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RESEARCH ARTICLE

HISTOCHEMICAL INVESTIGATION OF TWO MEDICINAL PLANTS IN MAHARASHTRA

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ABSTRACT

The histochemical studies of leaves and wood of Butea *monosperma* Lam and *Madhucaindica* Gmel are medicinal important plants in Maharashtra. For histochemical studies the free hand sections of leaves and wood were taken and treated with the respective reagent in localize components, viz. starch, protein, tannin, saponin, fat, glucosides and alkaloids in the tissues.

Key words: Histochemistry, starch, protein, tannin, saponin, fat, glucosides and alkaloids

INTRODUCTION

Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. The knowledge about the use of medicinal plants has been acquired through centuries and such plants are still valued even today. Medico scientist practicing allopathy and research minded vaidyas, Hakims have contributed valuable knowledge regarding efficacy of reputed medicinal plants indigenous to India. Establishment of herbal forms in well selected localities will excersie scientific control over the cultivation of medicinal herbs [1].

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 [2]. Starch and proteins are the principal ergastic substances of the protoplast [3]. Tannin is the heterogeneous group of phenol derivatives, usually related to glucosides .Tannins is particularly abundant in the leaves (xylem) of many plants [4]. Saponin is the rare occurrence. Fats are widely distributed in the plant body and they probably occur in small amount in every plant cell [5]. Fats are common reserve material in seeds, spores and embryos in meristematic cells. Glucosides are the degradation product of the carbohydrates. Alkaloides are the degradation product of protein. Many woody plants contain medicinally important secondary product [6]. Therefore, we have attempted to histochemical investigations of different plant parts of *Butea monosperma* Lam and *Madhucaindica* Gmel are medicinal plants in Maharashtra.

MATERIALS AND METHODS

Temporary and permanent mounts of sections were employed for the test of histochemical studies. For study of isolated different tissues, small pieces of material were macerated in Jeffery's fluid. For the histochemical studies free hand sections of the organs to be studies, were taken and treated the respective reagent to localize component , Viz. starch, protein, tannin, saponin, fat, glucosides and alkaloids in the tissues [7].

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 the solution was added on the section, washed 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 acid. Section was kept in for an hour. They section were washed with 60% alcohol and few drop of aqueous $Fecl_3$ were added .Blue color 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 concentration 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 saturation barium hydroxide solution for about 24 hours. Sections were washed with calcium chloride, the 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 (Goignard'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

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carbonate. A red colour of the section with hydrochloric acid reveals the glucosides. For the localization, section were placed in solution composed of 20 parts of 2o% 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 Fecl3 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 indicated the presence of glucosides.

7) Test for Alkaloids - Transverse sections of the different plants were treated with the following 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 2g 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 gives details about the occurrence of ergastic content or secondary metabolites, Viz starch, protein, fat, Tannin, saponin, glucoside and alkaloids in leaves and stem.

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 C6H12O5. 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 pharmaconostically up to a limited extent [3].

Starch deposition occur widely n 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 Butea monosperma Lam (Table 1) , Madhuca*indica* Gmel (Table 2).

Protein:

Protein are the major constituents of the living protoplast, but they also occur as temporarily inactive erastic substance, Erastic protein is knows as a storage material and is 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 and lower epidermis, scattered cells of mesophyll of leaves, pith parenchyma and cortical parenchyma in the stem of *Butea monosperma* Lam (Table 1) and *Madhucaindica* Gmel (Table 2).

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 [3-8]. No tissue, however, appears to lack tannins entirely. They may be found in meristematic cells too. Sometimes tannins containing cells are conspicuously associated with a vascular tissue terminates beneath storage tissue or secretory cells of necaries. The monocotyledons are notably poor in tannins [8].

Tannins also show distributions, occurring mostly in epidermis, mesophyll, and cortical as well as parenchymatous tissue, associated with conductive tissue. Tannins were observed in the leaves of *Butea monosperma* Lam (Table 1) and *Madhucaindica* Gmel (Table 2).

Saponin:

The saponin are 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 *Butea monosperma* Lam(Table 1) and *Madhucaindica* Gmel (Table 2). Saponin was observed in the cells of mesophyll and xylem paremchyma of stem of *Butea monosperma* Lam (Table 1) *Madhucaindica* Gmel (Table 2).

Fat:

Fat 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 [5].

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 [9]. They occur as solid bodies or, more frequently, as fluid droplets of various size either dispersed in the cytoplasm or aggregated in large masses fatty substance are thought to be elaborated directly by the cytoplasm and also by elaioplast.

In taxa under study, fat was found in cells of mesophyll and phloem parenchyma (leaves and stem) of Butea *monosperma*Lam (Table 1) and *Madhucaindica* Gmel (Table 2).

Glucoside:

Glucosides are the degradation production of carbohydrates glucosides were observed in the epidermis, pith parenchyma of leaves vascular bundles and scattered cells of medullar ray of stem *Butea monosperma* Lam (Table 1) and *Madhucaindica* Gmel (Table 2)

Alkaloids:

Alkaloids are degradation of protein they were investigated by using two methods, namely; Meyer's reagent and

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Wagner's reagent. In Mayer's reagent alkaloids were observed in the scattered cells of mesophyll of leaves and pith parenchyma of stem .In winger's reagent, alkaloids were found in the cells of mesophyll and cells of cortex parenchyma and pith parenchyma of stem of *Butea monosperma* Lam (Table 1) and *Madhucaindica* Gmel (Table 2).

Table 1: Histochemical test for fresh section of leaves and stem of ***Butea monosperma***Lam

Sr. no	Ergastic content	Reaction		Localization	
		Leaves	wood	Leaves	Stem
1	Starch	$^{+}$ve	$^{+}$ve	Scattered cells of mesophyll, Mid-rib pith parenchyma,	Cortical parenchyma, Madullary rays, Vascular bundle, and Pith parenchyma
2	Protein	-do-	-do-	Epidermis, Scattered cells of mesophyll, mid-rib Pith parenchyma	Epidermis, Scattered cells of Cortex and Pith parenchyma, and Phloem parenchyma.
3	Tannin	-ve	-do-	-----	Scattered cells of Cortex and Pith parenchyma
4	Saponin	-ve	-do-	----	Epidermis, Scattered cells of Cortex parenchyma, and Pith
5	Fat	-do-	-do-	Upper & lower epidermis, Scatterend cells of Mesophyll cells and Mid -rib	Cortical parenchyma, Medullary rays, Scattered cells of Pith parenchyma.
6	Glucoside	-ve	-ve	-----	----
7	Alkaloids	-do-	-do-		
	a) Meyers reagent	-do-	-do-	Cells of Mesophyll, Mid –rib	Cortex, Xylem parenchyma, and Pith parenchyma.
	b)Wagner's reagent	-do-	-do-	Upper and lower Epidermis, Mid –rib parenchyma.	Epidermis, Cortical parenchyma, Medullary rays and Vascular bundle and Pith parenchyma

Table 2: Histochemical test for fresh section of leaves and stem of ***Madhucaindica*** Gmel

Sr. no	Ergastic content	Reaction		Localization	
		Leaves	wood	Leaves	Stem
1	Starch	$^{+}$ve	$^{+}$ve	Upper and lower epidermis, Scattered cells of Mesophyll, Mid-rib Parenchyma, Pith parenchyma	Xylem and Phloem parenchyma and Scattered cells of Medullary ray. and Scattered cells of Cortex
2	Protein	-do-	-do-	Upper and lower epidermis, cells of mesophyll cells, Xylem parenchyma, Scattered cells of medullar rays and Pith parenchyma.	Epidermis, Scattered cells of cortex parenchyma, Xylem and Phloem, Scatteredcells of medullary rays, Pith parenchyma
3	Tannin	-do-	-do-	Scattered cells of mesophyll and Pith cells	Vascular bundle and Scattered cells of medullary ray, and Pith parenchyma
4	Saponin	-do-	-do-	Mesophyll cells , Pith parenchyma,	Scattered cells of cortex parenchyma and Pith region, Xylem parenchyma
5	Fat	-do-	-do-	Scattered cells of epidermis , Mesophyll cells and mib- rib Pith parenchyma	Vascular bundle and Scattered cells of medullary rays and Pith parenchyma
6	Glucoside	-ve	-do-	-----	Scattered cells of cortex , Medullary rays and Vascular bundle
7	Alkaloids	-do-	-do-		
	a) Mayers reagent	-do-	-do-	Upper and lower epidermis, Scattered cells of mesophyll cells.	Hypodermis, Xylem parenchyma ,Pith
	b)Wagners reagent	-do-	-do-	Upper and lower epidermis , Scattered cells of mesophyll cells, Medullary rays , Mid-rib, Pith parenchyma	Epidermis, Scattered cells of cortical parenchyma, Medullary rays, and Vascular bundle

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