The Potential Use of Selected Organic Natural Products, Individually and in Combination, as Antifungal and Antibacterial Agents Against *Candida albicans*

By
Bobby Patel
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Dr. Laura H. Twersky, Professor of Biology

Thesis Supervisor

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Dedication

To all the microbes and fungi that died for this research. You will not be forgotten.

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Abstract

The purpose of this research was to determine the antifungal and antibacterial properties of organic raw honey, organic castor oil, and holy basil (*Ocimum tenuiflorum)*. The fungus, *Candida albicans,* and microbes found in common places were used to conduct the experiment. These organic solutions possess antibacterial and antifungal properties. There were several groups observed in this experiment: the control, honey vs. fungi, honey vs. bacteria, castor vs. fungi, castor vs. bacteria, honey/castor mix vs. fungi, honey/castor mix vs. bacteria, holy basil vs. fungi, and holy basil vs. bacteria. The petri dishes were first made by making nutrient agar. Afterwards, the *C. albicans* samples were spread onto the plates and incubated for several days. Once the fungus grew, the organic solutions were placed onto them and left alone for one day in the incubator. The same steps were taken for the bacterial part of the experiment. Results showed that all the plates with honey and castor oil had the best effect against the fungi. Castor oil by itself had zones of inhibition indicating no growth around the areas where the oil was placed. The honey was only effective in high dosages. Lastly, the holy basil was effective in regard to its antibacterial and antifungal activity. This study helped investigate which solution was more suitable to use in modern medicine to fight fungal and bacterial infections, more specifically, Candidiasis.

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Introduction

Candidiasis is a fungal infection that is well-known for causing infections in the skin. There are over one hundred fifty species of *Candida* which exist today. The most predominant species are *Candida albicans*. The process of transmitting Candidiasis is developed when the skin becomes infected, in other cases it can be transmitted through skin on skin contact, personal items or from animals. Since the fungus naturally lives on the skin, it begins to dramatically multiply which causes the infection to arise. Overall, most fungal infections are on the surface of the skin; there are different types of antifungal treatments that can be used such as over the counter products, prescription medications or other antifungal products (Vengurlekar et al., 2012). However, certain antifungal treatments tend to have negative side effects which could cause a skin irritation. Also, there are some people who prefer the use of natural remedies rather than using chemicals. Since fungal cells are like human cells, it is difficult to have a specific treatment which targets the correct cells. Rather than killing the fungal cells, which requires quite high specificity and may lead to the emergence of resistance, inhibiting growth and virulence factors in fungal cells represents a good alternative for the development of new antifungal drugs (Li et al., 2015). For this reason, natural antifungals have been considered as one of the best options for treating yeast or fungal related infections. Some of the most common antifungal treatments are caprylic acid, coconut oil, garlic, grapefruit seed, olive oil and oregano oil (Freire et al., 2017).

C. albicans must be placed in a warm environment where they can grow. An ideal temperature would be around twenty-five to thirty degrees Celsius (Mayer et al., 2013). In addition, there are other factors that are involved with the growth of *C. albicans*. Ambient pH influences fungal filamentous growth. When exposed to high pH, anything greater than seven,

growth is severely induced (Xiaoyong et al., 2010). At low pH, less than six, *C. albicans* predominantly grow in yeast form. Like many other species, *C. albicans* need a source of fuel to survive. For instance, it is found that they have an affinity for the transition metal, iron. *C. albicans* can adapt to extremes of iron availability, such as the iron replete environment of the gastrointestinal tract and iron deficiency during systemic infection. *C. albicans* have developed a complex and effective regulatory circuit for iron acquisition and storage to circumvent iron limitation within the human host (Flourie et al., 2018). In other words, the infection can survive in hosts even if they do not have an adequate amount of iron within them. Copper and manganese are also essential for fungal growth; however, the mechanisms by which *C. albicans* acquires these metals is currently poorly understood and there has not been any research related to this yet (Baek, 2008). Researchers and mycologists are unsure of how much iron is needed for the fungus to survive on but there have been a few experiments where scientists used tiny trace amounts. However, the exact amount remains a mystery.

The rate of fungal infection has significantly increased over the years. This is due to the increase in antimicrobial resistance and the restricted antifungal drugs. As a result, *Candida* species are one of the major human fungal pathogens to cause mucosal and deep tissue infections (Sardi et al., 2013). Previous research has shown that most infections are associated with biofilm growth. Biofilm production involves a high level of antimicrobial resistance of specific organisms. Therefore, studies have been done on plants as an alternative form of drug development because it provides a wide variety of drug options; were extensively used in folk medicine are easily accessible and can be used for different diseases (Sardi et al., 2013). Hence, plants are an excellent source for developing new antifungal treatments.

In addition, preliminary research was conducted on the antifungal effect of coconut oil, probiotics *Lactobacillus*, and 0.2% chlorhexidine to treat decayed, non-cavitated or cavitated lesions in children that are three years or younger (Heidrich, 2019). Studies have shown that high levels of cariogenic microorganism, dental plaque and enamel defects in the primary teeth are due to a diet of high sugar and carbohydrates. Hence, predominant microorganisms found in dental plaque are *Streptococcus mutans*, *Lactobacilli*, and *C. albicans*. Results showed, *C. albicans* to be the highest colonization site because it provides an ecological niche for the microorganisms in the tooth.

Overall, ketoconazole is an antifungal of the imidazole compound which has significant activity against superficial and systemic infections caused by pathogenic yeasts, dermatophytes, and filamentous fungi, including *C. albicans* (Beena et al., 2016). Furthermore, in a previous study on *Candida* species isolated from HIV-positive patients, it has been shown that there has been an increased resistance to antifungal agents and the toxicity of the drugs have reduced the effectiveness of antibiotic treatments. For this reason, natural antibiotics have shown alternative therapies for the treatment of *Candidiasis* (Kabir & Ahmad, 2012). With this intention, there have been many natural products that are used for alternative therapies in treating Candidiasis. Amongst these products are essential oils which have antimicrobial properties. For instance, castor oil and honey have antimicrobial properties against a wide variety of bacteria and fungi.

To evaluate the effectiveness of these natural antifungals, essential oils and honey were used against strains of *Candida* from HIV-positive patients. To adequately compare the most potent antifungal treatment a generally used prescription drug amphotericin B and vitamin D3 were evaluated amongst essential oils and honey. Results showed that vitamin D3 had the best performance at eliminating the *Candida* isolates (Bouzid, 2017). However, this exact

effectiveness was also present in castor oil and honey. Although, amphotericin provided great results in controlling *Candida* species a limitation that it has is the toxicity limits the use of the drug. For this reason, alternative therapies for treating patients is recommended and necessary for treating Candidiasis. In fact, a synergistic effect of some essential oils and a combination of amphotericin B could be a potential alternative that may further be researched (Oro et al., 2015). Scientists have also experimented with Vitamin A and studied its properties against fungal infections. Vitamin A is known to play an essential role in multiple biological processes, including reproduction, embryogenesis, maintenance of body tissues and augmentation of the immune system. Vitamin A has been tested on *C. albicans* and was shown to be ineffective. However, Vitamin A was able to increase human monocytes to combat Candidiasis and induce further growth of the infection (Klassert et al., 2014). No other vitamins and minerals have been experimented with to combat *C. albicans.*

With the results and research that has been conducted on antifungals in the past a centralized focus on castor oil and honey will be evaluated to identify the positive effects that both constituents have individually and in combination. Generally, there have been very few articles that have tested the antifungal properties of castor oil and honey. However, there have been a few articles which have tested honey's antifungal activity. For instance, honey is known for having additional phytochemicals which can enhance the antibacterial activity, but the nature of these components is yet to be identified (Ahmad et al., 2017). Since fungal infections are increasing in both the community and the hospital environments, they often lead to oral infections, vaginal yeast infections, and several skin fungal infections.

Although this study demonstrates the antifungal effect of honey *in vitro* there are some practical considerations for its use *in vivo*. Firstly, honey is limited to topical treatments, and

could not be used to treat Candidemia, the most serious form of candidiasis (Estevinho et al., 2011). However, as the leading risk factor for bloodstream infection is colonization or infection of external sites, such as indwelling catheters, or the oral or vaginal mucosa, honey may be used prophylactically to prevent more serious infections. Whole honey placed directly around catheters was found to be at least as effective as povidone iodine 26 or mupirocin 27 in preventing exit site infection (Irish et al., 2006). Secondly, as honey is water soluble, it may be diluted or removed by body fluids, particularly saliva in the oral cavity. A pilot study found a significant reduction in mean plaque scores and bleeding sites in patients given a chewable 'honey leather'; this same technique could be applied for the treatment of oral candidiasis. At other body sites, regular application of 100% honey would maintain a concentration well above the desired minimum inhibitor concentration. Honey could also be incorporated into a pessary for the treatment of vaginal candidiasis. Another practical issue is the presence of catalase in body fluids that has the potential to reduce hydrogen peroxide activity. However, case reports and clinical trials suggest enough activity is retained to allow honey to be effective in the clinical setting. The results of the current study argue for controlled clinical trials to establish honey as a topical antifungal agent. Most scientific research has only used castor oil as a base for other chemicals when studying effects on *Candida* (Drobot & Thom, 2000). It has not been used by itself when experimented with *Candida*. Essential oils, such as castor oil, may have antifungal and antibacterial properties due to the fatty acids it may contain (Mahilrajan et al., 2014). Castor oil can be used internally and externally. If applied externally, castor oil may trigger the T-cells in the skin to activate a general immune system reaction throughout the lymphatics to help combat against skin infections such as those of Candidiasis (Bakker, 2015).

There are also several plant species that possess antibacterial and antifungal properties. The use of medicinal plants in traditional medicine dates to thousands of years ago. There are many advantages and benefits associated with the use of medicinal plants as they are cost-effective and can easily be taken care of. There are also minimal to none amount of side effects present in most medicinal plants (Mallikarjan et al., 2016). Among the medicinal plants, aromatic herbs are a rich source of biologically active compounds useful both in agriculture and medicine. Of these, *Ocimum tenuiflorum*, also known as *Ocimum sanctum*, Tulsi, or Holy Basil from the family Lamiaceae has been described as the "Queen of plants" and the "mother medicine of nature" due to its perceived medicinal qualities (Yamani et al., 2016). The medicinal properties of Tulsi have been studied in hundreds of scientific studies including *in vitro,* animal and human experiments. These studies reveal that Tulsi has a unique combination of actions that include antiviral, antifungal, antiprotozoal, antimalarial, anthelmintic, and potentially even antibacterial properties (Cohen, 2014). However, literature pertaining to the antimicrobial activity of Tulsi on bacteria has not been thoroughly addressed. Therefore, the present study was conceptualized as the initial step to comprehensively report the antimicrobial potential of Tulsi that it may or may not possess.

Materials and Methods

Preparation of Solutions

Four vials of *C. albicans* and petri dishes were purchased from Carolina Biological Supply Company. The fungi were maintained in the petri dishes containing Agar nutrient (a medium used for many organisms to grow on). Kirkland's raw organic honey, Sky Organic's Castor oil, and Healing Solutions Organic Holy Basil Essential oil were purchased online from Amazon.

Preparation of Tulsi Essential Oil

The concentrations of 4.5% and 2.25% were decided, based on Yamani et al. (2016) contribution, where *C. albicans* and bacteria were exposed to concentrations in those range. Any concentration that was lower than 2.25% did not show any antibacterial nor antifungal activity. The essential oil concentration was prepared by combining 90 µl of the essential oil and 10 µl of dimethyl sulfoxide (DMSO) were added to a sterile microcentrifuge tube and then vortexed. The oil mixture was further diluted in the test (1:2) by the bacterial suspension, resulting in a solution containing of 4.5% essential oil in the first sample.

Fungus Care and Maintenance

The experiment began with the fungi grown at a high rate so that there was enough fungi culture to be experimented on with the organic solutions. Their growth was contingent to a temperature of 30-35 degrees Celsius. All other experimental variables were maintained at a constant throughout the course of the experiment. One petri dish was used as a control group. The rest of the groups had three petri dishes that contained different amounts of organic solutions. An inoculation loop method was used to spread the fungi onto the plates. Between

each plate, the loop was sterilized to prevent any cross-contamination. A Bunsen burner was used to sterilize the loop.

Bacterial Care and Maintenance

No specific type of bacteria was used to spread onto the petri dishes. Common bacteria that is found anywhere was used. A non-sterilized loop was smeared in a zig-pattern onto the nutrient agar.

Figure 1. The Setup

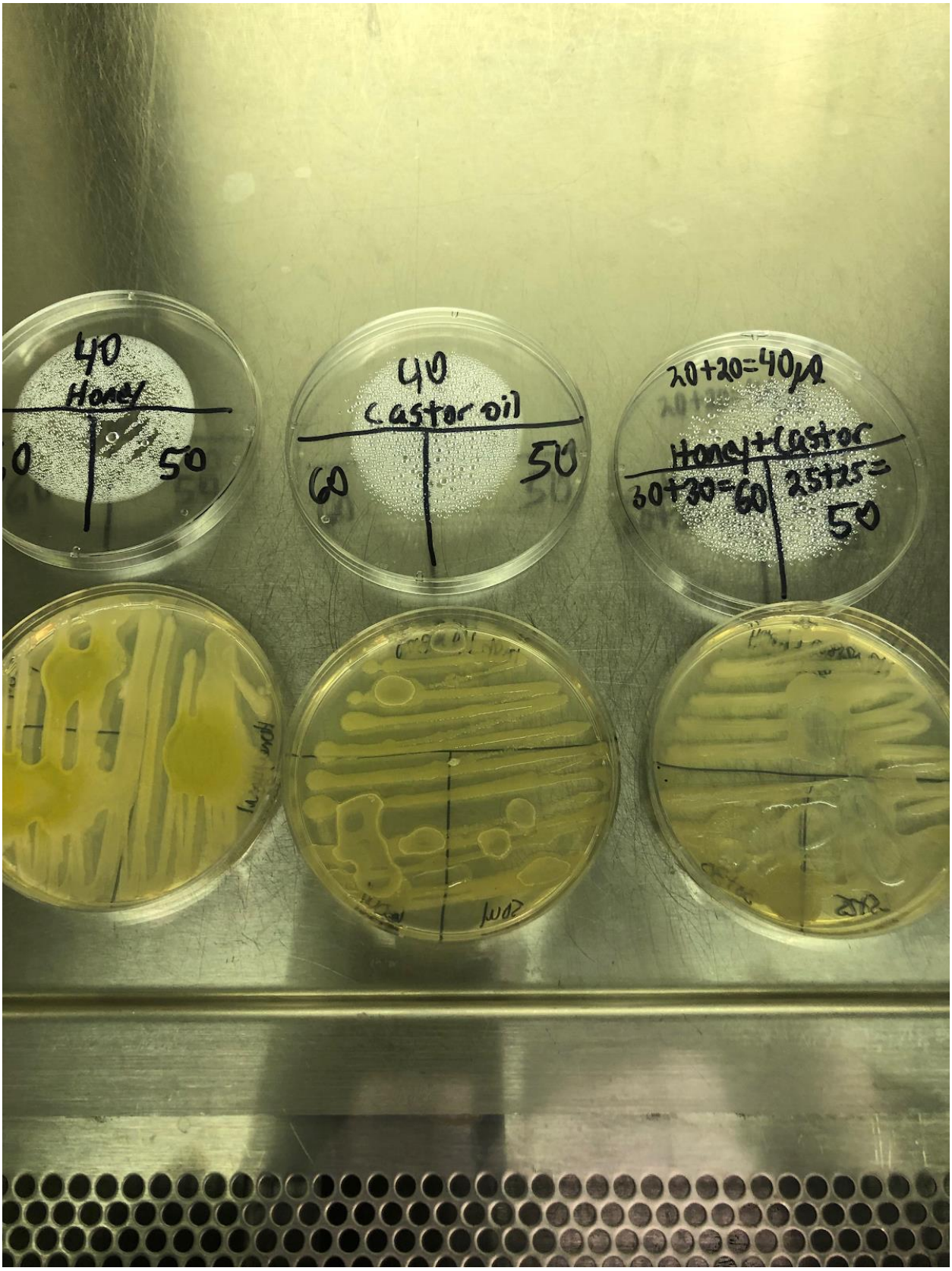
From left to right: control, honey+fungus, castor oil+fungus, and honey+castor oil+fungus. As clearly depicted, the plates are in four groups and are sectioned off indicating different amounts of solution in them.

The bacterial plates are not shown due to shutdown of campus facilities because of COVID-19.

Preparation

Two 50 mL beakers were thoroughly cleaned and disinfected with ethanol before use. About 10 mL of honey and castor oil were measured out. The petri dishes were broken into three different sections that indicated different amounts of solution in them. For example, plate 1 contained 10µL, 20µL, and 30µL. Plate 2 contained five more microliters than plate 1. Lastly, plate 3 had 10 more microliters than plate 1. The plates that contained both the honey and castor oil were made using half of each solution. For instance, plate 1 contained 5µL of honey and 5µL of castor oil that added up to be a total of 10µL. A micropipette was used to distribute the organic solutions onto the petri dishes. Once the solutions were distributed onto the plates, they were left to incubate for twenty-fours in the incubator set at thirty degrees Celsius.

Figure 2. A Close-Up of the Petri Dishes



Incubation of Fungus (Growing It)

For a complete understanding of the antifungal properties of the organic solutions on the fungi, the fungi were exposed to the experimental conditions during their peak growth. The petri dishes were placed in a thirty degrees Celsius incubator where the fungi would grow.

Incubation of Bacteria (Growing It)

The petri dishes with bacteria were also placed in an incubator and placed at the same temperature as the fungi plates.

Collection of Data

Frequent use of photography was used as a qualitative measurement to observe the decay of the fungi. This help determined if the organic solutions were effective or not. A sample from one the control group was taken and observed under the highest power (400x) using a Swift® M3600 Series Educational microscope. A picture of each of the four groups were taken with an Iphone camera after the organic solutions were placed onto them.

Figure 3. A Microscope Slide Under High Power (400x)

A sample was taken from the control and placed under the microscope to make sure that the fungus was on the plates.

Figure 4. Honey+Fungus Results

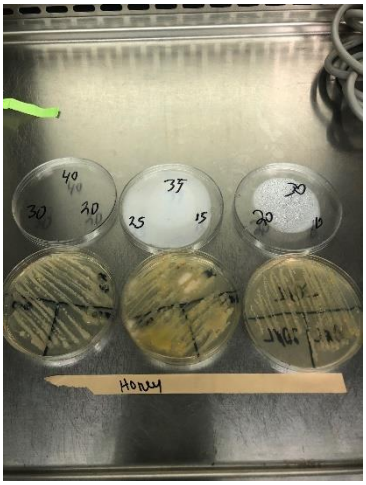
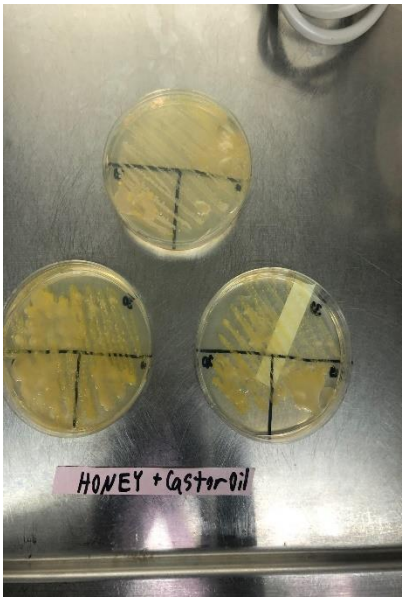
The results of the honey and fungus show that the organic honey is effective when it was used in higher doses. 

Figure 5. Castor Oil+Fungus Results

The castor oil plates show zones of inhibition indicating that the fungus was not growing around it.

Figure 6. Honey+Castor Oil+Fungus Results



It is shown that when both the organic solutions are combined, they are very effective against the fungus.

Discussion

Upon completing this research, several conclusions were made after meticulously analyzing the results and data. It was shown that the petri dishes which contained both the organic solutions combined had the best results. There was no growth or any presence of *C. albicans* in the areas where the mixed organic solutions were placed. In figure (6), it was clearly portrayed that there was barely any growth present. Organic castor oil by itself was effective as well. Petri dishes that contained organic castor oil had zones of inhibition present in the areas where the organic solution was planted. The zones of inhibition are circular areas around the spots of the antifungal agent in which the fungi colonies do not grow. In figure (5), there were several zones of inhibition in the organic castor oil plates. Lastly, the plates with the organic honey were not as effective as the organic castor oil ones. This could be due to their different chemical properties. In figure (4), the plates with higher doses of honey were more effective than the ones with the lower amounts. The plates with the Holy Basil (Tulsi) essential oil proved to have antibacterial and antifungal properties as there were zones of inhibition present in the plates where the drops were added with a sterile disposable 1 mL pipette. Upon completion of the experiment, it was safe to say to conclude that this experiment was a success due to the fact that the organic solutions were effective against *C. albicans* and bacteria. Future studies would involve performing more trials, using a different fungi strain, or even using different natural remedies as antifungal and antibacterial agents such as turmeric or apple cider vinegar.

Natural medicine is safer than most chemical based ones as they possess little to no side effects. Naturally based medicines should be incorporated for people who dislike chemicals or those w suffer allergic reactions or negative side effects from them. Plant kingdoms are the rich source of organic compounds, many of which have been used for medicinal purposes. There are many natural crude drugs from plants that have the potential to treat many disease and disorders

and one of them is *Acrocomia aculeata,* a species of palm native to tropical regions of the Americas. "A phytopharmacological review by Jena and Gupta in 2012, revealed that, *A. aculeata* has proven to possess antimicrobial activities as they were used against dermatophytic and pathogenic bacterial strains *S. aureus, P. aeruginosa* as well *as K. pneumoniae* and *E. coli.* Also, anti-fungal activity of the leaf was potent against *Candida albicans*" (Suurbar et al., 2017). This plant and many others alike can be used to make natural antiseptics as well. Hand sanitizer can sometimes lead to irritation and dryness of the skin because the active ingredient, ethyl alcohol, can cause burning sensations in areas where scrapes are present. Alcohol has the tendency to dry up so it could potentially cause people's hands to dry up too much leaving minimal moisture. Some natural antiseptics such as eucalyptus, tea tree oil, and lavender can prevent dryness and irritation.

Experimental error was bound to have occurred throughout the course of this research experiment. Human error such as using the wrong amount of solutions could have possibly led to different results. For instance, using too much or too little of organic honey on a petri dish than a certain amount would lead to different visual results. When measuring the solutions, a range was set of ± 5µL to 10µL. When a centrifuge was used to prepare the Tulsi essential oil, it was imperative that a balance tube was placed at the opposite end to prevent damage to the sample and the machine. Another source of error would be contamination. When conducting this experiment, it was important that everything was as sterile as possible to avoid contamination. The workstation was sanitized before and after with ethanol. Gloves were always worn and changed after each use. If they were not changed after each use, then the materials that came in contact with the used gloves would become contaminated. Most of these sources of error were kept at a minimum because the data and results were not impacted as much. More trials should

have been performed to obtain consistent results. However, due to the Coronavirus, facilities on campus were shut down and prevented continuation of the research. The antibacterial portion of the experiment was partially incomplete because of this. Nevertheless, enough data and results were obtained.

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