Research Article

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MICRO-MORPHOLOGICAL AND PHYSICOCHEMICAL STANDARDISATION OF *OPUNTIA ELATIOR* MILL. CLADODE: AN UNEXPLORED TRADITIONAL PRICKLY PEAR CACTUS PLANT FROM MAHARASHTRA, INDIA

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ABSTRACT

Opuntia elatior Mill. (Cactaceae) is a significant medicinal herb In Maharashtra. In Ayurveda, it is referred to as Nagphani, and in English, prickly pear. Its fruits and cladode have long been used to remedy wounds, boils, coughs, fever, inflammation, and asthma. Despite its significant therapeutic potential, there isn't much information about standardised criteria for the species. Consequently, the current project was started to produce a comprehensive report on *Opuntia elatior* Mill's quality control and standardisation criteria. The current study aimed to highlight this traditional herbal crude, which would make it easier to identify fresh and dried samples using macro, microscopic, fluorescence, and physicochemical parameters in accordance with WHO guidelines. The present study's compilation of botanical characteristics can serve as a benchmark for the herbal medicine industry in identifying adulterations in market samples utilised for manufacturing different herbal remedies. These studies will provide reference information for accurately classifying *Opuntia elatior* Mill among closely related species within the same genus and family. They will also aid in identifying adulteration in market samples utilised to produce a range of herbal remedies.

Keywords: Cactaceae, Micro-Morphology, Nagphani, *Opuntia Elatior* Mill. Cladode, Spines And Glochids

INTRODUCTION

Nowadays, natural-sources drugs are gaining popularity, and it is estimated that they will account for 25% of all prescribed medicines on the global market. According to the World Health Organization (WHO), traditional medicine is preferred by more than 80% of the world's population as their first choice of medication treatment. The human body quickly absorbs phytomedicines and sometimes has a lesser adverse effect than synthetic compounds[1]. Since ancient times, medicinal plants have been essential to treating many disorders and diseases in traditional medicine. Proper characterisation and quality assurance of raw material is crucial in ensuring consistent quality of herbal medication, which will allow us to establish its safety and efficacy[2]. To prevent possible adulterations as well as the misuse of incorrect medications, the authenticity of any raw herbal substance must be tested using established processes. One of the most essential techniques in the botanical identification of an herbal medication is macroscopic and microscopic research[1]. A critical obstacle that has hindered the promotion of the use of alternative medicines in developed countries is the lack of documentation and the absence of stringent quality control measures.

Microscopy is a traditional pharmacopeial method used for plant identification. Microscopic verification of commercial herbal medicines would, therefore, help to raise public awareness about the degree of adulteration and the importance of maintaining consumer safety against globalisation's problems. Because of the advantages of a low amount of sample required, rapidity, consistency, simplicity, and low prices, microscopy has often been used to identify herbal drugs in many nations, as documented in various pharmacopoeias. In addition, histochemical techniques have been employed to identify tissue structure and cellular characteristics, which can be used as species identification markers[3].

Opuntia is a large genus of the Cactaceae family that grows in a dry and semi-arid environment and is a remarkable medicinal herb with over 2000 species. *Opuntia* has about 250 species recorded worldwide[4,5]. The name "*Opuntia*" derives from an old Greek village (Opus), where Tour Fort observed a spiny shrub resembling American *Opuntia*[6]. It's native to northern and southern South America. In 1800, it was introduced to Indonesia, India, and South Africa, where it expanded rapidly[6]. In the desert (less than 250 millimetres annual precipitation) and semi-arid (250-450 mm annual precipitation) locations, many kinds of cactus are grown as a wild plant[7].

This plant has excellent ecological adaptability since it can survive in a wide range of climatic conditions, including drought, high temperatures, and so on[8,9]. Declining water

availability and global deserts may render *Opuntia spp.* much more important as a food production system that includes both fruits and cladode in the future[9]. Humans have been using *Opuntia* species for various purposes for thousands of years[10]. The *Opuntias* are believed to have been accidentally brought into India and other eastern countries by early European travellers who carried them as vegetables to avoid scurvy on lengthy journeys[11]. Seven to eight species have been introduced into India, but only two or three have been neutralised. *Opuntia dilenii* Haw is primarily found in the country's southern regions. *Opuntia Vulgaris* Mill. (syn. *Opuntia monacantha* Haw) is found mainly in northern India. *Opuntia elatior* Mill. is a species that is found in western India[11].

Opuntia elatior Mill. is a well-known xerophytic plant from the Cactaceae family. Nagphani, Phadya Nivdung, and Prickly Pear are some of the popular names for this plant. This is a widespread prickly pear in western India, but it's also been found in South-eastern India, Punjab, Uttar Pradesh, Madhya Pradesh, and Orissa[4].

Opuntia is gaining popularity as a sustainable food production system with vegetative and fruit components[9]. The cladodes, as well as the fruits of the *Opuntia elatior,* have been revealed to have various health-promoting properties. Such as, the ripe fruit pulp is eaten to relieve stomach burning and to provide a cooling effect[12], baked fruit is given internally once a day for patients with asthma[13], and warmed cladode pulp is applied externally to abscesses and boils for quick healing[14]. According to reports, *Opuntia elatior* has antibacterial, analgesic, anti-inflammatory, antidiarrheal, antispermatogenic, antimicrobial, and antidiabetic characteristics[15].

For standardisation, we use different techniques and methodologies to achieve our goal stepwise, e.g., pharmacognostic and phytochemical studies. These steps and processes are helpful in the identification and standardisation of the plant material. Correct characterisation and quality assurance of starting material are essential steps to ensure the reproducible quality of herbal medicine, which will help us justify its safety and efficacy.

Though the primary microscopy of *Opuntia elatior* has been reported, four extensive micro-morphology along with physicochemical standards have not been reported previously on this plant species, thus making this first report, which provides detailed micro-morphology along with the physicochemical standard inclusive pharmacognostic profile of O*puntia elatior* and thereby will be helpful for correct identification and authentication of the species for future studies. The microscopy of the other species of *Opuntia,* like *Opuntia dillenii* and *Opuntia littoralis,* is reported, but not *Opuntia elatior* [7,8].

In this context, the present study aims to find out the micromorphological characteristics and physicochemical parameters of Opuntia elatior growing in the Khandesh region of Maharashtra, India, which would be of immense use in differentiating it from *Opuntia dillenii* and other species of *Opuntia ficus indica, Opuntia ficus amyclaea*, *Opuntia vulgaris* Mill. (syn. *Opuntia monacantha)* .These parameters differ from cultivar to cultivar and are heavily influenced by the surrounding environment[8].

Taxonomical classification

Kingdom: Plantae
Division: Magnoliophyta (Angiosperms)
Class: Magnoliopsida (Dicotyledons)
Subclass: Archichlamydeae
Order: Caryophyllales (Cactales)
Family: Cactaceae
Subfamily: Cereoideae, Opuntioideae, Pereskioideae
Tribe: Opuntieae
Genus: *Opuntia*
Species: *Opuntia elatior* Mill.

MATERIALS AND METHODS

Procurement and authentication

Developing cladodes (continuous parts of the stem) of *Opuntia elatior* were collected from the village Mubarakpur, District Nandurbar, Maharashtra, India, in November 2022 from its natural habitat. The plant was authenticated by Dr. S.R. Kshirsagar, Taxonomist. Dept of Botany, S.S.V.P.S. College of Science Dhule. Maharashtra, India.

Micro-morphological evaluation

Fresh and healthy plants of *Opuntia elatior* were assessed for their external characteristics. Different macroscopic parameters of cladode, flowers, and fruits were noted. Detailed micromorphological characters were studied[16]. (Figures 1-6 and Tables 1-3).

Microscopic evaluation

Fresh *Opuntia elatior* cladode was collected and microscopically examined by freehand. Thin transverse sections were taken by following standard guidelines[17,18].

Powdered microscopy

Powdered microscopy of Fresh *Opuntia elatior* cladode was carried out by following standard guidelines[17,18].

Physicochemical evaluation

Physicochemical parameters such as the percentage of total ash, water-soluble/acid-insoluble ash values, moisture content, and determination of extractive values were determined according to the official methods[17-19].

Phytochemical evaluation

100 g of freshly collected coarse powder was extracted using 70% alcohol till clearance. The extract was filtered and subjected to a qualitative test to identify various phytochemical constituents[17,18].

Fluorescence analysis

A small amount of dried and finely powdered cladode sample was placed on a clean microscope slide placed inside the UV viewer chamber and exposed to visible light, as well as short (254 nm) and long (365 nm) ultraviolet radiations. The colours produced when various reagents were applied to different radiations were recorded [20].

RESULTS

Macro and micromorphology of *Opuntia elatior* mill.

Opuntia elatior is a wide, succulent, upright-growing shrub with a length of 15 to 26 cm and a width of 9 to 15 cm that grows to a height of 1 to 3 metres. (Figure 1) The stems are joined by a narrow, dark green cladode. Cladodes are fleshy, flattened branches of a single internode with restricted growth potential. The flattened cladodes of the *Opuntia* plant that perform photosynthetic functions in place of leaves[9]. Cladode is thickened up to 1-2 cm in diameter and coated with smooth and fine cuticles, which is referred to as "waxiness of surface." In extreme climatic conditions, wax coating is helpful for water retention. In the centre raw, cladode has 6 to 7 greyish brown coloured areoles. (Figure 2)

The name "areoles" refers to the area on the cactus body where spines, hairs, glochids, and flowers develop. Areoles are a unique characteristic of cacti that makes them different from other succulent plants. There are 6 to 7 spines on each areole. These spines are important for identifying *Opuntia elatior* from other *Opuntia* species. The spines of *Opuntia elatior* are generally straight or semierect and thin, whereas those of *Opuntia dilenii* are curved and thick[21]. The longest spine in *Opuntia elatior* is up 4 to 5 cm long. The matured spine is grey, while the young spine is yellowish-brown. The spine's surface has been observed to be grooved. The central spine is twisted and flexible. Each areole has a different number of glochids depending on its age. Glochids-small hair-like spines or short prickles have a brownish-yellow colouration. Glochids are fine barbed bristles. (Figure 3)

Each cladode of this species bears 5 to 6 flowers with lengths varying from 3 to 4 cm. The flower's colour changes from immature to mature. The juvenile flower bud is reddish pink, whereas the fully grown flower is yellowish orange; according to reports, the *Opuntia elatior* has been misidentified as *Opuntia dilenii*, distinguished by its yellow flowers that soon turn to rose-pink with red stripes or outer tepals red with inner dirty yellow[11]. The style is white, and the stigma is greenish-yellow, with six lobes on each flower. (Figure 4)

Depending on their development and age, cladodes can have six to seven fruits. Green unripe fruits turn reddish pink when fully ripe. Fruits are naturally juicy, containing 150 to 200 seeds in each ovary. The fruits are elliptic to oval, with light brown glochids on the surface. Raw fruits are consumed for their medicinal properties. (Figures 5 and 6) The detailed morphological characters of *Opuntia elatior* are shown in Tables 1-3.

Table 1: The Detailed Micromorphological Characters of *O. elatior* Plant and Cladode

Parts	Parameters	Observation
Plant	Plant growth	Upright manner
	Plant height	1 to 3 meters
	Plant width	1 ft to 1 meter
	Length	15 to 26 cm
	Width	9 to 15 cm
	Shape	Narrow obviate
Cladode	Colour	Green to Dark green
	Thickness of cladode	1 to 1.5 cm
	Waxiness	Medium
	Pubescence of surface	Slight
	Unduration of margin	Absent
	No. of areoles in central row	6 to 7 aeroles
	Colour of areoles	Grey to brown
	No. of spines per areoles	6 to 8 spines
	Length of longest spine	4 to 5 cm
	Spine colour	Immature - Yellow Mature – Grey to Brown
	No. of colour in the spine	Yellow and Brown
	Spine surface	Grooved surface
	Spine attitude	Semi erect
	Central spine flexibility	Flexible
	Central spine twisting	Present
	No. of glochids per areole	Many in one areole
	Colour of glochids	Yellowish-brown

Table 2: The Detailed Micromorphological Characters of *O. Elatior* Flower and Fruit

Parts	**Parameters**	**Observation**
Flower	No. of flowers per cladode	6 to 7 flowers
	Flower length	3 to 4 cm
	Colour of petals	Unmatured -reddish pink Matured -yellowish-orange
	Colour of style	White
	Colour of stigma	Greenish-yellow
	No. of stigma	6 lobes
	Length	4 to 5 cm
	Width	2 to 3 cm
	Colour of fruit	Unripe fruit – green Ripened fruit – reddish pink
	Shape in longitudinal section	Elliptic
	No. of fruits per cladode	6 to 7 fruits
Fruit	Maximum diameter	2 to 3 cm
	No. of glochids per fruit	20 to 25
	Colour of glochids	Light brown
	Depression of receptacle scar	0.6 to 1 cm
	Diameter of receptacle scar	1.5 to 2 cm
	Thickness of peel	0.2 to cm
	Colour of flesh	Dark pink
	No. of seeds per fruit	150 to 200

Table 3: Organoleptic Characters of *O. Elatior* Cladode.

Parameters	Observations
Colour	Light green
Odour	Characteristic
Taste	Mucilaginous
Texture	Coarse

Table 4: The Physicochemical Characteristics of *O. Elatior* Cladode

Physicochemical Parameters	Result (%)	
	Fresh drug	Dry drug
Total ash	3.70 ± 0.30	3.65 ± 0.40
Acid insoluble ash	2.60 ± 0.15	2.52 ± 0.10
Water soluble ash	1.56 ± 0.35	1.47 ± 0.01
Moisture content	11.00 ± 0.10	4.52 ± 0.12
1% pH	6.97 ±0.01	7.11±0.080
10% pH	5.95 ±0.08	5.91±0.008
Extractive value		
Alcohol soluble	16.36 ± 0.28	30.33 ± 0.24
Water soluble	28.96 ± 0.20	32.20 ± 0.32

Table 5: Preliminary Phytochemical Test of *O. Elatior* Cladode

Phytochemical test	Reagents	Results
Carbohydrates	Molisch test Fehling's test	+ +
Flavonoids	Shinoda test NaOH	+ +
Tannins	$FeCl_3$ Lead acetate	+ +
Phenols	Ellagic acid $FeCl_3$	+ +
Alkaloids	Mayer's Dragendorff's	- -
Glycosides	Kellar-Killiani H_2SO_4	- -
Proteins	Biuret test Millon's test	- -

The table shows the results of present (+) and absent (-) of phytochemicals.

Table 6: Fluorescence Analysis of *O. Elatior* Cladode Powder.

Reagents	Visible light	UV (254)	UV (365)
Powder drug	Light green	Pale green	Light green
Powder drug + Distilled water	Light green	Green	Brown
Powder drug + Ammonia	Cream yellow	Dark brown	Light green
Powder drug + Conc. sulfuric acid	Yellow	Dark yellow	Yellowish brown
Powder drug + Sulphuric acid +water	Light green	Light yellow	Brown
Powder drug + Conc. HCl	Pale green	Olive green	Greyish black
Powder drug + HCl +water	Light green	Yellowish green	Brown
Powder drug + Conc. nitric acid	Yellow	Pale yellow	Yellowish brown
Powder drug + Nitric acid +water	Light green	Yellowish brown	Brown
Powder drug + Iodine	Brown	Greenish black	Yellowish black
Powder drug + Picric acid	Yellow	Dark yellow	Brownish yellow
Powder drug + Picric acid +water	Light yellow	Yellow	Yellowish green
Powder drug + Glacial acetic acid	Cream yellow	Light yellow	Light brown
Powder drug + Chloroform	Light orange	Pale green	Light brown
Powder drug + Methanol	Light green	Olive green	Greenish brown
Powder drug + Ethyl acetate	Cream yellow	Dark yellow	Yellowish orange

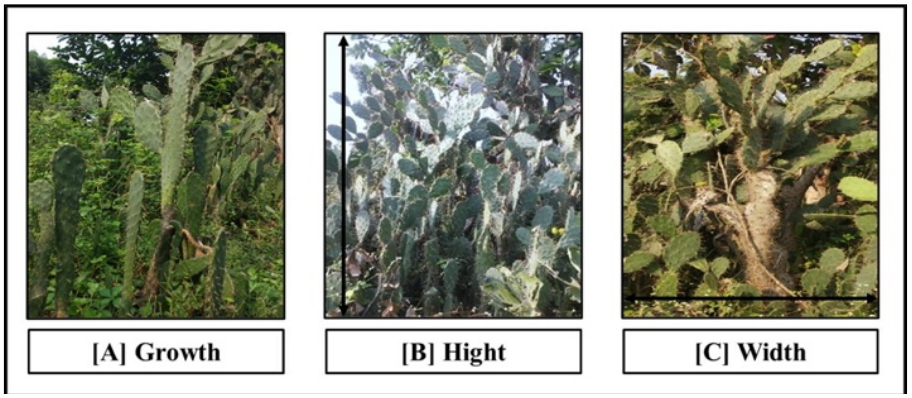

Figure 1: Macromorphological Study of *O. elatior* showing plant [A] Growth, [B] Height, [C] width.

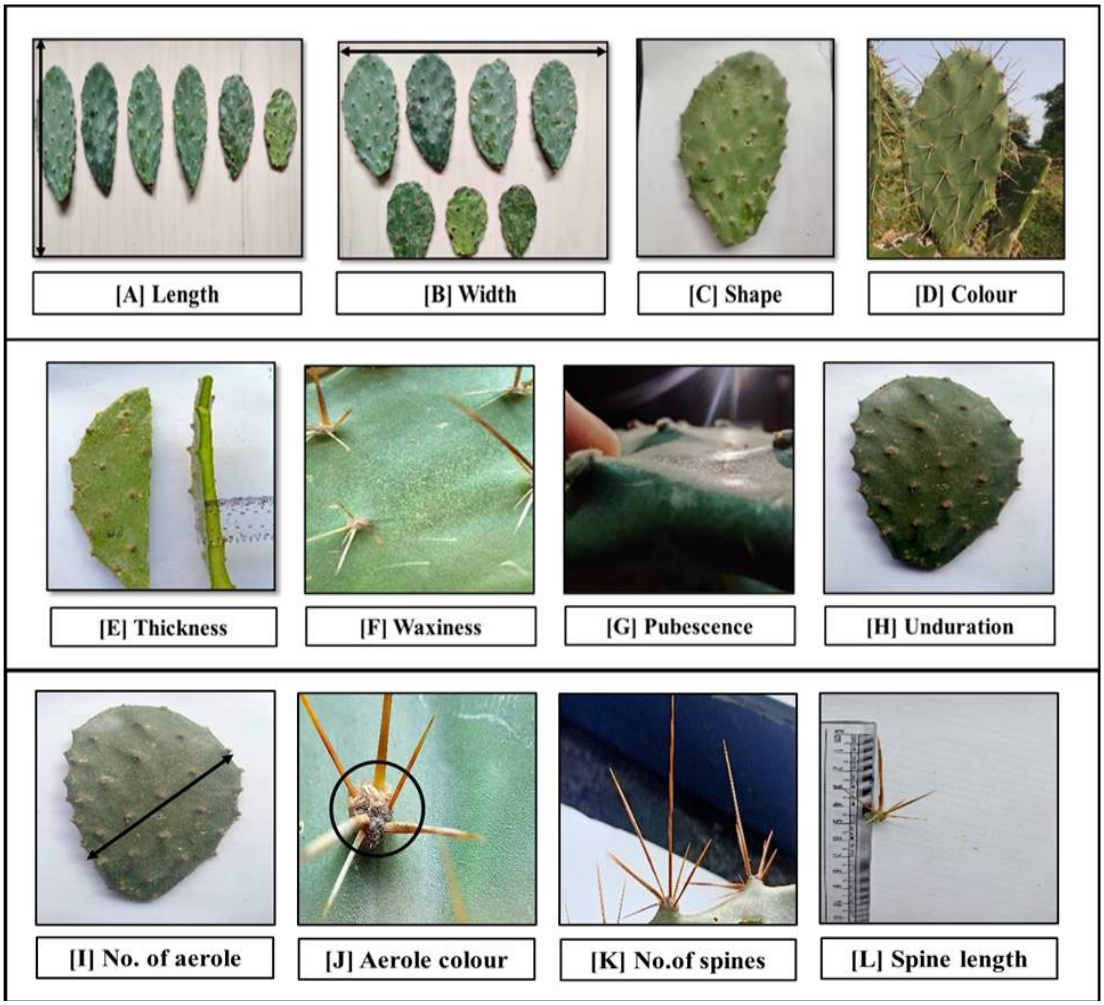

Figure 2: Macro morphological Study of *O. elatior* showing cladode [A] Length, [B] Width, [C] Shape, [D] Colour, [E] Thickness, [F] Waxiness, [G] Pubescence, [H] Undulation, [I] No. of aerole, [J] Aerole colour, [K] No. of spines, [L] Spine length.

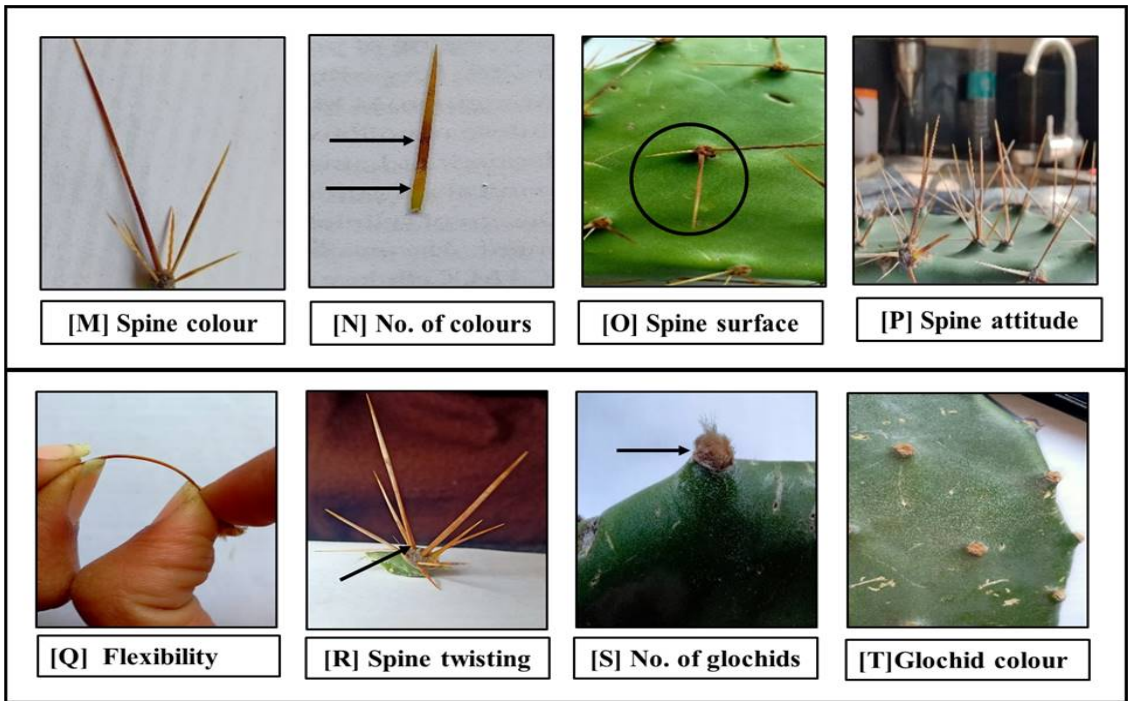

Figure 3: Macromorphological Study of *O. elatior* showing spine [M] Colour [N] No. of colour, [O] Surface [P] Attitude [Q] Flexibility [R] Twisting, [S] No. of Glochids [T] Glochids colour

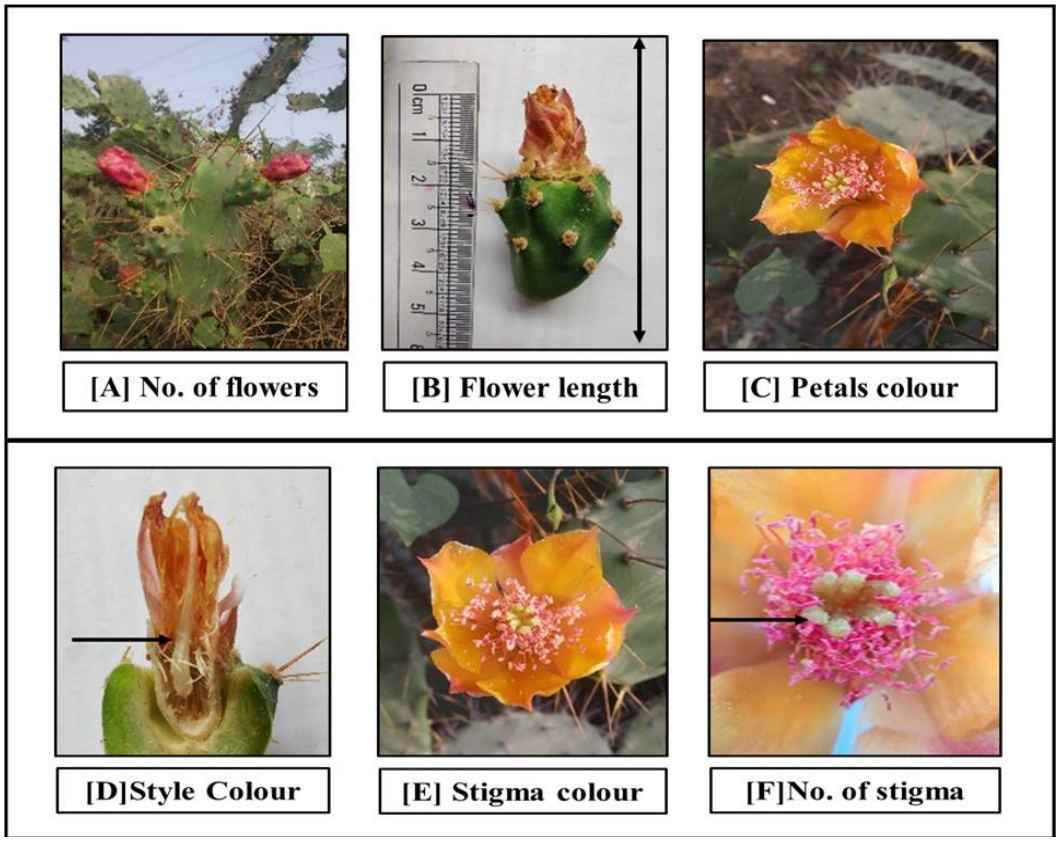

Figure 4: Macromorphological Study of *O. elatior showing* flower [A] No. of Flower, [B] No. of Flower length, [C] Petals colour, [D] Style colour, [E] Stigma colour, [F] No. of Stigma

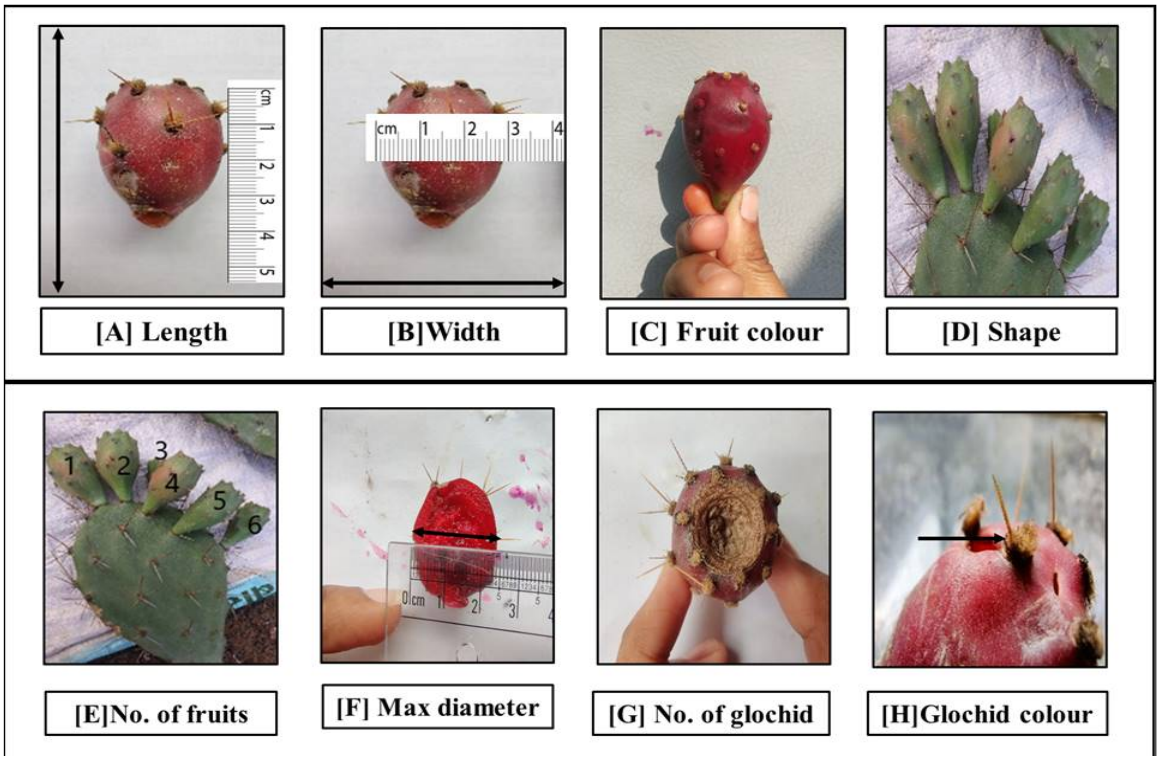

Figure 5: Macromorphological Study of *O. elatior* showing fruits [A] length, [B] width, [C] colour, [D] Shape, [E] No. of Fruit, [F] Max. Diameter, [G] No. of Glochid [H] Glochid colour

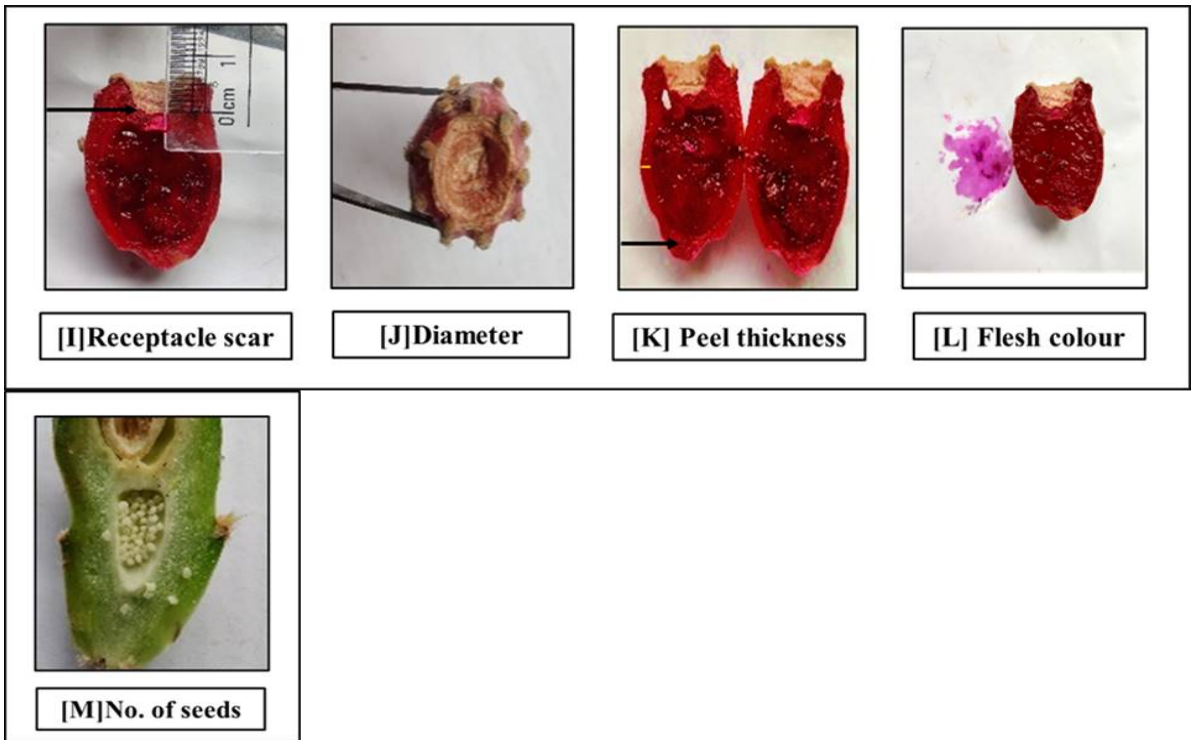

Figure 6: Macromorphological Study of *O. elatior* showing seed [I] Receptacle scar, [J] diameter, [K] Peel Thickness, [L] Seed Flesh colour, [M] No. of seeds.

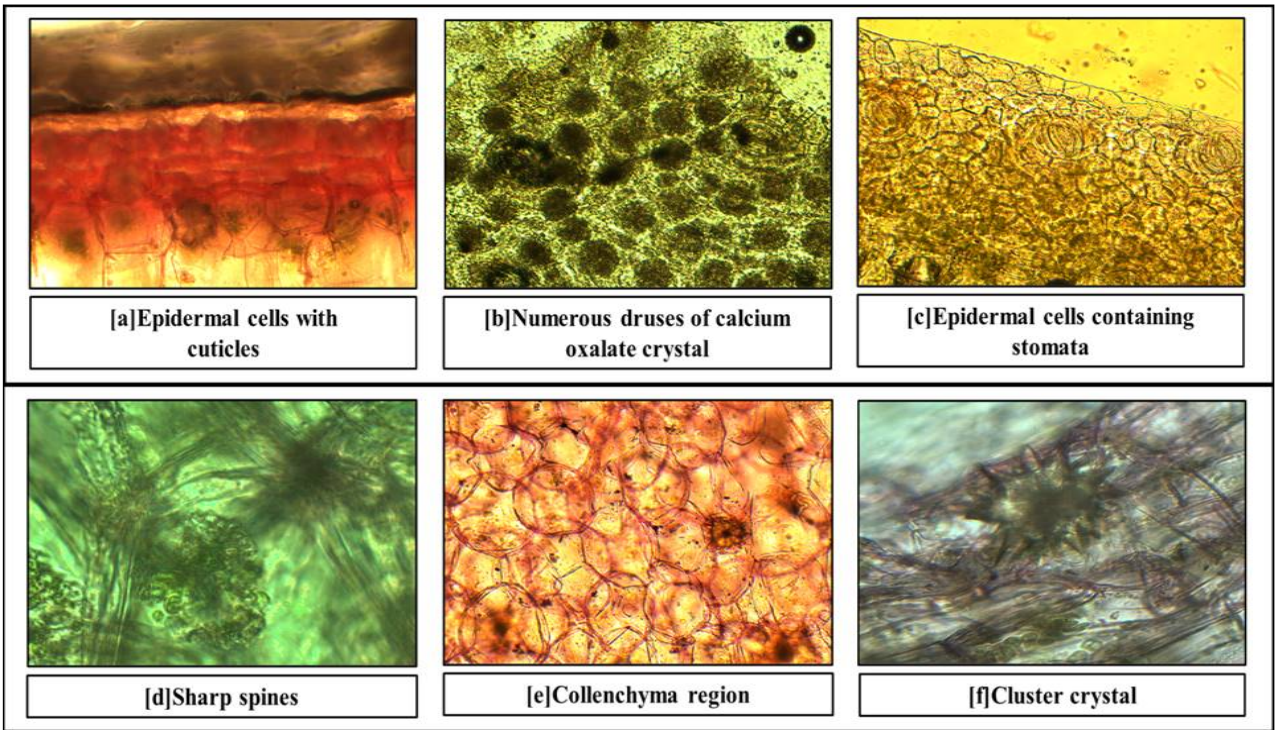

Figure 7a: Microscopical Evaluation of *O. elatior*. [a] thicker walls epidermal cells with cuticles, [b] Numerous druses of Calcium oxalate crystal, [c] Epidermal cells containing stomata, [d] Sharp spines, [e] Collenchyma region, [f] Cluster crystal

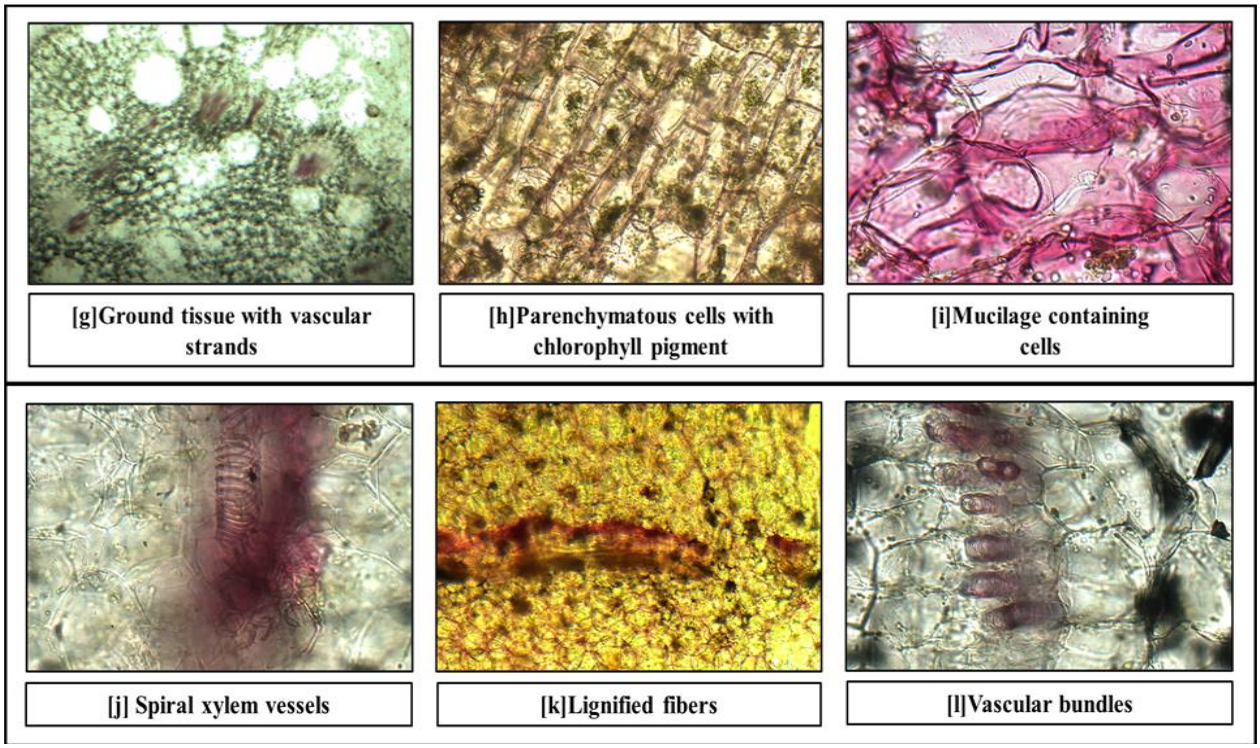

Figure 7b: Microscopical Evaluation of *O. elatior* [g] Ground tissue with vascular strands, [h] Parenchymatous cells with chlorophyll pigment, [i] Mucilage containing cells, [j] Spiral xylem vessels, [k] Lignified fibres, [l]Vascular bundles.

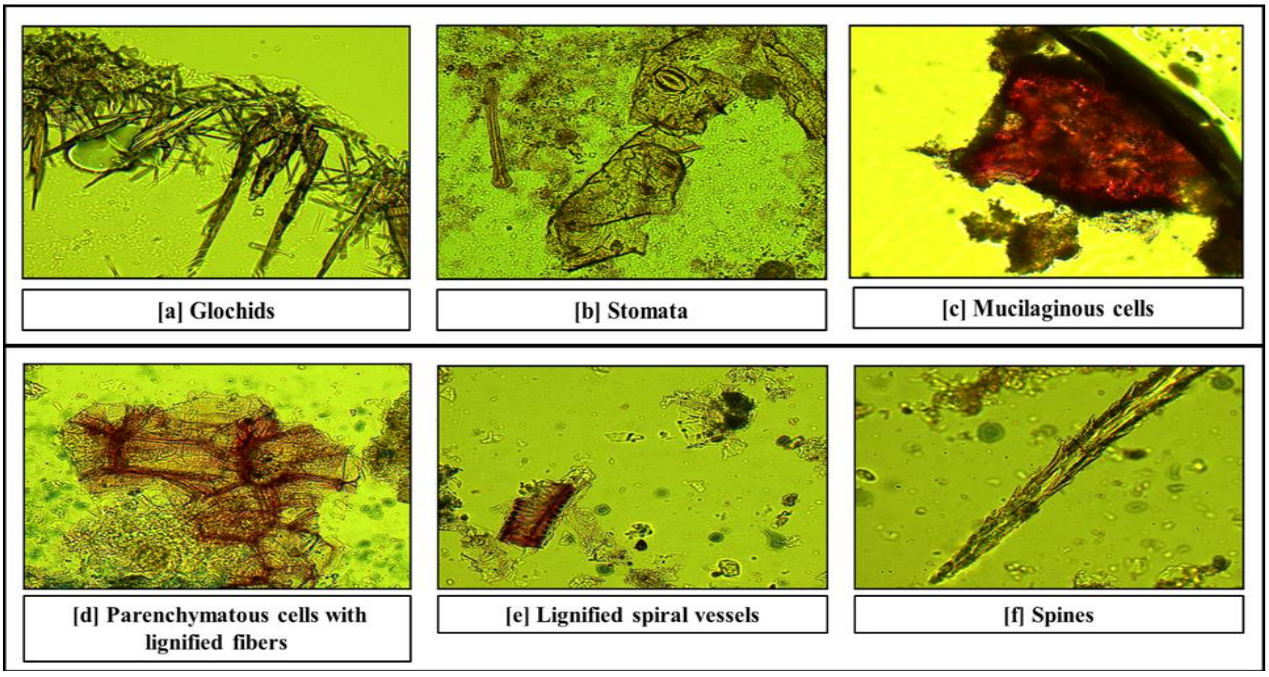

Figure 8a: Powdered Microscopy of *O. elatior*. [a] Glochids, [b] Stomata, [c] Mucilage cells, [d] Parenchymatous cells with lignified fibres, [e] Lignified spiral vessels [f] Spines.

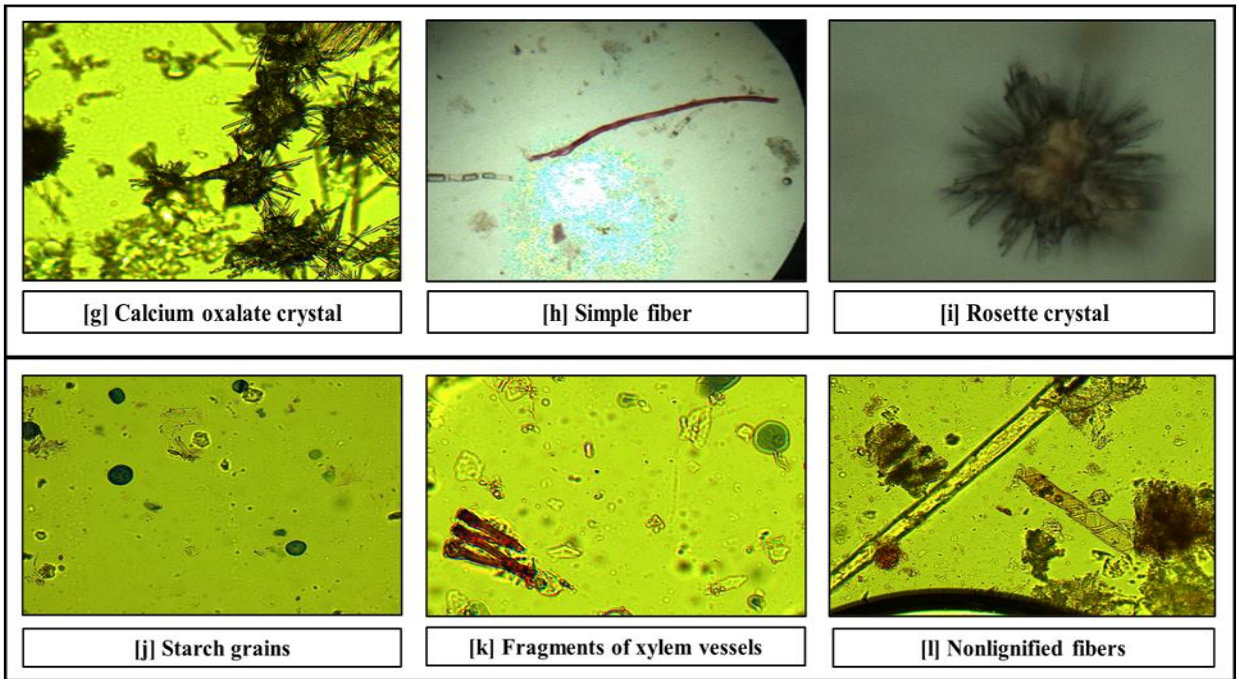

Figure 8b: Powdered Microscopy of *O. elatior* [g] Calcium oxalate crystal, [h] Simple fibre, [i] Rosette crystal, [j] Starch grains, [k] Fragments of xylem vessels [l] Nonlignified fibres.

Microscopical Evaluation

T.S of *Opuntia* cladode

The current study demonstrates most of the micromorphological characteristics of *Opuntia elatior* cladodes. T.S. of *Opuntia elatior* represents the outer epidermis, hypodermis, central ground tissue, and sharp spines.

The epidermis is made up of a single layer of barrel-shaped cells with chloroplasts, while the hypodermis is comprised of 2-3 layers of rectangular to irregularly shaped cells among many calcium oxalate druses. The epidermis of a typical cactus stem is usually uniseriate, with square or rectangular cells in the transverse section[22]. A thick hydrophobic cuticle covers the epidermis, which contains a hypodermis of three or four layers of cells with thicker walls[23]. The cladode *Opuntia elatior* Mill. contains significant amounts of calcium oxalate crystals as druses, which are present in all tissues. Calcium crystals in cacti promote herbivore protection and/or reflect excessive sunlight, thus further avoiding destruction to the chlorophyll parenchyma[24-27]. Calcium is important in the water retention of succulent tissues, which helps regulate osmotic pressure in the cells. It has been demonstrated that the size of these crystals increased with maturation[28].

The hypodermis helps to protect the inner tissues from the outer extreme conditions. Similarly, water stress and high temperature have been reported to cause an increase in the number of plant cuticles[29]. A surface view of a transverse section of *Opuntia* shows epidermal cells, composed mainly of several stomata, mainly paracytic stomata were present above the epidermal cells and appear bigger than the other tissues, although they are sunken.

Sharp spines and glochids (a cluster of fine spine hairs) were observed on both sides of the stem. The thorns and hairs have grown from the so-called "areoles." One or two unicellular thorns, which are larger than hairs, were observed in this areole. A wide area of rounded to unevenly shaped parenchyma cells was filled by collenchyma ground tissue cells comprising rosette/cluster crystals of calcium oxalate) and vascular strands, parenchymatous cells have chlorophyll pigmentation and are organised in uneven rectangular patterns.

The mucilage that holds a large amount of water that has been absorbed.[30] The vascular layer comprises xylem vessels with spiral lignification and a few lignified fibres. Furthermore, the vascular system that runs through this tissue comprises 6 to 7 circularly arranged vascular bundles, each of which includes exterior and internal phloem but no secondary structure. Phloem is made up of sieve ailments fibres, whereas xylem is made up of xylem parenchyma and its fibre. (Figures 7a and b)

The presence of numerous crude cladodes powder characteristics such as parenchymatous cells, paracytic stomata, glochids, spines, mucilaginous cells, xylem vessels, rosette crystal, starch grains, calcium oxalate crystals, and simple fibres were identified by microscopic observation of the powder, as shown in following (Figures 8a and b).

Results of physicochemical parameters like moisture content ash value are given in Table 4. The preliminary phytochemical screening results of *Opuntia elatior* cladodes revealed the presence of flavonoids, carbohydrates, tannins, and phenolic compounds. Table 5. The detailed fluorescence analysis of *O. elatior* cladode powder is shown in Table 6.

DISCUSSION

To distinguish between different species of *Opuntia cacti* at the microscopic level, we would typically need to examine various features, including the structure of their spines, trichomes and specific cellular characteristics. *Opuntia elatior* Mill. (Cactaceae), known as a Nagaphani or Hathlothore, is a sub arborescent or shrubby, having 3 meters high or more. The obtained microcharacters of the stem and flowers of *Opuntia dillenii* and *Opuntia monacantha* are considered diagnostic at the generic and specific levels. Some distinguishing

morphological features observed were elliptical to obovate cladodes. The studied taxa's pollen and stem anatomical characters are considered highly diagnostic at the generic and species levels. The epidermis has irregular and wavy cells, with straight to sinuate wall patterns and paracytic stomata. Pollen grains appear as pantoporate and prolate spheroidal, reticulating to perforate-reticulate sculpturing, while exine semi-tectate to tectate.

Spine morphology of different *Opuntia* species may have spines with different shapes, sizes, and numbers of barbs. They have prominent areoles in another variety, the cushion-like structures from which spines emerge. In *Opuntia elatior, s*harp spines and glochids (a cluster of fine spine hairs) were observed on both sides of the stem. The thorns and hairs have grown from the so-called "areoles." One or two unicellular thorns, which are larger than hairs, were observed in this areole. It was observed that the *Opuntia ficus-indica* spine has a slimmer and sharper tip than *Opuntia ficus amyclaea* and *Opuntia dillenii*. Retrorse barbs are narrower and more protruding in *Opuntia ficus-indica* than in *Opuntia ficus amyclaea,* while in *Opuntia dillenii,* the epidermal cell is similar to flat and wide plates tightly fitted, with a small pointy protuberance at the end. They are considerably narrower and elongated in *Opuntia ficus-indica* and *Opuntia ficus amyclaea* than in *Opuntia dillenii.* 1-7 spines per areole and presence of glochidia in *Opuntia dillenii* while in *Opuntia elatior,* 6-7 spines.

The fruit spines of *Opuntia ficus-indica and Opuntia ficus amyclaea* are thin and sharp projections. Perhaps *Opuntia ficus amyclaea* epidermal cells are a little longer than *Opuntia ficus-indica.* Nevertheless*, Opuntia dillenii* prominences are less projected outward glochids. Cladode and fruit glochids are similar. It has been observed that the retrorse barbs in glochid epidermic cells of *Opuntia dillenii* and *Opuntia ficus-indica* are longer than those of *Opuntia ficus amyclaea*. *Opuntia. ficus-indica* has a slightly sharper tip. However, glochids of *Opuntia dillenii* are easy to differentiate by their widest aspect and because they have more projections. *Opuntia ficus-indica* and *Opuntia ficus amyclaea* exhibit similar areoles, while *Opuntia dillenii* shows very different ones. However, *Opuntia ficus amyclaea* spines have a high resemblance with *Opuntia dillenii*. This results in a big difference between the spines of the cultivated and grown wild forms of *Opuntia ficus indica.*[31-32]

Cellular structure includes the arrangement of cells in the epidermis, cortex, and vascular bundles. The arrangement and characteristics of these cells can differ between species. The vascular layer comprises xylem vessels with spiral lignification and a few lignified fibres. Phloem is made up of sieve ailments fibres, whereas xylem is made up of xylem parenchyma and its fibre in *Opuntia elatio*r xylem made by xylem parenchyma and its fibres and phloem made up of sieve aliments fibres. Glochids of some *Opuntia* species have small, glochid spines, which are often more hair-like and contain small barbs. Examine these structures for differences in shape and size.

Epidermal features of *Opuntia elatior* include leaves that are 7.5 mm long, subulate, recurved, and reddish at the tips. Characteristics of the epidermis, such as the presence of waxy coatings or unique epidermal cell shapes, can also vary among species. A surface view of a transverse section of *Opuntia* shows epidermal cells, composed mainly of several stomata paracytic stomata that were present above the epidermal cells and appear bigger than the other tissues, although they are sunken. Epidermal cells of *Opuntia elatior* are also more elongated and downwardly oriented than in the other opuntias. In contrast, *Opuntia dillenii* has irregular and wavy cells, with straight to sinuate wall patterns and paracytic stomata. Results of *Opuntia elatior* showed the presence of epidermal cells loaded with rosette crystals of calcium oxalate, hypodermis loaded by simple starch grain and central ground tissue, and ground tissue cells consisting of rosette and cluster crystals of calcium oxalate and simple starch grain. The structure and arrangement of areoles, small, raised areas on the stem segments from which spines and glochids emerge, can differ between species. Microscopic examination can reveal the presence of unique structures or patterns in the areoles.

It's important to note that microscopic identification can be challenging, and it's often more accurate when combined with macroscopic observations, including the overall growth habit, flower characteristics, and geographic location.

Opuntia littoralis plant has thin-walled cuticles outside the epidermal cells; however, the new cladode had a layer of meristematic cells like the phelloderm that produces two layers of cells like that of the cork, but non-lignified and they were transparent. It was found that the epidermal cells were composed mainly of several stomata; sharp spines were found on both sides of the stem mixed with glochids (a cluster of hairs).Areole one or two unicellular thorns. *Opuntia vulgaris* Mill**.** Subshrubs, fleshy branches, areoles woolly, spines to 2.5 cm. Leaves subulate or absent. Flowers solitary, sessile; calyx lobes 0.7 cm, ovate, pubescent, tube adnate to ovary; corolla 6 cm across; stamens many, filaments 0.5 to 2 cm; ovary inferior, 3 cm long; ovules many, style to 2 cm, stigma 3-5 lobed. Berry 5 x 3.5 cm, obovoid. The study was conducted on *Opuntia lasiacantha* and *Opuntia zedowskii* species and found that both are different species.[33]

Opuntia ficus-indica and *C. bigelovii* are evident in the significantly smaller junctions of *C. bigelovii* and the mechanics of the vascular bundles, whose stiffness and tensile strength are 25- to 200-fold smaller compared with those of *O. ficus-indica*[34] floral traits must be used for species differentiation in contrast to past Opuntia treatments that were usually based on vegetative morphology alone. Morphometric variables with genetic, ecological, and environmental characteristics and geographic distribution of the species will help disentangle this complex.[35-36]

The global invasive spread of *Opuntia stricta, Opuntia monacantha* and *Opuntia ficus-indica* has been facilitated by their purposeful introduction for crop cultivation and other human uses, as well as several key ecological traits that promote their dominance.[37]

CONCLUSION

Estimating crude drugs is essential in determining the correct identification of medicinal plant material. The microscopic and macroscopic characteristics of *Opuntia elatior* cladode have been examined.

To avoid possible adulterations and the misuse of incorrect medications, the authenticity of any raw herbal material must be standardised using established processes. One of the most essential techniques in the botanical identification of herbal medicines is macroscopic and microscopic research. Along with these primary morphological characters, this study also provided a variety of anatomical features that can be employed as helpful identification markers. The present research work was confined to the macro microscopic features of the crude along with powdered drug, physicochemical, phytochemical and fluorescence analysis.

The presence of a paracytic type of stomata, mucilaginous cells, cluster crystals, sharp spines, vascular bundles, and calcium oxalate crystals was found in the transverse section of *Opuntia*. This could be the difference between other species of *Opuntia,* like *Opuntia dillenii* and *Opuntia vulgaris* Mill. (syn. *Opuntia monacantha) and Opuntia littoralis.*

Physicochemical parameters are also vital for standardising and quality control of herbal drugs. The acid insoluble ash measures the sandy (silica) matter and plant body parts, including calyx, leaves, etc., which contain a higher non-combustible acid insoluble matter.

A phytochemical examination of the plant extract revealed the existence of various secondary metabolites. To conclude, this research could be employed as a diagnostic tool for standardising this medicinal plant and will contribute to the characterisation of the crude drug.

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