Pharmacognostical studies on leaves of Stephania japonica var. Timoriensis


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Abstract
Stephania japonica (Thunb). (Syn S. harnendifolia) is a species under the genus of climbers belonging to family Menispermeceae. It is used in traditional medicinal practices and is locally known as Tubuki lota or Goldua. Having high medicinal value, roots of Stephania japonica are used for treatment of fever, diarrhea, dyspepsia and urinary diseases. The alkaloid akanidine shows significant anti-spasmodic activity on uterine spasms. The leaves of the plant are employed in treatment of convulsions, skin diseases, cough, asthma like symptoms and kidney disorders. Even though this plant has gained scientific importance recently, there is a need for the pharmacognostic standardization. Hence, in the present work the leaf and root part of the plant were subjected to various microscopic and physical evaluations. In the microscopic studies, the different cell structures and arrangements were studied and in physical evaluation the ash values and extractive values were studied. The various pharmacognostical constants were obtained which could help in the development of a suitable monograph for the plant.

INTRODUCTION

The Plant Stephania japonica var. timoriensis have been claimed to possess various medicinal properties. A juice of the whole plant is employed in treatment of convulsions, skin diseases, cough, asthma like symptoms and kidney disorders. In Japan and Taiwan, decoction of the plant is used as a drink to treat malaria. In Indonesia, the roots are used to provide relief in stomach aches convulsions.

From the ethnomedical information and folk claims it is observed that the plant Stephania japonica var. timoriensis have medicinal properties related to urolithiasis and convulsant which have not been scientifically validated, and only some of the phytochemical studies have been carried out and reported for the presence of Alkaloids, Flavonoids, Tannin, Saponins with ability to produce stable foam and steroids. The Present investigation is concerned with the widely distributed indigenous medicinal plant Stephania japonica var. timoriensis. Herbs show a number of problems in when quality aspect is considered. This is because of nature of the herbal ingredients & different secondary metabolites present therein. It is also due to variation in the chemical profile of herbs due to intrinsic & extrinsic factors like growth, harvesting, geographical source, storage & drying etc. Majority of the crude drugs come from wild sources and it is collected by poor, illiterate tribal without any attention to botanical identification and authentication.

Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant. To ensure reproducible quality of herbal medicines, proper control of starting material is utmost essential the first step towards ensuring quality of starting material is authentication followed by creating numerical values of standards for comparison. Pharmacognostical parameters for easy identification like leaf constants, microscopy & physico chemical analyses are few of the basic protocol for standardization of herbs.

MATERIALS AND METHODS

Plant material

The plant specimen for the proposed study were collected from Trichy in the month of July 2010, the plant material was identified and authenticated by Dr. P. Jayaraman, Plant Anatomy Research Centre, Pharmacognosy Institute, Chennai.

Microscopical studies

Transverse section of leaf

Free hand sectioning was done for fresh leaf and root to obtain a thin section. Phloroglucinol and hydrochloric acid in the ratio 1:1 was used as a stain and mounted on a glass slide and focused under a microscope.
Powder microscopy

Shade dried leaf and roots were powdered with the help of an electric grinder till a fine powder was obtained. This fine powder of the leaf and root were subjected to powder microscopy, as per standard procedures mentioned.

Determination of leaf constants

The different parameters like stomatal number, stomatal index, vein islet number and vein termination number was determined as per standard procedure.

Fluorescence analysis

Powdered leaf and root parts were subjected to analysis under ultra violet light after treatment with various chemical and organic reagents like ethanol, 50% sulphuric acid, 10% sodium hydroxide and dilute hydrochloric acid.

Proximate analysis

The various physicochemical parameters like ash values, and extractive values were performed as per the standard procedures.

RESULTS AND DISCUSSION

Leaf

The leaf is distinctly dorsiventral and uniquely differentiated into adaxial and abaxial side (Fig. 1 & 2). The adaxial and abaxial layers are thin with small squanish cells. The adaxial part of the lamina consists of a less prominent slightly thick part with a small vascular strand. This part represents the midrib. The midrib portion has 7-9 layers of fairly larger compact cells. The inner most of the adaxial multilayered part comprises short, cylindrical bone shaped palisade layer. The abaxial part of the leaf consists of two or three layers of large thin walled compact cells. The two adaxial and abaxial zones are widely separated large air chambers which are partitioned from each other by thin vertical partition filaments (Fig.1). The total thickness of the leaf in the midrib region is 1.1mm; thickness of the abaxial midrib is 300µm. The large part is 100µm thick.

Leaf Margin

The leaf margin is bluntly conical in sectional view. As in the middle part of the leaf, the marginal part also has a thin epidermal layer, three or four layers of compact parenchyma cells for the leaves and root of this plant, the diagnostic microscopic features and the numerical standards reported in this work could be useful for the compilation of a suitable monograph for its proper identification.

and an innermost layer of short, bone shaped palisade cells. In between the upper and lower part large, vertically rectangular thin walled cells occur in 2-4 layers. The leaf margin is 300µm thick. Calcium Oxalate Crystals are located in the adaxial epidermal cells (Fig.14). The drugs are occur each epidermal cells and are in uniseriate horizontal row. The drugs are 20µm thick. The crystals are not evident in other parts of the lamina.

Powder Microscopy

Peelings are seen in the powder. The adaxial epidermis consists of squarish thin walled angular cells with straight walls. The adaxial epidermis possesses stomata; The stomata are cyclocytic type, five or six subsidiary cells as an circle. A stomata cuticular striatious are visible on the epidermis.

Determination of leaf constants

The results obtained from leaf constants are tabulated in Table No1. The results show the vien termination of 10-16.

Fluorescence analysis

The coloured fluorescence obtained for the leaf and root powders are tabulated in table no.3.

Proximate analysis

The results obtained for the leaf and root are tabulated in Table no. 2. Physalis angulata is used for the treatment of various physiological conditions. But so far the plant has not been standardized Pharmacognostically. The detailed pharmacognostic studies like microscopical studies, determination of leaf constants, fluorescence analysis and proximate analysis would be a useful for compilation of a suitable monograph for its proper identification and will help in establishing some biological indices. For easier identification of powdered crude drugs UV estimation for both leaf and root powder will be helpful. The Pharmacognostic constants

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomatal index (up.epidermis)</td>
<td>9-10-11</td>
</tr>
<tr>
<td>(low.epidermis)</td>
<td>6-7-7.5</td>
</tr>
<tr>
<td>Vien islet number</td>
<td>8-13 (avg 10.5)</td>
</tr>
<tr>
<td>Vienlet termination</td>
<td>10-16 (avg 13)</td>
</tr>
</tbody>
</table>

Table 1. Leaf constants of Stephania japonica

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>(LOD) 4.5%</td>
</tr>
<tr>
<td>Total ash</td>
<td>2.2%</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>1.5%</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>1.9%</td>
</tr>
<tr>
<td>Alcohol-soluble extractive</td>
<td>18%</td>
</tr>
<tr>
<td>Water-soluble extractive</td>
<td>15%</td>
</tr>
</tbody>
</table>

Table 2. Proximate analysis of leaf
### Table 3. Fluorescence Analysis of Whole Plant Powder

<table>
<thead>
<tr>
<th>S.No</th>
<th>Reagents</th>
<th>Day Light</th>
<th>UV Light</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Whole Plant Powder</td>
<td>Green</td>
<td>Yellow</td>
</tr>
<tr>
<td>2</td>
<td>Powder + 1 N NaOH (aq)</td>
<td>Yellowish green</td>
<td>Brown</td>
</tr>
<tr>
<td>3</td>
<td>Powder + 50% HNO₃</td>
<td>Brown</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>4</td>
<td>Powder + 50% H₂SO₄</td>
<td>Reddish brown</td>
<td>Pale green</td>
</tr>
<tr>
<td>5</td>
<td>Powder + Methanol</td>
<td>Dark brown</td>
<td>Light green</td>
</tr>
<tr>
<td>6</td>
<td>Powder + NH₃ soln</td>
<td>Yellowish green</td>
<td>Grayish yellow</td>
</tr>
<tr>
<td>7</td>
<td>Powder + 1₂ solution</td>
<td>Reddish brown</td>
<td>Dark green</td>
</tr>
<tr>
<td>8</td>
<td>Powder + Picricacid solution</td>
<td>Yellow</td>
<td>Greenish yellow</td>
</tr>
<tr>
<td>9</td>
<td>Powder + FeCl₃</td>
<td>Yellowish green</td>
<td>Pale green</td>
</tr>
<tr>
<td>10</td>
<td>Powder + Glacial acetic acid</td>
<td>Pale green</td>
<td>Green</td>
</tr>
</tbody>
</table>

### Figure 1. T.S of Leaflet through the Lateral View

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### REFERENCES