

Pharmacognostic investigation of the leaves of *Otostegia limbata* (Lamiaceae)

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Abstract: Pharmacognostic investigation of the fresh, powdered and anatomical sections of the leaves of *Otostegia limbata* (Benth.) Boiss was carried out to determine its macro- and microscopical characters and also some of its physical constants. The leaves are symmetrical at the base, have an entire edge, an obtuse apex, and a pilose surface. Anisocytic stomata, unicellular, uniseriated-covering trichomes with swelling bases and acute apexes, a prism, clustered calcium oxalate crystals, and fibre components are all present internally. Proteins, starch grains, lignin, mucilage, lipids, and oils were all found by chemo-microscopy. The study's findings may help establish certain diagnostic criteria for the identification of the plant and the creation of a monograph on it.

Key words: pharmacognostic standardization, macro- and microscopical characters, leaf morphology of *Otostegia limbata* (Benth.) Boiss.

1. Introduction:

The *O. limbata* belongs to the Lamiaceae family having 40–60 cm tall, heavily branching shrub. Stems are pubescent with short, spreading eglandular hairs, quadrangular on younger shoots, and pale yellow or brown. The leaves are grouped, whole, oblong-oblongate, 10–20 x 5-8 mm, coarsely glandular-punctate, and covered in short, adpressed hairs, they are scattered across the stem and merge into the bracts; and they have spines that are around 10 mm long in the axils. They are 6–10 flowering, remote verticillasters. Multiple, hard-spiny, spreading-erect bracts that are about 10 mm long and pale in colour. Calyx: heavily pubescent to pilose, with sessile oil globules; tube tubular-infundibuliform 7-8 mm, densely bearded in throat; limb straw-colored, reticulate nerved, broadly turbinate, asymmetrically lobed, often with intermediate irregular smaller lobes; all apiculate or toothed; larger lobes up to 7-8 mm; smaller 4-5 mm. Upper lip is about 12 mm long, falcate, vinous, and long-bearded at margin; corolla is 15-20 mm long, yellow or orange-yellow; tube is about 7 mm included within calyx. 3.5 x 2.5 mm, oblong, rounded at the apex, nontrigonal, and frequently only one developing nutlets [1]. The

Lamiaceae, formerly called Labiatae, Which are the largest family of flowering plants in the Lamiales order is the mint family, which contains 236 genera and more than 7,000 species. Nearly all species of the Lamiaceae family are grown for their scented leaves and beautiful blooms. Humans place a great deal of value on the family of herb plants because they have flavour, aroma, or therapeutic benefits. (True epiphytes) as well .The family Lamiaceae includes one of the well-known genera *Otostegia*, which is geographically widespread around the world. There are roughly 4000 species and 220 genera in this group of flowering plants. *Otostegia limbata*, also known as *Rydingia limbata* (Benth.) Scheen & V. A. Albert and *Ballota limbata* is a significant medicinal plant of this genus [2]. The common names "Spin aghzai," "Chiti booti," "Chitti jharri," "Spin azghay "and" Bui" are used to identify certain plant species. The plant's distinctive features include a cluster of pale yellow flowers, oblong leaves with a thick pointed tip, pointy bracts, and a tiny petiole. Plant species contain a variety of chemical components, including the acids ballotenic and ballodiolic, limbatolide A, B, C, and D [3]. The *Otostegia limbata* is commonly used as an ethnomedicine in Pakistan for a variety of ailments, including jaundice, cancer, scabies, boils, goitre, ulcer, cuts, wounds, dental issues, and animal diseases [4]. Traditional healers use fresh leaf infusion to treat conditions including acidity, hypertension, depression, ulcer, jaundice, gum disease, and ocular infection in Pakistan's Azad Jammu & Kashmir, KPK, Punjab, and Himalayan regions [5]. Herbs can be annual or perennial, subshrubs or shrubs on occasion, and are typically aromatic. Branch and stem angles are typically four. There are no stipules on the opposite, infrequently whorled or alternate, simple to pinnately dissected, or compound, leaves. Typically compound inflorescences, occasionally with single, axillary flowers; verticillasters with two to many blooms, covered in leaves or bracts. Flowers can be bracteolate or not, bisexual, zygomorphic, or subactinomorphic. The calyx is persistent, 5-toothed, and 2-lipped. The upper lip is 3-toothed or complete (deciduous in *Scutellaria*), while the lower lip is 2- or 4-toothed. The inside of the tube is occasionally hairy and annulate. Usually, the corolla limb has two lips, with the top lip having two lobes and the lower having three. Less frequently, the upper lip is entire and the lower has four lobes. Stamens epipetalous, 4 or 2, free, rarely filaments connate, sometimes one staminodial; anther 1- or 2-celled, usually dehiscent longitudinally; disc persistent Ovary superior, 2-celled, with each cell having two ovules, and with the style subterminal, or ovary 4-parted, with each lobe Style gynobasic, 1-ovuled, and 2-cleft at the apex (from bases of ovary lobes). Fruit typically has four

dry nutlets, seeds that contain or lack endosperm. There are about 3500 species in 220 genera that are found all over the world, but primarily in the Mediterranean and SW Asia. China includes 96 genera and 807 species [6]. Traditional medicines made from *O. limbata* have a long history and are used for a variety of ailments. The plant's juice is a useful remedy for treating children's bleeding gum issues as well as for treating wounds and treating eye conditions. A local remedy for treating various eye infections is an extract made from crushed fresh leaves and little water [7].

2. Materials and methods

The plant sample was collected from district (Mansehra) during session June 2021 and Identified by Prof. Dr Ghulam Mujtaba Shah, Chairman, Department of Botany, Hazara University Mansehra KP, Pakistan. After identification the voucher Number (15060) was assigned to the plant species and specimen were deposited in the Herbarium of Hazara University (HUP) for permanent record.

2.1. Macroscopical Features

The macroscopical observations of the leaves and organoleptic characters i-e odour, taste, texture along with colour size, shape, fracture, fracture surface, presence or absence of rootlets, margin, venation, apex, duration, type, phyllotaxis, presence and absence of petiole and stipule were analyzed by following the standard procedure proposed by [8]. Morphological observations of the stem leaves and flowers of both the plants were examined by digital microscope (Portable USB Digital Microscope 20x-800x).

2.2. Anatomical Features

Microscopic and anatomical features of the leaves of selected research plants were examined by free hand section cutting. Leaves were preserved in the fixative i-e Formalin Acetic Acid (FAA) for 24-48 hours. Fixative composition was: FAA = 5:5:90 5ml Formalin, 5ml Acetic acid, 35 90 ml Ethanol. Transverse free hand-section cutting of the leaves of *O. limbata* was carried out from fresh material. Using a sharp microtome and 1% safranin for two to three minutes, thin slices were created; afterwards these sections were gradually dehydrated by treating with different alcoholic grades for 2-3 minutes in each grade i-e 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and finally 100% alcohol (ethanol). Section of plants after dehydration were placed on the petri dish and treated with a drop of light green for 2 minutes.

Afterwards the sections were mounted on a permanent glass slides, with a drop of Canada balsam [9].

2.3. Powdered Microscopy

Powdered drug analysis of *O. limbata* was supported by succeeding the standard method of [10, 11], with certain modifications to study of the physical and microscopical characteristics of the drug samples. Physical parameters includes the organoleptic characteristic such as taste, colour and odour, although microscopic studies was done to analyze different structures present in the plant's powder. A touch of fine powder was taken in a test tube and boiled for 1-2 minutes, afterwards 70% Chloral Hydrate was added and heated again for 2 mins and extra water was poured off from the test tube and again treated with iodine solution in the same test tube and boiled for 1-2 mins. A drop of this solution is placed on a glass slide and analyzed by using research microscope having objective lenses of 10X, 40X and 100X for observation of different structures, and microphotographs were captured with Nikon microscope fitted with camera.

2.4. Stomatal Number (SN) and Stomatal Index (SI)

The (SN) and (SI) are most specific and distinguishing character for authentication of leaf of crude drugs. Average number of stomata present per square mm of both the surfaces of leaves (abaxial and adaxial) is called the stomatal number and stomatal index in the stomatal percentage to the whole stomatal number found in the epidermis of leaf [12, 13, 14].

Technique

Fresh leaf surfaces from the abaxial and adaxial surfaces were chopped into small pieces, cooked in 30% nitric acid with 2.0 g of potassium chloride for 2 to 3 minutes, and then rinsed with distilled water. After drying, both epidermis layers were peeled off with a forcep and kept in a solution of 60% NaOH for two hours. The peeled parts were then mounted on glass slides and covered with cover slips after being bathed in lactic acid. Size, shape and number of stomata per square mm were observed under 100x 37 magnifications. A standard formula proposed by was used to find out the stomatal index [15, 16].

3. Results

3.1 Organoleptic Characters (Macroscopic)

Organoleptic approach was used to macroscopically observe and record the powder drug's flavour, consistency, aroma, and colour. The outcomes of *Otostegia limbata*'s organoleptic

tests have been noted and described in (Fig 4.2.1, table 4.2.1). The leaf powder resembles green, has a fine texture, a flavour that is slightly bitter, and a fragrant scent.

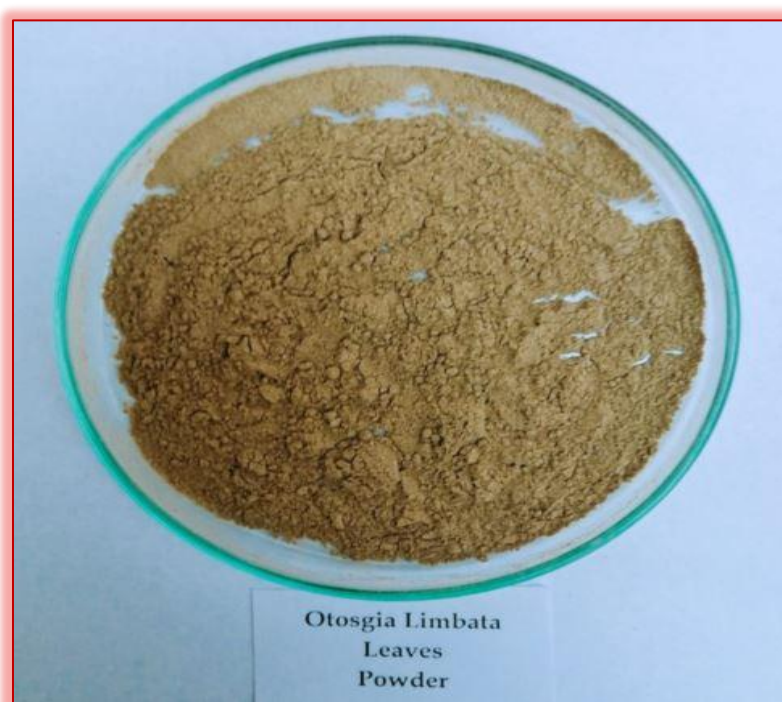


Fig 1. Powder of leaves of *Ostegia limbata* (Benth.) Boiss.

Table 3.1 Macroscopic characters of powder drug of *Ostegia limbata* Leaf

S. No.	Characters	Observations
1	Odor	Aromatic
2	Color	Dark Brown
3	Texture	Fine
4	Taste	Slightly bitter

3.2 Microscopical Characters

A microscope was used to observe the powdered drug made *Ostegia limbata* leaves under (10X- 40X) and different type of cells like epidermis cell, parenchyma cell, mesophyll fragments of leaf, reticulate tracheid, non-granular trichome, and reticulate parenchyma were observed. In addition to taking photos of these cells, their lengths and widths were also measured using a micrometer. (Fig 1, Table 3.2).

Table 3.2. Microscopical Characters of *Otostegia limbata*

Cell types	Length in (μm)	Width in (μm)
Parenchyma cell	57 μm	14 μm
Epidermis cell	42 μm	32 μm
Mesophyll fragments of leaf	55 μm	33 μm
Reticulate tracheid	59 μm	20 μm
Non granular trichome	26 μm	12 μm
Reticulate parenchyma	40 μm	20 μm

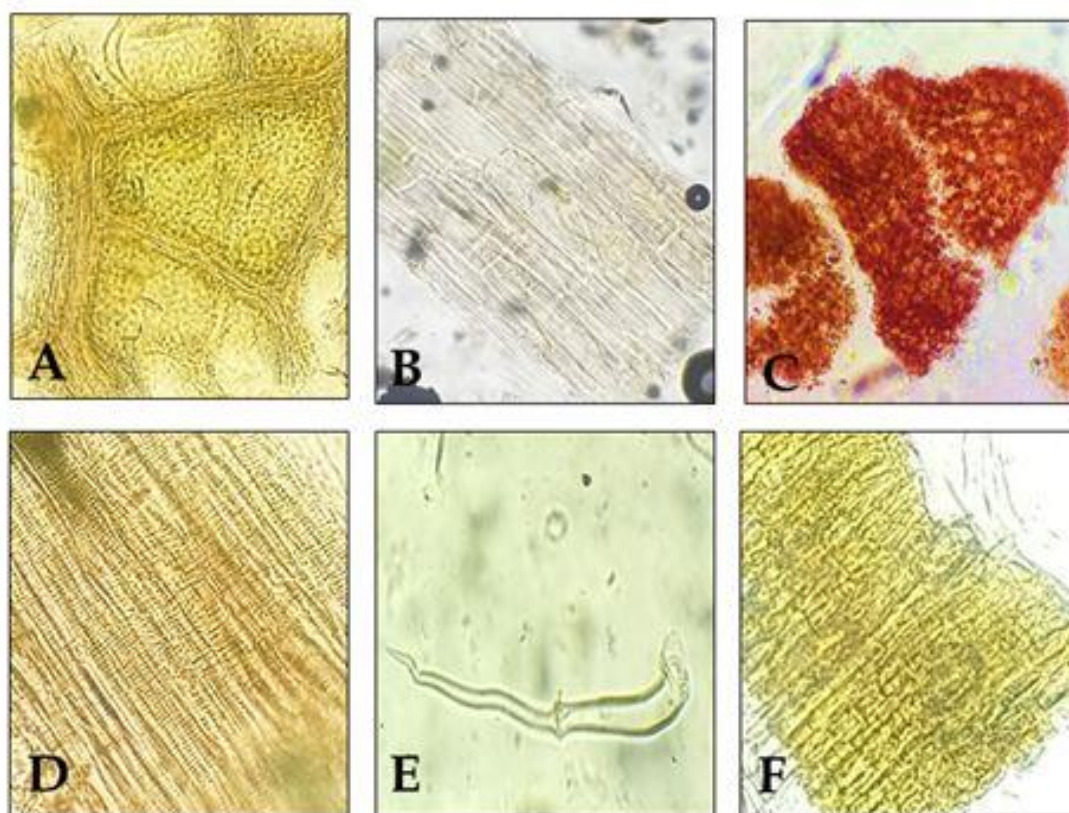
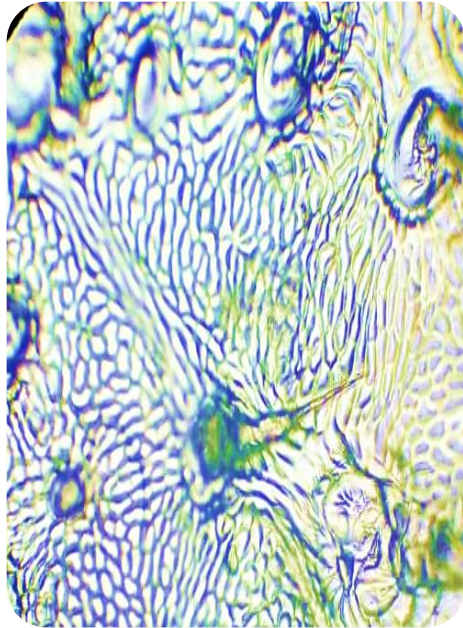


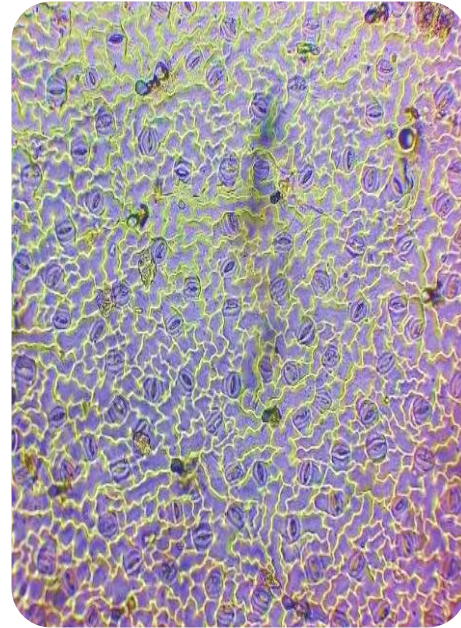
Fig 2.2. Powdered drug of leaves A- Epidermis cell B- Parenchyma cell C- Mesophyll fragment of leaf D- Reticulate tracheid E- Non granular trichome F- Reticulate parenchyma

3.3. Epidermal anatomy

The abaxial and adaxial surfaces of epidermal anatomy were determined.



Adaxial surface



Abaxial surface

3.3.1. Qualitative analysis

The qualitative study revealed that the stomata on the abaxial and adaxial surfaces were anomocytic. Stomata were present on both the abaxial and adaxial surfaces. Compared to the other surfaces, the abaxial surface showed more stomata. There were stomatal guard cells with a bean form. Trichomes were visible on both surfaces, as well.

3.3.2. Quantitative analysis

The number of stomata and epidermis were determined using a quantitative analysis. Stomata counted 135 while epidermal cells totaled 290. Under a 10x power microscope, the length and width of the epidermal cells, guard cells, and trichomes were measured in millimetres.

Table 3.3.1. Qualitative characters of *Otostegia limbata*

S. No.	Qualitative character	Observations
1.	Layer of epidermis	Double
2	Shape of epidermis cell	Undulate
3	margins of Epidermal cell	Wavy
4	Types of Trichome	Non-granular, uniseriate
5	Trichome	Present
6	Presence and absence of stomata	Present on both sides
7	Shape of guard cell	Bean shape
8	Stomata types	Anomocytic
9	Margins of subsidiary cells	Undulating

Table 3.4. Quantitative characters of *Otostegia limbata*

S. No.	Qualitative Character	Observation in μm
1	No. of epidermal cells /area	290 μm
2	Length of epidermis cell	40 μm
3	Width of epidermis cell	20 μm
4	No of Stomata/area	135 μm
5	Width of stomata	15 μm
6	Length of Stomata	13 μm
7	Length of guard cells	25 μm
8	Width of guard cells	10 μm
9	Length of trichomes	200 μm
10	Width of trichomes	10 μm
11	Subsidiary cell length	20 μm
12.	Subsidiary cell width	25 μm

3.4. Stomatal Number (SN) and Stomatal index (SI) analysis

There were stomata in both the upper and lower epidermis. Based on the quantity of stomata and the quantity of epidermis cells, the stomata index was determined (Table 3.5)

Table 3.5. Stomatal Index

Numerical data	
Number of epidermal cell	290 μm
Number of stomata	135 μm
Stomata index	31.75 μm

Stomatal index (SI) = $S \times 100 / S + E$

- SI = Stomatal index
- E = Number of epidermal cells per unit area
- S = Number of stomata per unit area

3.6. Section cutting of leaf

Under a microscope, the transverse section of leaf was observed at 4X and 10X which show the presence of following different cells (Fig 3).

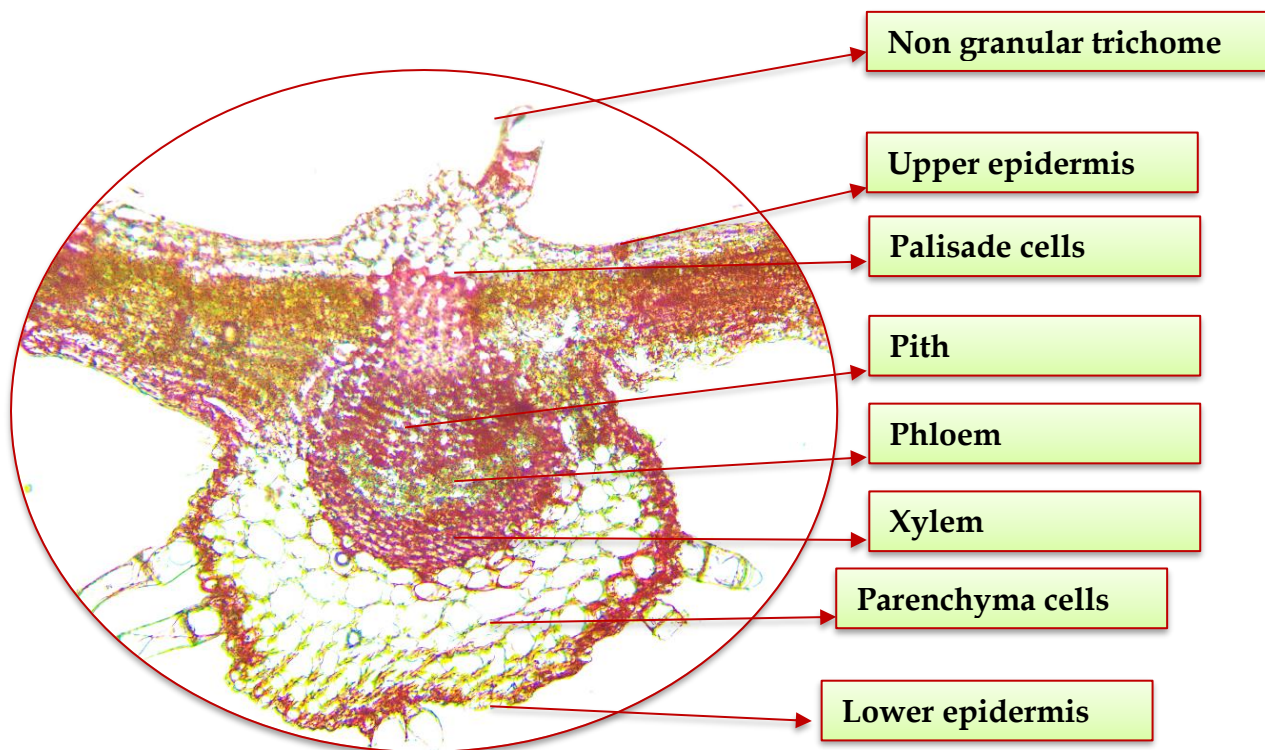


Fig .3. Transverse section of *Otostegia limbata* leaf

Discussion

Pharmacognosy is a scientific knowledge of identification and classification of crude drugs. For the identification of crude drugs, pharmacognostic study constitutes unique importance. In the current work pharmacognostic study of *Otostegia limbata* was carried out to document the macroscopic study of leaf of selected plants, microscopic study that is anatomy of leaves (Transverse sections of leaf), epidermal anatomy, stomatal index, powdered drug and phytochemical analysis of leaf of selected research plants was studied for, while the different extracts of the various parts studied plants were analyzed for bioassays and ethanolic extract of the aerial parts of the *Otostegia limbata*. Powdered drug study of *Otostegia limbata* along with microscopy, transverse section of leaf as well as leaf constant values such as stomatal index was analyzed. Stomatal number and stomatal index value for *Otostegia limbata* is 290 μm , 135 μm and 31.75 μm (Table: 3.5).). These studied the stomatal number and Stomatal index of *Skimmia laureola* and *Zanthoxylum armatum* of the family Rutaceae [17]. In case of *Otostegia limbata* stomatal number and stomatal index values were in the range of 120 to 140 (130) and 10 to 13 (11.5) per mm^2 . Transverse section of leaf of selected research plants is Abaxial and adaxial surface of the leaves are composed of closely packed epidermal cells, a waxy cuticle covers the outer walls of the epidermal cells which is thicker on adaxial surface as compare to abaxial surface. Schizogenous canal glands are also prominent which is the characteristics feature of the members of family rutaceae for secretion of essential oils. Schizogenous gland sac on leaves is the characteristics for the members of Rutaceae family [18]. *Otostegia limbata* leaf section cuttings were examined under a microscope, and the transverse section of the leaf was studied at 4X and 10X, revealing the presence of the following distinct cells (Fig .3). The powder drug's flavour, consistency, aroma, and colour were macroscopically examined and noted using the organoleptic approach. The organoleptic results of *Otostegia limbata* are shown in (Fig.1, table.1)). The leaf powder has a fine texture, a flavour that is slightly bitter, and an aromatic scent. It seems to be green in colour. The powder drug's flavour, consistency, aroma, and colour were macroscopically examined and noted using the organoleptic approach. Other

researchers examined several plants from various families of plants that were powdered drugs [19]. They are performed the histochemical analysis of the various plants of family Solanaceae for authentication and classification of medicinal plants [20], performed the powdered drug analysis of leaf of *Tridax procumbens*, analyzed epidermal cells, fibers, trichomes and calcium oxalate crystals of *Ficus bengalensis* in powdered drug [21]. The plant were studied the powdered drug of *Calotropis procera* [22]. They are revealed the presence of xylem vessels, pitted xylem fibers, xylem parenchyma, calcium oxalate crystals and starch grains and multiseriate medullary rays in powdered drug analysis of *Sesbania sesban* [23]. They are carried out the powdered drug analysis of root of *Chenopodium album* and differentiated different cells i-e starch grains, different type of vessels, fibers and parenchyma cells [24]. The plants were reported the powdered drug analysis of *Atropabelladoona* root and found the parenchyma cells, medullary rays and starch grains studied the powdered drug of *Datura stramonium* leaves and identified the stomata, non-glandular trichomes, crystals of calcium oxalate palisade parenchyma and spongy parenchyma [25].

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