



STUDIES ON PHARMACOGNOSY, MICROMORPHOLOGY AND HISTOCHEMICAL LOCALIZATION OF FEW PHYTOCHEMICALS IN MEDICINAL PLANTS IN THE LATERITIC BELT OF WEST BENGAL.

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Abstract

The present study was focused to analyze some phytochemicals along with its Pharmacognosy, micromorphology and histochemical localization of those phytochemicals in different economically important parts in relation to drug production in three important medicinal plants in lateritic belt of West Bengal, India. Among the plants, *Holarrhena antidysenterica* Wall. (Apocynaceae) was found an adjunctive therapy for Inflammatory Bowel Disease (IBD); *Paederia foetida* Linn. (Rubiaceae) was shown useful for diarrhoea, piles, gout etc. and *Rauwolfia serpentina* (L) Benth ex Kurz. (Apocynaceae) was noticed as very important traditional medicine to cure Rheumatic pain, snake and scorpion bite and treated a number of diseases like hypertension and mental disorder. The presence of different phytochemicals like alkaloid, starch, tannin, reducing sugar, protein, flavonoids and amino acids were localized through colourization using different reagents. These were further confirmed through colour development due to the specific chemical reaction tests. The phytochemicals were identified prominently in different locations of the stem, leaf and root of *H. antidysenterica*, *P. foetida* and *R. serpentina* respectively. Observation on micromorphological features revealed the standardization of these traditional drug sources.

Key words: Phyto-pharmacognostical, histochemical, phytochemicals, medicinal plants

Introduction

In India many indigenous plants are used in herbal medicine to cure diseases, heal injuries and also herbal drugs are in great demands than ever before. Now a day's public are also of general awareness in regarding the safety and efficacy of herbal drugs. Hence development of new drugs without any side effect is the urgent need of the society (Hussain and Sheikh, 2008). Quality control studies on plant material are essential to ensure the reproducible quality of the herbal products. The initial step to ensure quality of any starting material is authentication (Lalithrani *et. al.* 2011). WHO suggests that the macroscopic and microscopic description of a plant material is the primary step in establishing its identity (WHO report, 1998). So it is very necessary that the plant material should be proved before developing any formulation or using in any disease condition.

Different important medicinal plants are found in the

lateritic belt of W.B., India. Among them, *Holarrhena antidysenterica* Wall (family Apocynaceae, local name in west Bengal, India Kurchi; *Rauwolfia serpentina* (L) Benth ex Kurz (Family Apocynaceae local name in West Bengal, India- Sarpagandha) and *Paederia foetida* Linn (Family Rubiaceae; local name in West Bengal- Gandal are very popular and well-known medicinally important plants of the locality. *H. antidysenterica* is a reputed plant in Ayurvedic system of medicine; it has antioxidant (Kavitha *et. al.* 2004) antiamebic (Nadkarni, 1982), antidiarrhoeal (Sing, 1986) activity. *R. serpentina* (L) Benth ex Kurz has long been used in India for the treatment of snake bites, hypertension, high blood pressure, mental illness, gastrointestinal diseases, circulatory disorders, pneumonia, fever, malaria, asthma, skin diseases, AIDS, rheumatism and body pain etc. (Dey and De, 2011). In India *P. foetida* Linn is a medicinal plant and reported to be used in gout, vesical calculi, diarrhoea, dysentery, piles, inflammation of the liver and emetic (Blatter and Caius, 1981). Considering the above

views on importance of these medicinal plants, an attempt was made to study the anatomy of the different parts and to find out histochemical localization of crude drugs, characters of these phytochemicals and micromorphology of *H. antidysenterica*, *R. serpentina* and *P. foetida* respectively.

Material and Methods

Collection of plant material:

Plants materials (Fig.1) *i.e.* *Holarrhena antidysenterica* Wall (Apocynaceae), *Paederia foetida* Linn. (Rubiaceae) *Rauwolfia serpentina* (L) Benth ex Kurz (Apocynaceae) for the present study were collected from medicinal plant garden of Rampurhat College, Rampurhat, Birbhum, West Bengal, India, located in the lateritic belt of W.B. The plants had been carefully identified with the help of different Floras (Maheshwari, 2000, Panigrahi and Murthy, 1989, Verma, 1981, Paria, 2005). After authentication of stem, leaf and root of *H. antidysenterica*, *R. serpentina* and *P. foetida* respectively were collected in bulk and washed under running tap water to remove adhering dirt.

Study on anatomy:

For microscopical studies on the stem of *H. antidysenterica*, leaf of *P. foetida* and root of *R. serpentina*, free hand sections were done, cleared and stained with safranin according to the prescribed method (Kokate, 1994, Kokate *et. al.* 2008) and observed under 10X × 10X Olympus microscope (Fig 2a, 2b, 2c)

Study on Pharmacognosy:

Powder microscopy is an important parameter for identification of the drug. After shade drying the plant specimens and the materials were made into a coarse powder by grinding in mechanical grinder. A judicious quality of powder (stem, leaf and root of *H. antidysenterica*, *P. foetida* and *R. serpentina* respectively) was taken on a glass slide. A few drops of chloral hydrate were added on the slide which was heated for 1-2 minutes after placing a coverslip; care should be taken to avoid the air bubbles. Lignified tissues are to be confirmed by staining. To the powder a few drops of mixture of phloroglucinol with concentrated hydrochloric acid was added and after 3-4 minutes and observed under microscope. The well known microscopic characters

were determined under 10X × 10X Olympus microscope (Fig. 3-5).

Study on microchemicals:

For qualitative test and histochemical localization of the phytochemicals or secondary metabolites such as alkaloids, sugars, flavonoids, tannins, reducing sugars, amino acids, lignins, powder of the different materials added with 90% methanol following the method of Jana *et al.* 2009. The extracts were used for different colour reaction tests for identification of different phytochemical groups (table 1 and table 2). General reaction in this analysis revealed the presence or absence of these compounds in crude extract tested (Brindha and Saraswathy. 1981).

Results and Discussion

The plant *Holarrhena antidysenterica* Wall. *Paederia foetida* Linn. and *Rauwolfia serpentina* (L) Benth ex Kurz. were found important folk medicine as well as in modern medicine system. The stem of *H. antidysenterica*, leaf of *P. foetida* and root of *R. serpentina* were mainly explored rather than other parts of these mentioned plants. So the economically important parts in relation to phytomedicine were investigated.

Table 1: Phytochemical analysis of methanol extracts of stem of *H. antidysenterica*, leaf of *P. foetida* and root of *R. serpentina* respectively.

Reagents	Colour	Secondary metabolite	<i>H. antidysenterica</i>	<i>P. foetida</i>	<i>R. serpentina</i>
Wagners' brown	Dark	Alkaloid	+++	++	+++
KI+I ₂ Iodine solutions	Blue black	Starch	++	++	+++
10% aqueous Lead Acetate	Yellow	Tannin	++	+	+
Benedict's	Brick red	Reducing Sugar	++	+	++
Lugol's	Dark Brown	protein	++	+	+
10% NaOH	Yellow	Flavonoids	+++	++	+++
Millon's	Yellowish brown	Protein	++	+	+
Fehling's (A+B)	Brick red	Reducing Sugar	++	+	+
2% Ninhydrine	Lemon yellow	Amino acids	++	+	+

+, ++, +++ represent the degree of intensity of the colour change *i.e.* presence of phytochemical groups and – represents no change of colour *i.e.* absence of phytochemical groups.

Table 2: Histochemical localization of secondary metabolites in stem of *H. antidysenterica*, leaf of *P. foetida* and root of *R. serpentina* respectively

Secondary metabolite / Plant species	Alkaloid	Starch	Tannin	Reducing sugar	Protein	Flavonoids	Amino acid
Stem of <i>H. antidysenterica</i>	Bark, hypodermis	Bark, hypodermis	Bark	Bark, hypodermis	Bark, hypodermis	Bark, hypodermis	Bark, epidermis, hypodermis
Leaf of <i>P. foetida</i>	Xylem, epidermis	Hypodermis	Absent	Xylem, Phloem	Xylem, Phloem	Xylem, Phloem	Xylem
Root of <i>R. serpentina</i>	Cork, Xylem, Phloem	Xylem, pith	Cork, xylem	Cork, xylem	Cork, xylem	Xylem	Cork, xylem

Anatomy and micromorphology of stem of *H. antidysenterica*, leaf of *P. foetida* and root of *R. serpentina*:-

The stem of *H. antidysenterica*: The transverse section of *H. antidysenterica* showed complete cork layer. Phelloderm cells were parenchymatous and contained calcium oxalate crystals. Cortex layer was merged with Phelloderm; vascular tissues showed secondary wood formation and starch grains, sclerides were also present in the cortical layers (Fig. 2a)

The powder features showed presence of cork

tissues, complex starch grains, non functional and functional sieve tissues, medullary ray cells, perforated vessels and companion cells etc (Fig. 3a -3d).

The leaf of *P. foetida*: The transverse section of the leaf of *P. foetida* showed prominent midrib and lamina. Both upper and lower epidermis was consisted with epidermal multicellular trichomes. Mesophyll composed of single layered palisade cells and 3-4 layered spongy tissues. The margins of the leaf were consisted with the thick walled cells. Midrib was composed of single layered epidermis covered with cuticle and ground tissue



Fig. 1. Photographs of investigated plants.

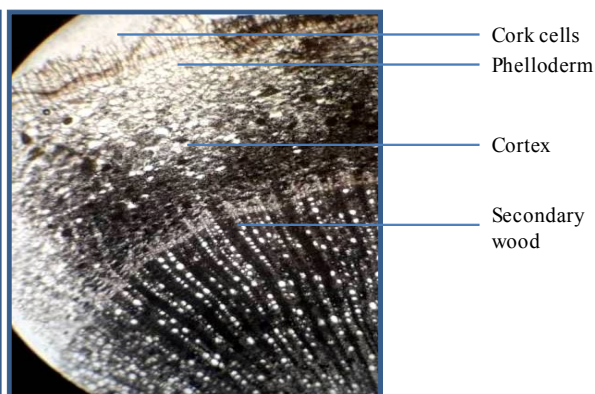


Fig. 2a. T.S. of the stem of *Holarrhena antidysenterica*.



Fig. 2b. T.S. of the leaf of *Paederia foetida*.

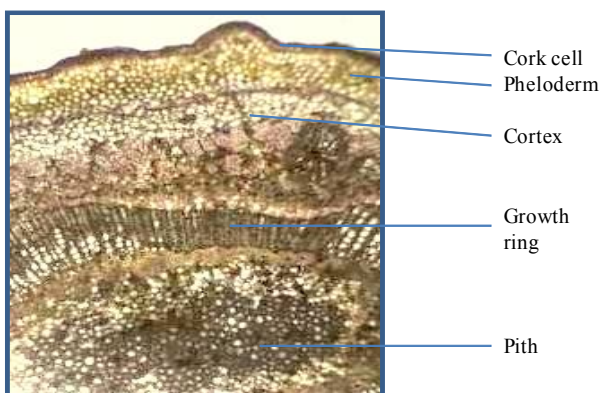


Fig. 2c. T.S. of the root of *Rauwolfia serpentina*.

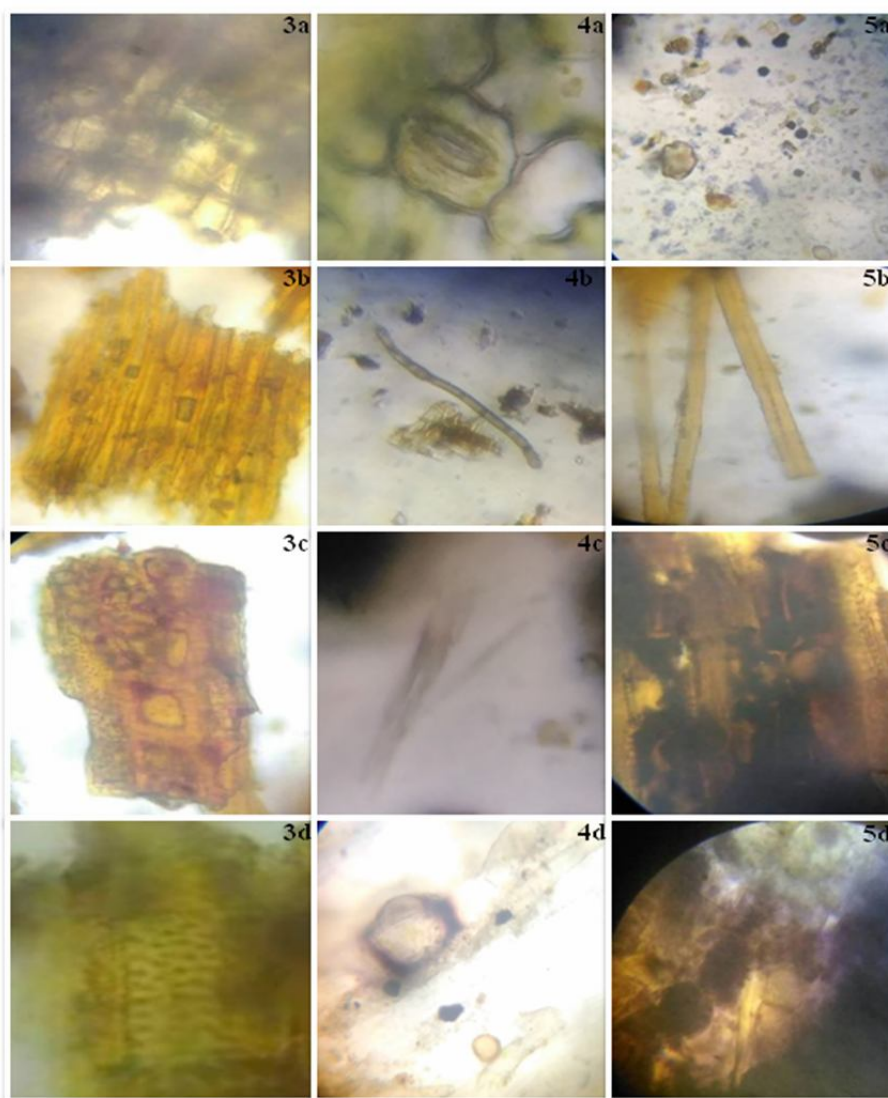


Fig. 3-5. Micromorphology of stem of *H. antidysenterica* (Fig. 3) Leaf of *P. foetida* (Fig. 4) and Root of *R. serpentina* (Fig. 5)

3a. Cork tissue 3b. Sieve tubes 3c. Medullary ray cells 3d. Perforated vessel
 4a. Paracytic stomata 4b. Uniseriate hair 4c. Acicular raphides 4d. Glandular hair
 5a. Starch grains and calcium oxalate crystals 5b. Fibres 5c. Pitted vessels
 5d. Tracheids with xylem parenchyma

consisting of 2-5 layered collenchyma towards upper and lower side. A large vascular bundle with xylem towards upper side and phloem towards lower side was also present in the centre of the midrib (Fig 2b).

Powder anatomy of *P. foetida* showed epidermal fragments with paracytic stomata and multicellular hairs. The few mesophylls and few palisade parenchymas consisted of cluster of needle shaped raphides of calcium oxalate (acicular raphides). The hairs were uniseriate, 6-9 celled, bluntly pointed and smooth. Glandular hairs were also present in epidermal cell. The secretion cells contained volatile oil.

The root of *R. serpentina*: The transverse section of root *R. serpentina* showed thin cork layers followed by cortex and massive secondary tissues surrounding the large pith. In the secondary xylem there was distinct growth ring. The cortical cells were filled with granular secretions which stained yellow with iodine; starch granules were also present in cortical cells (Fig. 2c).

Powder anatomy of *R. serpentina* showed abundant starch grains, simple to compound prism shaped calcium oxalate crystals, non lignified fibres, polygonal cork cells and elongated pitted vessel with oblique end walls.

Phytochemicals and their localization of stem of *H. antidysenterica*, leaf of *P. foetida* and root of *R. serpentina*:

It was observed from the Table. 1 that *H. antidysenterica* contained high quantity of alkaloids giving dark colouration with Wagner's and 10% NaOH respectively, but starch, tannin, reducing sugar, protein and amino acids remained lower giving light colouration with iodine, 10% lead acetate, Benedict's and Fehling's reagent, Millon's reagent and 2% ninhydrin respectively. In case of *P. foetida*, all of the chemicals except alkaloid, starch and flavonoids were present in low quantity. *R. serpentina* gave light colouration for tannin, protein and reducing sugar and amino acid but gave dark colouration for alkaloids, starch and flavonoids which are the main source of valuable drugs.

Presence of Alkaloids: It is found from the table 1 that the alkaloids were present with dark brown colouration by the Wagner's reagents in bark and hypodermis of *H. antidysenterica* but in case of *P. foetida* leaf alkaloids were found in xylem and epidermis. On the other hand cork, xylem and phloem of *R. serpentina* root contained alkaloids. Similar types of results were obtained by Panda *et al.* 2012.

Presence of Starch: Through iodine test (blue black colouration), it was found that starch grains were located only in hypodermis of *P. foetida* leaf; bark and hypodermis of *H. antidysenterica* stem and the xylem and pith cells of *R. serpentina* root. Similar types of results were obtained by some other workers in other plants in table. 2. (Mandal and Mandal, 2012, 2014).

Presence of Tannins: Yellow colouration was found by using 10% lead acetate only in bark of *H. antidysenterica*, xylem of *P. foetida* leaf and cork of *R. serpentina* root indicated the moderately presence of tannins in the three taxa under study. Kumar *et al.* 2009 also was found similar type of result (table. 2).

Presence of Reducing sugar: It is observed from table. 2 that reducing sugar was present in bark and hypodermis of *H. antidysenterica* stem, in xylem and phloem in of *P. foetida* leaf and in the cork and xylem cells of *R. serpentina* root with brick red colouration by Benedict's and Fehling's reagents both. Trease and Evans, 1983, observed similar types of results and chemical analysis in medicinal plants.

Presence of Proteins: Through the test with Lugol's and Millon's reagents dark brown and yellowish brown colouration were observed respectively in bark and hypodermis of *H. antidysenterica* stem; in xylem and phloem of *P. foetida* leaf and cork and xylem of *R. serpentina* root. Similar types of chemical analysis of

secondary metabolites were present in some medicinal plants in table. 2 (Harborne *et al.* 2000, Mandal and Mandal, 2014).

Presence of Flavonoids: Yellowish brown colouration was found in bark and hypodermis of *H. antidysenterica* stem; in xylem and phloem of *P. foetida* leaf and only in xylem of *R. serpentina* root using 10% NaOH. Harisaranraj *et al.* 2009 also found similar type of result.

Presence of Amino acids: Amino acids were observed only in the xylem of *P. foetida* leaf and bark, epidermis and hypodermis of *H. antidysenterica* stem and cork and xylem of of *R. serpentina* root with lemon yellow colouration using 0.2% ninhydrin reagent. Similar types of results were observed in Asclepiadaceae by Krishnamurthy and Kannabiran, 1970.

The presence of these phytochemicals in the three studied plants indicates their medicinal properties. The alkaloids and flavonoids are used as basic medicinal agents for their analgesic, antispasmodic and bacterial effects (Stray, 1998). Tannins have stringent properties, hasten the healing of wounds and inflamed mucous membranes. These perhaps explain why the traditional medicine healers. India often uses these plants in treating many disorders (Okaka and Okaka, 2001). Preliminary qualitative chemical tests were performed, which showed that the studied plants were credited with alkaloid, starch, tannin, reducing sugar, protein, flavonoids and amino acids. Microscopic, macroscopic and other chemical values and parameters will help to identify the correct species of the plant. Among the three plant, *R. serpentina* (L) Benth ex Kurz. is almost on the tract of extinction and is categorized as an endangered species. Therefore it is necessary to preserve this species for its valuable drug source.

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