

ESSENTIAL OILS: DISTILLATION & EXAMINATION OF ANTIMICROBIAL ACTION

Text Reference: CH. 7

NAME _____

INTRODUCTION

Essential oils are volatile oils that are extracted from plants, and are classified as secondary metabolites; that means that they are organic compounds not directly involved in the normal plant growth or development. These oils often possess the same flavor or aroma as the plant. They have long been used for a variety of purposes: the Romans crushed rose petals and placed the fragments into wine, both as a flavoring agent, and as an air freshener; many indigenous peoples of South America still use crushed plants in a variety of medical uses; in India and Sri Lanka, these plant oils became the primary ingredients for many food dishes. These oils were, and in many cases, still are the primary ingredients in many perfumes: ever heard of rose water? Most consumers are familiar with certain essential oils, although they probably do not realize it: lemon grass oil (citronella, Fig. 1), orange oil (*d*-limonene, Fig. 2), oil of wintergreen (methyl salicylate, Fig. 3), peppermint oil (menthol, Fig. 4), tea tree oil (*Melaleuca*), and almond (benzaldehyde).

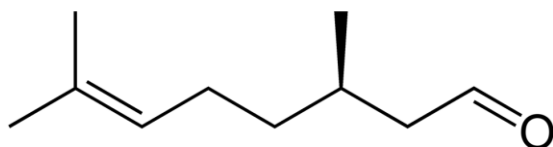


Fig. 1

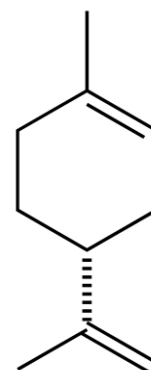


Fig. 2

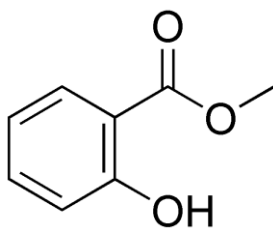


Fig. 3

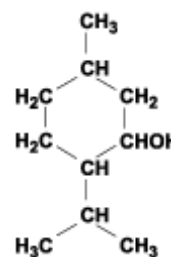
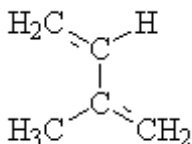


Fig. 4

Most of the oils contain isoprenoids, a chemical with the general formula, C₅H₈:



So, these oils often smell nice, and they can certainly add flavor to a variety of dishes, but what other purposes do they serve? What else have they done for past civilizations, and what are they capable of doing in the future?

PURPOSE

The purpose of this experiment is to distill and isolate* an essential oil, and then test it for possible antimicrobial activity.

* *Pure samples of oils, if available, may be used if distillation is not an option because of time or equipment.*

REFERENCES

1. Duke, J. A. *CRC Handbook of Medicinal Herbs*; CRC Press: Boca Raton, FL, 1985.
2. Cannon, J., et al. "Investigation of Secondary Metabolites in Plants." *J. Chem. Ed.* 2001, 78(9): 1234- 1237.
3. Duke, J. A. *Handbook of Biologically Active Phytochemicals and Their Activities*; CRC Press: Boca Raton, FL, 1992.
4. Mayo, Dana W., Ronald M. Pike and Peter K. Trumper. *Microscale Organic Laboratory: with Multistep and Multiscale Syntheses*, 3rd Ed. Wiley: New York, NY, 1994.

MAIN REACTION(S) & EQUATIONS

N/A.

SAFETY CONCERNS

- See the MSDS materials for detailed information on each chemical used in this experiment.

TABLE OF REACTANTS

Compound	Formula	Molar Mass (g/ mol)	Molar Concentration (mol/ L)	Density (g/ mL)	m.p./ b.p. (°C)	Moles Used/ Made	Mass/ Volume Used	Mass/ Volume Made
Water, distilled	H ₂ O	18.01	-----	≈ 1	100			
Dichloromethane	CH ₂ Cl ₂	84.932	-----	1.3266	-97.2/ 40			
Methanol	CH ₃ OH	32.042	-----	0.7914	-97.3/ 64.6			
Hexane	C ₆ H ₁₄	86.175	-----	0.6606	-95.34/ 68.73			
Silica gel	SiO ₂	60.085	-----	2.196	1713/ 2950			

MATERIALS

Goggles & apron
Whatman® filter paper
96- well spot plate
Staph. aureus culture
Methanol
Silica gel (drying agent)
125- mL side arm flask
Nutrient agar
Cuvettes

Electric blender or coffee bean grinder
Büchner funnel
Incubator
Pseudomonas aeruginosa culture
Hexane
Gas chromatograph/ Mass spectrometer (GC-MS)
250 mL separatory funnel
Sabouraud agar

Stir plate + stir bar
100- mL grad. cylinder
Dichloromethane
Candida albicans culture
Plant samples or extracts
Micropipette + tips
Spectrophotometer

PRE- LAB QUESTIONS

None.

PROCEDURES

Part A: Distillation (May be omitted if extracts are used)

1. Measure out a 5- g sample of the ground plant material, and place this into a 125 mL side arm flask. Cover the sample with approx. 40 mL of the dichloromethane, and then add a Teflon[®]-coated stir bar to the flask. Stopper the flask.
2. In a fume hood, attach one end a vacuum hose to the flask and the other end of the hose to a vacuum line. If a vacuum line is not available, use a water aspiration set- up.
3. Set the flask on a stir plate, begin stirring the mixture on a medium setting while applying the vacuum to the flask's contents. Continue the mixing for twenty (20) minutes.
4. Filter the flask's contents, keeping the solvent in a separate flask. Place the solid filtrate back into the side- arm flask, add another aliquot of the dichloromethane, and repeat *Steps 2 & 3*, then filter the solid, saving the solvent in the other flask.
5. Add 2- 3 Scoopula[®]- full portions of the silica gel to the flask containing the aliquot portions. Swirl the flask and its contents for 1- 2 minutes, then filter the contents, saving the filtrate. Repeat this procedure twice. (This removes any water that was present in the plant material)
6. Repeat *Steps 2- 5* using methanol instead of the dichloromethane. Add the filtrates collected to the filtrates from *Step 5*.
7. Place the combined filtrate collections into the separatory funnel and then add an equal volume of hexane. Wash the filtrates three times using the hexane. Make sure to save the aqueous portions since these contain the majority of the extracts.

Part B: Anti- Microbial Studies

1. Using a spectrophotometer (Spec- 20[®]), determine the optical density of each culture.
2. Inoculate the wells in the spot plate with the appropriate growth media.
3. Designate row #1 as the **Gram (+) Control**; label row #2 as the **Gram (+) Treated**; label row #3 as the **Gram (-) Control**; label row #4 as the **Gram (-) Treated**; label row #5 as the **Fungal Control**; label row #6 as the **Fungal Treated**.
4. Inoculate rows 1 & 2 with the *Staph. aureus* culture; inoculate rows 3 & 4 with the *Pseudomonas aeruginosa* culture; inoculate rows 5& 6 with the *Candida albicans* culture.
5. Using the micropipette, inoculate the **Treated** rows with 5 μ L of the plant extract, then incubate the plate for 24 hours.
6. After the 24 hour incubation period, determine the optical density of the wells in each row.

OBSERVATIONS

Data Table

	Initial Optical Density	Optical Density After Incubation	Bacterial Population
<i>Staph. aureus</i>			
<i>Pseudomonas aeruginosa</i>			
<i>Candida albicans</i>			

ANALYSIS & CONCLUSIONS

1. Using the initial optical density data, calculate the microbial concentration of each species used.

2. Calculate the microbial concentrations after the 24 hour incubation period.

3. What effect, if any, did the plant extract have on the three tested microbes? **Explain** your answer.

4. What is the active component(s) in your particular plant extract? Do you think this would be an effective treatment in humans? **Explain.**

Instructor's Notes

1. Ideally, the plants used should be obtained the summer before the school year begins; this will allow you time to dry them in the shade or indoors (sunlight can cause photodegradation). For a class of approximately 20 students, a total of 1- 2 kg of dried plant material will be needed.
2. Once dried, the plants can be placed into a sealed plastic bag, and then stored in a freezer until needed.
3. The plants will need to be ground before undergoing extraction; this can be done using either a blender or, more preferably, a coffee bean mill.
4. Some plant samples that can be used include basil, dill, fennel, ginger, lemon grass, orange, and lemon- all of which are available in the produce section of the grocery store. Dried, ground spices do not work well since they usually have undergone chemical processing.
5. More than one plant extract can be used for comparative studies.
6. <http://www.essentialoils.co.za/essential-oils/index.htm>
7. In *Step 7*, a silica gel column can be used in place of adding the gel to the flask.
8. In **Part B**, *Step 1* the well plates can be poured prior to their use.