

## ORIGINAL ARTICLE

# Standardization of homoeopathic drug: *Buxus sempervirens* L.

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### ABSTRACT

**Background:** *Buxus sempervirens* L. (Buxaceae), is a small tree, used in Homoeopathy for acute pain, increase in pulse rate and nausea. Leaves and stems are used in the preparation of medicine.

**Objective:** The pharmacognostic and physico-chemical studies are carried out to facilitate use of correct species and standard raw materials.

**Material and Methods:** Pharmacognostic studies of leaf and stem of authentic samples of *Buxus sempervirens* have been carried out. Physico-chemical parameters of the raw drug, namely, extractive values, ash value, formulation besides weight per milliliter, total solids, alcohol content, High Performance Thin Layer Chromatography (HPTLC) and Ultraviolet (UV) studies are given for the mother tincture.

**Results:** The leaves are nearly sessile, opposite, entire, narrowly lanceolate or ovate and up to 2.5 cm. The stomata are paracytic and confined to the abaxial side. Unicellular conical hair and peltate scaly hair occur on the adaxial side near the base. The midvein is ribbed on either sides. Crystalliferous idioblasts occur towards the abaxial side at the midvein and lamina. Secretory canals occur in the mesophyll. A single vascular bundle is present in the midvein. The stem is quadrangular. The vascular tissue is present as a cylinder with four cortical bundles, one each in the angles. The microscopical and organoleptic characteristics of the powder are provided.

**Conclusion:** The powder microscopic features and organoleptic characters along with anatomical and physico-chemical studies are diagnostic to establish the standards for ensuring quality and purity of the drug.

**Keywords:** *Buxus sempervirens* L., High performance thin layer chromatography, Homoeopathy, Pharmacognosy, Physico-chemical, Spectroscopy, Standardization

### INTRODUCTION

*Buxus sempervirens* L., popularly known as “boxwood” in English; “Bachsbaum” in German; “Chikri” in Punjab and Kashmir; “Shamshad” in Urdu and Persian, is a small tree belonging to the family *Buxaceae*.<sup>[1]</sup> It is a native of Western and Southern Europe and also occurs in Western Asia and North. Africa. In India, it is found in Himalayas and Punjab.<sup>[2]</sup> In Himalayas, it is found in Kumaon to Shimla, Punjab and Bhutan.<sup>[3]</sup> It is also cultivated as a hedge in gardens at Kodaikanal in Tamil Nadu.<sup>[1]</sup>

Since it is grown at an altitude of 1600-2800 m, it is not found in other states.

The tincture of the young leaves and twigs are used as medicine in homoeopathy in the treatment for forcing pains (e.g., labor), frequent desire to urinate, increase in pulse rate and slight nausea.<sup>[4]</sup> In unani system of medicine, the leaves are used for the treatment of headache, pain and prolapse ani. The leaves are bitter, purgative, diaphoretic and useful in rheumatism and syphilis.<sup>[2]</sup>

In homoeopathy, its authority was mentioned in

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Br. Jour. Hom. 11, 158, for the effects of an infusion taken for the purpose of producing an abortion which it failed to do.<sup>[4]</sup>

Chemically the leaves are reported to contain buxenine-G (an alkaloid), sitosterol, stigmasterol, cycloartenol, lupeol, germanicol,  $\beta$ -amyrin, (+)-semperviramidine, (-) buxadienine (steroidal alkaloid), (+)-sempervirine, (-)-31-acteyl-cyclomicrophylline-A and (-)-benzoylbuxidienine from this plant.<sup>[5-8]</sup>

A review of the literature reveals that no pharmacognostic standards have been recorded for the drug except for the anatomical review.<sup>[9]</sup> In view of the importance of the drug, pharmacognostic and physico-chemical studies of leaves and stem are carried out to lay down standards.

## MATERIAL AND METHODS

### Plant Material

The plant material of *Buxus sempervirens* L., was supplied by the Survey of Medicinal Plants and Collection Unit, The Nilgiris, Tamil Nadu. The leaves and young stems were fixed in formaldehyde-acetic acid-alcohol, dehydrated through the alcohol-xylene series, embedded in paraffin wax. Sections cut between 8 and 10  $\mu$ m were stained in crystal violet – basic fuchsin combination.<sup>[10]</sup> The epidermal peels were obtained by gently scraping and peeling with a razor blade. The microscopic characters of powder were studied by boiling the powdered drug in distilled water, stained in saffranin and mounted in glycerine.

### Preparation of Extracts

The air dried leaves and twigs of the drug were coarsely powdered to 10/44 (sieve size) and were subjected to the determination of moisture content (loss on drying at 105°C), total ash, water soluble ash, acid insoluble ash, extractability in different solvents, physico-chemical constants, thin layer chromatography (TLC) and ultraviolet aspects of mother tincture following official methods.<sup>[11]</sup> Mother tincture was prepared as per Homoeopathic Pharmacopoeia of India. 100 g of coarse powder of the drug was suspended in 680 mL of 95% alcohol and 350 mL of purified water for 24 hour at room temperature. It was filtered and made up to 1000 mL using same solvent ratio. Percolation method<sup>[11,12]</sup> was used for the preparation of mother tincture.

### High Performance Thin Layer Chromatography (HPTLC) Analysis

25 mL mother tincture was evaporated on water bath to remove alcohol. The residue was extracted with 3 mL  $\times$  25 mL chloroform. Concentrated chloroform extract was used for the HPTLC study. The concentrated chloroform extract was spotted in the form of the band of width 4 mm with a Camag microliter syringe on pre-coated silica gel aluminium plate 60F-254, (5 cm  $\times$  10 cm with 0.25 mm thickness; Merck, Darmstadt, Germany) using a Linomat IV sample applicator (Camag, Muttenz, Switzerland, Supplied by Anchrom Technologists, Mumbai). A constant application rate of 6 mL/s was employed. The slit dimension was kept at 4 mm  $\times$  0.45 mm and 20 mm/s scanning speed was employed. The mobile phase consisted of chloroform: methanol (9:1 v/v) and 10 mL of mobile phase was used for chromatography. Linear ascending development was carried out in a 10 cm  $\times$  10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase at room temperature for 20 minutes. The length of the chromatogram run was 8 cm and subsequent to the development, the TLC plates were dried in a current of air with the help of hot air dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed (Camag TLC scanner III) at 254 nm and 366 nm by reflectance scanning and operated by Win Cats software (v 4.05, Camag) resident in the system.<sup>[13-15]</sup>

## OBSERVATIONS AND RESULTS

### Morphology

A much branched shrub or small tree. Leaves are nearly sessile, opposite, narrowly lanceolate or ovate, up to 2.5 cm, entire, usually obtuse. Flowers small, yellow to green, strongly scented in small axillary heads or spikes. The terminal flowers are usually female, others being male. Capsule ovoid, 1.3 cm long, 3-horned, seeds 3-6, small.<sup>[2]</sup>

### Macroscopy

Leaves nearly sessile, opposite, narrowly lanceolate or ovate, up to 2.5 cm entire, usually obtuse, dark green above and pale beneath, glabrous. Stem quadrangular, 1.6-1.8 mm thick, covered by conical and occasionally few peltate scaly hair.

### Microscopy

#### Leaf

In surface view, epidermal cells are polygonal

isodiametric in outline and have sides thin to slightly thick and straight to curved [Figure 1a-c]. The cells show scanty to slightly dense contents and are 5130/mm<sup>2</sup> (abaxial) and 7260/mm<sup>2</sup> (adaxial). The costal cells are anisodiametric to linear, parallelly oriented and are present on primary and secondary veins. The stomata are paracytic [Figure 1c], and 260/mm<sup>2</sup> with stomatal index: 4.8; size 16-25 μm (19) long and 11-16 μm (14) wide.

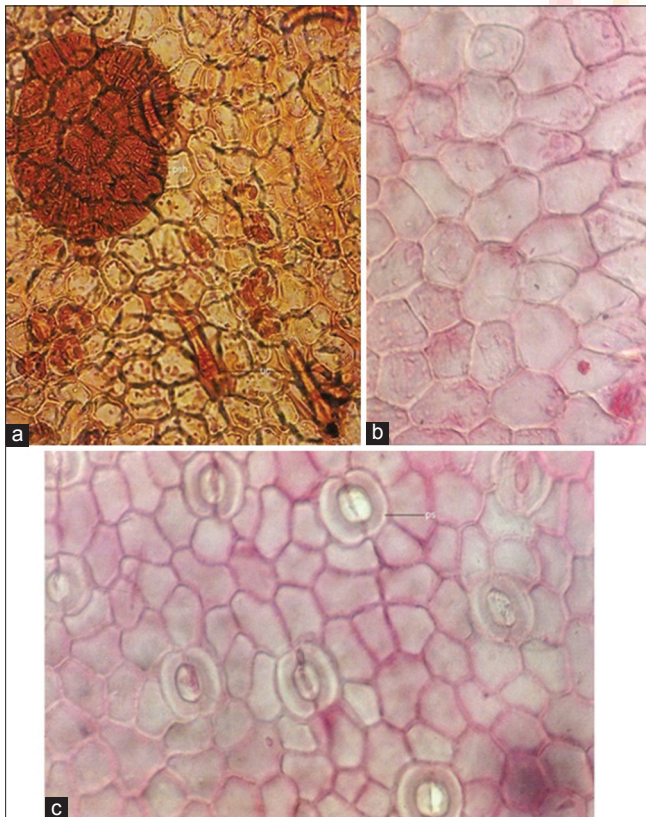
Trichomes are of two types: (1) Unicellular conical hair and (2) peltate scaly hair. The former are confined to the base, midvein and margins on adaxial [Figure 1a] and the latter are few and present more towards base, sides of midvein and near margins [Figure 1a].

In a Transverse Section (TS), the midvein is ribbed on either sides and 216-346 μm (305) thick. The lamina is dorsiventral, 184-216 μm (200) thick [Figure 2a]. The margins are pointed and slightly bent inwards.

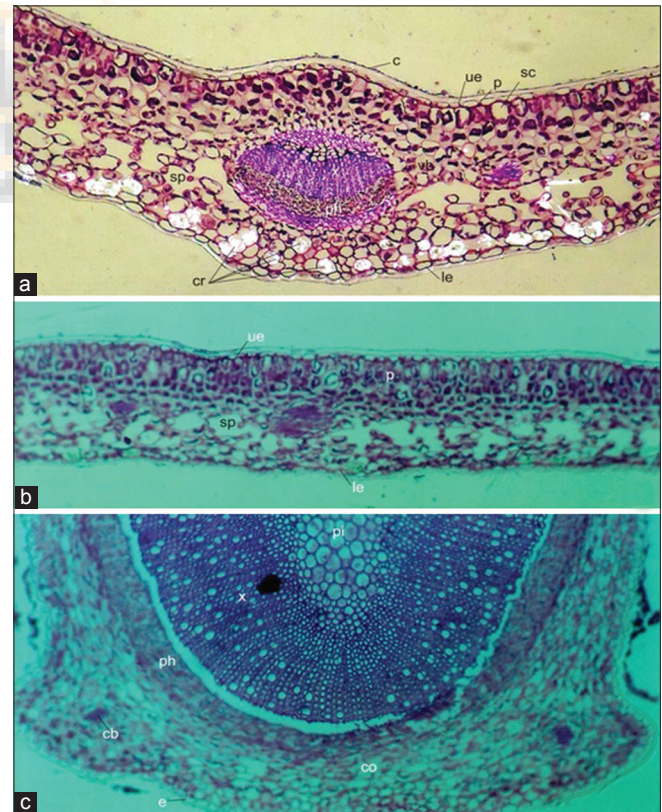
Epidermis is single-layered and composed of mostly barrel shaped cells, some isodiametric, oval to

spherical. The epidermal cells are covered by a thick cuticle. Stomata are confined to lower surface of the leaf and flushed with epidermis. Unicellular trichomes are present on the adaxial surface and restricted to the base. Mesophyll is dorsiventral. Palisade is two-layered but at places three-layered, extending into midvein, cells 20-38 μm (30) long and 14-22 μm (17) wide, walls thin, contents dense with chloroplasts, occasionally with sphaeraphides. Spongy parenchyma is predominant with upper 3-4 layers of closely packed and lower portion loosely arranged with large intercellular spaces often interspersed with sphaeraphidial idioblasts. Some large secretory pores also occur in the mesophyll [Figure 2a and b].

The ground tissue at midvein consists of 1-2 layered collenchyma on abaxial followed by 4-5 layers of parenchyma while the collenchyma on adaxial as a group of cells followed by 3-4 layers of parenchyma. Collenchyma cells are 8-16 μm (13) in diameter and angular. Parenchyma cells 8-25 μm (18) in diameter, often contain chloroplasts and interspersed with sphaeraphidial idioblasts mostly towards abaxial near



**Figure 1:** (a) Upper epidermis at the base with trichomes ×417. (b) Upper epidermis in surface (enlarged) ×364. (c) Lower epidermis in surface (enlarged) ×630 (Psh: Peltate scaly hair, uc: Unicellular conical hair, ps: Paracytic stomata)



**Figure 2:** (a) Transverse section (TS) of leaf at midvein ×162. (b) TS of leaf lamina ×132. (c) TS of stem ×145 (c: Cuticle, ue: Upper epidermis, le: Lower epidermis, p: Palisade tissue, vb: Vascular bundle, x: Xylem, ph: Phloem, cr: Crystal, sp: Spongy tissue, e: Epidermis, cb: Cortical bundle, pi: Pith, sc: Secretory canal)

vascular bundle, 14-36  $\mu\text{m}$  (27) in diameter. Besides, few cells contain rhombic or hexagonal prismatic crystals of calcium oxalate [Figure 2a].

The vascular bundle is single, large, oval, 227-281  $\mu\text{m}$  (254) long and 162-205  $\mu\text{m}$  (181) wide, endarch, conjoint, collateral and capped by lignified sclerenchyma. A layer of endodermis and pericycle encloses the vascular bundle. The xylem elements are numerous and arranged in radial rows. Phloem is extensive on the abaxial [Figure 2a].

#### Stem

In TS, the stem is quadrangular with winged angles, covered by unicellular conical hair, papillate hair and peltate scales. The epidermis is single-layered covered by thick cuticle; hypodermis is collenchymatous as a group of cells, angular or lamellar in few with chloroplasts. Cortex is 8-10 layered in the interangles and 12-16 at the angles; cells polygonal to spherical and tangentially long; those at periphery collenchymatous, few with yellowish contents. Crystalliferous idioblasts occur in the phloem besides few in cortex. Cortical spherical vascular bundles are present one each in the angles [Figure 2c].

Vascular tissue is in the form of thick secondary xylem cylinder. It is enclosed by an endodermis followed by pericycle. The phloem is precocious, external, encloses the xylem. A small amount of primary xylem is present close to pith. The xylem consisting of vessels or tracheids, fibres and xylem parenchyma are arranged in radial rows. In longisection, the vessels and tracheids show helical thickenings, besides few scalariform and bordered pits. Pith parenchyma is centrally present. Prismatic and rhombic crystals of calcium oxalate occur in the cortex close to cortical bundles while sphaeraphidal cells are close to the phloem [Figure 2c].

#### Powder Microscopy

1. Pieces of adaxial epidermis.
2. Pieces of abaxial epidermis with stomata.
3. Unicellular hairs, broken, few.
4. Rhombic and prismatic crystals of Calcium oxalate.
5. Pieces of stem epidermis with parallelly oriented cells and attached unicellular hairs.
6. Fragments of tracheary tissue with fibres.
7. Fragments of leaf with epidermis and underlying palisade.
8. Few sphaeraphidal crystals broken.

#### Organoleptic Characters

Colour: Moss green.

Touch: Smooth.

Odour: Slightly pungent.

Taste: Bitter.

#### Physico-chemical Studies

The determined data under the physico-chemical study for the raw drug is summarized in Table 1 and that of mother tincture preparation and its standardization in Tables 2 and 3 respectively.

Results of physico-chemical studies are summarized in Tables 1-3.

**Table 1: Standardisation of raw drug**

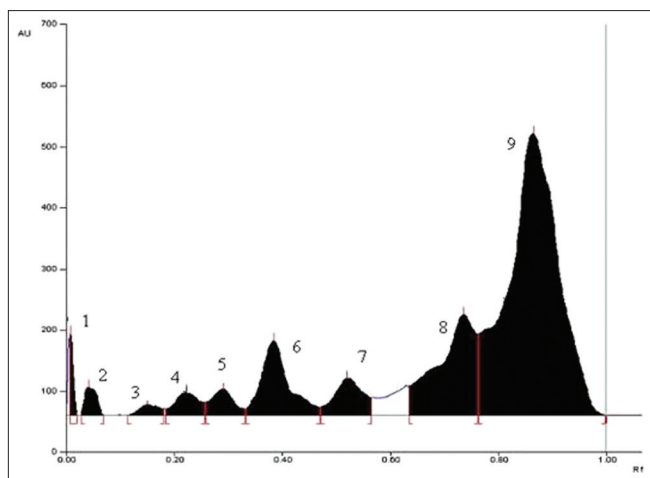
Parameters	Quantitative values (% w/w)
Moisture content (loss on drying at 105° C)	Not more than 4.5
Total ash	Not more than 6.6
Acid insoluble ash	Not more than 0.49
Water soluble ash	Not more than 0.94
Alcohol soluble extractive	Not less than 17.5
Water soluble extractive	Not less than 21.9
Extractive values in	
Hexane	Not less than 2.8
Chloroform	Not less than 5.9
Methanol	Not less than 20.6

**Table 2: Formulation of mother tincture (Percolation technique used)**

Alcohol	65% v/v
Drug strength	1/10
Preparation	
<i>Buxus sempervirens</i> in coarse powder	100 g
Strong alcohol	680 mL
Purified water	350 mL
To make 1,000 mL of the mother tincture	

**Table 3: Standardization of the mother tincture**

Parameters	Observations
Organoleptic profile	
Appearance	Clear, non-viscous, foam on shaking
Colour	Blackish brown
Odour	Characteristic
Sediments	Absent
Weight per mL	Not more than 0.89 g
Total solids	Not less than 1.9% w/v
Alcohol content	61-65% v/v
pH	5.0-6.0
$\lambda$ max	318 (0.20), 278 (0.25), 202 (1.06)



**Figure 3:** High performance thin layer chromatography densitogram (chloroform: methanol [9:1 v/v]) of *Buxus sempervirens* mother tincture scanned at 254 nm

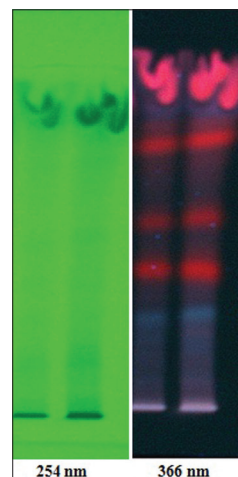
### HPTLC Finger Printing

The profile of chromatographic separation scanned at 254 nm, reveals seven spots [Figures 3 and 4] out of which seven, six and four spots possess maximum composition with  $R_f$  at 0.86, 0.74 and 0.38 respectively. On the other hand scanned chromatogram at 366 nm revealed seven spots with seven, six and five spots showing maximum composition at  $R_f$  0.89, 0.85 and 0.72 respectively. It is evident from the data that these are characteristic for the studied drug, which will help in the identification and authentication of the mother tincture. This is considered as valuable standards in pharmacopoeia. At 254 nm, seven spots appeared at  $R_f$  0.15, 0.22, 0.29, 0.38, 0.52, 0.74 and 0.86 [Figures 3 and 4] with various concentrations while at 366 nm, seven spots at  $R_f$  0.13, 0.27, 0.38, 0.51, 0.72, 0.85 and 0.89.

## DISCUSSION

### Leaf

The stomata have been reported to be confined to the lower surface and surrounded by rosettes of subsidiaries in *Buxus*.<sup>[9]</sup> However, presently they are hypostomatic as has been reported earlier but presently they are distinctly paracytic in *Buxus sempervirens* studied. Further, the guard cells are strongly crested as also reported earlier.<sup>[9]</sup> The number of stomata are 260/mm<sup>2</sup> and the stomatal index is 4.8. The trichomes in *Buxus* were reported as unicellular conical<sup>[9]</sup> which is presently confirmed and are found restricted to the basal region near the midvein on the adaxial side. Besides, a few peltate scaly hairs are also found near the base on the adaxial side in



**Figure 4:** High performance thin layer chromatography finger printing (chloroform: methanol [9:1 v/v]) of *Buxus sempervirens* mother tincture scanned at 254 nm and 366 nm

the present species. Idioblasts containing prismatic crystals and sphaeraphides occur more on the abaxial side and also in the midvein as also reported earlier.<sup>[9]</sup> Some secretory canals are present in the mesophyll as also reported.<sup>[9]</sup>

### Stem

In TS, the stem is quadrangular with winged angles. The epidermis is reported as thickly cutinized and papillate<sup>[9]</sup> which is presently confirmed. Besides, the epidermis possesses unicellular conical hair and rarely peltate scaly hair over its surface. The cortex is chlorenchymatous in its outer layer as also reported earlier.<sup>[9]</sup> The vascular tissue is a continuous vascular cylinder with four cortical bundles, one each in the angular wings. The presence of 1-2 layers of sclerenchyma fibres enclosing the cortical bundles has been described,<sup>[9]</sup> which is now confirmed. The xylem is a continuous cylinder exhibiting some secondary growth. The phloem is external and often possesses idioblasts with prismatic, rhombic and cluster crystals as also earlier reported.<sup>[9]</sup> The pith is quite abundant with thick walled pitted cells [Figure 2c] as also been reported earlier.<sup>[9]</sup>

### Physico-Chemical

Herbal medicines are composed of many constituents, therefore, amenable to variation. The physico-chemical analysis of *Buxus sempervirens* is presented in Tables 1-3. HPTLC fingerprinting is a precise and accurate method for herbal identification and can be used in authentication and characterization of this important medicinal plant. Hence, it is very important to obtain reliable chromatographic fingerprints that represent therapeutically active and chemically characteristic

components of the herbal medicines. Thus, the developed chromatogram will be specific with selected solvent system and  $R_f$  value and serve as a good standardization tool for *Buxus sempervirens*.

Physical parameters include colour, appearance, odour, viscosity, moisture content, pH, sedimentation and ash values. Chemical parameters include limit tests, extractive values, chemical assays, etc. These standards along with the preparation of mother tincture furnished in Homoeopathic Pharmacopoeia of India have been meticulously adhered to in our study.

## CONCLUSION

The macro and microscopical, organoleptic characters along with the anatomical and methodology used for the studies are diagnostic and establish the standards. HPTLC analysis of *Buxus sempervirens* L. leaves and twigs can provide standard finger prints and will be used as reference tool for identification, authentication, quality control and standardization of this important medicinal plant.

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**पृष्ठभूमि:** बक्सस सेम्पर्वयरेंस एल. (बक्सेसी) एक छोटा पेड़ है, जिसका इस्तेमाल होम्योपैथी में तीव्र दर्द, नाड़ी स्पन्दन गति और उबकाई में किया जाता है। औषधि तैयार करने में इसकी पत्तियों और डंठलों का इस्तेमाल किया जाता है।

**उद्देश्य:** मानक कच्चे माल और सही उपजाति के इस्तेमाल में सुविधा प्रदान करने के लिए भेषज-विज्ञान संबंधी और भौतिकरासायनिक अध्ययन किए गए हैं।

**सामग्री और पद्धतियाँ:** बक्सस सेम्पर्वयरेंस के प्रामाणिक नमूनों की पत्तियों और डंठलों के भेषज-विज्ञान संबंधी अध्ययन किये गये हैं। मदर टिंचर के लिए कच्ची औषधि के भौतिक-रासायनिक मापदंड, नामतः निष्कर्षणात्मक मूल्य, भस्म मूल्य, प्रति मिली मीटर भार के अलावा फार्मूलेशन, कुल ठोस, एल्कोहल की मात्रा, उच्च-निष्पादन पतली परत वाली क्रोमाटोग्राफी (एच पी टी एल सी) और पराबैंगनी (यूवी) अध्ययन किये गए हैं।

**परिणाम:** पत्तियाँ, निकटता से अवृन्त, विपरीत, पूरी, निकटता से बल्लम-नोक या अण्डाकार और 2.5 से.मी. तक होती हैं। रंग, पारासाइटिक और अपाक्ष साइड तक परिरुद्ध रहते हैं। आधार के निकट अपाक्ष साइड पर एककोशिक शंक्वाकार बाल और ढालाकार पपड़ीले बाल होते हैं। दोनों तरफ मध्यवर्ती शिरा जुड़ी होती है। मध्यवर्ती शिरा और पत्रदल पर अपाक्ष साइड की ओर क्रिस्टलीफेरस इडिओब्लास्ट होता है। पूर्णमध्यक में स्रावी नालिकाएं बन जाती हैं। मध्यवर्ती शिरा में एक एकल संवहनी पुलिंदा मौजूद होता है। डंठल चतुष्कोणीय होता है। संवहनी तंतु चार कॉर्टिकल बंडलों के साथ एक सिलेंडर के रूप में प्रत्येक कोण में मौजूद होता है। पाउडर की माइक्रोस्कोपियल और ऑरगेनोलेप्टिक विशेषताएं प्रदान की गई हैं।

**निष्कर्ष:** शरीररचना-विज्ञान संबंधी और भौतिक-रासायनिक अध्ययनों के साथ-साथ पाउडर सूक्ष्मदर्शी विशेषताओं और जैव-तत्व संबंधी गुण, औषधि की गुणवत्ता और परिशुद्धता सुनिश्चित करने के लिए मानक स्थापित करने हेतु नैदानिक है।