EXTRACTION AND APPLICATION OF NATURAL DYE FROM *AVERRHOA CARAMBOLA* L. AS A PLANT HISTOLOGICAL STAIN

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DECLARATION

I hereby declare that the dissertation entitled" **Extraction and application of natural dye from *Averrhoa carambola* L. as plant histological stain"** submitted to Mahatma Gandhi University , Kottayam in partial fulfillment of the requirements for the award of the degree of Master of Science is a bonafide record of the original project work done by me under the supervision and guidance of Dr. Tintu Jose Manicketh, in Department of Botany, St. Teresa's College(Autonomous), Ernakulam and that it has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title or recognition to any candidate of the university.

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Place: Ernakulam ADHITHYA MADHAVAN K S

Date:

ABSTRACT

The staining ability of *Averrhoa carambola* L. extract as a natural plant histological stain was investigated across various plant tissues. According to the study, it revealed that *A. carambola* L. has a higher affinity towards cell walls, chloroplasts and parenchymatous regions. Through hydrogen bonding with cellulose molecules and hydrophobic interactions with anthocyanins, the extract consistently imparted a pinkish -red coloration to diverse tissues including epidermis, parenchyma and vascular tissues in gymnosperms, angiosperms and pteridophytes. *A. carambola* L. stain is a viable substitute for synthetic dyes due to its affordability, sustainability and eco-friendliness. The study highlights the versatility and potential of *A. carambola* L. extract in enhancing histological staining techniques, providing opportunities for further exploration in both plant and animal tissues

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CHAPTER -1

GENERAL INTRODUCTION

1.DYES

Dyes are organic compounds that, when applied to a substrate, produce color by changing the colored substances' crystal structures at least temporarily. These compounds are widely used in the textile, pharmaceutical, food, plastics, photography, and paper sectors due to their significant coloring potential (Manzoor, & Sharma, 2020). Water readily dissolves dyes ,which have particle size distribution of 0.025-1.0 μm (Ardila-Leal et al., 2021). A good dye should have color retention under various conditions such as heat, light, moisture, diluted acids, washing soaps, and more. Fast dyes are those that adhere to fiber surfaces permanently, whereas fugitive dyes fade or wash off (Shindy, 2017).

1.2. HISTORY OF DYES

Dyes have been an integral part of human history, dating back thousands of years, and continue to hold a significant place in contemporary society (Affat, 2021). Plants, fruits, vegetables, flower, and insects were the sources of natural color for textiles, wood, and clay as early as 3500BC, according to a number of civilization (Phan et al., 2021). In the Ancient Stone Age, various colored powders made up of colored minerals were used by the people for applying them in to their hair and body as a protection during hunting and also for occasional dressings. People have always been interested in colors, the art of dyeing has a long history (Yusuf et al., 2017). Otto Unverdorben prepared aniline for the first time from the destructive distillation of Indigo. Henry Perkin went on to create Mauveine, the first synthetic color. It was produced from coal tar, in 1854. Coal tar-derived ingredients allowed for the development of new colors, and by1869, low cost synthetic dyes had replaced some natural dyes like Alzarin (Ardila- Leal et al ., 2021).

1.3. CHEMISTRY OF DYES

Dyes are complex unsaturated chemical compounds what give visible area color by absorbing light and imparting it. They consist of two major structural components, auxochrome and chromophore, whose presence gives them distinctive characteristics

(Rahman et al., 2020). The dye's active location is called the chromophore. It is composed of many atom groups, the most frequent being nitro(-N02), azo(-N=N-), nitroso(-N=O), thiocarbonyl(-C=S), carbonyl(-C=O), and alkenes(-C=C-). The molecule which contains chromophore are called chromogenic. The chromogenic molecule shows dyeing properties only by the adding additional atom groups known as auxochrome. Auxochromic groups allows proper fixation and modification of dyes. These groups can be acidic or basic (Benkhaya et al., 2020).

1.4. CLASSIFICATION OF DYES

Dyes are classified in to two categories based on their origin,

- Natural dyes
- Synthetic dyes

Natural dyes, which have been existed since ancient times, they are derived mainly from plants and **Synthetic dyes** are artificially synthesized from chemical compounds (Slama et al., 2021). Natural dyes are also known as natural pigments, are mainly obtained from plant sources, animals, or natural-coloured ores. Natural dyes are biodegradable, non-carcinogenic, non- toxic and renewable (Pizzicato et al., 2023).
Synthetic dyes are usually unsaturated organic molecules (Srivatsav et al., 2020). Synthetic dyes are obtained from petroleum products and coal tar (Salauddin et al., 2021)

1.5_CLASSIFICATION OF NATURAL DYES

1.5.1 BASED ON PLANT SOURCE

Dyes are naturally obtained from plants in earlier time onwards. There were about 500 plant species are identified as the dye sources. These are high biodegradability and pharmaceutical as well as health benefits also. The content and the amount of coloring material is mainly depended on the age and harvesting time of the plant (Chungkrang et al., 2020).

Table :1

Plant source	**Useful parts**	**Colour produced**	**References**
Acacia catechu	Bark	Brown	Samant et al., (2022)
Adathoda vasica	Leaf	Yellow	Chaudhari et al., (2023)
Aegle marmelos	Fruit	Yellow	Sobh et al., (2024)
Bougainvillea glabra	Fresh flowers	Yellow	Rasool et al., (2023)
Cassia fistula	sap wood	Red	Samanta, & Singhee, (2023)
Curcuma longa	Rhizome	Yellow	Kusumawati et al., (2020)
Indigofera tinctoria	Leaves, seeds	Blueish black,indigo	Das et al., (2021)
Tectona grandis	Stem,	Yellow	Tibkawin et al., (2022)

1.5.2 BASED ON COLOUR

Table:2

Colour	**Plant source**	**References**
RED DYES	Saf flower Caesalpinia Madder	Sanda & Liliana, (2021)
YELLOW DYES	Bougainvillea Golden rod Teak Marigold	Baig et al., (2020)
GREEN DYES	Tulsi Bougainvillea Lily	Aggarwal, (2021)
BROWN DYES	Caesalpinia Marigold	Sanda & Liliana, (2021)

BLUE DYES	Indigo Woad Suntberry Pivet	Kumar & Prabha, (2018)
BLACK DYES	Lac Rof blamala Sappan wood	Aggarwal, (2021)
ORANGE / PEACH DYES	Bougainvillea Balsam	Kumar & Prabha, (2018)

1.5.3 BASED ON ANIMAL SOURCE

These are the dyes obtained from animal sources, secretion of insects and dried bodies of insects are the major sources for natural dye (Chungkrang et al., 2020)

Table:3

Animal source	**Colour of dye**	**References**
Leaccifer lacca	Red	Baqri, (2023)
Coccus ilicis	Scarlet	Baqri, (2023)
Coccus cacti	Pink,purplish–red, grayish violet,scarlet	Baqri, (2023)
Murex species	Tyrian purple	AlAshkar&Hassabo ,(2021)

1.5 .4 BASED ON MINERAL SOURCE

These are the dyes obtained from inorganic metal salts and metal oxides. (Yusuf et al.,2017)

<u>Table:4</u>

Colour	**Dye**	**Dye source**	**References**
RED	• Cinnabar, • Red Ochre • Red lead • Realgar	Mercury sulphide Hydrated iron oxide Pb_3O_4 Arsenic sulphide	Sharma & Singh,(2021)
YELLOW	• Yellow Ochre • Raw Sienna • Orpiment • Litharge	$Fe_2O_3 \cdot H_2O$ Ironoxide, Manganese oxide Arsenic sulphide lead oxide	Mastrotheodoros.& Beltsios,(2022)
GREEN	• Terre-Verte (Green Earth) • Malachite • Vedgiris	Fe, Mg, Al, and K Copper carbonate hydroxide Acetate of copper	Sharma & Singh,(2021)
BLUE	• Ultramarine Blue • Azurite	lapis lazuli Copper ore	Švarcová et al .,(2021)

1.6 DISADVANTAGES OF SYNTHETIC DYES

- Synthetic dyes are extremely harmful and can lead to cancer (Islam et al., 2022).
- Dyes can prevent sunlight from entering water and also harm aquatic life (Bal & Thakur, 2022).
- It can also result in significant harm to the liver, kidney, reproductive system, brain, and central nervous system. It can cause irritations to skin, allergies, cancer, neurological problems, and other human health disorders like nausea, vomiting, and paralysis (Ayele et al., 2021).
- Synthetic dyes are not biodegradable, they accumulate on lands, rivers and other waterbodies and it causes ecological problems (Affat, 2021).

- When synthetic dyes degrade, it's byproducts can cause health risks to human beings directly or indirectly (Manzoor & Sharma, 2020).

1.7 ADVANTAGES OF NATURAL DYES

- Natural dyes are biodegradable and renewable,non-allergic and non-carcinogenic, non-toxic (Chungkrang et al., 2020).
- Natural dyes are abundant and their extraction process is relatively easy (Omar et al., 2020).
- Natural dyes present no harmful health hazards, rendering them inherently safe for use (Rahman Bhuiyan et al., 2018).
- Natural dyes have medicinal value as well as antimicrobial and UV protective character (Samanta et al., 2018).
- Less chemical reactions are involved during dye preparation (Choudhury, 2018).
- Natural dyes have some inherent insect repellent properties (Salauddin et al., 2021)

1.8 APPLICATIONS OF NATURAL DYES

Natural dyes have diverse applications in contemporary industries, highlighting their benefits and creative potential. Natural dyes are used in various fields such as follows,

- Textiles (Ahsan et al., 2020).
- Food colourant (Bora et al., 2019).
- Dye sensitized solar cells (Verma & Gupta, 2017).
- Anti-microbial property,UV resistant (Singh et al., 2021).
- Histological stains and pH indicators (Dulo et al., 2021).
- Cosmetics (Bujak et al., 2022).
- Fluorescent natural dyes are used for cell imaging. A beetroot extracted fluorescent dye is an example (Yadav et al., 2023).
- Natural dyes are used as photo initiators for design of photo initiating species for polymerization (Noirbent & Dumur, 2021).
- Natural dyes are used as marker ink, example marker ink from mangosteen leaves (Mohd Basri et al., 2021).

1.9 RELEVANCE OF THE PRESENT STUDY

The growing environmental consciousness has made it crucial to utilize eco-friendly, non-allergic, non-toxic and natural dyes. *Averrhoa carambola* L. is readily available in nature and it contains pigments suitable for dyeing purpose. The present study mainly focused on the exploration on the use of dyeing potential of the selected plant.

1.10 OBJECTIVES OF THE STUDY

- To identify and extract the dye from flowers of *Averrhoa carambola* L.
- To examine the staining potential of *Averrhoa carambola* as a plant histological stain.

CHAPTER – 2

EXTRACTION AND APPLICATION OF NATURAL DYE FROM *Averrhoa carambola* L. AS A PLANT

HISTOLOGICAL STAIN

2.1 INTRODUCTION

2.1.1 HISTOLOGY

Histology is the study of analyzing the cells and tissues of both plants and animal section by cutting and staining then after viewing under light or electron microscope. Histological studies can be applicable in different fields such as forensic investigation, diagnosis, pharmaceutical, academics etc. (Hartika et al.,2021)

2.1.2 HISTOLOGICAL STAINING

Histological staining is a series of steps used in stain sample tissues with histological stains in order to prepare them for microscopic inspection. Fixation, processing, embedding, sectioning and staining are the five crucial phases in histological staining (Alturkistani et al., 2016)

Various stain types have been developed corresponding to different biological features to be highlighted. Haematoxylin and eosin made more contrast staining in both nuclei and extracellular tissue matrix are the common example (Bai et al., 2023). In histological staining mainly two types of stains are used, synthetic dye which is obtained from chemical reaction and natural stain acquired from natural sources. Synthetic dyes are more effective for staining tissues but it has harmful side effects (Dina et al., 2021).

2.1.3 HISTORY OF STAINING

In ancient times histologists were used substances like Tyrian purple, alizarin, carmine, saffron etc. to stain the tissues. But later in 17th century scientists like Leeuwenhoek and Robert Hooke were used Cochineal to stain the tissues. In 1825, a scientist named Raspail used iodine to stain plant cells to identify the starch contents then after German scientists used carmine to stain plant tissues to find out the structures of cells and tissues (Dibal et al., 2022).

Later synthetic dye such as aniline dye was discovered and it was very useful to histological staining. Along with this synthesis of basic fuchsin, aniline blue, eosin and methylene blue were discovered in 1858,1862,1871 and 1876 (Dibal et al., 2022).

In 1879, Haematoxylin, a natural dye has been discovered by Cook. It was obtained from logwood tree. Extraction method was done using alum, copper sulphate extractions to remove the hematoxylin from logwood. Later it was applied in plant tissues and it showed more results in staining cytoplasm and nuclei. In 1896, sudan III were discovered to stain lipids in tissues. At the beginning of 20^{th} century new stains and staining techniques were developed and also the modification of old ones were also be done (Dibal et al., 2022).

2.1.4 MECHANISM OF STAINING

The up taking of stain is mainly due some interactions. The interactions may be dye – tissue or reagent-tissue interactions. In addition, there are other elements that influence the interactions between dye and tissue,solvent interactions (hydrophobic effect), reagent -reagent interactions ,reagent -tissue interactions(vander waals' forces) , hydrogen and covalent bonding.

Vander waal's forces includes intermolecular attractions like dipole -dipole, dipole induced dipole, dispersion forces etc. These interactions can be commonly occurring in all reagents and tissues.

Hydrogen bonding is the contact occurs between a dye and tissue when a hydrogen atom is covalently bound to only one of two electronegative atoms.

Hydrogen bonds are not much important for stain- tissue affinity when the aqueous solvents are used, except in case of staining with connective tissue fibers. Covalent bonding is also present in between the tissue and stains. The polar covalent bonding between metal ions and mordants are an example (Horobin, 2008).

CHAPTER -3

REVIEW OF LITERATURE

Hematoxylin was extracted from the logwood of the tree *Haematoxylon campechianum* and can be used as a histological stain for staining the cell nuclei of plant cells with blue-black colour (Gamble & Wilson, 2008).

The brilliant red colored extract produced from dried calyces of *Hibiscus sabdariffa* L using aqueous extraction method can be used as an alternative for hematoxylin (Benard, 2008).

According to (Sikhruadong et al., 2009) dye extracted from *Morus alba* was capable for staining the chromosomes in *Crinum asiaticum L.*

(Akinloye et al., 2010) analyzed the ability of herbal dye extracts from *Bixa orellana*, *Curcuma domestica*, *Lonchocarpus cyanescens* and *Pterocarpus osun* to stain fibre and vessel elements of wood sections.

A yellow pigment that was taken out of the dried stem powder of *Berberis Pachyacantha can* be used as histological staining agent for angiospermic plants (Jan et al., 2011). Effective staining was observed in the stem tissues of monocots and dicots.

Dye was extracted from dry leaves of *Lawsonia inermis* were used for staining the angiospemic stem tissues of *Helianthus annuus* L., and *Zea mays* (Jan et al., 2011).

The staining capacity of *Melastoma malabathricum* fruit was tested by (Deepak&Omman, 2013) for staining stem sections of dicot, monocot, and pteridophytes. The results demonstrate that the stems xylem, collenchyma, and sclerenchyma tissues all had outstanding differential staining.

Aqueous extracts from plants like *Lowsonia inermis, Hibiscus rosa-sinensis,Rubia tictorium L., Butea monosperma,Rosa indica,and Bougainvillea glabra* were used to stain angiospermic stem tissues(Deepali et al., 2014),this can be serve as a substitute for artificial stains.

Red dye was obtained from the red dragon fruit (*Hylocereus castaricensis)* and was applied on plant tissues. The plant tissues were stained with different concentrations to analyze the high contrasting concentration (Wagiyanti & Noor, 2017).

The intense yellow colored dye extracted from *Curcuma longa* can be applied in place of eosin stain (Suryawanshi et al., 2017).

A study conducted by (Sudhakaran et al., 2018) showed that the extracts from the rhizome of Zingiber *officinale* and *Curcuma longa can* be used as an alternative stain to eosin.

The extraction of yellow dye from *Curcuma longa* were applied on nine different plant and animal tissues for comparing the staining effect with different concentration.

(Cruz et al., 2018) has studied the capability of the dye extracted from *Ixora coccinea* can act as an alternative for eosin. It has proved that the extract contains cyanidin, flavonoids, and anthocyanins which imparts good colour to the material.

The dye was extracted from *Erythrina crista-galli* L. and was applied on *Piper betle* L. stem. The anthocyanin present in the dye gave the colour. (Susetyarin et al., 2020).

The extracts from *Allium cepa* and S*orghum bicolor* were identified as a good counter stain for hematoxylin (Krampah et al., 2021).

A substitute stain for eosin was carried out by extracting dye from *Lawsonia inermis* and *Hibiscus sabdariffa* using aqueous extraction method. The stain showed high effectiveness for staining cytoplasm (Joshua et al., 2021).

Dicot and monocot stem sections were stained using the dye extracted from *curcuma longa* (rhizome)and *Nyctanthes arbortristis* (corolla tube) (Shet Verenkar et al., 2021).

Efficacy of a dye extracted from red dragon fruit *(Hylocercus costaricensis*) and its application to stain the chromosomes of onion root tip was carried out by (Sujjaritthurakarn et al., 2022).

According to (Chingangbam et al., 2023), the dye extracted from *Bixa orellana* and *Strobilanthes cusia* shows better stainability while applied on root tip cells of *Allium ascalonicum*. It showed better nuclear stainability as good as carmine stain.

CHAPTER -4

MATERIALS AND METHODS

4.1 PLANT MATERIAL

4.1.1 SYSTEMATIC POSITION

Scientific name: *Averrhoa carambola* L.

Kingdom : Plantae

Division : Magnoliophyte

Class : Magnoliopsida

Subclass : Rosidae

Order : Geraniales

Family : Oxalidacea

Genus : *Averrhoa*

Species : *carambola*

Part used : Flower

Fig: 1 A. Habit , B. Flower

4.1.2 ORIGIN AND DISTRIBUTION

Star fruit have originated in Ceylon and Moluccas. For hundreds of years, it has been cultivated in Southeast Asia and Malaysia. It is also distributed in Taiwan, Thailand, Israel, Florida, Brazil, Philippines China, Australia, Indonesia, in the warmer parts of India and (Ferrara, 2018).

4.1.3 BOTANICAL DESCRIPTION

Averrhoa carambola L. is a tiny, showy, slow growing evergreen tree that grows between 5 and 7 meters in height and diameter about 20-25 ft. Leaves are long, alternate, spirally arranged, ovate-oblong in shape. They are 15-25 cm long. Leaflets are smooth usually in 5 pairs. Panicles are small, axillary and bell shaped. Flowers are red or pink and white appear on bare branches. Petals are purple to bright purple. Fruit is fleshy with greenish to yellow in color. Growing season is from August-March (Gowrishankar et al., 2018).

4.2 PLANTS SELECTED FOR STAINING

Staining was done by selecting members from each plant groups.

Table -5 Plant material selected for staining

Group	Plant material	Part used for staining	Type of preparation
Algae	*Cladophora* sp.	Whole material	Whole mount
Bryophyte	*Marchantia* sp.	Thallus	T.S of thallus
Pteridophytes	*Psilotum* sp.	Stem	T.S of stem
	Selaginella sp.	Stem	T.S of stem
	Equisetum sp.	Stem	T.S of stem
	Pteris sp.	Stem	T.S of stem
Gymnosperms	*Cycas* sp.	Rachis, Leaflet	T.S of rachis, leaflet
	Gnetum sp.	Stem	T.S of stem
Angiosperms	*Eupatorium* sp.	Stem	T.S of stem
	Cyperus sp.	Stem	T.S of stem

4.3. METHODS

4.3.1 SELECTION AND SCREENING OF PLANT

Averrhoa carambola L. is a widely distributed perennial tree. The Plant material was selected for the current study is based on its abundance and intense red to pinkish colored flowers.

4.3.2 COLLECTION AND PREPARATION OF SELECTED PLANT

Plants were identified and authenticated from St. Teresa's college, Ernakulam, Kerala. It was collected, washed with tap water to remove the impurities and stored for further analysis

4.3.3 EXTRACTION OF STAIN

Fresh flowers of *A. carambola* L. were harvested. Flowers were washed in tap water. The extract was prepared by hot aqueous extraction method. 10 gms of plant material were boiled in 100 ml of distilled water for 20 minutes. The solution was cooled and filtered using Whatmann No.1 filter paper. The extract was stored airtight bottles in freezer for further study.

4.3.4 pH ANALYSIS

The pH of the extract was determined using digital pH meter.

4.3.5 FREEHAND SECTIONING AND STAINING

The easiest technique for getting specimens ready for microscopic examination is a freehand section. It is a suitable method for variety of plant materials from soft herb to small woody twigs (Yeung, 1998). Algae and stem, leaves sections of different plants were selected for tissue staining. Very thin free hand sections of these materials were stained using the extract made from *A. carambola* L. for 15 minutes. The stained sections were mounted on glycerin. The prepared sections were observed and analyzed using compound light microscope (XSZ-N107T) under the power of 4X, 10X ,40X

CHAPTER -5

RESULTS AND DISCUSSION

5.1 pH ANALYSIS

The extraction of dye from *A. carambola* L. exhibited promising results in its application as a plant histological stain. Initially, the pH of the dye was 4.2 which has a purple color. The purple colour of the dye was due to the presence of anthocyanin, a natural pigment found in many plants. Anthocyanins are mainly in the form of 2- phenyl-flavylium cations, which give the solution its red color and remain stable under acidic condition (Sachdev et al., 2021). There are many factors which influence the stability of anthocyanins. pH is one of the important factors among them. Anthocyanins are more stable at low pH (Enaru et al., 2021). Due to this reason, the pH was adjusted to 2 which gave a pinkish color similar to the safranine, a synthetic dye. The lower pH not only intensified the purple color but also facilitated better adhesion and penetration of the dye in to tissues. It was observed that the extraction from *A. carambola* L. was stable at low pH and retained the shelf life for 3 months at freezing temperature of 4°C.

Fig :3 Original pH - 4.

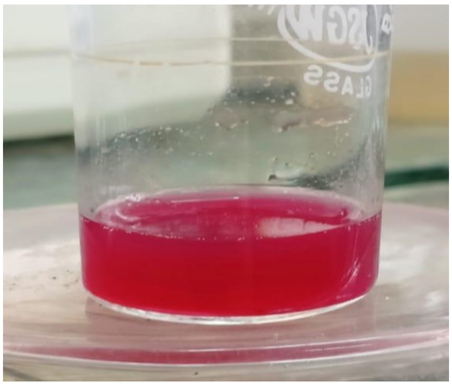


Fig :4 pH - 2

5.2 STAINING POTENTIAL OF Averrhoa *carambola*

The staining efficiency of *A. carambola* L. in different plant groups were shown in the Table :6

<u>Table: 6</u> staining potentials of *A. carambola* L. in different plant group

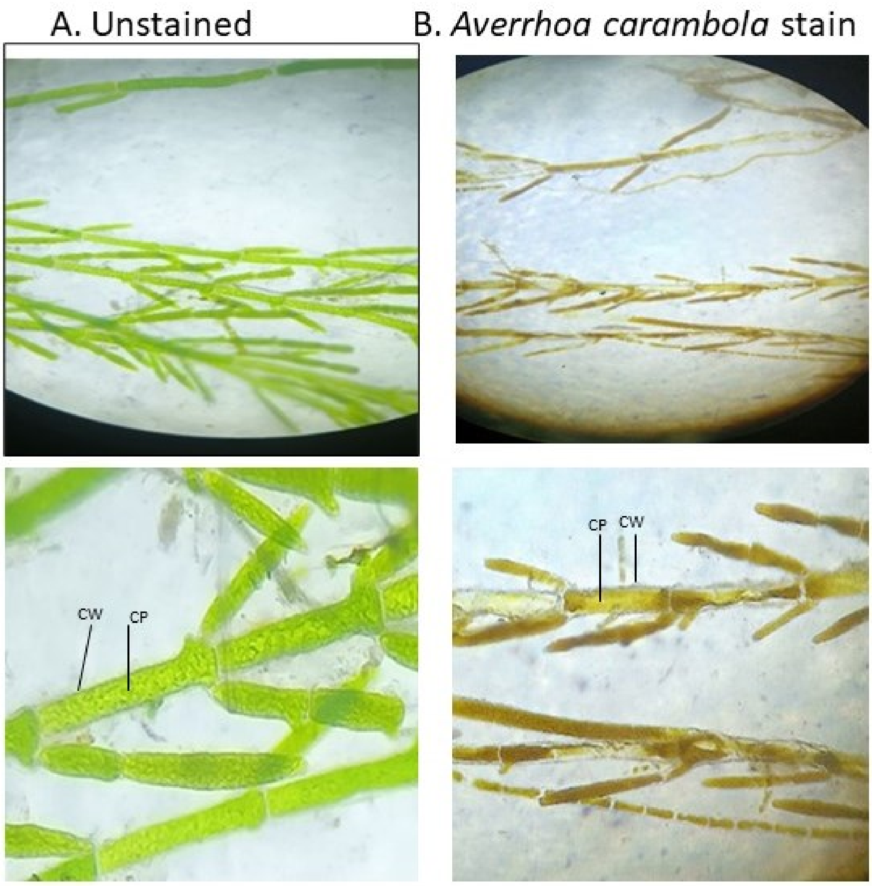
Group	Genus name	Region	*Averrhoa carambola* L. extract
Algae	*Cladophora* filament	Cell wall Chloroplast Pyrenoids	+ + -
Bryophytes	*Marchantia* thallus	Epidermis Chlorenchyma Parenchyma	- - +
Pteridophytes	*Psilotum* stem	Cuticle Epidermis Sclerenchyma Parenchyma Xylem Phloem Pith	+ + + + + + +
	Selaginella stem	Cuticle Epidermis Sclerenchyma hypodermis Parenchyma cortex Xylem Phloem	+ + + + + +
	Equisetum stem	Cuticle Epidermis Sclerenchyma Parenchymatous cortex Xylem Phloem	+ + + + + +
	Pteris rachis	Epidermis Sclerenchymatous hypodermis Xylem Phloem	+ + + +

Gymnosperm	*Cycas* leaflet	Cuticle Epidermis Sclerenchymatous hypodermis Xylem Phloem	+ + + + +
	Cycas rachis	Epidermis Sclerenchymatous hypodermis Xylem Phloem	+ + + +
	Gnetum stem	Cuticle Epidermis Parenchymatous cortex Sclerenchyma Xylem Phloem Pith	+ + + + + + +
Angiosperms	*Eupatorium* stem	Cuticle Epidermis Collenchymatous hypodermis Parenchymatous cortex Sclereids Xylem Phloem Pith	+ + + + + + +
	Cyperus stem	Epidermis Sclerenchyma Xylem Phloem	+ + + +

*+ Stained, - Not stained

Stain obtained from *Averrhoa carambola* shows better staining on the cell wall and cytoplasm of *Cladophora* (Plate 1). The cell wall and chloroplast of *Cladophora* is mainly composed of cellulose. Cellulose is a polysaccharide composed of linear β – (1,4)- linked glucan chains. Cellulose molecules aggregate to form micorofibrils, which are long, parallel chains of cellulose held together by hydrogen bond. These microfibrils provide structural support and rigidity to the cell wall (Nicolai & Preston, 1952; Dawes 1966; Zhang et al., 2021). The result showed that the enhanced staining capacity of *Averrhoa carambola* was due to the high affinity of anthocyanins towards cellulose present in the cell wall and chloroplast of *Cladophora.*

Plate 1: Staining of *Cladophora* filament

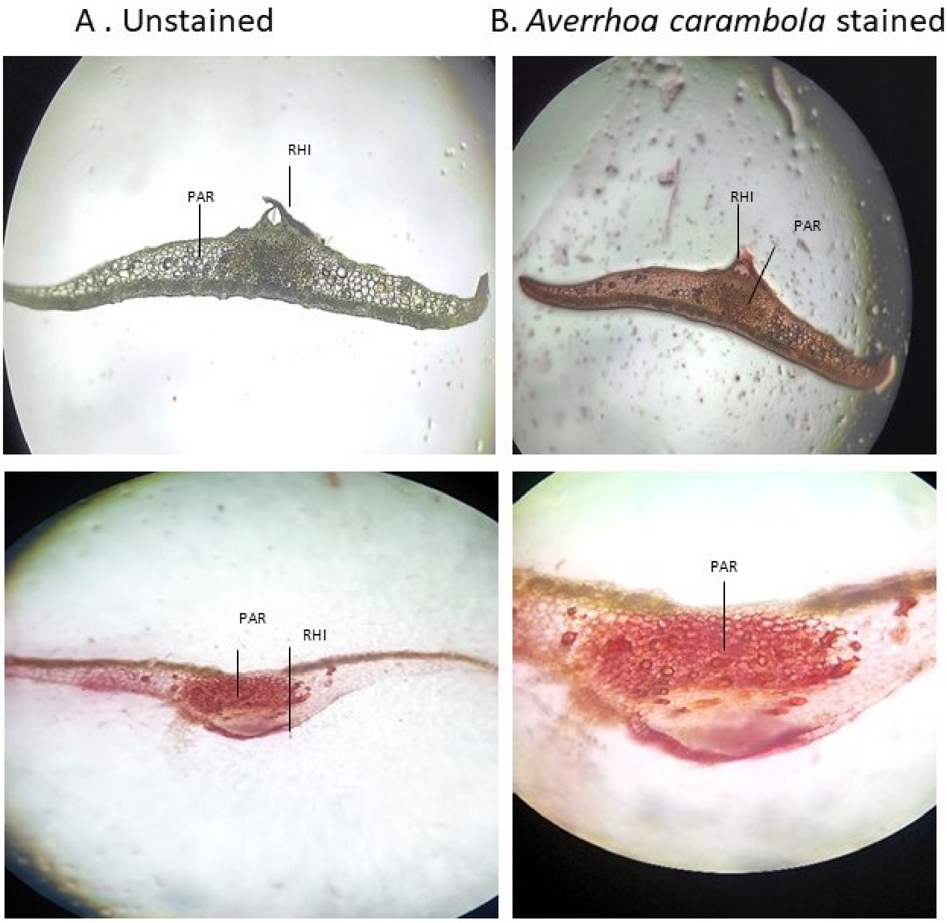


Cladophora filaments (10X) showing cell wall (CW), Chloroplast (CP). A. Unstained, B. *Averrhoa carambola* stain.

Another reason for the stainability was due to the hydrogen bonding between the dye molecules and cellulose. The dye molecules within the stain form hydrogen bond with hydroxyl groups on the glucose unit of cellulose chain. These bonds allow the dye molecule to adhere to the cellulose molecule, leading the formation of stable complexes (Sahin & Arslan, 2008; Zanjanchi et al., 2013; Hubbe et al., 2019; Pruś et al., 2022).

Dye from *Averrhoa carambola* stain gave a pinkish red color to the parenchymatous tissues of storage region in *Marchantia* thallus (Plate 2). This is because of the presence of cell components like cellulose and pectin present in that region. Parenchymatous region is rich in cellulose and pectin (Richter, 2011). Pectin is hydrophilic in nature, which can aid in retaining staining solution within the cell wall and also promote cell to cell adhesion (Roberts e t al., 2012; Roig-OliRoig-Oliver et al., 2021; Henry, 2021; Pfeifer et al., 2022).

Plate 2: Staining of *Marchantia* thallus

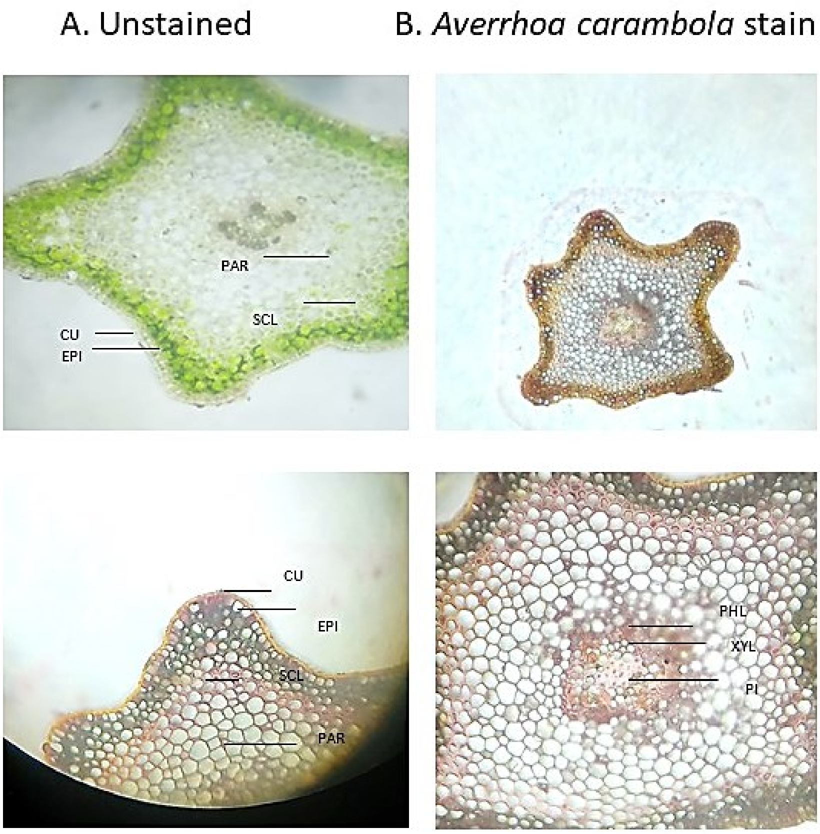


T. S of *Marchantia* thallus(10X,40X) showing parenchymatous region A.Unstained B. *Averrhoa carambola* stain , Parenchyma (PAR), rhizoids (RHI)

The application of *Averrhoa carambola* dye shows excellent stainability to the epidermal tissues, sclerenchyma, parenchyma, xylem and phloem in Pteridophytes (Plate3, 4, 5, 6). Parenchyma and phloem cells contain cellulose, hemicellulose and pectin. Sclerenchyma and xylem tissues contains cellulose, hemicellulose and lignin (Baldacci-Cresp et al., 2020; Liao et al., 2022). Lignin molecules contain various functional groups such as phenolic hydroxyl groups and aromatic rings. These chemical structures provide potential binding sites for dye molecule (Cauley & Wilson, 2017). Parenchyma and phloem tissues contains cellulose in their cell wall. Anthocyanins bind to cellulose molecules through hydrophobic interactions (Padayachee et al., 2012). Similar result was observed with the dye from M*elastoma malabathricum* (Deepak and Omman, 2013). Gymnosperms and angiosperms also stain pink colour to the parenchyma, sclerenchyma and vascular tissues (plate 7,8,9,10,11). Similar result was also observed with the dye extracted from *Berberis pachyacantha* (Jan et al., 2011).

All the above results showed that *Averrhoa carambola* stain acts as an excellent plant histologicall stain which imparted pink colour to the various plant tissues similar to that of safranine.

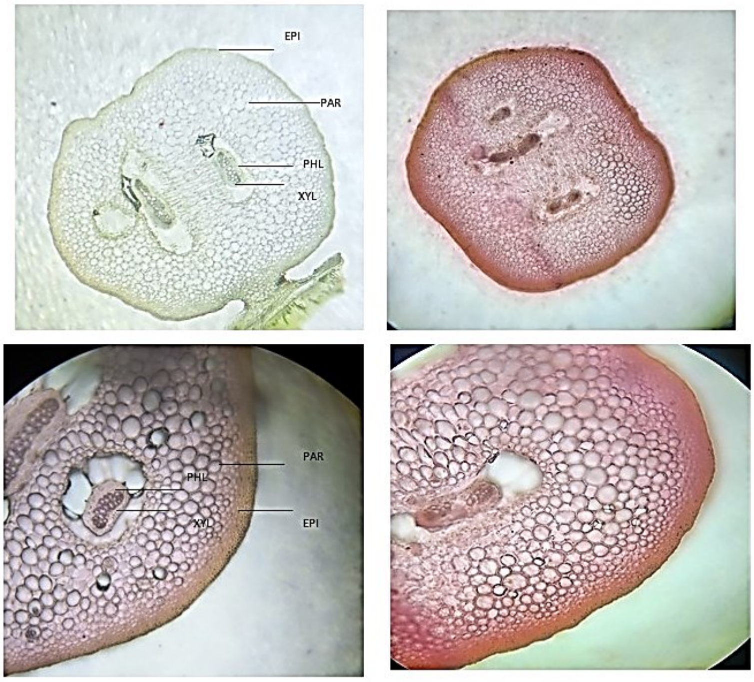
Plate 3: Staining of *Psilotum* stem



T.S of *Psilotum* stem (10X, 40X) with epidermis (EPI), sclerenchyma (SCL), parenchyma (PAR), xylem, (XYL) phloem (PHL)

Plate 4: Staining of *Selaginella* stem

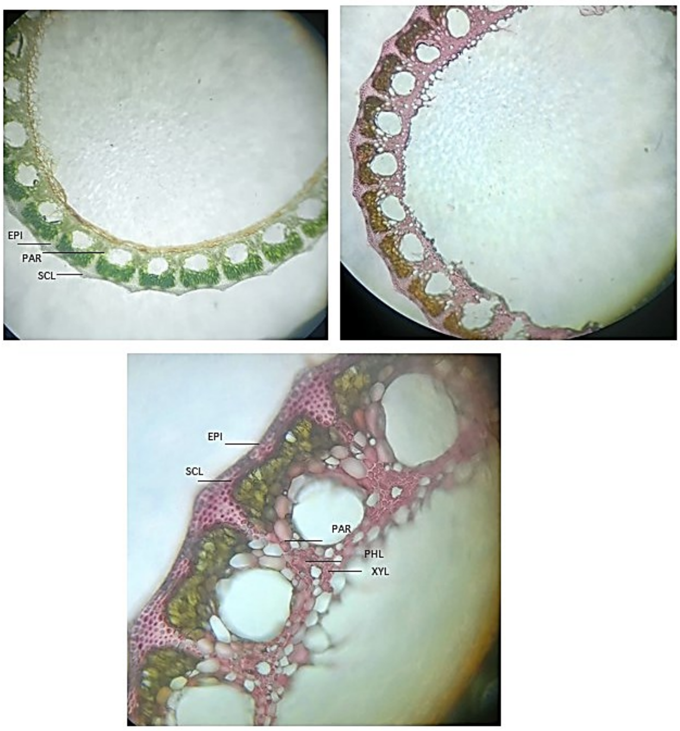
A.Unstained B .*Averrhoa carambola* stain



T.S of *Selaginella* stem (10X,40X) showing parenchyma (PAR), sclerenchyma (SCL), xylem (XYL) phloem (PHL).

Plate5: Staining of Equisetum stem

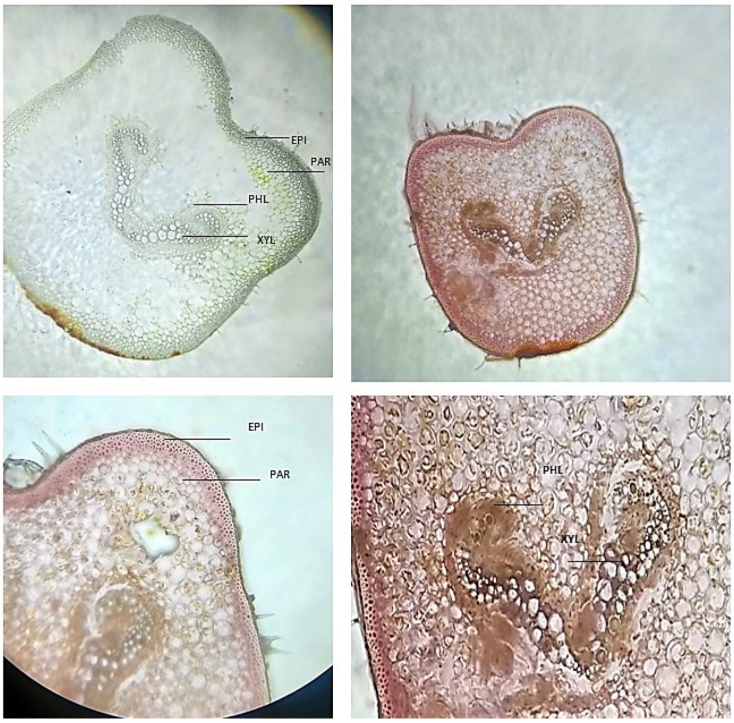
A.Unstained B. *Averrhoa carambola* stain



T.S of *Equisetum* stem (10X,40X) showing parenchyma (PAR), sclerenchyma (SCL), xylem (XYL) phloem (PHL)

Plate 6: Staining of Pteris *rachis*

A.Unstained B.*Averrhoa carambola* stain



T.S of *Pteris* stem (10X,40X) showing parenchyma (PAR)sclerenchyma (SCL), xylem (XYL), phloem (PHL)

Plate7: Staining of *Cycas* leaflet

A.Unstained B.*Averrhoa carambola* stain

T.S of *Cycas leaflet* (10X,40X) showing parenchyma (PAR), sclerenchyma (SCL), xylem (XYL) phloem (PHL).

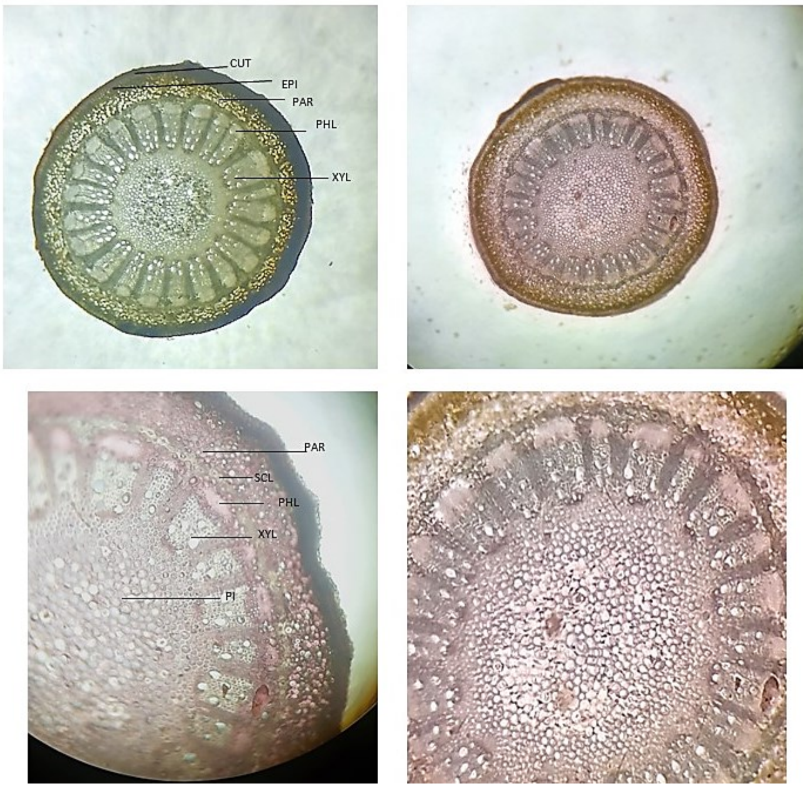
Plate 8: Staining of *Cycas* rachis

A.Unstained B.*Averrhoa carambola* stain

T.S *Cycas* rachis (10X,40X) showing parenchyma (PAR), sclerenchyma (SCL), xylem (XYL) phloem (PHL).

Plate9: Staining of *Gnetum* stem

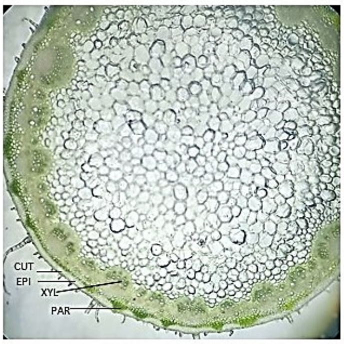
A.Unstained B.*Averrhoa carambola* stain



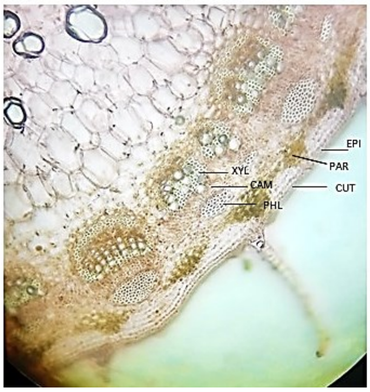
T.S of *Gnetum* stem (10X,40X) showing parenchyma (PAR) sclerenchyma (SCL), xylem (XYL) phloem (PHL).

Plate10: Staining of *Eupatorium* stem

A.Unstained



B.*Averrhoa carambola* stain

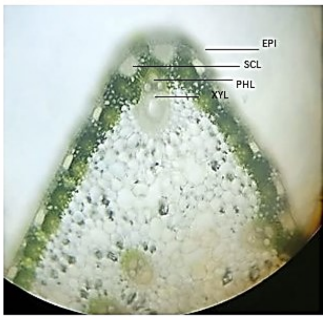


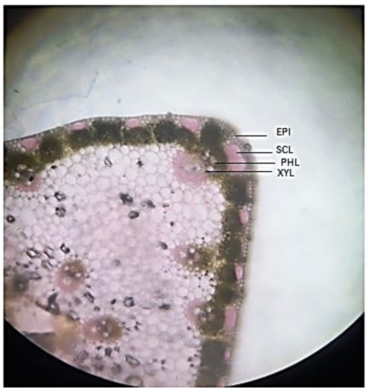
T.S of *Eupatorium* stem (10X,40X) showing parenchyma(PAR), sclerenchyma(SCL), xylem(XYL) phloem(PHL).

Plate11: Staining of *Cyprus* stem

A.Unstained

B.*Averrhoa carambola* stain





T.S of Cyprus stem (10X,40X) showing parenchyma (PAR), sclerenchyma (SCL), xylem (XYL) phloem (PHL).

CHAPTER - 6

CONCLUSION

Averrhoa carambola L. extract was derived from a natural plant source and offers more environmentally benign staining solution. The findings of current study suggests that *A. carambola* L. plant extract holds great ability for staining various plant tissues. Its ability to produce vivid and consistent staining across different tissue types including epidermis, parenchyma, vascular tissues highlight its versatility and applicability for a wide range of histological applications. One of the primary advantages of using this stain is its eco-friendliness and sustainability. It could be used as an alternative histological stain as they are non -toxic, non -carcinogenic, easy to use and cost effective compared to synthetic dyes. This was the first report of the use of *A. carambola* L. extract as a natural plant histological stain. Further studies are required to understand more staining ability of *A. carambola* L. in plant tissues as well as in animal tissues.

CHAPTER -7

REFERENCES

1. Affat, S. S. (2021). Classifications, advantages, disadvantages, toxicity effects of natural and synthetic dyes: a review. *University of Thi-Qar Journal of Science*, *8* (1), 130-135
2. Aggarwal, S. (2021, February). Indian dye yielding plants: Efforts and opportunities. In *Natural Resources Forum* (Vol. 45, No. 1, pp. 63-86). Oxford, UK: Blackwell Publishing Ltd.
3. Ahsan, R., Masood, A., Sherwani, R., & Khushbakhat, H. (2020). Extraction and application of natural dyes on natural fibers: an eco-friendly perspective. *Review of Education, Administration & LAW*, *3*(1), 63-75.
4. Akinloye, A. J., Illoh, H. C., & Olagoke, A. O. (2010). Screening of some indigenous herbal dyes for use in plant histological staining. *Journal of Forestry Research*, *21*, 81-84.
5. AlAshkar, A., & Hassabo, A. G. (2021). Recent use of natural animal dyes in various field. *Journal of Textiles, Coloration and Polymer Science*, *18*(2), 191-210.
6. Alturkistani, H. A., Tashkandi, F. M., & Mohammedsaleh, Z. M. (2016). Histological stains: a literature review and case study. *Global journal of health science*, *8*(3), 72.
7. Ardila-Leal, L. D., Poutou-Piñales, R. A., Pedroza-Rodríguez, A. M., & Quevedo-Hidalgo, B. E. (2021). A brief history of colour, the environmental impact of synthetic dyes and removal by using laccases. *Molecules*, *26*(13), 3813.
8. Ayele, A., Getachew, D., Kamaraj, M., & Suresh, A. (2021). Phycoremediation of synthetic dyes: an effective and eco-friendly algal technology for the dye abatement. *Journal of Chemistry*, *2021*, 1-14.

9. Bai, B., Yang, X., Li, Y., Zhang, Y., Pillar, N., & Ozcan, A. (2023). Deep learning-enabled virtual histological staining of biological samples. *Light: Science & Applications*, *12*(1), 57.

10. Baig, U., Khatri Hossain, S., Jalil, M. A., Kamal, S. A. B., & Kader, A. (2021). A natural dye extracted from the leaves of Mimusops elengi Linn and its dyeing properties on cotton and silk fabrics. *The Journal of the Textile Institute*, *112*(3), 455-461.

11. Bal, G., & Thakur, A. (2022). Distinct approaches of removal of dyes from wastewater: A review. *Materials Today: Proceedings*, *50*, 1575-1579.

12. Baldacci-Cresp, F., Spriet, C., Twyffels, L., Blervacq, A. S., Neutelings, G., Baucher, M., & Hawkins, S. (2020). A rapid and quantitative safranin-based fluorescent microscopy method to evaluate cell wall lignification. *The Plant Journal*, *102*(5), 1074-1089.

13. Baqri, S. S. R. (2023). Dye-Yielding Insects. *Commercial Insects*, 218-237.

14. Benard, S. A. (2008). Iron-roselle: A progressive nuclear stain substitute for hematoxylin. *Journal of Histotechnology*, *31*(2), 57-59.

15. Benkhaya, S., M'rabet, S., & El Harfi, A. (2020). A review on classifications, recent synthesis and applications of textile dyes. *Inorganic Chemistry Communications*, *115*, 107891

16. Bora, P., Das, P., Bhattacharyya, R., & Barooah, M. S. (2019). Biocolour: The natural way of colouring food. *Journal of Pharmacognosy and Phytochemistry*, *8*(3), 3663-3668.

17. Bujak, T., Zagórska-Dziok, M., Ziemlewska, A., Nizioł-Łukaszewska, Z., Lal, K., Wasilewski, T., & Hordyjewicz-Baran, Z. (2022). Flower extracts as multifunctional dyes in the cosmetics industry. *Molecules*, *27*(3), 922.

18. Cauley, A. N., & Wilson, J. N. (2017). Functionalized lignin biomaterials for enhancing optical properties and cellular interactions of dyes. *Biomaterials science*, *5*(10), 2114-2121.

19. Choudhury, A. K. R. (2018). Eco-friendly dyes and dyeing. *Advanced Materials and Technologies for Environmental*, *2*, 145-76.

20. Chingangbam, D. S., Singh, T. A., & Singh, O. B. (2023). High potential biological stains from the traditional dyes of Manipur, India.

21. Chungkrang, L., Bhuyan, S., & Phukan, A. R. (2020). Natural dye sources and its applications in textiles: a brief review. *International Journal of Current Microbiology and Applied Sciences*, *9*(10), 261-269

22. Cruz, P. E., De Vera, A. P., & Villa, A. D. (2018). The efficiancy of Santan flower (Ixora occinea Linn.) as an alternative stain to eosinY in Wright-Giemsa stain. *LPU-Laguna Journal of Allied Medicine*, *3*(1), 11-21.

23. Das, B. C., Reji, N., & Philip, R. (2021). Optical limiting behavior of the natural dye extract from Indigofera Tinctoria leaves. *Optical Materials*, *114*, 110925.

24. Dawes, C. J. (1966). A light and electron microscope survey of algal cell walls. II, Chlorophyceae.

25. Deepak, M. S., & Omman, P. (2013). Use of dye extract of Melastoma malabathricum Linn. for plant anatomical staining. *Acta Biologica Indica*, *2*(2), 456-460.

26. Deepali, K., Lalita, S., Deepika, M., Stem, M., & Hibiscus, F. O. (2014). Application of aqueous plant extracts as biological stains. *International Journal of Scientific & Engineering Research*, *5*(2), 1586-1589.

27. Dibal, N., Garba, S., & Jacks, T. (2022). Histological stains and their application in teaching and research. *Asian Journal of Health Sciences*, *8*(2), ID43.

28. Dina H. Sadiq, Abdul M. Ghalib, Diyar Mohammad Hussein, Hiba Huthaim Qasim. (2021). Histologystaning with an Alternative Natural Dye (Daucus Carota L.). *Annals of the Romanian Society for Cell Biology*, 6080–6084.
29. Dulo, B., Phan, K., Githaiga, J., Raes, K., & De Meester, S. (2021). Natural quinone dyes: a review on structure, extraction techniques, analysis and application potential. *Waste and Biomass Valorization*, *12*(12), 6339-6374.
30. Enaru, B., Drețcanu, G., Pop, T. D., Stănilă, A., & Diaconeasa, Z. (2021). Anthocyanins: Factors affecting their stability and degradation. *Antioxidants*, *10*(12), 1967.
31. Ferrara, L. (2018). Averrhoa carambola Linn: is it really a toxic fruit?. *International Journal of Medical Reviews*, *5*(1), 2-5.
32. Gamble, M., & Wilson, I. (2008). The hematoxylins and eosin. *Theory and practice of histological techniques*, *6*, 121-34.
33. Gowrishankar, N. L., Farsena, A., Mubashireen, R., Rameesa, K., VP, S. S., & Sinara, N. S. (2018). A complete review on: Averrhoa carambola. *Journal of Pharmacognosy and Phytochemistry*, *7*(3), 595-599
34. Hartika, G., Zulharmita, Z., & Asra, R. (2021). Utilization of natural dyes substances for histological staining: a review. *Asian Journal of Pharmaceutical Research and Development*, *9*(1), 149-158.
35. Henry, J. S. (2021). *Studies on cell wall composition in bryophytes across taxa, tissue, and time*. Southern Illinois University at Carbondale.
36. Horobin, R. W. (2008). How do histological stains work. *Theory and Practice of Histological Techniques. 6th ed. Philadelphia: Churchill Livingstone Elsevier Ltd*, 105-18.

37. Hubbe, M. A., Chandra, R. P., Dogu, D., & Van Velzen, S. T. J. (2019). Analytical staining of cellulosic materials: a review. *BioResources*, *14*(3), 7387-7464.

38. Islam, M. T., Islam, T., Islam, T., & Repon, M. R. (2022). Synthetic dyes for textile colouration: process, factors and environmental impact. *Textile and leather review*, *5*, 327-373.

39. Jan, H. U., Shinwari, Z. K., & Marwat, K. B. (2011). Influence of Herbal Dye Extracted From Dry Wood of Indigenous Berberis Pachyacantha Kochine In Plant Histological Staining. *Pak. J. Bot*, *43*(5), 2597-2600

40. Joshua, B. I., Ndam, K. S., Danjuma, G. D., & Gunya, D. Y. (2021). Assessing the use of Lawsonia Inermis and Hibiscus Sabdariffa Aqueous Extracts as a Possible Substitute for Eosin Stain in Paraffin-embedded Tissues. *International Journal of Medical Sciences and Academic Research*, *2*(2), 1-12.

41. Krampah, C., Nyanzu, F., Quaye, A., Adu, P., Asiamah, E. A., Aboagye, B., & Simpong, D. L. (2021). Crude extracts from Allium cepa skin and Sorghum bicolor seed can provide as non-toxic and eco-friendly cytoplasmic stains. *Practical Laboratory Medicine*, *26*, e00239.

42. Kumar, V., & Prabha, R. (2018). Extraction and analysis of natural dye. *J. Nat. Prod. Plant Resour*, *8*(2), 32-38.

43. Kusumawati, N., Samik, A. B., & Muslim, S. (2020). New natural dyes development: Caesalpinia Sappan L.-Curcuma Longa blended dyes. *Rasayan Journal of Chemistry*, *13*(2), 992-999.

44. Liao, K., Han, L., Yang, Z., Huang, Y., Du, S., Lyu, Q., ... & Shi, S. (2022). A novel in-situ quantitative profiling approach for visualizing changes in lignin and cellulose by stained micrographs. *Carbohydrate Polymers*, *297*, 119997.

45. Manzoor, J., & Sharma, M. (2020). Impact of textile dyes on human health and environment. In *Impact of textile dyes on public health and the environment* (pp. 162-169). IGI Global.

46. Mastrotheodoros, G. P., & Beltsios, K. G. (2022). Pigments—Iron-based red, yellow, and brown ochres. *Archaeological and Anthropological Sciences*, *14*(2), 35.

47. Mohd Basri, M. S., Liew Min Ren, B., A. Talib, R., Zakaria, R., & Kamarudin, S. H. (2021). Novel Mangosteen-Leaves-Based Marker Ink: Color Lightness, Viscosity, Optimized Composition, and Microstructural Analysis. *Polymers*, *13*(10), 1581.

48. Nicolai, E., & Preston, R. D. (1952). Cell-wall studies in the Chlorophyceae. I. A general survey of submicroscopic structure in filamentous species. *Proceedings of the Royal Society of London. Series B-Biological Sciences*, *140*(899), 244-274.

49. Noirbent, G., & Dumur, F. (2021). Photoinitiators of polymerization with reduced environmental impact: Nature as an unlimited and renewable source of dyes. *European Polymer Journal*, *142*, 110109.

50. Omar, A., Ali, M. S., & Abd Rahim, N. (2020). Electron transport properties analysis of titanium dioxide dye-sensitized solar cells (TiO2-DSSCs) based natural dyes using electrochemical impedance spectroscopy concept: A review. *Solar Energy*, *207*, 1088-1121.

51. Padayachee, A., Netzel, G., Netzel, M., Day, L., Zabaras, D., Mikkelsen, D., & Gidley, M. J. (2012). Binding of polyphenols to plant cell wall analogues–Part 1: Anthocyanins. *Food chemistry*, *134*(1), 155-161.

52. Pfeifer, L., Mueller, K. K., & Classen, B. (2022). The cell wall of hornworts and liverworts: innovations in early land plant evolution?. *Journal of Experimental Botany*, *73*(13), 4454-4472.

53. Phan, K., Raes, K., Van Speybroeck, V., Roosen, M., De Clerck, K., & De Meester, S. (2021). Non-food applications of natural dyes extracted from agro-food residues: A critical review. *Journal of Cleaner Production*, *301*, 126920.
54. Pizzicato, B., Pacifico, S., Cayuela, D., Mijas, G., & Riba-Moliner, M. (2023). Advancements in sustainable natural dyes for textile applications: A review. *Molecules*, *28*(16), 5954.
55. Pruś, S., Kulpiński, P., Matyjas-Zgondek, E., & Wojciechowski, K. (2022). Eco-friendly dyeing of cationised cotton with reactive dyes: mechanism of bonding reactive dyes with CHPTAC cationised cellulose. *Cellulose*, *29*(7), 4167-4182.
56. Rahman Bhuiyan, M. A., Ali, A., Islam, A., Hannan, M. A., Fijul Kabir, S. M., & Islam, M. N. (2018). Coloration of polyester fiber with natural dye henna (Lawsonia inermis L.) without using mordant: a new approach towards a cleaner production. *Fashion and Textiles*, *5*, 1-11
57. Rasool, W., Adeel, S., Batool, F., Ahmad, S. A., Mumtaz, S., & Hussaan, M. (2023). Environmental friendly silk and cotton dyeing using natural colorant of Bougainvillea (Bougainvillea glabra) flowers: the sustainable approach towards textile industry. *Environmental Science and Pollution Research*, *30*(8), 21863-21871.
58. Rehman, A., Usman, M., Bokhari, T. H., ul Haq, A., Saeed, M., Rahman, H. M. A. U., ... & Nisa, M. U. (2020). The application of cationic-nonionic mixed micellar media for enhanced solubilization of Direct Brown 2 dye. *Journal of Molecular Liquids*, *301*, 112408.
59. Richter, S., Müssig, J., & Gierlinger, N. (2011). Functional plant cell wall design revealed by the Raman imaging approach. *Planta*, *233*, 763-772.

60. Roberts, A. W., Roberts, E. M., & Haigler, C. H. (2012). Moss cell walls: structure and biosynthesis. *Frontiers in Plant Science*, *3*, 25695.
61. Roig-Oliver, M., Douthe, C., Bota, J., & Flexas, J. (2021). Cell wall thickness and composition are related to photosynthesis in Antarctic mosses. *Physiologia Plantarum*, *173*(4), 1914-1925.
62. Sahin, H. T., & Arslan, M. B. (2008). A study on physical and chemical properties of cellulose paper immersed in various solvent mixtures. *International Journal of Molecular Sciences*, *9*(1), 78-88.
63. Salauddin Sk M, Mia R, Haque MA, Shamim AM. Review on Extracton and Applicaton of Natural Dyes. Textle & Leather Review. 2021; 4(4):218-233.
64. Samant, L., Jose, S., Rose, N. M., & Shakyawar, D. B. (2022). Antimicrobial and UV protection properties of cotton fabric using enzymatic pretreatment and dyeing with Acacia catechu. *Journal of Natural Fibers*, *19*(6), 2243-2253
65. Samanta, A. K., & Singhee, D. (2023). Sources, Application, and Analysis of Natural Colorants: An Indian Perspective. *Handbook of Natural Colorants*, 103-159.
66. Samanta, P., Singhee, D., & Samanta, A. K. (2018). Fundamentals of natural dyeing of textiles: pros and cons. *Curr. Trends Fashion Technol. Textile Eng*, *2*(4)..
67. Sanda, B., & Liliana, I. (2021). Natural dye extraction and dyeing of different fibers: a review. *Innovative and Emerging Technologies for Textile Dyeing and Finishing*, 113-135.
68. Sanda, B., & Liliana, I. (2021). Natural dye extraction and dyeing of different fibers: a review. *Innovative and Emerging Technologies for Textile Dyeing and Finishing*, 113-135.
69. Sharma, A., & Singh, M. R. (2021). A Review on Historical Earth Pigments Used in India's Wall Paintings. *Heritage*, *4*(3), 1970-1994.

70. Shet Verenkar, N. G., & Sellappan, K. (2021). Evaluation of natural dyes Curcuma longa and Nyctanthes arbor-tristis with different mordants on plant tissues under fluorescence microscopy. *Microscopy Research and Technique*, *84*(5), 902-911.
71. Shindy, A. (2017). Problems and solutions in colors, dyes and pigments chemistry: a review. *Chem. Int*, *3*(2), 97-105.
72. Sikhruadong, S., Tanomtong, A., Wonkaonoi, W., & Gomontean, B. (2009). Chromosome staining of crinum lily (Crinum asiaticum L.) using natural dyes. *Cytologia*, *74*(1), 17-22.
73. Singh, M., Vajpayee, M., & Ledwani, L. (2021). Eco-friendly surface modification of natural fibres to improve dye uptake using natural dyes and application of natural dyes in fabric finishing: A review. *Materials Today: Proceedings*, *43*, 2868-2871.
74. Slama, H. B., Chenari Bouket, A., Pourhassan, Z., Alenezi, F. N., Silini, A., Cherif-Silini, H., ... & Belbahri, L. (2021). Diversity of synthetic dyes from textile industries, discharge impacts and treatment methods. *Applied Sciences*, *11*(14), 6255
75. Sobh, N., Elshemy, N., Nassar, S., & Ali, M. (2024). New insights into the role of color extraction from (Aegle Marmelos leaf) using a non-traditional heating source. *Pigment & Resin Technology*.
76. Srivatsav, P., Bhargav, B. S., Shanmugasundaram, V., Arun, J., Gopinath, K. P., & Bhatnagar, A. (2020). Biochar as an eco-friendly and economical adsorbent for the removal of colorants (dyes) from aqueous environment: A review. *Water*, *12*(12), 3561
77. Sudhakaran, A., Hallikeri, K., & Babu, B. (2018). Natural stains Zingiber officinale Roscoe (ginger) and Curcuma longa L.(turmeric)–A substitute to eosin. *AYU (An international quarterly journal of research in Ayurveda)*, *39*(4), 220-225.

78. Sujjaritthurakarn, P., Buatip, S., Kraiprom, T., Chamnien, A., Pohma, A., & Jantarat, S. (2022). Natural Dyes from Red Dragon Fruit (Hylocercus costaricensis) on Plant Chromosome Staining. *Agriculture Reports*, *1*(2), 1-7.

79. Suryawanshi, H., Naik, R., Kumar, P., & Gupta, R. (2017). Curcuma longa extract–Haldi: A safe, eco-friendly natural cytoplasmic stain. *Journal of Oral and Maxillofacial Pathology*, *21*(3), 340-344.

80. Susetyarini, E., Wahyuni, S., Kharoir, I., Husamah, H., & Setyawan, D. (2020, April). Influence of Erythrina crista-galli L. extract natural dye in plant histology staining. In *AIP Conference Proceedings* (Vol. 2231, No. 1). AIP Publishing.

81. Švarcová, S., Hradil, D., Hradilová, J., & Čermáková, Z. (2021). Pigments—copper-based greens and blues. *Archaeological and Anthropological Sciences*, *13*, 1-29.

82. Tibkawin, N., Suphrom, N., Nuengchamnong, N., Khorana, N., & Charoensit, P. (2022). Utilisation of Tectona grandis (teak) leaf extracts as natural hair dyes. *Coloration Technology*, *138*(4), 355-367.

83. Verma, S., & Gupta, G. (2017). Natural dyes and its applications: A brief review. *International Journal of Research and Analytical Reviews*, *4*(4), 57-60.

84. Wagiyanti, H., & Noor, R. (2017). Red dragon fruit (Hylocereus costaricensis Britt. Et R.) peel extract as a natural dye alternative in microscopic observation of plant tissues: The practical guide in senior high school. *JPBI (Jurnal Pendidikan Biologi Indonesia)*, *3*(3), 232-237.

85. Yadav, S., Tiwari, K. S., Gupta, C., Tiwari, M. K., Khan, A., & Sonkar, S. P. (2023). A brief review on natural dyes, pigments: Recent advances and future perspectives. *Results in Chemistry*, *5*, 100733.

86. Yeung, E. C. (1998). A beginner's guide to the study of plant structure. *Tested studies for laboratory teaching*, *19*, 125-141.

87. Yusuf, M., Shabbir, M., & Mohammad, F. (2017). Natural colorants: Historical, processing and sustainable prospects. *Natural products and bioprospecting*, *7*, 123-145.

88. Zanjanchi, F., Hadipour, N. L., Sabzyan, H., & Beheshtian, J. (2013). Theoretical investigation of azo dyes adsorbed on cellulose fibers: 1. Electronic and bonding structures. *Journal of the Iranian Chemical Society*, *10*, 985-999.b

89. Zhang, B., Gao, Y., Zhang, L., & Zhou, Y. (2021). The plant cell wall: Biosynthesis, construction, and functions. *Journal of Integrative Plant Biology*, *63*(1), 251-272