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Histochemical studies of some plant parts of *Aegle marmelos*

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Abstract: The histochemical studies of leaves and wood of *Aegle marmelos* are medicinally important plant in India. 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, glycosides and alkaloids in the tissues.

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

I. INTRODUCTION

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 (Kadam, 1999). Starch and proteins are the principal ergastic substances of the protoplast (Kuster, 1956). Tannin is the heterogeneous group of phenol derivatives, usually related to glycosides' (Vaidya, 1972).

Tannins are particularly abundant in the leaves (xylem) of many plants (Kadam et al., 1996). Saponin are the rare occurrence. Fats are widely distributed in the plant body and they probably occurs in small amount in every plant cell (Seifriz, 1934). 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 woody plants contain medicinally important secondary product (Dhar et al., 1968). Therefore, we have attempted to histochemical investigations of different plant parts of *Aegle marmelos.* 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 (Johansen, 1940).

Bael has great religious significance. In Hindu tradition, the leaves and the fruit of the plant are offered to god during prayer, especially god Shiva. Its leaves are also used to worship Parvati and Viva Rupra. The fruit is used in religious ceremonies and rituals and its mentioned is also seen in Vedas and Mahabharata. Plants are an important part of our everyday diet, their constituents and nutritional value has been intensively studied for decades.

Aegle marmelos (L.) Corr. is slow growing, medium sized tree, 25 to 30 feet tall. The stem is short, thick, soft, flaking bark and spreading, sometimes spiny branches, the lower ones dropping. There are sharp, axial one inch long spikes on this tree. The leaflets are oval or lancet shaped, 4-10 cm long, 2-5 cm wide. Leaves composed of 3-5 leaflets in it. The lateral leaflets are without petiole and the terminal one has a long one. The petiole is 1 to 2.5 inch long. Mature leaves emit a peculiar fragrance when bruised. Flowering occurs in April and May.

Pushpendra, et al. (2012), evaluated the medicinal uses and pharmacological activity of the plant Aegle marmelos (L.) Corr. in India. The Aegle marmelos (L.) Corr. is beneficial in different health problems like cancer, heart related diseases, diabetes, increase in cholesterol level, constipation, respiratory infection, diarrhoea and dysentery. The aim of study was to improve axillary branching by using nodal sector of plant. Remya, et al. (2009), investigated antifertility effect of leaves of Aegle marmelos.

Dhankar, et al. (2011), reported the biological and phytochemical evaluation in the literature for the importance of Aegle marmelos. They reported it has used in ethanomedicine as a antidiabetic, antiulcer, antioxidant, antimalarial, anti-inflammatory, anticancer, readioprotective, antihyperlipidaemic, antifungal, antibacterial and antiviral activities. Aegle marmelos plant was used in the treatment of wide range of diseases in all Ayurveda, Siddha and folk medicines Ariharan, et al. (2013).

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II. 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 (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 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 in for an hour. They section were washed with 60% alcohol and few drop of aqueous Fecl3 were added .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 and observed 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 concentration H2So4 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 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 glycerine for observations. Blue, red, pink, precipitate indicated the presence of fat.

Glycoside- (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 of Glycosides. For the localization, section were 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 FeSo4 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 glycosides.

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 HgCl2 and 50 g of KI, were dissolved in one litre 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.

III. 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 secondary metabolites, viz., starch, protein, fat, tannin, saponin, glycoside and alkaloids in leaves and Wood.

Starch: Starch is the principal agrestic substance of the protoplast. Starch is composed of long chain molecules, whose basic units are anhydrous glucose residues of the formula CH12O5. 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 morph metric 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 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. In the present work, for the taxa under study, starch was present in leaves (Scattered cells of mesophyll, mid-rib pith parenchyma) and wood (Cortical parenchyma, Medullary rays, vascular bundle, and pith parenchyma) (Table 1).

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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 the taxa under investigation. Protein were observed in the leaves of upper and lower Epidermis, scattered cells of mesophyll and wood of Pith parenchyma, and cortical parenchyma (Table .1)

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 secretary cells of nectarines. The monocotyledons are notably poor in tannins. Tannins also show distributions, occurring mostly in Mesophyll cells, epidermis, and parenchymatous tissues of leaves and wood of scattered cells of cortex (Table 1)

Saponin: The saponin is of rare occurrence and wherever present, they apparently remain to one or two organs. Saponin were observed in the scattered cells of mesophyll and xylem fibres of leaves and Cells of cortex parenchyma, pith parenchyma and xylem fibres of wood (Table 1)

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 mire wood tic 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 spongy cell of leaves and scattered cells of cortex parenchyma of wood.

Glycoside: Glycosides are the degradation production of carbohydrates glycosides were observed in the Upper and lower epidermis, mid – rib parenchyma of leaves and Cortex, xylem parenchyma, and pith parenchyma of wood (Table 1)

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 Upper and lower epidermis, scattered cells of mesophyll, mid-rib parenchyma, pith parenchyma of leaves and epidermis and cortical parenchyma of wood .In Wagner's reagent, alkaloids were found in the cells of mesophyll and midrib parenchyma and Cells of cortex parenchyma and pith parenchyma of wood (Table 1)

Table 1-Histochemical test for fresh section of leaves and wood of *Aegle marmelos*

SR. NO.	ERGASTIC CONTENT	REACTION		LOCALIZATION	
		LEAVES	WOOD	LEAVES	WOOD
1	STARCH	$+^{ve}$	$+^{ve}$	Scattered cells of mesophyll, mid-rib pith parenchyma	Cortical parenchyma, Medullary rays, vascular bundle, and pith parenchyma
2	PROTEIN	$+^{ve}$	$+^{ve}$	Upper and lower Epidermis, scattered cells of mesophyll	Pith parenchyma, and Cortical parenchyma
3	TANNIN	$+^{ve}$	$+^{ve}$	Mesophyll cells, epidermis, parenchymatous tissues	Scattered cells of cortex
4	SAPONIN	$+^{ve}$	$+^{ve}$	Scattered cells of mesophyll and xylem fibres.	Cells of cortex parenchyma, pith parenchyma and xylem fibres
5	FAT	$+^{ve}$	$+^{ve}$	Cells of mesophyll and spongy cell.	Scattered cells of cortex parenchyma
6	GLYCOSIDE	$+^{ve}$	$+^{ve}$	Upper and lower epidermis, mid – rib parenchyma	Cortex, xylem parenchyma, and pith parenchyma

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7	ALKALOID				
	A)Mayer's reagent	+ve	+ve	Upper and lower epidermis, scattered cells of mesophyll, mid-rib parenchyma, pith parenchyma	Epidermis and cortical parenchyma
	B)Wagner's reagent	+ve	+ve	cells of mesophyll, mid-rib parenchyma	Cells of cortex parenchyma and pith parenchyma

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