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Research Article

ASSESMENT OF THE POTENTIAL OF NATURAL DYES AS BOTANICAL HISTOCHEMICAL STAINS

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ABSTRACT

This study reports the use of natural dyes as histochemical stains in microscopic investigations. Preparation of *Rubia cordifolia, Curcuma longa, Bixa* and *Nyctanthes* powder has been described. The histochemical property of natural dyes has been displayed. The effect of different solvents like methanol, ethanol and acetone in increasing the binding efficiency of the stain has been determined. The use of natural dye, a low cost and as an effective nuclear stain has been experimented. Experiments carried out in this regard with *Allium cepa* proved that natural dyes can be excellent nuclear stain. The metachromacy of natural dyes in differentially staining various tissue components has been brought out. An alternative staining procedure using natural dyes for mitotic studies and chromosome analysis has been described. The ecofriendly dyes can be recommended as an alternative to the popularly used, costly and harmful synthetic dyes.

INTRODUCTION

Botanical histochemistry, although relatively more recent, is a fast developing area of research combining histology and analytical biochemistry. There are strong indications for its establishment as an independent discipline, with a theoretical background of its own. Many hundreds of techniques have been developed and use on plant tissues after initial successful trials on animal tissues. These techniques have added sufficiently significant information to our knowledge on plant structure, its complexity and function (Krishnamurthy, 1999).

Histochemistry is a branch of science that deals with the composition of the cells or tissues in terms of the chemical elements and their compounds present in the material. Diachromes which are dyes to be examined by transmitted light and flurochromes which are dyes having the property of fluorescence. Some dyes like Eosin, Congo Red and Basic Fuschin are both fluorochromes and diachromes. The increased use of fluorochromes in microscopic staining has added a higher order of sensitivity and selectivity to the histochemical staining process. Dyes, which are fluorescent, even if they are colourless or weakly coloured in visible region of the spectrum, can be used for advantage in the non-visible range of the spectrum.

Many natural dyes have been known since time immemorial. They were obtained from animal and vegetable sources. Today, however, practically almost all dyes are synthetic. They are prepared from aromatic compounds, for which the only available source is coal tar (Anonymous, 1994).

Many natural dyestuff and stains are obtained mainly from plants and dominate as sources of natural dyes, producing different colours like red, yellow, blue, black, brown and a combination of these (Chandramouli,1995). Almost all parts of the plants like root, bark, leaf, fruit, wood, seed and flower produce dyes (Siva, 2003).

The dye is generally prepared by boiling the crushed powder with water, but sometimes it is left to steep in cold water. The solution then obtained is used generally to dye coarse cotton fabrics. Alum is generally used as a mordant (Krishnamurthy, 1993) Several studies are being carried out in dye yielding plants like madder, woad, indigo, saffron and sappan to improve their dye yielding quality and also to disclose their histological properties.

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Natural dyes are being tested for their usage as biological stains. Most of the biological stains used today are synthetic ones. Recent research has found out that sappan a natural dye is more efficient than the synthetic stains and has differentially staining principles (Vinodhini Jochebed, 2000).

MATERIALS AND METHODS

Extraction of Dyes Using Organic Solvents

Bixa

5g of bixa seeds were soaked for 15 minutes in 10ml of ethanol solvent (50% conc. of dye). Then it was boiled for 10 minutes and dye was filtered. Similarly bixa dye was prepared with methanol and acetone solvents. The filtered dye was stored in dark bottle for future use.

Madder Root

5g madder root was taken and powdered. This powder was soaked for 15 minutes in 10ml of ethanol solvent (50% conc. of dye). Then it was boiled for 10 minutes and dye was filtered. In the same way madder dye was prepared with methanol and acetone solvents.

Turmeric

5g of turmeric powder soaked for 15 minutes in 10ml of ethanol solvent (50% conc. of dye). Then it was boiled for 10 minutes and dye was filtered. In the same way turmeric dye was prepared with methanol and acetone solvents.

Nyctanthes

5g of dried Nyctanthes flower corolla tube & were macerated by hand. Then it was soaked in 10 ml of ethanol solvent (50% conc. of dye) for 10 minutes followed by filtration. Similarly the dye was extracted with methanol and acetone solvents.

Selected Experimented Plants for Histochemical Studies

Cyperus sp (mat sedge), *Pedilanthus* leaves, dicot stem (*Albizzia* sp) were collected from Sri Parasakthi College for women Courtallam. Healthy and disease free materials were selected for the study.

Anatomical and Cytological Studies

Free hand sections, leaf peeling of the materials selected, were taken and stained with the respective natural dye. The staining time however, varied between 30min – 1 hour depending on the material. The stained sections / leaf peelings were mounted in glycerine and water and observed under a binocular microscope.

Root Tip Squash Method

Root tips of *Allium cepa* were fixed using glacial acetic acid and absolute alcohol in the ratio of 3:1. Fixed root tips were left undisturbed for 24 hrs. The fixed root tips were washed well with distilled water and then hydrolyzed using 1 N HCl for 20 min at room temperature. After hydrolyzing they were washed 2 to 3 times in distilled water and mordanted in 4% Ferric Ammonium Sulphate. Then, the tips were stained in 50% natural dye for about 30min. Stained root tips were washed in water prior to squashing. Then it was mounted with 45% acetic acid. The squash was then observed under the microscope (Marimuthu and Subramaniam, 1964)

RESULTS

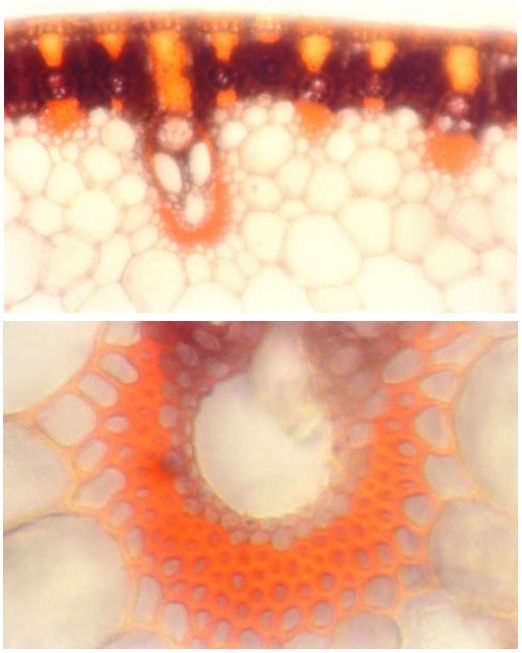
Indians have been considered as forerunners in the art of natural dyeing. Natural dyes find use in the colouring of textiles, drugs, cosmetics, etc. Owing to their non-toxic effects they are also used for colouring various food products. In India, there are more than 450 plants that can yield dyes. In addition to their dye-yielding characteristics, some of these plants also possess medicinal value. Though there is a large plant resource base, little has been exploited sofar. Due to lack of availability of precise technical knowledge on the extracting and dyeing technique, it has not commercially succeeded like the synthetic dyes (Siva, 2003)

Natural dyes are environment-friendly, for example, turmeric, the brightest of naturally occurring yellow dyes is a powerful antiseptic which revitalizes the skin, while indigo gives a cooling sensation (Debajit and Diwari, 2005)

The principle compound present in the madder has been identified as alizarin. It was used in the treatment of jaundice. Madder is known for various uses its utilization in the field of bacteriological, histological and botanical histochemistry has now been experimented in the present study. Using the microscopic facilities preliminary studies were made in characterizing the histochemical properties and dyeing ability of madder, bixa, turmeric and *Nyctanthes*.

Madder dye was also found to be useful in localizing the stomata, nucleus, cellwall and starch grains by its differential staining property (Fig.1). The presence of alizarin, gives a red colour. Similarly Turmeric, Bixa, Nyctanthes were also used as a histochemical stain.

Cross Section of Cyprus pangorei culm stained with madder



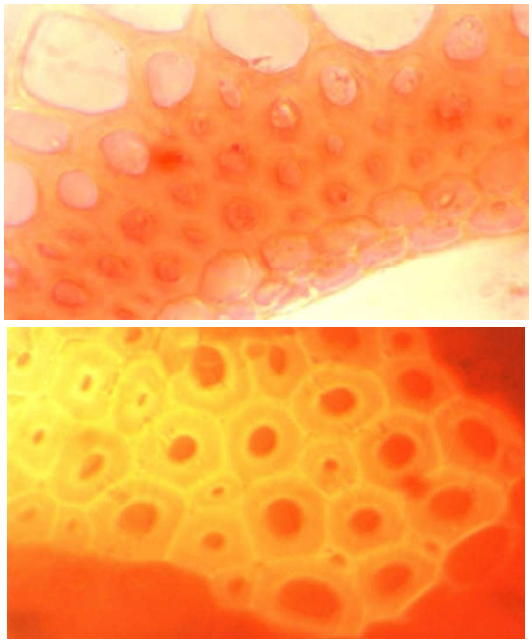
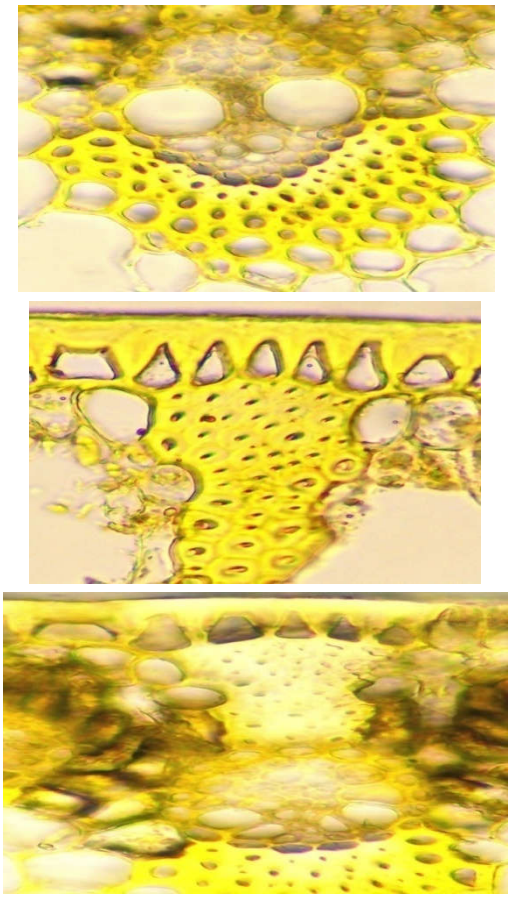


Figure 1 Cross section of Cyprus pangorei stained with Madder methanol extract, vascular bundle, hypodermal sclerenchyma, fluorescence

Turmeric makes a yellow colour due to the presence of the active substance of "Curcumin"(Fig. 2). A recent study involving mice has shown that turmeric slows the spread of breast cancer into lungs and other body parts (Sharma *et al*., 2005).

Cross Section of Cyprus pangorei culm stained with turmeric



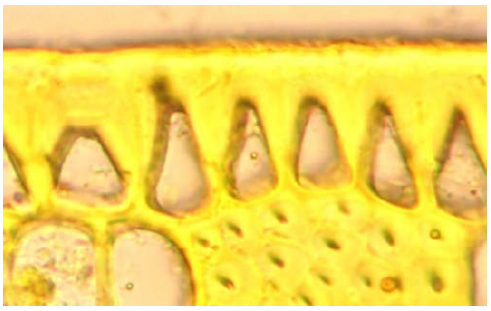


Figure 2 Cross section of *C. pangorei* stained with turmeric methanol extract. Thick walled cells, fibrous sheath, the vascular bundle, epidermal cells are all stain yellow

Cross Section of Cyprus pangorei stained with Bixa

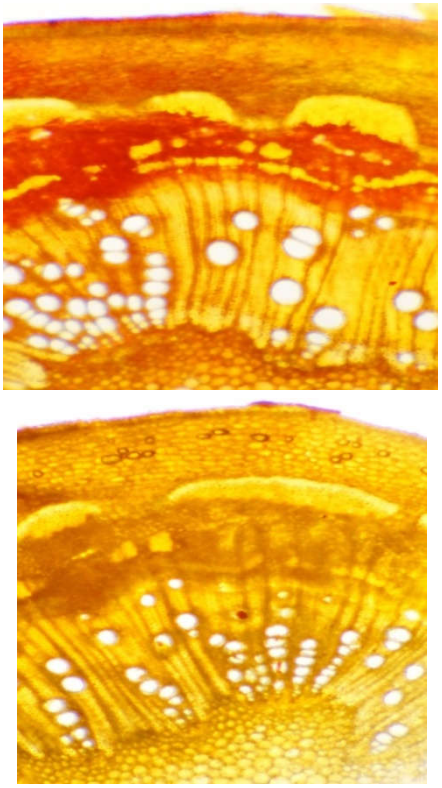


Figure 3 Cross Section of *Albizzia* stem stained with *Bixa (Acetone extract)*

Nyctanthes corolla tube gives orange yellow colour due to the iridoid nyctanthoside and also used for rheumatism and fever (Fig.4).

Cross Section of Albizzia Stem stained with Nyctanthes

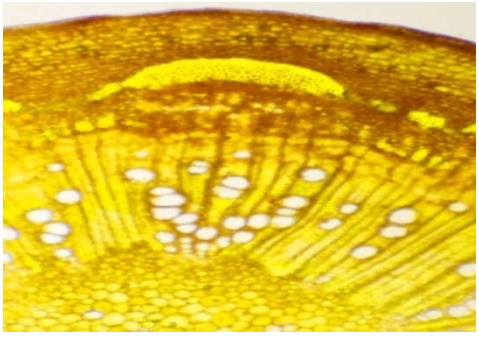


Figure 4 Cross Section of *Albizzia* Stem stained with *Nyctanthes (Acetone extract)*

These studies made us to realize that madder, bixa, turmeric, *Nyctanthes* could be a potential stain for botanical histochemistry including the possibilities of a nuclear stain. Standardization of obtaining these dyes in powder form or as an extract has been carried out as a pre-requisite of this investigation. The detailed method has been described in the materials and methods section.

Using the bright field and fluorescence microscopic facilities preliminary studies have been made in characterizing the histochemical properties and dyeing ability of Sappan dye.[10]

Sappan dye seems to have both dichromatic and metachromatic principles and thus help differentiate various kinds of cells and tissue components that includes parenchyma, collenchyma, bundle sheath cells, xylem, phloem, metaxylem, protoxylem and fibre sheath. Further sappan was also found to be useful in localizing the accumulation of cellulose, lignin, pectin, cutin, suberin and waxes by its differential staining property (Sree Devi, 2001)

The present investigation has described two methods to understand the staining property of madder, turmeric bixa and nyctanthes, (i) by staining epidermal peeling for the localization of nucleus and (ii) by staining of monocot (*Cyperus*) stem, dicot stem (*Albizzia*) and also onion root tip (The binding efficiency of these dyes has been enhanced by treating with organic solvents).

Based on the results and observations a simple staining procedure for the nucleus and mitotic chromosomes are being described employing these dyes.

Nuclear Staining Potential of Natural Dyes

To understand the nuclear staining capacity of natural dyes, a variety of plants consisting of monocots and dicots were subjected to test the staining potential of natural dyes. Natural dye could stain the nucleus of both lower and upper epidermal cells either alone or in conjunction of both lower and upper epidermal cells. Natural dyes strongly binds with nucleus and nucleolus and well differentiates the cytoplasmic contents. Apart from staining the nuclei of the epidermis they also stain the nuclei of stomatal apparatus with same intensity (Fig.5). The incubation period required for staining the epidermal nuclei varies between 30-80min. Increasing the incubation period leads to over staining of the nucleus. Cell walls in *cyperus* stem appear red when stained with madder in solvents in contrast treatment with turmeric makes the nuclei appear with yellowish tinge. In the same way Bixa and Nyctanthes make the nuclei appear orange and light or pale yellow in colour (Fig.6). This treatment helps in differentiating the nucleus and the nucleolus.

Leaf epidermal peeling of Pedilanthus

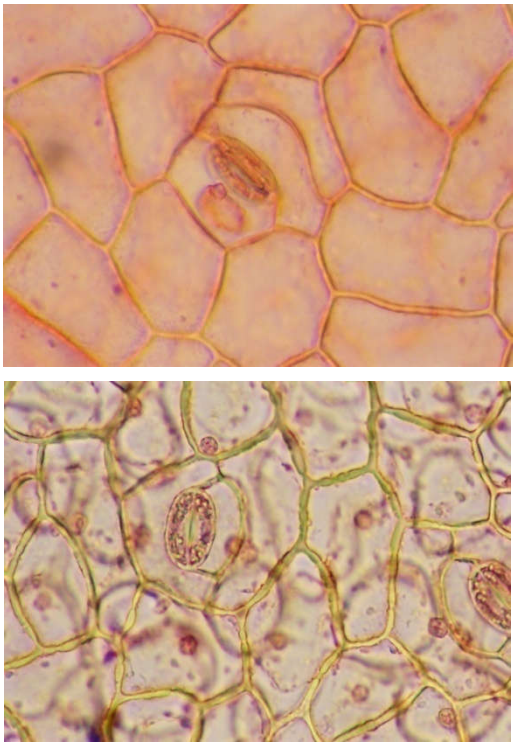


Figure 5 The nuclei of the stomatal apparatus stained with Madder, *Bixa*

Onion root tip squash

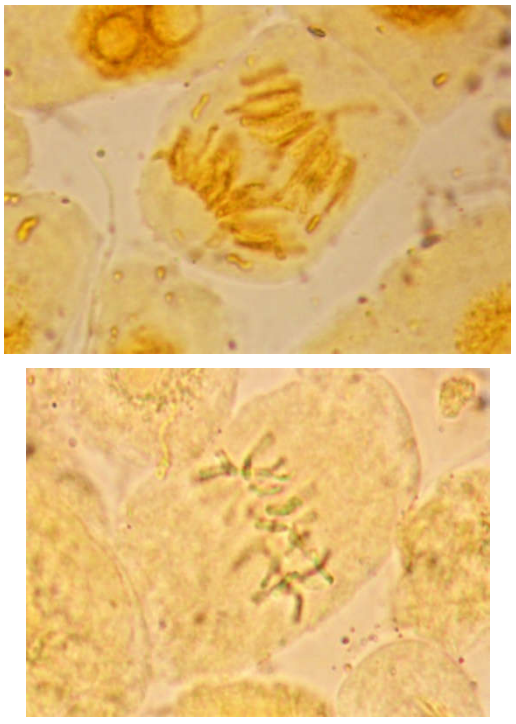


Figure 6 Cell in telophase stained with Madder, Cell in metaphase stained with turmeric

Similarly observations were also made with that of *Albizzia lebbeck*. Cell wall in *Albizzia* stem section appear orange colour when stained with *Bixa orellana*. In the same way other dyes such as turmeric, madder, nyctanthes makes the colour yellow blood red and pale yellow. Using these dyes the chromosomal index, size measurements, shape and karyotypic analysis could also be carried out.

The nuclei in *Pedilanthes* leaf epidermal peelings were also stained following the same procedure. The plant cell wall and nuclei take up the light stain. Increasing the incubation period leads to overstaining of the nucleus.

CONCLUSION

In classroom teaching and research the conventional chromosome stains, viz., haematoxylin and acetocarmine are being used to demonstrate both mitotic and meiotic chromosomal stages. The present study has employed natural dye is as equivalent as that of haematoxylin and acetocarmine. Experiments carried out with root tip squash of *Allium cepa* for chromosomal staining at mitotic stages proved significant binding efficient with chromosomes at all the stages. The staining intensity could be enhanced because the dyes prepared with different solvents like methanol, ethanol and Acetone. In case of onion the chromosomes could bind more efficiently with turmeric, bixa and madder stains. The present study has explored the possibility of using plant dyes as biological stains in routine histochemical and cytological works.

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