



## HISTOCHEMICAL SCREENING OF DIFFERENT PARTS OF TWO TRADITIONAL MEDICINAL PLANTS IN THE VICINITY OF MARATHWADA REGION

Dr. Kamble Rajabhau Anantrao\*

Associate Professor, Department of Botany, Shri Hawagiswami College, Udgir, Dist.-Latur.(Maharashtra) India.

**Corresponding Author: Dr. Kamble Rajabhau Anantrao**

Associate Professor, Department of Botany, Shri Hawagiswami College, Udgir, Dist.-Latur.(Maharashtra) India.

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### ABSTRACT

The histochemical studies of leaves and wood of Sesbania and Caster are medicinally 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 its varied biodiversity, it has a diverse cultural heritage too. Though at present Marathwada health care delivery consists of both traditional and modern 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 secondary product (Dhar et.al.1968). Therefore, we have attempted to histochemical investigations of different plant parts of Sesbania and Caster 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, fats, glucosides and alkaloids in the tissues (Johansen, 1940).

### MATERIAL 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 (Johansen, 1940). For the histochemical studies free hand sections of the organs to be studied, were taken and treated with 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 with water and observed under microscope.

**Protein:** 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, stain 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<sub>3</sub> were added. Blue colour indicates the presence of proteins.

**Tannin:** Sections were treated with dilute acidic FeCl<sub>3</sub> 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<sub>3</sub> plus little Na<sub>2</sub>CO<sub>3</sub>; blue green colour is given by tannin.

**Saponins:** Sections were placed directly in one drop of conc. H<sub>2</sub>SO<sub>4</sub> 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.

**Fat:** 0.5 gm 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.

**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 carbonate. A red colour of the section with hydrochloric acid reveals the of Glucosides. For the localization, section were placed Sin 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<sub>4</sub> and 20% aqueous FeCl<sub>3</sub> 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.

**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<sub>2</sub> 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 Wood.

**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<sub>6</sub>H<sub>12</sub>O<sub>5</sub>. Starch has an ordinary arrangement of molecule and, therefore, shows optical anisotropy and double refraction. In starch granules the molecule is

radically arranged, therefore, in polarized light a cross pattern is seen. The morphometric Variation of starch grain is so extensive that they may be used taxonomically and pharmacognostically up to a limited extent (Kuster, 1956). 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 wood and roots, tuber, rhizome and corms. (Kadam 1999) In the present work, for the taxa under study, starch was present in leaves and wood of viz., *Sesbania sesban* (L.) Merr. (Table 1) *Ricinus communis* Linn (Table 2)

**Protein:** Protein are the major constituents of the living protoplast, but they also occur as temporarily inactive ergastic substance. Ergastic protein is known 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, and cortical parenchyma in the wood of *Sesbania sesban* (L.) Merr. (Table 1) *Ricinus communis* Linn (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 (Kuster, 1956). No tissue however, appears to lack tannins entirely. Sometimes tannins containing cells are conspicuously associated with a vascular tissue terminates beneath storage tissue or secretory 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 *Sesbania sesban* (L.) Merr. (Table 1) *Ricinus communis* Linn (Table 2).

**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 wood *Sesbania sesban* (L.) Merr. (Table 1) *Ricinus communis* Linn (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. 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 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 *Sesbania sesban(L.)Merr.*(Table 1) *Ricinus communis Linn*(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 medullary ray of wood *Sesbania*

*sesban(L.)Merr.*(Table 1) *Ricinus communis Linn*(Table 2).

**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 wood .In Wagner's reagent, alkaloids were found in the cells of mesophyll and cells of cortex parenchyma and pith parenchyma of wood of *Sesbania sesban(L.)Merr.*(Table 1) *Ricinus communis Linn*(Table 2).

**Table 1: Histochemical test for fresh section of leaves and wood of *Sesbania sesban(L.)Merr.***

Sr. No.	Ergastic Content	Reaction		Localization	
		Leaves	Wood	Leaves	Wood
1	Starch	+Ve	+Ve	Cells of mesophyll, pith and 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 *Ricinus communis 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	-do-	-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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