Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading

Pharmacognostic evaluation of leaf and root bark of *Holoptelea integrifolia* Roxb.

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ARTICLE INFO

Article history:
Received 11 August 2011
Received in revised form 4 September 2011
Accepted 28 September 2011
Available online 28 March 2012

Keywords:
Holoptelea integrifolia
Microscopy
Organoleptic
Fluorescence analysis
Identification
Standardization
Pharmacognostic character

ABSTRACT

Objective: To evaluate the pharmacognostic characters of an important medicinal plant, *Holoptelea integrifolia* (*H. integrifolia*) Roxb. **Methods:** The pharmacognostic studies were carried out in terms of organoleptic, microscopic, macroscopic and fluorescence analysis. **Results:** The characteristic microscopic features of leaves were observed as trichomes, multicellular trichomes, xylem cells, phloem cells, collenchyma, vascular bundles, spongy parenchyma and palisade cells. The characteristic microscopic features of root bark included cork cambium, primary cortex, phloem fibers, medullary rays, endodermis, pericycle and lignified fibers in the transverse section and longitudinal section. The characteristic microscopy of root bark powder showed the presence of cortex cells, sieve tubes, calcium oxalate crystals and lignified fibers. Macroscopic study showed that leaf shape-oval, apex-acute, base-cordate and leaf margin was entire with glabrous surface, bitter taste and characteristic odour. The morphological features of root bark showed deep fissured, rough and firm surface with rhitydome and the periderm parallel to cambium. **Conclusions:** Various pharmacognostic characters observed in this study help in the identification and standardization of *H. integrifolia*.

1. Introduction

Over the last decade there has been a growing interest in drugs of plant origin in contrast to the synthetics that are regarded as unsafe to human and environment[1]. *Holoptelea integrifolia* (*H. integrifolia*) is a roadside tree of family Ulmaceae and commonly known as Indian elm. It has gray bark covered with blister peeling in corky scales on old trees. In traditional system of medicine, bark and leaves are used as bitter, astringent, acrid, thermogenic, anti-inflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent, and in rheumatism[2,3]. The bark of *H. integrifolia* can be easily adulterated with low grade material if the supply of crude drug is inadequate[4]. This adulteration can be prevented by means of various evaluation parameters like microscopic study. Microscopy is an important tool for authentification of crude drugs and study of powdered drugs[5]. It is important to interpret morphological and anatomical descriptions of crude drugs as well as characteristic features of drugs and adulterants of commercial significance[6]. Establishment of the pharmacognostic, morphological and microscopical characters of leaves and bark of the plant will assist in standardization, which can guarantee quality, purity and identification of samples.

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Foundation Project: This work was financially supported by University Grants Committee, New Delhi (grant No. 39-955/2010SR).

2. Materials and methods

2.1. Chemicals

All the chemicals used were of analytical grade and were obtained from E. Merck Limited India and Hi-Media Laboratories, Mumbai, India.

2.2. Procurement of plant materials

Fresh leaves and root bark of *H. integrifolia* were

collected from Ch. Devilal Herbal Nature Park, Chuharpur, Yamunanagar. Identification of the plant was done by Dr. HB Singh, NISCAIR under reference number (NISCAIR/RHMD/Consult/-2010-11/1574/172).

2.3. Organoleptic evaluation

Various sensory parameters of the plant material (such as colour, odour, size, shape, and taste) were studied by organoleptic evaluation.

2.4. Macroscopic evaluation

Various macroscopic characters of fresh leaves of *H. integrifolia* were recorded such as duration, type of leaf base, presence or absence of petiole and characters of lamina. Lamina consists of characteristic features such as composition, incision, shape, venation, margin, apex, base, surface and texture. The root bark is morphologically studied for its size, shape, surface, fracture and configuration[7].

2.5. Microscopic evaluation

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.

2.5.1. Qualitative microscopy

In this study, transverse sections of leaf and root bark as well as longitudinal section of root bark were studied under photomicrograph. Staining reagents (such as phloroglucinol-HCl and methyl orange) were used as per standard procedures[8-11]. The various identifying characters were studied with or without staining and recorded.

2.5.1.1. Leaf microscopy

In this study, leaf was dipped in chloral hydrate solution for several hours until it lost its colour and pigments. The cube of pith was selected and vertically cut, leaf was inserted and fine sections were obtained. Fine sections mounted on glass slide with help of glycerin without any staining reagent used were placed under microscope. Staining of the fine sections with methyl orange and phloroglucinol was done. Various identifying characters, such as type of trichomes[12] and cell composition[13] were recorded and then photomicrography was done.

2.5.1.2. Root bark microscopy

The root bark was placed in a test tube containing sufficient water and was boiled for few minutes. The softened bark was transversally and longitudinally sliced into fine sections. These fine sections were subjected to staining reagent 0.1% w/v phloroglucinol followed by concentrated hydrochloric acid. The stained and unstained sections were observed under microscope[13,14]. Different layers of cells and identifying characters were observed and then photomicrography was done.

2.5.1.3. Powder microscopy

The dried root 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. A little quantity of root bark powder was taken onto 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. The presence of starch grain and calcium oxalate crystal was detected by the formation of blue colour on addition of 2-3 drops of 0.01 M iodine solution[15]. The characteristic structures and cell components were observed and their photographs were taken using photomicrography.

2.5.2. Quantitative microscopy

2.5.2.1. Determination of stomatal number and stomatal index

Stomatal number is the average number of stomata per square millimeter of epidermis. The percentage proportion of the ultimate divisions of the epidermis of a leaf which can be converted into stomata is termed as stomatal index. Stomatal index can be calculated by using following equation:

$I = S / E + S \times 100$, where, I = stomatal index, S = number of stomata per mm^2 and E = number of ordinary epidermal cells per mm^2. A piece of leaf was cleaned and the upper and lower epidermis was peeled out separately by means of forceps. It was kept on slide and mounted in glycerin water. Camera lucida was attached and drawing board was placed for drawing the cells. A square of 1 mm by means of stage micrometer was drawn on it. The slide with cleared leaf was placed on the stage and the epidermal cells and stomata were traced. The number of stomata and the number of epidermal cells in each field were counted. The numbers of stomata were counted as stomatal number and the stomatal index using the above formula was calculated separately for upper and lower surface.

2.5.2.2. Determination of vein-islet and vein termination number

Vein islet is the minute area of photosynthetic tissue encircled by the ultimate division of the conducting strands. Vein termination number is the number of veinlet terminations per mm of leaf surface. A piece of the leaf was cleared by boiling in chloral hydrate solution and camera lucida and drawings board were arranged and 1 mm line was drawn with help of stage mm. A square was constructed on this line in the centre of the field. The slide was placed on the stage. The veins included within the square were traced