



# STAINING EFFECT OF DYE EXTRACTED FROM LEAVES OF *Lawsonia inermis* L., & *Eclipta prostrata* L., ON ANGIOSPERMIC STEM TISSUES

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**Abstract:** Natural dyes are colored substances found in various plants, including fruits, stems, leaves, bark, and roots. Commercially, these biodegradable dyes were used to color textiles, pharmaceuticals, cosmetics, leather, and paint. Natural dyes are extremely beneficial as alternatives to artificially manufactured dyes. The current work analyzes the extraction of plant dyes from fresh and dry leaves of *Lawsonia inermis* and *Eclipta prostrata* using ethanol and water, as well as their efficiency as a stain for angiospermic stem tissues. A 10% w/v crude extract of *Lawsonia inermis* and *Eclipta prostrata* fresh and dried leaves in ethanol and water was used to stain the stem cross section of angiospermic plants such as *Boerhavia chinensis* L. (Dicot) and *Stenotaphrum secundatum* Kuntze (Monocot). Dye extracted from *Lawsonia inermis* and *Eclipta prostrata* fresh and dry leaves in ethanol profusely stained the vascular bundle of the angiospermic stem cross section of *Boerhavia chinensis*, but ground parenchyma was only slightly stained. Dye extracted from *Lawsonia inermis* and *Eclipta prostrata* leaves in 10% w/v ethanol through stained the sclerenchyma of *Stenotaphrum secundatum* Kuntze stem cross section more effectively than parenchyma in *Boerhavia chinensis*. Xylem cells were highly stained within each vascular bundle, but the cortex and medulla were less so. This opens up a new channel of research and further inquiries to uncover better methods of extracting the dye in concentrated form in more diverse solvents and better methods of creating stains.

**keywords –** *Natural plants dyes, Mordants, Stem Tissues, plant anatomy.*

## INTRODUCTION

Nature has lots of wonderful resources for humans. Color is one of the elements of nature that make our lives more aesthetic and fascinating. The majority of stains that are used in pathology are made chemically. The advantages of these chemically synthesized stains are their good fastness properties, easy availability at an economical price, and broad variety of color, but they also cause skin allergies and other harmful effects on humans, animals, plants, and soil. Therefore, it is advisable to use more eco-friendly, biodegradable, and economical alternatives, which can be effectively obtained from plants and other natural sources.

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.

These days, a wide range of dyes are employed for histological staining depending on the goal of the investigation. The study of cells and tissues under a light microscope is known as histology. The ability to visualize or identify histological structures is frequently enhanced through the use of histological stains. They give contrast to the tissues as well as highlight particular features of interest. There are two types of dyes that are classified by their origins: synthetic and natural dyes. The natural dyes are obtained from natural sources such as plants, insects, animals, clays and minerals (Carleton *et al.*, 1976). Some of the plants are rich in important bioactive phytochemical constituents such as alkaloids, essential oils, flavonoids,

tannins, terpenoids, saponins, and phenolic compounds (Solecki & Shanidar, 1975). Plants are the potential source of natural dyes that yield different colors like red, yellow, blue, black, brown, and a combination of these. Primarily, they are present in parts of the plant like the root, bark, leaf, fruit, wood, seed, flowers, etc. Natural dyes are eco-friendly, non-toxic, non-carcinogenic, and biodegradable. Natural dyes have been extensively used in textile, food, and other industries (M.S. Deepak & Philpote Omman, 2013). Natural dyes are said to mostly come from plants.

*Lawsonia inermis* Linn, commonly known as Henna, is a shrub plant belonging to the Lythraceae family. It is seen as a perennial shrub in sub-tropical. One of its large-scale uses includes those for cosmetic purposes as pigments to color hair and nails, imparting a reddish yellow tint. Other uses are, namely, in the textile industries for dyeing wool and nylon, traditionally practiced medicinal plants for the treatment of various ailments. The presence of naphthaquinone (hannotanic acid), mannite, tannic acid, gallic acid, crysophanic acid, etc. is revealed by phytochemical screening of henna leaves. The staining property of henna is mainly attributed to anaphthoquinone compound in the dried leaves. The compounds in these dried leaves impart a brown color and chemical properties that are analogous to tannic acid, hence its name, hennotannic acid.

A prostrate, frequently branching annual or perennial herb, *Eclipta prostrata* (L.) is a member of the Asteraceae family. It is used medicinally for a number of conditions, including lung infections, coughs, jaundice, gastrointestinal issues, and urinary infections. There have been several reports of this plant's health advantages and antivenomous qualities. Many Indian farmers view *eclipta* as a weedy plant. Many people utilize the entire plant as a medicinal source for skin allergies, fever, wounds, and hair coloring. Alkaloids, saponins, phenolic compounds, and tannins were discovered when the dried *Eclipta* source's phytochemical composition was examined. According to the research on the *Eclipta* Species' ability to dye hair, the presence of a black precipitate containing ferric chloride as a mordant is what gives the dye its potential.

Despite the biotechnological advance in medical science today, biological stains are vital in laboratory diagnosis, and the Gram's staining method remains an important simple diagnostic tool in diagnostic and research laboratories. The commonly used counterstain in Gram's staining reaction is safranin. Safranin is also used as a counterstain in endospore and capsule staining. Acid-fast staining used in identifying all bacteria in the Genus *Mycobacterium*, including the two important disease producers, *Mycobacterium tuberculosis* and *Mycobacterium leprae*, uses carbol fuchsin as the primary stain and methyl blue as the counterstain (Chukwu *et al.*, 2011).

The systematically studied dye for histology was stated in the second half of the 19<sup>th</sup> century by Weigert C., J. Gerlach, P. Ehrlich, and H. Gierke. By then, coloring materials were mostly of natural origin, like carmine, cochineal, haematoxylin, and indigo. Gradually, the use of natural dyes has decreased due to the introduction of synthetic dyes, which were first invented by William Henry Perkin in 1856. They are widely applied in many fields, such as the food, cosmetic, and textile industries. Many dyes, techniques, and procedures are utilized to stain various types of animal tissues. At present, hematoxylin and eosin stain is widely used as a light microscopical stain in histology and histopathology. It is a combination of the natural hematoxylin stain and the synthetic eosin stain. Haematoxylin stains the cell nuclei dark blue, while eosin stains the cell cytoplasm, most connective tissue fibers, and matrices in varying shades of pink and red. In the last 10 years, natural dyes have been studied for their potential use in various fields, including histological staining (Araya Suebkhampet and Nattakarn Naimon, 2014). The present report describes the basic information about plant dye classification based on their chemical structures, methods of dye extraction, mordants, and some of their applications for tissue staining. This is available and can easily be processed. Therefore, in the present study, we investigated the efficacies of two herbal dyes in a bid to obtain non-toxic, eco-friendly, and cheap stains for use in plant histological studies.

## MATERIALS AND METHODS

### PLANT MATERIAL:

Fresh *Lawsonia inermis* L. and *Eclipta prostrata* L. plants were collected from the fields in the Tamil Nadu district of Sullur, Coimbatore. A specimen of the discovered plant was preserved for future uses.

**DYE EXTRACTION:**

The plant of *Lawsonia inermis* L. and *Eclipta prostrata* (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 water and ethanol.

**TISSUE STAINING:**

The staining properties of stem tissue from *Boerhavia chinensis* L. and *Stenotaphrum secundatum* Kuntze., angiosperms, were investigated. The stem sections were taken *Stenotaphrum secundatum* Kuntze and *Boerhavia chinensis* stem tissues were colored with *Lawsonia inermis* and *Eclipta prostrata* (L.) leaf extracts in water and ethanol.

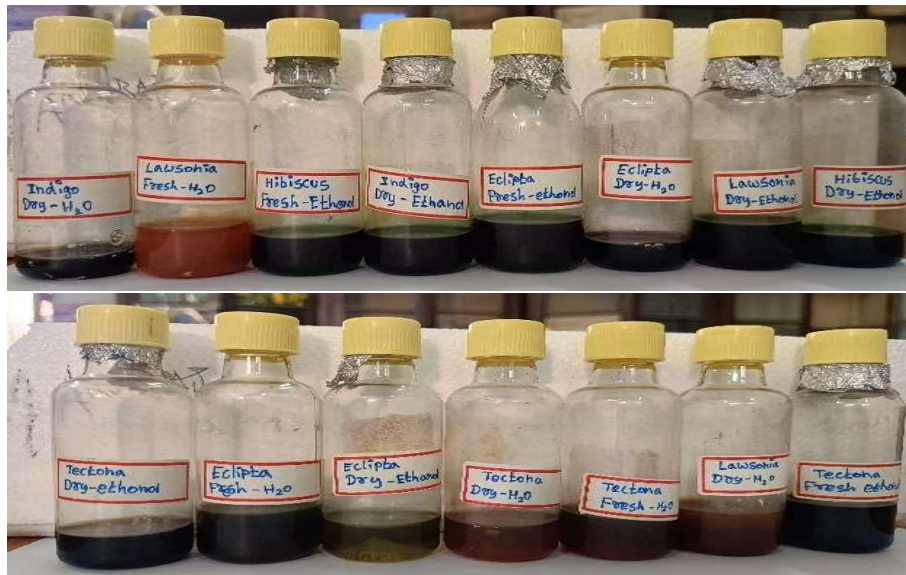
**MICROSCOPY:**

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).

**Scientific Classification:**

<i>Kingdom</i>	: <i>Plantae</i>	<i>Kingdom</i>	: <i>Plantae</i>
<i>Division</i>	: <i>Magnoliophyta</i>	<i>Division</i>	: <i>Magnoliophyta</i>
<i>Class</i>	: <i>Magnoliopsida</i>	<i>Class</i>	: <i>Magnoliopsida</i>
<i>Order</i>	: <i>Asterales</i>	<i>Order</i>	: <i>Asterales</i>
<i>Family</i>	: <i>Asteraceae</i>	<i>Family</i>	: <i>Asteraceae</i>
<i>Genus</i>	: <i>Eclipta</i>	<i>Genus</i>	: <i>Eclipta</i>
<i>Species</i>	: <i>prostrata</i> L.	<i>Species</i>	: <i>prostrata</i> L.
<i>Botanical name</i>	: <i>Eclipta prostrata</i> L.	<i>Botanical name</i>	: <i>Eclipta prostrata</i> L.

## DYE EXTRACTION

**RESULT AND DISCUSSION:****Fresh and dry leaves of *Lawsonia inermis* L. Dye Extracts**

A solution of dye prepared from the crude extract of *Lawsonia inermis* 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 *Lawsonia inermis* leaves with water was orange (fresh) and light reddish brown (dry), while that extracted with ethanol was dark blackish green (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 *Lawsonia* 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 Parenchyma, but not as profusely as the results of *Lawsonia* 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 *Lawsonia* 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 *Lawsonia inermis* leaves with ethanol imparted greenish color to the sclerenchyma and parenchyma 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 *Lawsonia* dye in ethanol was found to be more effective in staining sclerenchyma but less effective in the parenchyma and vascular bundle of dicotyledonous and monocotyledons in fresh dye ethanol. A dye extract of *Lawsonia* in fresh leaves of ethanol was found to be less effective at staining dicot and monocot stem tissue.



Table: 1

Staining effect of *Lawsonia inermis* L., leaves dye on stem tissues of *Boerhavia chinensis* L

<i>Lawsonia inermis</i> L.	Leaves extracts	Tissue Stained	Intensity of staining
Fresh Leaves	<b>Water</b>		
	1%	-	-
	5%	Parenchyma	+
	10%	Sclerenchyma	++
	<b>Ethanol</b>		
	1%	-	-
Dry Leaves	5%	Sclerenchyma, Vascular bundle Xylem & Phloem	+++
	10%		+++
	<b>Water</b>		
	1%	-	-
	5%	Parenchyma, Sclerenchyma	++
	10%	Vascular bundle	+
Dry Leaves	<b>Ethanol</b>		
	1%	-	-
	5%	Sclerenchyma Xylem,	+++
	10%	Phloem Tissue	+++

Table: 2

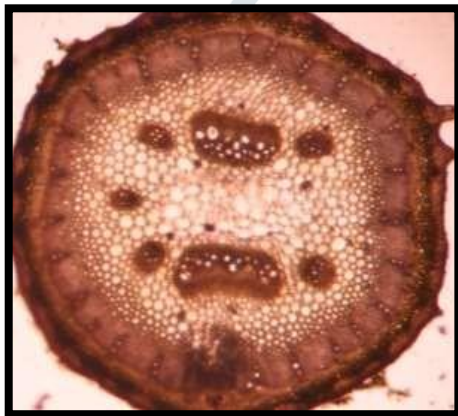
Staining effect of *Lawsonia inermis* L., leaves dye on stem tissues of *Stenotaphrum secundatum* Kuntze

<i>Lawsonia inermis</i> L.	Leaves extracts	Tissue Stained	Intensity of staining
Fresh Leaves	<b>Water</b>		
	1%	-	-
	5%	Parenchyma	+
	10%	Vascular bundle	++
	<b>Ethanol</b>		
	1%	-	-
Dry Leaves	5%	Parenchyma, Sclerenchyma	+
	10%	Vascular bundle	+++
	<b>Water</b>		
	1%	-	-
	5%	Parenchyma	+
	10%	Sclerenchyma, vascular tissues	++
Dry Leaves	<b>Ethanol</b>		
	1%	-	-
	5%	Sclerenchyma	+++
	10%	Vascular Tissues	++

STAINING EFFECT OF DYE FROM FRESH LEAVES O *LAWSONIA INERMIS L.*,

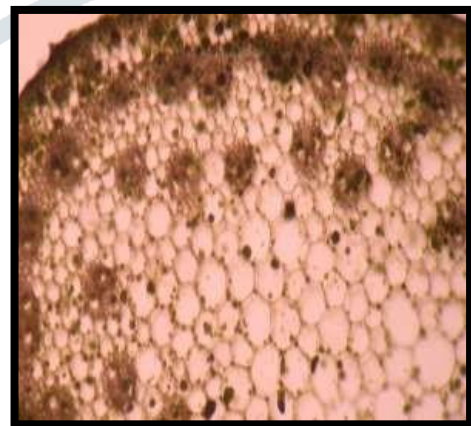
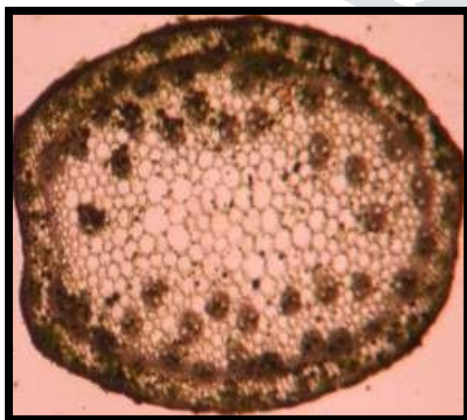


Stem Cross Section of (Dicot) *Boerhavia chinensis L.*, stain with extract of *Lawsonia inermis L.*, in Water



Stem Cross Section of (Dicot) *Boerhavia chinensis L.*, stain with extract of *Lawsonia inermis L.*, in Ethanol

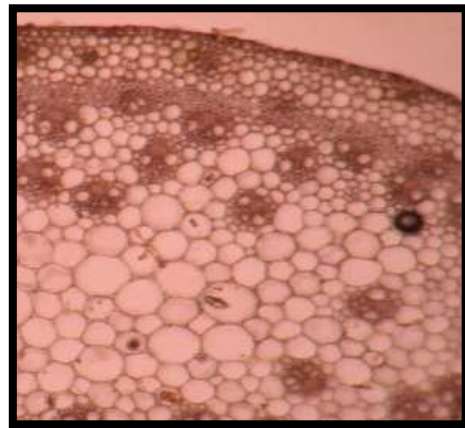
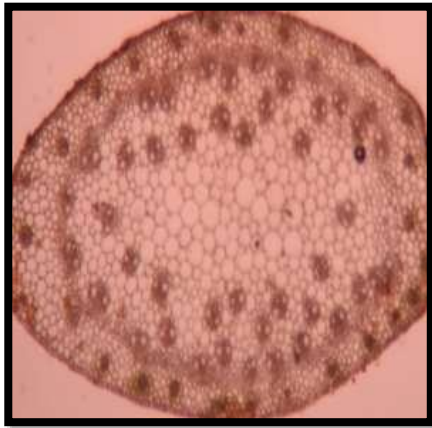
STAINING EFFECT OF DYE FROM FRESH LEAVES OF *LAWSONIA INERMIS L.*,



Stem Cross Section of (Monocot) *Stenotaphrum secundatum Kuntze.*, stain with extract of *Lawsonia inermis L.*, in Water

Stem Cross Section of (Monocot) *Stenotaphrum secundatum Kuntze.*, stain with extract of

*Lawsonia inermis* L., in Ethanol



STAINING EFFECT OF DYE FROM DRY LEAVES OF *LAWSONIA INERMIS* L.,

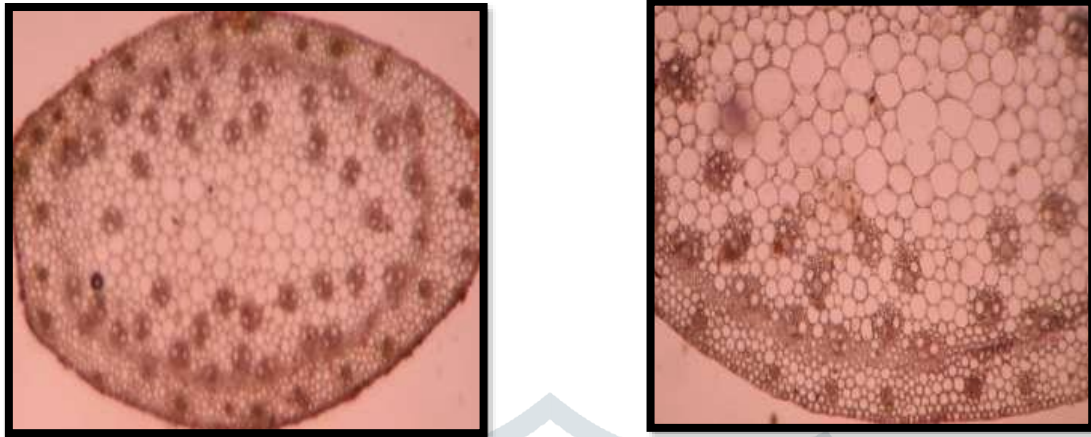


Stem Cross Section of (Dicot) *Boerhavia chinensis* L., stain with extract of *Lawsonia inermis* L., in Water

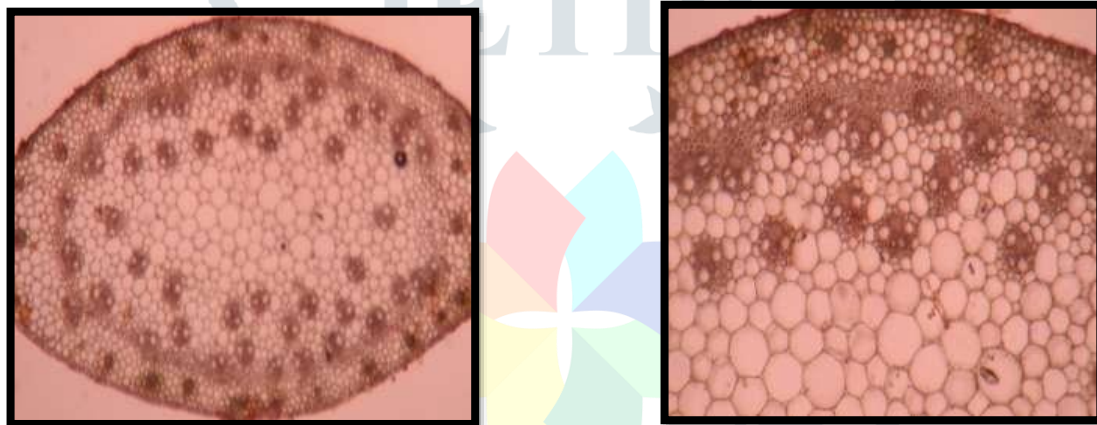


Stem Cross Section of (Dicot) *Boerhavia chinensis* L., stain with extract of *Lawsonia inermis* L., in Ethanol



STAINING EFFECT OF DYE FROM DRY LEAVES OF *LAWSONIA INERMIS* L.,

Stem Cross Section of (Monocot) *Stenotaphrum secundatum* Kuntze., stain with extract of *Lawsonia inermis* L., in Water



Stem Cross Section of (Monocot) *Stenotaphrum secundatum* Kuntze., stain with extract of *Lawsonia inermis* L., in Ethanol

Fresh and dry leaves of *Eclipta prostrata* L. Dye Extracts

A solution of dye prepared from the crude extract of *Eclipta prostrata* 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 *Eclipta prostrata* leaves with water was dark green (fresh) and dark brown (dry), while that extracted with ethanol was dark brownish green (fresh) and light greenish (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 *Eclipta prostrata* 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 *Eclipta prostrata* 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 1 and 2). The fresh dye extract of *Eclipta prostrata* 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.

## Table: 3

Staining effect of *Eclipta prostrata* L. Leaves dye on stem tissues of *Boerhavia Chinensis* L.,



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

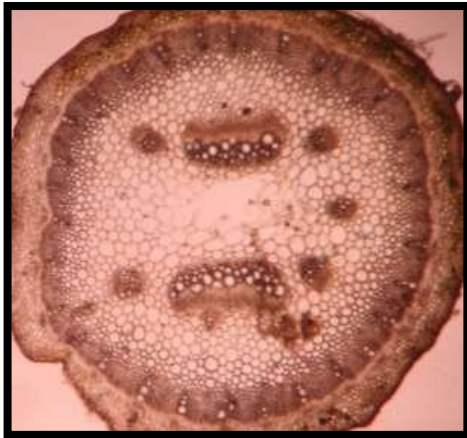
A 10% fresh and dry dye extract of *Eclipta prostrata* leaves with ethanol imparted greenish color to the sclerenchyma, parenchyma, and vascular bundle of dry leaf dye extract of monocotyledons stem cross section (*Stenotaphrum secundatum*) and produced a very interesting stem vascular tissue. Although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

#### STAINING EFFECT OF DYE FROM FRESH LEAVES OF *ECLIPTA PROSTRATA* L.,

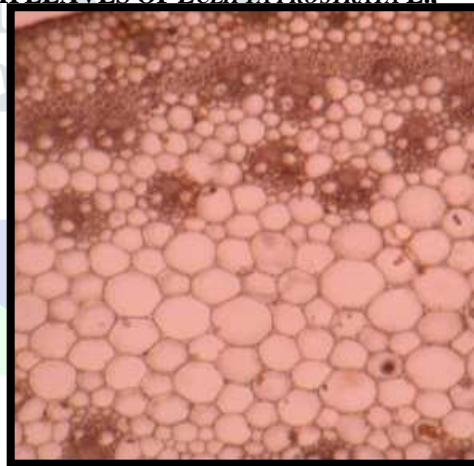
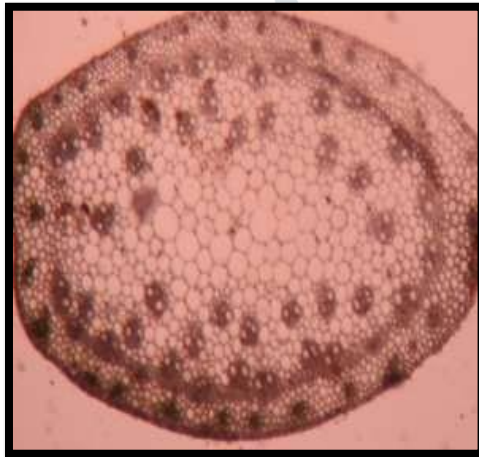


Stem Cross Section of (Dicot) *Boerhavia coccinea* L.,  
*Eclipta prostrata* L., in Water

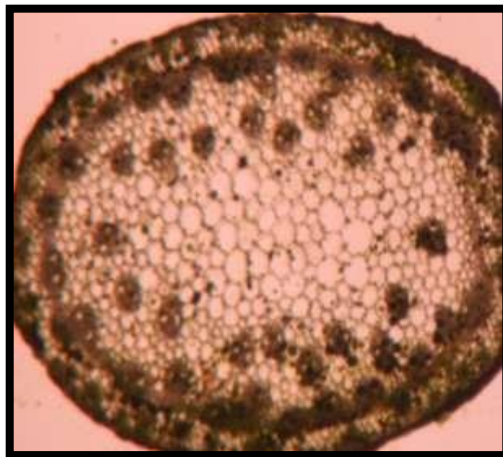
**Stem Cross Section of (Dicot) *Boerhavia chinensis* L., stain with extract of *Eclipta prostrata* L., in Ethanol**



**STAINING EFFECT OF DYE FROM FRESH LEAVES OF *ECLIPTA PROSTRATA* L.,**



**Stem Cross Section of (Monocot) *Stenotaphrum secundatum* Kuntze., stain with extract of *Eclipta prostrata* L., in**



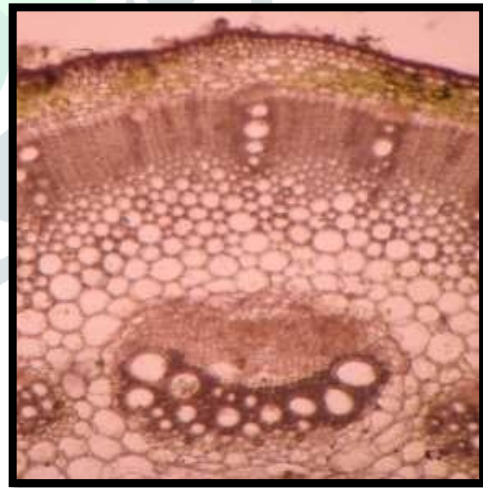
**Stem Cross Section of (Monocot) *Stenotaphrum secundatum* Kuntze., stain with extract of *Eclipta prostrata* L., in Ethanol**

Table: 4

Staining effect of *Eclipta prostrata* L. Leaves dye on stem tissues of *Stenotaphrum secundatum* Kuntze

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

STAINING EFFECT OF DYE FROM DRY LEAVES OF *ECLIPTA PROSTRATA* L.,



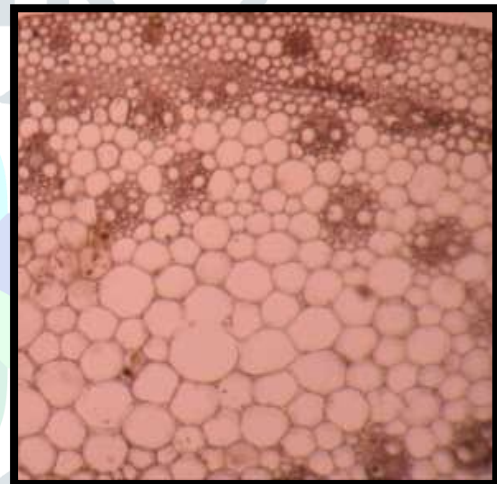
Stem Cross Section of (Dicot) *Boerhavia chinensis* L., stain with extract of *Eclipta prostrata* L., in Water



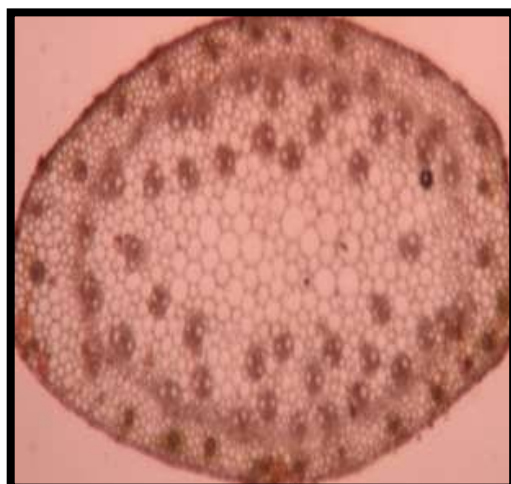
**Stem Cross Section of (Dicot) *Boerhavia chinensis* L., stain with extract of *Eclipta prostrata* L., in Ethanol**



**STAINING EFFECT OF DYE FROM DRY LEAVES OF *ECLIPTA PROSTRATA* L.,**



**Stem Cross Section of (Monocot) *Stenotaphrum secundatum* Kuntze., stain with extract of *Eclipta prostrata* L., in Water**



**Stem Cross Section of (Monocot) *Stenotaphrum secundatum* Kuntze., stain with extract of *Eclipta prostrata* L., in Ethanol**

## SUMMARY AND CONCLUSION

Natural dyes have been an excellent supply of pigments and color to enhance our daily life. This article offered a quick summary of the natural colors of *Lawsonia inermis* and *Eclipta prostrata*, as well as the dyeing technique and future developments. Natural dyes have several advantages for human use, but they are especially helpful to the environment and health. Despite its widespread appeal and overwhelming good effects, it still has a few downsides that must be addressed, and more research is needed to close the gap and support sustainability. Natural dyes demand more attention and effort, and there is a long road ahead of them. Natural dyes should be chosen with care; therefore, adequate attention should be taken when selecting colors based on requirements and intended application. People say that the most commonly used plant parts for dye extraction include seeds, flowers, leaves, berries, stems, barks, and roots. This study shown that herbal stains from *Lawsonia inermis* and *Eclipta prostrata* can be successfully used in plant histology. Dye extracts from *Lawsonia inermis* and *Eclipta prostrata* are effective substitutes for safranin O, phenol-glucinol, and any stain that may color fiber and vessel elements. The results also demonstrated that using ethanol as a solvent for dye extraction can result in good performance. This discovery will go a long way toward eliminating reliance on poisonous, expensive, and unavailable exotic stains.

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