ORIGINAL ARTICLE

Pharmacognostic standardization of Homoeopathic drug: Juniperus virginiana L.

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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 Homeopathy.

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

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 Neveda to British Columbia. 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. Its history and authority about the proving of drug is mentioned in Homœopathic Pharmacopœia of the United States [HPUS]. Chemically, the plant is reported to contain volatile oils, cedrenol, pseudocedrenol, cedrene isomers, bicyclic sesquiterpenes, podophyllotoxin, oleoresins, cedrol [Figure 1], thujopsene and juniperin.

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 µm were stained in crystal violet and basic fuchsin combination as per the Johansen method. 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. Mother tincture was prepared as per Homoeopathic Pharmacopoeia of India [HPI] by percolation method.

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 × 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 µL/s was employed. The slit dimension was kept at 4 mm × 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 × 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.
**OBSERVATIONS AND RESULTS**

**Morphology**

Evergreen tree up to 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.

**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 sclereidal 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 interangled 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, sclereidal fibers, sieve cells and medullary rays. Phloem parenchyma cells possess sphaeraphides, prismatic crystals and starch grains. Sclereidal 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.
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 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.

### Table 1: Standardization of raw drug

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Quantitative values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying at 105°C</td>
<td>Not more than 18.55% w/w</td>
</tr>
<tr>
<td>Total ash</td>
<td>Not more than 6.73% w/w</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>Not more than 0.60% w/w</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>Not more than 1.03% w/w</td>
</tr>
<tr>
<td>Alcohol soluble extractive</td>
<td>Not &lt;20.0% w/w</td>
</tr>
<tr>
<td>Water soluble extractive</td>
<td>Not &lt;15.5% w/w</td>
</tr>
<tr>
<td>Extractive values in [15]</td>
<td></td>
</tr>
<tr>
<td>Hexane</td>
<td>Not &lt;5.82% w/w</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Not &lt;9.23% w/w</td>
</tr>
<tr>
<td>Methanol</td>
<td>Not &lt;22.3% w/w</td>
</tr>
</tbody>
</table>

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 fingerprint 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
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 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 *Rf* 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.

**Acknowledgment**

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Nil.

**Conflicts of Interest**

There are no conflicts of interest.

**REFERENCES**


effect: Juniperus virginiana L., a method of pharmacognosy. The plant is known for its

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