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HISTOCHEMICAL INVESTIGATIONS OF DIFFERENT ORGANS OF THREE ENDANGERED MEDICINAL TAXA OF SOUTH GUJARAT FORESTS

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The histochemical studies of leaves and stem of *Semecarpus anacardium* Linn., *Mallotus philippensis* Muell.-Arg., and *Pterocarpus marsupium* Roxb. are medicinally important and extremely rare of South Gujarat Forests. 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 :Alkaloids; Fat; Glucoside; Histochemistry; Protein; Saponin; Starch; Tannin.

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 the stem and root, tubers, rhizomes, and corn[1]. Starch and proteins are the principal ergastic substances of the protoplast[2]. Tannin is the heterogenous group of phenol derivatives, usually related to glucosides[3]. Tannins are particularly abundant in the leaves (xylem) of many plants[4]. Saponin are the rare occurance. Fats are widely distributed in the plant body and they probably occurs in small amount in every plant cell[5].

Fats are common reserve material in seeds, spores and embryos in meristematic cells and occasionally in differentiated tissue of the vegetable body[6]. Glucosides are the degradation product of the carbohydrates. Alkaloides are the degradation product of protein. Many woody plants contain medicinally important secondary product[7]. Little is known about the primary bio-product and nutritive values of medicinally important trees. Therefore, We have attempted to histochemical investigations of different plant parts of *Semecarpus anacardium* Linn, *Mallotus philippensis* Muell.-Arg., and *Pterocarpus marsupium* Roxb., three endangered medicinal taxa of South Gujarat Forests.

The plant material of *Semecarpus anacardium* Linn, *Mallotus philippensis* Muell.-Arg., and *Pterocarpus marsupium* Roxb. were collected from the Dangs forest. The 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 & alkaloids in the tissues[8]. The test employed are as follow:

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

2. *Proteins* : 0.1 gm of potassium ferrocyanide dissolved in 20ml water and 100ml glacial acetic acid. Sections were washed with 60% alcohol and few drops of aqueous $FeCl_3$ were added. Blue colour indicates the presence of proteins.

3. *Tannis* : 10% aqueous $FeCl_3$ plus little Na_2CO_3. A drop of solution was added on the section and observed under microscope. The blue-green colour indicates the presence of tannin.

4. *Saponins* : 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 indicates the presence of Saponins.

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

6. *Glucosides (Goignard's test)* : 20 ml of 20% aqueous KOH and 80 ml of 90% alcohol for few minutes. In a small watch glass mixture of 2.5% aqueous $FeSO_4$ and 20% $FeCl_3$ solution were 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 precipitate indiacted the presence of glucosides.

7. *Alkaloids*

a. Mayer's reagent : 13.55 gm of $HgCl_2$ and 50 gm of Kl were dissolved in one litre of distilled water. Presence of grey colour in the section reveals the presence of alkaloids.

b. Wagner's reagent : 1 gm iodine and 29gm pottassium iodide were dissolved in 50 ml of distilled water. Sections were kept in the solution. Presence of golden yellow colour reveals the presence of akaloids.

Starch was present in leaves and stem of all the texa, viz. *Semecarpus anacardium* (Table 1A), *Mallotus philippensis* (Table 1B) and *Pterocarpus marsupium* (Table 1 C). Proteins were observed in the upper and lower epidermis, scattered cells of mesophyll of leaves, pith parenchyma and cortical parenchyma in the stem of *Semecarpus anacardium, Mallotus philippensis,* and

Table 1.A. Histochemical test for fresh sections of leaves and stem of *Semecarpus anacardium* Linn.

Sr. No.	Ergastic content	Reaction		Localization	
		Leaves	Stem	Leaves	Stem
1.	Starch	+ve	+ve	Scattered cells of mesophyll.	Xylem and phloem parenchyma
2.	Protein	-do-	-do-	Upper and lower epidermis, scattered cells of mesophyll.	Epidermis, cortical parenchyma and pith parenchyma.
3.	Tannin	-do-	-ve	Mesophyll	-
4.	Saponin	-do-	+ve	Mid-rib pith parenchyma	Cortex and pith parenchyma
5.	Fat	-do-	-do-	Cells of mesophyll, phloem parenchyma	Pholem parenchyma
6.	Glucoside	-do-	-do-	Epidermis and mid-rib, pith parenchyma	Vascular bundle and scattered cells of medullary ray.
7.	Alkaloid				
	(a) Mayer's reagent	-do-	-do-	Scattered cells of mesophyll	Pith parenchyma
	(b) Wagner's reagent	-do-	-do-	Cells of mesophyll	Cells of cortex parenchyma and pith parenchyma

Table 1.B. Histochemical test for fresh sections of leaves and stem of *Mallotus philippensis* Muell. Arg.

Sr. No.	Ergastic content	Reaction		Localization	
		Leaves	Stem	Leaves	Stem
1.	Starch	+ve	+ve	Cells of mesophyll, xylem and phloem parenchyma	Hypodermis, xylem, cortex and phloem parenchyma
2.	Protein	-do-	-do-	Scattered cells of mesophyll	Medullary ray and pith parenchyma
3.	Tannin	-ve	-ve	-	-
4.	Saponin	+ve	+ve	Cells of mesophyll	Xylem parenchyma, pith
5.	Fat	-do-	-do-	Phloem parenchyma	Xylem parenchyma
6.	Glucoside	-ve	-ve	-	-
7.	Alkaloid				
	(a) Mayer's reagent	-do-	-do-	-	-
	(b) Wagner's reagent	-do-	-do-	-	-

Table 1.C. Histochemical test for fresh sections of leaves and stem of *Pterocarpus marsupium* Roxb.

Sr. No.	Ergastic content	Reaction		Localization	
		Leaves	Stem	Leaves	Stem
1.	Starch	+ve	+ve	Cells of mesophyll	Cortex, xylem and phoem parenchyma
2.	Protein	-do-	-do-	Upper and lower epidermis	Medullary ray, scattered cells of cortical parenchyma and pith parenchyma
3.	Tannin	-do-	-do-	Cells of mesophyll	Xylem and phloem parenchyma
4.	Saponin	-do-	-do-	Cells of palisade and spongy tissue	Scattered cells of cortical parenchyma and vascular bundle
5.	Fat	-ve	-ve	-	-
6.	Glucoside	+ve	+ve	Spongy parenchyma and mid-rib pith parenchyma	Epidermis and scattered cells of cortex parenchyma
7.	Alkaloid				
	(a) Mayer's reagent	-do-	-do-	Palisade parenchyma	Cells of cortex and medullary ray
	(b) Wagner's reagent	-do-	-ve	Cells of mesophyll, phloem parenchyma	-

Pterocarpus marsupium (Table 1 A, B, C) respectively. Tannins are particularly abundant in the leaves of many plants[2,4]. Tannins were observed in the leaves of mesophyll cells and absent in the stem of *Semecarpus anacardium*(Table 1.A). However, tannins were not detected in leaves and stem of *Mallotus philippensis.* Tannins were observed in the cells of mesophyll, xylem parenchyma and phloem parenchyma of stem of *Pterocarpus marsupium* (Table 1.C).

Saponins were observed in the mid-rib parenchyma of leaves and cortex and pith parenchyma of stem of *Semecarpus anacardium* (Table 1.A). Saponins were observed in the cells of mesophyll and xylem parenchyma of stem of *Mallotus philippensis* (Table 1.B). Saponins were also observed in the leaves and stem of *Pterocarpus marsupium* (Table 1.C). The fatty substances are thought to be elaborated directly by the cytoplasm[6]. Fat was found in the cells of mesophyll and phloem parenchyma of leaves and stem of *Semecarpus anacardium* (Table1.A). In leaves and stem of *Mallotus philippensis* fat was obsered on the phloem parenchyma and xylem parenchyma. Fat was not detected in leaves and stem of *Pterocarpus marsupium*. Glucosides were observed in the epidermis, pith parenchyma of leaves, vascular bundles and medullary rays of stem of *Semecarpus aacardium* (Table1.A). Glucosides were not found in the *Mallotus Philippensis*. Glucosides were found in the leaves and stem of *Pterocarpus marsupium* (Table1.C). In Mayer's reagent alkaloids were observed in the mesophyll of leaves and pith parenchyma of stem of *Semecarpus anacardium* (Table1.A). *Alkaloids* were not found in the leaves and stem of *Mallotus philippensis.* In Mayer's reagent alkaloids were found in the leaves and stem but in Wagner's reagent alkaloids were observed only in leaves and were not found in stem of *Pterocarpus marsupium* (Table1.C).

Therefore, it is suggested from the present observations that, all the seven chemicals were found in the parenchymatous cells of both the organs of *Semecarpus anacardium;* except the tannins were absent from the stem. Proteins (contain in the epidermis of leaves and stem) and glucosides (contain in the epidermis of leaves) were noted in the epidermal cells and mid-rib pith cells of leaves showed restricted accumulation of saponin of *Semecarpus anacardium.* Leaves and stem of *Mallotus philippensis* were devoid of glucosides, tannin and alkaloids, whereas starch, protein, saponin and fat were noted in the parenchymatous cells of leaves and stem of *Pterocarpus marsupium* do not show any fat. However other six substances were found in the parenchymatous cells as usual.

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