Isolation and Identification of an Essential Oil from Natural Source

In this experiment, you will steam distil and determine the structures of the main essential oil (**S**) from a given natural source and a product from its chemical conversion (unknown **Y**).

To determine the structures, you have to use organic qualitative analysis to identify any functional groups present in the compounds by using the reagents at your station. NMR data will be given only after the functional group test is completed.

Chemicals Available:

Sample (1 g in a vial)
Unknown **Y** (in a vial)

Anhydrous Na_2SO_4 (in a plastic vial)
Dichloromethane
Ceric ammonium nitrate solution
2,4-Dinitrophenylhydrazine (labelled as 2,4-DNP)
2% aq. NH_3
5% aq. $AgNO_3$
5% aq. HCl
5% aq. NaOH
5% aq. $NaHCO_3$
1% $FeCl_3$ in EtOH
0.2% aq. $KMnO_4$ Decolourised with easily oxidised functional groups.
Acetone (for washing)

Equipments and Glasswares

1.	Microscale kit	1 set
2.	Round bottomed flask, 25 mL	1
3.	Hotplate-stirrer/stand/clamps	1 set
4.	Sand bath	1
5.	Beaker (250 mL)	1
6.	Test tube	16
7.	Test tube rack	1
8.	Pasteur pipette	8
9.	Rubber bulb	1
10.	Microspatula	1
11.	Rubber tubing (1 m)	2
12.	Thermometer	2
13.	Wooden ring	2
14.	A bag of tissue paper	1
15.	A bag of cotton/a piece of paper	1 set
16.	Cotton gloves	1 pair
17.	Vial (for recovered dichloromethane)	1
18.	Wooden stick	1
19.	Ice (in a bucket in each lab)	

Procedure:

Apparatus. Assemble a distillation apparatus (as shown in the diagram 1) using a 25 mL round bottomed flask for distillation and a 10 mL round bottomed flask to collect the distillate. **Heat the sand bath to approximately 150 °C before proceeding the next step.**

Simplified Steam Distillation: Mix 1 g of ground sample with 15 mL of water in the 25 mL round bottomed flask and allow the sample to soak in the water for about 10 minutes before distillation. Do not forget to put in a magnetic bar, turn on the water in the condenser and stirring motor, heat the mixture (the temperature of the sand bath should not be below 170°C) to provide a steady rate of distillation. **At least 5 mL** of distillate must be collected. Hot plate must be turned off after distillation is finished. Disassemble the apparatus and rinse the condenser with acetone. **Be sure that the condenser is dry before using in the next step**

Q.1) Show the distillate to your demonstrator and ask for his or her Signature on your answer sheet before proceeding to the next Step.

Extraction of the Essential Oil: Transfer the distillate to a 15 mL capped centrifuge tube and add 1 mL of dichloromethane to extract the distillate. Cap the tube securely and shake vigorously, cool in ice. Allow the layers to separate.

Using a Pasteur pipette, transfer the dichloromethane layer to a 10 mL test tube. Repeat this extraction with fresh 1 mL dichloromethane twice and combine with the first extract.

Drying: Dry the dichloromethane extract by adding anhydrous Na_2SO_4 and stir occasionally for 10 minutes.

Evaporation: With a clean, dry cotton plugged Pasteur pipette transfer the organic layer to a dry 5 mL conical vial. Use approximately 1 mL of clean dichloromethane to wash Na_2SO_4 using the dry cotton plugged Pasteur pipette, then transfer into the vial. Be careful not to transfer any of the Na_2SO_4 into the vial. Use Hickman still head and **dry** condenser (see diagram 2) to distil the dichloromethane from the solution until the volume is reduced to 1 mL. Discard the distilled dichloromethane from the Hickman still head with a Pasteur pipette or a syringe to a vial (for recovered dichloromethane) and keep the residue for functional group analysis.

Functional Group Analysis: Carry out the functional group analysis of the residue solution (1 mL) by using the appropriate reagents at your station. (Note: dichloromethane is immiscible with water.)

Tollen's Reagent: add 1 drop of 5% aq. $AgNO_3$ in a small test tube followed by 1 drop of 5% aq. NaOH, brown precipitate will appear. Add 2% aq NH_3 to the tube until all the precipitate dissolved. The solution is ready for the test.

Q.2) Fill in your results in the answer sheet and indicate the functional group(s) present or not present.

Structure elucidation of the main essential oil (S): Reaction of the main essential oil (**S**) with CH_3I in the presence of K_2CO_3 gives compound **X** ($C_{11}H_{14}O_2$). Oxidation of **X** gives unknown **Y** ($C_{10}H_{12}O_4$) as the main product and CO_2.

Q.3) Identify the functional groups of unknown Y (provided in a conical vial) by using the reagents at your station and fill in your results in the answer sheet. Indicate the functional group(s) present or not present.

Hand in your copy of **answer sheet PART I (Demonstrator copy)** of functional group analysis with your signature **and ask for ^{1}H NMR spectra and answer sheet PART II**. ^{1}H NMR spectra will be given only when the functional group analysis is completed.

Q.4) Draw the structure which represents the main component in the essential oil (S) that was distilled from the sample. Assign each proton from the provided ^{1}H NMR spectra by labelling the peak number on the proton in the structure in the answer sheet.

Q.5) Draw the structures of compound X and unknown Y. Assign each proton of unknown Y from the provided ^{1}H NMR spectra in the same manner as Q.4.