HISTOCHEMICAL STUDIES OF SVENSONIA HYDEROBADENSIS (WALP.) MOLD – A RARE MEDICINAL PLANT TAXON

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ABSTRACT

Traditional drugs are prepared from a single plant or combination of plants. Histochemical studies are helpful in drug adulteration and systematic arrangement. The present study deals with the location or identification of phytochemicals like tannins, polyphenols, crystals and starch grains in various regions of leaf, stem and root of Svensonia hyderobadensis by using different chemicals or reagents (FeCl₃, Iodine solution, toluidine blue reagent and HCl). The results showed that the bluish black, purple or blue, bluish green and dark black colour indicates the presence of tannins, starch grains, polyphenols and crystals respectively in various regions like epidermis, endodermis, midrib, cortex and vascular bundle of leaf, stem and root of Svensonia hyderobadensis. These observations could be of immense value in the botanical identification and standardization of crude drug.

Keywords: Svensonia hyderobadensis, histochemical, tannins, polyphenols.

INTRODUCTION

Plants are the great sources of medicines, especially in traditional system of medicine, which are useful in the treating various diseases. Indian contribution to herbal market and emphasis on novel research is continuously increasing. The Svensonia hyderobadensis a delicate shrub belonging to the family Verbenaceae, it is commonly called as “Adavichiki” in Telugu language and listed under rare medicinal plants and also recorded highly threatened taxon in Sri Lanka [1]. The macroscopical studies revealed that the plant average is 0.5 to 2.0 M in length. Stem is green in colour, hard, branched, branchlets 4-angular, pubescent. Leaves are 6-8 cm long, 2-4 cm in broad. Opposite phyllotaxy, elliptic-ovate to obovate, coarsely serrate, acute, base rounded to decurrent, charataceous, lateral nerves 6 pairs. Flowers are pink-
purple in colour, small, terminal spikes, bracts linear-lanceolate, scarious. Calyx tubular, unequally 5-toothed, 5-ribbed, splitting along 2 longer-teeth. Corolla salver-form, slightly widened above, obscurely 2-lipped, lobes 5. Stamens 4, inserted at the dilated portion of corolla-tube, included, filaments hairy. Ovary bicarpellary, bilocular; ovule 1 per locule, basal, stigma bilobed. Fruits of 2 oblong, 1-seeded pyrenes \[2\]. The plant is used to treat hepatotoxic diseases \[3\] and has also been studied for biological synthesis of silver nanoparticles and antimicrobial activity \[4-5\], preliminary phytochemical screening \[6\], quantification of phytochemicals \[7\] and also isolation of \( \beta \)-sitosterol \[8\] and pharmacognostical studies \[9\].

Histochemistry or cytochemistry deals with localization of chemical compounds within the cells by means of specific colors of the compounds. Staining the cells with different stains or dyes, which render the compounds visible under the microscope, makes the specific color reaction compounds. The importance of histochemistry in solving critical biosystematic problems is as popular as the use of other markers. According to botanical literatures, the use of histochemical characters in taxonomic conclusions is now a common practice. For example, the presences of calcium oxalate crystals in various plant families have been reported by various scientists \[10\] reported that the size and shape of calcium oxalate crystals though variable in each species showed enough interspecific differences that may be used for taxonomic references in \textit{Vigna} species. This has been done in other groups of plants such as Dioscoreaceae \[11\], Icacinaceae \[12\], Nyctaginaceae, \[13\] and Verbenaceae \[14\]. The biosystematic importance and implications of histochemical features of ergastics, calcium oxalate crystals, nature of tannins and saponins have been investigated in various plants families such as Dioscoreaceae \[15\], Leguminosae-papilionoideae \[16\]. Identification of localization of secondary metabolites in plant parts which are using in the preparation of drug is an immense importance to prevent adulteration and also helpful in taxonomic hierarchy. Hence in the present study an attempt has been made to identification and localization of secondary metabolites in the medicinal plants.

**MATERIAL AND METHODS**

Fresh and healthy leaves, stems and roots of \textit{Svensonia hyderabensis} were collected from Mamandur forest area of Chittoor district, Andhra Pradesh, India during the year December, 2011. These specimens were initially fixed in FAA (1:1:18) glacial acetic: 40% formaldehyde: 70% ethanol (v/v) for 48-72 h after 72 h transverse (T.S) sections were taken.
using a rotary microtome (RMT-30). Anatomical staining was done by initially staining with few drops of alcian blue for 5 min and counter stained with safranin solution for 2 min. The slides were treated with FeCl₃, Iodine solution, tolidune blue reagent and HCl for identification of polyphenols, tannins, crystals and starch grains. Photomicrographs of the anatomical features were then taken from the slides using Nikhon Labhot 2 microscopic unit [17].

**Fluorescence studies**

The fluorescence studies were carried out as per the method of Bhattacharya and Zaman [18]. A small quantity of the leaf powder was placed on a grease free clean microscopic slide and added 1-2 drops of the freshly prepared reagent solution, mixed by gentle tilting the slide and waited for 1-2 min. Then the slides were placed inside the UV-viewer chamber and viewed in day light short (254 nm) and long (365 nm) ultraviolet radiations. The colours observed by application of different reagents in different radiations were recorded.

Behavior of the leaf powder with different chemical reagents was studied to detect the presence of major phytoconstituents with color changes under day light by reported method of Pratt and Chase [19].

**RESULTS AND DISCUSSION**

Histochemical color reactions were carried out through transverse sections of leaf, stem and root of *Svensonia hyderobadensis*. The results were showed in Table-1. Fig-1 showed various secondary metabolites are present in leaf with treatment of different reagents. The presence of tannins was indicated by the development of bluish black colour, when treated with ferric chloride (FeCl₃). The tannins were found mainly in the parenchyma tissue of the midrib region, whereas the starch grains were indicated by the development of blue color or purple, when treated with iodine solution. The starch grains were located in the epidermis and paranchymatous region of midrib. The presence of polyphenols were indicated by the development of bluish green color, when treated with toluidine blue reagent. The polyphenols were found surrounding the vascular bundle sheath. The presences of crystals were indicated by the development of dark black color, when treated with HCl, the crystals were present in the midrib region and vascular bundles.

Fig-2 showed the histochemical studies of stem, the presence of tannins were indicated by the development of bluish black color, when treated with ferric chloride (FeCl₃). The tannins
were presented in endodermis and cortex region. The starch grains were indicated by the
development of blue color or purple, when treated with iodine solution found in epidermis,
cortex and vascular bundles. The polyphenols were found mainly in the endodermis and
cortex region, which were indicated by the development of bluish green colour, when treated
with toluidine blue reagent. The presences of crystals were indicated by the development of
dark black color, when treated with HCl the crystals were present mainly in the cortex.

The histochemical analysis of roots showed in Fig-3 the presence of tannins were indicated
by the development of bluish black color, when treated with ferric chloride (FeCl₃) and these
were found in cortex and vascular bundle region. The polyphenols were found mainly in the
cortex region which were indicated by the development of bluish green color, when treated
with toluidine blue reagent. The presence of crystal indicated by the development of dark
black color, when treated with HCl the crystals were present mainly in the cortex. The starch
grains were indicated by the development of blue or purple colour, when treated with iodine
solution the starch grains were present mainly in the cortex and vascular bundle region.
Starch is mainly stored within xylem parenchyma ray tissue of underground organs. This
type of storage tissue can be considered to be expensive in terms of resource allocation as ray
parenchyma cells of wood are living and non-photosynthetic and require a high metabolic
demand to be both created and maintained [20-21].

Toluidine blue is a cationic dye that binds to negatively charged groups. An aqueous solution
of this dye is blue, but different colors are generated when the dye binds with different
anionic groups in the cell for example, a pinkish purple colour will appear when the dye
reacts with carboxylated polysaccharides such as pectic acid; green, greenish blue or bright
blue with polyphenolic substances such as ligning and tannins; and purplish or greenish blue
with nucleic acids [22]. Plants store glucose as the polysaccharide starch, it can be separated
into two fractions-amylose and amylopectin. Amylose forms a colloidal dispersion in hot
water, whereas amylopectin is completely insoluble. The structure of amylose consists of
long polymer chains of glucose units connected by an alpha acetal linkage. Amylose in
starch is responsible for the formation of a deep blue colour in the presence of iodine. The
iodine molecule slips inside of the amylose coil. When added the Iodine-KI reagent to a
solution or other materials blue colour is present. If starch amylose is not present, then the
colour will stay orange or yellow.
Fluorescence Analysis

Behavior of the leaf powder with different chemical reagents was studied to detect the presence of major phytoconstituents with color changes under day light and the results were showed in Table-2.

The fluorescence characteristics of leaf powder with different chemical reagents were summarized in Table-3. Although a change in color was observed by the addition of various reagents under day light, none of the reagents induced any fluorescence to the leaf powder under both short and long UV radiations. Under UV light dark brown and black colors were prominent. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents showed fluorescence in the visible range in the day light. The natural products (Alkaloids) produce fluorescence in UV light but do not produce fluorescence in visible day light. If the substances themselves are not fluorescent,
they often be converted fluorescent derivatives or decomposition products by applying different reagents \cite{23-24}.

The histochemical studies and behavior analysis of fluorescence studies of *Svensonia hyderobadensis* are useful to supplement the information with regard to its botanical identification and drug standardization. Moreover, it also helps in distinction from other allied species and adulteration.

**Fig-2: Histochemical studies of stem of *Svensonia hyderobadensis***

1) Starch grains, 2) Polyphenols, 3, 4) Tannins and 5) Crystals
Fig-3: Histochemical studies of root of *Svensonia hyderobadensis*

1) Starch grains, 2) Polyphenols, 3) Tannins and 4) Crystals

Table-1: Histochemical analysis of leaf, stem and root of *Svensonia hyderobadensis*

<table>
<thead>
<tr>
<th>S. No</th>
<th>Reagent</th>
<th>Color</th>
<th>Constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leaf</td>
<td>Stem</td>
</tr>
<tr>
<td>1.</td>
<td>Toluidine blue</td>
<td>Bluish green</td>
<td>Bluish green</td>
</tr>
<tr>
<td>2.</td>
<td>FeCl₃</td>
<td>Bluish black</td>
<td>Bluish black</td>
</tr>
<tr>
<td>3.</td>
<td>Iodine</td>
<td>Blue</td>
<td>Blue</td>
</tr>
<tr>
<td>4.</td>
<td>HCl</td>
<td>Dark black</td>
<td>Dark black</td>
</tr>
</tbody>
</table>
Table-2: Leaf powder behavior of the *Svensonia hyderobadensis* with different chemical reagents

<table>
<thead>
<tr>
<th>S.No</th>
<th>Reagent</th>
<th>Color/Precipitate</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Powder + Conc.H₂SO₄</td>
<td>Reddish</td>
<td>Steroids are present</td>
</tr>
<tr>
<td>2.</td>
<td>Powder + Aq.FeCl₃</td>
<td>Blackish</td>
<td>Tannins are present</td>
</tr>
<tr>
<td>3.</td>
<td>Powder + Iodine solution</td>
<td>Blue</td>
<td>Starch grains are present</td>
</tr>
<tr>
<td>4.</td>
<td>Powder + Dragendorff’s reagent</td>
<td>No precipitation</td>
<td>Alkaloids are absent</td>
</tr>
<tr>
<td>5.</td>
<td>Powder + Aq. HCl+ Trim-Hill reagent</td>
<td>Green</td>
<td>Terpenoids are present</td>
</tr>
<tr>
<td>6.</td>
<td>Powder + Aq. Lead acetate</td>
<td>Reddish brown</td>
<td>Flavonoids are present</td>
</tr>
<tr>
<td>7.</td>
<td>Powder + Distilled water</td>
<td>Foam formation</td>
<td>Saponins are present</td>
</tr>
</tbody>
</table>

Table-3: Fluorescence analysis of leaf powder of *Svensonia hyderobadensis*

<table>
<thead>
<tr>
<th>Powder + Reagent</th>
<th>Visible/ Day light</th>
<th>UV 254 nm</th>
<th>365 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder + 1M NaOH</td>
<td>Light brown</td>
<td>Black</td>
<td>Blackish Brown</td>
</tr>
<tr>
<td>Powder + Acetic acid</td>
<td>Dark brown</td>
<td>Black</td>
<td>Black</td>
</tr>
<tr>
<td>Powder + 1M HCl</td>
<td>Brown</td>
<td>Blue</td>
<td>Black</td>
</tr>
<tr>
<td>Powder + Dil HNO₃</td>
<td>Brown</td>
<td>Blue</td>
<td>Black</td>
</tr>
<tr>
<td>Powder + 5% Iodine</td>
<td>Yellowish brown</td>
<td>Black</td>
<td>Black</td>
</tr>
<tr>
<td>Powder + 5% FeCl₃</td>
<td>Greenish brown</td>
<td>Black</td>
<td>Black</td>
</tr>
<tr>
<td>Powder + HNO₃ + 25% NH₃</td>
<td>Light brown</td>
<td>Black</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Powder + Methanol</td>
<td>Brown</td>
<td>Dark brown</td>
<td>Brown</td>
</tr>
<tr>
<td>Powder + 50% HNO₃</td>
<td>Yellowish brown</td>
<td>Black</td>
<td>Black</td>
</tr>
<tr>
<td>Powder + 1M H₂SO₄</td>
<td>Reddish brown</td>
<td>Dark brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Powder + Dil. NH₃</td>
<td>Dark brown</td>
<td>Dark brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Powder + Con. HNO₃</td>
<td>Yellowish brown</td>
<td>Black</td>
<td>Black</td>
</tr>
</tbody>
</table>

REFERENCES