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1. Histochemical Investigation of Different Organce of Two Traditional Medicinal Plants

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Abstract

The histochemical studies of leaves and wood of Moringa and Tulsi aremedicinally important plants of Marathwada region 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.

Keywords: Histochemistry, starch, protein, tannin, saponin, fat, glucosides and alkaloids.

Introduction

Marathwada is a rich source of plant and animal wealth, which is due to its varied geographical and agro-climatic regions. Besides it's varied biodiversity, it has a diverse cultural heritage too. Though at present Marathwada health care delivery consists of both traditional and modem systems of medicines, both organized traditional systems of medicine like Ayurveda, Siddha and Unani and unorganized systems like folk medicine have been flourishing well. Ayurveda and Siddha are of Indian origin and accounted for about 60% health care delivery in general and 75% of rural Indian population depends on these traditional systems. These two systems of medicine use plants, minerals, metals and animals as source of drugs, plants being the major source. It is estimated that roughly 1500 plant species in Ayurveda and 1200 plant species in Siddha have been used for drug preparation (Jain, 1987, Krishnakumar and Sureshkumar, 1995). In Indian folk medicine use, about 7500 plant species are recorded as medicinal plants (Anonymous, 1996).

Many plants contain medicinally important secondaryproduct (Dhar et al.,1968). Therefore, we have attempted to histochemical investigations of different plant parts of Moringa and Tulsimedicinal plants of Marathwada region in Maharashtra. Free handsections were taken for the histochemical studies. Sections aretreated with the respective reagent to localize components, viz. starch, proteins, tannin, saponin, fat glycosides and alkaloids in the tissues (Johansen, 1940).

Materials and Methods

Temporary and permanent mounts of sections were employed for the test of histochemical studies. For study of isolated differenttissues, small pieces of material were macerated in Jeffery's fluid(Johansen, 1940). 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 (Johansen, 1940).

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.

Protein

Saturated aqueous solution of picric acid is an excellentprecipitating 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 mlglacial acid. Section was kept in for an hour. They section werewashed with 60% alcohol and few drop of aqueous Fecl3 wereadded .Blue colour indicates the presence of proteins.

Tannin

Sections were treated with dilute acidic Fecl3 solution (0.5%to 1 % of ferric chloride in 0.1 N HCL); mounted in clove oil andobserved under microscope for the presence of tannins. 10%aqueous Fecl3 plus little Na2co3; blue green colour is given by tannin.

Saponins

Sections were placed directly in one drop of concentrationH2So4 on a slide, which gives a characteristic sequence of colour reactions, beginning immediately with yellow, changing to red within30 minutes and finally becoming violet or blue green in a short time.

To determine localization of the saponin, sections were put instauration 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.

Fat

0.5 g of dye, Sudan III or Sudan IV was dissolved in 100ml of70% alcohol. Sections were kept in the stain for 20 minutes, rinsedquickly with 50% alcohol and mounted in glycerin for observations.Blue, red, pink, precipitate indicated the presence of fat.

Glucoside (Goignard's test)

Section were immersed in 1% of aqueous picric acid for 30minutes, washed with water and placed in a drop of 10% aqueoussodium carbonate. A red colour of the section with

hydrochloric acidreveals the of Glucosides. For the localization, section were placed in solution composed of 20 parts of 20% aqueous KOH and 80 partsof 90% alcohol for few minutes. In a small watch glass, mixture of2.5% aqueous Feso4 and 20% aqueous Fecl3 solution taken in equalproportion was heated to boiling and then the sections weretransferred to a slide holding a drop of 20% hydrochloric acid. Adeep blue precipitates indicates indicated the presence of glucosides.

Test for Alkaloids

Transverse sections of the different plants were treated withthe following with the following alkaloid reagent.

a) Mayer's Reagent

Potassium mercuric iodide solution; 13.55g of HgCl2 and 50 gof KI, were dissolved in one liter of distilled water. Presence of greycolour in the section reveals the presence of alkaloids.

b) Wagner's Reagent

1gm iodine and 2g potassium iodide were dissolving in 50mlof distilled water. Presence of golden yellow colour reveals thepresence of alkaloids.

Results and Discussion

Histochemical localization in different organs of the taxa understudy was made, using methods described elsewhere. The initialpresentation gives details about the occurrence of erastic content orsecondary metabolites, viz., starch, protein, fat, tannin, saponin, glucoside and alkaloids in leaves and Wood.

Starch : Starch is the principal ergastic substance of theprotoplast. Starch is composed of long chain molecules, whose basicunits are anhydrous glucose residues of the formula C6H12O5. Starchhas an ordinary arrangement of molecule and, therefore, shows optical anisotropy and double refraction. In starch granules themolecule is radically arranged, therefore, in polarized light a crosspattern is seen. The morph metric Variation of starch grain is soextensive that they may be used taxonomically andpharmacognostically up to a limited extent (Kuster, 1956).Starch deposition occurs widely n the plant body, but the particularlycommon places of its accumulation are seeds, the parenchyma ofthe secondary vascular tissue in wood and roots, tuber, rhizome andcorms.(Kadam 1999) In the present work, for the taxa under study, starch waspresent in leaves and wood of viz.,*Moringa oleifera* Lamk (Table 1) *Ocimum sanactum* Linn (Table 2)

Protein

Protein are the major constituents of the livingprotoplast, but they also occur as temporarily inactive erasticsubstance. Erastic protein is knows as a storage material and isfound deposited in amorphous and or crystalline forms. Like starchand cellulose, crystalline protein combine crystalline and colloidalproperties, therefore, the individual units of this material are spokenof as crystalloids (meaning crystal like) rather than as crystals.

This is also present in all the taxa under investigation. Proteinwere observed in the upper and lower epidermis, scattered cells ofmesophyll of leaves, and cortical parenchyma in the wood of*Moringa oleifera* Lamk (Table 1) *Ocimum sanactum* Linn (Table 2)

Tannin

Tannin is a heterogeneous group of phenolderivatives, usually related to glucosides. Tannins are particularlyabundant in the leaves of much plant; in the xylem, in the testa ofseeds and in pathological growth like galls (Kuster, 1956). No tissue,however, appears to lack tannins entirely. Sometimes tanninscontaining cells are conspicuously associated with a vascular tissueterminates 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 withconductive tissue. Tannins were observed in the leaves of*Moringa oleifera Lamk* (Table 1) *Ocimum sanactum* Linn (Table 2)

Saponin

The saponin is of rare occurrence and whereverpresent, they apparently remain to one or two organs. Saponin wereobserved in the mid-rib parenchyma of leaves and cortex and pith parenchyma of wood*Moringa oleifera* Lamk (Table 1) *Ocimum sanactum* Linn (Table 2)

Fat

Fat are widely distributed in the plant body, and theyprobably occur in small amounts in every plant cell. The term fat maybe used to described not only the fats proper (that is, ester of fattyacids with glycerol), but also related substances grouped under the name of lipids. As protoplast inclusion, fats are common reserve material inseeds, spores and embryos in meri wood tic cells and occasionally in differentiated tissue of the vegetable body. They occur as solidbodies 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 leucoplast. In taxa under study, fat was found in cells of mesophyll and phloem parenchyma (leaves and wood) of *Moringa oleifera* Lamk (Table 1) *Ocimum sanactum* Linn (Table 2)

Glucoside

Glucosides are the degradation production of carbohydrates glycosides were observed in the epidermis, pith parenchyma of leaves vascular bundles and scattered cells of medullar ray of wood*Moringa oleifera* Lamk (Table 1) *Ocimum sanactum* Linn (Table 2).

Alkaloids

Alkaloids are degradation of protein they wereinvestigated by using two methods, namely; Mayer's reagent andWagner's reagent. In Mayer's reagent alkaloids were observed in the scattered cells of mesophyll of leaves and pith parenchyma ofwood .In Wagner's reagent, alkaloids were found in the cells ofmesophyll and cells of cortex parenchyma and pith parenchyma of wood of *Moringa oleifera* Lamk (Table 1) *Ocimum sanactum* Linn (Table 2).

Table 1-Histochemical test for fresh section of leaves and wood of ***Moringa oleifera*** Lamk

Sr. No.	Ergastic Content	Reaction		Localization	
		Leaves	Wood	Leaves	Wood
1	Starch	+Ve	+Ve	Cells of mesophyll, pith parenchyma.	Vascular bundle and pith parenchyma
2	Protein	-do-	-do-	Epidermis, Cortex cell, pith parenchyma	Epidermis, cortical parenchyma and pith parenchyma
3	Tannin	-do-	-do-	Mesophyll and pith region	Xylem and phloem parenchyma.
4	Saponin	-do-	-do-	Epidermis,mesophyll cells	Cortex parenchyma and pith parenchyma
5	Fat	-do-	-do-	Upper and lower epidermis xylem and phloem parenchyma	Vascular bundle, scattered cells of pith
6	Glucoside	-Ve	-Ve	---------	---------
7	Alkaloids	—			
	a) Mayer's reagent	+Ve	+Ve	Epidermis and mesophyll cells, Mid-rib.	Pith parenchyma
	b) Wagner's reagent	-do-	-do-	Scattered cells of mesophyll Mid-rib.	Cortex parenchyma and pith parenchyma

Table 2-Histochemical test for fresh section of leaves and wood of ***Ocimum sanactum***Linn

Sr. No.	Ergastic Content	Reaction		Localization	
		Leaves	Wood	Leaves	Wood
1	Starch	+Ve	+Ve	Scattered cells mesophyll.	Xylem and phloem, hypodermis and scattered cells of cortex
2	Protein	-do-	-do-	Upper and lower epidermis mesophyll cells and pith.	Cells medullary ray and pith parenchyma

3	Tannin	-do-	-do-	Mesophyll,Mid-rib pith.	----------
4	Saponin	-do-	-do-	Upper and lower epidermis,pith	Xylem and phloem parenchyma
5	Fat	-Ve	-do-	----------	Scattered cells of pith
6	Glucoside	-Ve	+Ve	----------	Epidermis and cortex.
7	Alkaloids	—			
	a) Mayer's reagent	+Ve	+Ve	Epidermis and mesophyll cells	Scattered cells of cortex and hypodermis
	b) Wagner's reagent	-do-	-do-	Mesophyll Mid- rib parenchyma	Epidermis medullary rays vascular bundle and pith parenchyma.

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