Effectiveness Between Melaleuca Alternifolia Oil and Cocos Nucifera Oil Against Fungal Infections

Rachel Li
Parkland High School
Research Question

Which combination between components of Melaleuca Alternifolia Oil and Cocos Nucifera Oil is the most effective against Fungal Infections? Is there synergism between the most effective combination?
Background Information

- Fungi have cell membranes made of ergosterol
- Fungi - made up of eukaryotic cells
  - Yeast - single cells that reproduce by budding
    - Produces CO₂ as it reproduces
  - Fermentation - metabolic process that converts sugar to acids, gases, or alcohol
    - Yeast consumes sugar for energy
    - \( \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2 \text{C}_2\text{H}_5\text{OH} + 2 \text{CO} \)
Types of Results from a Combination of Drugs/Treatment

- Additive - Action of Drug A is added to action of Drug B with no increase in the sum effect
- Synergistic - Action of both drugs is greater than the sum of each individually
- Antagonism - Action of both drugs is less than expected

Isobologram Analysis

- A method to analyze drug combinations
- Antagonism - higher than the expected doses of both drugs that are needed to produce the target effect
Components of Melaleuca Alternifolia Oil (Tea Tree Oil)

- Terpinen-4-ol
  - Main antifungal component
  - Fungicidal at low concentrations
  - Can cross the cell wall and be inserted in the lipid bilayer which cause changes in its permeability
  - Inhibit the synthesis of ergosterol

- Alpha-terpineol
  - Affect cell wall synthesis and lead to disruption of the cell wall
  - Leakage of intracellular components
  - Fungicidal
Components of Cocos Nucifera Oil (Coconut Oil)

- Lauric Acid
  - Elevate the fluidity of the cell membrane and interfere with membrane-bound enzymes
  - Prevent from adhering to host cells
- Caprylic Acid
  - Inhibits respiratory metabolism and dehydrogenase activity
  - Interferes cell membrane through the essential enzymes to metabolism and growth

[Chemical structures of Caprylic acid, Capric acid, and Lauric acid]
Hypothesis

- If components combinations of Melaleuca Alternifolia Oil (terpinen-4-ol and alpha-terpineol) and Cocos Nucifera Oil (lauric acid and caprylic acid) at low and high concentrations are used against yeast, a fungus that produces CO₂ as it grows, terpinen-4-ol and caprylic acid would be the most effective drug combination and synergism to decrease the amount of living yeast and its production of CO₂.

- According to the multiple published research, terpinen-4-ol and caprylic acid are the most potent fungal component of each respective naturopathic medication.
Variables

- Independent Variables - combinations of components at 25%, 50%, 100%
  - Terpinen-4-ol + Lauric Acid
  - Terpinen-4-ol + Caprylic Acid
  - Lauric acid + Alpha-terpineol
  - Caprylic Acid + Alpha-terpineol
  - Terpinen-4-ol + Alpha-terpineol
  - Lauric Acid + Caprylic Acid

- Dependent Variable - the amount of CO₂ produced

- Control Group: Yeast tested without any components

- Controls: yeast came from the same container, consistent amount of water, scale, temperature, and strokes
Materials

- 1000 µl micropipette + tips
- 4 grams of yeast, 2 grams of sugar
- Erlenmeyer flask with one-hole stopper
- Clamp and ring stand
- 1000 ml beaker
- 100 ml beaker (+ multiple 50 ml beakers)
- 2 ml of Terpinen-4-ol, Caprylic Acid, Lauric Acid, Alpha-terpineol
- Graduated cylinder
- Thermometer
- Electric scale
Procedure - Creating a Gas Apparatus Collection

1. Fill the 1000 ml beaker with 800 ml distilled water.
2. Fill the 200 ml graduated cylinder with distilled water. Use a plastic cover to cover the opening of the cylinder.
3. Flip the 200 ml graduated cylinder upside down and lower it down to the water in the 1000 ml beaker.
4. Remove the plastic cover once the opening of the cylinder is completely submerged underwater.
5. Lift the graduated cylinder 6 cm from the bottom of the 1000 ml beaker.
6. Use the clamp to secure the graduated cylinder to the ring stand.
7. Attach the delivery tube to a flask with a one-hole stopper.
8. Put the other end of the delivery tube under the opening of the graduated cylinder.
Gas Collection Apparatus
Procedure - The Experiment

1. Put on gloves. Heat water to 40°C. Dissolve 2 grams of sugar in 60 ml of 40°C water in a beaker. Stir until dissolved (sugar solution).
2. Then add and mix 4 grams of yeast in the sugar solution when the water is 37°C. (This is the control) Pour in the flask.
3. Cap the flask and collect the CO₂ for 45 minutes.
4. Take the stopper off the tube to stop CO₂ collection.
5. Record how much CO₂ was collected by reading the graduated cylinder.
6. Pour the yeast solution in a quart and dispose it properly.
7. Wash the flask, delivery tube, and graduated cylinder.
8. Prepare the gas apparatus again.
9. Repeat steps 1-8, now testing the combinations: Terpinen-4-ol + Lauric Acid, Terpinen-4-ol + Caprylic Acid, Lauric acid + Alpha-terpineol, Caprylic Acid + Alpha-terpineol, Terpinen-4-ol + Alpha-terpineol, Lauric Acid + Caprylic Acid.

10. Each naturopathic medication will be tested on undiluted, 50% and 25% concentrations.
   a. Label the flask appropriately to the combination and its concentration.
   b. Add the combination to the sugar solution before adding the yeast.
   c. Terpinen-4-ol and Lauric acid (use the micropipette)
      i. Use 500 μl of terpinen-4-ol and 500 μl ml of lauric acid. (undiluted or 100%)
      ii. Mix 500 μl of terpinen-4-ol with 500 μl of lauric acid and 1000 μl of canola oil.
          1. Measure out 1000 μl mixture to use. (50%)
      iii. Take 1000 μl of 50% concentration (made in cii) and mix in 1000 μl of canola oil. Measure out 1000 μl of the terpinen-4-ol + lauric acid. Mix. (25%)
      iv. These same steps can be repeated for the other component combinations with its concentrations.

11. Do 3 more trials for each combination (100%, 50% and 25%) and the control.
12. After analyzing the data, find the most effective component combination. Find the MIC for its individual components and its combination.

13. Use a 25% concentration as the sample for the serial dilution.

14. Prepare 5 tubes with 9 ml of canola oil. Draw 1000 μl with the micropipette of the sample into the first tube. Then, draw 1000 μl from the first tube to put in the second tube. Continue on for the 5th tube. (dilutions of 1:10, 1:100, 1:1000, 1:10000, 1:100000)

15. For each dilution, draw 1000 μl to use and perform steps 1-8 again.

16. Repeat 13-15 for the rest of the component/combination. Do two more trials. Determine the MIC.
<table>
<thead>
<tr>
<th></th>
<th>Trial 1 (in ml)</th>
<th>Trial 2 (in ml)</th>
<th>Trial 3 (in ml)</th>
<th>Trial 4 (in ml)</th>
<th>Averages (in ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100%</td>
<td>50%</td>
<td>25%</td>
<td>100%</td>
<td>50%</td>
</tr>
<tr>
<td>Control</td>
<td>196</td>
<td>200</td>
<td></td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>Alpha-terpineol and Caprylic Acid</td>
<td>136</td>
<td>124</td>
<td>120</td>
<td>130</td>
<td>122</td>
</tr>
<tr>
<td>Lauric Acid and Alpha-terpineol</td>
<td>120</td>
<td>116</td>
<td>128</td>
<td>117</td>
<td>115</td>
</tr>
<tr>
<td>Lauric Acid and Caprylic Acid</td>
<td>128</td>
<td>110</td>
<td>97</td>
<td>125</td>
<td>112</td>
</tr>
<tr>
<td>Terpineol-4-ol and Lauric acid</td>
<td>172</td>
<td>108</td>
<td>212</td>
<td>170</td>
<td>105</td>
</tr>
<tr>
<td>Terpineol-4-ol and Alpha-terpineol</td>
<td>132</td>
<td>115</td>
<td>98</td>
<td>130</td>
<td>120</td>
</tr>
<tr>
<td>Terpinen-4-ol and Caprylic Acid</td>
<td>120</td>
<td>95</td>
<td>56</td>
<td>117</td>
<td>92</td>
</tr>
</tbody>
</table>
Graph

Legend
100% - blue
50% - red
25% - yellow
Control (yeast only) - green
<table>
<thead>
<tr>
<th></th>
<th>1:10</th>
<th>1:100</th>
<th>1:1000</th>
<th>1:10000</th>
<th>1:100000</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terpinen-4-ol</td>
<td>1.62*10^-1 mol/L</td>
<td>1.62*10^-2 mol/L</td>
<td>1.62*10^-3 mol/L</td>
<td>1.62*10^-4 mol/L</td>
<td>1.62*10^-5 mol/L</td>
</tr>
<tr>
<td>CO2 produced</td>
<td>187</td>
<td>197</td>
<td>199</td>
<td>198</td>
<td>199</td>
</tr>
<tr>
<td></td>
<td>190</td>
<td>197</td>
<td>198</td>
<td>200</td>
<td>201</td>
</tr>
<tr>
<td></td>
<td>192</td>
<td>195</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>189.66666667</td>
<td><strong>196.33333333</strong></td>
<td>199</td>
<td><strong>199.33333333</strong></td>
<td>200</td>
</tr>
<tr>
<td>Caprylic Acid</td>
<td>1:10</td>
<td>1:100</td>
<td>1:1000</td>
<td>1:10000</td>
<td>1:100000</td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.73*10^-1 mol/L</td>
<td>1.73*10^-2mol/L</td>
<td>1.73*10^-3 mol/L</td>
<td>1.73*10^-4 mol/L</td>
<td>1.73*10^-5 mol/L</td>
</tr>
<tr>
<td>CO2 produced</td>
<td>193</td>
<td>195</td>
<td>198</td>
<td>199</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td>194</td>
<td>196</td>
<td>199</td>
<td>200</td>
<td>199</td>
</tr>
<tr>
<td></td>
<td>196</td>
<td>194</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>194.33333333</td>
<td>195</td>
<td><strong>199</strong></td>
<td><strong>199.66666667</strong></td>
<td>199</td>
</tr>
<tr>
<td>Terpinen-4-ol and Caprylic Acid</td>
<td>1:10</td>
<td>1:100</td>
<td>1:1000</td>
<td>1:10000</td>
<td>1:100000</td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.67*10^-1 mol/L</td>
<td>1.67*10^-2 mol/L</td>
<td>1.67*10^-3 mol/L</td>
<td>1.67*10^-4 mol/L</td>
<td>1.67*10^-5 mol/L</td>
</tr>
<tr>
<td>CO2 produced</td>
<td>175</td>
<td>180</td>
<td>191</td>
<td>198</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>174</td>
<td>183</td>
<td>192</td>
<td>199</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td>176</td>
<td>184</td>
<td>193</td>
<td>201</td>
<td>199</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>175</td>
<td>183.33333333</td>
<td><strong>189</strong></td>
<td><strong>189.33333333</strong></td>
<td>189</td>
</tr>
</tbody>
</table>
Isobologram

Legend
- MIC of respective component
- Calculated MIC add
- MIC mix (synergism line)
Calculation of the FIC (Fractional Inhibitory Concentration)

\[
\Sigma FIC = \frac{\text{MIC of } A \text{ in Combination}}{\text{MIC of } A \text{ Alone}} + \frac{\text{MIC of } B \text{ in Combination}}{\text{MIC of } B \text{ Alone}}
\]

\[
\Sigma FIC = 0.0997
\]

- \(\Sigma FIC \leq 0.5 = \text{synergistic}\)
- \(0.5 < \Sigma FIC < 1 = \text{additive}\)
- \(1 < \Sigma FIC \geq 2 = \text{unrelated}\)
- \(\Sigma FIC > 2 = \text{antagonistic}\)

A = Terpinen-4-ol
B = Carpylic Acid

The \(\Sigma FIC\) shows a **synergistic effect** between Terpinen-4-ol and Caprylic Acid.
Conclusion

- Data does support the hypothesis
- Control: 198 ml CO₂
- Most effective: Caprylic Acid and Terpinen-4-ol at 25% = yeast CO₂ production: 80.5 ml
  - Caprylic Acid Interferes with respiratory metabolism
    - Acts on the surface component of yeast cells that target enzymes for metabolism and growth and eventually kills it
    - Intracellular components released outside (leaky)
  - Terpenes can insert itself in the lipid bilayer membrane to change the cell’s fluidly and permeability
    - Lead to disruption of membrane, reduction in adherence to host surfaces, cell lysis
    - Affect ergosterol and lipid biosynthesis
- Combination creates a synergistic effect because of the $\Sigma_{FIC} = 0.997$
  - Have similar modes of mechanism → target the cell membrane
  - Caprylic Acid aids in stopping reproduction, Terpinen-4-ol prevents spreading, and in the process causes mayhem in the cells
Least effective: Terpinen-4-ol and Lauric Acid at 25% which led to the most CO2 production: 213 ml

- Suspected antagonistic effect
- Theoretical: pharmacokinetic antagonism (affects the absorption of other) or competitive antagonism (receptors)
**Conclusion Cont.**

Further Experimentation

- Test synergism between essential oils
- Design a experiment to find more information about the mechanism.

**Improvements**

- More trials
- Use a more scientifically accurate method to determine the MIC
- Test the other component combinations for synergism

**Society**

- Raises awareness of fungal infections
- Fungal Resistance to OTC medications
- Aids in scientists in developing a new drug
Bibliography


