Morphoanatomical and physicochemical standardization of *Casuarina equisetifolia* L. stem bark

Dinesh Kumar a,*, Ajay Kumar a, Om Prakash b

a Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra 136119, India
b Manav Bharti University, Solan, H.P., India

**Abstract**

Objective: The present work was carried out to perform the morphoanatomical and physicochemical evaluation of *Casuarina equisetifolia* L. stem bark.

Method: The pharmacognostic studies were carried out in terms of organoleptic, microscopic, microscopic, fluorescence analysis and physicochemical parameters.

Results: The bark consists of channelled, curved, slightly quilled, usually 0.2–0.8 cm thick, lenticellate pieces with outer surface ash-grey to greyish-brown and internal surface light yellow to deep dirty brown coloured having no odour and astringent taste. The main microscopic characteristics of the bark include phellem (2–5 or more layers of cork), phellogen (3–5 layered) followed by 12–18 layered phelloderm. Other important microscopic components observed include phloem parenchyma, phloem fibre and stone cells. Stem bark powder showed thick walled oval to polygonal cork cells, hexagonal phelloderm cells, rectangular thin walled cortex cells, thick walled elongated phloem fibres, lignified stone cells and rhomboidal crystals of calcium oxalate. Further, physicochemical analysis of the bark powder showed loss on drying, total ash, water soluble ash, acid insoluble ash and sulphated ash as 2.3, 5.0, 1.6, 0.7 and 5.7% w/w respectively. The alcohol and water soluble extractives values of the stem bark were 17.10 and 11.0% w/w respectively.

Conclusion: Various pharmacognostic characters observed in this study helps in botanical identification and standardization of *C. equisetifolia* L. in crude form.

Copyright 2014, Beni-Suef University. Production and hosting by Elsevier B.V. All rights reserved.

* Corresponding author. Division of Pharmacognosy and Phytochemistry, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra 136119, Haryana, India. Tel.: +91 1744 239617, +91 9466772500; fax: +91 1744 238277.
E-mail address: dineshbarbola@yahoo.co.in (D. Kumar).

Peer review under the responsibility of Beni-Suef University
1. Introduction

Casuarina equisetifolia L. synonym Casuarina litorea L. (family: Casuarinaceae) popularly known as 'Junglisaru ' in Hindi and 'She-oak or Horse tail or Whistle pine' in English, is an exotic species to India and native to South-East Asia, Australia and Polynesia (Parrotta, 1993; Pinyopusarerk and House, 1993). It is a large erect evergreen tree up to 50 m height with drooping branches and needle-like branchlets and commonly found along the coast on beaches, rocky coasts, limestone outcroppings, dry hillsides and open forests in India, Sri Lanka and Australia (Han, 1998). In India, it is cultivated in coastal regions from Gujarat to Orissa, West Bengal and Andaman (Anonymous, 1992).

The plant is traditionally used to treat constipation, cough, diabetes, diarrhoea, dysentery, gonorrhea, nervous disorders, stomach ache, throat infections and ulcer (Weiner, 1971; Whistler, 1992; Prajapati et al., 2003). The plant is reported to possess various biological activities like antiasthmatic (Parekh et al., 2011), antioxidant (Chopra et al., 1956; Prakash et al., 2007; Zhang et al., 2010), antifungal (Han, 1998), hepatoprotective (Ahsan et al., 2009), nitrogen fixation (Li-Hua et al., 2009), antidiabetic (Shalini and Kumar, 2011), antiulcerogenic (Ahmed and Urooj, 2011), antidiabetic, antihyperlipidemic (SriRam, 2011), antilucerogenic (Shalini and Kumar, 2011). Phytoconstituents reported in C. equisetifolia include β-sitosterol, campesterol, stigmasterol, cholesterol, cholest-5-en-3β-ol derivatives, casuarine, catechin, citrulline, cupressavon, epicatechin, gallicin, gentisic acid, isoquercitrin, juglanin, kaempferol, proanthocyanidins, rutin, trifolin (Cambie and Ash, 1994; Nash et al., 1994).

A review of literature revealed that no pharmacognostic standards have been recorded for this crude drug. Owing to its ethnopharmacological importance, the present investigation has been undertaken with an objective to establish morphoanatomical and physicochemical standards for stem bark of C. equisetifolia so that authentic plant material could be explored for its therapeutic claim.

2. Materials and methods

2.1. Chemicals

All the chemicals used in the study were of analytical grade and were obtained from Rankem Limited India and Hi-Media laboratories, Mumbai, India.

2.2. Procurement of plant materials

Stem bark of the plant was collected from the campus of Kurukshetra University, Kurukshetra during May 2011 and authenticated by Dr. H.B. Singh, NISCAIR (No: NISCAIR/RHMD/Consult/-2012-13/2105/112).

2.3. Morphological evaluation

Various organoleptic and morphological characters of C. equisetifolia stem bark like colour, shape, size, taste, odour, fracture and configuration etc. were studied (Khatoon et al., 2009).

2.4. Anatomical evaluation

Anatomy allows one to discover convincing diagnostic characters for a specific plant species (of crucial importance in quality control), and it also allows one to observe the distribution of compounds in the plant matrix. In microscopic evaluation, studies were conducted on both grounds qualitatively and quantitatively. The model of microscope used for study of different characters was SKC-400, Suswox Optik, Sudheer Scientific Works, India.

2.5. Qualitative microscopy

In this study, transverse sections of stem bark were studied under photomicrograph. Staining reagents (such as phloroglucinol-HCl) were used as per standard procedures (Kokate, 2010; Bisth et al., 2011). The various identifying features of the drug were studied with or without staining and recorded.

2.6. Stem bark microscopy

The stem bark was dipped in a test tube containing sufficient water and was boiled for few minutes. The softened bark was transversally sliced into fine sections which were subjected to staining reagent 0.1% w/v phloroglucinol followed by concentrated conc. hydrochloric acid. The stained sections were observed under microscope (Ahmed and Urooj, 2011, Bhide et al., 2011). Different layers of cells and identifying characters were observed and thereafter, photomicrography was done.

2.7. Powder microscopy

The dried stem bark was powdered and studied under microscope. Different staining reagents (such as iodine for detection of starch grains and phloroglucinol for detection of lignified components) were used. To a little quantity of stem bark powder taken over a microscopic slide, 1–2 drops of 0.1% w/v phloroglucinol solution and a drop of concentrated hydrochloric acid were added and covered with a cover slip. The slide preparation was mounted in glycerol and examined under microscope (Khandelwal, 2011). The characteristic structures and cell components were observed and their photographs were taken using photomicrography.

2.8. Fluorescence analysis

Fluorescence study of stem bark powder was performed as per reported standard procedure (Kokashi et al., 1958). A small quantity of the bark powder was placed on a grease free clean microscopic slide and 1–2 drops of the freshly prepared reagent solution were added, mixed by gentle tilting the slide and waited for 1–2 min. Then the slide was placed inside the UV chamber and observed in visible light, short (254 nm) and long (365 nm) ultraviolet radiations. The colours observed by...
application of different reagents in different radiations were recorded.

2.9. **Physicochemical analysis**

In this study, air dried material was used for quantitative determination of physicochemical values like loss on drying, total ash, acid insoluble ash, water soluble ash, sulphated ash values and extractive values were determined as per reported method (WHO, 1992).

3. **Results**

3.1. **Macroscopic study of stem bark**

Morphological examination of the stem bark (Fig. 1) shows that the bark consists of channelled, curved, slightly quilled, usually 0.2–0.8 cm thick, lenticellate pieces which are smooth on small trunks, becoming rough and thick furrowed on older trees. Outer surface of the bark is ash-grey to greyish-brown and internal surface light yellow to deep dirty brown coloured with short fracture, odourless and astringent taste.

3.2. **Microscopic study of stem bark**

Transverse section study of the stem bark (Fig. 2) depicts that bark shows 2–5 layers of cork (phellem), consisting of polygonal thick-walled parenchymatous cells filled with reddish-brown content. Phellogen (cork cambium) is 3–5 layered thick having polygonal and tangentially elongated thin-walled parenchymatous cells. Secondary cortex (phelloderm) consists of 12–18 layers having oval to polygonal, tangentially elongated thin-walled parenchymatous cells. Beneath secondary cortex, a large group of oval to elongated stone cells arranged in a tangential manner forming a continuous or discontinuous band. Secondary phloem is composed of phloem parenchyma, phloem fibres and stone cells alternating with lignified stone cells. Lignified fibres are small thin-walled polygonal cells present in cork. A few stone cells are also found scattered in secondary cortex as in secondary phloem.

3.3. **Powder study**

Stem bark powder appears brownish showing thick walled oval to polygonal cork cells; hexagonal phelloderm cells; rectangular thin walled cortex cells; thick walled elongated phloem fibres; lignified stone cells and rhomboidal crystals of calcium oxalate. Powder characteristics of the bark have been shown in Fig. 3.

3.4. **Fluorescence analysis**

The fluorescence characteristics of the stem bark powder with different chemical reagents are summarized in Table 1.

3.5. **Physicochemical analysis**

In this study, various physicochemical parameters like loss on drying, total ash, acid insoluble ash, water soluble ash, sulphated ash and extractive values were determined in triplicate as mentioned in Table 2.

4. **Discussion**

Owing to the wide use of herbal drugs in traditional medicines, standardization becomes an important measure for ensuring quality, purity and authenticity of the crude drugs.
First step in this regard is the authentication of plant species. For this purpose, morphological and anatomical analysis is one of the simplest and cheapest methods to start with establishing the correct identification of the source materials (Nirmal et al., 2012; Kumar et al., 2012). As there is no pharmacognostic work recorded on this medicinally potent plant, the present work was undertaken to lay down the standards which could be useful for establishing its authenticity. The present study reports the morphoanatomical and physico-chemical characteristics of *C. equisetifolia* L. stem bark. Physicochemical studies of stem bark acts as a reliable tool for detecting adulteration (Evans, 2009; Zhao et al., 2011; Raj and Radhamany, 2012).

Studies of physicochemical constants can serve as a valuable source of information which is usually helpful in evaluation of purity and quality of a crude drug. The extractive values give an idea about the chemical constitution of the drug (Kumar et al., 2012). In the present study, the extractive value of methanol was maximum followed by water. The ash values determine the earthy matter or inorganic composition and other impurities present along with the drug. Fluorescence characteristics are an alternative rapid method for resolution of doubtful specimen. When physical and chemical methods are inadequate, the plant material may be identified and differentiated from their adulterants on the basis of fluorescence characteristics (Kumar et al., 2012). Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in daylight. The ultraviolet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter for pharmacognostical evaluation of crude drugs. Adulteration of the genuine raw material is the main cause of degradation of desired therapeutic effect of plant species used in various traditional systems of medicine. Thus, industries could utilize

---

**Table 1** Fluorescence analysis of *C. equisetifolia* stem bark powder.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Visible light</th>
<th>Under UV light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Short wavelength (254 nm)</td>
<td>Long wavelength (365 nm)</td>
</tr>
<tr>
<td>Powder</td>
<td>Yellowish brown</td>
<td>Light brown</td>
</tr>
<tr>
<td>Powder + 1N NaOH (aq.)</td>
<td>Light brown</td>
<td>Light brown</td>
</tr>
<tr>
<td>Powder + 1N NaOH (alc.)</td>
<td>Light brown</td>
<td>Light brown</td>
</tr>
<tr>
<td>Powder + Ammonia</td>
<td>Brown</td>
<td>Brown</td>
</tr>
<tr>
<td>Powder + Picric acid</td>
<td>Yellowish brown</td>
<td>Brown</td>
</tr>
<tr>
<td>Powder + Pet. ether</td>
<td>Light brown</td>
<td>Brown</td>
</tr>
<tr>
<td>Powder + 50% HCl</td>
<td>Light brown</td>
<td>Black</td>
</tr>
<tr>
<td>Powder + 50% H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Pinkish Brown</td>
<td>Black</td>
</tr>
</tbody>
</table>

---

**Table 2** Physicochemical analysis of *C. equisetifolia* stem bark.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value obtained on dry weight basis (% w/w)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying</td>
<td>2.3 ± 0.42</td>
</tr>
<tr>
<td>Total ash value</td>
<td>5.0 ± 0.21</td>
</tr>
<tr>
<td>Acid insoluble ash value</td>
<td>0.7 ± 0.28</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>1.6 ± 0.35</td>
</tr>
<tr>
<td>Sulphated ash</td>
<td>5.7 ± 0.53</td>
</tr>
<tr>
<td>Petroleum ether soluble extractive</td>
<td>1.4 ± 0.16</td>
</tr>
<tr>
<td>Chloroform soluble extractive</td>
<td>2.1 ± 0.08</td>
</tr>
<tr>
<td>Methanol soluble extractive</td>
<td>17.10 ± 0.39</td>
</tr>
<tr>
<td>Water soluble extractive</td>
<td>11.0 ± 0.48</td>
</tr>
</tbody>
</table>

<sup>a</sup> Average of three reading ± SEM.
the scientific background for identification of raw material and this work is not only beneficial to the industries but also enhance the credibility of Indigenous System of Medicine (Chumbhale and Upasani, 2012).

In the present paper, the morphoanatomical and physicochemical studies of the stem bark material help to determine the quality and purity of the C. equisetifolia L. Thus, the above finding will serve in the development of pharmacoepial standards for the future studies.

Acknowledgement

The authors express sincere thanks to UGC, New Delhi (39-955/2010 SR) for financially supporting the study.

REFERENCES

Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. New Delhi: Council of Scientific and Industrial Research (India); 1956.
Prajapati ND, Purohit SS, Sharma AK, Kumar T. Handbook of medicinal plants. Jodhpur: Agrobios (India); 2003.