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ISOLATION AND CHARACTERIZATION OF ALPHA-HUMULENE FROM TURPENTINE OIL

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1. ABSTRACT

Background: This invention is for finding the simplest method of isolation of α-humulele from turpentine oil. Present methods of isolation of α-humulele are industrial and required a large setup. α-humulele is mainly used as a marker compound for the standardization of formulation. Alpha Humulele is used in pharmaceutics, nutraceuticals, and cosmetics. **Method:** α-humulele is isolated with the help of a rotary evaporator. Various fractions are isolated from the turpentine oil at various temperatures and pressure. At 50 °C and 132.40 mmHg, α-humulele is isolated from turpentine oil.

2. KEYWORDS: Turpentine oil, alpha humulene, GCMS, RI.

3. INTRODUCTION

3.1 Turpentine Oil

Turpentine oil is used in the pharmaceutical. Because of its aroma it is used in perfume, food additive, and other chemical industries. Hippocrates, Galen, and Dioscorides were among the great physicians of ancient times who used terpenic oil to treat lung dysfunction and biliary lithiasis. In France, Thillenius, Pitcairn, Recamier, and Martinet proposed it as a used for blennorrhoea and cystitis. It was advised for neuralgias by Chaumeton, Kennedi, and Merat. Rheumatism, nephritis, dropand mercury salivation were all treated with terpenintine oil. a-humulene was the most common element, contributing for 34.99 to 43.60 percent of the total, accompanied by caryophyllene, longifolene, D3-carene, cymene, transpinocarveol, limonene, and b-humulene,. The compound percentage varies.

3.2 Rotary Evaporator for Vacuum Distillation

A rota evaporator[19] is an instrument used in chemical labs to evaporate liquids fromsamples in an efficient and friendly manner. It can also be used to separate compound.

Lyman C. Craig[20] created a simple rotational evaporator device. Büchi, a Swiss business, was the first to market it in 1957. Huge scale variations are used in pilot plantsin manufacturing of commercial chemical, whereas the 1L bench-top unit is the most common in research.

3.3 NEED AND SCOPE OF INVENTION

The existing all methods of isolation of Alpha-Humulene are mainly industrial. As they are industrial, they havesome limitations like

- The methods are time consuming; it takes 20 to 24 hr for fraction.
- The methods are industrial so they required large setup hence they are costly.
- Existing methods are multistep.
- Some methods give less purity as compare to its cost and time.

To overcome all this limitation we required the simple, easy, cost-effective method for isolation of Alpha-humulene.

4.0 MATERIAL AND METHOD

Turpentine Oil Ethanol Acetone Sulfuric Acid Hydrochloric Acid Sudan Red III
Rotary EvaporatorSAM-REV-0.25 Spire Automation, India
Turbomass with Autosystem XL GCMake: Perkin Elmer

4.1 Method

1. To find the appropriate temperature and pressure for the isolation of alpha-humulenewe tried the differenthypothesis a temperature and pressure.
2. Turpentine oil contain overall total of about 42 constituents. One components having boiling point less than α-humulene their name with boiling point listed in table.

4.2 Collection of turpentine oil

The oleoresins are used to make turpentine oil. The resin from pine tree collects andthen distilled to produce turpentine oil.

4.3 Method for isolation of alpha humulene from turpentine oil

Methodology A

- We Calculated pressure required to isolate these α-humulene at 30 °C using Pressure-TemperatureNomograph Interactive Tool.
- Set the temperature and pressure at rotary evaporator to isolate.
- To isolate α-Humulene, we try to isolate the oil.
- Only one fraction isolated at this temperature and pressure but RI & BP not matched with α-Humulene.

SR. NO.	Turpentine oil constituents	Boiling Point	Pressure (In mmHg)	Temperature (In °C)
1	a-humulene	106	54.50	30

Methodology B

- We Calculated pressure required to isolate these α-humulene at 40 °C using Pressure-TemperatureNomograph Interactive Tool.
- Set the temperature and pressure at rotary evaporator to isolate the fractions Yield obtains for at 40 °C but RI and BP not matching with α-humulene

SR. NO.	'urpentine oil constituents	Boiling Point	Pressure (In mmHg)	Temperature (In °C)
1	a-humulene	106	80.62	40

Methodology C

Optimized Method for Isolation of α-humulene

- We Calculated pressure required to isolate these α-humulene at 50 °C using Pressure-TemperatureNomograph Interactive Tool.
- Set the temperature and pressure at rotary evaporator to isolate the fractions
- Yield obtains for at 50 °C and RI and BP matching with α-humulene.

SR. NO.	Turpentine oil constituents	Boiling Point	Pressure (In mmHg)	Temperature (In °C)
1	a-humulene	106	132.40	50

5.0 Procedure and Characterization of Alpha-humulene

5.1 Boiling Point (By capillary tube method) Method

- Thermometer is attached to the stand by using thread or rubber and attach a minitest tube (TT) to it.
- 1 mL α-humulene, measured using a glass pipette and a bulb, is transferred in amini-TT.

- Line up the end of the thermometer with the α-humulene in the test tube, keepingthe two as near as possible.Place a capillary tube inverted in the TT with the open end looking down.
- Use paraffin oil-filled Thile's tube.
- In the tiles tube, place thermometer and TT.
- Slowly increase temperature of Thile’s tube by 10°C to 20°C every ten min by using burner, and keep a close eye on the liquid in the TT. Increase the heat by5°C every ten minutes once you notice infrequentbubbles in the liquid.
- Observe the capillary tube inside the TT. Note the temperature at which a rapidand continuous bubbles comesout of the capillary i.e. T1.
- Stop heating the tube and allow the Thile’s tube for cooling. Continue to observethe capillary as the number of bubbles produced reduces until there are none remain. In the capillary, the liquid will begin to ascend. Keep track of the temperature at which this happens. i.e. T2
- The boiling point od turpentine oil is obtained after average of T1 and T2.
- Boiling Point = T1 + T2/2
- **T1** = The temperature at which fast and continuous bubbles comes out of the capillary
- **T2** = The temperature at which the liquid will start to rise in the capillary

5.2 Refractive Index

- **Method**
- Abbe’s refractometer is used for the RI determination.
- Allow to dry after cleaning the prism surface with alcohol and then acetone with cloth. Put 3-4 drops of α-humulene on prisms, use dropper for it and press them together.
- Place the refractometer according to light source that light fall on mirror. Adjust the mirror so that the mostlight is reflected into the prism box.
- By sliding the lever, rotate the prism box until the boundary between dark and white light areas appears inthe field of vision.
- If a strip of colors appears in the light shade boundary, rotate the compensator to sharpen it. Adjust the leveruntil the light shade border crosses through the cross-wire’scenter.
- After adjusting this, the refractive index value of α-humulene appears on scale.

5.3 Density

- Density of the α-humulene is measure with the help of density bottle, and then comparewith the standard.
- Measure the mass of density bottle using physical balance. Then measure the mass ofdensity bottle withdistilled water and α-humulene.
- Calculate weight of empty density bottle
- Calculate weight of Density bottle + Distilled water
- Calculate weight of Density bottle + α-humulene
- Then calculate the volume of the density bottle by using the standard density of distilledwater
- Volume = Mass/ Density
- Use this volume to calculate the density of turpentine oilCalculate the density of turpentine oil
- **Density** = mass/volume

5.4 GCMS

Principle & Instrumentation

When two separate methods are effectively coupled to generate a gas chromatography stream, like gas chromatography (GC) and mass spectrometry (MS). The benefits of mass spectrometry were clear. While gas chromatography canisolate a range of volatile as well as semi-volatile chemicals, it cannot always look for them; similarly, mass spectrometry can detect multiple substances but cannot continuously identify them. The original problems with GC-MS were considerable pressure differences: the GC gas that reaches the system is around one atmosphere (760 torr), whereas the MS runs at about 10-5–10-6 torr.

Many solutions have been planned, which includes splitting the GC effluent so that only a small portion reaches the MS and the reaming is sold off, creating links between the GC and MS to lessen MS's strain. For packaging, a jet separator was frequently utilised. Column GC looks to be easily jammed and requires a significantamount of care.

Because the MS vacuum system operates at a high speed (about 100 litres per sec), all GS effluent may be transferred to the MS. The capillary column GC approach has been widely utilised. For capillary GC-MS, the capillary column is now inserted into the ion source directly. It's likely that the effluent entering the GCis at a temperature of up to 4008C. Join the

MS since the GC generally elutes the effluent (individual chemicals); most often detected by electron ionisation. To manufacture ions, an ion trap or flight time might be employed. They're currently being targeted by a flood of electrons, which is driving them to fracture. (M / Z) charging ratio The charge is almost always equal to 1, and the M/Z ratio is equal to the fragment's molecular mass. Each fragment is concentrated to the detector through a slit by a quadruple of electromagnets. These quadruples are a machine that exclusively controls those fragments. It has quadruples in software, which transport these pieces one by one (scan) to the next. The M/Z spectrum has been restored.

A mass spectrum, which is a graph of signal amplitude, is the outcome of this. (relative abundance) vs. M/Z ratios (basically molecular weight).

Every substance was a distinct fingerprint, and the application includes a database of unknown molecular spectra. 3D GC-MS is becoming increasingly prevalent. In conclusion, GC-MS is an excellent approach for both qualitative and quantitative analysis.

Organic substances that are volatile or semi-volatile in a range of specimens. It is conceivable to achieve a detection limit of sub-ng. For GC injection, the sample must be in solution. This might be as simple as dissolving into the solvent like dichloromethane.

Complete bench equipment is available for research and is commonly used in education. Approximately $50,000 (MS range 200 & 700 pounds). Advanced, accurate systems that are virtually solely utilised into research can cost up to $250,000, although they still accessible. The mass capacity varies between 20 and several thousand pounds.

6.0 Characterization of Alpha-humulene

The isolated compound was evaluated based on certain physicochemical parametersand findingsof these studies help in the identification of drug.

6.1 Physical Appearance

The physical appearance of alpha-humulene is evaluated on the basis of colour and odour.

Table: Physical Appearance of α-humulene.

Parameters	Standard	Observes
Colour	Pale-yellow	Pale-yellow
Odour	Turpentine odour	Turpentine odour

6.2 Boiling Point (By capillary tube method)

He boiling point of the alpha-humulene is determine by using capillary tube method with the help of boiling point apparatus and compared with standard.

Table: Boiling point of α-humulene.

Sr.No.	Observed	Standard
61	106	105-106

6.3 Refractive Index

The refractive index of the alpha-humulene as observed by abbe's refractometer along with refractive index reported in literature is given in table.

Table for RI of α-humulene

Sr. No.	Observed	Standard
1	1.5	1.503

6.4 Density

Density of the α-humulene is measure with the help of density bottle, and then compare with the standard.

Measure the mass of density bottle using physical balance. Then measure the mass of density bottle with distilled water and α-humulene.

Weight of empty density bottle = 14.56 gm

Weight of Density bottle + Distilled water = 23.74 gm

Weight of Density bottle + α-humulene = 22.38 gm

Mass of distilled water = 23.74-14.56 = 9.18

Mass of α-humulene = 23.36-15.52 = 7.82

Then calculate the volume of the density bottle by using the standard density of distilledwater i.e. 0.9982

Volume = Mass/ Density

= 9.18/0.9982

= 9.19 ml

Calculate the density of α-humulene

Density = mass/volume

= 7.82/9.19

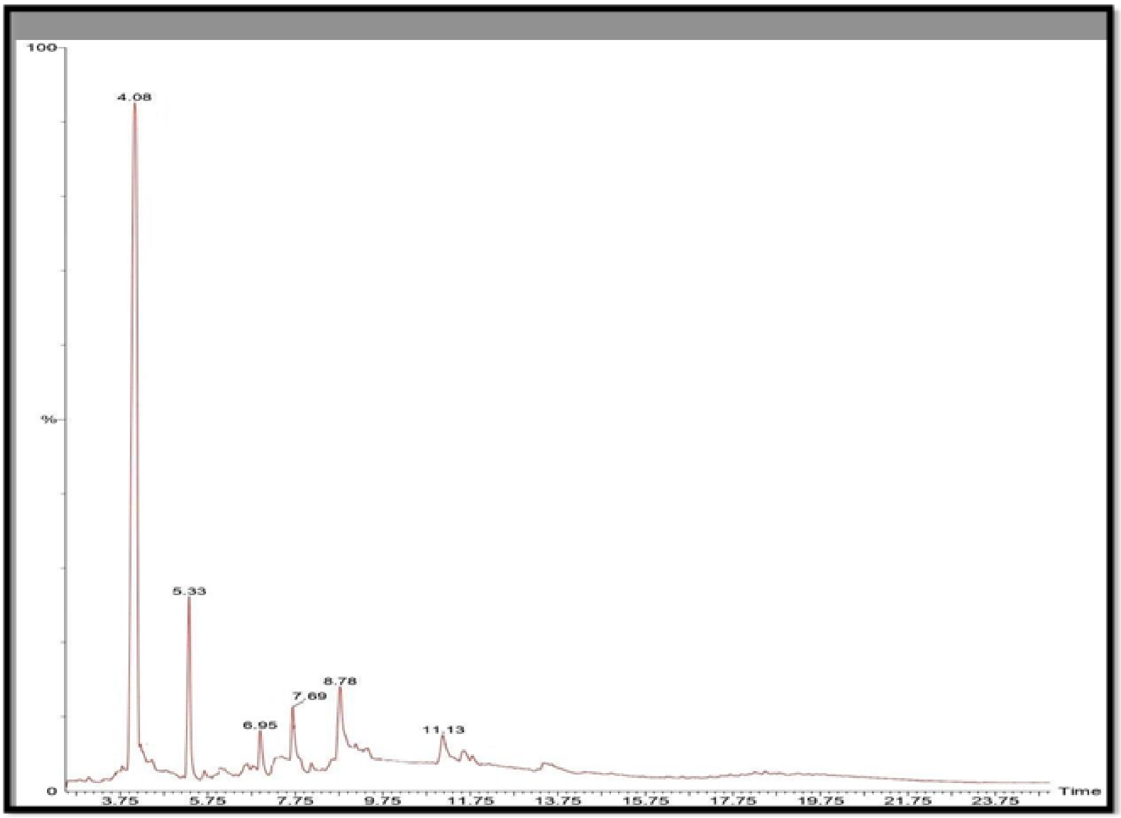
Density = 0.8590

Table for Density of α-humulene.

Parameter	Observed	Standard
Density	0.8590	0.8450-0.8860

6.5 Gas Chromatography-Mass Spectroscopy

After performing the GC-MS study of the fraction, α-humulene found in major concentration. α-humulene is found 85% in fraction along with the 3-carene, d-limonene, beta-humulene, camphene are observed concentration. α-humulene is retain at the 4.08 in graph. 3-carene is retain at 5.33, d-limonene at 6.95, beta-humulene 7.69 and camphene retain at 8.78.



RESULTS

According to the GC-MS study, α-humulele was found in major concentrations. α-humulele is found at 85% in fraction along with the 3-carene, d-limonene, beta-humulele, and camphene is observed concentration. α-humuleleis retained at the 4.08 RT in the graph. The density of α-humulele is 0.8509 gm/mL. Refractive index of the α-humulele as observed by abbe's refractometer.

1.46. BP was observed at 153-157 °C.

CONCLUSION

alpha-humulele was successfully isolated having purity up to 85% from turpentine oil by using a rotary evaporator and characterization is done by using GC-MS and FTIR.

SUMMERY AND CONCLUSION

- We have completed all the basic preliminary testing of isolated fraction such as colour, odourappearance.
- Perform the characterisation for isolated fraction such as Refractive index, Density, Boiling point,GC-MS, specific gravity.
- As per our project title we successfully isolated the alpha-humulene up to 85% purity from turpentineoilby using rotary evaporator.
- The use of rotary evaporator for the isolation of alpha-humulene is our novelty.
- Further study is required because author was unable to complete work due to instrumental problem.

Finally, it is suggested that, in future study work needed on the purity of alpha-humulene.

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