# Practical Pharmacognosy third stage lab.2

# The Isolation and Identification of Piperine from Black Pepper

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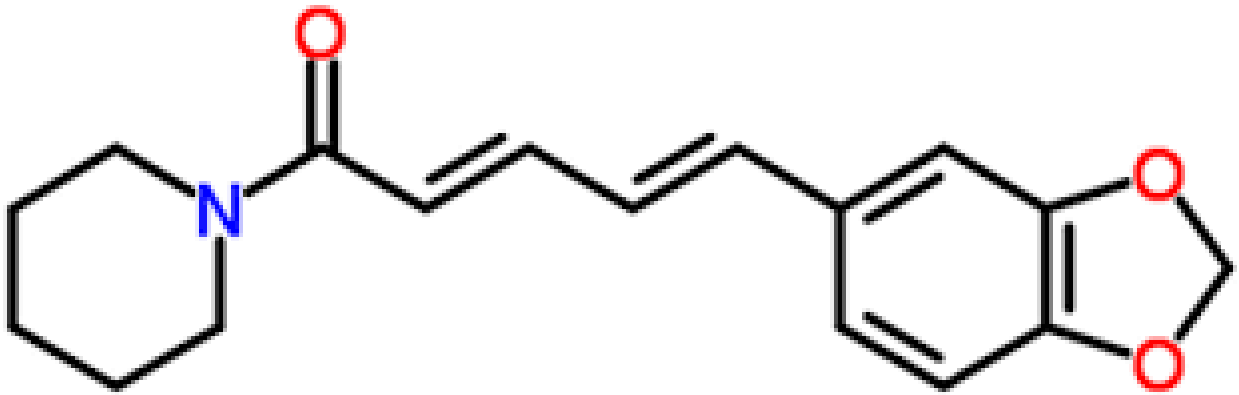
# The Isolation and Identification of Piperine from Black Pepper

- **1.Extraction:**

Aim: isolatation of the piperine alkaloid from black pepper seeds.




Piperine



# The Isolation and Identification of Piperine from Black Pepper





- **Equipments:**
- Large beaker & medium size beakers.
- Soxhlet instrument.
- Funnel & filter paper.
- Water bath.

- **Reagents:**
- 90% ethanol.
- 10% alcoholic potassium hydroxide.

# The Isolation and Identification of Piperine from Black Pepper

- Method of extraction: Soxhlet extraction.
- Plant used: Piper nigrum.
- Part used: Seeds

# Procedure

10 gm of fine powdered of black pepper in 150 ml of 90% ethanol for 2 hrs.In Soxhlet extractor.

↓ Hot filtration

30 ml of extract solution concentrated on water bath at 60°c.

↓ Add

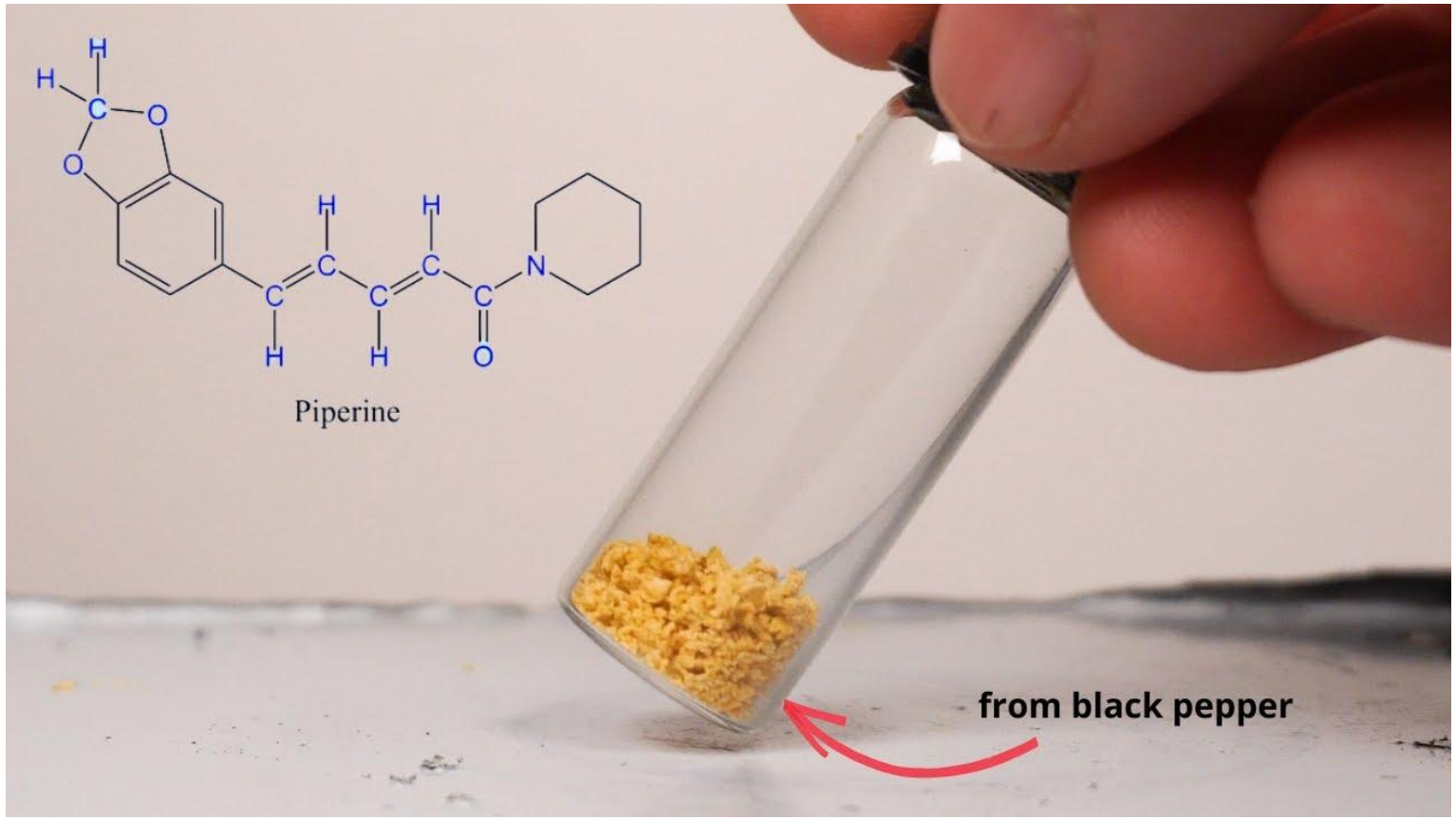
2 ml of 10% alcoholic potassium hydroxide (KOH)

↓

filtrate and alcoholic extract is left over night.

# Results:

Yellow needles with melting point of $125^0$C are deposited.
Yielding 0.3 gm of piperine alkaloids.



# Questions

1- why soxhlet is used instead of reflux apparatus ?

2- why 90% ethanol  is used ?

3- why we used alcoholic KOH ?

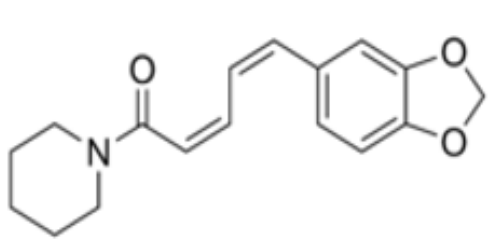
4- why alcoholic KOH and not aq.KOH?

# Discussion

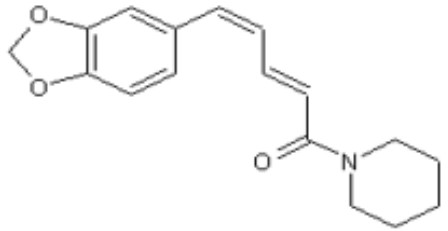
1. The plant is affected by heat; therefore, Soxhlet apparatus is used in its extraction.

2. The use of 90% ethanol is to extract both, the alkaloid and the alkaloidal salt that might be present. (This is true for the extraction of most alkaloids).

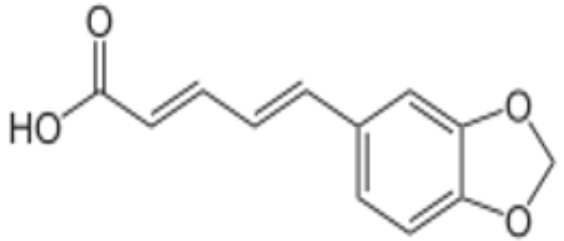
3. The use of alcoholic KOH is to precipitate the isomers of piperine that are chuvacine, isochuvacine and piperic acid.




***Chuvacine***



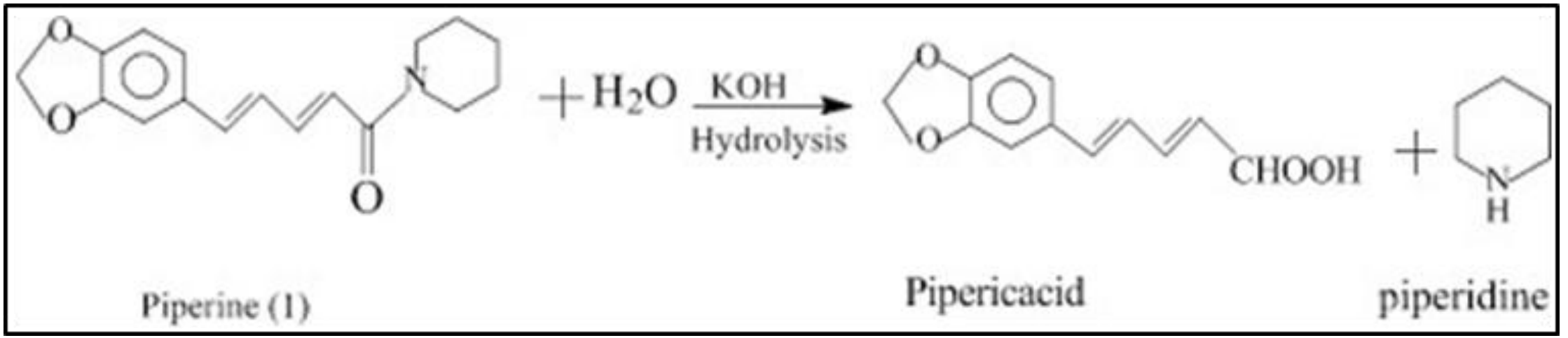

***isochuvacine***




***Piperic acid***

# Discussion

4.Alcohol was used in the preparation of KOH instead of water, since water will hydrolyze piperine  into piperidine and piperic acid as shown below:



# Quantitative and Qualitative Analysis of Piperine Alkaloid :

- **Quantitative Analysis:**
- This is done by weighing the crystals of piperine alkaloid.
- **Qualitative Analysis:**
- The General Chemical Tests :
- 1.Mayer 's Test
- 2.Wagner 's Test
- 3.Dragendorff's Test

# 1. Mayer 's Test:

- Aim: used for the detection of alkaloids in natural products. Mayer's reagent is freshly prepared by dissolving a mixture of mercuric chloride (1.36 g) and of potassium iodide (5.00 g) in water (100.0 ml)
- Equipments and Reagents:
- Petri dish.
- Ethanol.
- HCl.

# 1. Mayer 's Test:




- Procedure:
- Take few crystals of piperine alkaloid and dissolve in few mls of ethanol, in Petri dish then add 2 drops of HCl. Then add 2 drops of Mayer'sreagent.
- Result:
- White precipitate will occur.

# 2. Wagner 's Test:

- Aim: Phytochemical Screenings test for alkaloids.
- **Equipments and Reagents:**
- Petri dish.
- Ethanol.
- HCl.
- Wagner's reagent.
- **Procedure:**
- Take few crystals of piperine alkaloid and dissolve in few mls of ethanol, in Petri dish then add 2 drops of HCl. Then add 2 drops of Wagner's reagent.
- **Result:**
- Brown precipitate will occur.

# 3. Dragendorff's Test:



- Dragendorff's reagent is a color reagent to detect alkaloids in a test sample or as a stain for chromatography plates.
- **Equipments and Reagents:**
- Petri dish.
- Ethanol.
- HCl.
- Dragendorff's reagent.
- **Procedure:**
- Take few crystals of piperine alkaloid and dissolve in few mls of ethanol, in Petri dish then add 2 drops of HCl. Then add 2 drops of Dragendorff's reagent.
- **Result:**
- Orange precipitate will occur.