

ORIGINAL ARTICLE

Pharmacognostic standardization of Homoeopathic drug: *Juniperus virginiana L.*

P. Padma Rao, P. Subramanian¹, P. Sudhakar², P. R. Reddy³

ABSTRACT

Background: *Juniperus virginiana* L., commonly known as 'red cedar' in English is a well-known evergreen tree belonging to the family Cupressaceae. The leaves and young aerial shoots are used for preparation of medicine in Homoeopathy.

Objective: Standardization is the quintessential aspect which ensures purity and quality of drugs. Hence, the pharmacognostic and physico-chemical studies are carried out to facilitate the use of authentic and correct species of raw drug plant material with established parametric standards for manufacturing the drug.

Materials and Methods: Pharmacognostic studies on leaves and young aerial parts of authentic samples of *J. virginiana* L. have been carried out; physico-chemical parameters of raw drug viz., extractive values, ash values, formulation, besides weight per mL, total solids, alcohol content along with High Performance Thin Layer Chromatography (HPTLC) and ultraviolet visible studies have been worked out for mother tincture.

Results: The leaves are needles, narrow and sharp at tips; stems are round with greyish white to brown bark possessing small lenticels and covered by imbricate leaves. Epidermal cells in the surface have polygonal linear sides with pitted walls containing crystals and starch. Stomata exclusively occur on the adaxial surface in linear rows. Hypodermis of leaf in T.S. is marked with 1–2 layered lignified sclerenchyma. 2–4 secretory canals are present with one conspicuously beneath midvein bundle. The young terminal axis is sheathed by two closely surrounding leaves while the mature stem possess four leaf bases attached. Vascular tissue of stem possesses predominant xylem surrounded by phloem containing sphaeraphides, prismatic crystals and starch grains. Uniseriate rays occur in the xylem. Mature stem possess shrivelled cork, followed by the cortex. Physicochemical properties and HPTLC values of the drug are standardized and presented.

Conclusion: The powder microscopic features and organoleptic characters along with anatomical and physicochemical studies are diagnostic to establish the standards for the drug.

Keywords: High performance thin layer chromatography, *Juniperus virginiana*, Pharmacognosy, Secretory canal, Standardization

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INTRODUCTION

Juniperus virginiana L., commonly known as 'red cedar' in English is an evergreen tree belonging to family Cupressaceae. It is grown from Canada to Gulf of Mexico, Westward to Texas and Nevada to British Columbia.^[1] It is also found in Nilgiris, India. Leaves and young aerial parts are used for preparation of medicine in Homoeopathy. Medicinally, it is used in apoplexy, convulsions, strangury, tetanus, uterine haemorrhages and affection of eyes.^[2,3] Its history and authority about the proving of drug is mentioned in Homoeopathic Pharmacopœia of the United States [HPUS].^[4] Chemically, the plant is reported to contain volatile oils, cedrenol, pseudocedrenol, cedrene isomers, bicyclic sesquiterpenes, podophyllotoxin, oleoresins, cedrol [Figure 1], thujopsene and juniperin.^[5-8]

The pharmacognostic studies on leaves and young shoots were not conducted yet. A part from histo anatomical researches in Cupressaceae, Considering the medicinal importance of the drug in Homoeopathy and absence of earlier pharmacognostic studies, this study is undertaken.

MATERIALS AND METHODS

Pharmacognosy

The plant material *J. virginiana* was supplied by the Survey of Medicinal Plants and Collection Unit, Nilgiris, Tamil Nadu. The leaves and stems were fixed in formaldehyde acetic acid alcohol, dehydrated through xylene alcohol series, embedded in paraffin wax. The cross sections between 8 - 10 μ m were stained in crystal violet and basic

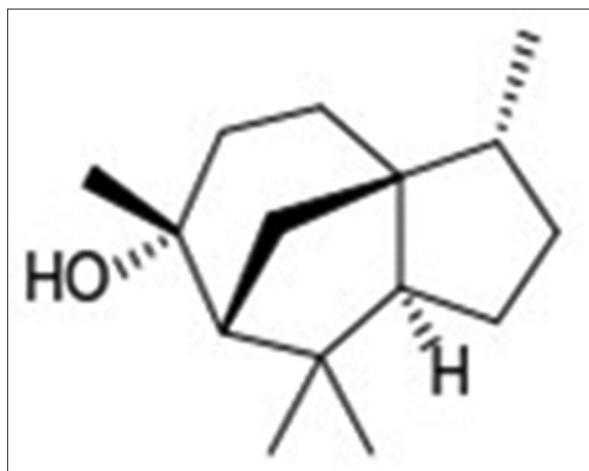


Figure 1: Chemical structure of cedrol

fuchsin combination as per the Johansen method.^[9] Epidermal peels were obtained by gently scraping and peeling by the razor blade. Then peels were stained in saffranine and mounted in glycerine. The photomicrography was done on Olympus BX-53 trinocular microscope (Japan) attached with digital Sony Camera.

Physicochemical

Air-dried leaves and stems were coarsely powdered to 10/44 (sieve size) and subjected to determination of loss on drying at 105°C, total ash, water soluble ash, acid insoluble ash, extractability in different solvents, physicochemical constants, ultraviolet (UV) aspects of mother tincture following official methods.^[10] Mother tincture was prepared as per Homoeopathic Pharmacopoeia of India [HPI] by percolation method.^[10]

High-Performance Thin Layer Chromatography Analysis

25 mL mother tincture was evaporated on a water bath to remove the alcohol. The residue was extracted thrice with 25 mL chloroform. The concentrated chloroform extract was used for the High Performance Thin Layer Chromatography (HPTLC) study. The concentrated chloroform extract was spotted in the form of band of width 4 mm with a Camag microliter syringe on precoated Silica gel aluminum 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 μ L/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- Camag Switzerland) at 254 nm and 366 nm by reflectance scanning and operated by Wincats software (Camag) resident in the system.^[11-13]

OBSERVATIONS AND RESULTS

Morphology

Evergreen tree upto 30 meter high; branches horizontal and surface of covered with minute knots; twigs covered with densely imbricated leaves, which increase in size and become broken and confounded with the bark; tree yields small bluish berries, male cones small, greenish yellow.

Leaf

Macroscopy

Leaves are green, needles sessile, 0.8–1.2 cm long and 1–1.5 mm wide, narrow, tip sharp, surface adaxially reticulate and abaxially ridged with a median simple vein.

Microscopy

Leaf - surface

Epidermal cells 5–6 sided, polygonal linear, sides slightly thick, straight to curved and few wavy on abaxial, often pitted; surface smooth on adaxial and striated on abaxial; contents dense with crystals of Calcium oxalate and starch grains in few. Distributed: Irregularly and, parallelly oriented [Figure 2b].

Stomata restricted to adaxial, anomocytic, subsidiaries 5 or 6, indistinct, guard cells linear reniform, contents dense, ledged. Distributed: in linear patches of 2–3 rows, parallelly oriented. Stomatal sizes 44–80 μm (58) long and 16–27 μm (22) wide [Figure 2a]. Uniseriate peltate scaly hair, few restricted to lower half of leaf on abaxial.

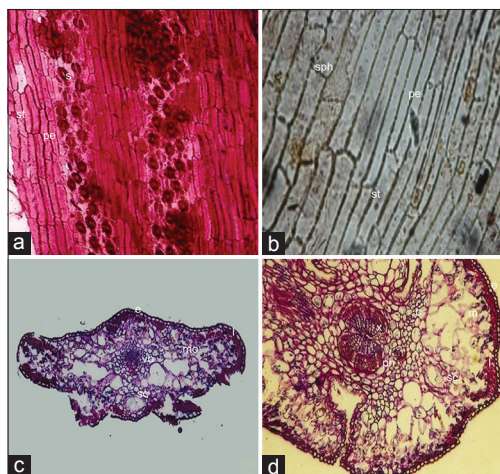


Figure 2: (a) Leaf adaxial surface $\times 111$, (b) Leaf abaxial surface $\times 486$, (c) T.S. of leaf $\times 132$, (d) T.S. of young stem axis $\times 149$. pe: Pitted epidermis, s: Stomata, st: Starch grains, sph: Sphaeraphides, e: Epidermis, l: Lignified hypodermis, sc: Secretory canal, v: Vascular bundle, m: Mesophyll, c: Cortex, ph: Phloem, x: Xylem

In T.S. the leaf is elliptic in outline, ribbed on either sides at midvein, margins obtuse; midvein 302–368 μm (336) and lamina 150–260 (198) μm thick. Epidermis is one layered, with few sunken stomata on adaxial side. Cells oval to spherical, oblong and barrel-shaped, walls slightly thick, contents scanty and in few dense with sphaeraphides of Calcium oxalate, covered by a thick cuticle. 1–2 layered stone cells are characteristic and occur as hypodermis. Mesophyll is 3 to 5 layered made of polygonal and elongated parenchyma cells towards adaxial containing chloroplasts and sphaeraphidal crystals. Cells in middle and abaxial sides are polygonal or rounded in often containing prismatic crystals and chloroplasts with large intercellular spaces. The mesophyll also possess 2–4 oil secretory canals, one conspicuously beneath the central vascular bundle [Figure 2c].

Vascular tissue consists of a central spherical vascular bundle 202–280 μm (220) in diameter, endarch, collateral with a sclerenchymatous layer enclosing the phloem. A layer of endodermis encloses the vascular bundle [Figure 2c].

Young Stem

Macroscopy

Rounded, up to 3 mm thick, surface, greyish to brown, rough, with four leaf bases fused at corners appearing quadrangular, internally creamish.

Microscopy

The epidermis at leaf base is one layered covered by a thick cuticle. Hypodermis consists of a layer of sclereid fibers, which are radially long. The mesophyll is 8–10 layered with some peripheral cells filled with sphaeraphidal crystals and a few with chloroplasts and starch grains. A spherical secretory canal occurs at the periphery in the hypodermal area enclosed by a 2–3 layered epithelium. The inter angled furrowed region has single layered epidermis with a thick cuticle. A 6 to 8 layered cortex with radially elongated cells is present. Secondary phloem consists of phloem parenchyma, sclereid fibers, sieve cells and medullary rays. Phloem parenchyma cells possess sphaeraphides, prismatic crystals and starch grains. Sclereid fibers occur in transverse bands. Medullary rays are uniseriate often containing crystals [Figure 3a].

Centrally the wood is abundant with tracheids and medullary rays. Tracheids are rectangular to tabular arranged in radial rows interrupted by uniseriate rays, which are 2–6 cells high often containing starch

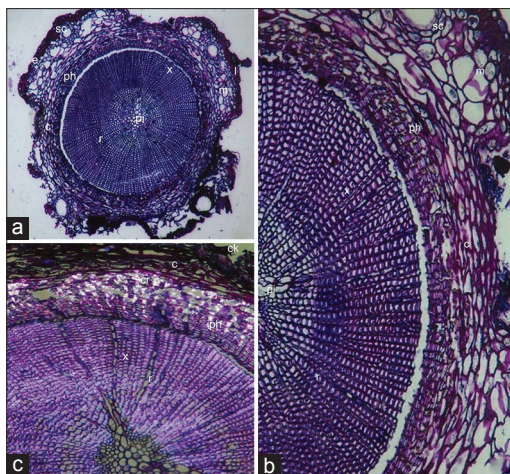


Figure 3: (a) T.S. of young stem $\times 62$, (b) T.S. of mature stem $\times 185$, (c) T.S. of mature stem with crystals $\times 158$. e: Epidermis, l: Lignified hypodermis, m: Mesophyll, sc: Secretory canal, c: Cortex, ph: Phloem, x: Xylem, r: Xylem rays, pi: Pith, cr: Crystals, ck: Cork

grains. The tracheids are long with circular bordered or field pits arranged in single rows. Centrally the pith is scanty, parenchymatous and appears radiating in 3–5 sides. Cells are polygonal, slightly larger with dense contents [Figure 3a].

T.S. mature stem

The stem is spherical in outline covered with remnants of the leaf. The structure of leaf is similar as described in the young stem. Few branch traces occur with precocious cortex. The stem consists of outermost shrivelled cork followed by the cortex which is scanty and compressed. The cortex possesses few crystalliferous cells. Secondary xylem or wood is as described in the young stem but more extensive. Centrally pith is scanty [Figure 3b and c].

Terminal Axis

Macroscopy

Slender, oblong in T.S. upto 1 mm thick, sheathed by young leaves, green.

Microscopy

In T.S. oblong, enclosed by two young leaves, which appear merged with the stem. The leaf mesophyll possesses sphaeraphides and prismatic crystals in few. Stem thin 216–302 μm (244) in diameter. Epidermis is one layered and followed by 3 to 4 layered cortex made of polygonal to elongated cells in tangential bands enclosing vascular tissue. The vascular tissue is surrounded by phloem which possess crystalliferous idioblasts [Figure 2d].

Physico-chemical Studies

The determined data under the physico-chemical

Table 1: Standardization of raw drug

Parameters	Quantitative values
Loss on drying at 105°C	Not more than 18.55% w/w
Total ash	Not more than 6.73% w/w
Acid insoluble ash	Not more than 0.60% w/w
Water soluble ash	Not more than 1.03% w/w
Alcohol soluble extractive	Not <20.0% w/w
Water soluble extractive	Not <15.5% w/w
Extractive values in ^[15]	
Hexane	Not <5.82% w/w
Chloroform	Not <9.23% w/w
Methanol	Not <22.3% w/w

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.

Qualitative Phytochemical Tests

Loss on drying reveals the presence of water in the plant and also some volatile organic matter. Results of physico-chemical studies are summarized in Tables 1-3.

High Performance Thin Layer Chromatography Fingerprinting

The profile of chromatographic separation scanned at 254 nm, reveals seven spots [Figures 4 and 5] out of which 1, 2, 5 and 7 spots possess maximum composition with R_f at 0.24, 0.36, 0.63 and 0.80 respectively. While, chromatogram scanned at 366 nm, revealed 9 spots with 6, 7, 8 and 9 spots showing the maximum composition at R_f 0.42, 0.51, 0.68 and 0.83 respectively. It is evident from the data that these are characteristic for the studied drug, which will help in identification and authentication of the mother tincture. These are considered valuable standards in Pharmacopoeia. At 254 nm, seven spots appear at R_f 0.24 (dark brown), 0.36 (dark brown), 0.45, 0.51 (light brown), 0.63 (dark brown), 0.74 (light brown) and 0.80 (dark brown) [Figure 6] with various concentrations while at 366 nm, nine spots appears at R_f 0.17, 0.23, 0.32 (light red), 0.42 (red), 0.45 (light red), 0.51 (blue), 0.68, 0.81 and 0.83 (red). These are a vital finger print parameters to ensure the reliability and reproducibility of the drug.

DISCUSSION

Pharmacognosy

Juniperus virginiana L. is a large evergreen tree with horizontal branches. The leaves are green, sessile

Table 2: Formulation of mother tincture (percolation technique used)

Alcohol	65% v/v (based on HPUS)
Drug strength	1/10
Preparation	
<i>Juniperus virginiana</i> in coarse powder	100 g
Strong alcohol	677 mL
Purified water	350 mL

To make 1000 mL of the mother tincture. HPUS: Homoeopathic pharmacopoeia of United States

Table 3: Standardization of mother tincture

Parameters	Observations
Organoleptic profile	
Appearance	Clear, nonviscous
Color	Blood red
Odor	Pleasant and fruity
Sediments	Absent
Weight per mL	Not more than 0.89 g
Total solids	Not <1.76% w/v
Alcohol content	61-64% v/v
pH	5.23
λ max	212, 230, 276, 332 nm

needles. The young stem is greyish, slender with minute knots and covered with leaf bases and appear quadrangular. The epidermal cells in the surface are polygonal linear with sides straight to curved and few wavy on abaxial and parallelly oriented. Leaves are epistomatic with stomata in 2–3 rows and anomocytic as also reported earlier. Few, uniseriate peltate scaly hair are present on leaf abaxial. 1–2 layered lignified hypodermis made of stone cells is characteristic as also reported.^[14] The assimilatory parenchyma was reported as reversed heterofacial bifacial in *J. virginiana*^[14] but presently it is undifferentiated.

The 2 to 4 secretory ducts are present in the leaf with one conspicuously beneath the central vascular bundle close to endodermis in leaf and confirms earlier studies.^[14] The presence of idioblasts with sphaeraphides and prismatic crystals in mesophyll is characteristic.

A spherical secretory canal occurs in hypodermal areas at four corners of the stem. Phloem parenchyma possess idioblasts with starch grains, sphaeraphides and prismatic crystals. Centrally the secondary xylem is abundant interrupted by unilayered medullary rays, which contain starch grains. Pith is scanty and appears stellate radiating on 3–5 sides. The mature stem has shrivelled cork

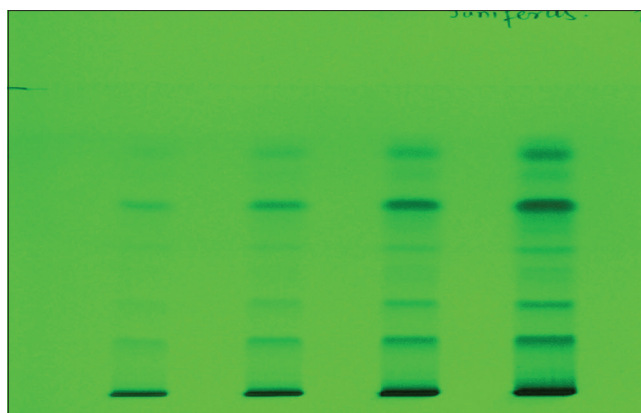


Figure 4: High Performance Thin Layer Chromatography finger printing (chloroform: Methanol 9:1 v/v) of *Juniperus virginiana* Φ scanned at 254 nm

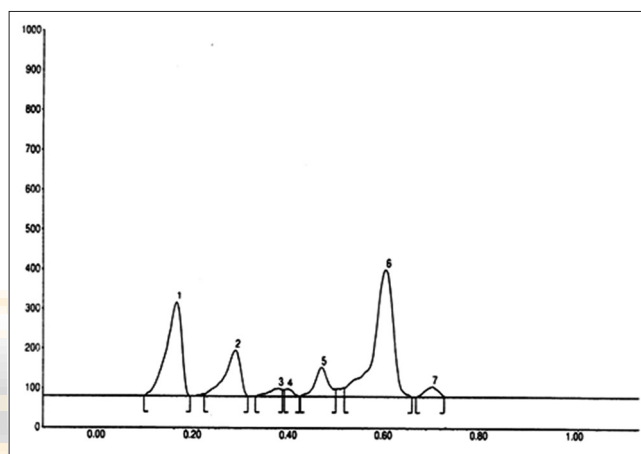


Figure 5: High Performance Thin Layer Chromatography finger printing (chloroform: Methanol 9:1 v/v) of *Juniperus virginiana* Φ scanned at 254 nm

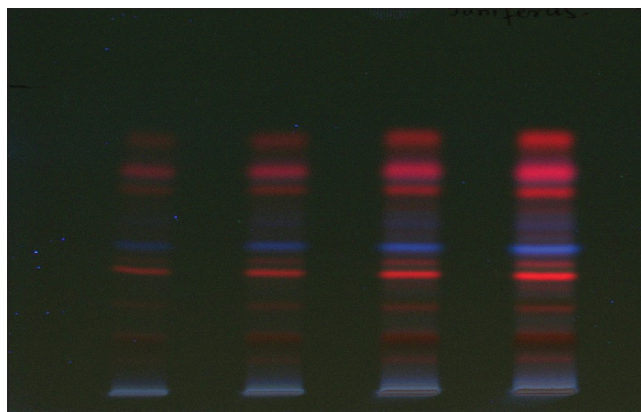


Figure 6: High Performance Thin Layer Chromatography finger printing (chloroform: Methanol 9:1 v/v) of *Juniperus virginiana* Φ scanned at 366 nm

followed by cortex toward the exterior. Secondary phloem is extensive interrupted by medullary rays. Secondary xylem is also extensive, and pith is reduced. The T.S. terminal axis (stem) is slender,

enclosed by the leaves, which appear merged. The vascular tissue is enclosed by phloem containing crystalliferous idioblasts.

Physicochemical

The phytochemical analysis using various reagents showed the presence of secondary metabolites like tannins and phenolic compounds, alkaloids, and volatile oils. Physico-chemical constants viz., ash and extractive values can be used as a reliable aid to check the identity, purity and strength.

Thin Layer Chromatography is done as an important tool for the qualitative and quantitative analysis of herbal drugs and formulations. The results obtained from the study could be utilized for scientific validation and formulating standards for the quality assurance of the drug. The physicochemical properties of *Juniperus virginiana* [Tables 1-3] help to identify and estimate the active compounds present in the drugs. In HPTLC fingerprinting, the developed chromatogram and R_f values of bands will be specific for the drug with the selected solvent system. UV spectroscopic study exhibits, four prominent peaks, which serve as characteristic standards.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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होम्योपैथी औषधि :जूनियेरेस वर्जीनियाना एल. का भेषज अभिज्ञानिक मानकीकरण

सार:

पृष्ठभूमि: जूनियेरेस वर्जीनियाना एल., जिसे अंग्रेजी में आमतौर पर 'रेड सीडर' (लाल देवदार) के नाम से जाना जाता है, क्यूप्रेसेसी कुल का एक प्रसिद्ध, सदाबहार वृक्ष है। होम्योपैथी में पत्तियों और कम आयु के वायवीय प्ररोहों का उपयोग औषधि के रूप में किया जाता है।

उद्देश्य: मानकीकरण वह सर्वोत्कृष्ट पक्ष है जो औषधियों की शुद्धता एवं गुणवत्ता सुनिश्चित करता है। अतः स्थापित प्राचलिक मानकों के साथ प्रामाणिक, अपरिष्कृत औषध पादप सामग्री के उपयोग को सुगम बनाने के लिए भेषज अभिज्ञानिक एवं भौतिक-रासायनिक अध्ययन किए जाते हैं।

सामग्रियां एवं विधियां: जे. वर्जीनियाना एल. के प्रामाणिक नमूनों की पत्तियों एवं कम आयु के वायवीय भागों पर भेषज अभिज्ञानिक अध्ययन किए गए हैं; अपरिष्कृत औषधि के भौतिक-रासायनिक प्राचलों, नामतः निष्कर्षी मान, भस्म मान, सूत्रीकरण, एवं भार प्रति मिली, कुल टोस, एल्कोहल की मात्रा, ज्ञात कर लिए गए हैं और साथ में मदर टिक्चर के लिए उच्च प्रदर्शन तनुस्तर क्रोमेटोग्राफी (एच.पी.टी.एल.सी.) एवं पराबैंगनी दृश्य अध्ययन कर लिए गए हैं।

परिणाम: पत्तियां सूच्याकार, संकीर्ण एवं शीर्ष पर तीक्ष्ण हैं; तने गोलाकार हैं एवं चर्म धूसर श्वेत से भूरी है जो छोटे-छोटे वातरंध्रों से युक्त एवं शल्कीय पत्तियों से आच्छादित हैं। सतह की अधिचर्मी कोशिकाओं के पार्श्व बहुभुजीय रैखिक हैं एवं उनकी भित्तियां गर्तयुक्त हैं जिसमें क्रिस्टल एवं मंड उपस्थित हैं। रंध्र केवल अभ्यक्ष सतह पर रेखीय पंक्तियों में उपस्थित हैं। पत्ती की अधश्चर्म की अनुप्रस्थ काट में 1-2 पतों वाला काष्ठीय दृढोत्तक दिखाई पड़ता है। 2-4 स्रावी नाल उपस्थित हैं जिनमें से एक मध्यशिरा बंडल के नीचे सुस्पष्टतः उपस्थित है। नवोदिभद अंत्याक्ष पर निकट से घेरा बनाती हुई दो पत्तियों का आवरण है जबकि परिपक्व तने पर आधार से जुड़ी 4 पत्तियां होती हैं। तने के संवहनी ऊतकों में प्रधान रूप से दारु ऊतक होता है जो स्फीराफाइड, प्रिज्मीय क्रिस्टल एवं मंड कणों से युक्त पोषवाह ऊतक से घिरा होता है। दारु में एकपंक्तिक किरणें होती हैं। परिपक्व तने में झुर्रीदार काग एवं तत्पश्चात बल्कुट होता है। औषधि के भौतिक-रासायनिक गुणधर्मों एवं एच.पी.टी.एल.सी. मानों को मानकीकृत करके प्रस्तुत किया गया है।

निष्कर्ष: चूर्ण की सूक्ष्मदर्शीय विशेषताएं एवं इंद्रियग्राही अभिलक्षण एवं साथ में आकारिकीय एवं भौतिक-रासायनिक अध्ययन, औषधि हेतु मानक स्थापित करने हेतु नैदानिक हैं।

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