

# UMBI\*SERVE

## Rural Solutions

WHO WE ARE



**Our Mission**—To research and develop preventative medicines derived from natural products

Young Scientist,

Diarrhea is the second highest cause of children's death worldwide. Poor sanitation increases children's interaction with the bacteria causing the diarrhea. About 1.5 billion children under the age of five die each year from dehydration, resulting from diarrhea. Local scientists have noticed, some people never seem to get diarrhea. They studied the diet and food preparation techniques of families with healthy children. UMBI is now aware that foods containing natural products can prevent bacterial infection and diarrhea.

As you can see, I am excited to hear you are interested in working in our research facility. We are investigating the antimicrobial properties of local foods in this area:

**Carrots**

**Tomatoes**

**Rosemary**

**Sage**

**Thyme**

**Lettuce**

Your task is to check the antimicrobial ability within these foods. You will be using the bioluminescent bacteria *Vibrio phosphoreum*. If the substance examined is an antimicrobial, the bacteria will no longer glow, indicating the bacteria are dead, and there is an antimicrobial affect. If the bacteria glow, they are living, and there is no antimicrobial effect. Your deadline is approaching. I am looking forward to the results of your experiment.



Sincerely,  
Dr. Stapleton

# Natural Products Lab

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Lab Investigator: \_\_\_\_\_



1. View the conical containing only bacteria in a darkened room. Glowing bacteria are alive.
2. What is a control?

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3. Identify an item at your workstation that can be used as a control in this experiment. \_\_\_\_\_

4. Identify two variables used in this experiment.

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5. What is the difference between a positive and negative control?

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6. Explain why it is beneficial to have a positive and negative control in an experiment?

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# Natural Products Lab

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7. Label 3 beakers with the plant types to be tested.
  - a. Positive control: \_\_\_\_\_
  - b. Experimental Sample #1: \_\_\_\_\_
  - c. Experimental Sample #2: \_\_\_\_\_



8. Label 4 test tubes with the plant types to be tested.
  - a. +
  - b. --
  - c. Sample #1
  - d. Sample #2

9. Using your micropipette, add 2000  $\mu\text{L}$  *V. phosphoreum* into the test tubes labeled.

- a. +
- b. --
- c. Sample #1
- d. Sample #2



10. Place cheese cloth over the beaker labeled Positive Control.
11. Strain the juice from the lemon into the beaker labeled Positive Control.
12. The cheese cloth will contain the plants and the beaker should only contain liquid.

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13. Strain the lemon juice by squeezing the cheese cloth.
14. Place the cheese cloth in the waste container.
15. Place 1.0 grams of your **Sample #1**, already measured at your station, into a mortar and pestle.



16. Take the Pipette Aide in one hand and hold the pipette tip with the other.
17. Insert the pipette tip into the Pipette Aide.
18. Use the Pipette Aide to add 10 mL of water to the mortar.
19. Use your pestle to grind the mixture into a solution.
20. Grind up the plant with the pestle until the liquid takes on the color of the water. There should be at least one minute of grinding, but not more than three minutes.

21. Place cheese cloth over the beaker labeled Sample #1.
22. Place the solution into the beaker labeled Sample #1.
23. Strain Sample #1's juice by squeezing the cheese cloth.
24. Place the cheese cloth in the waste container.

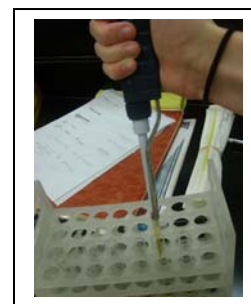


25. Place 1.0 grams of your **Sample #2**, already measured at your station, into a mortar and pestle.

# Natural Products Lab

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26. Use a Pipette Aide to add 10 mL of water to the mortar.
27. Use your pestle to grind the mixture into a solution.
28. Grind up the plant with the pestle until the liquid takes on the color of the water. There should be at least one minute of grinding, but not more than three minutes.
29. Place cheese cloth over the beaker labeled Sample #2.
30. Place the solution into the beaker labeled Sample #2.
31. Strain Sample #2's juice by squeezing the cheese cloth.
32. Place the cheese cloth in the waste container.
33. Use the Micropipette to measure 100  $\mu$ L of the strained lemon juice.
34. Place 100  $\mu$ L of the strained lemon juice into the test tube labeled +
35. Remember to change the tip on the Micropipette.
36. Use the Micropipette to measure 100  $\mu$ L of water.
37. Place this water into the test tube labeled --
38. Using your Micropipette, take 100  $\mu$ L of the Sample #1 solution and place it into the test tube labeled **Sample #1**.
39. Using your Micropipette, take 100  $\mu$ L of the Sample #2 solution and place it into the test tube labeled **Sample #2**.
40. Wait 5 minutes and observe results in a darkened room.
41. View your 4 samples and record your results on the **Observations Sheet**.
42. When all groups have viewed their samples place your data on board in the Class Data Chart.
43. Record the entire class's data on your **Observations Sheet**.



# Natural Products Lab

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## Student Observations

Did bacteria glow before the plant was added? \_\_\_\_\_

<b>Specimen</b>	<b>Results Circle the best answer</b>	<b>Conclusion</b>
<b>Positive Control</b> _____	<b>Glowed Did not Glow</b>	
<b>Negative Control</b> _____	<b>Glowed Did not Glow</b>	
<b>Carrots</b>	<b>Glowed Did not Glow</b>	
<b>Lettuce</b>	<b>Glowed Did not Glow</b>	
<b>Rosemary</b>	<b>Glowed Did not Glow</b>	
<b>Sage</b>	<b>Glowed or Did not Glow</b>	
<b>Tomatoes</b>	<b>Glowed Did not Glow</b>	
<b>Thyme</b>	<b>Glowed Did not Glow</b>	

## **Rosemary** (*Rosmarinus officinalis*)



Rosemary is a woody herb from the mint family. It has evergreen needle-like leaves. The plant contains borneol, tannic acid, bornyl acetate camphor and esters. It can be used as a tonic, an astringent, and a stimulant and has antibacterial, antifungal and antioxidant properties. Rosemary has been used historically to improve memory and treat gout. It is currently be research as a treatment for cancer.

## **Sage** (*Salvia officinalis*)



Sage is a small woody shrub with purple flowers, and is a member of the mint family. It is from the Mediterranean region and gets its name from the Latin root "salvere" which means "to heal". Sage contains camphor, borneol, thujone, cineole, rosmarinic acid and tannins. It has known antibacterial, antifungal and antiviral properties and has been historically used to treat a variety of ailments, from indigestion to insect bites.

## Thyme (*Thymus vulgaris*)



Thyme is a sub-shrub native to the European and Asian regions. It is a member of the mint family and is the most commonly used culinary herb, providing an excellent source of iron. It is composed of a variety of phenols. The major component of the essential oil of Thyme is thymol, the main ingredient in oral antiseptics. Historically, it was used to medicate bandages before common antibiotics

# Natural Products Lab

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## Student Extension Lab Observations

Now we can identify the concentration of the natural product with the best antimicrobial activity. We can identify the minimal concentration where the natural product solution will kill bacteria. This is called MIC. Continue working in your original lab groups for this activity.

1. Identify which natural product will be used in your serial dilutions to test MIC.
  - a. Rosemary
  - b. Thyme
  - c. Sage

**I will test the Minimum Inhibitory Concentration of the Antimicrobial** \_\_\_\_\_

2. Create serial dilutions of your **sample (rosemary, thyme, or sage)** into 7 new test tubes.
  - Label test tube #1 100% Sample
  - Label test tube #2 10% Sample
  - Label test tube #3 1% Sample
  - Label test tube #4 0.1% Sample
  - Label test tube #5 0.01% Sample
  - Label test tube #6 0.001% Sample
  - Label test tube #7 0% Sample (contains water instead of your sample)
3. In test tube #1, place 1100  $\mu\text{L}$  of the **sample: rosemary, thyme, or sage**.
4. Into test tubes #1  $\rightarrow$  #7, place 900  $\mu\text{L}$  of *V. phosphoreum* media.
5. Write a hypothesis determining what can be expected to occur on your Observation Sheet.

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6. Identify the test tube that will be used as a positive control: \_\_\_\_\_

7. Identify which test tube should be used as a negative control: \_\_\_\_\_

**8. REMEMBER: In test tube #1 you placed 1100  $\mu\text{L}$  of the sample: rosemary, thyme, or sage**

9. Use the micropipette to mix the solution. Take fluid in and out of the pipette.

10. Then take 100  $\mu\text{L}$  of the solution in test tube #1 and place it in test tube #2.

# Natural Products Lab

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11. Use the micropipette to mix the solution. Take fluid in and out of the pipette.
12. Then take 100  $\mu\text{L}$  of the solution in test tube #2 and place it in test tube #3.
13. Use the micropipette to mix the solution. Take fluid in and out of the pipette.
14. Then take 100  $\mu\text{L}$  of the solution in test tube #3 and place it in test tube #4.
15. Use the micropipette to mix the solution. Take fluid in and out of the pipette.
16. Then take 100  $\mu\text{L}$  of the solution in test tube #4 and place it in test tube #5.
17. Use the micropipette to mix the solution. Take fluid in and out of the pipette.
18. Then take 100  $\mu\text{L}$  of the solution in test tube #5 and place it in test tube #6.
19. Use the micropipette to mix the solution. Take fluid in and out of the pipette.
20. Change your pipette tip.
21. Take 100  $\mu\text{L}$  of water and place it in test tube #7
22. Wait 10 minutes.
23. Place all test tubes in a darkened room.
24. Record on your Observation Sheet, the antimicrobial activity at each concentrations using this scale: **5 very high activity  $\rightarrow$  0 no activity at all.**  
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**No glow at all  $\rightarrow$  Very bright glow**
25. Write a conclusion explaining the results of this investigation and why these observations may have occurred.

# Natural Products Lab

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## Student Observations for Extension Activity

Test tube \_\_\_\_\_ is the positive control because \_\_\_\_\_

Test tube \_\_\_\_\_ is the negative control because \_\_\_\_\_

Hypothesis:

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Test Tube # and Contents	Serial Concentration	Observations	
		scale 5 high activity NO GLOW	scale 0 low/no activity no BRIGHT GLOW
Test tube #1	100%		
Test tube #2	10%		
Test tube #3	1%		
Test tube #4	0.1%		
Test tube #5	0.01%		
Test tube #6	0.001%		
Test tube #7	0%		

Conclusion:

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