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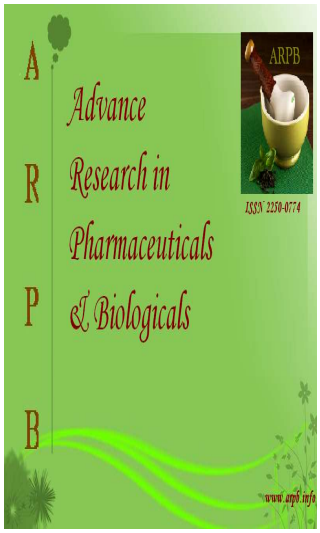

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HISTOCHEMICAL INVESTIGATION OF SOME MEDICINAL PLANTS

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ABSTRACT:

This research article is aiming at histochemical investigation of some medicinal plants. For this, we choose three species used in folk medicine to determine their histochemical investigation: *Adhatoda zeylanica*, *Ruta graveolence* and *Vitex negundo*. In general, these plants are used in folk medicine in the treatment of gonorrhoea, antiperiodic, bronchitis, infected wounds, scrotal swelling, synovitis, arthritis pain and rheumatic arthritis. For histochemical studies the free hand sections of leaves and stem were taken and treated with the respective reagent to localize components, *viz*. starch, protein, tannin, saponin, fat, glucosides and alkaloids in the tissues.

KEYWORDS: Histochemical, Gonorrhoea, Synovitis, Medicinal plants, Folk Medicine.

INTRODUCTION

In Marathwada, indigenous people traditionally use *A. zeylanica, R. graveolence* and *V. negundo* to maintain their health. These plants have anormous reservoirs of many secondary metabolites which exhibit some medicinal properties.

The different parts of *A. zeylanica* have been used in the indigenous systems of medicine. The root has been mentioned as useful in the treatment of used gonorrhoea, the flowers in Jaundice and Opthalmia. But leaves are more extensively used, particularly for the infection of respiratory tract such as chronic and acute bronchitis. The dried leaves are smoked as cigarettes in asthma and juice of fresh leaves have been used for diarrhoea and dysentery and also said to be a valuable antiseptic, antiperiodic and anthelmintic[1], essential oil isolated from the leaves shows anti-tubercular activity[2]. *R. graveolens* are traditionally used for the treatment of rheumatism, arthritis and other inflammatory conditions in the traditional medicine of India[3]. The leaves of *V. negundo* are best analgesic, anti-inflammatory, antibacterial, cleaning and healing wounds, hair tonic. Heated *V. negundo* leaves are tied over the affected part in headache, scrotal swelling, synovitis, arthritis pain and rheumatic arthritis. Decoction prepared from leaves is used for tub bath in endometritis, colitis and orchitis. It is also useful in the diseases of the eye, inflammations, leucoderma, enlargement of spleen, bronchitis, asthma, swelling of joints and painful teething of children[1].

Histochemical methods have been developed for qualitative and quantitative analysis of virtually all cellular components, including proteins, carbohydrates, lipids, nucleic acids and the range of ionic elements occurring in cell solutions[4,5,6]. These methods, in combination with various microscopic imaging techniques, can be utilized in the study of essential oil secretion in plants.

Several published reports demonstrate the use of histochemistry to locate essential oil, such as the localization of citral accumulation in *Cymbopogon citratus*[7], where the aldehyde-specific Schiff's reagent was used to detect the monoterpene aldehydes neral and geranial (citral), and the lipid stains Sudan red and Nile blue were used to locate essential oil in leaves of *Salvia aurea*[8].

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues. Starch deposition occurs widely in the plant body, but the particularly common places of its accumulation are seeds, the parenchyma of the secondary vascular tissues in the stem and root, tubers, rhizomes and corn[9]. Starch and proteins are the principal ergastic substances of the protoplast[10]. Tannin is the heterogeneous group of phenol derivatives, usually related to

glucosides. Tannins are particularly abundant in the leaves (xylem) of many plants[11]. Saponins are the rare occurrence. Fats are widely distributed in the plant body and they probably occurs in small amount in every plant cell[12]. Fats are common reserve material in seeds, spores and embryos in meristematic cells. Glucosides are the degradation product of the carbohydrates. Alkaloids are the degradation product of protein.

Many plants contain medicinally important secondary product[13]. Therefore, we have attempted to perform histochemical investigations of different plant parts of *Adhatoda zeylanica*, *Ruta graveolence* and *Vitex negundo*, three medicinal plants of Marathwada region in Maharashtra. Free hand sections were taken for the histochemical studies. Sections are treated with the respective reagent to localize components, *viz*. starch, proteins, tannin, saponin, fat glucosides and alkaloids in the tissues.

MATERIALS AND METHODS

Sample collection:

The Plant material of *Adhatoda zeylanica* Medicus (Acanthaceae), *Ruta graveolence* Linn. (Rutaceae) and *Vitex negundo* Linn. (Verbenaceae) were collected from the Dr. Babasaheb Ambedkar Marathwada University, campus and Milind Science College, University Road, Aurangabad. The plant materials (leaves, flowers) were identified using the Flora of Marathwada[14] at Department of Botany, Government institute of Science, Caves Road, Aurangabad (M.S.).

Temporary mounts of sections were employed for the test of histochemical studies. For study of different isolated tissues, small pieces of material were macerated in Jeffery's fluid. For the histochemical studies free hand sections of the organs to be studied, were taken and treated with respective reagent to localize component, *viz*. starch, protein, tannin, saponin, fat, glucosides and alkaloids in the tissues[15].

1) Starch: 0.3 g of iodine and 1.5 g of potassium iodide were dissolved in 100 ml of distilled water. A drop of solution was added on the section, washed with water and observed under microscope.

2) Protein: a) Saturated aqueous solution of picric acid is an excellent precipitating agent for protein, staining them an intense yellow. It was allowed to react with the reagent for 24 hours. b) Dilute eosin, stains protein red. c) To localize protein, reagent was prepared by mixing 0.1 g potassium Ferro cyanide dissolved in 20 ml water and 100 ml glacial acetic acid. Section was kept for an hour. The sections were washed with 60% alcohol and few drop of aqueous $FeCl_3$ were added. Blue colour indicates the presence of proteins.

3) Tannin: Sections were treated with dilute acidic $Fecl_3$ solution (0.5% to 1 % of ferric chloride in 0.1 N HCl); mounted in clove oil

and observed under microscope for the presence of tannins. 10% aqueous $FeCl_3$ plus little Na_2CO_3; blue green colour is given by tannin.

4) Saponins: Sections were placed directly in one drop of conc. H_2SO_4 on a slide, which gives a characteristic sequence of colour reactions, beginning immediately with yellow, changing to red within 30 minutes and finally becoming violet or blue green in a short time. To determine localization of the saponin, sections were put in saturated barium hydroxide solution for about 24 hours. Sections were washed with calcium chloride, then placed in potassium dichromate, yellow colour indicated the presence of saponins.

5) Fat: 0.5 g of dye, Sudan III or Sudan IV was dissolved in 100ml of 70% alcohol. Sections were kept in the stain for 20 minutes, rinsed quickly with 50% alcohol and mounted in glycerin for observations. Blue, red, pink, precipitate indicated the presence of fat.

6) Glucoside (Guignard's test): Section were immersed in 1% of aqueous picric acid for 30 minutes, washed with water and placed in a drop of 10% aqueous sodium carbonate. A red colour of the section with hydrochloric acid reveals the Glucosides. For the localization, section was placed in solution composed of 20 parts of 20% aqueous KOH and 80 parts of 90% alcohol for few minutes. In a small watch glass, mixture of 2.5% aqueous $FeSo_4$ and 20% aqueous $FeCl_3$ solution taken in equal proportion was heated to boiling and then the sections were transferred to a slide holding a drop of 20% hydrochloric acid. A deep blue precipitates indicates the presence of glucosides.

7) Test for Alkaloids: Transverse sections of the different plants were treated with the following alkaloid reagent.

a) Mayer's Reagent: Potassium mercuric iodide solution; 13.55g of $HgCl_2$ and 50 g of KI, were dissolved in one liter of distilled water. Presence of grey colour in the section reveals the presence of alkaloids.

b) Wagner's Reagent: 1gm iodine and 2 g potassium iodide were dissolving in 50ml of distilled water. Presence of golden yellow colour reveals the presence of alkaloids.

RESULTS AND DISCUSSION

Histochemical localization in different organs of the taxa under study was made, using methods described elsewhere. The initial presentation give details about the occurrence of erastic content or secondary metabolites, *viz*., starch, protein, fat, tannin, saponin, glucoside and alkaloids in leaves and stem.

1) Starch: Starch is the principal ergastic substance of the protoplast. Starch is composed of long chain molecules, whose basic units are anhydrous glucose residues of the formula $C_6H_{12}O_5$. Starch has an ordinary arrangement of molecule and, therefore, shows optical anisotropy and double refraction. In starch

granules the molecule is radially arranged, therefore, in polarized light a cross pattern is seen. The morph metric variation of starch grain is so extensive that they may be used taxonomically and pharmacognostically up to a limited extent[10].

Starch deposition occurs widely in the plant body, but the particularly common places of its accumulation are seeds, the parenchyma of the secondary vascular tissue in stem and roots, tuber, rhizome and corms. In the present work, for the taxa under study, starch was present in leaves and stem of all the taxa, *viz.*, *A. zeylanica* (Table 1), *R. graveolence* (Table 2), *V. negundo* (Table 3).

2) Protein: Protein is the major constituents of the living protoplast, but they also occur as temporarily inactive ergastic substance. Ergastic protein is known as storage material and found deposited in amorphous and or crystalline forms. Like starch and cellulose, crystalline protein combine crystalline and colloidal properties, therefore, the individual units of this material are spoken of as crystalloids (meaning crystal like) rather than as crystals.

This is also present in all the taxa under investigation. Protein were observed in the upper end; lower epidermis, scattered cells of mesophyll of leaves, and cortical parenchyma in the stem of *A. zeylanica* (Table. 1), *R. graveolence* (Table. 2), *V. negundo* (Table .3)

3) Tannin: Tannin is a heterogeneous group of phenol derivatives, usually related to glucosides. Tannins are particularly abundant in the leaves of much plant; in the xylem, in the testa of seeds and in pathological growth like galls[10]. No tissue, however, appears to lack tannins entirely. Sometimes tannins containing cells are conspicuously associated with a vascular tissue terminates beneath storage tissue or secretary cells of nectarines. The monocotyledons are notably poor in tannins.

Tannins also show distributions, occurring mostly in epidermis, mesophyll cortical as well as parenchymatous tissue, associated with conductive tissue. Tannins were observed in the leaves of *A. zeylanica* (Table1) *S. bisponisa* (Table 2), *V. negundo* (Table 3).

4) Saponin: The saponin is of rare occurrence and wherever present, they apparently remain to one or two organs. Saponin were observed in the mid-rib parenchyma of leaves and cortex and pith parenchyma of stem *A. zeylanica* (Table 1), *R. graveolence* (Table 2) and *V. negundo* (Table 3). Saponin was also observed in the cells of mesophyll and xylem parenchyma of stem of all studied taxa.

5) Fat: Fats are widely distributed in the plant body, and they probably occur in small amounts in every plant cell. The term fat may be used to described not only the fats proper (that is, ester of fatty acids with glycerol), but also related substances grouped under the name of lipids.

As protoplast inclusion, fats are common reserve material in seeds, spores and

embryos in meristematic cells and occasionally in differentiated tissue of the vegetable body. They occur as solid bodies or, more frequently, as fluid droplets of various sizes either dispersed in the cytoplasm or aggregated in large masses fatty substance are thought to be elaborated directly by the cytoplasm and also by leucoplast. In taxa under study, fat was found in cells of mesophyll and phloem parenchyma (leaves and stem).

6) Glucoside: Glucosides are the degradation production of carbohydrates glucosides were observed in the epidermis, pith parenchyma and vascular bundles of leaves and stem of *A. zeylanica* (Table1), *R. graveolence* (Table 2), *V. negundo* Linn (Table 3).

7) Alkaloids: Alkaloids are degradation of protein they were investigated by using two methods, namely; Mayer's reagent and Wagner's reagent. In Mayer's reagent alkaloids were observed in the scattered cells of mesophyll of leaves and pith parenchyma of stem. In Wagner's reagent, alkaloids were found in the cells of mesophyll and cells of cortex parenchyma and pith parenchyma of stem of *A. zeylanica* (Table 1), *R. graveiolence* (Table 2), *V. negundo* (Table 3).

Table 1: Histochemical Test for fresh section of leaves and stem of *Adhatoda zeylanica*

S. No.	Ergastic content	Reaction		Localization	
		Leaves	Stem	Leaves	Stem
1	Starch	+ve	+ve	Scattered cells of mesophyll, Mid –rib pith parenchyma,	Cortical parenchyma, Vascular bundle, and Pith parenchyma
2	Protein	+ve	+ve	Epidermis, Scattered cells of mesophyll,	Epidermis, Scattered cells of Cortex.
3	Tannin	-ve	+ve	--	Scattered cells of Cortex and Pith parenchyma
4	Saponin	+ve	+ve	Scattered cell of mesophyll	Epidermis, Scattered cells of Cortex parenchyma, and Pith
5	Fat	+ve	+ve	Upper and lower epidermis, Scattered cells of Mesophyll cells and Mid –rib	Cortical parenchyma, Scattered cells of Pith parenchyma.
6	Glucoside	+ve	+ve	Lower epidermis, Scattered cell of mesophyll	Epidermis and Scattered cells of Cortex and Pith parenchyma
7	Alkaloids				
a)	Mayer's reagent	+ve	+ve	Epidermis and cortical cells	Cortex, Xylem parenchyma and Pith parenchyma.
b)	Wagner's reagent	+ve	+ve	Upper and lower Epidermis, Midrib parenchyma.	Epidermis, Cortical parenchyma, Vascular bundle and Pith parenchyma

Table 2: Histochemical test for fresh section of leaves and stem of *Ruta graveolence*

S. No.	Ergastic content	Reaction		Localization	
		Leaves	Stem	Leaves	Stem
1	Starch	+ve	+ve	Upper and lower epidermis, Scattered cells of Mesophyll, Mid–rib Parenchyma, Pith parenchyma	Xylem and Phloem parenchyma and and Scattered cells of Cortex
2	Protein	+ve	+ve	Upper and lower epidermis, cells of mesophyll cells, Xylem parenchyma and Pith parenchyma.	Epidermis, Scattered cells of cortex parenchyma, Xylem and Phloem, Pith parenchyma
3	Tannin	+ve	+ve	Scattered cells of mesophyll and Pith cells	Scattered cells of Pith parenchyma
4	Saponin	+ve	+ve	Mesophyll cells, Pith parenchyma,	Scattered cells of cortex parenchyma and Pith region, Xylem parenchyma
5	Fat	+ve	+ve	Scattered cells of epidermis, Mesophyll cells and mib- rib Pith parenchyma	Vascular bundle and Scattered cells of Pith parenchyma
6	Glucoside	+ve	+ve	Epidermis and cortical cells	Epidermis, Scattered cells of cortex parenchyma
7	Alkaloids				
a)	Mayer's reagent	+ve	+ve	Upper and lower epidermis, Scattered cells of mesophyll cells.	Hypodermis, Xylem parenchyma ,Pith
b)	Wagner's reagent	+ve	+ve	Upper and lower epidermis , Scattered cells of mesophyll cells, Mid-rib, Pith parenchyma	Epidermis, Scattered cells of cortical parenchyma, and Vascular bundle

Table 3: Histochemical test for fresh section of leaves and stem of *Vitex negundo*

S. No.	Ergastic content	Reaction		Localization	
		Leaves	Stem	Leaves	Stem
1	Starch	+ve	+ve	Upper and lower epidermis, Mesophyll cell, Cortical cells, Pith parenchyma	Cortical parenchyma
2	Protein	+ve	+ve	Scattered cells of cortex, Mesophyll cells and Pith	Epidermis, Cortical parenchyma, Pith parenchyma
3	Tannin	+ve	+ve	Scattered cells of mesophyll, Mid-rib pith parenchyma.	Vascular bundle and Scattered cells of medullary ray.
4	Saponin	+ve	+ve	Upper and lower epidermis and Mid-rib pith	Scattered cells of cortex and Pith parenchyma
5	Fat	+ve	+ve	Mesophyll, cortical and in pith	Scattered cells of pith, Cortex, and Medullary rays
6	Glucoside	+ve	+ve	Epidermis and cortical cells	Scattered cells of cortex and Vascular bundle
7	Alkaloids				
a)	Mayer's reagent	+ve	+ve	Upper epidermis Scattered cells of mesophyll, Mid rib pith parenchyma	Scattered cells of cortex, and Vascular bundle
b)	Wagner' s reagent	+ve	+ve	Upper and lower epidermis, Scattered cells of mesophyll cells, Mid-rib, Pith parenchyma	Epidermis, Scattered cells of cortical parenchyma, and Vascular bundle

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