A Comparative Study of Homeopathic Alternatives vs Allopathic Treatments on Candida albican Testing Growth Inhibition and Fungicidal Properties

By

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Thesis Supervisor

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Lastly, I wish to thank my friends and family who have been there to support me through the late night writing sessions and fed me caffeine to keep going.

Abstract
Candida albicans is one of the most prominent forms of Candidiasis, accounting for more than 75% of all Candidal infections. Aside from its growing prevalence, it is also becoming one of the most resistant strains against antifungal medication. This increase in resistance to allopathic medication has fueled a homeopathic movement in medicine. Homeopathy has recently become a well-known alternative to traditional allopathic medication and has shown success in inhibiting fungal growth in certain strains. Due to the growing amount of resistant strains of fungi due to adaptation to antifungal medication mechanisms, seeking alternative treatments can prevent the rise of a fungal epidemic resistant to known treatments. In this study, 5 separate antifungal agents will be tested on their efficacy of inhibiting fungal growth and their fungicidal properties on cultured C. albicans on Sabouraud dextrose agar plates. The agents being tested are Eucalyptus globulus, Ocimum basilicum, Boiron Benzoicum Acidum 30c, Kali Iodatum 30c, and 100mg Ketoconazole. Growth inhibition rate and fungicidal properties were tested using individual treatment. Results showed that in the growth inhibition trial, Ocimum basilicum had the largest zone of inhibition with Eucalyptus globulus following. The allopathic group showed similar inhibition rates with Benzoicum Acidum and Kali Iodatum showing more inhibition than allopathic Ketoconazole. Fungicidal efficacy was tested in a similar protocol. Data showed that no individual agent had an effective fungicidal effect on Candida albicans, only causing a minimal reduction on the surface in the fungal colony. All data was analyzed using the ANOVA statistical analysis method, and all trials were replicated 10 times to minimize human error and obtain clear results.

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Introduction

Over one billion people have been impacted by fungal infections in various clinical manifestations, these include hair, nails, and skin among other types (Bongomin, et al. 2017). These infections can pose a threat to human organisms by causing damage to several organ
systems. More than 1.5 million deaths are accredited to fungal disease and infections (Bongomin, et al. 2017). Despite the large number of individuals affected by fungal infections, the topic is not prevalent in public health education (Bongomin, et al. 2017). With the few studies conducted it is known that fungal disease mortality is similar to that of tuberculosis and is three times the amount of malaria mortality (Bongomin, et al. 2017). The rise of fungal infections in immunocompromised patients holds a significant factor in the mortality of these cases, along with the acquired resistance of fungi to allopathic treatment (Matejuk, et al. 2010).

The severity of the infection varies by the fungi identified; some are mucocutaneous which target the mucosal membrane of the epidermis, and the other systemic fungi are life-threatening to immunosuppressed patients (Bongomin, et al. 2017). Severe infections include invasive candidiasis, histoplasmosis, aspergillosis which affect those with weakened immune systems by attacking blood flow and lung function (Center for Disease Control, 2019). Immunocompromised patients are most susceptible and at risk for fungal infection, these include patients suffering from AIDS, chemotherapy treatments, cystic fibrosis, and diabetes (Vengurlekar, et al. 2012). The lethal complications of these infections could be avoided with proper awareness and education towards the public. As of now the topic of fungus among people is not well known, and many do not know the severity of the matter. Of the nearly one billion people impacted by fungal infections, millions are affected by Candida derived infections such as life-threatening invasive candidiasis (Bongomin, et al. 2017). There are about 2 million incidences of oral candidiasis, 1.3 million cases of esophageal candidiasis, 750 thousand cases of invasive candidiasis a year, and 134 million cases of recurrent vulvovaginal candidiasis as a global figure (Bongomin, et al. 2017).
The number of estimated Candidal infections resulting in invasive candidiasis seems to be growing each year, in 2016 there were an estimated 5,142 cases in the United Kingdom (Bongomin, et al. 2017). Candidiasis is one of the most prominent forms of fungal disease, and also harbors a strain of the most anti-fungal resistant fungi: Candida albicans (Gupta, et al. 2015). There have been several studies conducted on Candidal infections obtained from human patients suffering from oral and vaginal Candidiasis and the effects of homeopathic treatment (Witt, et al. 2009; Gupta, et al. 2015). Studies show that recurrent vulvovaginal candidiasis affects 75% of women at least once and is recurrent in 5-8% of women in prime reproductive years, and the clinical management is problematic due to being unable to identify all factors causing the infection (Witt, et al. 2009). The most common strain responsible for vulvovaginal candidiasis is C. albicans (Witt, et al. 2009).

Previous studies have used a randomized trial of oral administration regimen of fluconazole, a well-known allopathic antifungal medication showed that regardless of chosen administration regime of the drug, a 50% relapse rate is shown in patients after stopping fluconazole treatment (Witt, et al. 2009). Azole antifungals like the aforementioned Fluconazole are the primary treatment for several Candidal infections, however, there are several studies and documentation of intrinsic and acquired resistance to azole antifungals among the Candida genus (Whaley, et al. 2017). Current antifungal drugs and treatment have limitations due to the increased occurrence of systemic fungal infections and rapidly developed fungal resistance (Vengurlekar, et al. 2012). It has been demonstrated that nosocomial Candidiasis evolved into virulent strains, as well as exhibiting the ability to mix with different bacteria found in the body (Gupta, et al. 2015) which can spike the chances of antifungal resistance. This same study sought to determine the inhibitory effect on human pathogenic C. albicans and found that a select
amount of homeopathic drugs in 200c and 30c potency exhibited inhibitory properties comparable to the effect of ketoconazole (Gupta, et al. 2015). Leading factors in raised incidences of fungal infections include excessive use of antimicrobial agents, immune system defects, and diseases that affect the human immune system (Klepser, et al. 1996). This shows that although allopathic treatment still holds the maximum inhibition rate, homeopathic drugs such as Acidum Benzoicum and Kali Iodatum are comparable and effective in eradicating the fungal infection. As well as inhibiting growth without risking the strain of fungus to become resistant. The growing homeopathic approach in medicine exhibits efficiency and success in clinical treatment as an alternative to allopathic medicine, as well as provides a method of combating antifungal resistance in fungal infections.

Due to the recent climb in acquired drug resistance in fungal infections, researchers have been looking into alternatives for traditional allopathic -azole antifungal treatments such as peptide-based (Matejuk, et al. 2010), and plant-derived essential oil-based (El kaoua, et al. 2017; Oxenham, et al. 2005). There has been a considerable amount of success and advancement in the use of peptide-based antifungal therapy found in both plant and animal kingdoms (Matejuk, et al. 2010). The utilization of natural sources and treatments have been imperative in developing new active molecules with unique chemical skeletons and bioactivities (Vengurlekar, et al. 2012). These studies conducted on natural sources have resulted in the discovery of potent antifungals found in nature such as plants, marine products, and microorganisms (Vengurlekar, et al. 2012).

The use of plant-derived essential oils such as *Eucalyptus globulus* and *Ocimum basilicum* is growing in antifungal treatment. The fungicidal properties found in Basil (*Ocimum basilicum*) oil have been studied in-vitro and showed that two chemotypes found in the essential
The chemotypes found, methyl chavicol and linalol, were found to be equally effective in the same amount of quantity administered, reducing fungal growth by 78% four days post-inoculation (Oxenham, 2005).

Eucalyptus globulus is a well-studied oil that shows fungicidal properties and is used in the treatment of other existing conditions such as Diabetes Mellitus (Bokaeian, et al. 2010). In a study using both normal and diabetic rats, the efficacy in antifungal properties was tested using Eucalyptus globulus essential oil, using sixty normoglycemic male rats randomly divided into six groups (Bokaeian, et al. 2010). Overall the study found that essential oil administration accounted for a reduction of hyperglycemia, polydipsia, polyphagia, and Candidal infection in the liver and kidneys (Bokaeian, et al. 2010). Past research focused on a different strain of eucalyptus but bore similar effects as the previously mentioned study. It tested Eucalyptus gomphocephala’s effects on microbial infection, and looked at the epidemiology and toxicity (El Kaoua, et al. 2017). The research concluded that E. gomphocephala bore a significant amount of inhibitory properties on microbial agents (El Kaoua, et al. 2017). Another study looked at the chemical composition of 8 Eucalyptus species harvested from various parts of Tunisia and their antimicrobial, antifungal, and antiviral activities (Elaiissi, et al. 2012).

These studies provide a precedent for a less impactful form of treatment than traditional oral and topical fungal treatments which can affect the liver and other organ systems. Additionally, homeopathic treatment and alternatives have been proven to be more cost-effective than traditional allopathic treatment, which is imperative with patient care in order to enable access to medical treatment (Colas, et al. 2015). This study expanded on previous studies of individual focus on homeopathic alternatives, and explored both the inhibitory growth rates and fungicidal effects on singular treatments between allopathic and homeopathic medicine. It is
hypothesized that homeopathic treatment and mother tinctures will hold efficient fungicidal properties equal if not more than allopathic treatment on an existing \textit{C. albicans} infection. Additionally, I hypothesize that homeopathic treatments and mother tinctures will have a substantial growth inhibitory effect on the colony, aiding in preventative measures against fungal infections.

**Materials & Methodology**

**Model Organism Care and Culturing**

\textit{Candida albicans} was used as the model organism in this study and was acquired from a commercial science company. This fungal organism was cultured in a lab using yeast malt agar at 25 degrees Celsius and packaged in tubes. Upon the arrival of the \textit{C. albicans} tube, proper inspection of the product was conducted to ensure no cross-contamination occurred by an accidental crack or break in the seal. The model organism enclosed in the tube was used within 3-5 days of receipt. Fungal culturing of \textit{Candida albicans} was conducted in the laboratory under sterile conditions to ensure an ample supply for the study and to provide additional nutrients. The culturing of \textit{C. albicans} was conducted in a sterile environment using proper sterile technique under a decontaminated hood. After Sabouraud dextrose agar (SDA) plates were made and chilled, they were cultured with \textit{Candida albicans} from a standard test tube. The fungi was inoculated using sterile tools such as an inoculating loop, and then sterilized using ethyl alcohol and Benchmark Scientific Infrared Micro Sterilizer. A total of 50 plates were created as a stock culture for future trials, they were left to culture for 7 days. It was decided that the use of Sabouraud dextrose broth (SDB)( Sigma Aldrich #S3306) would be most effective in achieving an even spread of fungal growth. SDB was made using Sigma Aldrich protocol in a sterile hood environment. To make the SDB 15g of SDB mix was measured on a scale, then poured into
500mL of distilled water in an Erlenmeyer flask. The SDB mix in the 500mL were heated and mixed using a stirring rod inside Erlenmeyer flask until it was entirely dissolved, afterwards it was autoclaved at 121 degrees Celsius. The flasks were then placed in a 50 degree Celsius water bath for ten minutes. This was cultured by obtaining fungal specimens from culture stock, and swirling it into the SDB in the Erlenmeyer Flask using an inoculating loop. After culturing it was left in a 25 degree Celsius incubator to allow for proper fungal growth. After the 7 day incubation period 1mL was pipetted from a 25mL pipette gun and then spread with a L-spreader.

**PETRI DISHES: SABOURAUD DEXTROSE AGAR**

In order to produce viable data and minimize human and statistical error, there will be 10 replications of each trial conducted. In order to conduct these trials, a total of 70 Petri dishes with Sabouraud dextrose agar (SDA) was prepared for the three rounds, as well as 10 more to conduct the aforementioned subculturing of *C. albicans*. There were 50 used in the primary trial where all agents are tested individually, allowing for 10 replications of the study. The Saint Peter’s University lab protocol was followed for agar plate production. For 10 Plates, mix 32.5g of Sabouraud dextrose agar (CBS # 786781) in 500mL sets, placed directly into 1L Erlenmeyer flasks, and covered with aluminum foil. This was autoclaved and flasks were placed in a 50 degree Celsius water bath for ten minutes. The media was then poured into Petri dishes and allowed to cool for 30 minutes. They were then placed in autoclave bags and left overnight in the hood, the following day they were taped and stored in the cold room until culturing stage.

**TRIALS**
Growth Inhibitory: This experiment was replicated 10 times to minimize human and statistical error. An individual analysis of each antifungal agent tested was conducted, including *Eucalyptus globulus*, *Ocimum basilicum*, Boiron Benzoicum Acidum 30c, Kali Iodatum 30c, and 100mg Ketoconazole. The application of each agent varies by the physical property it holds, in this study agents come in either oil or tablet form. Essential oils 2mm were pipetted using a P20 pipette and evenly distributed along the petri dish. Tablet agents were crushed using a mortar and pestle and then dispersed along with the petri dish.

Trial 1: For this experiment the prepared and cultured SDB broth was used but not previously cultured on the SDA plates. The SDB broth was cultured and incubated for 7 days to ensure fungal growth. Simultaneously all 5 agents were turned into an aqueous solution that was used to soak diffusion discs for 30 minutes. *Benzoicum Acidum* and *Kali Iodatum* were presented in pellet/solid form were crushed using a mortar and pestle until completely pulverized, and then 1mL of distilled water was added and mixed into a solution with a concentration of 1g/mL. Ketoconazole was acquired in powder form, so therefore it was measured out to .25g and 300uL of water was applied to great a solution. *Eucalyptus globulus* and *Ocimum basilicum* essential oils were acquired in aqueous form so 50uL were simply pipetted out onto a weighing boat recipient. One mL of the SDB broth was pipetted onto the SDA plate and spread with a L spreader simultaneously the soaked discs were placed in the middle of the SDA plate and then the plate was turned. This was repeated for all 50 individual plates, 10 for each agent.

Table 1: Table indicating the concentrations of solutions used for treatments in trial 1 of Growth Inhibition test. All concentrations were made to a volume of 50uL. Ten discs were soaked in these concentrations for 30 minutes.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration</th>
</tr>
</thead>
</table>
Fungicidal: Unlike the previous Growth Inhibitory experiment, this component only had one trial conducted that mimics trial one of the Growth Inhibition treatment. All five agents were treated to test their fungicidal efficacy on cultured *Candida albicans*. This experiment was completed over approximately a month-long period, and was monitored every Monday, Wednesday, and Friday. After the SDB was cultured it was incubated at 25 degrees Celsius for 7 days. This experiment testing fungicidal properties was carried out with two different methods, one with disc diffusion and another with pipetting the agent solution onto the SDA plate with the SDB culture growth on top of plate. Concentrations of solutions were made using the same method and protocol as the agents used in the growth inhibition treatment, but had different values (Table 2). For both protocols fungal cultures were grown on SDA plates using cultured SDB broth, in quantity of 1mL per plate and spread by L-spreaders and incubated at 25 degree Celsius for 5-7 days to ensure optimal growth. All was done in a sterile hood using aseptic technique.

Pipetting Solution: After the concentration solutions were prepared for each of the five agents, they were pipetted with a 20-100uL micropipette. Then 50uL of each respective solution was pipetted onto the center of the SDA plate, and then the plate was flipped. These were then

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Benzoicum Acidum</strong></td>
<td>1g/mL (50uL)</td>
<td></td>
</tr>
<tr>
<td><strong>Kali Iodatum</strong></td>
<td>1g/mL (50uL)</td>
<td></td>
</tr>
<tr>
<td><strong>Eucalyptus globulus</strong></td>
<td>50 uL</td>
<td></td>
</tr>
<tr>
<td><strong>Ocimum basilicum</strong></td>
<td>50 uL</td>
<td></td>
</tr>
<tr>
<td><strong>Ketoconazole</strong></td>
<td>.25g/300 uL (50uL)</td>
<td></td>
</tr>
</tbody>
</table>
covered with parafilm to seal the plate and reduce risk of contamination. Plates were stored in a 25 degree Celsius incubator for 7 days, being monitored each Monday, Wednesday, and Friday.

*Disc Diffusion:* The same solution concentrations were prepared for this protocol as in the pipetting solution. They were mixed thoroughly and then 10 diffusion discs were soaked in each agent solution for 15 minutes. Using sterile tweezers, each disc was placed directly in the middle of each SDA plate on top of the fungal growth. Plates were then stored upside down in a 25 degree Celsius incubator for 5-7 days. They were monitored each Monday, Wednesday, and Friday.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoicum Acidum</td>
<td>.8/1.5mL (50uL)</td>
</tr>
<tr>
<td>Kali Iodatum</td>
<td>.6g/1.5mL (50uL)</td>
</tr>
<tr>
<td><em>Eucalyptus globulus</em></td>
<td>50 uL</td>
</tr>
<tr>
<td><em>Ocimum basilicum</em></td>
<td>50 uL</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>.25g/300 uL (50uL)</td>
</tr>
</tbody>
</table>

**COLLECTION AND ANALYSIS OF DATA:**
Qualitative and quantitative data were collected throughout this study and its various trials. The qualitative assay of this experiment was measured by visual observation of the eradication of fungal colonies in separate trials. Colonies of *C. albicans* were measured at the start of trial and then remeasured two days post-treatment.

Quantitative data was collected using the vernier caliper, and then analyzed using Analysis of Variance (ANOVA) statistical analysis. This will determine the greatest inhibitor of fungal growth which will go on to be tested in the combination trial. One-way ANOVA statistical analysis tests differences between the multiple agents tested throughout this trial and will show the comparative rates of efficacy in inhibiting fungal growth. This method of statistical analysis is used for continuous data that is independent across groups, equally distributed, and homogenous (Pandis, 2015).

Formal observations were taken every Monday, Wednesday, and Friday with recorded measurements taken in (mm) quantities. It must be clarified that all measurements are taken in diameter x diameter, not excluding the diffusion disc which has a measurement of 5mm x 5mm (diameter x diameter). After the initial start of treatment when discs were applied simultaneously to the SDB broth, measurements were taken the following day to provide details as to how the experiment was running.

**Results**

**Growth Inhibition:** It was observed that *Ocimum basilicum* showed an immediate reaction with the fungi and it had already begun to inhibit growth in large quantities, compared to other agents (Figure 1). However, *O. basilicum* had a cloudy zone of inhibition (Figure 7). During treatment set up it was recorded that on multiple incidents, *O. basilicum* showed
corrosive properties to plastic, often corroding through plastic containers. It was also observed that ketoconazole did not harbor any results after one day. *Eucalyptus globulus* also began to show a zone of inhibition, with a very clear area of inhibition, unlike *O. basilicum* (Figure 3,7). Benzoicum Acidum and Kali Iodatum did not show any results after one day. Formal observations and measurements were recorded two days after the start of the trial (Table 3). It was noted that some diffusion discs had dislodged and were in other locations besides the initial center of the SDA plate (Figure 5). Benzoicum Acidum and Kali Iodatum had shown inhibition after two days of testing, with Benzoicum Acidum showing more inhibition. *Eucalyptus globulus* showed high inhibition zones, following behind *Ocimum basilicum* which showed the largest zone of inhibition. Ketoconazole showed approximately the same results as Benzoicum Acidum and Kali Iodatum (Table 2). Refer to tables for more detailed descriptions of measurements.

Table 3: Growth Inhibition Zone Measurements
Table showing the inhibition zone after 2 days of having diffusion discs soaked with agent solutions in the SDA plate. Diffusion disc measurement - 5mm.

<table>
<thead>
<tr>
<th></th>
<th>Benzoicum Acidum (T1)</th>
<th>Eucalyptus globulus (T2)</th>
<th>Kali Iodatum (T3)</th>
<th>Ketoconazole (T4)</th>
<th>Ocimum basilicum (T5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.1</td>
<td>10.8</td>
<td>10.1</td>
<td>13.1</td>
<td>62.1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>7.8</td>
<td>12.8</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>10.1</td>
<td>8</td>
<td>9</td>
<td>7.8</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>7.9</td>
<td>13</td>
<td>7</td>
<td>16</td>
<td>48.9</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>17.8</td>
<td>9</td>
<td>10</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>10.7</td>
<td>14</td>
<td>10.2</td>
<td>13.2</td>
<td>42.7</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>11</td>
<td>6.2</td>
<td>15</td>
<td>56</td>
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<tr>
<td>0</td>
<td>9.5</td>
<td>5</td>
<td>8.3</td>
<td>51</td>
<td></td>
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<tr>
<td>0</td>
<td>15</td>
<td>10.9</td>
<td>13.1</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>13.2</td>
<td>7</td>
<td>18</td>
<td>46</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: ANOVA analysis Growth Inhibition
The F-ratio value is 146.83566. The p-value is < .00001. The result is significant at p < .05.

<table>
<thead>
<tr>
<th></th>
<th>t1</th>
<th>t2</th>
<th>t3</th>
<th>t4</th>
<th>t5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>$\sum X$</td>
<td>63.8</td>
<td>112.3</td>
<td>82.2</td>
<td>127.3</td>
<td>495.7</td>
<td>881.3</td>
</tr>
<tr>
<td>Source</td>
<td>SS</td>
<td>df</td>
<td>MS</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>----------------------</td>
<td>-----------</td>
<td>-----</td>
<td>---------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between-treatments</td>
<td>13002.4412</td>
<td>4</td>
<td>3250.6103</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F = 146.83566</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within-treatments</td>
<td>970.735</td>
<td>45</td>
<td>21.5719</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>796.9588</td>
<td>36</td>
<td>22.1377</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Growth Inhibition Zone
The effect of each treatment’s growth inhibitory properties of Benzoic Acidum, Eucalyptus globulus, Kali Iodatum, Ketoconazole, and Ocimum basilicum on C. albicans. Ketoconazole is used as positive control. The p-value is < .00001. The result is significant at p < .05.

**Fungicidal:** This experiment was conducted and completed with only one trial, testing all 5 agents and their efficacy in treating an already present fungal infection. Observations were recorded one day after application of agents. Two protocols were used for this treatment, one
with pipette distribution of agent solution (50 uL) onto SDA plate, and another with diffusion discs soaked in agent concentrations for 15 minutes. During pipetting protocol, observations were taken and recorded after 1 day. Kali Iodatum, Benzoicum Acidum, and Ketoconazole had a slight zone of impact. Meaning, that instead of completely inhibiting all fungal growth there was only slight indentations in the fungal colony (Figure 8,9). *Eucalyptus globulus* and *Ocimum basilicum* showed no inhibition (Figure 2), but *Ocimum basilicum* showed corrosive properties that fused petri plates together, making it impossible to open up the plate for closer inspection. *Ocimum basilicum* plates showed no inhibition but they did show an oily residue on the surface of the fungal colony that reflected light (Figure 8). Measurements were repeated the following day (2 days post treatment), and again on Monday (5 days post treatment), and finally on Wednesday (7 days post treatment) and all showed no changes in fungicidal properties nor decrease in fungal colonies.

Treatment was started again using the diffusion disc method being mounted on top of the already treated SDA plates, observations were taken again after one day and subsequent days. There was no change noted for any of the days that it was observed, 1, 2, 3, 6, and 7 days post treatment. The plates were moved to room temperature storage from day 3 post treatment to day 7 post treatment, no results or changes were observed.

Table 5: Fungicidal Efficacy Measurements
Table showing the inhibition zone after 1 day of having diffusion discs soaked with agent solutions in the SDA plate. Diffusion disc measurement - 5mm
Table 6: ANOVA analysis Fungicidal Efficacy
The F-ratio value is 76.11744. The p-value is < .00001. The result is significant at p < .05

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between-treatments</td>
<td>1059.5062</td>
<td>4</td>
<td>264.8765</td>
</tr>
<tr>
<td>Within-treatments</td>
<td>173.4632</td>
<td>45</td>
<td>3.8547</td>
</tr>
<tr>
<td>Error</td>
<td>125.2743</td>
<td>36</td>
<td>3.4798</td>
</tr>
</tbody>
</table>
Figure 2: Fungicidal Efficacy Zone
The effect of each treatment’s fungicidal properties of Benzoicum Acidum, *Eucalyptus globulus*, Kali Iodatum, Ketoconazole, and *Ocimum basilicum* on *C. albicans*. Ketoconazole is used as positive control. The p-value is < .00001. The result is significant at p < .05.
Discussion

**Growth inhibition:** Results show that the growth inhibitory properties of all agents used are valid, but some surpass others. Results were noticed after only one day of disc application which accounts for the preventative properties of these agents. It was thought that the ketoconazole would have the most effective inhibitory characteristic, and prevent growth altogether due to its use as an allopathic treatment in already cultured fungal infections. However, ketoconazole did not have either the largest nor clearest zone of inhibition. Similarly, Benzoicum Acidum and Kali Iodatum did not yield the most effective inhibitory properties, although it was thought that because they are a homeopathic “drug” they would be clinically more efficient in preventing fungal growth. However, Benzoicum Acidum yielded the least results with the smallest zone of inhibition and Kali Iodatum was a close second worst. The agents that excelled in this treatment were the essential oils derived from *Eucalyptus globulus* and *Ocimum basilicum*. *O. basilicum* had the largest inhibition zone by far, and in some cases the fungal growth was very miniscule and sparsely located throughout the rim of the petri dish. These results correlate well with the observations taken during the application phase, where *O. basilicum* was found to have corrosive properties. Thus meaning that this was a harsh essential oil that could easily corrode plastic, and thus should be able to inhibit fungal growth. Although *O. basilicum* had the largest zone of inhibition, it did not have the clearest. The clearest inhibition zone belonged to the *Eucalyptus globulus* SDA plates, that were the 2nd largest zones and they showed absolutely no fungal growth or colonies inside their inhibition zone. Results indicate that the proposed hypothesis is accepted, and homeopathic drugs and tinctures are efficient in inhibiting fungal growth.
**Fungicidal:** This experiment tested the efficacy of each agent as a treatment for an existing and prevalent fungal infection. The results of this trial showed that none of the agents held an ability to efficiently treat or eradicate fungal growth and colonies. Initially the pipetting method was used but when that reared no viable results, diffusion disc testing was used. It was interesting to see the change in results with the change of methodology, when the pipetting method was used, the essential oils bore no results and the homeopathic drugs and allopathic medicine managed to create an indent in fungal colonies. This can be attributed to the higher quantity of solution that was distributed through the diffusion disc. When discs are used the concentrated solution is kept to one specific location and thus can be more effective in treating it.

**Conclusion:** The aforementioned experiments were conducted in order to provide a detailed observation in regards to the efficacy of homeopathic treatments and their inhibitory and fungicidal properties of *Candidal* infections. The inhibitory experiment showed that homeopathic substances, specifically plant derived oils were most effective in preventing fungal growth. Clinically, this is relevant because it shows that it can successfully prevent the growth of *Candida albicans* and therefore minimize the risk for infections that can result in a plethora of complications such as invasive candidiasis (Bongomin, et al. 2017). This is a cautionary treatment that can be conducted in the initial stages of fungal growth, and can prevent the chance of opportunistic fungal infections especially in immunocompromised patients (Matejuk, et al. 2010). The fungicidal experiment was conducted in order to find a homeopathic treatment as a substitute to drug resistant cases of *C. albicans* with recurrent exposure to Azole treatments. This hypothesis was disproved by multiple methods of application, where not even homeopathic drugs caused an effective treatment. The allopathic agent used also did not rear successful results, which calls for a re-evaluation and adjustment of protocol. The findings were consistent
with previous research done on homeopathic drugs Kali Iodatum showed more growth inhibition than Benzoicum Acidum (Gupta, et al. 2015). However, that study did not include mother tinctures, therefore the results of this study vary so as to include those agents. *Eucalyptus globulus* was found to have a larger zone of inhibition than that of *Ocimum basilicum* in past research, but that is not consistent with the data compiled in this experiment (Prajapti, et al. 2017).

Future research would include assessing the components of *E. globulus* and *O. basilicum* and attempt combination treatment for the most effective results. *Eucalyptus globulus* was found to have a clearer zone of inhibition, but *Ocimum basilicum* had the largest measurement therefore a combination may result in a more efficient treatment. In further stages of research more testing using raw plant mother tinctures of *E. globulus* and *O. basilicum* will be used to test which of these two oils would be the best preventative measure. Furthermore, a revision of the fungicidal treatment protocol is needed to provide more results and data. It may be possible that one dose of the medication is not sufficient to combat an already cultured and developed fungal colony. In a continuation of this study, repeated applications will be considered to test the same hypothesis again.
Appendices

Figure 3 - Ketoconazole - Growth Inhibition
SDA plate with cultured *Candida albicans* with diffusion disc soaked in ketoconazole.

Figure 4 - Kali Iodatum - Growth Inhibition
SDA plate with cultured *Candida albicans* with diffusion disc soaked in Kali Iodatum
Figure 5 - Benzoicum Acidum - Growth Inhibition
SDA plate with cultured *Candida albicans* with diffusion disc soaked in Benzoicum Acidum

Figure 6 - *Eucalyptus globulus* - Growth Inhibition
SDA plate with cultured *Candida albicans* with diffusion disc soaked in *Eucalyptus globulus*
Figure 7 - *Ocimum basilicum* - Growth Inhibition
SDA plate with cultured *Candida albicans* with diffusion disc soaked in *Ocimum basilicum*

Figure 8 - *Ocimum basilicum* - Fungicidal
Table showing the lack of fungicidal treatment zone after multiple days post treatment, under pipetting method. Cloudiness is attributed to the *O. basilicum* corroding plastic of SDA plate.
Figure 9 - Benzoicum Acidum - *Fungicidal*

Vernier caliper used to mark indentations on fungal colonies after B. Acidum application.
Bibliography


