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Extraction and utilization of plant dyes on plant tissues

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Abstract
Colour is what the eyes see when light is reflected off an object. Dyes are coloured substances which impart colour when applied to a substrate. This phenomenon is known as dyeing. The colouring chemicals are called stains. The types of the stains to be used depend upon the chemical nature of the material, pH value of the fixative and the reactivity of the stain with the material. Staining is the colouring of the fixed or sectioned material with suitable organic or inorganic dyes to brighten the contrast between different structures. Natural dyes are obtained from different plant parts like fruits, leaf, bark, roots and stem. The use of natural dyes as an alternative to chemically synthesized dyes has great importance. The present study investigates extracting plant dyes from the fruit of *Beta vulgaris* and the leaves of *Tectona grandis* and tested for its effect on staining with stem sections of dicot, monocot. The effectiveness of natural mordants in the process of dyeing was also investigated using fruit extract of *Citrus aurantifolia*. Very thin transverse sections were kept in the dye extract for 2-5 minutes and then passed through mordant solution. Excellent differential staining was noticed on sclerenchyma, collenchyma and xylem tissues of stem sections, but the cortex, medulla and pith region occupied by parenchyma were stained less effectively. More intensity in colour appearance on sclerenchyma tissues with mordant of *Citrus aurantifolia* was noticed. Naturally obtained dyes from plants are less expressive and have no side effects.

Keywords: natural dye, stain, mordant, *Beta vulgaris*, *Tectona grandis*, plant anatomy

Introduction

The term 'Dye' includes natural dyes, synthetic dyes and pigments. They may be defined as organic chemical compounds, which are used to colour materials permanently in such a manner that the imparted colour seems to be a part of the material and not merely applied to the surface as in painting (M. L. Gulrajani and Deepti Gupta, 1992) [5].

Plants are potential sources of natural dyes which yield different colours like red, yellow, blue, black, brown and a combination of these; primarily they are present in parts of the plant like root, bark, leaf, fruit, wood, seed, flower etc. (Siva R, 2007) [11]. Natural dyes are eco-friendly, non-toxic, non-carcinogenic and biodegradable (Samanta, 2009) [9], but many of the commercially used dyes cause carcinogenicity. Dyes are also used to colour paints, varnishes, inks, leather, paper, wood, furs, foods, cosmetics and medicines (Bendre kumar, 1998) [2]. Synthetic dyes, due to cheapness, brightness, easy usability, permanency and offering a wide range of colours, threatened to wipe out the natural dye industry (S. N Pandey, 2016) [7].

A mordant is a separate chemical that combines with the dye in such a way as to attach the coloring matter to the fiber by increasing affinity and/or strengthen interaction in some cases via a lasting chemical bond thereby making the color stand fast against light and washing (Bechtold and Mussak, 2009) [1].

Red dyes are obtained from Alkanna, Barwood, Brazilwood, Cudbear, Logwood, Safflower, Sappanwood and Sandalwood; yellow dyes are obtained from Annatto, Fustic, Gamboge, Henna, Osage Orange, Persian Berries, Quercitron, Saffron, Turmeric and Weld; blues from Cudbear, Indigo and Woad; greens from chlorophyll and lakao; brown from cutch. These more important natural colouring materials will be considered according to their morphological nature (O P Sharma, 1996) [10].

The red beetroot (*Beta vulgaris* L.) is a good source of red and yellow pigments known as betalains. Beetroot (*Beta vulgaris)* is a rich source of betalain pigments which can protect against age related diseases. Betalain pigments can be used as a natural additive for food, cosmetics and drugs in the form of beet juice. This vegetable is ranked as one of the ten most potent vegetables. It originates from the Mediterranean sea region and its ancestor is the beet *B. vulgaris* subsp. Beet root is a biennial plant, which belongs to the chenopodiaceae family. As the naturally obtained dyes from plants are less expressive and have no side effects.

Beetroot is the succulent, tuberous herb which represent the main source for the natural red dye. The main component of these natural red dye is betanin which are not found in plants containing anthocyanin pigments based upon their molecular structure. The pigment stability is influenced by enzymes, temperature, oxygen and pH. Betanin is the most abundant dyes and it originates from the schudel in 1918 (pucher Gw *et al*. 1938) [8]. The major commercially exploited betalain crop is red beetroot which contains 2 major pigments, namely betanin (a red beta cyanin) and vulgaxanthine I (Gengatharan *et al*.2015) [4].

Teak leaf extract (*Tectona grandis*) is promising to be used as a natural additive in order to avoid negative impacts of nitrile. *Tectona grandis* contains natural pigments called anthocyanin that produces maroon color. Teak leaf extract also contains antimicrobial compounds such as flavonoids, alkaloids, tannins, anthraquinone and naphthoquinone which inhibit the growth of bacteria. Altogether teak leaf extract is promising not only for staining but also for food preservations.

Materials and Methods

Fruit extract of Beetroot (Figure 1) and leaf extract of teak (Figure 2) was used in the present investigation. Fruits of *Beta vulgaris* are a good source of red and yellow pigments known as betalains. Betalains consist of betacyanins (red) and betaxanthins (yellow). Teak leaf extract contains natural pigment called anthocyanin, which produces maroon colour. Teak leaves also consists antimicrobial such as flavanoids, alkaloids, tannins and anthraquinone.

Dye extract was prepared by crushing and squeezing the fruit pulp of *Beta vulgaris* and leaves of *Tectona grandis* without using any solvents. Extract comes out due to squeezing was filtered with glass wool and collected. This extract was used as the source of natural stain. Natural mordant used in the present investigation are fruit extract of *Citrus aurantifolia*. Extract from the fruits of *Citrus aurantifolia* were prepared and used as mordant solutions. Stem sections of two plants were selected for tissue staining, *viz*. *Centella asiatica* (Figure 3) and Grass species (Figure 4) representing dicot and monocot respectively. Very thin free hand transverse stem sections of the above materials were taken and stained in the dye extract for 2-5 minutes and then kept in the mordant extract for 4-7 minutes. Observation of all the prepared slides were made and evaluated microscopically. The stained slides were studied under microscope and their staining intensity was identified and the photographs of selected preparations were taken using camera.

Fig 1: *Beeta vulgaris*

Fig 2: *Tectona grandis*

Fig 3: *Centella asiatica*

Fig 4: Grass sp.

Result and Discussions

In present investigation, extraction and utilization of natural dyes from *Beta vulgaris* and *Tectona grandis* on plant tissues of *Centella asiatica* and a Grass species were carried out.

Staining with and without mordant was recorded on *C. asiatica* [Figure 5(a)], specifically on collenchymatous hypodermal cells residing just beneath the epidermis. Also differential staining was noticed in the vascular xylem elements present in the bundles [Figure 5(b)]. Other tissue systems present in the stem consists of phloem tissue, medullary rays and inner most cortex composed of parenchyma were not stained characteristically.

In the stem section of grass, the dye extract of *Beta vulgaris* had some effect on sclerenchymatous bundle sheath that covers the vascular bundles and also on xylem components present inside the bundles [Figure 6 (b)]. Characteristic colouration was noticed upon treatment with mordants used

in the present investigation. The stelar region representing xylem cells were also stained dark red.

In the dicot and monocot stem sections, the dye extract of *Tectona grandis* had also some effects. For sections treated with natural mordant *Citrus aurantifolia*, the staining intensity was deepened in the outer region composed of collenchyma cells and cuticular layer system of the epidermal layer [Figure 7(b)]. Other tissue systems do not make any more drastic variation in staining which were obtained without mordant treatment. The colouration makes some marked variation in sclerenchymatous tissue system upon treatment with the mordant fruit extract of *C. aurantifolia* [Figure 8(b)]. In comparison with sections without mordant, sclerenchymatous cells have exhibited a pink colouration and also the collenchymatous layer's red colouration was deepened.

The mordant *Citrus aurantifolia* showed good mordant effect [Figure 8(b)], hence the outer tissue systems colour intensity has further increased. The efficiency of fruit extract as source of dye for staining plant sections was scarcely identified. The present finding on the competence of dye extract of *Beta vulgaris* and *Tectona grandis* recognized the fact that dye extract stain of *Beta vulgaris and Tectona grandis* could be successfully utilized for plant histological evaluation.

(Jan *et al*, 2011) [6] also had experimented and established the used dye extract from the leaves of *Lawsonia inermis* on sclerenchyma and xylem tissues of dicot and monocot stem. They pointed variation on staining property based on different solvent used in the extract procedure. In the present investigation no solvent was used for the extraction and thereby reduced the involvement of any chemicals in the procedure. Due to worldwide concern against synthetic hazardous chemicals, a great momentum has been achieved favouring the use of cost effective, eco-friendly and biodegradable materials, the use of natural dyes has got much attention and interest among scientists (Eom S, 2001) [3]. The recognition of exact dye component in *Beta vulgaris* and *Tectona grandis* which can stain mechanical tissues of plant system will open a way of research feature.

In the present investigation of extraction and utilization of plant dyes from plant parts used for anatomical staining on dicot and monocot plant, *Centella asiatica* and grass species respectively.

Fruits of *Beta vulgaris* and tender leaves of *Tectona grandis* contain ample quantity of dyes effective to stain tissues similar to safranin. Hence they can be used as alternative of safranin. Sclerenchyma and xylem cells have clear staining ability with *Beta vulgaris* extract. Dye extract of *Beta vulgaris* has characteristic staining on sclerenchyma and collenchyma cells. The *Beta vulgaris* has differential staining effect on dicot stem in presence of mordant. Sclerenchyma, xylem and cuticle cells was stained deep with leaf extract of *Tectona grandis*. The natural mordant have positive effect in the staining process and in certain situations original colour of the dye was little changed while using mordant.

By doing this study we can identified that natural dyes are more effective than synthetic dyes for plant anatomical staining.

1. Beetroot

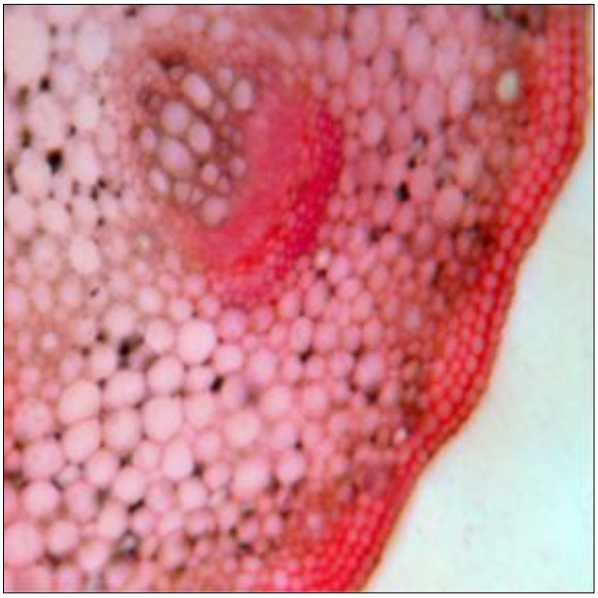

Fig 5 (a): Staining on dicot stem without mordant

Fig 5 (b): Staining on dicot stem with mordant *Lemon*

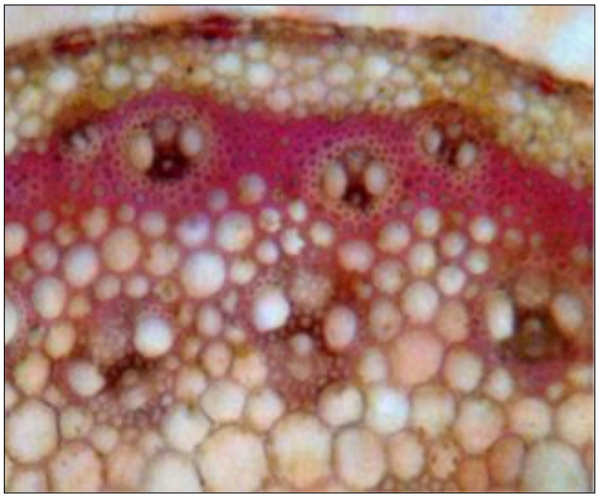

Fig 6 (a): Staining on monocot stem without mordant

Fig 6 (b): Staining on monocot stem with mordant *Lemon*

2. Tectona

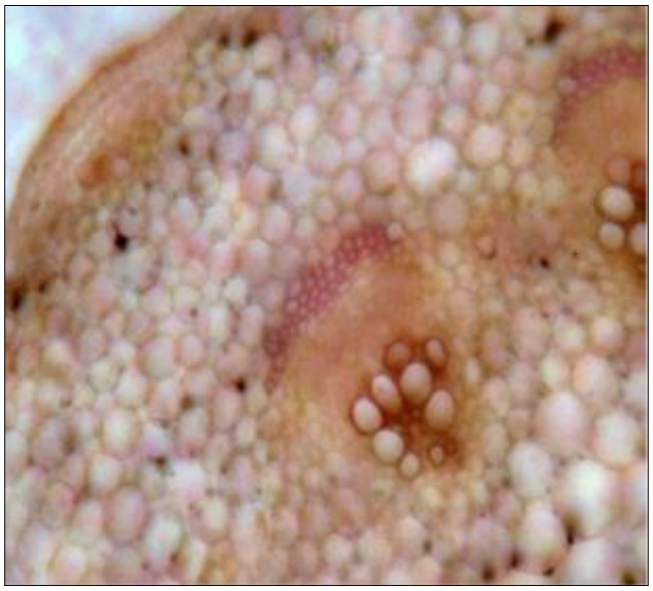

Fig 7 (a): Staining on dicot stem without mordant

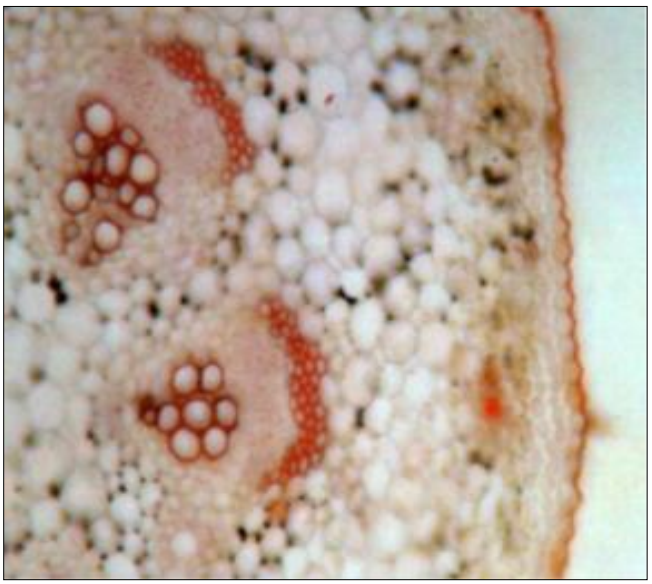

Fig 7 (b): Staining on dicot stem with mordant *Lemon*

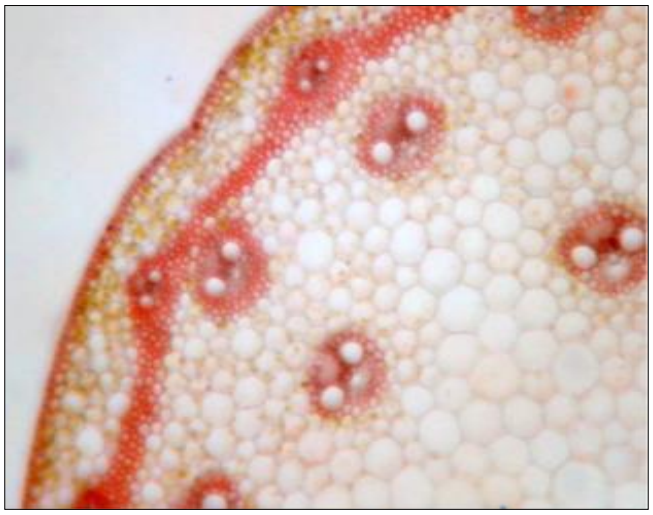

Fig 8 (a): Staining on monocot stem without mordant

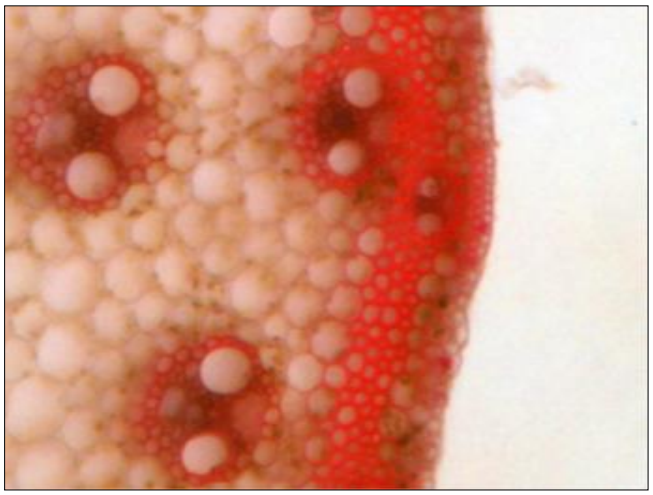

Fig 8 (b): Staining on monocot stem with mordant *Lemon*

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