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HISTOCHEMICAL INVESTIGATION OF TWO PLANT SPECIES VALUED AS MEDHYA RASAYANAS

D. A. DHALE*

Post-Graduate Department of Botany, S.S.V.P. Sansthas, L. K. Dr. P. R. Ghogrey Science College, Dhule-424005 (M.S.) India

***Corresponding author**

ABSTRACT

We chose two different taxa used in folk medicine to determine their histochemical investigation: *Bacopa monniri* Pennell. and *Centella asiatica* Linn. Both the plants widely known as Brahmi are considered as 'Medhya rasayanas' in Ayurveda meaning brain tonic. However, these plants are distinctly different. The name Brahmi, Jala-brahmi or water-brahmi is assigned to *Bacopa monnieri* whereas 'mandukaparni', often confused with Brahmi, refers to *Centella asiatica*. A detailed histochemical investigation of leaf and stem of both the drugs was carried out. In general, these plants are used in folk medicine in the treatment as medhya rasayanas or 'mental rejuvenatives. Brahmi is used to treat specific mental disorders such as insanity and epilepsy, while mandukaparni is a general rejuvenative tonic which improves mental health. Brahmi promotes fertility and sustains implantation of the embryo in the uterus, while mandukaparni tends to reject the embryo. For histochemical studies, the free hand sections of leaves and stem were taken and treated with the respective reagent in localize components, *viz*. starch, protein, tannin, saponin, fat, Sugar, glucosides and alkaloids in the tissues.

KEYWORDS

Histochemistry, Medicinal Plants, Medhya rasayanas and Folk Medicine.

INTRODUCTION

In India, indigenous people traditionally use *Bacopa monniri* Pennell. and *Centella asiatica* Linn. to maintain their health. These plants have anormous reservoirs of many secondary metabolites which exhibit some medicinal properties. *B. monnieri*, known as 'Brahmi', is revered in the indigenous system of medicine as a nerve tonic. In early literature, the name Brahmi was also used to refer to another plant species, *C. asiatica* Linn., known as Indian penniwort[1]. However, these plants are distinctly different. The name Jala-brahmi or water-brahmi assigned to *B. monnieri* in ancient Sanskrit writings provides the differentiation. Brahmi is found in marshy areas near streams and ponds throughout India especially in the North eastern regions[2]. The vernacular name 'mandukaparni', often confused with Brahmi, in fact refers to *C. asiatica*. Mandukaparni is commonly found as a weed in crop fields and other waste places throughout India. It is abundant in tropical and subtropical regions particularly in damp, shady places along marshy banks of rivers, streams, ponds, irrigated fields. In Himalayas, it grows wild in natural habitat all round the year[3]. The Charaka Samhita considers them both to be promoters of cognitive functions, but it suggests that Brahmi is superior to mandukaparni. Chemically both species are rich in saponins. Madecassoside and Asiaticoside are the important saponins of *C. asiatica* whereas *B. monnieri* contains bacosides A and B having biological activity[4]. Both are medhya rasayanas or 'mental rejuvenatives[5]. Brahmi is used to treat specific mental disorders such as insanity and epilepsy[6], while mandukaparni is a general rejuvenative tonic which improves mental health[7]. Brahmi promotes fertility and sustains implantation of the embryo in the uterus[8], while mandukaparni tends to reject the embryo[9]. This suggests that the plant materials have opposite effects on uterine functions.

Histochemical methods have been developed for qualitative and quantitative analysis of virtually all cellular components, including proteins, carbohydrates, lipids, nucleic acids and the range of ionic elements occurring in cell solutions[10,11,12]. These methods, in combination with various microscopic imaging techniques, can be utilized in the study of essential oil secretion in plants.

Several published reports demonstrate the use of histochemistry to locate essential oil, such as the localization of citral accumulation in *Cymbopogon citratus*[13], where the aldehyde-specific Schiff's reagent was used to detect the monoterpene aldehydes neral and geranial (citral), and the lipid stains Sudan red and Nile blue were used to locate essential oil in leaves of *Salvia aurea*[14].

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[15]. Starch and proteins are the principal ergastic substances of the protoplast[16]. Tannin is the heterogeneous group of phenol derivatives, usually related to glucosides. Tannins are particularly abundant in the leaves (xylem) of many plants[17]. Saponins are the rare occurrence. Fats are widely distributed in the plant body and they probably occurs in small amount in every plant cell[18]. 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.

Many plants contain medicinally important secondary product[19]. Therefore, we have attempted to histochemical investigations of different plant parts of two medicinal plants of Marathwada region of Maharashtra. Free hand sections were taken for the histochemical studies. Sections were treated with the respective reagent to localize components, *viz*. starch, proteins, tannin, saponin, fat, sugar, glucosides and alkaloids in the tissues. The information generated by this particular study would generate relevant data of plants to confirm identification and authentification of plants of these particular species for the benefit of taxonomists as well as common man.

MATERIALS AND METHODS

***Sample collection**:*

The Plant material of *Bacopa monniri* Pennell. family Scrophulariaceae and *Centella asiatica* Linn. family Umbelliferae (Apiaceae) were collected from the Botanical Garden of SSVP Sanshthas, L.K.Dr.P.R.G.Science College, Dhule (M.S., INDIA). The plant materials (leaves, flowers) were identified using the Flora of Dhule and Nadurbar District[20] at Post-graduate Department of Botany, S.S.V.P. Sanshthas, L. K. Dr. P. R. G. Science College, Dhule (M.S., INDIA).

Temporary mounts of sections were employed for the test of histochemical studies. For this study free hand sections of the organs were taken and treated the respective reagent to localize component, *viz*. starch, protein, tannin, saponin, fat, sugar, glucosides and alkaloids in the tissues[21].

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. The sections were washed with 60% alcohol and few drop 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 0.1N 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) Sugar**:*

A drop of 20% aqueus NaOH was placed on a slide, dissolved small quantity of Copper tartrate, section was placed there and cover slip was mounted. Fructose immediately gave yellow red precipitate of cuprus oxide. On gentle warming glucose gave cuprus oxide crystals. On heating the sections for about 20 minutes, dextrincaused formation of cuprus oxide crystals. No precipitated by sucrose noted upon addition of 95% alcohol. The fructose and glucose were dissolved leaving the insoluble dextrin in the tissue.

7) Glucoside (Guignard's test):

Sections 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 revealed the Glucosides. For the localization, section was 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 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.

8) 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 $HgCl_2$ 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.
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 gave details about the occurrence of erastic content or secondary metabolites, *viz*., starch, protein, fat, sugar, tannin, saponin, glucoside 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 molecule and, therefore, shows optical anitsotropy 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[16].

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 stem and roots, tuber, rhizome and corms. In the present work, for the taxa under study, starch was scattered in cells of mesophyll of leaves and cortical parenchyma and pith parenchyma of stem of the *B. monnieri* (Table 1). While starch is scattered in spongy cells of mesophyll in leaves and it is scattered in cells of pith parenchyma in *C. asiatica* (Table 2).

2) Protein:

Protein is 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 both the taxa under investigation. Protein were observed in the cells of mesophyll of leaves, and cortical parenchyma in the stem of *B. monnieri* (Table 1) while it is noted in the cells of mesophyll of leaves, and scattered in cells of hypodermis collenchymas and cortical parenchyma of *C. asiatica* (Table 2).

3) 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[16]. 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 of leaves of both species. While in stem, it is observed scattered in cells of cortex of both species (Table 1, 2) and in pith parenchyma of *C. asiatica* (Table 2).

4) Saponin:

The saponin is of rare occurrence and wherever present, they apparently remain to one or two organs. Saponin were observed Scattered in cell of mesophyll and xylem fibres of leaves and cells of cortex parenchyma, pith parenchyma and xylem fibres of stem *B. monnieri* (Table 1). It was also observed in the cells of spongy tissues in leaves and cells of cortical parenchyma and bundle cap of stem of *C. asiatica* (Table 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 describe 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 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 taxa under study, fat was found in cells of mesophyll and spongy cells of leaves while in stem it was scattered cells of cortical parenchyma and pith parenchyma of *B. monnieri* (Table 1) and cells of hypodermal collenchymas and cortical parenchyma *C. asiatica* (Table 2).

6) Sugar*:*

They are substances of universal occurrence and are much abundant in plants. Sugars were observed scattered in cell of mesophyll of leaves and cells of cortex parenchyma and pith parenchyma of stem *B. monnieri* (Table 1). It was also observed in the cells of mesophyll in leaves and cells of cortical parenchyma of stem in *C. asiatica* (Table 2).

7) Glucoside:

Glucosides are the degradation production of carbohydrates. Glucosides were observed only in the mesophyll of leaves and absent in stem of *B. monnieri* (Table 1). It is totally absent in leaves and stem of *C. asiatica* (Table 2).

8) 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 cortical parenchyma and hypodermis collenchyma of stem. In Wagner's reagent, alkaloids were found in the cells of mesophyll and cells of cortex parenchyma and pith

parenchyma and hypodermis collenchyma and vascular bundle of stem of *B. monnieri* (Table 1) and *C. asiatica* (Table 2).

Table 1
Histochemical test for fresh section of leaves and stem of Bacopa monniri

Sr. No.	Ergastic content	Reaction		Localization	
		Leaves	Stem	Leaves	Stem
1	Starch	+ve	+ve	Scattered cells of mesophyll	Cortical parenchyma and Pith parenchyma
2	Protein	+ve	+ve	Scattered cells of mesophyll	Scattered cells of cortical parenchyma
3	Tannin	+ve	+ve	Scattered cell of mesophyll	Scattered cells of cortex and Pith parenchyma
4	Saponin	+ve	+ve	Scattered cell of mesophyll and xylem fibres	Scattered cells of cortex parenchyma, pith parenchyma and xylem fibres
5	Fat	+ve	+ve	Scattered cells of mesophyll	Scattered cells of cortical parenchyma and pith parenchyma
6	Sugar	+ve	+ve	Scattered cells of mesophyll	Scattered cells of cortical parenchyma and pith parenchyma
7	Glucoside	+ve	-ve	Scattered cell of mesophyll	Absent
8	Alkaloids				
	a)Mayer's reagent	+ve	+ve	Scattered cell of Mesophyll	Scattered cells of cortical parenchyma
	b)Wagner's reagent	+ve	+ve	Scattered cell of mesophyll	cortical parenchyma and Pith parenchyma

Note: +ve = Component present; -ve = Component absent

Table 2
Histochemical test for fresh section of leaves and stem of Centella asiatica

Sr. No.	Ergastic content	Reaction		Localization	
		Leaves	Stem	Leaves	Stem
1	Starch	+ve	+ve	Scattered spongy cells of mesophyll	Scattered cells of pith parenchyma
2	Protein	+ve	+ve	Scattered cell of mesophyll	Scattered cells of hypodermis collenchymas and cortical parenchyma

3	Tannin	+ve	+ve	Scattered cells of mesophyll	Some scattered cells of pith parenchyma
4	Saponin	+ve	+ve	Cells of spongy tissues	Scattered cells of cortical parenchyma and cells of bundle cap
5	Fat	+ve	+ve	Cells of spongy tissues	Scattered cells of hypodermal collenchymas and cortical parenchyma
6	Sugar	+ve	-ve	Scattered cells of mesophyll	Scattered cells of cortical parenchyma
7	Glucoside	-ve	-ve	Absent	Absent
8	Alkaloids				
	a) Mayer's reagent	+ve	+ve	Scattered cells of mesophyll cells.	Cells of hypodermis collenchyma
	b) Wagner's reagent	+ve	+ve	Scattered cells of mesophyll cells	Cells of hypodermis collenchyma and vascular bundle

Note: +ve = Component present; -ve = Component absent

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