Analysis of the Therapeutic Potential of Essential Oils as Antimicrobial Agents

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A Thesis submitted to the Department of Biology in partial fulfillment of the requirements for the degree of Bachelor of Science

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4/29/2021

I pledge my word of honor that I have abided by the Washington College Honor Code while completing this assignment.

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Abstract

Essential oils (EOs) have been used as homeopathic remedies throughout history, primarily as inhalants or topical treatments. Essential oils are extracted from a variety of plants as volatile metabolites with high lipophilicity. Both *in vivo* and *in vitro* trials have shown positive results of antimicrobial activity using essential oils. In this review, recent findings on essential oils as antibacterial and antiviral treatments are analyzed, pointing to potential future directions for developing essential oils as antimicrobial agents. One result seen across multiple studies shows potential for lemongrass EO, an oil derived from *Cymbopogon citratus*. Lemongrass oil has shown promise as both an antiviral and antibacterial treatment, with high efficacy *in vitro* and *in vivo* studies.

Introduction

Antimicrobial therapy is the use of a pharmacological agent to treat infection of a microorganism. Since the early 20th century, research in antimicrobial therapies has led to impressive improvements in tactics. However, as microorganisms increasingly develop resistance to antimicrobial agents, it is necessary to continue researching alternative antimicrobial therapies, including those that can be used in place or in conjunction with modern pharmacological agents (Saga & Yamaguchi, 2009). Herbal agents, such as essential oils, have been studied for generations due to their antimicrobial properties. Archives dated as early as 4500 B.C.E provide evidence that ancient Egyptians developed essential oils and plant-based products for religious rituals, embalming practices, and healing treatments (Elshafie & Camele, 2017). A large proportion of these ancient remedies were inhaled, using the oils' aromatic nature. Other treatments were consumed, used as a fumigant, or applied externally to treat wounds (Cole, 2006). The written evidence remaining from the Egyptians and indigenous peoples has expanded the world's knowledge of essential oils and plant-based therapeutics. However, more research must be conducted to correctly identify mechanisms of action and the full therapeutic potential of these compounds.

Chemistry of Essential Oils

Essential oils are extracted from over 17,000 species of plants as volatile secondary metabolites. Secondary metabolites are organic compounds utilized by bacteria, fungi, or plants and are not directly involved with development. However, they are utilized in antagonistic environmental interaction (Asbahani et al., 2015). These compounds are usually synthesized in the cell cytoplasm and plastids of plant cells through malonic acid, mevalonic acid, and methyl-d-erythritol-4-phosphate (MEP) pathways (Figure 1). The oil is then stored in holding cells referred to as secretory granules. Secretory granules are located on the plant surface that undergoes exogenous secretion of EOs (Asbahani et al., 2015).

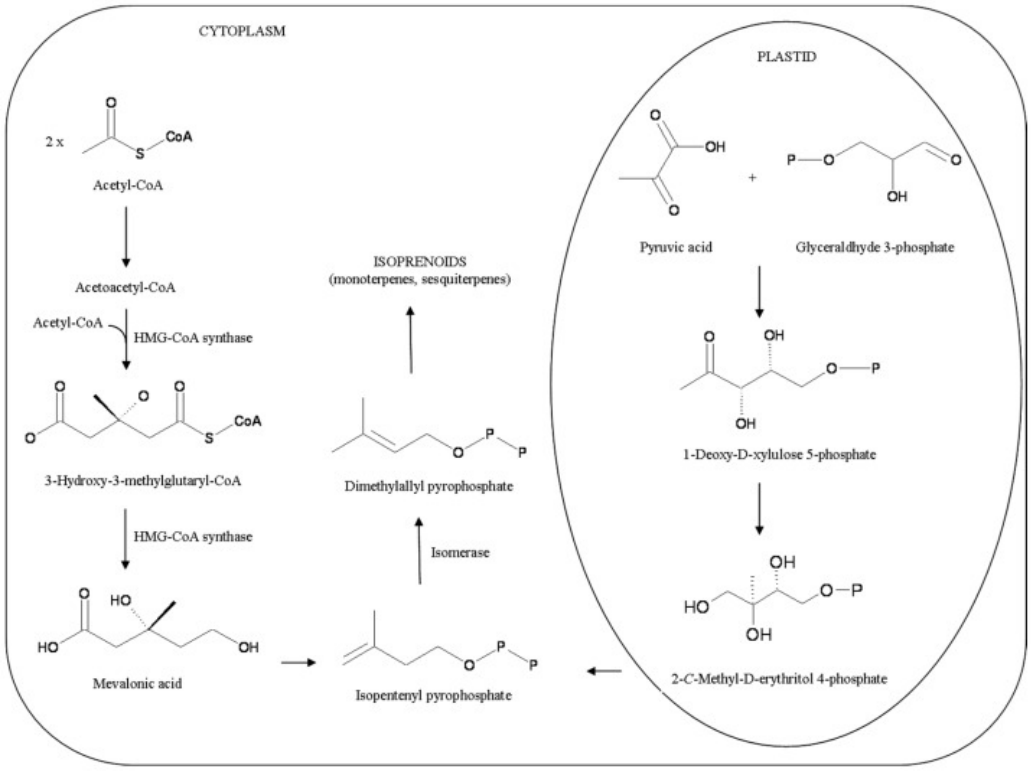

Figure 1. Terpenoid biosynthetic routes in a plant cell using the mevalonic pathway, located in the cytoplasm and 2-*C*-methyl-D-erythritol-4-phosphate in plastids. Pyruvic acid and Glyceraldehyde-3-phosphate interact in the plastic, starting the cascade of pathways of essential oil production (Sharifi-Rad et al., 2016).

Plant organs contain specialized cell systems and vacuoles that store and secrete essential oils and precursors (Asbahani et al., 2015). Exogenous secretory structures are found on flower petals, stems, and leaves. The tissues of these regions consist of epidermal papillae and secretory granules (also known as glandular trichomes or bristles). The secretion process is simple: the oil is produced and then secreted by the glandular trichomes, exiting in specialized ports in leaves, stems, flowers and fruits, bark, and roots of plants (Wińska et al., 2019)**.**

The synthesized oils are highly volatile compounds comprised mainly of hydrocarbon terpenes and terpenoids, which are aromatic phytochemicals that produce the aroma profile of plants, such as cannabis, lavender, and pine (Noriega, 2020). Terpenoids (also referred to as isoprenoids) are a group of lipids derived from five-carbon isoprene units. The difference between a terpenoid and a terpene is that a terpene is a hydrocarbon, with terpenoids containing oxygen, but the two terms are often used interchangeably to describe isoprenes (*Chemistry LibreTexts,* 2016). The abundance of terpenes results in the significant lipophilicity of essential oils. Alcohol groups are added to the terpene backbone to oxygenate the oil, contributing to metabolism and pharmacokinetics (Wińska et al., 2019). Common antimicrobial-associated compounds are eugenol, carvacrol, cinnamaldehyde, citral, and nerol (Sharifi-Rad et al., 2016).

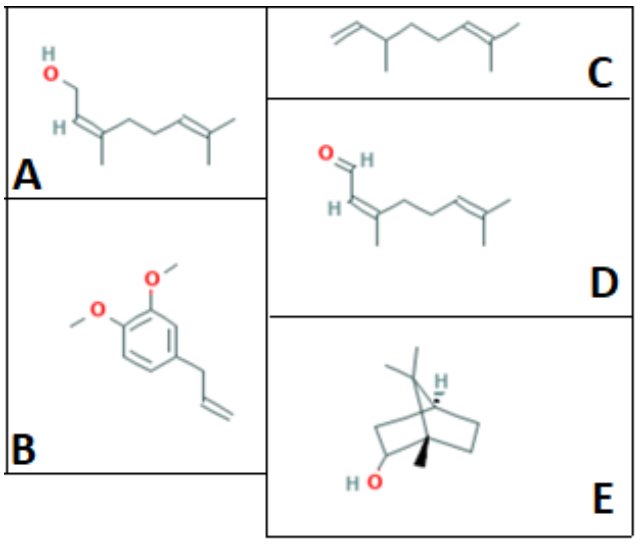

Figure 2. The main compound components of lemongrass oil ($C_{51}H_{84}O_5$) (PubChem, 2021). Lemongrass oil has been documented to exhibit antimicrobial effects, acting as both an antibacterial and antiviral agent (Minami et al., 2003; Ohno et al., 2003). Lemongrass EO is a popular essential oil for both the appealing scent and bioactive effects against microbes and inflammation. Primary chemical constituents include nerol (A), methyl eugenol (B), dihydromycene (C), neral (D), and l-borneol (E) (PubChem, 2021).

Due to the terpene backbone, essential oils are highly volatile compounds. Thus, the extraction can be challenging. The focus of oil quality involves the identity and purity of the oil (Elshafie & Camele, 2017). Extraction yields vary across species and organs, but usually, the yield remains very low (around 1%) across most species (Asbahani et al., 2015). Purity tests, chromatography, and distillation are all processes used to extract and analyze the oils. Gas-chromatography mass spectrometry (GC-MS) is commonly used to identify the chemical composition. Purity tests seek to identify the presence of polar substances, such as alcohols, glycols, esters, and glycerin acetates. Measurements of melting, boiling, and congealing points are also critical to identify purity and composition (Elshafie & Camele, 2017). Evaporation residue, which is the oil that is not volatile at 100°C, is another crucial indicator of purity.

The extracted essential oil can then be used for therapeutic benefits without further synthetic modification. Essential oils are currently marketed as over-the-counter natural remedies

for various indications, including emesis, migraines, anxiety, and sleep maintenance (Dagli et al., 2015). One example of a commonly used essential oil is Megaleion. Megaleion a non-FDA-approved oil blend consisting of balanos oil with burnt resin, myrrh, and cinnamon, which was used to relieve inflammation (Cole, 2006). Research has shown that both cinnamon and myrrh contain compounds that act as anti-inflammatory agents, such as eugenol (Gunawardena et al., 2014).

The safety and efficacy of essential oil therapy have been proven in numerous clinical trials, but there are still no FDA-approved uses of essential oils (FDA, 2020). This thesis will focus on current research on essential oils as antibacterial and antiviral agents. There is a need for environmentally friendly and non-toxic disinfectants and antimicrobials, and establishing safer alternatives is necessary for human and environmental health. Microbes are constantly evolving and gaining resistance against current therapies, driving the necessity of novel treatments.

Essential Oils as Antibacterial Agents

The antibacterial properties found in different essential oils have garnered significant attention in medical research. Certain EOs harbor their antibacterial effects through the presence of glycosides and phenols (such as eugenol, carvacrol, methyl-eugenol, and various tannins) (Ahiwar et al., 2018). An oil's method of bactericidal actions may occur through their antioxidant abilities, as well as the presence of the compounds, methyl-eugenol and estragole (Ahiwar et al., 2018; Sakkas & Papadopoulou, 2016). These compounds increase membrane permeability, causing a loss of membrane integrity, which leads to bacterial death (Cetin-Karaca, 2011).

Ahirwar et al., 2018: EO Efficacy Against a Triple Antibiotic Paste *in vivo*.

Research has focused on essential oils as antimicrobial agents for bacterial infections. The human oral cavity contains the body's second most abundant microbiota, contributing to a high risk of infection (Verma et al., 2018). One clinical trial compared the antimicrobial effects of *Ocimum sanctum* (tulsi) and a triple antibiotic paste (TAP) as an intracanal treatment of root canals of primary molars. The purpose of this study was to evaluate the aerobic and anaerobic antimicrobial efficacy of tulsi oil in treating aerobic and anaerobic polymicrobial infections (Ahirwar et al., 2018). This study demonstrates the antibacterial properties of tulsi-derived essential oils, implying future use of *O. Sanctum* as a potential remedy for oral infections.

Ahirwar et al.'s clinical trial was a longitudinal clinical trial. Two initial samples (one for aerobic testing and one for anaerobic testing) were collected from the canal's opening. Once the samples were taken, tulsi oil or TAP treatment was applied directly to the root canal. This process of sample extraction was repeated three times (Figure 3). The TAP used by the study team was a combination of metronidazole, ciprofloxacin, and minocycline at a 1:1:1 ratio, supplied in a paste mixed with propylene glycol for adhesion. The study team then took the

second round of microbiological samples from the patient's infected distal root canal at different stages of pulpectomy to analyze treatment efficacy. The collected samples were stored in a transport medium appropriate for the culture: brain-heart infusion broth for aerobic cultures and sodium thioglycolate broth for anaerobic cultures.

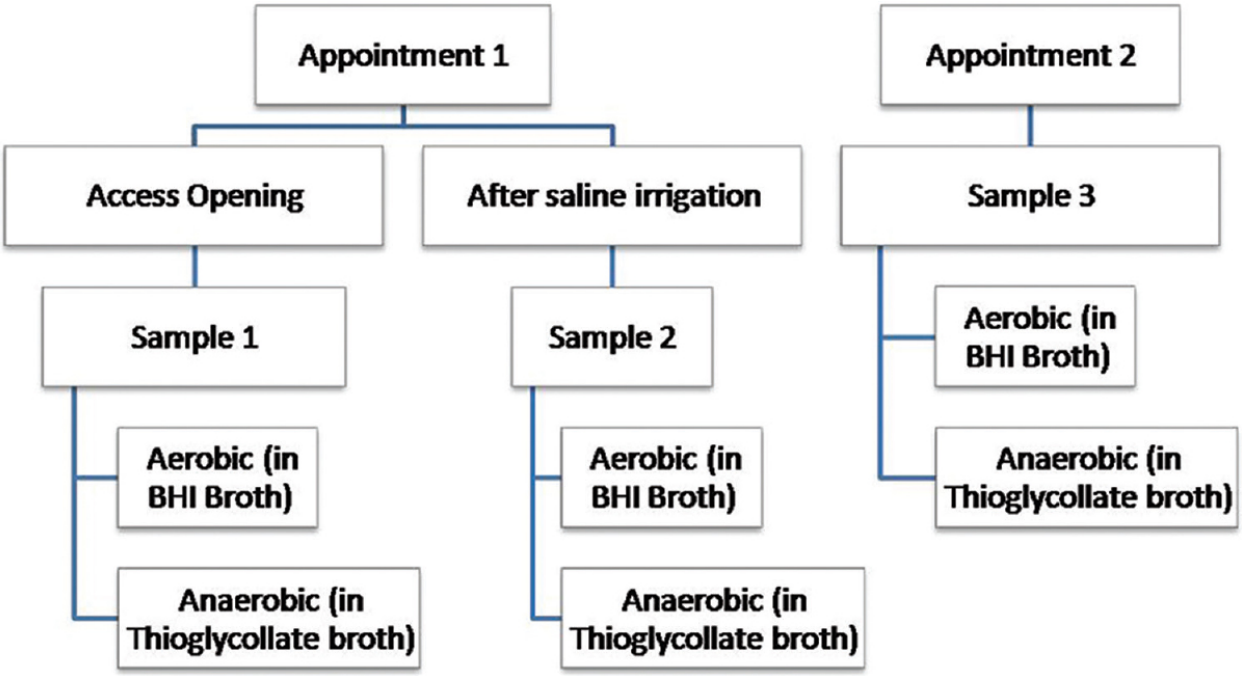

Figure 3. Sample collection flow chart, showing the division of samples for aerobic and anaerobic environment testing. Three total rounds of sampling were conducted from pediatric patients during the root canal process. Two samples were taken at each step of the treatment: aerobic testing and the other for anaerobic testing. The first samples were obtained from the initial opening of the canal before treatment or sanitization. The root canal was then cleaned via saline irrigation, and a sample was taken, then treatment was applied. The final round of sampling occurred 72 hours later.

Once stored and incubated, these samples were streaked on sheep's blood agar and cultured again. This process was repeated three times, with no changes to the protocol. The colony-forming units (CFU) were analyzed for each sample to quantify bacterial infection. The team utilized Tukey's Honest Significant Difference test in order to quantify CFUs. However, this report did not go in-depth on how data was quantified.

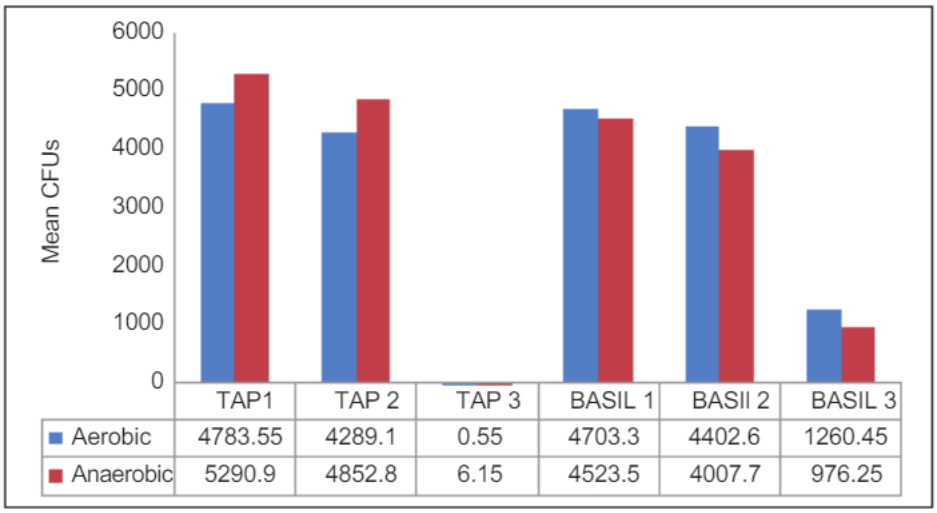

Figure 4. The mean values of colony-forming units in both TAP alone and experimental EO group. Samples collection was done via pulpectomy during a patient's root canal removal, then being cultured in both anaerobic and aerobic settings. The TAP-treated group had significantly higher numbers of CFUs at p compared to day 3 of treatment (TAP3) (*p-value < 0.0005)*. The tulsi-treated group had a similar and significant decrease in the mean CFUs at day 3 of treatment (BASIL 3) compared to day 1 (BASIL 1) (*p-value <0.0005)*.

By utilizing HSD test, it was found that there was a significant reduction of CFU counts in both aerobic and anaerobic cultures in patients treated with tulsi, as compared to pretreatment (Figure 4). Both treatment groups had reported a significant reduction in CFUs, demonstrating that tulsi is a suitable alternative to TAP for treating infected root canals (Figure 4*)*. Justification of quanitifications are missing from this study.

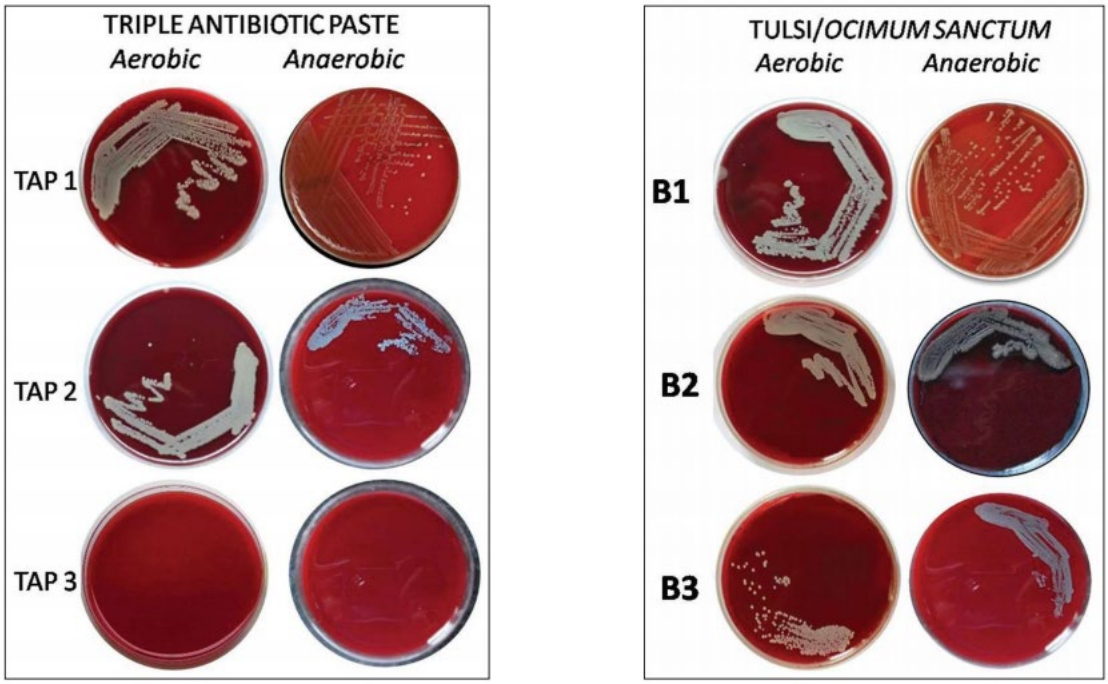

Figure 5. The comparison of the aerobic and anaerobic bacterial concentrations via quadrant streak on sheep's blood agar. A illustrates aerobic and anaerobic samples collected after access opening (TAP1), after saline irrigation (TAP2), and three days post TAP treatment in root canals (TAP3). It is seen that the addition of TAP after saline irrigation effectively works as a bactericidal agent. B also demonstrates the same pattern of collection, with the difference being the addition of *O. sanctum* EO in the root canal.

Although there were positive results seen in this study, several microbiological techniques and protocols were completed incorrectly, potentially resulting in skewed results. For example, samples were inappropriately stored in a liquid nutrient medium which was then cultured and incubated before plating and colony count. This pre-incubation changes the original bacterial counts, altering the number of bacteria present (due to early incubation of samples) and clouding results. Quantification of results also raises questions, as it is impossible to calculate a CFU analysis from quadrant streaks and HSD. In order to successfully analyze CFU count, a series of dilutions must be created and analyzed. While one can visualize differences in general bacterial concentration through a quadrant streak (Figure 4), this does not provide exact,

quantitative results to prove exact efficacy and a mechanism of action. While this study is lacking in some respects, it still provides some insight into applications of EOs for oral infections.

Ohno et al., 2018: EO Efficacy against *H. pylori* infections *in vivo* and *in vitro.*

Helicobacter pylori, the causative bacterial agent of peptic ulceration and other gastroduodenal diseases, has been a subject of interest when it comes to new therapeutics. As *H. pylori* infections can cause severe complications, organic treatments could potentially provide relief. Ohno et al.'s 2003 study examined bactericidal efficacies of different essential oils *in vivo* and *in vitro*. Five total experiments were run to determine the effects of pH, antimicrobial resistance, and bactericidal effects.

Seven *H. pylori* strains were utilized in this experiment, with all strains showing pathogenicity through the presence of the *vacA* s1-m1 genotype. Oils from the study included *Cupressus sempervirens* (Cyprus), *Juniperus communis* (juniper), *Melaleuca alternifolia* (tea tree), *Ocimum basilicum album* (tropical basil), *Mentha piperita* (peppermint), *Origanum majorana* (marjoram), *Eucalyptus globulus* (eucalyptus), *Ravensara aromatica* (ravansara), *Lavandula latifolia* (lavender), *Citrus Limonum* (lemon), and *Cymbopogon citratus* (lemongrass), with samples being purchased commercially.

Experiment 1: The first experiment aimed to identify the minimum inhibitory concentration (MICs) of antibiotics to use as a control group. The antibiotics tested included amoxicillin, clarithromycin, metronidazole, and tetracycline. Brain heart infusion (BHI) agar was dosed with 64 to 1.0 μg/mL of the chosen antibiotic; one plate was created for each type of antibiotic. The antibiotic-combined plates were inoculated with 1x 10^6 *H. pylori*. The samples were incubated for five days at 37 °C. MICs were recorded and then used as a control for

experiment two. Strains ATCC43504 and CPY2052 were susceptible (MIC < 1 μg/ml) to all antibiotics used in the study. Three strains—KP142B, KP143B, and KP206B—proved resistant to metronidazole (MIC: 4, 2, and 64 μg/ml, respectively). KP146B was resistant to metronidazole (MIC 2 μg/ml) and clarithromycin (16 μg/ml). These tests established that there was resistance against the antibiotic.

Experiment 2: The objective of this experiment was to establish the antibacterial activity of essential oils. Broth microdilution methods were used, as there is no established protocol to identify antibacterial activity in *H.* pylori. Dimethyl sulfoxide (DMSO), which enhances oil solubility, was supplemented to BHI broth with 0.2% (v/v) of DMSO. DMSO has no antibacterial activity and dissolves polar and non-polar compounds, allowing the oil to disperse easier. 0, 0.1, and 0.01% (v/v) concentrations of essential oils were created in 10mL broth cultures containing roughly 1 x 10^7 CFU of *H. pylori.* The tubes were incubated for 48 hours under a gas mixture containing 80% N_2, 15% CO_2, and 5% O_2 on a reciprocal shaker. Following incubation, 100μL samples of the mediums were obtained and then plated on blood agar plates. These plates were cultured for five days under the same conditions as the antibiotic tests, and colonies were counted post-incubation.

It was found that an *in vitro* antimicrobial effect was seen from all 13 oils tested. It was demonstrated that all EOs completely inhibited *H. pylori* growth of all strains at a concentration of 0.1% (v/v). Lemongrass oil specifically inhibited growth from all strains at 0.01% (v/v). Lemon also inhibited the growth of five out of seven strains utilized at a concentration of 0.01% (v/v).

Experiment 3: This experiment aimed to identify the effects of pH on the tested oils' antibacterial activities in acidic and basic environments. Two altered pH environments were

created. An added buffer had adjusted pH to a pH 5.2. Another buffer was added to create an environment of pH 7. *H. pylori* (strain ATCC43504) were inoculated into 10mL of liquid BHI broth, with various concentrations of EOs of 0.0025 to 0.1% (v/v) added to individual tubes. These tubes were incubated, and twenty samples were taken at 0-, 15-, 30-, and 60-minutes post-inoculation to enumerate the number of viable cells. Specific strain sensitivity was seen in strain ATCC43504 in both altered pH environments. Lemongrass oil showed a concentration-dependent bactericidal effect at a pH of 4.0 and 5.0, but not at a pH of 6 or 7 (Figure 2). At a pH of 4, visible colonies were reduced after 60 minutes of incubation. Sharp decreases were seen in the pH 4.0 group, specifically with the 0.02% oil concentration group, seeing a drastic drop in the colony count.

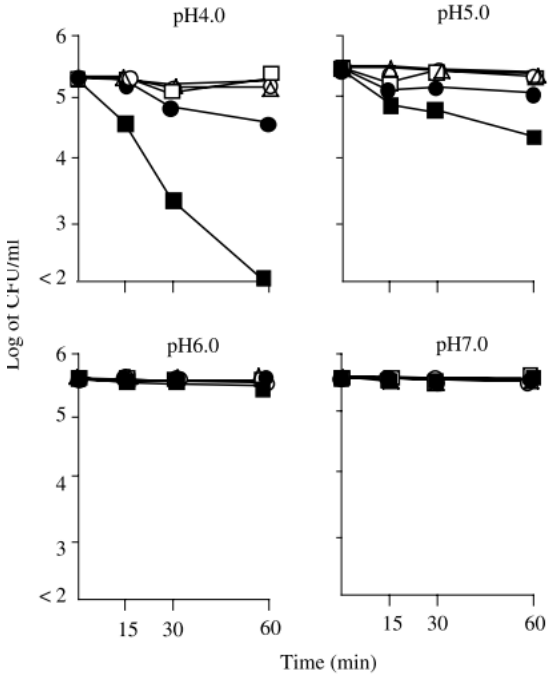

Figure 6. The effect of lemongrass oil on the growth of *H. pylori* at tested pHs. Oil concentrations utilized were 0.02 (■), 0.01 (●), 0.005 (△), 0.0025 (○) and 0 (□)% (v/v). pH altered buffers were added to a BHI broth, altering the environment to a range of pH values in single increments from pH 4.0 to pH 7.0. Concentration-dependent bactericidal effects are seen

in more acidic environments (pH 4.0 and pH 5.0), seeing fewer visible colonies 60 minutes post-incubation.

Experiment 4: Testing the development of resistance to the EOs was another task performed by the study team. Clarithromycin-sensitive *H. pylori* strains (ATCC43504, CPY2052, KP142B, and KP143B) were utilized in these assays. Agar dilution assays were performed using lemongrass oil in a serial two-fold dilution series, ranging between 0.0025% to 0.635% (V/V) concentrations again in BHI broth. Clarithromycin ranging in concentration from 0.01 to 8 μg/ml was added to agar, with or without EOs administered. Agar plates were inoculated with *H. pylori* in $1x10^7$ CFU concentrations and incubated for five days. MIC (minimum inhibitory concentration) was determined through plate colony counting, and colonies surviving at the maximum concentrations were passaged ten times to similarly created plates. Colonies that survived despite antibiotics are deemed to have some sort of resistance to the target antibiotic present in the medium. After replication, strains showed signs of antibiotic resistance, but the team found no resistance acquired across any *H. pylori* strains regarding the lemongrass oil, even after ten passes.

Experiment 4: *In vivo* experiments using mice were also utilized in this study. *H. pylori* CPY2052 were colonized in the stomachs of mice through oral inoculation through the infected broth. For 15 months after inoculation, mice were infected until treatment. Specific pathogen-free mice were also used as a control group, maintained on sterile food and water to prevent infections.

Infected mice were split into three groups, one receiving 0.5ml of lemongrass oil (0.1% v/v) dissolved in distilled water with DMSO, group two, a control group, was given only 0.5mL distilled water with DMSO via an orogastric tube for 14 days, and the third group was receiving nothing as an added control group. Lemongrass oil was chosen as the experimental treatment due

to its strong antibacterial effect in *vitro* experiments. After two weeks of treatment, the mice were sacrificed, and stomach samples were homogenized and plated on BHI agar plates. The plates contained a cocktail of horse blood, vancomycin, polymyxin B, trimethoprim, and amphotericin B. Plates were incubated again for five days at 37◦C.

One of ten mice in the treatment group was completely cured of the *H. pylori* infection (Figure 7), with others seeing improvement. It was seen that there was little to no improvement seen in mice in the control groups. *H. pylori* colonies recovered from the mice's stomachs treated with lemongrass oil saw significantly fewer colonies (Figure 5) compared to mice given only distilled water and those who have not received any treatment.

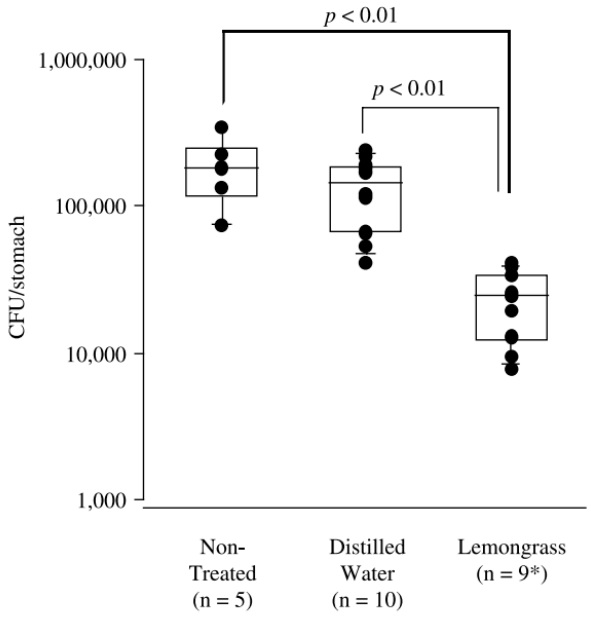

Figure 7: The effect of lemongrass essential oil on *H. pylori* colonization in mice. The end of each box indicates 25th and 75th percentiles, with the line inside marking the median CFU. All circles mark data, with bars indicating 10th and 90th percentiles. It is seen that the treated group has the lowest number of CFUs/stomach, indicating a bactericidal effect. The group that did not receive treatment saw little to no reduction of CFUs. The median number of colonies was 2.45 x 10^4 CFU/stomach in the lemongrass group, 1.45 x 10^5 CFU/stomach for distilled water, and 1.82 x 10^5 CFU/stomach for nontreated mice.

Overall, this study identifies that oils, like lemongrass oil, have potential bactericidal properties, proving efficacy *in vivo* and *in vitro*. Between all oils tested *in vitro,* a complete

inhibitory property was seen at oil concentrations of 0.1% (v/v). Both lemongrass and lemon verbena showed the highest amount of antibacterial action. It was found that the lemongrass oil did not contribute to resistance against any strains of *H. pylori*, opening possibilities of new antibacterial agents using lemongrass EO, especially in *H. pylori* infections. Curbing resistance while effectively removing pathogens are the hallmarks of a suitable treatment for these ulcer-causing infections. While efficacy was seen in other oils tested, none compared to the reportedly high rates of what was seen in the lemongrass oil groups. Further research of this type should focus on incorporating lemongrass oil into pharmaceutical agents and a more in-depth understanding of mechanisms of action.

Essential Oils as Antiviral Agents

Besides their antibacterial properties, essential oils have also been studied as antiviral agents. *In vitro* studies have identified antiviral activities of tea tree and eucalyptus oils against herpes simplex virus-1 (HSV-1) and -2 (HSV-2). In one study, plaque reduction assays using RC-37 cells showed that both essential oils had potent viricidal activity at noncytotoxic levels (Schnitzler et al., 2001). However, the mechanisms underlying the antiviral properties have yet to be elucidated.

Minami et al., 2003: EO Virucidal Effects Against HSV-1 *in vivo* and *in vitro*

A larger-scale study by Minami et al., 2003 expanded upon these results by testing twelve different essential oils for virucidal efficacy against samples of HSV-1 using *in vitro* plaque reduction assays. The essential oils used in this study are shown in Table 1.

Genus and Species	Common name
Cupressus sempervirens	Cyprus
Juniperus communis	Juniper
Melaleuca alternifolia	Tea Tree
Ocimum basilicum	Basil
Mentha piperita	Peppermint
Origanum majorana	Marjoram
Eucalyptus globulus	Rosemary
Cymbopogon citratus	Lemongrass

Table 1. Types of essential oils utilized by Minami et al., 2003. Oils were sourced from Laboratoire Sanoflore, Ltd, located in (Lozeron, France). This company also provided the same EO sets to Ohno et al., 2003. Oils were diluted in Eagle's minimum essential medium (MEM) supplemented with 5% fetal calf serum (FCS) and dissolved in dimethyl at a final concentration of 0.2%.

Oils were tested for efficacy against the HSV-1 Amakata strain, which was clinically isolated from a patient with herpetic ocular disease. Subject oils were diluted in minimum Eagle's essential medium (MEM) until a 0.2% concentration of oil was present. Once the virus was propagated, the virus was titrated on monolayered Vero cells (African green monkey kidney

cells/RC-37 cells) with mixtures of the essential oils. Plaque formation was counted at each point in the oil concentration dilution series (1%, 0.1%, 0.05%, 0.005% respectively) (Minami et al., 2003). The results show that all but three essential oils inhibited the growth of HSV-1 at a minimum concentration of 0.1% (Table 2).

Concentration of essential oil (%)	1	0.1	0.05	0.005
	Percentage of plaque formation (%)			
Control	100±18.0		100±12.2	
Cypress	42.8±12.3	88±22.9	121±20.8	95±5.0
Juniper	119±4.1	71.4±7.1	85±5.0	105±13.2
Tropical basil	73.8±4.1	90.4±16.4	78.3±5.7	81.6±12.5
Tea tree	0.0±0.0	90.4±10.9	90±13.2	86.6±7.6
Peppermint	0.0±0.0	80.9±25.0	86.6±2.8	86.6±11.5
Marjoram	0.0±0.0	92.8±21.4	88.3±15.2	91.6±15.2
Eucaryptus	0.0±0.0	69±4.1	103±7.6	103±15.2
Ravansara	0.0±0.0	92.8±7.1	81.6±7.6	93.3±7.6
Lavender	0.0±0.0	80.9±10.9	86.6±16.0	88.3±12.5
Lemon	0.0±0.0	88±4.1	103±16.0	80±18.0
Rosemary	0.0±0.0	90.4±8.2	76.6±10.4	76.6±12.5
Lemongrass	0.0±0.0	0.0±0.0	21.6±2.8	56.6±10.4

Table 2. The virucidal effects of chosen EOs in *in vitro* samples of HSV-1 Amakata strain. HSV-1 viral particles were obtained via patients with herpetic ocular disease and subjected to incubation in both the presence and absence of oil (ensuring a control). Cells underwent four days of incubation, and viral titers were measured via the plaque reduction method. Plaque percentage number was found through comparison of plaque numbers in the control and experimental groups. High rates of plaque reduction and total plaque elimination were seen, especially in lemongrass. Total elimination was seen at all 1% oil concentrations except cypress, juniper, and tropical basil.

Vero cells were treated with essential oils either before or after HSV-1 adsorption to determine antiviral activity mechanisms. Timing of the EO application is essential, as there is a possible time-dependent virucidal effect between the presence of the oil in the absorption period or the adsorption period. There was no antiviral activity in pretreatment conditions, suggesting that essential oils' antiviral properties are due to a direct interaction between the oil and the viral particle (Table 2). Similar results of antiviral activity were seen in the study performed by Schuhmacher et al. 2003, in which the team looked at the effects of peppermint essential oil on RC-37 cells and HSV-1/-2. This study had also concluded that cell treatment with the oil prior to

replication period saw no antiviral effect, but when added during the adsorption period (when molecules bind to the surface), there was an 80% decrease in plaque formation (Schuhmacher et al., 2003). These findings suggest that there is a presence of a time-sensitive window where these compounds can produce an antiviral effect during the adsorption period post-infection.

The timing of application could be due to binding at the viral envelope or glycoprotein (Minami et al., 2003). A significant result from the present study corroborates previous research showing that lemongrass has high viricidal activity at lower concentrations than other essential oils (Table 3).

A

	Percentage of plaque formation (%)
Control	100±14.6
Cypress	89.8±13.8
Juniper	90.6±12.6
Tropical basil	92.3±18.3
Tea tree	115.2±11.4
Peppermint	89.8±15.6
Marjoram	110.1±13.1
Eucaryptus	94.5±15.3
Ravansara	77.9±12.3
Lavender	92.3±13.4
Lemon	96.5±14.0
Rosemary	94.0±8.3
Lemongrass	87.9±18.0

B

	Percentage of plaque formation (%)
Control	100±12.2
Cypress	93.4±9.8
Juniper	89.8±12.5
Tropical basil	92.0±12.7
Tea tree	98.5±11.2
Peppermint	97.8±3.6
Marjoram	92.0±7.4
Eucaryptus	99.2±7.4
Ravansara	96.3±9.6
Lavender	97.1±11.8
Lemon	89.1±11.5
Rosemary	91.3±9.9
Lemongrass	96.3±14.1

Table 3. The effect of essential oils on HSV-1 replication before (A) and after (B) viral adsorption. (A) Cells were pre-treated with 0.1% essential oil mixtures for 24 hours at 37°C before viral adsorption, then being infected with HSV-1. 4-days post-incubation, titers were measured. Results are presented as % of plaque in EO-treated cells in nontreated control (mean+/-SD) in six different replicates. It is seen that there is minimal virucidal action occurring, as plaque formation was consistently above 77%. (B) Vero cells were infected with HSV-1 and treated with 0.1% EO at 37°C after viral absorption, with titers being measured 4-days post-incubation. Results are represented as the percentage of plaque in experimental cells in nontreated control cells (mean +/-SD) in six replicates.

Even though this study focused on HSV-1, the results provide essential groundwork and information about the effect of lemongrass on enveloped viruses. Future studies should focus on applying lemongrass to other enveloped viruses, such as varicella-zoster or SARS-CoV-2. Studies are in progress to analyze potential oils to combat SARS-CoV-2, so a potential treatment with EO compounds might be produced in the future. Currently, there are studies underway

looking at this possibility, notably looking at efficacies of geranium and lemon EOs (Senthil Kumar, 2020).

Future studies on essential oils should focus on elucidating the mechanisms of viricidal action. This can be done through large-scale studies comparing the efficacy of essential oils on different classes of viruses. Additionally, essential oils should be studied as a part of a cocktail therapy with traditional antiviral medications. Combinatory therapies can provide synergistic actions and far greater efficacy than single-drug therapies, possibly aiding the curb of resistance. Thus, *in vivo* animal studies must identify the mechanisms, toxicity, and therapeutic potentials of essential oils as antiviral therapies. Overall, the aforementioned research presents exciting possibilities for essential oils as antimicrobial therapies.

Conclusion

The findings summarized in this review suggest a potential use of essential oils as antibacterial and antiviral agents. *In vivo* studies of lemongrass oil showed a significant decrease in *Helicobacter pylori* infection post-treatment (Ohno et al., 2003), making this EO a potential agent for antimicrobial remedies. Lemongrass was efficacious for treatment against HSV-1 viral infection (Minami et al., 2003) and in *H. pylori* infections (Ohno et al., 2013). While these results show excellent preliminary efficacy, there is not enough research to ultimately justify the use of essential oils as antimicrobial treatments in a clinical setting. Further preclinical animal studies must be performed to generate dose-response data to identify the therapeutic index, which is the drug concentration at which there is efficacy without toxicity (NIH, 2020).

Additionally, essential oils must be studied against a variety of classes of bacterial and viral agents. Biochemical studies can be done *in vitro* to determine the mechanism of action of these EOs. If the mechanism can be determined, this will help define the types of infections treated with essential oils. Finally, any candidate essential oil therapies must be tested for additional safety when used in conjugation with prescription antimicrobial agents. This is crucial, as essential oils could be a valuable component of a combinatory drug regimen that treats infection while preventing microbial resistance. Thus, the safety and toxicity of the agents must be further analyzed in both *in vivo* and *in vitro* studies before pharmaceutical development for use. In conclusion, essential oils have exciting potential as antimicrobial agents, and continuing research will be crucial for finding the safest and most efficacious use of these compounds.

Acknowledgments

This review would not have existed without the help of my dear family and friends, with their constant support and validation. Without my parents' encouragement, line edits, and scientific influences, I would most certainly be in a different spot than I am today.

My grandparents also deserve a shoutout – thank you, Gramma and Poppa for helping finance and continue my education; I hope I made you proud. Mammam, thank you for always being a call away when things get too stressful.

Thank you to Luke for your constant support and encouragement to keep going and keeping me from getting (too) distracted when doing work (and this thesis). I love you.

I would like to thank Colleen, Samina, and Teresa for being incredibly supportive throughout our journey stumbling through science and academia. Late nights in the filtration labs (@ you Teresa) and late nights studying in that circular table on the bio floor in Toll will always be in my heart. Thank you all for the collective and constant sending of science memes and similar panic in response to a due date or application.

To my professors, thank you for dealing with me for four years. I know I've cried in pretty much everyone's office at one point, and so thank you for putting up with me. Hopefully, I've made you proud.

Finally, this goes out to the girl reading this <3

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