Isolation of Citral in Lemongrass: Steam Distillation, Spectroscopy, and GC-MS

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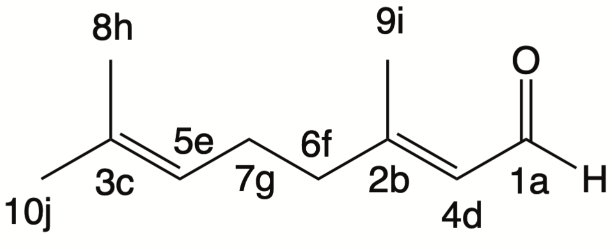
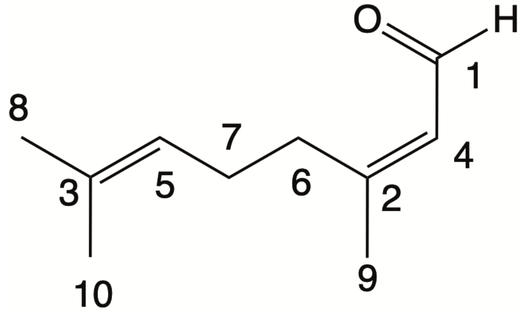
Introduction

- Citral is an acyclic monoterpene aldehyde
- Sweet lemon-like scent
  - Also found in fruits such as lemons & oranges, and in plants such as melissa and verbena.
- In lemongrass, citral is found as a mixture of two isomers Neral (Z-isomer) and Geranial (E-isomer).

(Dudai et al., 2005)



Geranial



Neral

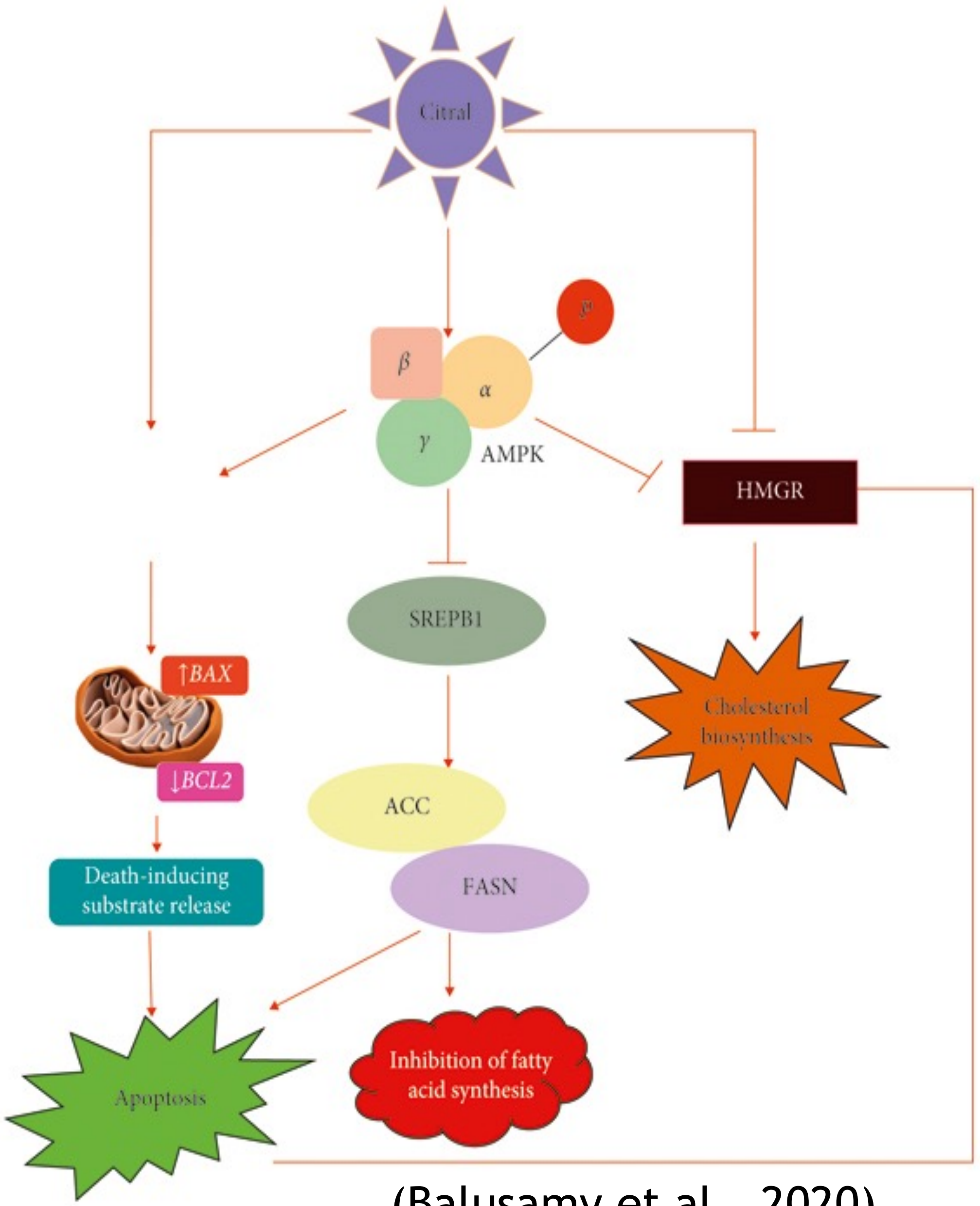
Health

- **Uses:**
  - Cosmetics
  - Cleaning Agent
- **Health Benefits:**
  - Antioxidant
  - Antimicrobial
  - Antifungal
  - Anti-inflammatory
  - Anticancer: triggers apoptosis in various types of cancer cells by binding to enzymes that participate in forwarding tumor growth.

(Bouzenna et al., 2017)

Citral's Anticancer Property

- Citral's aldehyde functional group:
  - Binds to MARK4, an enzyme that is important to the G0/G1 phase of the cell cycle and stop tumor growth in glioma cells (Rovina et al., 2014).
- Citral can alter lipogenesis pathway which is involved prostate cancer cell growth to trigger apoptosis (Balusamy et al., 2020).
- Citral is also non-cytotoxic, which means that it does not hurt healthy cells when treating things like cancer (Naz et al., 2018).
- **Importance:** natural source for lowering cancer growth in combination with chemotherapy treatments. (Maruoka et al., 2018)



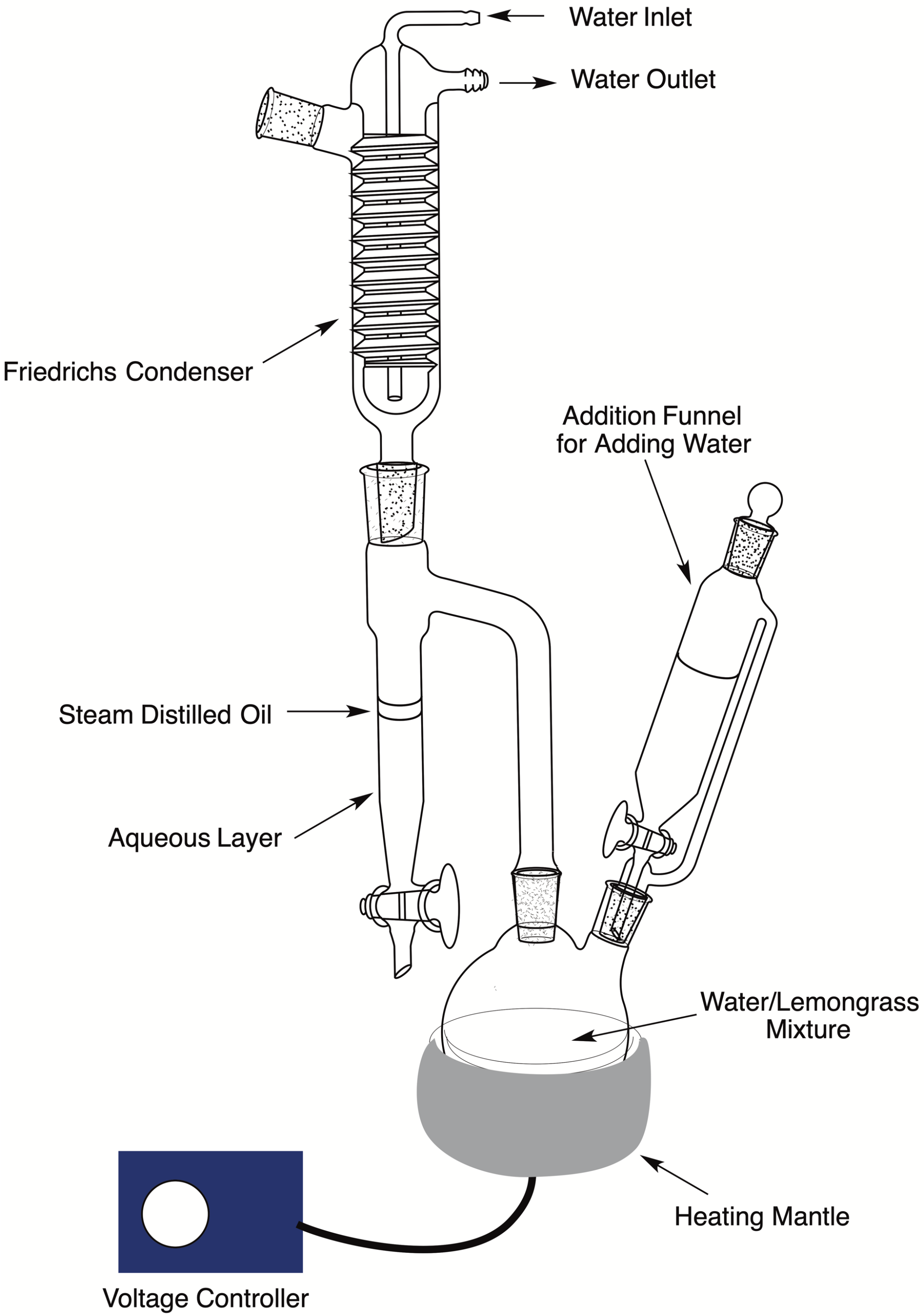
(Balusamy et al., 2020).

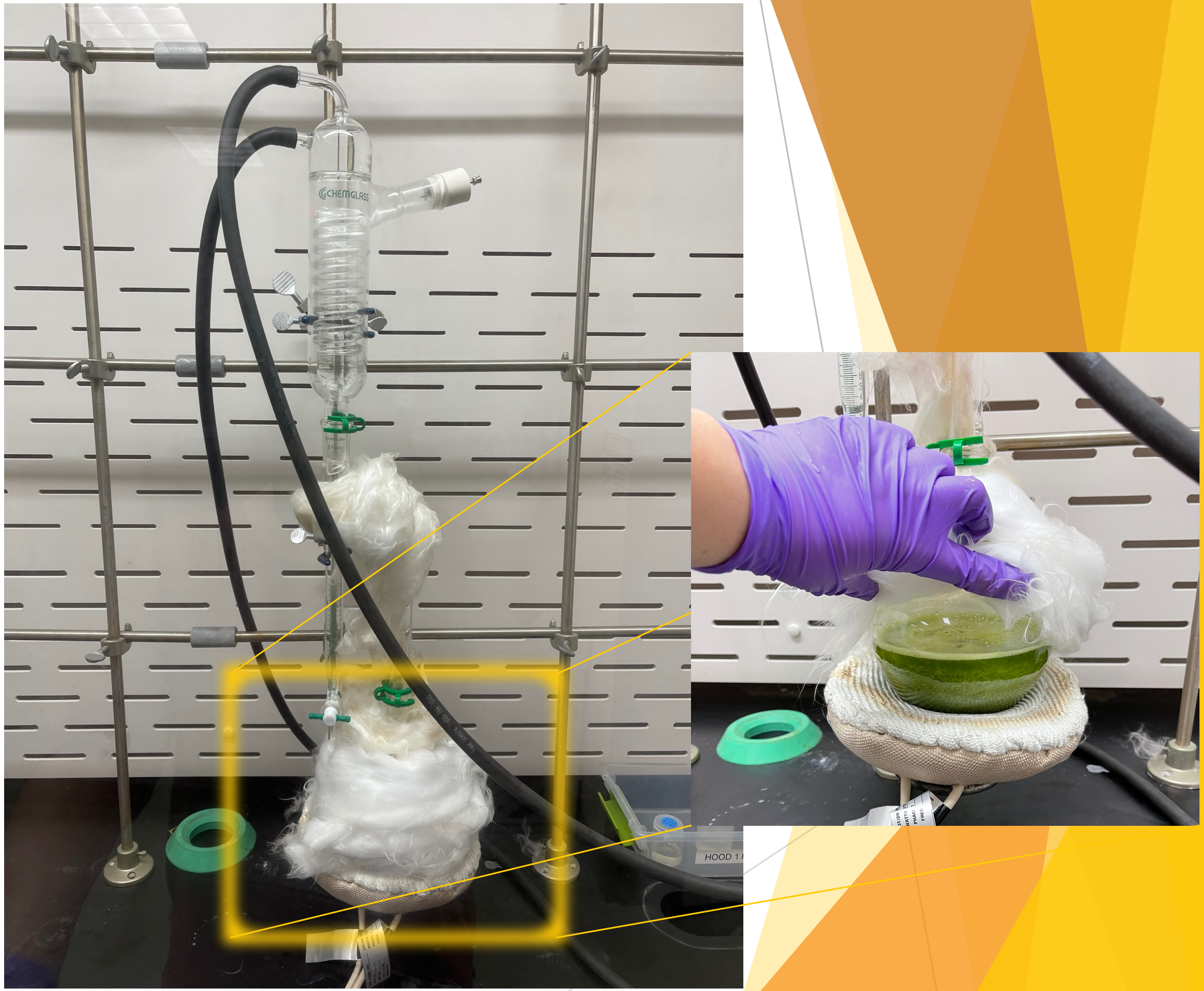
Objectives and Hypotheses

- The purpose of this experiment is to isolate citral oil from fresh lemongrass via steam distillation and analyze it by ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy, Infrared (IR) spectroscopy, Gas Chromatography–Mass Spectrometry (GC-MS), and Thin-Layer Chromatography (TLC).
- **Hypotheses:**
  - Steam distillation is an appropriate way to extract lemongrass oil.
  - Lemongrass stems contain more lemongrass oil than leaves.

Methods

- Steam Distillation: a vertical apparatus with Friedrichs condenser and Dean Stark apparatus. Several runs had to be done to get an adequate amount of lemongrass oil.
  - Rotary Evaporator (Rotovap)
- TLC (Thin-Layer Chromatography)
- GC-MS (Gas Chromatography Mass Spectrometry)
- IR (Infrared) Spectroscopy
- ^{13}C NMR (Carbon 14 Nuclear Magnetic Resonance) Spectroscopy





Steam Distillation with Dean Stark Apparatus

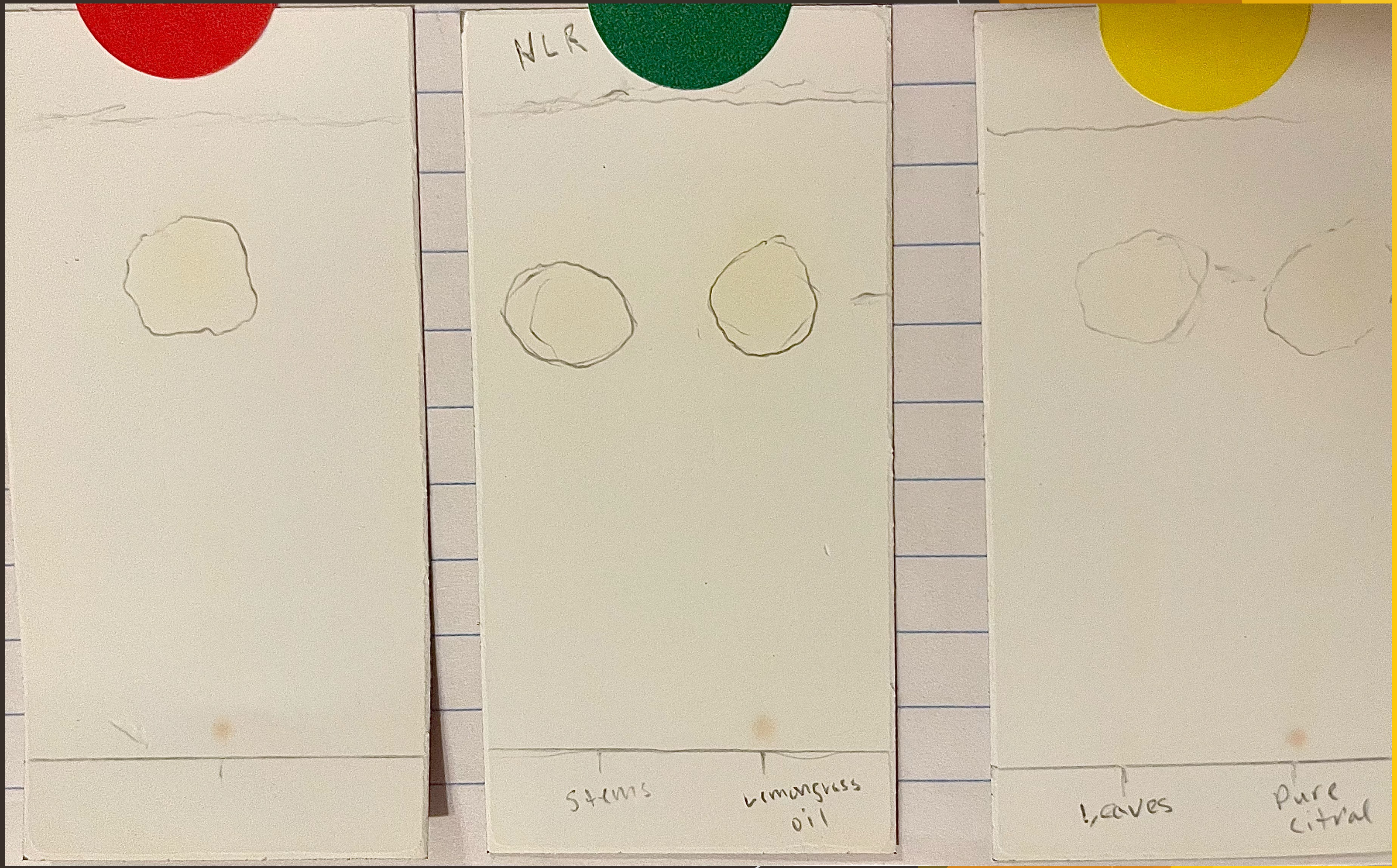
GC-MS

IR Spectrometer

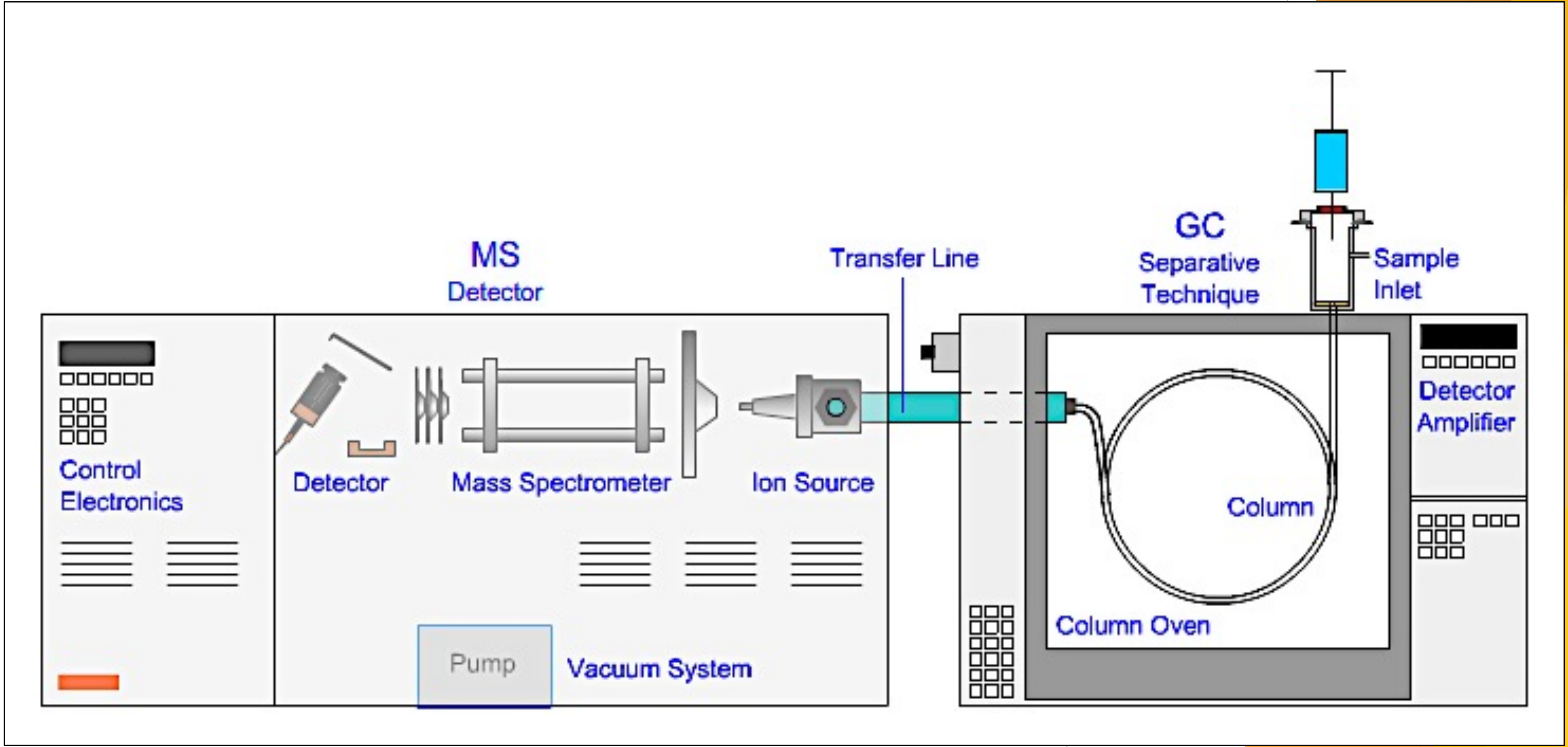
NMR Spectrometer

Results: Thin-Layer Chromatography

- Silica gel plates were run with a 4:1 ratio of hexane and ethyl acetate as a solvent.
- Samples included pure citral, lemongrass oil purchased from Pure Majestic Cosmeceuticals, and oil distilled from fresh lemongrass were compared.
- All three showed that citral was present (Rf = 0.7)

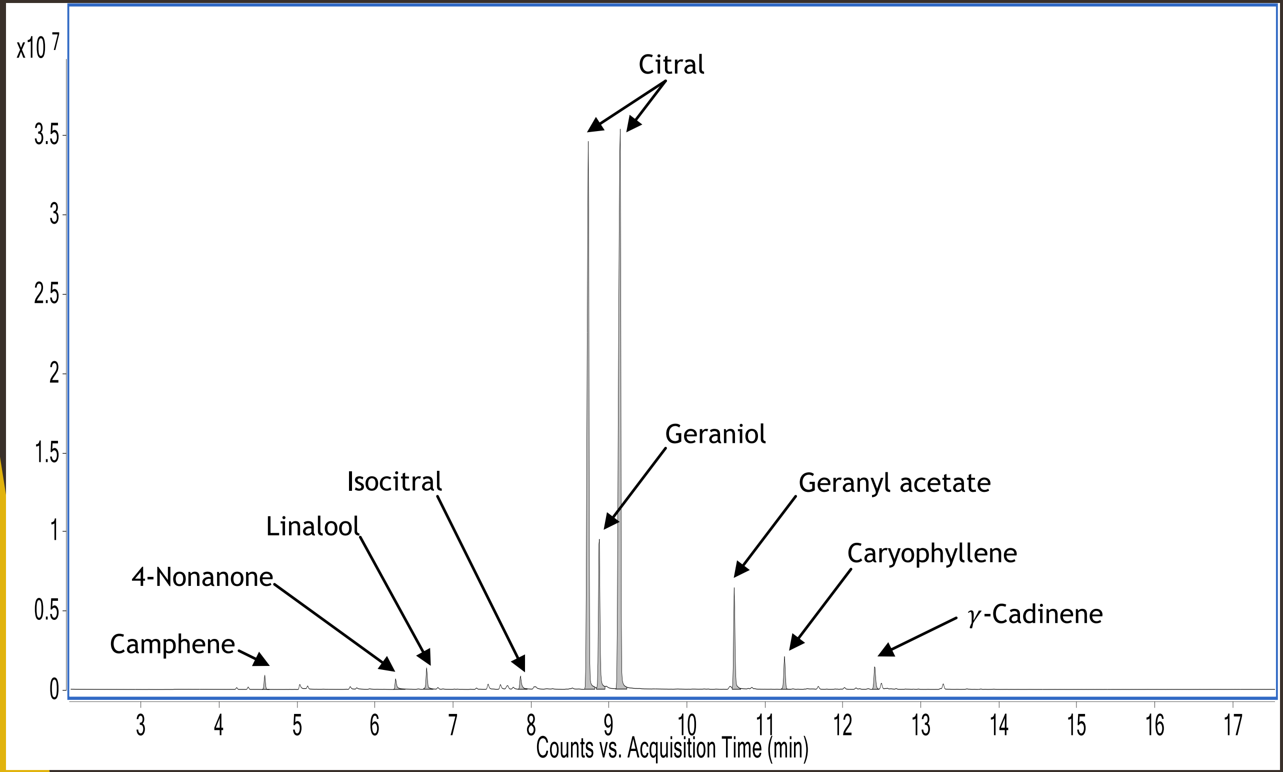


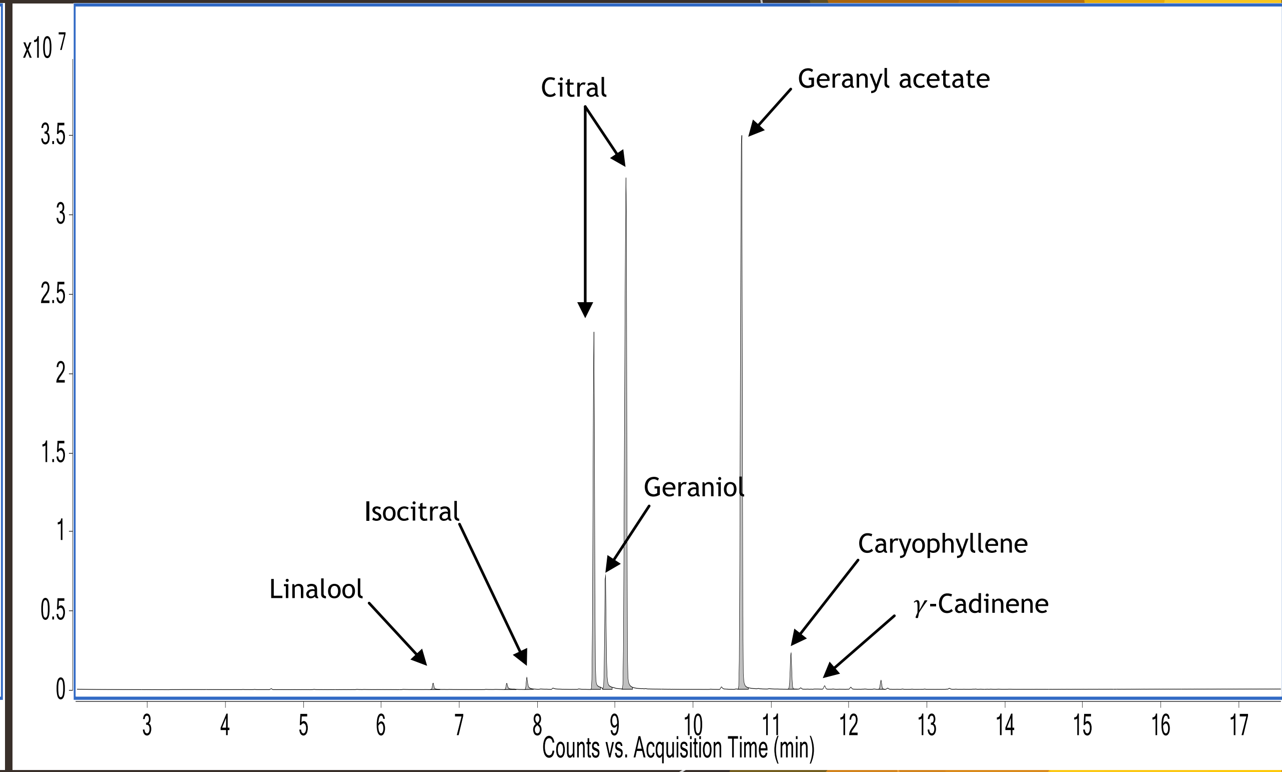
GC-MS Diagram



https://www.chromacademy.com/channels/gc-ms-training-courses/principles/gc-ms-introduction/

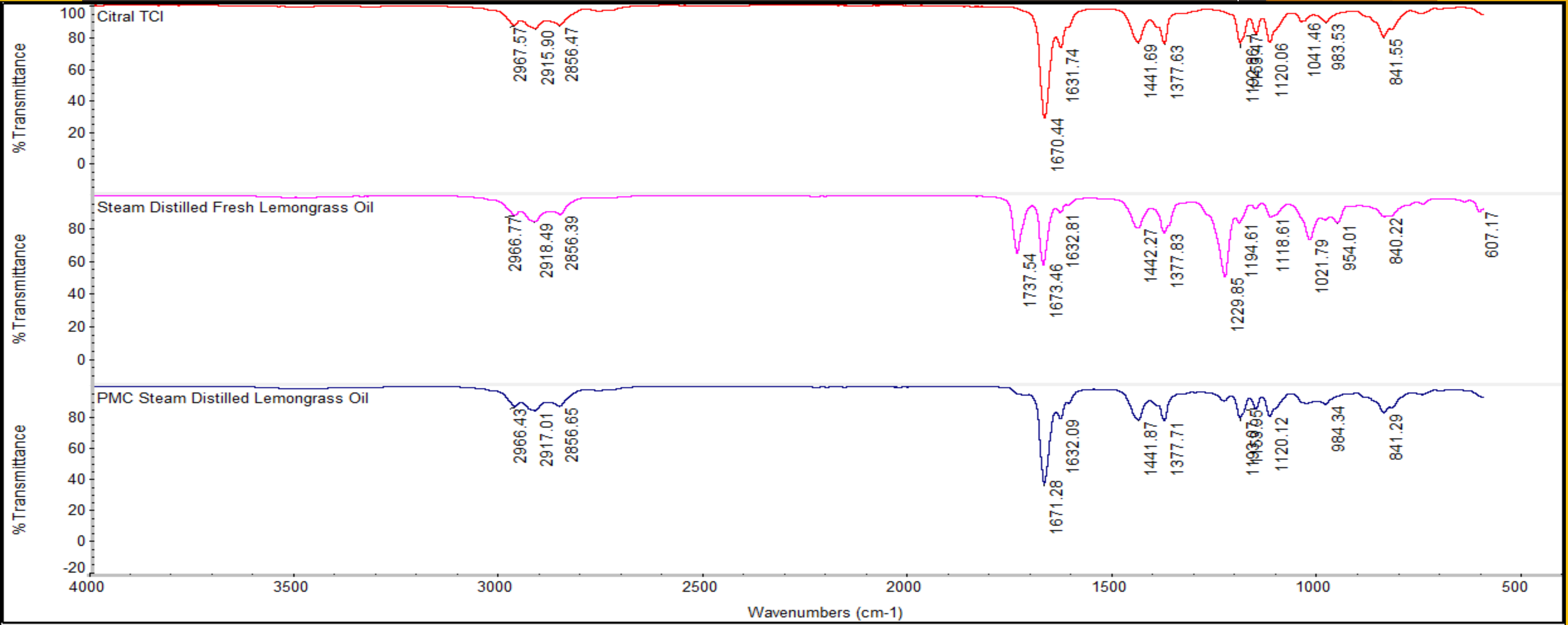
Results: GC-MS



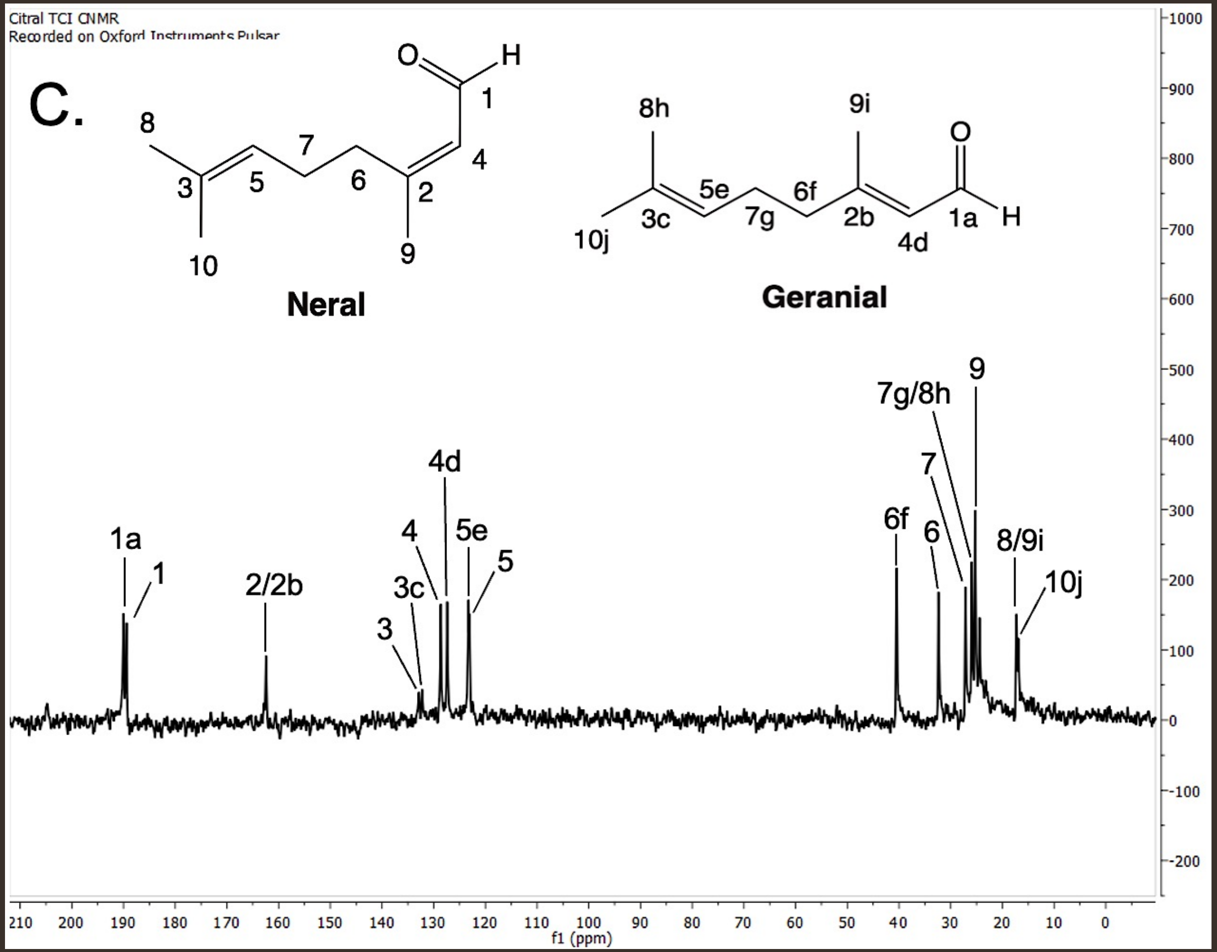


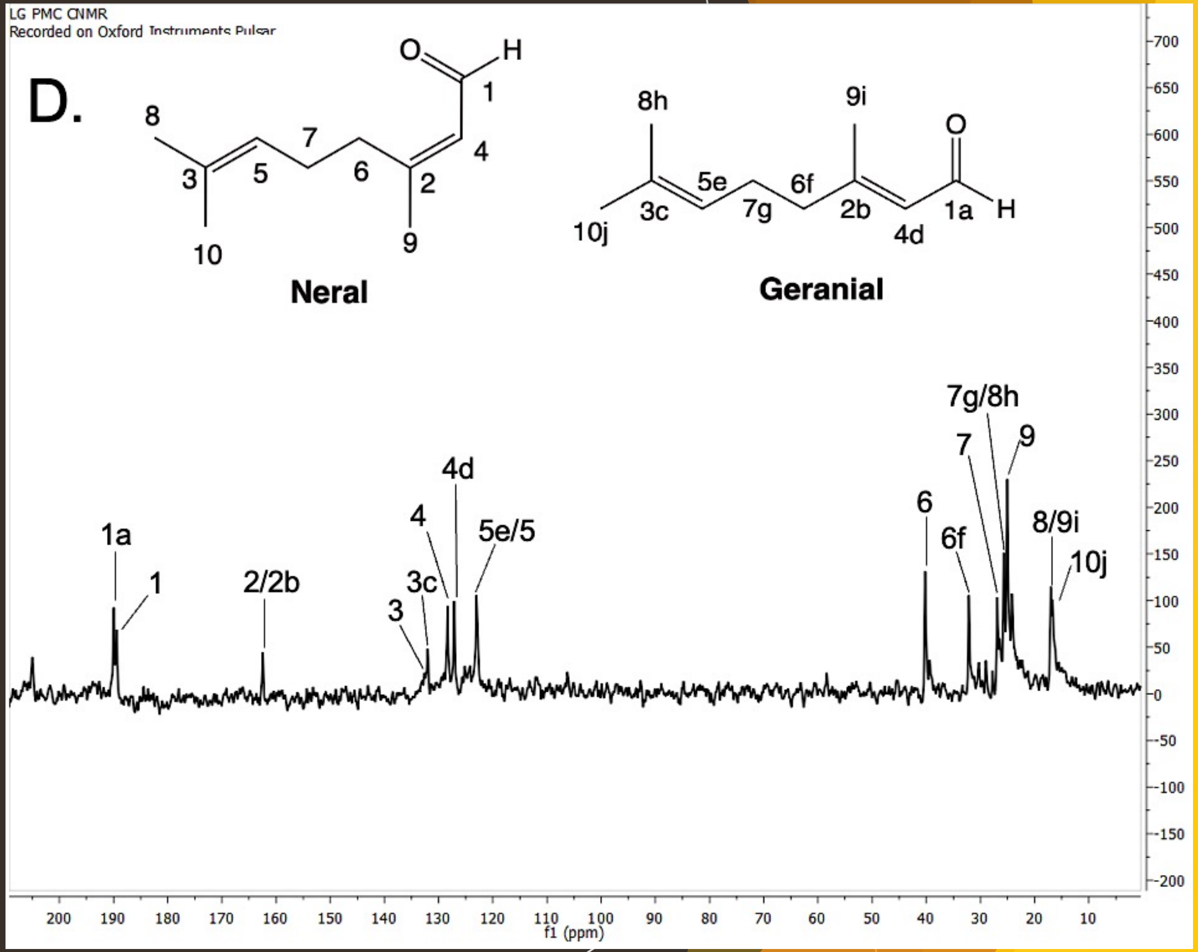
Gas chromatograms of a) PMC lemongrass oil and **b)** of distilled fresh lemongrass oil

Results: Infrared Spectroscopy



Results: ^{13}C NMR Spectroscopy





^{13}C NMR spectra of a) pure citral (TCI) and b) steam distilled lemongrass oil (PMC)

Discussion

- Oil extracted
- Citral was observed but did not appear to be the major component
- Contains more geranyl acetate
  - Different varieties of lemongrass
  - Plants are complex chemically
  - Age (Ganjewala & Luthra, 2009)
- The lemongrass oil from PMC also consisted of trace amounts of two different chemicals not found in the lemongrass used in this experiment (4-nonanone and camphene).

Conclusion

- More research needed on the properties and uses of citral and lemongrass oil.
- Different varieties of lemongrass need to be further studied to see which has the most citral content.
- Steam distillation with Dean Stark apparatus is an appropriate method of extracting oil from lemongrass, however it must be performed on a larger scale to provide an adequate amount of oil.
  - Also, depending on the variety or age, the use of leaves rather than stems might yield more oil.

References

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