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Histochemical Investigation of Some Medicinal plants of genus *Terminalia* of (Combretaceae) in Maharashtra

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Abstract

The histochemical studies of leaves and wood of *Terminalia cuneata Terminalia bellerica, Terminalia chebula* and *Terminalia catappa* are medicinally 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.

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

Contents

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1. 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 (Kritikar and Basu ,1975).

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 glucosides. 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. Alkaloides are the degradation product of protein. *Terminalia cuneata* Roth., *Terminalia bellerica* Roxb., *Terminalia chebula* Retz. and *Terminalia catappa* Linn. are most important plants from the family Combretaceae. All these plants contain chemical ingredients of a great importance in medical care, in agriculture and they have their great importance in physiology, biochemistry and even in taxonomy also.

Terminalia cuneata Roth. popularly known as Arjuna, Arjan, White mudra , Sadada, Orjun etc. have great medicinal properties, the bark of the trees contain calcium salts, magnesium salts and glucosides, B-Sitosterol, anthraquinone, glycoside, terchebin tanic acid , oleic , linoteic etc., its mature and immature fruits and bark is useful in diseases like wounds, ulcers, inflammation and so on .It has some cultural importance also. Arjuna is one of the sacred trees of India.

*Terminalia bellirica (*Roxb.) Behada, Belleric myrobalan, Bahura is one of the oldest medicinal herbs of India have an anti-inflammatory, dyne, styptic acid thermo genicproperties. It is also an ingredient of Triphala churn; the principal chemical constituents are triterphnoids, cardiac glycoside saponins, bellericoside, bellericanin, tannin, ellagic acid etc. It also contains galloyl glucose and number of free sugars. The seeds contain proteins and oxalic acid, while bark contains tannin and its oil contain plasmatic, oleic and linoleic acids as a major fatty acids.

Terminalia chebula Retz. Haritak, is an active ingredient of a Hirda formulation Triphala churn, chebulin, ellagic acid, 2, 4-Chebulyl –B-D-glucopyranosc, chebulic acid, gallic acid, ethyl gallate, bunicalagin, terflavin A, tanic acid, chebulic acid etc. are the major chemical constituents of this plant. It is an astringent, anthelminticner vine, expectorant toxic, carminative, laxative, rejunanative. *Terminalia catappa* Linn., an Indian almond or umbrella tree, is an ornamental tree or cultivated tree, having anti carcinogenic properties, antioxidant as well as anticastogenic characteristics. It contains hydrolysable tannins, flavonoids, triterphnoids etc. The seed is very rich in proteins (19-22%) and oil (50-52 %) (Muhammad and Oloyede , 2004). The bark contains Catappanin A, novel complex tannin, seven ellagic tannins (Lin and Hsu ., 1999).

Many woody plants contain medicinally important secondary product (Dhar et.al.,1968). Therefore, we have attempted to histochemical investigations of different plant parts of *Terminalia cuneata* Roth., *Terminalia bellerica* Roxb., *Terminalia chebula* Retz. and *Terminalia catappa* Linn. are most important plants from the family Combretaceae in Maharashtra

2. Materials and Method

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).

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 carbonate. A red colour of the section with hydrochloric acid reveals the of 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.

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.

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.

3. 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, proteins, fat, tannin, saponin, glucosides in leaves 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 $C_6H_{12}O_5$. Starch has an ordinary arrangement of molecules and therefore, shows optical anisotropy and double refraction. In starch granules, the molecules are 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 taxonomically and pharmacognostically up to a limit. (Kuster, 1956). Deposition of starch occurs widely in the plant body, but the particularly common places of its accumulation in seeds, the parenchyma of the secondary vascular tissue in wood and roots, tuber, rhizome and corns. In the present work, starch was present in leaves and wood of all the taxa studied, viz. *Terminalia cuneata* Roth. (Table-a), *Terminalia bellerica* Roxb. (Table-b), *Terminalia chebula* Retz. (Table-c) and *Terminalia catappa* Linn.(Table-d).

Protein:

Proteins are the major constituents of the living protoplast, but they also occur as temporarily inactive in 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 proteins 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. Proteins were observed in the upper and lower epidermis, scattered cells of mesophyll of leaves, pith parenchyma and cortical parenchyma in the wood of, *Terminalia cuneata Roth.* (Table-a) *Terminalia bellerica* Roxb. (Table-b), *Terminalia chebula* Retz. (Table-c) and *Terminalia catappa* Linn.(Table-d).

Tannin:

Tannin is a heterogeneous group of phenol derivatives, usually related to glucosides. Tannin found abundant in the leaves of much plants; in the xylem, in the testa of seeds and in pathological growth like galls (Kuster, 1956; Sperlich, 1939). No tissue, however, appears to lack tannins entirely. They may found in meristematic cells too. Sometimes tannins containing cells are conspicuously associated with a vascular tissue terminates beneath storage tissue or secretary cells of nectaries. The monocotyledons are notably poor in tannins (Sperlich, 1939). Tannins also showed distributions, occurring mostly in epidermis, mesophyll cells, cortical cells as well as parenchymatous tissue, associated with conductive tissue. Tannins were observed in the leaves of *Terminalia cuneata Roth.* (Table-a), *Terminalia bellerica* Roxb.(Table -b), *Terminalia chebula* Retz.(Table-c) and *Terminalia catappa* Linn.(Table-d).

Saponin:

The occurrence of saponin is rare and wherever present, they apparently remain to one or two organs, saponin is observed in the mid-rib parenchyma of leaves and cortex and pith parenchyma of wood of *Terminalia cuneata* Roth. (Table-4a and Plate-8), *Terminalia bellerica* Roxb. (Table - 4b and Plate - 16), *Terminalia chebula* Retz. (Table No. 4c and Plate - 24) and *Terminalia catappa* Linn. (Table - 4d and Plate-32). Saponin were observed in the cells of

mesophyll and xylem parenchyma of wood of *Terminalia cuneata* Roth., *Terminalia bellerica Roxb,*, *Terminalia chebula* Retz. and *Terminalia catappa* Linn.

Fat:

Fats 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 (Seifriz, 1936). 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 (Sharp, 1934). They occur as solids bodies or, more frequently, as fluid droplets of various sizes 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 the present taxa under study, fats are found in the cells of mesophyll and phloem parenchyma (leaves and wood) of *Terminalia cuneata* Roth.(Table - a), *Terminalia bellerica* Roxb. (Table - b), *Terminalia chebula* Retz. (Table - c). and *Terminalia catappa* Linn. (Table-d).

Glucoside:

Glucosides are the degradation product of carbohydrates, glucosides were observed in the epidermis, pith parenchyma of leaves, of *Terminalia cuneata* Roth .(Table- a), The test was negative regarding *Terminalia bellerica* Roxb. (Table - b), Glucosides were found in epidermis and cortical parenchyma of the wood of *Terminalia chebula* Retz (Table - c) and cortical parenchyma of wood of *Terminalia catappa* Linn. (Table - d).

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 cell of mesophyll, pith, upper and lower epidermis and cells of cortex parenchyma and pith parenchyma of wood of *Terminalia cuneata* Roth.(Table - a) , *Terminalia bellerica* Roxb. (Table - b) , *Terminalia chebula* Retz.(Table-c) and *Terminalia catappa* Linn.(Table- d).

Table 1: Histochemical tests for fresh sections of leaves and wood of *Terminalia cuneata* Roth.

S. 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 parenchymal
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	-do-	-do-	----------	Epidermis, scattered cells of cortex parenchyma, and pith
5	Fat	-do	-do-	Upper and lower epidermis, scattered cells of mesophyll cells and mid-rib	Cortical parenchyma, medullary rays, scattered cells of pith parenchyma.
6	Glucoside	-ve	-ve	----------	----------
7	Alkaloids				
	a) Mayer's reagent	+ ve	+ ve	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 tests for fresh sections of leaves and wood of *Terminalia bellerica Roxb*

S.No	Ergastic content	Reaction		Localization	
		Leaves	Wood	Leaves	wood
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, scattered cells of mesophyll, cortical parenchyma, scattered cells of medullary rays and pith parenchyma.	Epidermis, scattered cells of cortex parenchyma, xylem and phloem, scattered cells of medullary rays, pith parenchyma
3	Tannin	-ve	-do-	----------	Vascular bundle and scattered cells of medullary ray, and pith parenchyma
4	Saponin	-do-	-do-	----------	Scattered cells of cortex parenchyma and pith region, xylem parenchyma
5	Fat	+ve	-do-	Scattered cells of epidermis, mesophyll cells and mid-rib, pith parenchyma	Vascular bundle and scattered cells of medullary ray, and pith parenchyma.
6	Glucoside	-ve	-ve	----------	----------
7	Alkaloids				
	a)Mayer's reagent	-do-	-do-	Upper and lower epidermis, scattered cells of mesophyll cells	Hypodermis, xylem parenchyma, pith
	b)Wagner's reagent	-do-	-do-	Upper and lower epidermis, scattered cells of mesophyll, mid-rib parenchyma, pith parenchyma	Epidermis, scattered cells of cortex parenchyma, medullary rays, and vascular bundle

Table 3: Histochemical tests for fresh sections of leaves and wood of *Terminalia chebula* Retz.

S. No	Ergastic content	Reaction		Localization	
		Leaves	Wood	Leaves	wood
1	Starch	+ve	+ve	Upper and lower epidermis, mesophyll cell, cortical cells, pith parenchyma	Medullary rays, cortical parenchyma
2	Protein	-do-	-do-	Scattered cells of cortex, mesophyll cells and pith	Epidermis, cortical parenchyma, pith parenchyma
3	Tannin	-do-	-do-	Scattered cells of mesophyll, mid-rib pith parenchyma.	Vascular bundle and scattered cells of medullary ray.
4	Saponin	-do-	-do-	Upper and lower epidermis and mid-rib pith parenchyma	Scattered cells of cortex medullary rays, and pith parenchyma
5	Fat	-ve	-do-	--------	Mesophyll cells and phloem parenchyma
6	Glucoside	-do-	-do-	--------	Epidermis and cortical parenchyma
7	Alkaloids				
	a)Mayer's reagent	+ ve	-do-	Upper and lower epidermis, scattered cells of mesophyll, mid rib pith parenchyma	Scattered cells of cortex, and vascular bundle
	b)Wagner's reagent	-do-	-do-	Upper and lower epidermis, pith parenchyma	Scattered cells of cortex, medullary rays, vascular bundle

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