

EXPERIMENT NO-4

THE MORPHOLOGICAL, HISTOLOGICAL, POWDER CHARACTERISTICS, EXTRACTION AND DETECTION OF CLOVE BUDS.

Aim of the experiment :

To study the morphological, histological, powder characteristics, extraction and detection of Clove buds.

Requirements:

Apparatus:

Compound Microscope, Petri plate, clove slip, Glass-Slide, beaker, dropper, filter paper, forceps, tripod stand, wire gauze, dissecting needle, sharp razor, etc.

Chemicals:

Conc. HCL, Fluroglucinol, Glycerine, Ethyl acetate, toluene, conc. HNO₃, potassium hydroxide, methanol, benzene, etc.

Theory:

Clove

Synonyms: Clove buds; Clove flower; Lavang (Hindi)

Biological source: It consists of the dried flower buds of *Eugenia caryophyllus*.

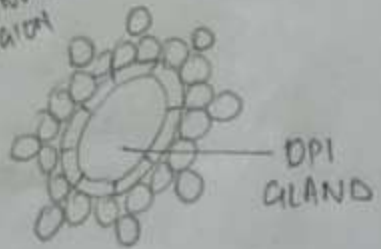
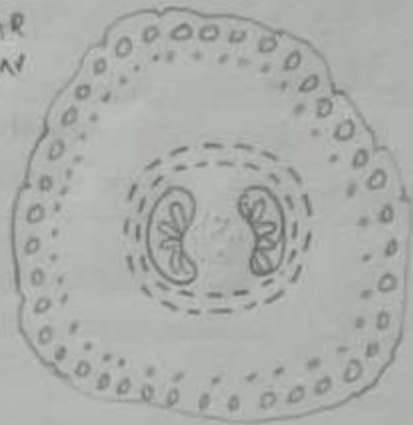
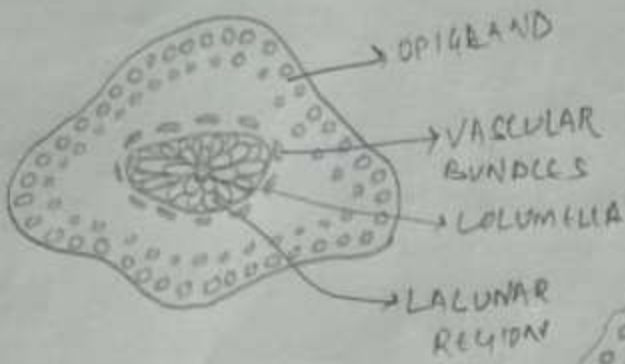
Family: Myrtaceae.

Chemical Constituents: Volatile oil (16-21%), Phenol chiefly Eugenol (80-88), acetyl eugenol (10- 15%), α and β -Caryophyllene. Tannins (10-13%) Pyrogallol tannins and other substances are methyl furfural and dimethyl furfural

Uses: It is used in Ayurvedic medicine, Chinese medicine, and dentistry where the essential oil is used as an anodyne (painkiller) for dental emergencies. Cloves are used as a carminative, antiseptic, stimulant, clove oil and zinc oxide are used in temporary filling of dental cavities.

MACROSCOPICAL CHARACTERS:

- 1) **Size & Shape:** Length varies from 12 to 17 mm. Sub-cylindrical slightly flattened shape.
- 2) **Type:** Actinomorphic, bisexual, epigynous. The flower bud has a spherical head and a sub cylindrical hypanthium tapering at the lower end.
- 3) **Calyx:** Polysepalous, 4 hard and thick sepals with oil glands
- 4) **Corolla:** Polypetalous, 4 petals imbricate, enclose the stamens and forms the head of the bud.
- 5) **Androecium:** numerous stamens, free and introrsely;



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6) **Gymnasium:** Binocular, inferior with ovules stamens, free placentation axial

7) **Style:** Single and erect about 3mm long

8) **Colour:** Dark brown

9) **Odour:** Aromatic, spicy, Strong

10) **Taste:** Pungent, aromatic.

MICROSCOPICAL CHARACTERS:

1) **Epidermis:** Single layered small cells with straight walls and has a very thick cuticle. Epidermal layer gets intercepted by Ranunculaceous type of stomata.

2) **Cortex:** The three distinct zones or regions in the cortex can be made out.

1. The peripheral region containing 2 to 3 layers of big, ellipsoidal, schizo-lysigenous Oil glands embedded in the radially elongated parenchymatous cell

2. The middle region containing 1 or 2 rings of bicollateral vascular bundles associated with a few pericyclic fibres, embedded in thick walled parenchyma and

3. The inner region made of loosely arranged parenchyma, composed of air spaces, separated by lamellae.

3) **Columella:** Forms the central cylinder containing thick walled parenchyma with a ring of bicollateral vascular bundles towards the periphery of the cylinder. Numerous sphaeraphides are seen scattered throughout the columella and to a certain extent in the middle cortical zone.

POWDER CHARACTERISTICS:

Oil glands: Fragments of parenchyma showing long oval schizolysigenous oil glands.

Aerenchyma: Option of loose parenchyma with air spaces.

Pollen grains: Grains having diameter of 15 to 20 μ , small, biconvex, rounded or tri-angular in shape

Fibres: Sclerenchymatous fibres with parenchymatous cells.

Anther: Fibrous layer of anther with reticulated cells

Calcium oxalate crystals-Sphaerophides

EXTRACTION OF VOLATILE OIL:

Firstly choose the Clevenger apparatus for volatile oil extraction. Take the accurately weighted quantity of clove buds and extract clove oil by water distillation method. As clove oil is heavier it forms lower layer collect that for further isolation of eugenol. Dissolve 10 ml oil with 100 ml solvent ether and the mixture was extracted with three portions of 10% KOH solution. Separate aqueous alkaline layer and wash with fresh ether, acidify with mineral

Micro chemical test

Sl. No.	Reagents	Observation	Characteristics
1	Phloroglucinol - Conc. HCl (1:1)	Red/pink	vascular bundle x fibres
2	Strong KOH solution	needles shaped pt. crystalline crystals	Benzenal
3	Dil. HCl	crystals soluble	Calcium oxalate crystals
4	Sulphuric acid (60%)	Calcium sulphate crystal formation	Calcium oxalate crystals
5	Sudan Red - III	Pink	o/t gland

Calculation

$$\textcircled{1} \text{ \% yield} = \frac{\text{Essential oil wt.}}{\text{Sample wt.}} \times 100$$

$$= \frac{0.783}{100} \times 100$$

$$= 0.783$$

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

$$= \frac{3.0}{4.7}$$

$$= 0.63$$

acid. The acidic solution is again extracted with two successive portion of solvent ether combine all the mixture and wash with water and lastly distilled off ether to get liquid

CHEMICAL TEST

Sr. no	Tests	observation	Interference
1	Ag. extract + Lead acetate solution	White ppt	Tannins
2	Clove oil + alcohol + 5% ferric chloride	Blue colouration	Eugenol
3	Aq. extract + 5% ferric chloride	Dark colour	Tannins

DETECTION BY TLC :

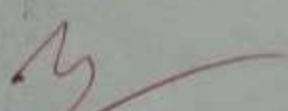
Volatile oil is identified by thin layer chromatography by using silica gel as stationary phase and Ethyl acetate: toluene (93:7) as mobile phase. After drying the TLC plate, the spot was detected after spraying vanillin- sulphuric acid solution. Calculate the Rf value by using formula.

Result:

From the above experiment the morphological, histological & powder characteristics of clove bud were studied.

The percentage yield of volatile is from clove bud was found to be 0.783%.

The extracted volatile oil were identified by TLC method
Rf value is 0.63


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