

EXPERIMENT NO-3

THE MORPHOLOGICAL, HISTOLOGICAL, POWDER CHARACTERISTICS, EXTRACTION AND DETECTION OF SENNA LEAVES

Aim of the experiment : To study the morphological, histological, powder characteristics, extraction and detection of Senna leaves.

REQUIREMENTS:

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

2. Chemicals: Conc. HCL, Fluroglucinol, Glycerine, n-propanol, ethyl acetate, glacial acetic acid, conc. HNO₃, calcium chloride, denatured spirit, potassium hydroxide, methanol, benzene, etc.

THEORY:

SENNA

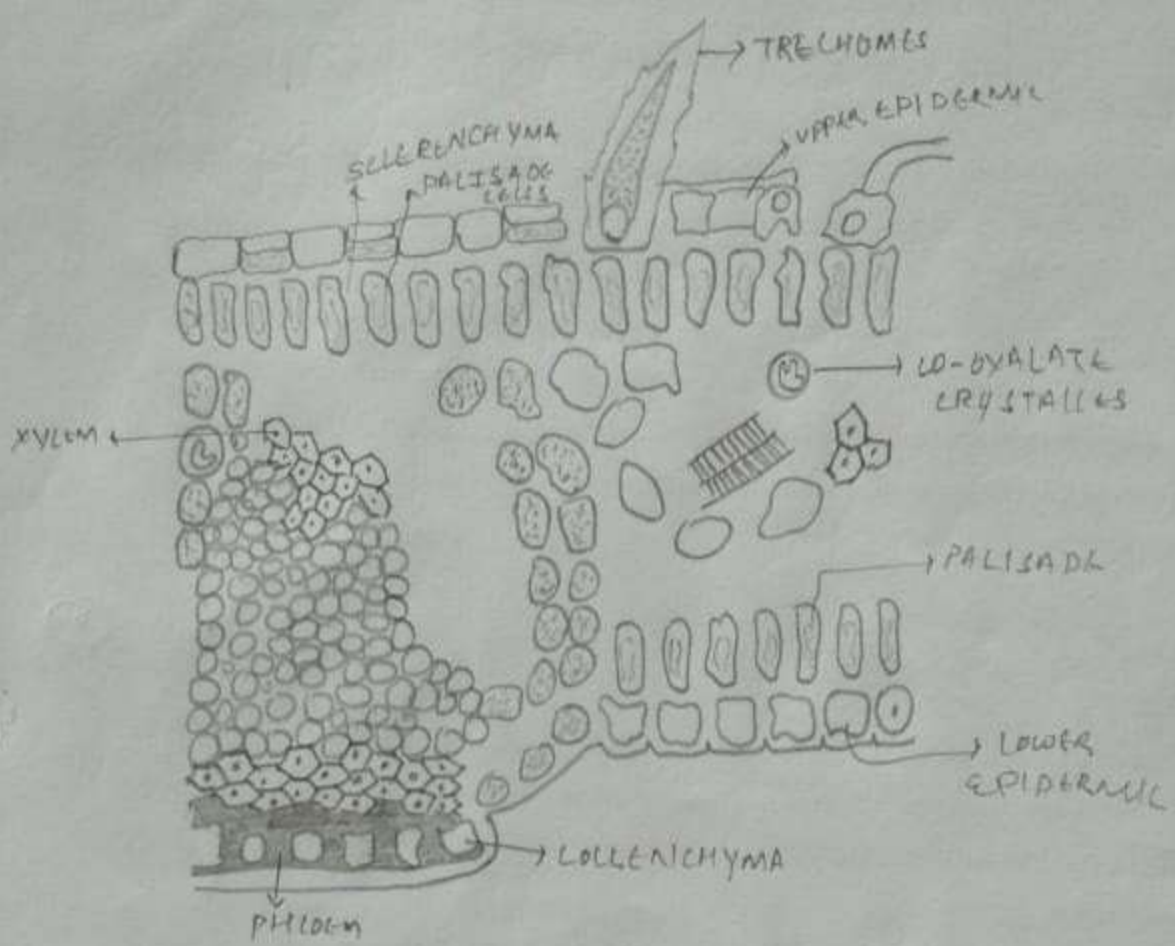
Synonyms: Senna leaf, Sennace folium, Tinnevelley Senna, Indian Senna

Biological Sources: It consist of dried leaflets of *Cassia acutifolia* (Alexandria senna) or of *Cassia angustifolia* (Indian or Tinnevelley Senna) belonging to the family Leguminosae.

Chemical constituents: Dianthrone glycosides (1.5-3 %) Sennosides A, B, C and D. Free anthraquinones are also present and several other glycosides such as palmidin A and aloe-emodin dianthrone diglycosides are also present. Senna also contains flavanols such as kaempferol (yellow color) and isorhamnetin. Traces of chrhanic acid, saponin, salicylic acid and volatile oils have also been found.

Uses: Senna is usually employed as purgative in habitual constipation. The anthraquinones irritate and stimulate the colon thereby enhancing its peristaltic movements causing bulky and soft excretion of faeces. The inherent action of senna is associated with appreciable griping, and therefore, it is generally dispensed along with carminatives so as to counteract the undesired effect.

T.S of Senna leaf



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MORPHOLOGICAL CHARACTERS:

Features	Alexandria senna	Tinnevelley senna
Colour	Pale greyish green	Yellowish green
Odour	Slightly characteristic	Slightly characteristic
Taste	Mucilaginous and slightly bitter	Mucilaginous, bitter and characteristic
Size	Length - 2-4 cm	Length - 2.5-5cm
Width	7-12 mm	3-8 mm
Shape	Ovate -lanceolate	Lanceolate
Texture	Thin and brittle	Thin and flexible

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MICROSCOPICAL CHARACTERS:

The transverse section of a senna leaflet exhibits isobilateral structure under the microscope. The following tissues are observed in the lamina and midrib region:

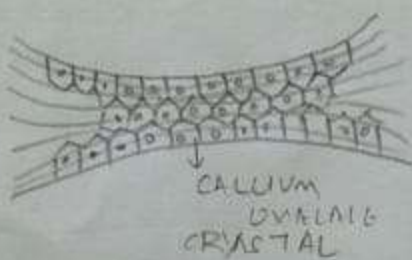
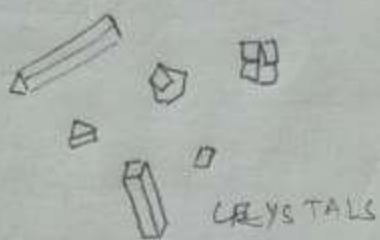
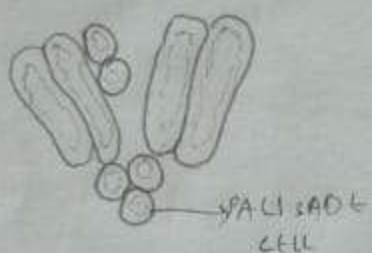
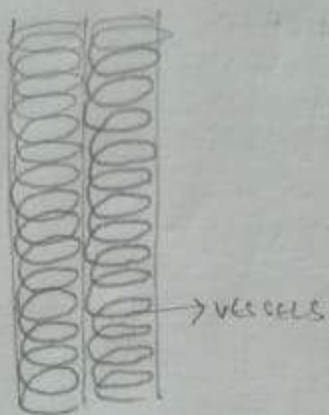
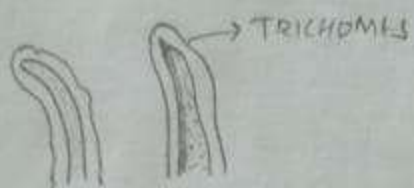
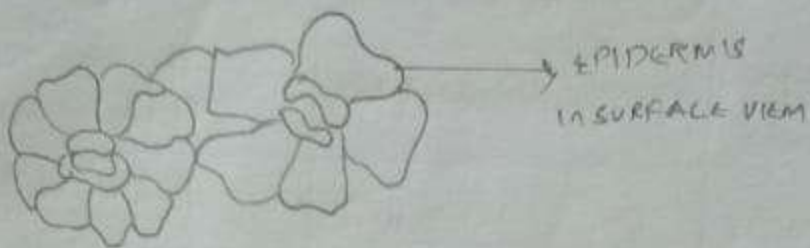
a) Lamina:**I) Upper Epidermis:**

It is composed of polygonal cells arranged in a single layer, covered on the outer side with prominently thick, warty cuticle. Few epidermal cells contain mucilage and straight anticlinal walls. The epidermis bears only nonglandular covering trichomes which are unicellular, short, thick walled, conical, non-lignified, warty and often curved at the bulbous base or with papillose walls. Paracytic stomata are seen at regular intervals.

II) Mesophyll:

It is differentiated into palisade and spongy parenchyma. Isobilateral structure exhibits presence of upper palisade below the upper epidermis and lower palisade placed above the lower epidermis:

I. Upper Palisade:



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It is a single layer of elongated, narrow, columnar cells with chloroplasts. The upper epidermis also continues over the midrib region.

2. Spongy Parenchyma:

It is made up of loosely arranged parenchymatous cells and contains rosette or prismatic crystals of calcium oxalate.

3. Lower Palisade:

It extends to somewhat limited area, i.e. to the lamina region only. Cells are small and loosely arranged and have wavy walls. Cells possessing prominent cuticle and sunken stomata are seen. These cells are somewhat shorter than those of the upper epidermis walls and have slightly wavy. Non-glandular trichomes are also found on the lower

b) Midrib:

The transverse section through the midrib region exhibits a flat ventral surface and convex dorsal surface. The epidermal layers are in continuation over the midrib also. The lower epidermis possesses small cells with thick cuticle. The upper palisade is also made-up of smaller cells particularly in the midrib region. The lower palisade is absent in the midrib portion, and a group of collenchymatous cells is seen. At the centre, a group of collateral vascular bundles with xylem on the upper side and phloem beneath. The both ventral and dorsal sides by an arc of lignified vascular bundles are covered on sclerenchymatous fibres. These patches of fibres are somewhat ovate in shape and crescent shaped below. This fibrous arc is characteristic as these fibres are encircled by a layer of parenchyma, with cells of most of it containing prisms of calcium oxalate crystals

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POWDER CHARACTERISTICS:

Epidermal cells:

Polygonal, straightwalled cells with paracytic stomata with subsidiary cells around the guardcells.

Covering trichomes:

Unicellular, thick warty walls, acute apex, bulbous base, narrow lumen and conical in shape lie appressed to the epidermis. Length 70-260 μ and width 12-25 μ .

Xylem vessels:

Angular thickening and lignified.

Calcium oxalate:

Crystals isolated or in parenchymatous cells. Very abundant, occur as prisms and also as clusters

Microchemical test

Sl. No.	Reagents	Observation	Characteristics
1	Phloroglucinol + conc. HCl (1:1)	Red / pink	Signified tissue xylem (vessels & tracheids)
2	Ruthenium Red	Dark	cleavage cells
3	dil. Acetic acid	Crystals insoluble	Calcium oxalate crystals
4	Dil. HCl	crystal soluble	Calcium oxalate crystals
5	Sulphuric acid (6%)	Calcium sulphate crystal formation	Calcium oxalate crystals
6	Sudan Red (III)	Pink	cutin / cuticle

Identification Test

⑥

Sl. No.	Test	Observation
1.	Over the surface of the stem, a thin layer of white powder is present. This powder is soluble in water. Add a few drops of dil. sulphuric acid (1:1) to the powder. A white precipitate is formed. This precipitate is soluble in water. Add a few drops of dil. sulphuric acid to the solution. A white precipitate is formed. This precipitate is soluble in water. Shake slightly, the precipitate is soluble.	Lower epidermal layer shows pink/red colour.

Calculation

$$\% \text{relat.} = \frac{\text{essential oil wt.}}{\text{Sample wt.}} \times 100 = \frac{0.985}{110} \times 100 = 0.656\%$$

$$\% \text{value} = \frac{\text{Distance travelled by solvent}}{\text{Dist. travelled by solvent}} = \frac{2.6}{5.0} = 0.56$$

EXTRACTION OF SENNOSIDES:


The weighted quantity of dried powdered senna leaves was extracted with benzene for 30 min on electric shaker, filtered in vacuum and benzene get distilled off. The left over marc was dried at room temperature and extracted with 70% methanol for three hrs. and filtered under vacuum. The marc was re-extracted with 50 ml of 70% methanol for 15min, filtered and the methanolic extracts get combined. The methanolic extract was concentrated to 1/4th volume, acidified to pH 3.2 by adding HNO₃ with constant stirring. It was set aside for 15 min. at 5°C, filtered and 1g of anhydrous calcium chloride in 10 ml of denatured spirit was added with constant stirring. The pH of the solution was adjusted to 8 by addition of potassium hydroxide (KOH) and set aside for 15 min. filter and collect the precipitate obtained in as calcium sennoside dried and weighed. The percentage yield was also calculated.

IDENTIFICATION BY TLC :

Sennosides is identified by thin layer chromatography by using silica gel as stationary phase and n-propanol: ethyl acetate: water: glacial acetic acid (40:40:29:1) as mobile phase. After drying the TLC plate, Sennosides is detected at a particular R_f value after spraying reagent used is HNO₃-KOH reagent.

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RESULT: From the above experiment the morphological, histological & powder characteristic of senna leaf was studied.
The percentage yield of senna leaf was found to be 0.55%.
The extracted senna leaf were identified by TLC method
x R_f value = 0.52


Signature of Faculty

Name: - Jenish Patel

Regd. No: - 1703252052

Group: - C