3.1 INTRODUCTION

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Plant anatomy mainly concerns with contents, structure, development of cells and tissues. Plant anatomy plays an important role in all aspects of research in plant sciences such as physiology, ecology, taxonomy, morphogenesis, genetics, evolution, reproduction etc. Plant anatomy as a part of pharmacognostic study plays an important role to discriminate right and desirable species from the spurious ones in an attempt to prevent adulteration and to assure the quality of drug. The anatomical evaluation is the identification tool for medicinal plants and it is one of the important parameter in monograph. Necessity of anatomical investigation of medicinal plants for the authenticity and quality control of the drugs has been claimed by many earlier workers Banerjee and Mukherjee and Gupta et al.

Pharmacognosy as a subject of pharmaceutical curriculum focused on those natural products employed in the allopathic system of medicine. Coincident with the increasing attractiveness of alternative therapies and the tremendous range of herbal products are now available to the public. Monograph are now available giving description, test for identity and purity and assay of the active constituents.

Pharmacognosy is the study of medicine derived from natural source. It is the study of the physical, chemical, biochemical and biological properties of drug substances of natural origin as well as the search of new drug from natural origin.
Pharmacognosy is interdisciplinary which includes phytochemistry, ethanobotany, ethanopharmacology and phytotherapy.

Standardization of medicinal plants is global perspective. Due to their heterogenous composition, standardization of natural products are a complex task because in the form of whole plant, plant parts or extracts obtained 4,5. There is a need for proper control of starting material to ensure reproducible quality of herbal products. Authentication is the first step to ensure the quality of starting material. Despite the modern techniques, pharmacognostic studies are more reliable for the identification of plant drugs. For the purpose of identification of a medicinal plant and also to control the purity of prepared medicine, World Health Organization has laid much emphasis on the macroscopic and microscopic studies before any test of that plant product (Anonymous 1996) 6.

3.2 MATERIALS AND METHODS

3.2.1 Specimen collection

The Decalepis hamiltonii root for proposed study was collected from local market, Chennai, Tamil Nadu, India. Identification and authentication of roots were done at Plant Anatomy and Research Center, Chennai, Tamil Nadu, India. To select healthy root, care was taken. The roots were fixed in formalin acetic acid (5 ml formalin + 5 ml acetic acid + 90 ml 70% ethyl alcohol). After fixing for 24 hours, specimen undergoes dehydration with tertiary butyl alcohol in a graded series as per Sass, 1940. Specimen infiltration was done by slowly adding paraffin wax (melting point 58 to 60°C) till super saturation of TBA solution occurs. Specimens were cats in a paraffin blocks.
3.2.2 Sectioning

The specimens were fixed with paraffin and were sectioned by Rotary Microtome. The section thickness were 10 – 12 μm. Dewaxing of sections were done by a customary procedure (Johansen, 1940)\(^7\). Then sections were stained with toluidine blue by method published by O’Brien et al 1964\(^8\). Toluidine blue was a polychromatic stain so staining was remarkably good and cytochemical reactions may also obtained. Dye gives pink color to cellulose walls, dark green to suberin, violet to mucilage, blue color to lignified cells and protein bodies etc. The sections were also stained with safranin and fat green and IKI (for starch) wherever necessary.

3.2.3 Photomicrographs

Microscopic tissue description was supplemented with micrograph wherever necessary. Photographs were also taken for different magnifications with Nikon labphoto 2 necessary units. Bright field were used for normal observations. Polarized light were employed to study lignified cells, crystals and starch grains. Since this structure has birefringent property, they visible bright in dark background under polarized light. Figure magnification was indicated by scale-bars. Descriptive terms of anatomical features were given in standard anatomy books (Esau, 1964)\(^9\).

3.2.4 Physicochemical analysis

The physicochemical analysis of powdered drug like pH, total ash content and moisture content and extractive values were performed.

3.2.4.1 Determination of pH

The pH was measurement of H\(^+\) ion activity and indicates acidity. It may be measured by determining electric potential between glass and reference electrodes,
using commercial apparatus standardized against primary standard pH buffers\textsuperscript{10}. [Appendix 1]

3.2.4.2 Determination of Ash content

Ash content was determined by AOAC method of analysis\textsuperscript{11} [Appendix 2]

3.2.4.3 Determination of Moisture content

Moisture content was determined by AOAC method of analysis\textsuperscript{12} [Appendix 3]

3.3 RESULTS

3.3.1 Morphological features

The dried roots of Decalepis hamiltonii were subjected to morphological study. The studies revealed that the roots are thick, fleshy and soft, storage; surface dark brown or black; strong aromatic aroma and tastes sweet; periderm peels into their flakes.

3.3.2 Microscopic features

The root of Decalepis hamiltonii has narrow, superficial undulate, smooth periderm (Fig 3.1). The periderm has thin, tabular phellem cells; about four layers of phellem cells towards the interior were intact while outer layers were collapsed into less distinct zone (Fig 3.2). Phelloderm was not evident.

Cortex

The cortex was wide, comprising of several layers of parenchyma cells; the cells towards the periphery were compressed forming dark patches (Fig 3.1).
Reddish brown amorphous inclusions were seen filling the entire cells in outer
cortical zone (Fig 3.2). The deposits were tannin contents.

Secondary phloem

Secondary phloem was extensive and wide. It gradually merges with cortex
and no demarcation can be seen between the cortex and phloem. Secondary phloem
consists of outer zone of crushed sieve elements, dilated parenchyma cells and
laticiferous canals (Fig 3.3). Both cortical cells and phloem parenchyma were densely
loaded with starch grains (Fig 3.5 and 3.6). A narrow innermost zone of Hon collapsed
phloem with intact sieve elements was seen outside the xylem cylinder. No starch
grains or laticifers were seen in the intact phloem region (Fig 3.4).

Secondary xylem

Secondary xylem was seen as a narrow, solid central core of cylinder. It
includes central primary xylem and outer secondary xylem (Fig 3.4). Secondary
xylem consists of wide, circular, thin walled, solitary vessels and xylem fibers.

The vessels were narrow in the centre and become wider towards the periphery.
They were in the radial arms with wide sclerochymatous gaps in-between. The
widest vessels 150 μm wide and narrow vessels were 50μm wide.

Xylem fibers were narrow, thin wall and lignified. They were arranged in
regular radial files.

Powder microscopic observation

The powder of the root tuber exhibits fragmentary xylem elements broken
pieces of laticiferous tubes and scattered starch grains.
Laticifers

Laticifers or the latex producing tubes which were wide septate and branched. These type laticifers were called articulated anastomosing laticifers (Fig 3.7 and 3.8). The laticifers were 40 – 60 µm wide. They contained minute granular latex.

Parenchyma cells

Mixed with other elements were seen spherical or polyhedral parenchyma cells (Fig 3.9). These cells were thin walled and have granular inclusions.

Starch grains

Starch grains were abundant in the powder. They included both solitary simple type and large compound type (Fig 3.10 and 3.11). The starch grains were concentric type with central hilum and X- shaped dark marks when viewed under polarized light microscope. The simple starch grains 50 µm wide; the compound grains were 100 µm or more in size.

Fig 3.1: T.S of Tuberous root – Outer part

[Co - Cortex; Pe - Periderm; Ta –Tanniniferous cells]
Fig 3.2: Outer part showing dense tannin filled cortical cells

[Co - Cortex; Pe - Periderm; Ta - Tanniniferous cells]

Fig 3.3: Outer secondary phloem showing crushed sieve elements and diluted phloem parenchyma with dense starch grains

[Co - Cortex; SPh - secondary phloem; Ve - Vessel]
Fig 3.4: Central core of primary and secondary xylem with wide radial arms of vessel surrounded by intact secondary phloem tissue.

[Co - Cortex; Px - Primary xylem; SPh - Secondary phloem; Sx -Secondary xylem; Ve- Vessel]

Fig 3.5: Starch grains seen under bright field microscope

[Co - Cortex; SG - Starch grains]
Fig 3.6: Starch grains seen under polarized light microscope

[SPh - Secondary phloem]

Fig 3.7: Laticiferous tubes embedded in parenchyma tissue

Lf- Laticiferous tube

Fig 3.8: A portion of laticifer enlarged

Lf- Laticiferous tube
Fig 3.9: Broken xylem element and isolated parenchyma cells

[Pa - Parenchyma cells; XE- Xylem element]

Fig 3.10: Starch grains stained with IKI

[CSG - Compound starch grains; SSG - Simple starch grains]

Fig 3.11: Starch grains under polarized light microscope

[CSG - Compound starch grains; SSG - Simple starch grains]
3.3.3 Physicochemical analysis

The physicochemical analysis like pH, total ash content and moisture content and extractive values of various extracts of Decalepis hamiltonii root was showed in Table 3.1.

Table 3.1: Physicochemical analysis of Decalepis hamiltonii root

<table>
<thead>
<tr>
<th>Physicochemical constants</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.8</td>
</tr>
<tr>
<td>Total ash content</td>
<td>5.04%</td>
</tr>
<tr>
<td>Moisture content</td>
<td>13.5%</td>
</tr>
<tr>
<td>Extractive values in g/50g of crude root</td>
<td></td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>3.137</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.437</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.420</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.299</td>
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<tr>
<td>Acetone</td>
<td>3.313</td>
</tr>
<tr>
<td>Methanol</td>
<td>13.30</td>
</tr>
<tr>
<td>Ethanol</td>
<td>12.42</td>
</tr>
<tr>
<td>Aqueous</td>
<td>0.52</td>
</tr>
</tbody>
</table>

3.4 DISCUSSION

The majority of the crude drugs that were of plant origin were used by Indian systems of medicine such as Ayurveda and Siddha. There was a need to set standards to control and check the plant identity and for quality ascertainment before use. A detailed and accurate pharmacognostic evaluation was therefore highly essential.
It was imperative that any crude drug for pharmacological or phytochemical use needs to be subjected to scruling for botanical identity. Correct botanical identity based on the external morphology was possible when a complete plant specimen was available. The correct identification was done with the help of standard floras\textsuperscript{13,14}. Anatomical characters can also help the identification when morphological features were indistinct. Thus in addition to chemical, morphological studies, anatomical studies could help in checking adulteration in crude drugs of similar appearance.

Based on World Health Organization 1998, initial step towards developing identity and degree of purity of such materials were microscopic and macroscopic evaluation of medicinal plant and should be carried out before any tests were undertaken\textsuperscript{15,16}.

Microscopic quantitative characters had been attributed with diagnostic values by the earlier pharmacognostists\textsuperscript{17,18}. Anatomical perspective of medicinal plants in an integral component of pharmacognosy, especially while proposing diagnostic protocols for establishing the botanical identity and ascertaining the quality control of raw materials\textsuperscript{19}.

Accurate microscopic and macroscopic description of medicinal plants must be carried out to maintain standards for safety and quality of herbal products and to authenticate crude drugs properly\textsuperscript{21}. Most of crude drugs were extracted from roots, leaves, barks, rhizomes, so it is difficult to identify crude drugs from macroscopic appearance only, so it should complemented by microscopic characterization.
3.5 REFERENCES


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