# CHEM253 Exp. 09 Hydrodistillation of Essential Oils

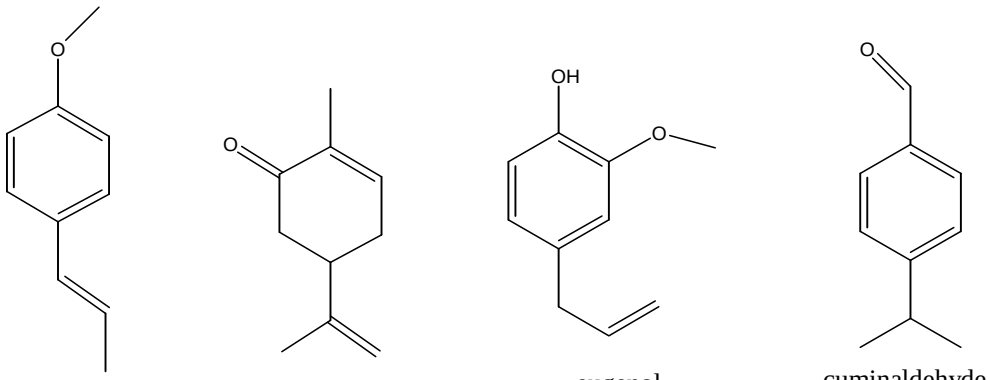
In the fifteenth century Theophrastus Bombastus von Hohenheim, otherwise known as Paracelsus, urged all chemists to make essential oil extracts for medicinal purposes rather than try to transmute base metals into gold. He believed every essential oil extract had a quintessence that was most effective in accomplishment of cures. These oils occur in all living parts of the plant; they are often found concentrated in twigs, flowers and seeds. Essential oils are complex mixtures of relatively volatile and odorous compounds immiscible with water. Among early essential oils were those of camphor, cloves, cedarwood, terebinth (turpentine), cinnamon, balsam (benzoin) and myrrh. Oil of clove is a time-honored remedy for toothache.

Currently the biggest market for essential oils is for perfumes. Aromatherapy is an alternative approach to medical care based on the therapeutic properties of fragrant essential oils extracted from herbs, flowers, and fruits.

In this lab, we will isolate the essential oils from different spices by hydrodistillation. Hydrodistillation is a simple form of steam distillation which is often used to isolate non-water-soluble, high boiling natural products. The advantage of this technique is that the desired material distills at a temperature below 100 °C. The essential oils of the distillate will then be extracted and analyzed.

> Variety's the very spice of life,
> That gives it all its flavour.
> **William Cowper**, The Task, Book ii, `The Timepiece',

Zubrick, Chapters 19, 20 and 35 contain background information on how to set up and run a distillation.




anethole (anise) | carvone (caraway) | eugenol (cloves) | cuminaldehyde (cumin)

TYPICAL ESSENTIAL OILS

A series of power point slides of the (old school) Essential Oil lab 2001 is found at:
http://domin.dom.edu/faculty/jbfriesen/chem254/steam.ppt
More recent material at OChemOnline
http://ochemonline.pbworks.com/w/page/6122857/Steam-Distillation-of-Essential-Oils

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Procedure:

## Part I: Hydrodistillation

1) Set up a steam distillation apparatus as shown below. Place your separatory funnel filled with distilled water in the two-neck adapter. You may use a 250 mL round bottom as your receiving flask.

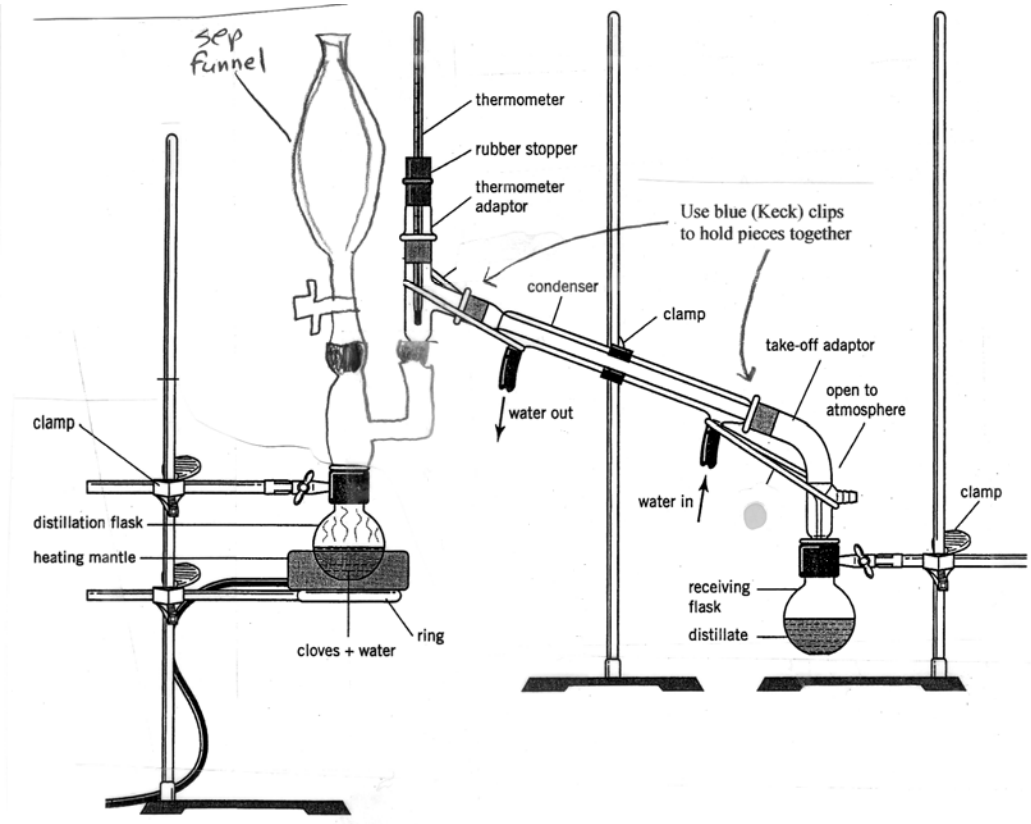
2) Obtain about 25g spice. If necessary, grind the spice in a mortar and pestle or grind it in a spice grinder. Add the ground spice to your 500 mL round-bottom flask with a plastic powder funnel.

3) Fill the flask half full with distilled water. Add a couple of boiling chips. The round-bottom flask with the spice mixture will be the distilling flask.

4) Rest your 500mL distilling flask on your heating mantel. Do not use a plastic clamp to hold your distilling flask to the adapter. Heat the distilling flask slowly. You may add water sparingly from your separatory funnel so the slurry inside doesn't dry out and burn.

5) Stop the distillation when you have about 100 mL of distillate, or you have been collecting distillate for one hour.

6) Record the volume of your distillate.



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Part II extraction of the essential oil:

1. Extract the distillate three times with 15 mL aliquots of dichloromethane. [a. add distillate to sep funnel, b. add 15 mL of $CH_2Cl_2$, c. shake, d. drain lower layer, e. repeat steps b. through d.] Combine and save the organic (dichloromethane) layers.

2. Use drying agent such as magnesium sulfate to dry the combined dichloromethane extracts. Filter off (gravity) the drying agent. Collect the filtrate in a pre-weighed beaker.

3. Remove the solvent by heating over a hot water bath in the fume hood. Use a boiling stick to facilitate boiling. Do not heat to dryness – let the final bit of solvent evaporate with the beaker off of the hot plate.

4. Obtain the mass of your product.

Analysis:

- UV/vis may be run during the laboratory time
- IR analysis may be run during the laboratory time.
- GC-FID will be done with the autosampler

5. Hand in product in an appropriately labeled vial

**DISPOSAL PROCEDURES**

All liquid and solid wastes can be placed in the hazardous waste container.

Checklist for completing the "Prelab" section: (refer to Laboratory Syllabus for complete directions)

____ *Title*.

____ *Purpose*. (1 point)

*Structures and equations*.

(1 point) Choose a spice to hydrodistill. Identify the principle component of the essential oil from your spice. Write down the chemical and physical characteristics of the principle component from your spice including but not limited to structural formula, molecular weight, molecular formula, boiling point, and melting point. Reference your findings (at least two sources).

- Anise (*Pimpinella anisum*).
- Fennel (*Foeniculum vulgare*).
- Cumin (*Cuminum cyminum*).
- Cloves (*Eugenia caryophyllus*).
- Oregano (*Origanum vulgare*)

_____(1 point) Consult the OChemOnline 2008 & 2009 student comments on < http://ochemonline.pbworks.com/w/page/6122857/Steam-Distillation-of-Essential-Oils>. Which comment did you find most helpful?

___ *Flowchart.* (1 point)

**Safety/Health Question**: (1 point)

What is “Multiple Chemical Sensitivity”? Can a person with Multiple Chemical Sensitivity lead a normal life?

*Physical constants of reagents*.

| Name | Formula | M.W. g/mole | m.p. $^{o}$C | b.p. $^{o}$C | Density g/mL |
|---|---|---|---|---|---|
| dichloromethane (methylene chloride) | $CH_2Cl_2$ | 84.93 | -95 (MI) | 39.75 (MI) | 1.32 (MI) |
| water | $H_2O$ | 18.02 | 0 | 100 | 1.00 g/mL at 19°C |
| magnesium sulfate | $MgSO_4$ | 120.4 | | | |

| Name | Solubility | Safety Information |
|---|---|---|
| dichloro methane | immiscible with water<br>miscible with alcohol<br>miscible with nonpolar | Warning – respiratory irritant<br>Caution – skin irritant<br>Warning – eye irritant<br>Caution – possible carcinogen<br>Dispose of a Hazardous Waste |
| water | miscible in alcohols,<br>immiscible with nonpolar | Safe |
| magnesium sulfate | soluble in water,<br>some soluble in alcohol<br>insoluble in nonpolar | Caution – respiratory irritant |

References: 1) Merck Index, 11th ed.
2) www.chemfinder.com
3) Hazardous Chemical Desk Reference, Lewis and Sax, 1987
4) Aldrich catalog online

**Experimental Observations and Data**:

Hand in a copy of your experimental observations and data before you leave lab. (3 points)

*Experimental Observations*: Refer to Laboratory Syllabus for guidelines.

Raw Data: *Refer to Laboratory Syllabus for guidelines.*

Lab report:

*Results.*

____ (1 point) % yield of product → mass of recovered product x 100/mass of starting material
____ (1 point) Interpret the UV/vis spectrum of your product that was obtained during the lab.
____ (1 point) Interpret the IR spectrum of the principle component of your essential oil.
____ (1 point) Interpret the GC-FID of your product done with the autosampler. Standard chromatograms of possible components on the course webpage.

*Literature Questions*: J Ethnopharmacol. 2009, 124, 151-153
Antibacterial activity and chemical composition of essential oil of *Pamburus missionis*.
Pavithra PS, Sreevidya N, Verma RS

____ (1 point) How where the chemical components of the essential oil identified?
____ (1 point) How was the essential oil prepared for the antibacterial assay?
____ (1 point) Draw the chemical structures of the two major components of the essential oil.

*Discussion and Conclusion.*

____ (1 point) Propose 2 ways that the percent yield could be increased.
____ (1 point) What commercial and/or medicinal value does the essential oil from your spice have? Cite your sources.
____ (1 point) What is one other way (besides hydrodistillation) one could extract essential oils from plant material?
____ (1 point) How could one measure the purity of an essential oil, which often is a mixture of similar compounds, that will be used for commercial or therapeutic products?
____ (1 point) What is a favorite spicy dish at a local restaurant? Be specific!

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When You're Feelin' Sad And Low
We Will Take You Where You Gotta Go
Smiling Dancing Everything Is Free
All You Need Is Positivity

Colours Of The World
Spice Up Your Life
Every Boy And Every Girl
Spice Up Your Life
People Of The World
Spice Up Your Life

Yellow Man In Timbucktoo
Colour For Both Me And You
Kung Fu Fighting Dancing Queen
Tribal Spaceman And All That's In between

Colours Of The World
Spice Up Your Life
Every Boy And Every Girl
Spice Up Your Life
People Of The World
Spice Up Your Life

Flamenco Lambada But Hip Hop Is Harder
We Moonwalk The Foxtrot Then Polka The Salsa

**Spice Girls**

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Ethnopharmacological communication

# Antibacterial activity and chemical composition of essential oil of *Pamburus missionis*

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ABSTRACT

*Aim of the study:* The aerial parts of *Pamburus missionis* (Wight) Swingle (Rutaceae) are traditionally used in the treatment of swelling, chronic rheumatism, paralysis, and puerperal diseases. The aim of this work was to investigate the chemical composition and antibacterial activity of essential oil of *Pamburus missionis* against different bacterial strains.
*Materials and methods:* The essential oil was obtained from fresh leaves by hydro distillation method and its chemical composition was analyzed by GC and GC–MS. The antibacterial activity was determined by disc diffusion and micro broth dilution assay.
*Results:* GC–MS analysis of the essential oil revealed the presence of 1-tridecanol (38.4%), *n*-hexadecanoic acid (16.1%), oxygenated monoterpenes (14.4%), monoterpene hydrocarbons (3.1%) and eugenol (1.9%) as the major components. The oil exhibited antibacterial activity at 100 and 450 μg against the test organisms with inhibition zones of 7–25 mm and minimal inhibitory concentrations values in the range of 10–100 and >100 mg/ml.
*Conclusion:* The present study reveals the antibacterial potency of essential oil of *Pamburus missionis*. The use of *Pamburus missionis* in the treatment of various ailments and puerperal diseases can be attributed to its antibacterial property.



## 1. Introduction

The genus *Pamburus* (Rutaceae) comprises the type species, *Pamburus missionis* (Wight) Swingle. It is a small thorny shrub with thick leaves and is native species of India and Sri Lanka. *Pamburus missionis* is also known as *Limonia missionis* or *Atalantia missionis* (Swingle and Reece, 1916). *Pamburus missionis* is found in the woody sylvan campus of Indian Institute of Technology Madras (IITM), Guindy, Chennai. The IITM campus has a rich biodiversity of flora and fauna, and has about 298 plant species. The fruits of *Pamburus missionis* yield fragrant oil which is traditionally used in the treatment of chronic rheumatism and paralysis (Somasundaram, 1967). The leaves of this tree are traditionally used for the treatment of swellings, fractures, piles and fistula. Decoction of leaves is given internally for phlegm and puerperal diseases (Jayaweera, 1982). Several compounds have been isolated from different parts of *Pamburus missionis*, e.g. indole alkaloid (Kumar et al., 1994), coumarins (Barua et al., 1974, 1980; Kumar et al., 1994), flavones and diterpenes (Dreyer and Park, 1975). Imperatorin and xanthyletin isolated from the stem bark of *Pamburus missionis* are reported for insecticidal activity (Kumar et al., 1994). However, there are no reports on the chemical composition of essential oil of this plant. In this report we describe the chemical composition of essential oil isolated from the leaves of *Pamburus missionis* and its antibacterial activity.

## 2. Materials and methods

### 2.1. Plant material

Fresh leaves of *Pamburus missionis* were collected from the forest of IITM Campus, Guindy, Chennai. The plant material was identified and authenticated by a plant taxonomist, Dr. K. Narayana Nair, NBRI Lucknow, and a voucher specimen was deposited to their herbarium.

### 2.2. Extraction, isolation and analysis of essential oil

Fresh leaves were subjected to hydro distillation for 6 h in Clevenger apparatus and a yield of 0.01% (w/w) oil was obtained. The oil was stored in sealed vial at 4 °C for further testing. The composition of oil was analyzed by GC–MS (g-Jeol MSGC-Mate II) instrument with capillary column of HP-5 (30 mm × 0.321 mm; film thickness 0.25 μm). The inlet oven temperature was kept at 60 °C initially. The detector and injector temperature were set at 250 and

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220 °C, respectively, and the column temperature was maintained at 70–240 °C at a rate of 4 °C/min. 1 μl of oil was injected into GC–MS instrument for analysis. Helium gas was used as carrier gas at flow rate of 1 ml/min. The chemical components of essential oil were identified by comparing their retention indices (RI) and mass fragmentation patterns with those on the stored NIST library (National Institute of Standards and Technology).

### 2.3. Microorganisms tested

*Bacillus subtilus* NCIM 2718, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aurginosa* ATCC 27853, *Klebseilla pneumonia* ATCC 70063 and *Escherichia coli* ATCC 25922 were obtained from NCIM (National Collection of Industrial Microorganisms), Pune, India. All the strains were maintained on nutrient agar at 4 °C and were subcultured every month in our laboratory.

### 2.4. Antibacterial activity assay

Antibacterial activity of the essential oil was determined by disc diffusion and micro broth dilution method. Briefly, bacterial cultures were diluted ($OD_{620}$ = 0.1) to obtain a bacterial suspension of $10^8$ CFU/ml. Petri plates containing 20 ml of nutrient agar were inoculated with 200 μl of bacterial culture and were allowed to dry in sterile chamber. Filter paper discs (6 mm in diameter) impregnated with 100 and 450 μg/disc of essential oil were placed on the inoculated agar surface. Gentamycin (10 μg/disc) and methanol were placed as controls. The plates were incubated at 37 °C for 24 h. The antibacterial activity against each test organism was quantified by determining the zone of inhibition around the paper discs in millimetres. Tests were performed twice and average diameter of the zone was determined. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the oil were determined by micro broth dilution assay. The oil was 10-fold serially diluted in DMSO to obtain concentrations from 100 to 0.01 mg/ml. 10 μl of the serially diluted essential oil was added to nutrient broth in each tube. 10 μl of $10^6$ bacterial suspensions were added to the tubes. The tubes were incubated at 37 °C for 24 h. Lowest concentrations of the essential oil, which inhibited the bacterial growth after 24 h, were recorded as MIC. Minimum bactericidal concentration (MBC) was determined by sub-culturing 10 μl of the MIC test solutions on nutrient agar plate at 37 °C for 24 h. The highest dilution that yielded no bacterial growth was taken as MBC (Rios et al., 1988).

## 3. Results and discussion

### 3.1. Chemical composition

GC–MS analysis of the essential oil of *Pamburus missionis* revealed the presence of 20 compounds. The essential oil of *Pamburus missionis* is composed of oxygenated monoterpenes (14.13%), monoterpenes (3.12%) and eugenol (1.89%). 1-tridecanol (38.28%) and *n*-hexadecanoic acid (16.08%) were the major components in the oil. Ricinoleic acid (7.40%), non-isoprenoid components (8.89%) and esters (10.01%) were other constituents of the essential oil (Table 1). The essential oil had the similar smell of the plant with a characteristic sweet odour. The compound responsible for the sweet odour was identified as nonanal.

**Table 1**
Chemical composition of leaf essential oil of *Pamburus missionis*.

| Compounds | RI[a] | (%)[b] |
|---|---|---|
| Hexane, 2,4-dimethyl | 688 | 0.38 |
| Pinene | 936 | 2.67 |
| l-Linalol | 973 | 2.47 |
| γ-Terpinene | 1013 | 0.45 |
| Nonanal | 1104 | 5.66 |
| Terpinen-4-ol | 1164 | 2.96 |
| Nerol | 1210 | 3.82 |
| Dodecane | 1214 | 0.35 |
| Citral | 1236 | 3.37 |
| Thymol | 1267 | 1.51 |
| Eugenol | 1331 | 1.89 |
| 10-Undecnoic acid methyl ester | 1371 | 1.73 |
| Benzoic acid 2,3-dimethyl | 1376 | 3.47 |
| 1-Tridecanol | 1556 | 38.28 |
| Heptadecane | 1711 | 0.38 |
| 1-Hexadecanoic acid | 1863 | 16.08 |
| Nonadecane | 1901 | 2.50 |
| 9-Octadecenic acid methyl ester | 2085 | 3.66 |
| Tetradeconic acid dodecyl ester | 2773 | 0.86 |
| Ricinoleic acid | 2337 | 7.40 |

[a] Retention index.
[b] Relative percentage obtained from peak area.

### 3.2. Antibacterial activity

The results of antibacterial activity of essential oil of *Pamburus missionis* against different microorganisms are tabulated in Table 2. The oil exhibited antibacterial activity at 450 and 100 μg against the test microorganism with zone sizes ranging from 7 to 25 mm. The antibacterial activity of oil at 450 μg against *Bacillus subtilus* was comparable to standard antibiotic gentamycin. The activity of essential oil of *Pamburus missionis* against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* was less than that of standard antibiotic gentamycin. The most susceptible bacterium was *Bacillus subtilus* with 25 mm zone of inhibition, and the most resistant bacterium was *Klebsiella pneumoniae*, with 7 mm zone of inhibition. The minimum inhibitory concentrations of the essential oil ranged from 10 to 100 mg/ml and >100 mg/ml against all the test organisms. The oil was bactericidal against *Bacillus subtilus*, *Staphylococcus aureus* and *Escherichia coli*. Higher concentration of oil was needed for the bactericidal action against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

The antibacterial activity present in the essential oil of *Pamburus missionis* can be due to the presence of tridecanol, hexadeconic

**Table 2**
Antibacterial activity of leaf essential oil of *Pamburus missionis*.

| Organisms | Inhibition zone (mm)[a] | | | Minimal concentration[b] | |
|---|---|---|---|---|---|
| | Essential oil | | Gentamycin | MIC | MBC |
| | 450 μg | 100 μg | 10 μg/disc | | |
| *Bacillus subtilus* | 25 | 10 | 21 | 10 | 10 |
| *Staphylococcus aureus* | 10 | 9 | 20 | 100 | 100 |
| *Escherichia coli* | 12 | 10 | 25 | 10 | 100 |
| *Pseudomonas aeruginosa* | 11 | 9 | 15 | 100 | >100 |
| *Klebsiella pneumoniae* | 7 | 0 | 13 | >100 | >100 |

[a] Includes diameter of disc (6 mm).
[b] Values in mg/ml.

acid, γ-terpinene, pinene (monoterpenes hydrocarbons), l-linalol, thymol, nerol, citral, terpinen-4-ol (oxygenated monoterpenes) and eugenol. It has been reported earlier that the monoterpene composition of the essential oil is responsible for the antibacterial activity. These compounds destroy the cellular integrity by inhibiting the respiration process in the microbial cell (Helander et al., 1998). However, the antibacterial activity of the essential oil may also be correlated to synergistic effect of all the chemical components present in the oil (Dorman and Deans, 2000).

## 4. Conclusions

The results from present study reveal for the first time that essential oil of *Pamburus missionis* have antibacterial activity and attribute its significance in the traditional use for the treatment of various ailments and puerperal diseases.

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