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HISTOCHEMICAL INVESTIGATION OF *CASSIA TORA* LINN.

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ABSTRACT

Cassia tora Linn. (Caesalpinaceae) is a semi wild annual herb grown widely in different places of India. This plant species is well known for having potential in traditional medicine practices for the treatment of a variety of disorders and ailments ranging from cough, hypertension, antimicrobial, antioxidant antidiabetic. This paper encompasses a comprehensive review on histochemical and biological aspects of *Cassia tora* Linn. For histochemical studies the freehand sections of leaves, stem and roots 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

Nature has provided the storehouse of remedies to cure all ailments of mankind.The traditional herbal medicines are still practiced in large part of our country mostly in tribal and rural areas. In many developing countries large section of population relies on traditional practioners, who are dependent on herbal folk medicines for their primary health care and has deep faith in it. Since the usages of these herbal medicines are increased, the issues regarding their safety, quality and efficacy in industrialized and developing countries are cropped up. Plant based traditional are well known for their potential health benefits in a variety of human disorders or ailments since time immemorial. According to the world health organization, it has been estimated that 80% of the world's population is still dependent on traditional medicines for maintaining their health and combating various diseases (Bent S., 2008). Besides, 56% of world's populations in the rural areas rely chiefly on herbal medicine and supplementation for their primary health care needs (Planta et.al., 2000).

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, root, tubers, rhizomes and corn (Kadam, 1999). Starch and proteins are the principal ergastic substances of the protoplast (Kuster, 1956). Tannins are particularly abundant in the leaves (xylem) of many plants (Kadamet.al. 2015). Saponin is the rare occurrence. Fats are widely distributed in the plant body and they probably occurs in small amount in every plant cell (Seifriz, 1936).

Young and tender leaves and stems are eaten as a vegetable and in soups. The unripe fruits are also cooked and eaten. The seeds can be introduced as a protein rich food for livestock. Other applications of *Cassia tora* Linn. are in abnormal child birth, vermicide, cold, epilepsy, night blindness, scabies, scorpion bite, stomachache and in bone fracture (Jain S K, 1968). The seed extracts of *Cassia tora* have been used in Chinese medicine as an aperients, anti-asthenic and diuretic agent and also to improve visual activity (Asolkar et al., 1992).

MATERIALS AND METHODS

Temporary and permanent mounting of sections were employed for the various test for histochemical studies of *Cassia tora*. For study of isolated different tissues, small pieces of material were macerated in Jeffery fluid (Johansen, 1940). Micro chemical tests were performed by following method described by Johansen (1940) and Gurr (1965). The different tests performed are listed in Table No.1.

Table No. 1 - Histochemical Tests

Sr. No	Erastic Content	Chemical Test	Reference:
1	Starch	Iodine, Potassium Iodide soln. (IKI Solution.)	Johansen (1940) and Gurr (1965)
2	Protein	Potassium Ferro Cyanide, Glacial AcetiAcid	Johansen (1940)
3	Tannin	10% Ferric Chloride Solution (aq)	Johansen (1940) and Gurr (1965)
4	Saponin	Sulphuric Acid	Johansen (1940)
5	Fats	Sudan III , Sudan IV	Johansen (1940)
6	Glycosides	Phloroglucinol in 90% Alcohol + 20% Hydrochloric acid	Johansen (1940) and Gurr (1965)
7	Alkaloids	Idonine solution	Johansen (1940)
		Nitric Acid	Johansen (1940)

For the histochemical studies free hand section of plant parts were studied, they were taken and treated the respective reagents to localize component, viz. starch, protein, tannin, saponin, fat, glucosides and alkaloids etc. in the tissues (Johansen, 1940). The tests were employed are as follows:

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

2) **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. Dilute eosin, stain protein red. To localize protein, reagent was prepared by mixing 0.1 gm. Potassium Ferro cyanide dissolved in 20ml water and 100ml glacial acetic acid. Sections were kept in this solution for an hour. Then sections were washed with 60% alcohol and few drops of aqueous $FeCl_3$ were added. Blue colour 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) **Saponin** -Sections were placed directly in one drop of concentration H_2SO_4 on a slide, which gives a characteristic sequence of colour reaction, 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 saturated barium hydroxide solution for about 24 hours. Sections were washed with calcium chloride, then placed in potassium dichromate, yellow colour will appear, which indicate the presence of saponins.

5) **Fat** - 0.5 gm. of dye and Sudan III or Sudan IV was dissolved in 100 ml of 70% alcohol. Then sections were kept in this stain for 20 minutes, rinsed quickly with 50% alcohol and mounted in glycerine for observation. Blue, red, pink, precipitate indicates the presence of fats.

6) **Glucoside** (Grignard's test) -Sections were immersed in 1% of aqueous Picric acid for 30 minutes, then washed with water and placed in a drop of 10% aqueous sodium carbonate, red colour of the section with hydrochloric acid reveals the presence of glucosides. For the

localization, sections 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 $FeSO_4$ and 20% aqueous $FeCl_3$ solution was taken in equal proportion, it was heated up to boiling and then the section were transferred to a slide holding a drop of 20% hydrochloric acid. Deep blue precipitation indicates the presence of glucosides.

7) **Test for Alkaloids**- Transverse sections of the different plants were treated with the following alkaloid reagents.

Mayer's Reagent- Potassium mercuric iodide solution; 13.55gm of $HgCl_2$ and 50gm of KI, were dissolved in one liter of distilled water. Presence of grey colour in the sections reveals the presence of alkaloids.

Wagner's Reagent -1gm iodine and 2gm 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, proteins, fat, tannin, saponin, glucosides in leaves and alkaloids in leaves and stem.

1) 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 corms (Radley, 1954). In the present work, for the taxa under study, starch was present in leaves of cells of mesophyll, pith parenchyma cells of spongy tissue. Starch was present in stem of epidermis, medullary rays, vascular bundle and pith parenchyma.

Starch was present in root of cortical parenchyma, vascular bundle and pith parenchyma (Table No.2).

2) 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.

Proteins were observed in epidermis, scattered cells of mesophyll, pith parenchyma of leaves. Epidermis, scattered cells of cortex, pith parenchyma in the stem and epidermis, cells of cortex, pith parenchyma and phloem parenchyma of root of *Cassia tora* Linn (Table No.2).

3) 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 *Cassia tora* was scattered cells of mesophyll, lower epidermis, phloem parenchyma. Stem of scattered cells of cortex, medullary rays and pith parenchyma and root of scattered cells of cortex, pith parenchyma and phloem parenchyma(Table No.2).

4) Saponin: The occurrence of saponin is rare and wherever present, they apparently remain to one or two organs, saponin is observed in the upper and lower epidermis, midrib parenchyma of leaves. Epidermis, scattered cells of cortex and phloem parenchyma of stem of *Cassia tora* .Saponin was observed in the cells of epidermis, scattered cells of cortex parenchyma of *Cassia tora* (Table No.2).

5) 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 scattered cells of mesophyll, phloem parenchyma of leaves and stem of xylem and phloem parenchyma, medullary rays, scattered cells of pith parenchyma. Fats are found in the cells of cortical parenchyma, medullary rays and scattered cells of pith parenchyma of root of *Cassia tora* (Table No.2).

6) Glycoside: Glucosides are the degradation product of carbohydrates, glucosides were observed in the upper and lower epidermis and midrib pith parenchyma of leaves of *Cassia tora*. Glucosides were found in the scattered cells of medullary rays and vascular bundle of stem and also found in the cells of cortex parenchyma and pith parenchyma cells of root of *Cassia tora*(Table No.2).

7) 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 cells of mesophyll, mid-rib and phloem parenchyma of leaves; cortex, xylem parenchyma, vascular bundle and scattered cells of medullary rays of stem and cortex, xylem parenchyma and pith parenchyma of root of *Cassia tora*(Table 1). In Wagner's reagent, alkaloids were found in the cell of epidermis, midrib parenchyma of leaves; epidermis, cortical parenchyma, vascular bundle and pith parenchyma of stem and epidermis, cortical parenchyma, medullary rays, pith parenchyma and vascular bundle of root of *Cassia tora.* (Table No.2).

Table No. 2- Histochemical tests for fresh sections of leaves, stem and root of *Cassia tora* L.

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

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