Extraction and Identification of the Major Components of Essential Oils by Gas Chromatography – Mass Spectrometry (GC-MS) February 7 - 13, 2025

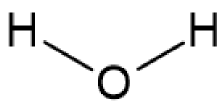
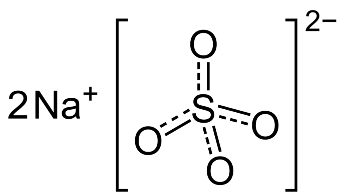
LAB2-AC2 Group E - Calubaquib, Reyes, Sanguyo

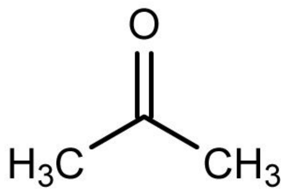
[Francis Sanguyo's copy]

Objectives:
This experiment aims to:

1. extract the essential oils (EOs) present in fresh lemongrass via steam distillation, and
2. determine the mass spectra, possible chemical structures, and relative abundance of major components of lemongrass EOs using GC-MS with electron ionization (EI).

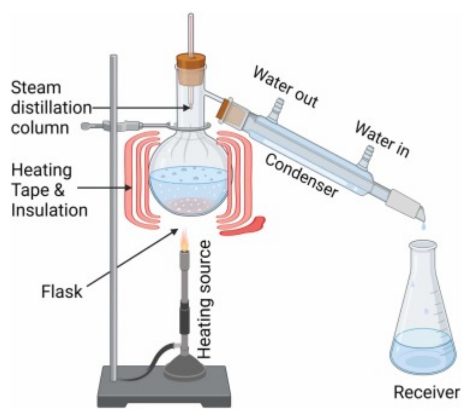
Reagents

Reagent Name and Structure	***Physical and Chemical Properties***	***Safety Precautions***
Distilled Water (H_2O) 	- colorless, odorless liquid - MW = 18.02 g/mol	Non-irritant, generally safe to handle Disposal: sink
Lemongrass Sample	- solid sample - contains liquid oil which may be extracted via steam distillation	- clean lemongrass samples before and after handling - handle lemongrass samples with washed, clean hands
Anhydrous Sodium Sulfate (Na_2SO_4) 	- white solid with characteristic odor - hygroscopic; absorbs surrounding moisture - clumps together in solution after absorbing moisture - soluble in water - MW = 142.04 g/mol	- chemical is not considered hazardous by 2012 OSHA Disposal: D499
n-Hexane (C_6H_{14}) 	- clear, colorless liquid with characteristic odor - non-polar solvent; dissolves many oils - MW = 86.18 g/mol - volatile and low BP (69 °C); serves as solvent for GC	- causes skin irritation - may be fatal if it is swallowed or if it enters airways - toxic to aquatic life with long-lasting effects Disposal: G704

Acetone (C_3H_6O) 	- clear, colorless liquid with pungent odor resembling of nail polish - polar aprotic solvent; dissolves many oils - MW = 58.08 g/mol - volatile and low BP (56 °C); serves as solvent for GC	- causes serious eye irritation - may cause drowsiness or dizziness - highly flammable liquid and vapor - Disposal: G704
Helium Gas (He)	- colorless, odorless gas - MW = 4 g/mol - serves as mobile phase for GC	- may displace oxygen and cause suffocation - contains gas under pressure; may explode if heated

Equipment:

- Glassware for Steam Distillation Setup
- Water Circulating Pump
- Heating Mantle
- Pasteur pipet
- Gas Cylinder for Helium Carrier Gas
- GC-MS Setup



Procedure:

I. Extraction of EOs from Lemongrass via Steam Distillation

a. Weigh 50 g of fresh lemongrass. Carefully cut the sample into 1-cm-long bits.
b. Transfer the lemongrass sample to a 1 L round bottom flask (RBF).
c. Add 500 mL distilled water
d. Assemble the steam distillation setup, as shown in the figure above.
e. Turn on the water circulating pump. Make sure there are no leaks.
f. Place the RBF on the heating mantle, and turn on the heating.
g. <u>Distill the lemongrass mixture for two hours.</u>
h. Observe carefully the appearance of the distillate (aqueous layer + top oil layer)

i. Once finished with distillation, turn off the heating mantle.
j. Collect lemongrass EO from the top oil layer by using a Pasteur pipette and sample vial.
k. Add anhydrous Na_2SO_4 to the EO samples and allow the oil to dry.
l. Store the oil at a cold temperature of 4 °C before GC-MS analysis.

II. GC-MS Analysis of Lemongrass EOs

a. Estimate the amounts of solvent required to dilute lemongrass EO by 1000-fold.
b. Dilute the EO by 1000-fold using a selected solvent (may be hexane, acetone, etc.)
c. Turn on the following in order: Helium carrier gas, GC system, MS system, and finally the assigned computer for GC-MS.
d. Perform auto start-up to initialize the vacuum pump and ion source temperature.
e. <u>Leave the vacuum to stabilize for at least two hours.</u>
f. Perform leak change and functioning.
g. Open a GC-MS method, and download the method.
h. Inject solvent headspace to determine the solvent cut-off time.
i. Analyze the essential oil solution that was prepared.

Literature Data

I. Notable Components Found in Lemongrass EOs

Table 1. Components of Lemongrass Oil Extract According to Carlson *et al.* (2001)

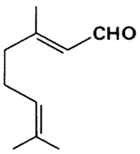
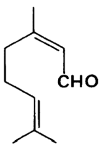
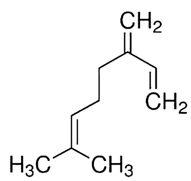
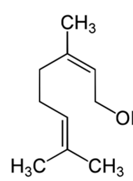
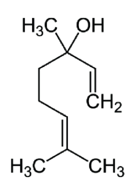
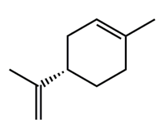
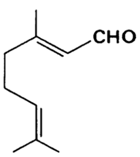
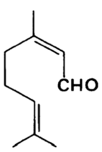
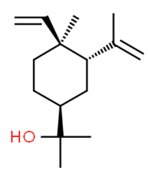
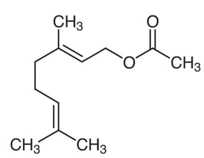
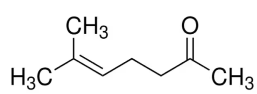
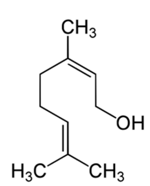
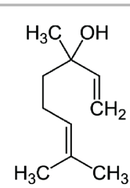
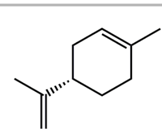
Component Name	*Structure*	*Area% at 90 bar and 23 °C*	*Area% at 85 bar and 50 °C*
Gerenial		49.79	51.07
Neral		27.47	31.32
Myrcene		5.29	5.01
Geraniol		2.31	1.82
Pentacosane	$CH_3(CH_2)_{23}CH_3$	1.06	0.13
Linalool		0.96	1.27
Heptacosane	$CH_3(CH_2)_{25}CH_3$	0.75	0.16
Limonene		trace	trace

Table 2. Components of Lemongrass Essential Oil in Underground and Above-Ground Shoots According to Kieltyka-Dadasiewicz *et al.* (2021)

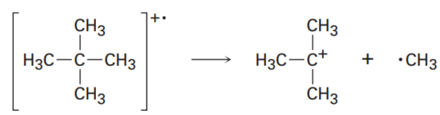
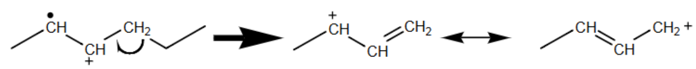
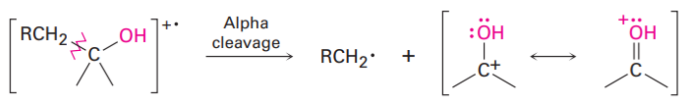
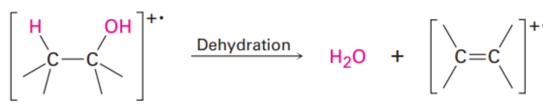
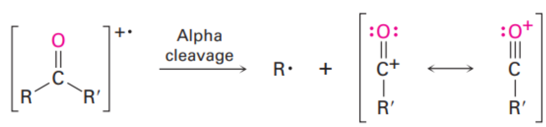
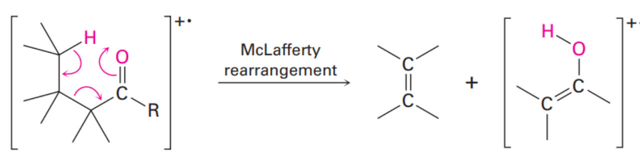
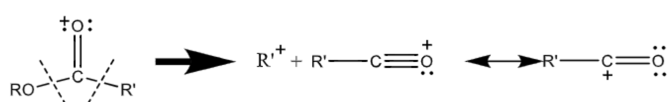
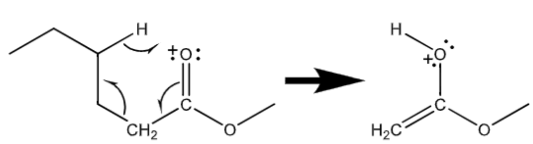
Component Name	*Structure*	*Relative Percentage in Underground Shoots*	*Relative Percentage in Above-Ground Shoots*
Gerenial		41.1	48.0
Neral		31.3	34.6
Elemol		14.0	0.8
Geranyl Acetate		2.1	0.5
6-methyl-5-heptene -2-one		1.3	1.6
Geraniol		1.2	0.4
Linalool		0.5	0.7
Limonene		0.2	0.4

II. Reference Tables for MS Fragmentation

Table 3. Summary of Detected Fragments and Lost Fragments in MS Spectra of Organic Compounds

Decrease in m/z by	Radical lost	Class of compound		m/z of ion	Positive ion	Class of compound
1	H·	any		15	$[CH_3]^+$	methyl
14	$NH_2\cdot$	amine		29	$[CH_3CH_2]^+$	ethyl
15	$CH_3\cdot$	methyl		29	$[CHO]^+$	aldehyde
17	OH·	alcohol		31	$[CH_2OH]^+$	primary alcohol
28	CO·	carbonyl		31	$[OCH_3]^+$	ester
29	$CH_3CH_2\cdot$	ethyl		43	$[COCH_3]^+$	ketone
29	CHO·	aldehyde		43	$[CH_3CH_2CH_2]^+$	propyl
31	$CH_2OH\cdot$	primary alcohol		45	$[COOH]^+$	carboxyl
31	$OCH_3\cdot$	ester		57	$[C(CH_3)_3]^+$	tertiary alkyl
43	$COCH_3\cdot$	ketone		57	$[C_2H_5CO]^+$	ketone/ester
43	$CH_3CH_2CH_2\cdot$	propyl		57	$[C_4H_9]^+$	butyl
45	COOH·	carboxyl		71	$[C_3H_7CO]^+$	ketone/ester
77	$C_6H_5\cdot$	phenyl		77	$[C_6H_5]^+$	phenyl

Table 4. Summary of Mechanisms Involved in Fragment Production (Dunnivant, 2017; McMurry, 2016)

#	*Functional Group*	*Mechanism*	*Example*
1	Branched Alkane	Sigma Cleavage	
2	Alkene	Allylic Cleavage	
3	Alcohol	Alpha Cleavage	
4	Alcohol	Dehydration	
5	Aldehyde or Ketone	Alpha Cleavage	
6	Aldehyde or Ketone	McLafferty Rearrangement	
7	Carboxyl Ester	Alpha Cleavage	
8	Carboxyl Ester	McLafferty Rearrangement	

Data and Observations:

References:

Carlson, L., Machado, R., Spricago, C., Pereira, L., and Bolzan, A. (2001). Extraction of lemongrass essential oil with dense carbon dioxide. *Journal of Supercritical Fluids*, *21*, 33-39. https://doi.org/10.1016/S0896-8446(01)00085-7

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Kieltya-Dadasiewicz, A., Ludwiczuk, A., Teraseviciene, Z., Michalak, M., Glowacka, A., Baj, T., Krecisz, and Krochmal-Marczak, B. (2021). Chemical and Nutritional Compounds of Different Parts of Lemongrass (Cymbopogon citratus (DC) Stapf.) Cultivated in Temperate Climate of Poland. *Journal of Oleo Science, 70*(1), 125-133.

McMurry, J. (2016). *Organic Chemistry* (9th ed.). Cengage Learning.

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