

STAINING EFFECT OF DYE EXTRACTED FROM LEAVES OF *Hibiscus rosa-sinensis* L., & *Tectona grandis* L., ON ANGIOSPERMIC STEM TISSUES

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Abstract: Natural dyes are colored compounds present in many plants, including fruits, stems, leaves, bark, and roots. Commercially, these biodegradable dyes were used to color fabrics, pharmaceuticals, cosmetics, leather, and paint. Natural dyes are incredibly advantageous as alternatives to synthetic dyes. The current study examines the extraction of plant dyes from fresh and dry leaves of *Tectona grandis* and *Hibiscus rosa-sinensis* with ethanol and water, as well as their efficacy as a stain for angiospermic stem tissues. A 10% w/v crude extract of *Tectona grandis* and *Hibiscus rosa-sinensis* fresh and dried leaves in ethanol and water was used to stain the stem cross section of angiospermic plants such *Boerhavia chinensis* L. (Dicot) and *Stenotaphrum secundatum* Kuntze (Monocot). The vascular bundle of the angiospermic stem cross section of *Boerhavia chinensis* was heavily stained with dye derived from *Tectona grandis* and *Hibiscus rosa-sinensis* fresh and dried leaves in ethanol, whereas the ground parenchyma was very mildly stained. The dye extracted from *Tectona grandis* and *Hibiscus rosa-sinensis* leaves in 10% w/v ethanol stained the sclerenchyma of *Stenotaphrum secundatum* Kuntze stem cross section more effectively than the parenchyma of *Boerhavia chinensis*. Within each arterial bundle, xylem cells were heavily stained, although the cortex and medulla were not. This opens up a new avenue for research and additional investigation into improved methods of extracting the dye in concentrated form in a wider range of solvents, as well as better means of generating stains.

Keywords – Natural plants dyes, Mordants, Stem Tissues, plant anatomy.

INTRODUCTION

India, with its abundant plant biodiversity, is ranked 11th in the world for biodiversity. With almost 490,000 species, the plant kingdom is unquestionably a treasure trove of varied natural goods (Neha Grover et al., 2011). Dye is one such natural substance. In art and craft, pigment derived from leaves, fruits, seeds, wood, and roots was used as paint and as a textile dye. Compared to other colorants, natural dyes are more lasting, hygienic, user-friendly, and environmentally beneficial. Because natural dyes can be used to achieve a variety of colors, they may eventually be replaced by synthetic dyes. Because they are harmless, natural dyes and colorants made from flora are thought to be safe. More than 500 plant species that produce dyes are a gift from nature. These plants' roots, leaves, bark, trunks, or fruits are the sources of their coloring ingredients. Plants provide all of the rainbow's colors. Natural dyes are often more environmentally friendly and have more biodegradability. Nowadays, almost all dyes are made from synthetic substances. Compared to synthetic dyes, natural dyes yield incredibly unusual, calming, and delicate hues (Lakshmana Naik R et al., 2019).

Dyes are colored and impart color when applied to a substrate. This phenomenon is known as dyeing. The coloring chemicals are called stains. The types of stains to be used depend upon the chemical nature of the material,

the pH value of the fixative, and the reactivity of the stain with the material. Staining is the coloring of the fixed or sectioned material with suitable organic or inorganic dyes to brighten the contrast between different structures.

The father of microbiology, Antonie van Leeuwenhoek, is credited with using saffron, a natural color made from Saffron crocus, for the first time in histology. The process known as staining is typically used to add color to plant, animal, microbial, and spore tissues in order to make them visually distinguishable. Despite being visible under a light microscope, microbes require fixing and/or staining in order to enhance their visibility, highlight their morphological characteristics, and occasionally preserve them for further study. In Africa, particularly in Nigeria, there are numerous natural dye plants that are capable of being cultivated, just as they have been cultivated in India and the United Kingdom. These widely available dye plants have been utilized as histology stains for several tissue components in recent investigations, with promising results. The root, root bark, leaves, flowers, stem, stem bark, fruit skins, and nut shells all contain these dyes. It was discovered that a few locally available natural herbal dyes were effective at staining plant materials without causing any harm to the environment. Additionally, they provide inexpensive stains for use in plant histology.

Hibiscus rosa-sinensis L., belonging to family Malvaceae. It is renowned for its vibrant, eye-catching blossom *Hibiscus* are rich in anthocyanin, ascorbic acid, and hibiscus acid. The bright color of the *Hibiscus* is by virtue of the presence of anthocyanin. Flowers extracts of plant have been used extensively in the textile industry because of their vibrant and long a lasting color. The calyx also been used as food in food products like jams, juices and in pharmaceuticals syrups. Several studies have reported that secondary metabolites derived from flowers extract, leaf extracts and root extracts contain, among others: tannins, saponins, flavonoids, terpenoids and polyphenols, which can provide benefits including: as an antibiotic, antifungal, anti-inflammatory, antioxidant, anticoagulant and antidiabetic.

Tectona grandis leaf extract 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. Additionally, flavonoids, alkaloids, tannins, anthraquinone, and naphthoquinone—compounds that prevent the growth of bacteria are present in teak leaf extract. Altogether teak leaf extract is promising not only for staining but also for food preservations. Teak plant roots, bark, and new leaves can be used to make dyes that give reddish-brown or yellow-brown hues that are used to make paper goods, matting, and clothing. The textile industry has used teak tree dyes to print vibrant designs on cotton and wool garments. In the textile business, teak leaves have been used to add color to cotton and woolen materials. Also, recent studies have demonstrated that fungal structures such as mycelium stained reddish-brown with *Tectona grandis* leaves extract and stood out well against a suitable background stain.

Natural dyes are used in the textile industry for a variety of purposes, such as coloring yarns that are subsequently woven into cloth, carpets, or other usable forms; coloring previously woven fabrics; block printing, which involves printing textile materials using printing blocks; and Kalamkari, which involves using a pen, or "Kalam," to create beautiful designs on fabric (Gopi, 2004). Even while it is doubtful that all dyestuffs will be made entirely of plants, it is an intriguing and thrilling possibility that someday a portion of common colors may come from natural sources. The plant possesses many medicinal properties. According to Kroes et al. (1990), flowers are the most potent fermentation agent employed in Ayurvedic therapy. Many investigations revealed that the use of combination of mordants in varying ratios gives different shades and different colour fastness results (Kumaresan, Palanisamy & Kumar, 2011). The flowers, which contain much of tannin, are Flame coloured and yield red/pink/brown/flame colour shades of dye (depending upon the fabric used) in large amounts, therefore, utilized throughout India for commercially dyeing textiles and silk. Light fastness of many natural dyes, particularly which are extracted from flower parts are found to be poor to medium (Samanta & Agarwal, 2009). Prior until recently,

India was a significant exporter of herbal dyes due to the developed world's ban on the manufacture of several synthetic dyes and intermediates countries due to pollution problem (Gaur, 2008).

In order to replace the costly, hazardous, and hard-to-find synthetic exotic stains used in plant histology, it would be beneficial to investigate the effectiveness of locally produced, environmentally friendly, and non-toxic herbal stains for staining plant tissues. Additionally, the current study will be beneficial and offer comprehensive details regarding the main pigments and their significance in naturally existing plants that produce dyes. It would be beneficial to the advancement of a fresh approach to the investigation and preparation of stains made from natural dyes.

Objectives :-

- To collect the selected medicinal plants.
- To select the plants *Hibiscus rosa-sinensis* L. and *Tectona grandis* L.
- To the extracts of the plant of fresh and dry leaves of *Hibiscus rosa-sinensis* L. and *Tectona grandis* L. leaf using different solvents.
- To prepare the dye extraction methods of dry and fresh leaf extracts.
- To the staining properties of stem tissue from *Boerhavia chinensis* L. and *Stenotaphrum secundatum* Kuntze., angiosperms.

MATERIALS AND METHOD**a. Plant material:**

Fresh *Hibiscus rosa-sinensis* L and *Tectona grandis* L. plants were gathered from the fields in the Tamil Nadu district of vagarayampalayam, Coimbatore. A specimen of the discovered plant was preserved for future use.

b. Dye extraction:

The plant of *Hibiscus rosa-sinensis* L. and *Tectona grandis* (L.) was dried in shade for five days at room temperature. The fresh and dry leaves are grinded and powdered mechanically for effective extraction. Dye was extracted with solution using solvents, that is water and ethanol.

c. Tissue staining:

The staining properties of stem tissue from *Boerhavia chinensis* L. and *Stenotaphrum secundatum* Kuntze., angiosperms, were investigated. The stem sections were microtome-cut using water. *Stenotaphrum secundatum* Kuntze., and *Boerhavia chinensis* stem tissues were colored with *Hibiscus rosa-sinensis* and *Tectona grandis* (L.) leaf extracts in water and ethanol.

d. Microscopy:

Stained slides of *Stenotaphrum secundatum* Kuntze., and *Boerhavia chinensis* were studied under simple light microscope (Olympus BX51) and their staining intensity were identified (Lux et al., 2005)

***Hibiscus rosa-sinensis* L.**



Scientific Classification:

Kingdom : Plantae
 Division : Tracheophyta
 Class : Magnoliopsida
 Order : Malvales
 Family : Malvaceae
 Genus : Hibiscus
 Species : rosa-sinensis
 Botanical name : *Hibiscus rosa-sinensis* L.

***Tectona grandis* L.**



Scientific Classification:

Kingdom : Plantae
 Division : Magnoliophyta
 Class : Magnoliopsida
 Order : Lamiales
 Family : Verbenaceae
 Genus : Tectona
 Species : grandis
 Botanical name : *Tectona grandis* L.

RESULT AND DISCUSSION

Fresh and dry leaves of *Hibiscus rosa-sinensis* L. Dye Extracts

A solution of dye prepared from the crude extract of *Hibiscus rosa-sinensis* L. leaves in water and ethanol was found to stain the vascular tissue of the stem in *Boerhavia chinensis* (Dicot) and *Stenotaphrum secundatum* (Monocot). Ethanol extract showed significant effects as compared to water dye extracts of fresh and dry leaves. The color of the dye extracted from *Hibiscus rosa-sinensis* leaves with water was dark green (fresh) and light brownish (dry), while that extracted with ethanol was dark greenish (fresh) and dark brownish (dry). The dye extracted in water imparted a grayish-green colour to the parenchyma and vascular bundle stem tissue of the cross section.

A 10% (w/v) fresh and dry extract of dye from *Hibiscus* in water was found to be effective in staining (*Boerhavia chinensis*) dicotyledonous stem tissues. In each vascular bundle, xylem cells were stained very effectively with dry leaf dye extracts. But the 10% (w/v) extract of dye in water was found to be more effective in staining sclerenchyma, but not as profusely as the results of *Hibiscus rosa-sinensis* in ethanol, and less effective in staining parenchyma of dicotyledonous in fresh dye extract. The same extract of dye also showed a slight effect on

monocotyledonous stem tissues (Tables 1 and 2). The fresh dye extract of *Hibiscus* leaves in water has less effect on the stem vascular tissues of angiosperms. The solubility of the dye in water and ethanol was quite evident.

A 10% fresh and dry dye extract of *Hibiscus rosa-sinensis* leaves with ethanol imparted greenish color to the sclerenchyma, parenchyma, and vascular bundle of dry leaf dye extract of monocotyledons stem cross section (*Stenotaphrum secundatum*) and produced a very interesting stem vascular tissue and would be a useful addition in new research studies. Although the staining effects were more prominent on the former tissue (sclerenchyma), A 10% (w/v) extract of *Hibiscus* dye in ethanol was found to be more effective in staining sclerenchyma but less effective in the parenchyma of dicotyledonous and monocotyledons in fresh dye ethanol. A dye extract of *Hibiscus* in fresh leaves of ethanol was found to be less effective at staining dicot and monocot stem tissue.

Table: 1 Staining effect of *Hibiscus rosa-sinensis* L., leaves dye on stem tissues of

***Boerhavia chinensis* L.,**

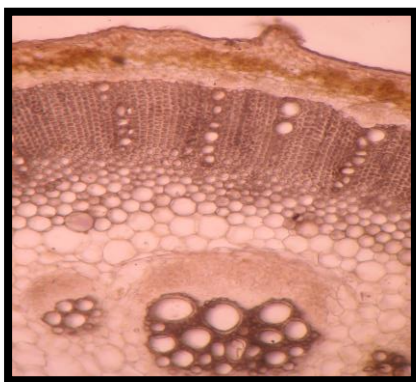
<i>Hibiscus rosa-sinensis</i>	Leaves extracts	Tissue Stained	Intensity of staining
Fresh Leaves	Water		
	1%	-	-
	5%	Parenchyma	+
	10%	Sclerenchyma	+
	Ethanol		
	1%	-	-
	5%	Parenchyma,	++
Dry Leaves	10%	Sclerenchyma	++
		Vascular bundle	
	Water		
	1%	-	-
	5%	Parenchyma, Sclerenchyma	++
	10%	Vascular bundle	++
	Ethanol		
	1%	-	-
	5%	Sclerenchyma	+++
	10%	Xylem, Phloem Tissue	+++

Table: 2 Staining effect of *Hibiscus rosa-sinensis* L., leaves dye on stem tissues of *Stenotaphrum secundatum* Kuntze

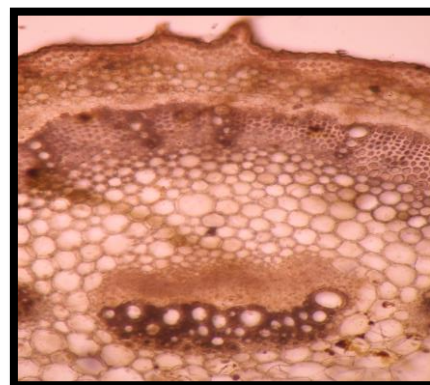
<i>Hibiscus rosa-sinensis</i>	Leaves extracts	Tissue Stained	Intensity of staining
Fresh Leaves	Water		
	1%	-	-
	5%	Parenchyma	+
	10%	Vascular bundle	+
	Ethanol		

	1%	-	-
	5%	Parenchyma,	+
	10%	Sclerenchyma	++
		Vascular bundle	
Dry Leaves	Water		
	1%	-	-
	5%	Parenchyma	+
	10%	Sclerenchyma	++
	Ethanol		
	1%	-	-
	5%	Sclerenchyma	+++
	10%	Vascular Tissues	+++

**STAINING EFFECT OF DYE FROM FRESH LEAVES OF
HIBISCUS ROSA-SINENSIS L., STEM CROSS SECTION OF DICOT (*Boerhavia chinensis* L.)**

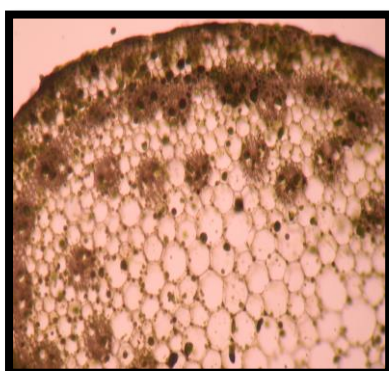


WATER



ETHANOL

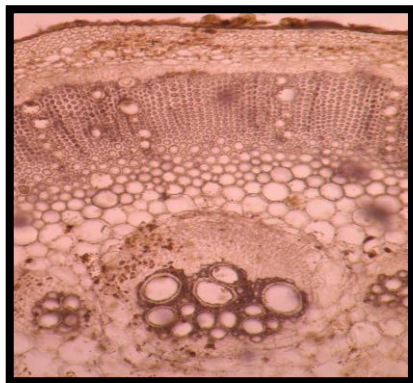
**STAINING EFFECT OF DYE FROM FRESH LEAVES OF
HIBISCUS ROSA-SINENSIS L., STEM CROSS SECTION OF MONOCOT (*Stenotaphrum secundatum*)**



STAINING EFFECT OF DYE FROM DRY LEAVES OF

HIBISCUS ROSA-SINENSIS L., STEM CROSS SECTION OF DICOT (*Boerhavia chinensis* L.)

WATER



ETHANOL



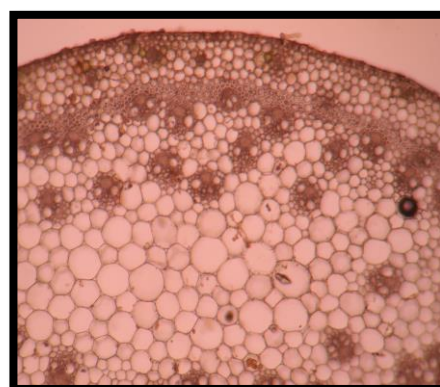
STAINING EFFECT OF DYE FROM DRY LEAVES OF

HIBISCUS ROSA-SINENSIS L., STEM CROSS SECTION OF MONOCOT (*Stenotaphrum secundatum*)

WATER



ETHANOL



Fresh and dry leaves of *Tectona grandis* L. Dye Extracts

A solution of dye prepared from the crude extract of *Tectona grandis* L. leaves in water and ethanol was found to stain the vascular tissue of the stem in *Boerhavia chinensis* (Dicot) and *Stenotaphrum secundatum* (Monocot). Ethanol extract showed significant effects as compared to water dye extracts of fresh and dry leaves. The color of the dye extracted from *Tectona grandis* leaves with water was light brownish (fresh) and light reddish (dry), while that extracted with ethanol was dark brownish (fresh) and dark blackish (dry). The dye extracted in water imparted a grayish-green colour to the parenchyma and vascular bundle stem tissue of the cross section.

A 10% (w/v) fresh and dry extract of dye from *Tectona grandis* in water was found to be effective in staining (*Boerhavia chinensis*) dicotyledonous stem tissues. In each vascular bundle, xylem cells were stained very effectively with dry leaf dye extracts. But the 10% (w/v) extract of dye in water was found to be more effective in staining sclerenchyma, but not as profusely as the results of *Tectona grandis* in ethanol, and less effective in staining parenchyma of dicotyledonous in fresh leaf dye extract. The same extract of dye also showed a slight effect on

monocotyledonous stem tissues (Tables 3 and 4). The fresh dye extract of *Tectona grandis* leaves in water has less effect on the stem vascular tissues of angiosperms. The solubility of the dye in water and ethanol was quite evident.

A 10% fresh and dry dye extract of *Tectona grandis* leaves with ethanol imparted greenish color to the sclerenchyma, parenchyma, and vascular bundle of dry leaf dye extract of monocotyledons stem cross section (*Stenotaphrum secundatum*) and produced a very interesting stem vascular tissue. Although the staining effects were more prominent on the former tissue (sclerenchyma), A 10% (w/v) extract of *Tectona grandis* dye in ethanol was found to be more effective in staining sclerenchyma but less effective in the parenchyma of dicotyledonous and monocotyledons in fresh dye ethanol. A dye extract of *Tectona grandis* in fresh leaves of ethanol was found to be less effective at staining dicot and monocot stem tissue.

Table: 3 Staining effect of *Tectona grandis* L. Leaves dye on stem tissues of

Boerhavia Chinensis L.,

<i>Tectona grandis</i>	Leaves extracts	Tissue Stained	Intensity of staining
Fresh Leaves	Water		
	1%	-	-
	5%	-	-
	10%	Parenchyma	+
	Ethanol		
	1%	-	-
	5%	Parenchyma,	+
	10%	Sclerenchyma	++
		Vascular bundle	
Dry Leaves	Water		
	1%	-	-
	5%	Parenchyma	+
	10%	Sclerenchyma, Xylem & phloem	++
	Ethanol		
	1%	-	-
	5%	Sclerenchyma	++
	10%	Vascular Tissues	+++

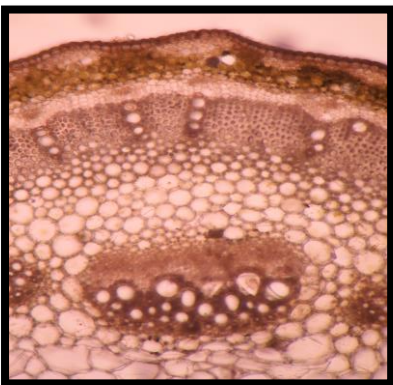
Table: 4 Staining effect of *Tectona grandis* L. leaves dye on stem tissues of

Stenotaphrum secundatum Kuntze

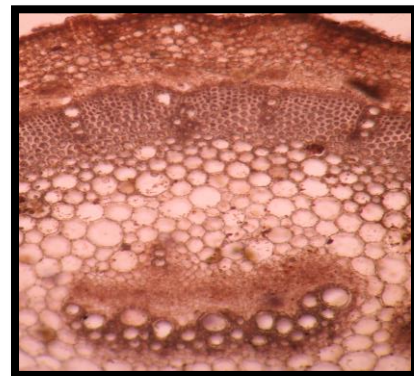
<i>Tectona grandis</i>	Leaves extracts	Tissue Stained	Intensity of staining
	Water		
	1%	-	-
	5%	-	-

Fresh Leaves	10%	Parenchyma	++
	Ethanol		
	1%	-	-
	5%	Parenchyma,	++
Dry Leaves	10%	Sclerenchyma Vascular bundle	++
	Water		
	1%	-	-
	5%	Parenchyma	++
Dry Leaves	10%	Sclerenchyma, Vascular tissue	++
	Ethanol		
	1%	-	-
	5%	Parenchyma, Sclerenchyma	+++
Dry Leaves	10%	Vascular Tissues	++

**STAINING EFFECT OF DYE FROM FRESH LEAVES OF
TECTONA GRANDIS L., STEM CROSS SECTION OF DICOT (*Boerhavia chinensis* L.)**

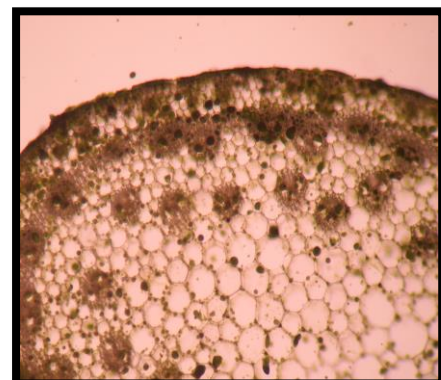


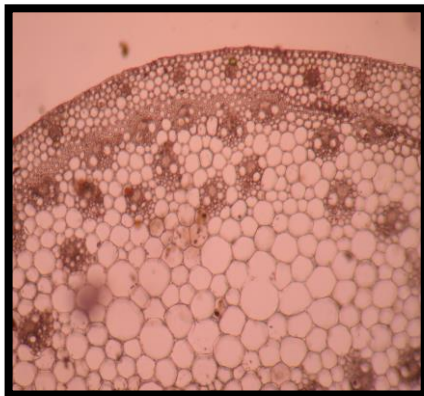
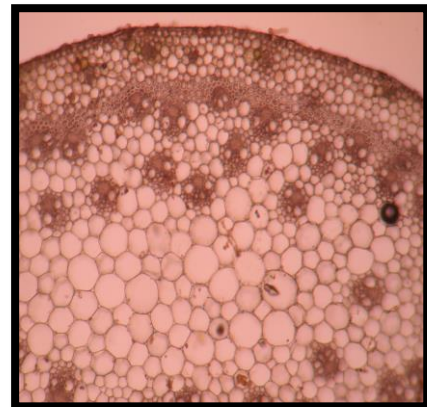
WATER



ETHANOL

**STAINING EFFECT OF DYE FROM FRESH LEAVES OF
TECTONA GRANDIS L., STEM CROSS SECTION OF MONOCOT (*Stenotaphrum secundatum*)**



STAINING EFFECT OF DYE FROM DRY LEAVES OF***TECTONA GRANDIS* L., STEM CROSS SECTION OF DICOT (*Boerhavia chinensis* L.)****WATER****ETHANOL****STAINING EFFECT OF DYE FROM DRY LEAVES OF*****TECTONA GRANDIS* L., STEM CROSS SECTION OF MONOCOT (*Stenotaphrum secundatum*)****WATER****ETHANOL****SUMMARY AND CONCLUSIONS**

The natural dyes have long been used for various purposes, including tissue staining. However, their application has decreased since the synthetic dyes were developed. Haematoxylin is an example of a natural dye that is widely used in histology, histopathology, and histochemistry. At present, many commercial and synthetic and some natural dyes used for tissue staining are available on the market. However, the hazardous effects of synthetic dyes on humans and the environment caused scientists to be concerned about using natural dyes instead of synthetic dyes. Therefore, alternative natural dyes have been studied for their potential use in histological staining. There are many studies that have investigated plant dye staining in diverse tissues. Studied the staining effect of dry extract from fresh and dry leaves of *Hibiscus rosa-sinensis* and *Tectona grandis* on histological sections of angiospermic stem. Although using natural dyes has many advantages, there are still some limitations that should be considered. It is difficult to standardize the dye and its application because the dye collected from similar plants or natural sources

varies due to climate, soil, maturity period, cultivation methods, etc. Moreover, most natural dyes require mordants to fix them to the stained tissues. The widely used metallic mordants that are applied in the dye solutions may cause health and disposal problems. The known chemical structures and properties of some plant dye extracts are also important and useful for determining the optimal extraction and tissue staining procedures. More detailed studies are needed to evaluate the potential and availability of natural dye-yielding resources in many other plants. In addition, biotechnology and various fields of study are required to increase the quantity and improve the quality of plant natural dyes.

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