Use of dye extract of *Melastoma malabathricum* Linn. for plant anatomical staining

M.S. Deepak, Philpose Omman

Post Graduate Research Department of Botany, Catholicate College, Pathanamthitta, Kerala, India,
Email: philiposeomman@yahoo.com

ABSTRACT

Natural dyes are coloring compounds present in different plant parts like fruits, leaf, bark, roots and stem. Commercially these biodegradable dyes were used for coloring textiles, drugs, cosmetics, leather and paintings. 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 *Melastoma malabathricum* and tested for its effect on staining with stem sections of dicot, monocot and pteridophyte. The effectiveness of natural mordants in the process of dyeing was also investigated using fruit extract of three plants *Garcinia cambojia*, *Citrus aurantifolia* and *Averrhoa carambola*. 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. Slight variations in colour intensity on tissues treated with mordants *Garcinia cambojia* and *Averrhoa carambola* have also been observed. Application of plant dyes as source of dyes for staining plant micro technique was however, very limited and any serious attempts on characterization of plant dyes would help in commercialization and promotion of some of the promising indigenous plants as alternative crops for farmers, thereby enhancing their economy.

Keywords: Natural dyes, *Melastoma malabathricum*, mordants, dicot, plant anatomy

INTRODUCTION

Plants are the potential source 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. [1-3]. Natural dyes are eco-friendly, non-toxic, non-carcinogenic and biodegradable nature [4,5] but many of the commercially used dyes causes carcinogenicity [6]. Natural dyes have been used extensively in textile, food and other industries [7-10]. Azo dyes are mostly employed in textile dyeing that may be harmful to human health and environment [8] and hence, a great awareness has been emerged among scientific community on the use of alternative greener substances [11]. More recently natural dyes were used efficiently to trap solar energy, where natural dye particles used to develop dye-sensitized solar cells [12-14].

Plant dyes were also extracted and used in micro technique observations. The natural stain Haematoxylin, used in histopathology is obtained from South African tree *Haematoxylon campachianum* L. [15] and another red dye obtained from *Pterocarpus osun* was also used [16]. Abubakar et al. [17] have used organic dye from *Hibiscus sabdariffa* Linn. for staining fungal mycelia. Dye extracts of *Berberis pachyacantha* Kochne was found to be effective on plant

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histological differential staining on dicot stem [18]. Studies done by Suabakyong [19] have suggested the use of dye from Black Plum fruit (*Syzygium cumini*) as an alternative dye for histological staining of animal tissues. Staining effect of dye extracted from dry leaves of *Lawsonia inermis* Linn. on angiospermic stem tissue was proved by Jan et al. [18].

Melastoma malabathricum Linn. is a shrub that belongs to the family Melastomaceae. The flowers have a cup shaped green calyx with five narrow reddish sepals and five purplish petals. The fruits are berry-like and they break open irregularly, bearing numerous non endospermous seeds [20] surrounded with purplish pulp. The plant has been used to treat several diseases such as dysentery, scar prevention [21-24] and as an anti-infection agent [23]. Also some people used the crude extract of roots and shoots as aspirin to relief toothaches, treat leucorrhea [24] and young roots are eaten to reduce high blood pressure and diabetes, while the roots are given to postpartum women to aid healing and womb strengthening [25,26]. As the naturally obtained dyes from plants are less expensive and have no side effect, the present investigation was carried out to find out usefulness of dye extract from *Melastoma malabathricum* fruits on plant micro technique to differentially stain different tissues.

MATERIALS AND METHODS

Fruits extract of *Melastoma malabathricum* Linn. (Figure 1a) was used in the present investigation. Fruits of *M. malabathricum* is a rich source of flavonoid, such as anthocynanins [27,28]. Anthocynanins are colouring pigments in flowering plants and have antioxidant, phytoalexin and antibacterial activities [29]. Dye extract was prepared by crushing and squeezing the fruit pulp of *M. malabathricum* 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 mordants used in the present investigation are fruit extract of three plants *Garcinia cambojia*, *Citrus aurantifolia* and *Averrhoa carambola*. Extracts from the fruits of the above plants were prepared as described above and used as mordant solutions.

Stem sections of three plants were selected for tissue staining, viz. *Centella asiatica*, Grass species and *Selaginella* species representing dicots, monocots and pteridophytes 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 Olympus microscope and their staining intensity was identified and the photographs of selected preparations were taken using Canon micro photographic unit attached with the microscope.

RESULTS AND DISCUSSION

Staining without mordant was mainly recorded on dicot stem (*C. asiatica*), specifically on collenchymatous hypodermal cells residing just beneath the epidermis. Also differential staining was noticed in the bundle cap composed of sclerenchymatous tissues and the vascular xylem elements present in the bundles (Figure 1b). Other tissue systems present in the stem consists of phloem tissue, medullary rays and inner most cortex composed of parenchyma were not stained characteristically.

For sections treated with natural mordant *Garcinia cambojia*, the staining intensity was deepened in the outer region composed of collenchyma cells and cuticular layer system of the epidermal layer (Figure 1c). 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 tissues system upon treatment with the mordant fruit extract of *C. aurantifolia* (Figure 1d). In comparison with sections without mordant, sclerenchymatous cells have exhibited a

violet colouration and also the collenchymatous layer's red colouration was deepened. But the treatment with fruit extract of *A. carambola* as mordant, the collenchymatous hypodermal system turned to violet colour and the bundle cap sclerenchymatous system's colour intensity increased many fold (Figure 1e).

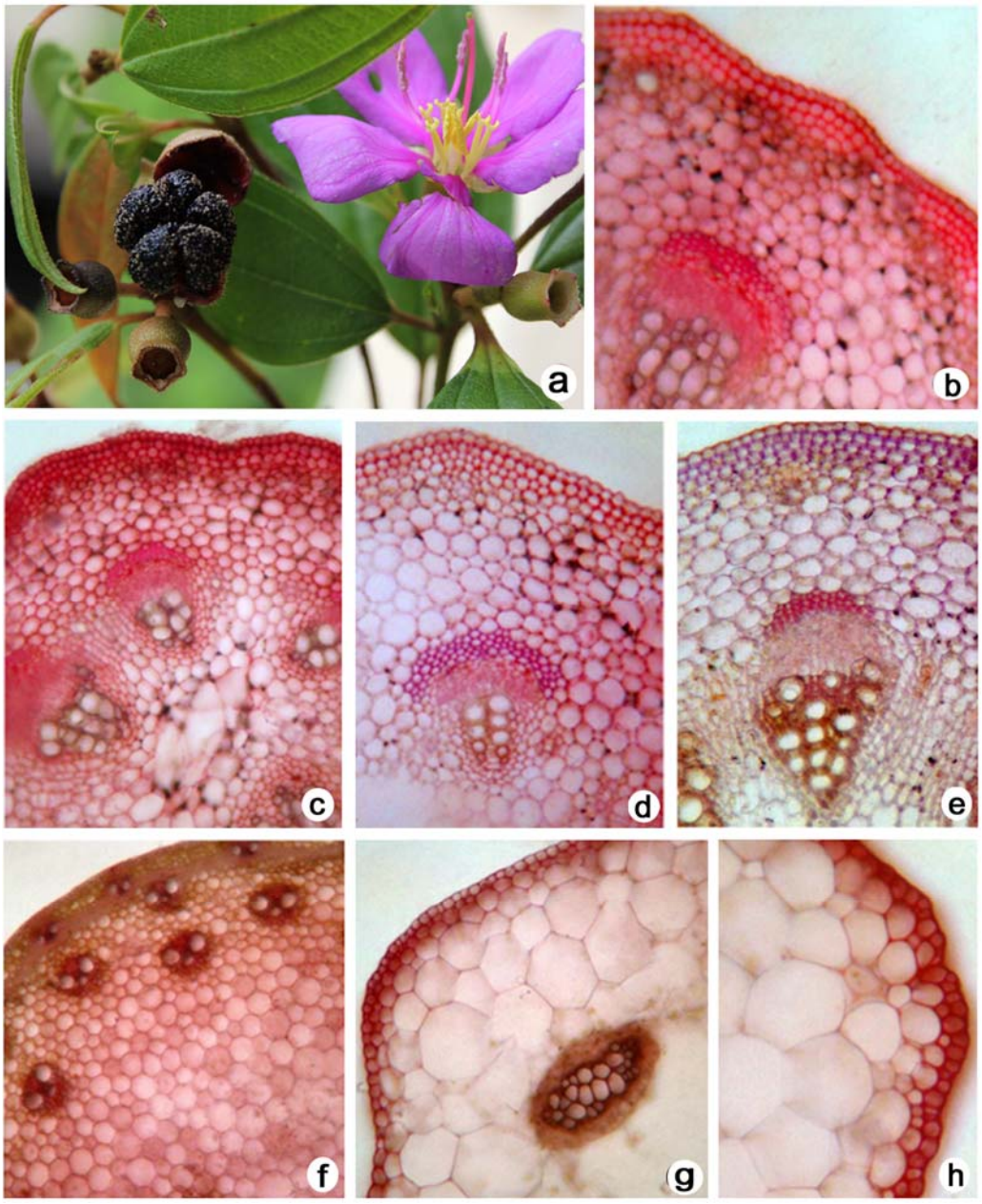

Figure 1. Stainability of dye extracts on plant sections. a-Fruit and flower of *M. malabathricum* Linn.; b-e: staining on dicot (*C. asiatica*) stem; b-without mordant; c- with mordant *G. cambojia*; d- with mordant *C. aurantifolia*; e- with mordant *A. carambola*; f- staining on monocot stem without mordant; g- staining on Selaginella stem without mordant; h- staining on Selaginella stem with mordant *A. carambola*.

In the stem section of grass, the dye extract of *M. malabathricum* had some effect on sclerenchymatous bundle sheath that covers the vascular bundles and also on xylem components present inside the bundles (Figure 1f). No characteristic colouration was noticed upon treatment with mordants used in the present investigation.

The sections of *Selaginella* had distinguishable red staining on outer epidermal and sub epidermal collenchymatous cells (Figure 1g). The stelar region representing xylem cells were also stained dark red. The mordant *A. carambola* showed good mordant effect (Figure 1h), hence the outer tissue systems colour intensity has further increased.

M. malabathricum is a potential source of many valuable chemical compounds having diversified ethno medicinal uses [20]. The fruits of *M. malabathricum* are rich source of anthocyanins [27,28] and the anti microbial effect of the fruit extract was variously tested in wide range of microorganisms [30]. The efficiency of fruits extract as source of dye for staining plant sections was scarcely identified. The present finding on the competence of dye extract of *M. malabathricum* recognized the fact that dye extract stain of *M. malabathricum* could be successfully utilized for plant histological evaluation. Jan et al. [18] 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 micro technique 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 [31,32]. The recognition of exact dye component in *M. malabathricum* which can stain mechanical tissues of plant system will open a way of research feature.

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