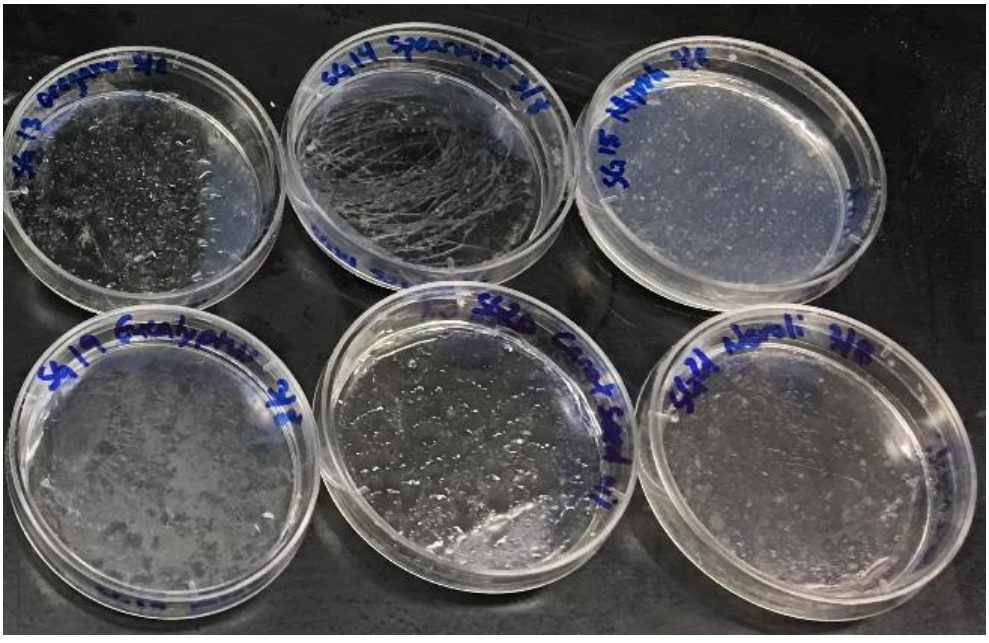
Appendix V: Plates from Trial 1



Appendix VI: Plates from Trial 2







Appendix VII: Plates from Trial 3