EXTRACTION OF NATURAL DYES FROM SELECTED PLANTS AND ITS POTENTIAL AS TEXTILE DYES AND BIOLOGICAL STAINS

Dissertation submitted to the
Goa University in partial fulfilment for
the requirement of

THE DEGREE OF MASTER OF

SCIENCE IN BOTANY

By

MISS AISHWARYA ASHOK GAUDE

Under the guidance of

PROF. S. KRISHNAN, M.Phil., Ph.D.

DEPARTMENT OF BOTANY
Goa University, Goa-403206
May 2022

CERTIFICATE

This is to certify that the dissertation entitled **"Extraction of Natural Dyes from Selected Plants and its Potential as Textile Dyes and Biological Stains"** submitted by **Miss Aishwarya Ashok Gaude** in partial fulfilment for the degree of **Master of Science in Botany, Goa University** is an authentic record of the dissertation carried out by her under my supervision and guidance.

Date:

Signature of Guide

(Prof. S. Krishnan)

DECLARATION

I hereby declare that this dissertation entitled **"Extraction of Natural Dyes from Selected Plants and its Potential as Textile Dyes and Biological Stains"** is an authentic work done by **Miss Aishwarya Ashok Gaude**, student of M.Sc., Botany, Goa University, in partial fulfilment of the requirement of **Degree of Master of Science in Botany** for the University and no part has been presented before any other degree or diploma in any University.

Date: (**Miss Aishwarya Ashok Gaude)**

ACKNOWLEDGEMENTS

It gives me immense pleasure and deep feeling of satisfaction to extend my sincere thanks to all those who have helped me in the completion of my dissertation.

I express my sincere gratitude to my guide Dr. S. Krishnan, Professor and Head, Department of Botany, for his guidance, constant support and help throughout my dissertation work and also provided the facilities to carry out the research work.

I thank all the teachers Prof. B. F. Rodrigues, Prof. P. K. Sharma, Prof. M. K. Janarthanam, Prof. Vijaya Kerkar, Dr. Rupali Bhandari and Dr. Siddhi Jalmi for their encouragement, help and support during my dissertation work.

My sincere thanks to Mr. Paresh C. Porob, Assistant Conservator of Forest, Goa Forest Department for giving valuable information on local dye yielding plants of Goa.

I am thankful to the non-teaching staff for their help during the completion of my dissertation work. I would also like to thank the Research scholars for their help and advice.

I am immensely thankful to my parents and my friends for their help and great support in successful completion of my dissertation work.

(**Miss Aishwarya Ashok Gaude)**

CONTENTS

LIST OF TABLES

LIST OF PLATES

Heliotropium indicum; d. Ethyl acetate extract of *Phyllanthus emblica*, e. Ethyl acetate extract of *Artocarpus lakoocha*, f. Ethyl acetate extract of *Heliotropium indicum*; g. Aqueous extract of *Phyllanthus emblica*, h. Aqueous extract of *Artocarpus lakoocha*, i. Aqueous extract of *Heliotropium indicum*.

33. TLC plates showing separation of phenols. 1. Under visible light; 2. Under long UV; a. Methanol extract of *Phyllanthus emblica*, b. Methanol extract of *Artocarpus lakoocha*, c. Methanol extract of *Heliotropium indicum*; d. Ethyl acetate extract of *Phyllanthus emblica*, e. Ethyl acetate extract of *Artocarpus lakoocha*, f. Ethyl acetate extract of *Heliotropium indicum*; g. Aqueous extract of *Phyllanthus emblica*, h. Aqueous extract of *Artocarpus lakoocha*, i. Aqueous extract of *Heliotropium indicum*. 60

LIST OF FIGURES

1. INTRODUCTION

1.1. Natural dyes

Natural dyes can be obtained from different sources such as plants, animals, insects and fungi. From the plants, dyes can be extracted from different parts such as roots, stem, leaves, fruits and flowers. Natural dyes were the main textile dyes till the 19th century, later they were substituted by synthetic dyes. Synthetic dyes gained popularity because of their easy application, availability and good fastness properties (Bechtold *et al*., 2006).

Production of natural dyes by cultivating the crops can be expensive. Natural dyes can also be obtained from the by-products of other agricultural activities, like the bark of dye yielding plants from the timber industry and from the food and beverage industry wastes that contain the dyes (Bechtold *et al*., 2003).

The use of natural dyes for textile dyeing declined during the second half of the nineteenth century, upon the discovery of synthetic dyes. Synthetic dyes offered a lot of advantages over the natural dyes. They were easy to produce and also their application was easier. They showed good fastness properties on the fabrics. They were available in varied shades. All these benefits of synthetic dyes replaced the natural dyes.

Synthetic dyes were far superior to natural dyes in their availability and application but they had a negative impact on the environment. Many of the synthetic dyes contained carcinogenic substances (Kulkarni *et al.*, 2011). Based on researches on synthetic dyes it has been observed that this dyes contain various toxic elements such as pentachlorophenol, formaldehyde, lead, zinc, benzidine, cadmium, aryl amine, mercury and halogen carriers (Singh & Srivastava, 2017).

Azo dyes are synthetic dyes which are stable in aerobic conditions but in anaerobic conditions they form aromatic amines. These azo dyes are associated with several health problems including cancer and birth defects (Ahlström *et al*., 2005). These dyes are therefore banned in China, Japan, India, Vietnam and European Union. Such kinds of toxic dyes also remain in the dye bath and leads to pollution of the environment (Rai et al., 2005). Due to these reasons, the natural dyes again gained the popularity and developed interest amongst the researchers to study the dye extraction from natural sources (Kiumarsi *et al*., 2009) for their commercialization.

1.2. Difference between dye and pigment

Dyes are the substances used to colour textiles, paper and other materials. Dyes should have some inherent characteristics such that apart from imparting colour, they should be able to resist any change due to washing, exposure to light and other factors (Stothers *et al*., 2019). Pigments and dyes are different from each other. Pigments are insoluble while the dyes are soluble in water. Dyes are mostly organic whereas pigments are mostly inorganic. Dyes get absorbed on the materials they are applied, while pigments are adsorbed onto the surface. Dye molecules are finer compared to the pigments. Pigments exhibit better light fastness than the dyes.

1.3. History

Natural dyes have been used from as early as Indus Valley period (2500 BC), ruins of garments dyed with madder dye have been obtained from Mohenjodaro and Harappa (Siva, 2007). They were used in cave paintings, evidence of their use have been obtained from the paintings of Ajanta, Ellora and Sithannvasal caves (Singh, 2019). Fabrics dyed with natural dyes have been obtained from Egyptian tombs which were more than 4000 years old (Stothers *et al*., 2019). In the past people used to dye their clothes with locally

available material. These dye yielding plants were selected based on the colour which they left on the soil or when touched accidentally. Natural dyes started to decline because of the emergence of synthetic dyes.

1.4. Characterization of natural dyes

A molecule of dye consists of two chemical groups, viz. chromophores and auxochromes. Chromophores impart the colouring property to the dye and they are mostly composed of an aromatic ring, while auxochrome plays a role in attaching the dye molecule to the substrate. Chromophores shows the presence of unsaturated double bonds and the number of these unsaturated double bonds determine the intensity of the colour developed by the dye molecule (Krishnamurthy, 1999).

Primary characterization of natural dyes can be done by phytochemical analysis, UV-Visible spectroscopy and thin layer chromatography (TLC). For detailed characterization methods like High Performance Liquid Chromatography (HPLC), Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography and Mass Spectrometry (GC-MS) are employed. UV-Vis spectroscopy helps in the characterization of colour based on the absorption maximum and the colour components can be identified by thin layer chromatography (Algarni, 2020) whereas methods like FTIR, GC-MS gives detailed information about the components of the dye.

1.5. Mordants

Mordants improve the binding of the dye with the fabric by forming complexes with the fibers and the dye molecules. Mordants also improve the fastness properties of the dyed fabrics. The functional groups in the dye molecules react with the metal ions in the

mordants to form insoluble compounds (Vankar, 2000). Mordants improve the dyeing procedure and also provide a range of shades for the fabrics.

In the past dyeing was carried out by boiling the plant parts in water to extract the dye and then applying it on textile fabrics. This method did not give good fastness of the dye to the fabrics; later people discovered that use of salt, vinegar from fermenting fruits and natural alum enhanced the fastness properties of dyes. These substances were used as mordants for many centuries to improve the efficiency of dyes. Salt fixes the dyes and vinegar dispels the carbonates in hard water and improves all dyes of red and purple colour (Singh, 2019).

Myrobalan and chitosan are the natural mordants that have been used traditionally in the dyeing. Myrobalan is natural tannin and chitosan is a linear polysaccharide formed by deacetylation of chitin (Kasiri & Safapour, 2014). These mordants improve the affinity between the dye and the fabric, thus improving the fastness properties of dyes.

Metallic mordants like potassium dichromate, copper sulphate, ferrous sulphate, ferric chloride, stannous chloride are used for mordanting. Oil mordants are also used. Metallic and oil mordants are sometimes used together. This combination of mordants gives better wash fastness as the metal atom and oil forms a complex and binds firmly to the dye molecules (Vankar, 2000).

1.6. Dyeing cotton and silk fibers with natural dyes

Cotton cultivation for commercial textiles is thought to have begun from Harappa civilisation by using Asiatic cotton (Rajendran et al., 2005). Making of cotton clothing have been recorded for over 7,000 years (Gray, 2014). Cotton is widely used in the textile industry because of its good absorbency; it can hold water 24-27 times its own weight.

Cotton fibers are soft, strong, can withstand abrasion and high temperature and gives comfortable feel (Hosseini Ravandi & Valizadeh, 2011). Cotton provides half of the world's requirement of fibers (Ali & Sarwar, 2010).

Silk is obtained from the cocoons of *Bombyx mori*. Production of silk and its weaving originated in China. The Silk Road brought Chinese silk to Syria and Rome and later on it spread to other parts of the world (Britannica, 2019). Silk fibers are used in the textile industry because of its softness, lustre and good draping ability (Roy & Choudhury, 2017). Silk shows good heat resistance and high tensile strength. Natural dyes tend to have better affinity for protein fibers, like silk than the fibers containing cellulose, such as cotton (Haji, 2019).

1.7. Biological stain

Natural dyes have the potential to be used as stains for microbes, spores, fungal, plant and animal tissues. Majority of the stains that are being used currently are chemically synthesized and they are mostly toxic to humans as well as the environment. Hence, natural dyes serve as better alternatives to be used as biological stains. Natural dyes were used as biological stains before the use of synthetic stains; haematoxylin is an example of such natural dye. It is a nuclear stain that is obtained from the Mexican tree *Haematoxylon campechianum* (Suryawanshi *et al*., 2017). Natural dyes have the ability to show differential staining of the plant and animal tissues (Suryawanshi *et al*., 2017). The stain obtained from madder can be used to stain the nuclear material; to study the different phases of cell cycle (Manimekalai *et al*., 2018).

1.8. Classification of dyes

Dyes can be classified based on their chemical structures as follows (Vankar, 2000; Algarni, 2020):

- Anthraquinone class: these dyes have anthraquinone structure. They are red in colour and usually show good light and wash fastness. These can be obtained from plants and insects, such as madder, lac, kermes and cochineal.
- Flavones: these are hydroxyl and methoxy derivatives of flavones and isoflavones. They give yellow colour. Weld dye is a flavone.
- Carotenoids: these dyes have long chain conjugated double bonds. They give orange colour. These dyes can be obtained from saffron and annatto.
- Indigoid class: These occur as glucoside indicant in the plants. Indigo blue and tyrian purple dyes belong to this class.
- Alpha naphthoquinone class: these dyes have alpha naphthoquinone structure such as 2-hydroxy 1-4-naphthoquinone. They are obtained from henna, lawsone and juglone.
- Dihydropyrans: these dyes produce dark black shade. These dyes are extracted from logwood and sappan wood.
- Anthocyanidins: these dyes give blue and orange shade. Carajurin shows the presence of anthocyanidins.

1.9. Benefits of natural dyes

- The most significant benefit of natural dyes is that they are environment friendly and biodegradable compared to synthetic dyes which usually produce toxic wastes which are non-biodegradable.

- Most of the plants used for the extraction of natural dyes show medicinal properties and have antimicrobial properties (Hussein *et. al*., 1997). Clothes dyed with natural dyes are used in Ayurveda, they are referred to as 'Ayurvastra'.
- Extraction and purification of natural dyes is easier. They are obtained from renewable resources (Algarni, 2020).
- Fabrics dyed with natural dyes shows better UV protection. These fabrics show enhanced absorption of UV rays, thus protecting the skin (Grifoni *et al*., 2011).
- They are suitable for sensitive skin as they are obtained from nature. They do not cause allergic reactions.

1.10. Limitations of natural dyes

- The shade of the natural dye obtained from plants varies according to the conditions of growth, the time of harvest of the plant part for extraction and maturity of that part.
- Mordants are required to enhance the binding of dye to the fabric and some of the metallic mordants are hazardous (Vankar, 2000).
- Standardization of natural dyes is difficult and the dyeing procedure is lengthy (Affat, 2021).

1.11. OBJECTIVES

The present work was carried out with the following objectives:

1. Extraction of natural dyes from different plant species.
2. Optimization of extraction conditions.
3. Dyeing of silk and cotton fibers with/without mordants and the fastness properties of the dyed fibers.
4. Use of dye as biological stain for monocot and dicot plant stem.
5. Phytochemical analysis of the dye and its characterization by UV-Visible Spectroscopy and Thin Layer Chromatography.

2. REVIEW OF LITERATURE

2.1 Natural dyes

Bechtold *et al*. (2006) extracted natural dyes from coloured plant wastes released from the food and beverage industry for textile dyeing. They extracted the dye in boiling water from the wastes of pressed berries, pressed grapes, distillation residues from liquor production and peels from vegetable processing. They used wool yarn for dyeing with and without mordants and performed the colour fastness tests.

Kulkarni *et al.* (2011) extracted and purified the natural dye from pomegranate peel by solvent extraction method and used it for dyeing scoured cotton cloth using copper sulphate and ferrous sulphate as the mordants. They also estimated the production cost of pomegranate peel dye.

Geetha and Sumathy (2013) extracted the natural dyes from five plants, namely, *Caesalpinia pulcherima, Bougainvillea glabra, Beta vulgaris, Brassica oleracea* and *Allium cepa* and used it for dyeing cotton fabric. They performed the fastness tests on the dyed fabrics and also performed IR spectrophotometry on the extracted dye.

Arora *et al*. (2017) extracted natural dyes from different plants like *Curcuma longa, Terminalia chebula*, *Carthamus tinctorius*, *Berberis lyceum*, etc. and used it for dyeing cotton, silk and wool fabrics. For dyeing three mordants, namely, alum, copper sulphate and ferrous sulphate were used and pre-, post- and simultaneous mordanting techniques were employed. They obtained a rainbow of natural dyes by using different combinations of plants and mordants. The dyes were extracted under acidic, neutral and alkaline conditions. They found that alkaline medium showed better dye extraction.

Manicketh and Francis (2019) extracted the natural dyes from *Araucaria columnaris*, *Macaranga peltata* and *Averrhoa bilimbi* and studied its application on textiles. They extracted the dye by aqueous method and checked its dyeing ability on cotton, silk and polyester yarns with and without mordants. They also performed UV-Visible spectral analysis and pH test for the extracted dyes. They found that the extracted dyes from the selected plants showed good fastness and the dyeing could be achieved at room temperature on the selected yarns.

Jiang *et al*. (2021) studied the photo fading of alizarin which is the main dye molecule of madder. They studied the photo fading process of alizarin dyed fabrics by UV, HPLC, HPLC-MS and Datacolor software. In the presence of light, alizarin is oxidized which leads to its fading. They also studied the effect of pre-mordanting and liquid rate on the photo fading of alizarin dyed fabrics.

Kandasamy *et al*. (2021) extracted the dye from *Pterocarpus indicus* Willd. sawdust using ultrasound assisted technique and studied its dyeing properties on cotton and silk fabrics. They pre-treated the fabrics with chitosan and myrobalan to study their effect on dyeing and they also used various mordants such as alum, stannous chloride, copper sulphate, gallnut, pomegranate rind and gooseberry. They found that the natural mordants showed fastness properties comparable to metallic mordants. The pre-treatments improved the dyeing and the fastness properties of the dye.

Andriamanantena *et al*. (2021) worked on five plants from Madagascar which yield red dyes and studied their antimicrobial activity. They used pressurized liquid extraction method for the extraction of dye from the bark and adventives roots of *Acridocarpus excelsus, Ceriops tagal, Rhizophora mucronata, Woodfordia fructicosa* and *Xylocarpus*

granatum. They found that these dyes had good pH stability in the pH range of 3-9 and also great thermal stability up to 200°C.

Dyes are extracted from the mesocarp and exocarp of *Cocos nucifera* for textile dyeing by Rodiah *et al*. (2022). The dye was extracted using different concentrations of sodium hydroxide and different concentrations of tannic acid were used for mordanting. They found that the dye extraction was optimum in 0.8 M NaOH and increasing concentration of tannic acid improved the fastness of the dye on the fabric.

2.2 Methods of extraction

In order to enhance extraction of dye from various plant parts, different techniques are being employed. Some of these methods not only enhance the process of extraction but also improve the fastness properties of dyes. Methods used in dye extraction are given below (Algarni, 2020):

- Aqueous extraction: the plant material is powdered, soaked in water and then boiled to extract the dye. It is then filtered to remove the debris. This method has a disadvantage, some of the dye components may decompose due to boiling.
- Acid and alkali extraction: the dyes are extracted in acidic or alkaline medium. Dyes with phenolic groups are extracted by alkaline extraction method.
- Ultrasonic microwave extraction: ultrasonic and microwave waves are used to produce vibrations in the extraction medium in order to extract the dye. There are several advantages of this method; the dye components are not decomposed as the dye is extracted at low temperature, amount of solvent required is less and also extraction is better.
- Fermentation: the plant materials are fermented by using bio enzymes to extract the dye. Indigo and annatto dye is extracted by this method.

- Solvent extraction: organic solvents are used in the extraction. Solvents like acetone, ether, chloroform, ethanol, etc. can be used.

Sivakumar *et al*. (2011) studied the extraction of dye from green wattle bark, marigold flowers, pomegranate rinds, 4'o clock plant flowers and cocks comb flowers by using ultrasound. Magnetic stirring process was used as control. They carried out UV-Vis spectrophotometry and gravimetric analysis to check the dye extraction. They found that, there was 13-100% improvement in the extraction efficiency of dye obtained by ultrasound.

Taghizadeh Borujeni *et al*. (2021) extracted the dye from *Reseda luteola* L. using membrane processes and studied its dyeing properties on wool fibers. They used ultra-filtration and nano-filtration membranes and reverse osmosis for extraction of dye. The extracted dyes showed better colour strength, light fastness, rubbing fastness and wash fastness. The dyes showed better fastness properties because they were extracted by physical processes, so that no chemical changes occurred in the dye extracts.

2.3 Natural dyes as biological stain

Saffron has been used as a stain for animal tissue sections from since 1719 by Leeuwenhoek (Clark & Kasten, 1983). Conn (1953) evaluated the use of saffron for staining the connective tissue and differential staining of glandular cells of stomach. Stain obtained from saffron is used in fluorescence microscopy. Trigoso & Stockert (2005) stained the chicken, rat, horse and human blood smears with saffron and observed that under violet-blue excitation filter the acidophilic granules in the cytoplasm of mammalian eosinophils and chicken heterophils fluoresces bright yellow-green. These results indicated that saffron dye can be used as a fluorochrome.

Jan *et al*. (2011) evaluated the role of dye extracted from dry wood of *Berberis pachyacantha* in staining of plant tissues. They used the stem sections of *Zea mays* and *Helianthus anuus* to study the staining effect. They extracted the dye in three different solvents, *viz.* clove oil, ethanol and water. They observed that monocot stem was stained most effectively by dye extracted in clove oil whereas dicot stem was stained by the dye extracted in ethanol.

Tousson & Al-Behbehani (2011) studied the use of *Morus nigra* dye as stain for animal tissues. They used this dye for staining the whole mount and transverse sections of Fasciola (adult liver fluke). Carmine, hematoxyllin and eosin staining method were used as control. They also used the beet root extract for staining. They found that beet root and *Morus nigra* dye stained sections can help in the identification and differentiation between the parasites.

Saiki *et al*. (2011) extracted the dye from *Syzygium cumini* (black plum) fruit and used it as a histological stain on rat hepatic tissue. They extracted the dye from both fresh and oven dried fruits; in distilled water and 45% glacial acetic acid. They studied its staining properties at different time duration. They also used 1% ferric alum as a mordant and studied its effect on staining. They observed that the extract stained the nucleus and cytoplasm in violet colour. Optimal staining results were shown by dried fruit extract in distilled water, when stained for 15 minutes. These results indicate that *Syzygium cumini* fruit dye can be used for histological staining and cytotoxicity testing in cosmetic.

Deepali *et al*. (2014) extracted the dyes by aqueous extraction from six plants; *Lawsonia inermis, Hibiscus rosa-sinensis, Rubia tinctorium, Butea monosperma, Rosa indica* and *Bougainvillea galabra* and studied their property as biological stain on different specimens such as fungus, paramecium, plant and animal tissues. They observed that rose

and bougainvillea extracts were the best for staining fungus and plant tissues and for animal tissues; rose, hibiscus and henna showed better staining.

Eosin, a synthetic stain is used as a counter stain with hematoxylin, to search for a natural substitute to eosin, Suryawanshi *et al*. (2017) explored the use of *Curcuma longa* extract as a counterstain for hematoxylin. They dried the turmeric rhizomes and powdered them. The dye was extracted by dissolving the powder in alcohol and centrifuging. They used the supernatant as a counter stain for eight different tissues such as epithelium, keratin, collagen fibres, muscles, adipocytes, blood vessels and red blood cells. Turmeric stain was found to be comparable with the eosin stain for epithelium, keratin, muscle, adipocytes, blood vessels and red blood cells however for cartilage, collagen fibres and bone, it did not show good staining. It showed distinct shades of yellow for the above mentioned five tissues. Epithelium and keratin was stained deep yellowish orange, collagen and muscle were stained dull yellow, red blood cells were bright yellow and bone was stained deep yellow. These results indicated that *Curcuma longa* stain can be efficiently used as a counter stain for hematoxylin and as an alternative for eosin.

Shet Verenkar & Sellappan (2021) extracted the dye from *Curcuma longa* and *Nyctanthes arbour-tristis* and evaluated its potential as a stain for plant tissue under fluorescence microscope along with different mordants. They found that the colour and intensity varied along with the mordants used. Fluorescence was better in plant sections stained with *Nyctanthes arbour-tristis* extract compared to the *Curcuma longa* extract.

2.4 Benefits of textiles dyed with natural dyes

Most of the plants that yield dye are medicinal; hence, they exhibit antimicrobial activities. Singh *et al.* (2005) studied the antimicrobial activity of natural dyes from five plants namely, *Acacia catechu, Kerria lacca, Quercus infectoria, Rubia cordifolia* and

Rumex maritimus against *Escherichia coli, Bacillus subtilis, Klebsiella pneumonia, Proteus vulgaris* and *Pseudomonas aeruginosa*. They found that *Quercus infectoria* dye showed maximum antimicrobial activity. The antimicrobial activity of the dyed textile material was less compared to the dye powder.

Selvam *et al*. (2015) extracted natural dyes from *Curcuma longa, Trigonella foenum graecum* and *Nerium oleander* and studied their phytochemical and pharmacological characteristics. The dye extraction was carried out using aqueous, acidic, alcoholic and alkaline extraction techniques. They found that dyes from turmeric and fenugreek showed antibacterial activity and *Nerium oleander* dye showed antifungal activity.

Mansour *et al*. (2022) evaluated the UV protection and dyeing properties of wool fabrics dyed with aqueous extracts of madder roots, chamomiles, pomegranate peels and apple tree branches barks. They studied the effect on UV protection and dyeing with and without mordants and found that UV protection increased after natural dyeing which was further enhanced by mordanting with ferrous sulphate.

2.5 Characterization of natural dyes

Lee *et al*. (2013) characterized the natural dyes from Korean silk fabric by surface analytical techniques. They used the techniques like time-of-flight secondary ion mass spectrometry (TOF-SIMS), X-ray photoelectron spectroscopy (XPS) and Fourier transform Infrared Spectroscopy (FTIR) to study the constituents of the dye, from the surface of silk fabric dyed with natural dyes. TOF-SIMS spectra showed the presence of molecular ions from the plant dyes, element ions from metallic mordants and specific fragments ions. These kinds of methods can be employed for the characterization of dye, which does not

require the extraction of dye as is required for characterization of by UV-Visible and chromatography technique.

Espinosa Morales *et al*. (2012) extracted the dye from *Justicia spicigera* and characterized it by UV-Visible and FTIR spectroscopy and GC/MS chromatography techniques. UV-Vis analysis indicated the presence of anthocyanins and it was confirmed by FTIR and GC-MS, which showed the presence of polar hydroxybenzoic acids and phenolic compounds which are the components of anthocyanins.

Characterization of dyes can be useful in identifying the plants used for dyeing the ancient textiles. Liu *et al*. (2013) analyzed the natural textiles from Yingpan (archeological site on the Silk Road) using high performance liquid chromatography with diode array and mass spectrometry. They removed the dyes from the textiles using soft extraction method. They were able to identify plants *Rubia tinctorum* and *Rubia cordifolia* as dye sources in textiles having red and brown colour, *Phellodendron* spp. as the dye source in yellow and green silk threads. Based on their results they proposed that the textile dyes found at Yingpan indicate the merging of Easten and Western practices during the early years of the Silk Route.

3. MATERIALS AND METHODS

3.1 Collection of plants

The plants selected for the extraction of the dye were *Artocarpus lakoocha, Calliandra surinamensis, Cymbopogon citratus, Heliotropium indicum, Piper betle* and *Phyllanthus emblica*. The plants were collected from different parts of Ponda taluka. Bark of *Artocarpus lakoocha* and *Calliandra surinamensis* and leaves of *Cymbopogon citratus, Heliotropium indicum, Piper betle* and *Phyllanthus emblica* were used for dye extraction.

3.2 Extraction of dye

The collected plant parts were washed thoroughly and dried in the shade. The dried material was then ground into fine powder. The dye was then extracted in distilled water by boiling the powdered sample.

3.3 Optimization of dye extraction parameters

Extraction time and temperature was optimized by keeping the material to liquor ratio constant.

3.3.1 Optimization of extraction time

The dye was extracted by adding 1g of the powdered plant sample to 10 ml of distilled water and kept in water bath at 60°C for varied time duration viz. 60 min, 120 min, 180 min and 240 min. The extracts were filtered using Whatman filter paper Grade-A. The optimum dye extraction time was determined by taking the optical density of the filtered extracts in the range of 200-800 nm by UV-Visible spectrophotometry. The time duration with highest optical density value was considered as optimal. The readings were taken in replicates of three.

3.3.2 Optimization of extraction temperature

Based on the optimum extraction time and with constant material to liquor ratio, the dye was extracted at varying temperatures viz. 40° C, 60° C and 80° C. The extracts were filtered using Whatman filter paper Grade – A. The optimum dye extraction temperature was determined by taking the optical density of the filtered extracts in the range of 200 -800 nm by UV-Visible spectrophotometry. The temperature with highest optical density value was considered as optimal. The readings were taken in replicates of three.

3.4 Pantone matching system (PMS) chart

The colour of the extracted natural dyes was determined by comparing with corresponding colour code on PMS chart (https://www.pantone-colours.com/).

3.5 Dyeing of cotton and silk fibers with natural dyes

3.5.1 Pre treatment of cotton fibers and silk fibers

Prior to dyeing the cotton and silk fibers were treated with sodium carbonate solution (2g/l) for 30 minutes at 60°C in hot water bath. The fibers were then washed with water to remove the traces of sodium carbonate. The pre-treated fibers were then dried at room temperature and used for dyeing.

3.5.2 Dyeing of cotton and silk fibers

The cotton and silk fibers were dyed with the natural extracts of *Artocarpus lakoocha, Calliandra surinamensis, Cymbopogon citratus, Heliotropium indicum, Piper betle* and *Phyllanthus emblica*. Six mordants were used for dyeing, namely, ferrous sulphate ($FeSO_4$), copper sulphate ($CuSO_4$), ferric chloride ($FeCl_3$), stannous chloride

($SnCl_2$), potassium alum ($KAl(SO_4)_2.12H_2O$) and potassium dichromate ($K_2Cr_2O_7$). Mordant solution was prepared by adding 1 g of mordant to 100 ml of distilled water.

Dyeing was carried out by pre-, post- and simultaneous mordanting methods (Samanta & Agarwal, 2009). In pre-mordanting the fibers were first treated with the mordant for one hour and then placed in the dye solution for one hour. In post-mordanting the fibers were first placed in the dye solution for one hour and then transferred to the mordant solution for one hour. In simultaneous mordanting fibers were dyed in the dye bath containing both the dye and the mordant.

The colour obtained on the dyed fabrics were determined by comparing with the ISCC-NBS (Inter-Society Color Council – National Bureau of Standards) colour system (Kelly and Deane, 1976).

3.5.3 Colourfastness tests of the dyed fibers

The dyed fibers were subjected to colour fastness to rubbing, light and washing. The colour change after the fastness tests was determined by comparing with the standard gray scales (ISO 105-A02).

Colourfastness to rubbing: The dyed cotton and silk fibers were placed between the tissue paper and rubbed manually for 5 minutes.

Colourfastness to light: the dyed fibers were kept in the direct sunlight for 6 hours.

Colourfastness to washing: the dyed fibers were washed with sodium carbonate solution (2g/L) for about 10 minutes and then washed with water and allowed to dry.

The photographs of the dyed cotton and silk fibers were taken with Nikon digital camera.

3.6 Extraction of dye for biological staining

The dye was extracted by adding 10 g of powdered sample in 50 ml of distilled water and boiling in a water bath. The extract was then filtered through Whatman filter paper Grade-A. the filtered extract was then dried over the water bath. 1 g of dye powder was dissolved in 1 ml of distilled water and was used as a stain.

3.6.1 Sectioning of plant material

The stem of monocot and dicot plant was selected for sectioning. For monocot plant *Eleusine indica* stem was chosen and for dicot *Hibiscus rosa-sinensis* was selected. Free-hand thin sections were taken using a sharp blade in water. The thin sections were chosen and used for staining.

3.6.2 Staining of plant sections

The sections were stained for 5, 10, 15 and 20 minutes to find the optimum staining time. After staining the excess stain was removed by washing the sections in distilled water. The sections were mounted on a clean slide in a drop of glycerine.

The sections were observed under bright field microscope (Nikon Y-TV55) and the staining was observed. The sections were also observed under fluorescence microscope in three filters viz. Ultraviolet excitation filter (UV-2A) (330-380 nm), violet excitation filter (V-2A) (380-420 nm) and blue excitation filter (FITC) (465-495 nm). The photographs of the sections were taken with the photographic unit attached with the microscope.

3.7 Qualitative phytochemical screening of natural dyes

The powdered plant samples were evaluated for the presence or absence of different phytochemicals. Extraction was carried out by maceration using three solvents, methanol, ethyl acetate and water. 10 g of powdered plant sample was added to 100 ml of the solvent and kept on the rotary shaker for three days. The extract was then filtered using Whatman filter paper Grade-A. The filtered extract was dried using rotary evaporator. The powdered extract was used for the phytochemical tests.

3.7.1 Test for Alkaloids

Wagner's test: 50 mg of powdered extract is dissolved in 4 ml of dilute HCl and filtered. To few ml of the filtrate, few drops of Wagner's reagent were added. Appearance of reddish brown precipitate indicates the presence of alkaloids (Wagner, 1993).

3.7.2 Test for Anthraquinones

Add 2 ml of benzene to 50 mg of the extract and filter. To the filtrate add 1 ml of ammonia solution and shake vigorously for 30 seconds. Pink, red or violet colour in the ammonia phase indicates the presence of anthraquinones (Gul *et al*., 2017).

3.7.3 Test for Carbohydrates

Benedict's test: 50 mg of extract is dissolved in 5 ml of distilled water and filtered. To 0.5 ml of the filtrate 0.5 ml of Benedict's reagent is added and kept in water bath for 2 minutes. Reddish brown precipitate indicates the presence of carbohydrates (Sofowora, 1993).

3.7.4 Test for Coumarins

NaOH test: 50 mg of the plant extract is dissolved in 5 ml of distilled water and filtered. To 1 ml of filtrate add 10% NaOH and chloroform. Appearance of yellow colour indicated the presence of coumarins (Jagessar and Cox, 2010).

3.75 Test for Flavonoids

Lead acetate test: 50 mg of the plant extract is dissolved in 5 ml of distilled water and filtered. To 5 ml of the filtrate add 3 ml lead acetate solution. Appearance of yellow precipitate indicates the presence of flavonoids (Vimalkumar *et al*., 2014).

3.7.6 Test for Proteins

Biuret test: 50 mg of the plant extract is dissolved in 5 ml of distilled water and filtered. 2 ml of filtrate is treated with one drop of 2% copper sulphate solution. To this 1 ml of 95% ethanol is added followed by excess of potassium hydroxide pellets. Pink colour in the ethanolic layer indicated the presence of proteins (Gahan, 1984).

3.7.7 Test for Saponins

Foam test: 50 mg of the plant extract is dissolved in 5 ml of distilled water and filtered. To 5 ml of the filtrate add 20 ml of distilled water and shake. Formation of stable foam indicates the presence of saponins (Auwal et al., 2014).

3.7.8 Test for Steroids

Liebermann Burchard reagent test: 50 mg of the extract is added to 2 ml of acetic anhydride followed by adding 2 drops of concentrated H_2SO_4 along the sides of the test tube. Appearance of red, blue to green colour indicates the presence of steroids (Cook, 1961).

3.7.9 Test for Tannins

Ferric chloride test: 50 mg of the plant extract is dissolved in 5 ml of distilled water and filtered. To 2 ml of the filtrate add few drops of 10% ferric chloride solution. Occurrence of blackish blue colour indicates the presence of gallic tannins and a green-blackish colour indicates the presence of catechol tannins (Auwal *et al*., 2014).

3. 7.10 Test for Terpenoids

5 g of powdered extract is dissolved in ethanol and filtered. To 2 ml of the filtrate add 1 ml acetic acid and few drops of concentrated H_2SO_4 along the sides of the test tube. Appearance of pink to violet colour indicates the presence of terpenoids (Sofowora, 1993).

3.8 Characterization of natural dyes

The methanolic, ethyl acetate and aqueous extracts of the selected plants, *Artocarpus lakoocha*, *Heliotropium indicum* and *Phyllanthus* emblica were used for the characterization.

3.8.1 UV-Visible spectroscopy

The extract of each sample was taken in a quartz cuvette and the blank was set by using the respective solvent. The spectrum of the extract was taken in the 200 - 800 nm range using UV-1800 Shimadzu UV spectrophotometer. The peaks obtained in the spectrum indicate the maximum absorption by a particular component at that wavelength.

3.8.2 Thin Layer Chromatographic analysis

Thin Layer Chromatography (TLC) was used for the separation of flavonoids and phenolics. Silica gel slurry was prepared and coated on the glass slide to prepare the TLC plate. The TLC plates were then activated by keeping in the oven at 60°C for 2 hours. 10µl

of each extract was loaded above 2 cm from the edge of the plate and allowed to dry. The plate was then developed in the chromatography chamber containing the particular solvent system. The solvent system used for separation of flavonoids was methanol:chloroform: hexane (7:2:1) (v/v/v). For phenols toluene:acetone:formic acid (4.5:4.5:1) (v/v/v) solvent system was used. The solvent was allowed to travel three forth of the plate. The plates were then removed and the solvent front was marked.

Resolution of TLC plates: the plates were exposed to ammonia fumes and then visualized under long UV using UV trans-illuminator. The bands visualized on each plate were photographed and their Rf values were calculated.

4. RESULTS

4.1 Selection of dye yielding plants

The plants selected for dye extraction were *Artocarpus lakoocha, Cymbopogon citratus, Calliandra surinamensis, Heliotropium indicum, Piper betle* and *Phyllanthus emblica*. The plant part used for dye extraction and the Pantone Matching System (PMS) colour code of the dye obtained is provided in Table 1; Plate 1-2.

Table 1. Plant name, part used and PMS colour code of the extracted dye.

Plant	Plant part used	Colour code of the dye
Artocarpus lakoocha	Bark	Pantone 1805
Calliandra surinamensis	Bark	Pantone 1795
Cymbopogon citratus	Leaves	Pantone 188
Heliotropium indicum	Leaves	Pantone 490
Phyllanthus emblica	Leaves	Pantone 412
Piper betle	Leaves	Pantone 173

4.2 Optimization of extraction parameters

Extraction time and temperature were optimized for the extracted dyes based on the optical density values.

4.21 Optimization of time

The optimum time duration for dye extraction varied for different plants. For *Calliandra surinamensis* and *Heliotropium indicum* the optimum time was 60 minutes,

Cymbopogon citrates, *Piper betle* and *Phyllanthus emblica* showed maximum extraction at 120 minutes while for *Artocarpus lakoocha* optimum time for dye extraction was 240 minutes (Table 2).

Table 2. Optimization of extraction time.

Plant species	**Wavelength λ_{max} (nm)**	**Absorbance at different time (min)**			
		60	**120**	**180**	**240**
Artocarpus lakoocha	274	0.654 ±0.01	0.655±0.05	0.709±0.03	**0.825±0.04**
Cymbopogon citratus	270	0.281±0.02	**0.445±0.04**	0.298±0.05	0.216±0.01
Calliandra surinamensis	279	**1.435±0.05**	1.295±0.04	1.194±0.05	0.993±0.04
Heliotropium indicum	271	**1.072±0.04**	0.881±0.01	0.486±0.02	0.398±0.03
Piper betle	279	0.291±0.01	**0.313±0.01**	0.216±0.004	0.173±0.02
Phyllanthus emblica	268	1.118±0.01	**1.485±0.01**	1.239±0.03	0.964±0.03

4.2.2 Optimization of extraction temperature

Based on the readings of the optimum extraction time, the optimum extraction temperature was determined for each plant. *Cymbopogon citratus*, *Calliandra surinamensis*, *Heliotropium indicum* and *Piper betle* showed maximum extraction at 60°C. Optimum extraction temperature for *Artocarpus lakoocha* and *Phyllanthus emblica* were 40°C and 80°C respectively (Table 3).

Table 3. Optimization of extraction temperature

Plant species	Wavelength λ_{max} (nm)	Absorbance at different temperature (min)		
		40	**60**	**80**
Artocarpus lakoocha	274	**1.124±0.02**	0.827±0.03	0.549±0.01
Cymbopogon citratus	270	0.216±0.007	**0.473±0.009**	0.171±0.02
Calliandra surinamensis	279	1.304±0.02	**1.426±0.04**	1.106±0.03
Heliotropium indicum	271	0.358±0.01	**0.853±0.03**	0.282±0.03
Piper betle	279	0.392±0.03	**0.801±0.05**	0.261±0.004
Phyllanthus emblica	268	0.767±0.008	0.866±0.03	**0.993±0.04**

4.3 Dyeing of cotton fibers

4.3.1 Dyeing of cotton fibers with *Calliandra surinamensis* dye

Dyeing with *Calliandra surinamensis* dye produced moderate orange colour. Ferrous sulphate mordant produced dark gray, copper sulphate produced moderate orange yellow, ferric chloride developed brownish black, stannous chloride developed pale orange yellow and alum mordant produced pale yellow colour. The colours produced were same for pre-, post- and simultaneous mordanting method except for potassium dichromate mordant which produced dark yellowish brown in pre and simultaneous mordanting and moderate brown colour in post mordanting method (Plate 3).

4.3.2 Dyeing of cotton fibers with *Cymbopogon citratus* dye

Dyeing with *Cymbopogon citratus* dye produced moderate yellow colour. Ferrous sulphate mordant in pre-mordanting produced dark yellowish brown colour, in post-mordanting it gave moderate yellowish brown colour and in simultaneous mordanting there was production of grayish yellowish brown colour. Copper sulphate mordant in pre-mordanting developed moderate yellow colour while in post and simultaneous mordanting dark yellow colour was produced. Ferric chloride mordant gave grayish brown colour in pre-mordanting, dark grayish yellow in post-mordanting and grayish yellow colour in simultaneous mordanting. Stannous chloride and potassium dichromate mordant produced pale yellow colour in all three mordanting types. Alum produced moderate yellowish brown in pre-mordanting and dark grayish yellow in post and simultaneous mordanting (Plate 4).

4.3.3 Dyeing of cotton fibers with *Piper betle* dye

Dyeing with *Piper betle* dye produced moderate orange yellow colour. Ferrous sulphate mordant produced moderate yellowish brown colour in pre-mordanting and grayish yellowish brown in post- and simultaneous mordanting. Copper sulphate produced moderate brown in pre-mordanting and grayish yellowish brown in post- and simultaneous mordanting. Ferric chloride mordant produced brownish gray in pre-mordanting and grayish yellow in post- and simultaneous mordanting. Stannous chloride and alum produced light yellowish brown colour in all three mordanting types. Potassium dichromate developed yellowish white in pre- and simultaneous mordanting and dark orange yellow in post-mordanting (Plate 5).

4.3.4 Dyeing of cotton fibers with *Artocarpus lakoocha* dye

Cotton fibers dyed with *Artocarpus lakoocha* dye produced brownish orange. The colour of the fibers did not change after rub, light and wash fastness. In pre-mordanting method, the dye developed grayish brown colour with ferrous sulphate and after the fastness tests the colour changed to dark gray. The dye with copper sulphate gave moderate brown colour to the fibers and it did not change after light and wash fastness, but showed slight fading after rub fastness. Ferric chloride with the dye produced brownish gray colour, which after fastness tests changed to dark gray. The fibers dyed with stannous chloride and dye developed deep reddish orange colour and it changed to deep reddish brown colour after exposure to light. Strong brown colour was obtained with alum and not much change was observed after fastness tests. Potassium dichromate with the dye produced moderate brown colour which exhibited slight fading after light and wash fastness (Plate 6).

In post mordanting, grayish brown colour was obtained with ferrous sulphate, which changed to brownish gray after the fastness tests. Dye with copper sulphate produced brownish orange colour and did not show any alteration after the fastness tests. Application of ferric chloride mordant with the dye developed dark grayish yellowish brown colour which changed to dark gray after the rub, light and wash fastness tests. Stannous chloride mordant with dye gave light orange colour which was unchanged after rubbing. However, after light and wash fastness the colour changed to moderate orange. Brownish orange colour was developed with alum which was unchanged after light and wash fastness, but after rubbing colour changed to moderate orange. The use of potassium dichromate mordant with the dye produced moderate brown colour to the fibers which remain unchanged after the fastness tests (Plate 7).

In simultaneous mordanting, dye with ferrous sulphate produced brownish gray colour which remain unchanged after exposure to light. It changed to dark gray after light and wash fastness. Brownish orange colour was obtained with copper sulphate mordant which changed to moderate brown after the fastness tests. Ferric chloride along with dye developed moderate brown colour which later changed to brownish gray after rub, light and wash fastness. Dye along with stannous chloride imparted moderate orange colour to the fibers which changed to light orange on rubbing and brownish orange after light and wash fastness. Brownish orange colour was developed with alum mordant which remained unchanged after light and wash fastness. However, it changed to moderate orange on rubbing. Potassium dichromate along with dye produced moderate brown colour which remained unchanged after rub fastness and it changed to light brown after light and wash fastness test (Plate 8).

4.341 Colour fastness results of the dyed fibers

The fibers dyed only with *Artocarpus lakoocha* dye showed no colour change after the fastness tests. In pre-mordanting the use of mordants showed the gray scale ratings between 5 – 4 indicating that the dye along with the mordants showed good colour fastness properties. The lowest rating of 4 was obtained for potassium dichromate mordant (Table 4).

Table 4. Gray scale ratings of colour fastness of cotton fibers dyed with *Artocarpus lakoocha* dye (Pre-mordanting).

Dyes and mordants used	Rub fastness (5 minutes)	Light fastness (6 hours)	Wash fastness
Artocarpus lakoocha	5	5	5
Dye + $FeSO_4$	5	4/5	4/5
Dye + $CuSO_4$	5	5	5

Dye + $FeCl_2$	4/5	4/5	4/5
Dye + $SnCl_2$	5	5	5
Dye + Alum	4/5	5	5
Dye + $K_2Cr_2O_7$	5	4	4

In post-mordanting the gray scale ratings ranged from 5 – 3. Stannous chloride mordant showed poor fastness to rubbing. All other mordants showed good colour fastness (Table 5).

Table 5. Gray scale ratings of colour fastness of cotton fibers dyed with *Artocarpus lakoocha* dye (Post-mordanting).

Dyes and mordants used	**Rub fastness (5 minutes)**	**Light fastness (6 hours)**	**Wash fastness**
Dye + FeSO4	4/5	4	4
Dye + $CuSO_4$	5	4	4/5
Dye + $FeCl_2$	4	4	5
Dye + $SnCl_2$	3	5	5
Dye + Alum	4	5	5
Dye + $K_2Cr_2O_7$	4/5	4	4

In simultaneous mordanting the ratings ranged between 5 – ½. Potassium dichromate mordant showed very poor colour fastness to light and washing. Ferrous sulphate, copper sulphate and ferric chloride showed good fastness to colour while stannous chloride and alum showed moderate colour fastness (Table 6).

Table 6. Gray scale ratings of colour fastness of cotton fibers dyed with *Artocarpus lakoocha* dye (Simultaneous mordanting).

Dyes and mordants used	Rub fastness (5 minutes)	Light fastness (6 hours)	Wash fastness
Dye + FeSO4	5	5	4/5
Dye + $CuSO_4$	5	5	5
Dye + $FeCl_2$	5	4/5	4/5
Dye + $SnCl_2$	3/4	5	5
Dye + Alum	3	4/5	4/5
Dye + $K_2Cr_2O_7$	4/5	1/2	1/2

4.3.5 Dyeing of cotton fibers with *Heliotropium indicum* dye

Dyeing with *Heliotropium indicum* dye produced moderate brown colour which changed to moderate yellowish brown after the rub, light and wash fastness. In pre-mordanting, ferrous sulphate with the dye developed grayish brown colour which later changed to brownish gray after the fastness tests. Grayish brown colour was obtained with copper sulphate mordant which turned to light brownish gray after the fastness tests. Ferric chloride mordant imparted moderate yellowish brown colour to the fibers which changed to grayish yellowish brown after the fastness tests. Stannous chloride gave moderate brown colour with the dye which after the fastness tests changed to moderate yellowish brown. Moderate yellow colour was obtained with alum mordant which remained unchanged after exposure to light. However, after rub and wash fastness it changed to grayish yellow. Potassium dichromate along with dye imparted grayish yellow colour which later changed to yellowish gray after the fastness tests (Plate 9).

In post mordanting, ferrous sulphate produced moderate brown colour which changed to brownish gray after rub, light and wash fastness. Copper sulphate along with dye imparted strong yellowish brown colour which changed to moderate yellowish brown after washing and grayish yellowish brown after rub and light fastness test. Dark grayish yellowish brown colour was obtained with ferric chloride which on rub, light and wash fastness test changed to grayish yellowish brown. Application of stannous chloride imparted moderate yellow colour which on rubbing changed to pale yellow and grayish yellow after light and wash fastness test. Alum with dye produced moderate yellowish brown colour which remained unchanged after light and wash fastness test. However, on rubbing the colour changed to dark grayish yellow. Potassium dichromate along with the dye developed grayish yellow colour which changed to yellowish gray after the fastness tests (Plate 10).

In simultaneous mordanting, ferrous sulphate with dye developed grayish yellow colour which changed to yellowish gray after the fastness tests. Copper sulphate mordant produced dark yellowish gray colour which on rub, light and wash fastness changed to dark grayish yellow. Moderate yellowish brown colour was obtained on application of ferric chloride along with the dye which remained unchanged on washing. However, the colour changed to grayish brown yellow after rub and light fastness test. Stannous chloride mordant developed moderate yellow orange colour which changed to grayish yellow after the fastness tests. Alum mordant produced moderate yellow colour which remained unchanged on light exposure. It changed to grayish yellow after rub and wash fastness test. Grayish yellow colour was obtained with potassium dichromate mordant which changed to yellowish gray after the fastness tests (Plate 11).

4.3.5.1 Colour fastness results of the dyed fibers

The fibers dyed with *Heliotropium indicum* extract showed moderate colour fastness ratings. In pre-mordanting the ratings varied from 4 – ½ for the fibers dyed along with mordants. For ferrous sulphate, copper sulphate, ferric chloride and stannous chloride the colour fastness varied between 4 - 3. For alum it ranged between 4 – 2/3. Potassium dichromate mordant showed very poor rating of ½ for the colour fastness tests (Table 7).

Table 7. Gray scale ratings of colour fastness of cotton fibers dyed with *Heliotropium indicum* dye (Pre-mordanting).

Dyes and mordants used	**Rub fastness (5 minutes)**	**Light fastness (6 hours)**	**Wash fastness**
Heliotropium indicum	4	4	4
Dye + $FeSO_4$	3	3	3/4
Dye + $CuSO_4$	4	3/4	4
Dye + $FeCl_2$	4	3/4	3/4
Dye + $SnCl_2$	4	3/4	3
Dye + Alum	2/3	4	2/3
Dye + $K_2Cr_2O_7$	1/2	1/2	1/2

In post-mordanting, the gray scale ratings ranged between 4/5 – 3/4 for copper sulphate and ferric chloride mordant. Ferrous sulphate showed good colour fastness. Alum showed good fastness to light and washing while the colour faded after rubbing. The lowest gray scale ratings of 1/2 - 1 was obtained for potassium dichromate indicating that potassium dichromate has least colour fastness (Table 8).

Table 8. Gray scale ratings of colour fastness of cotton fibers dyed with *Heliotropium indicum* dye (Post-mordanting).

Dyes and mordants used	Rub fastness (5 minutes)	Light fastness (6 hours)	Wash fastness
Dye + FeSO4	4	4	4/5
Dye + $CuSO_4$	3/4	4/5	4/5
Dye + $FeCl_2$	3/4	4/5	4
Dye + $SnCl_2$	1/2	3	3/4
Dye + Alum	3	5	5
Dye + $K_2Cr_2O_7$	1/2	1	1

In simultaneous mordanting all the mordants showed moderate colour fastness to rubbing, light and washing except for potassium dichromate which showed the lowest ratings of colour fastness (Table 9).

Table 9. Gray scale ratings of colour fastness of cotton fibers dyed with *Heliotropium indicum* dye (Simultaneous mordanting).

Dyes and mordants used	Rub fastness (5 minutes)	Light fastness (6 hours)	Wash fastness
Dye + FeSO4	3/4	4	4
Dye + $CuSO_4$	4	4	4
Dye + $FeCl_2$	3/4	4/5	4/5
Dye + $SnCl_2$	3/4	4	4
Dye + Alum	3	4	3
Dye + $K_2Cr_2O_7$	1/2	1	1

4.3.6 Dyeing of cotton fibers with *Phyllanthus emblica* dye

Dyeing with *Phyllanthus emblica* dye developed moderate brown colour which changed to brownish gray after the fastness tests. In pre-mordanting, dye with ferrous sulphate developed black colour which remained unchanged after the fastness tests. Copper sulphate with the dye gave brownish orange colour which showed change to moderate brown after the fastness tests. Dark gray colour was obtained with ferric chloride. It remained unchanged after was fastness, while it changed to black colour after rub and light fastness. Application of stannous chloride with dye gave light yellow colour to the fibers which changed to moderate yellow after the fastness tests. Alum mordant along with the dye imparted moderate yellow colour to the fibers which changed to grayish yellow after the colour fastness tests. Grayish yellow colour was produced by potassium dichromate mordant which changed to dark grayish yellow after the fastness tests (Plate 12).

In post-mordanting, ferrous sulphate mordant with the dye produced dark bluish gray colour which later changed to blackish blue colour after the fastness tests. Copper sulphate along with the dye imparted moderate yellow colour which turned to dark grayish yellow after the rub, light and wash fastness. Dark gray colour was developed with ferric chloride mordant which after the fastness tests changed to bluish black. Stannous chloride and alum mordant showed similar colours. Using these mordants produced moderate yellow colour which later changed to grayish yellow after the rub, light and wash fastness. Dark grayish yellow colour was obtained by potassium dichromate mordant which remained unchanged after the fastness tests (Plate 13).

In simultaneous mordanting, ferrous sulphate developed black colour which later changed to bluish black after the fastness tests. Copper sulphate mordant along with the

dye produced dark grayish yellow which turned to grayish yellow after the fastness tests. Dark gray colour was obtained with ferric chloride mordant which after the rub, light and wash fastness changed to medium gray colour. Application of stannous chloride with dye imparted moderate orange yellow colour to the fibers which changed to grayish yellow after the fastness tests. Alum mordant along with the dye developed grayish yellow colour which changed to yellowish gray after rub, light and wash fastness. Moderate yellow colour was produced by potassium dichromate mordant which changed to yellowish gray after the fastness tests (Plate 14).

4.3.6.1 Colour fastness results of the dyed fibers

The *Phyllanthus emblica* dyed cotton fibers showed the ratings between 3/4 - 3, indicating moderate fastness to colour. In pre-mordanting the fastness ratings varied between 5 – 3 indicating moderate to good colour fastness (Table 10).

Table 10. Gray scale ratings of colour fastness of cotton fibers dyed with *Phyllanthus emblica* dye (Pre-mordanting).

Dyes and mordants used	Rub fastness (5 minutes)	Light fastness (6 hours)	Wash fastness
Phyllanthus emblica	3/4	3	3
Dye + $FeSO_4$	4/5	4	4/5
Dye + $CuSO_4$	3/4	3/4	3/4
Dye + $FeCl_2$	5	5	4/5
Dye + $SnCl_2$	3/4	4	4
Dye + Alum	4	3	4
Dye + $K_2Cr_2O_7$	4/5	3	3

In post-mordanting ferrous sulphate and ferric chloride showed good fastness to colour with ratings between 5 – 4/5 while other mordants showed moderate colour fastness. Alum and potassium dichromate showed the gray scale ratings between 3/4 – 2/3 (Table 11).

Table 11. Gray scale ratings of colour fastness of cotton fibers dyed with *Phyllanthus emblica* dye (Post-mordanting).

Dyes and mordants used	**Rub fastness (5 minutes)**	**Light fastness (6 hours)**	**Wash fastness**
Dye + $FeSO_4$	5	4/5	4/5
Dye + $CuSO_4$	4	3/4	4
Dye + $FeCl_2$	5	4/5	4/5
Dye + $SnCl_2$	3	4	4/5
Dye + Alum	3/4	3	2/3
Dye + $K_2Cr_2O_7$	3/4	3	3/4

In simultaneous mordanting, the colour fastness varied from 4 – 1. Potassium dichromate showed the least colour fastness (1/2 – 1). Most of the mordants showed moderate to poor colour fastness (Table 12).

Table 12. Gray scale ratings of colour fastness of cotton fibers dyed with *Phyllanthus emblica* dye (Simultaneous-mordanting).

Dyes and mordants used	**Rub fastness (5 minutes)**	**Light fastness (6 hours)**	**Wash fastness**
Dye + $FeSO_4$	3/4	4	4
Dye + $CuSO_4$	2/3	2/3	3
Dye + $FeCl_2$	3	3/4	3

Dye + $SnCl_2$	2	3/4	3/4
Dye + Alum	1/2	1/2	1/2
Dye + $K_2Cr_2O_7$	1/2	1	1

4.4 Dyeing of silk fibers

4.4.1 Dyeing of silk fibers with *Calliandra surinamensis* dye

Dyeing with *Calliandra surinamensis* dye produced yellowish white colour. Ferrous sulphate mordant developed bluish gray in pre- and post-mordanting and dark grayish olive in simultaneous mordanting. Copper sulphate mordant produced yellowish white in pre- and post-mordanting and grayish yellow in simultaneous mordanting. Ferric chloride produced olive gray and alum produced yellow white in all three mordanting types. Stannous chloride produced pale orange yellow in pre- and simultaneous mordanting and pale yellow in post-mordanting. Potassium dichromate produced pale orange yellow in pre-mordanting, brownish orange in post-mordanting and moderate brown colour in simultaneous mordanting (Plate 15).

4.4.2 Dyeing of silk fibers with *Cymbopogon citratus* dye

Dyeing with *Cymbopogon citratus* dye produced grayish yellow colour. Ferrous sulphate produced olive gray in pre-mordanting, grayish olive in post-mordanting and light olive gray in simultaneous mordanting. Copper sulphate produced moderate greenish yellow; ferric chloride produced light grayish olive and stannous chloride developed pale yellow colour in all three mordanting methods. Alum produced moderate yellow in pre- and simultaneous mordanting and yellowish white in post-mordanting. Potassium

dichromate produced dark grayish yellow in pre- and simultaneous mordanting and moderate yellow in post-mordanting (Plate 16).

4.4.3 Dyeing of silk fibers with *Piper betle* dye

Dyeing with *Piper betle* dye produced grayish yellow colour. Ferrous sulphate produced yellowish gray, copper sulphate produced yellowish white, stannous chloride produced pale yellow and alum produced yellowish white colour in all the mordanting types. Ferric chloride produced yellowish white in pre-mordanting and grayish yellow in post- and simultaneous mordanting. Potassium dichromate produced yellowish white in pre-mordanting and strong yellow in post- and simultaneous mordanting (Plate 17).

4.4.4 Dyeing of silk fibers with *Artocarpus lakoocha* dye

Dyeing with *Artocarpus lakoocha* dye produced moderate orange yellow colour which remained unchanged after rub and light fastness, but changed to pale orange yellow colour on washing.

In pre-mordanting, ferrous sulphate mordant with dye developed black colour which changed to dark gray on rubbing, light gray on exposure to light and medium gray on washing. Copper sulphate mordant gave moderate orange colour which remained unchanged after the fastness tests. Ferric chloride with dye imparted dark gray colour which did not change after light and rub fastness. However, on rubbing it changed to moderate gray. Light orange yellow colour was obtained with stannous chloride which remained same on rubbing and changed to moderate orange on exposure to light and washing. Moderate orange yellow colour was developed with alum which did not change after the fastness tests. Potassium dichromate mordant along with the dye produced dark brown colour which remained the same after fastness tests (Plate 18).

In post-mordanting dark gray colour was developed with ferrous sulphate which remained unchanged after light and wash fastness but changed light gray on rubbing. Copper sulphate mordant produced dark orange yellow colour which remained unchanged after rub fastness, changed to pale yellow on exposure to light and moderate yellowish brown on washing. Ferric chloride mordant along with the dye gave brownish gray colour which remained unchanged after the fastness tests. Light orange colour was obtained with stannous chloride mordant which remained unchanged after the rub, light and wash fastness tests. Alum mordant with dye developed yellow white colour which remained same after rubbing and changed to pale yellow after light and wash fastness tests. Potassium dichromate mordant gave dark brown colour which remained unchanged after the fastness tests (Plate 19).

In simultaneous mordanting, ferrous sulphate mordant produced dark gray colour which remained same after light and wash fastness but changed to medium gray on rubbing. Copper sulphate developed moderate brown colour which did not change after the rub, light and wash fastness tests. Olive gray colour was obtained with ferric chloride which did not change after the fastness tests. Stannous chloride mordant produced pale orange yellow colour which remained unchanged after the rub test, however changed to moderate orange yellow after light and wash fastness tests. Alum mordant along with the dye produced pale yellow colour which remained unchanged after rubbing but changed to light yellow after light and wash fastness tests. Dark brown colour was developed with potassium dichromate mordant which remained unchanged after the fastness tests (Plate 20).

4.4.4.1 Colour fastness of the dyed fibers

The silk fibers dyed with *Artocarpus lakoocha* dye showed good colour fastness to rubbing and light with gray scale ratings of 5 but showed poor fastness to washing with 1/2 gray scale value. In pre-mordanting the ratings were between 5 - 3/4 for all the mordants except ferrous sulphate which showed the ratings between 4/5 – 2/3. Most mordants provided good colour fastness to the fibers (Table 13).

Table 13. Gray scale ratings of colour fastness of silk fibers dyed with *Artocarpus lakoocha* dye (Pre-mordanting).

Dyes and mordants used	Rub fastness (5 minutes)	Light fastness (6 hours)	Wash fastness
Artocarpus lakoocha	5	5	1/2
Dye + $FeSO_4$	4/5	2/3	3
Dye + $CuSO_4$	5	5	5
Dye + $FeCl_2$	4/5	5	5
Dye + $SnCl_2$	3/4	5	5
Dye + Alum	4/5	5	5
Dye + $K_2Cr_2O_7$	5	5	4/5

In post-mordanting the gray scale values ranged between 5 – 3 suggesting good colour fastness after rubbing, exposure to light and washing the silk fibers (Table 14).

Table 14. Gray scale ratings of colour fastness of silk fibers dyed with *Artocarpus lakoocha* dye (Post-mordanting).

Dyes and mordants used	Rub fastness (5 minutes)	Light fastness (6 hours)	Wash fastness
Dye + $FeSO_4$	4/5	5	5

Dye + $CuSO_4$	4/5	3	5
Dye + $FeCl_2$	5	5	5
Dye + $SnCl_2$	3/4	5	4/5
Dye + Alum	4/5	5	5
Dye + $K_2Cr_2O_7$	5	5	4/5

In simultaneous mordanting the fastness values varied between 5 – 2/3. Potassium dichromate and copper sulphate showed moderate colour fastness while other mordants showed good colour fastness (Table 15).

Table 15. Gray scale ratings of colour fastness of silk fibers dyed with *Artocarpus lakoocha* dye (Simultaneous mordanting).

Dyes and mordants used	**Rub fastness (5 minutes)**	**Light fastness (6 hours)**	**Wash fastness**
Dye + $FeSO_4$	3	4	4
Dye + $CuSO_4$	2/3	4/5	4/5
Dye + $FeCl_2$	4/5	5	5
Dye + $SnCl_2$	4	5	5
Dye + Alum	3/4	5	5
Dye + $K_2Cr_2O_7$	3/4	2/3	2/3

4.4.5 Dyeing of silk fibers with *Heliotropium indicum* dye

Dyeing with *Heliotropium indicum* dye produced light greenish yellow colour which remained same after exposure to light but changed after rub and wash fastness tests to pale greenish yellow. In pre-mordanting, ferrous sulphate mordant along with the dye

produced olive gray colour which remained unchanged after rub and wash fastness tests, however changed to light olive gray on exposure to light. Copper sulphate mordant developed grayish yellow colour which remained same after the fastness tests. Ferric chloride mordant gave light brownish gray colour which remained unchanged after the fastness tests. Grayish yellow colour was obtained with stannous chloride mordant which changed to pale yellow after rub, light and wash fastness. Alum mordant produced dark grayish yellow colour and potassium dichromate produced yellow white colour which remained unchanged after the fastness tests (Plate 21).

In post-mordanting, ferrous sulphate with dye produced grayish olive colour and copper sulphate produced light grayish olive colour which remained the same after the fastness tests. Ferric chloride mordant developed dark grayish yellow which remained unchanged after washing and changed to grayish yellow after exposure to light and rubbing. Grayish yellow colour was obtained with stannous chloride mordant and yellowish white colour was obtained with alum mordant which remained unchanged after the fastness tests. Potassium dichromate mordant gave strong yellow colour which remained after rubbing and changed yellowish white after light and wash fastness tests (Plate 22).

In simultaneous mordanting, ferrous sulphate mordant produced light grayish olive colour, copper sulphate developed greenish white which remained unchanged after rub, light and wash fastness tests. Ferric chloride mordant with dye gave moderate yellowish brown colour which remained same after rub and light fastness tests but changed to dark grayish yellowish brown after washing. Yellowish gray colour was obtained with stannous chloride mordant which remained unchanged after rubbing and changed to yellow white after light and wash fastness tests. Alum mordant produced grayish yellow colour which remained unchanged after the fastness tests. Potassium dichromate mordant with dye

developed moderate yellow colour which remained same after rubbing and changed to pale yellow after light and wash fastness tests (Plate 23).

4.4.5.1 Colour fastness of the dyed fibers

The fibers dyed with *Heliotropium indicum* dye showed good fastness to light, moderate fastness to rubbing and poor fastness to washing. In pre-mordanting the gray scale values ranged between 4/5 – 1/2. Except ferrous sulphate all other mordants showed poor colour fastness (Table 16).

Table 16. Gray scale ratings of colour fastness of silk fibers dyed with *Heliotropium indicum* dye (Pre-mordanting).

Dyes and mordants used	Rub fastness (5 minutes)	Light fastness (6 hours)	Wash fastness
Heliotropium indicum	3/4	4/5	2/3
Dye + $FeSO_4$	4/5	3	4
Dye + $CuSO_4$	1/2	4/5	1/2
Dye + $FeCl_2$	2/3	2/3	2/3
Dye + $SnCl_2$	1/2	2/3	1/2
Dye + Alum	2/3	3/4	3/4
Dye + $K_2Cr_2O_7$	1/2	1	1

In post-mordanting ferrous sulphate, stannous chloride and alum showed moderate colour fastness (4/5 – 2/3) while other mordants showed poor fastness to colour with ratings ranging from (4/5 – 1) (Table 17).

Table 17. Gray scale ratings of colour fastness of silk fibers dyed with *Heliotropium indicum* dye (Post-mordanting).

Dyes and mordants used	Rub fastness (5 minutes)	Light fastness (6 hours)	Wash fastness
Dye + $FeSO_4$	3	4	4
Dye + $CuSO_4$	2/3	2/3	3
Dye + $FeCl_2$	1/2	3	5
Dye + $SnCl_2$	3	3/4	4
Dye + Alum	4/5	3	2/3
Dye + $K_2Cr_2O_7$	4/5	1/2	1

In simultaneous mordanting silk fibers with ferrous sulphate, copper sulphate, ferric chloride, stannous chloride and alum showed good colour fastness. Potassium dichromate showed poor colour fastness (Table 18).

Table 18. Gray scale ratings of colour fastness of silk fibers dyed with *Heliotropium indicum* dye (Simultaneous mordanting).

Dyes and mordants used	Rub fastness (5 minutes)	Light fastness (6 hours)	Wash fastness
Dye + $FeSO_4$	3	4	4/5
Dye + $CuSO_4$	5	5	5
Dye + $FeCl_2$	4/5	5	5
Dye + $SnCl_2$	4/5	3/4	3/4
Dye + Alum	3/4	4/5	4/5
Dye + $K_2Cr_2O_7$	4	1/2	1/2

4.4.6 Dyeing of silk fibers with *Phyllanthus emblica* dye

Dyeing with *Phyllanthus emblica* dye produced light olive brown colour which changed to moderate yellow after the fastness tests. In pre-mordanting ferrous sulphate mordant with dye gave dark gray colour, copper sulphate mordant developed light olive brown colour, black colour was obtained with ferric chloride mordant and stannous chloride mordant produced dark orange colour. All these colours remained unchanged after the rub, light and wash fastness tests. Dark yellow colour was obtained with alum mordant which changed to moderate yellow after the fastness tests. Potassium dichromate produced dark grayish yellow colour which changed to grayish yellow after the fastness tests (Plate 24).

In post-mordanting, ferrous sulphate produced black colour which remained unchanged after rubbing and changed to dark gray after light and wash fastness tests. Copper sulphate mordant with the dye developed light olive brown colour, ferric chloride imparted black colour to the fibers, moderate yellow colour was obtained with stannous chloride mordant and dark grayish yellow colour was produced by alum mordant. All these colours remained unchanged after the rub, light and wash fastness tests. Potassium dichromate mordant along with the dye produced strong yellowish brown colour which remained same after rub fastness test and changed to moderate yellow after light and wash fastness tests (Plate 25).

In simultaneous mordanting, ferrous sulphate mordant with the dye developed black colour which changed to light gray after exposure to light and dark gray after rub and wash fastness tests. Copper sulphate mordant produced deep yellowish brown colour which remained unchanged after the fastness tests. Ferric chloride mordant gave dark gray colour and stannous chloride mordant produced dark orange yellow colour which remained

unchanged after the fastness tests. Alum with dye produced strong yellowish brown colour which changed to dark orange yellow colour after the fastness tests. Grayish yellow colour was obtained with potassium dichromate mordant which remained unchanged after rubbing, however changed to yellow white after light and wash fastness tests (Plate 26).

4.4.6.1 Colour fastness of dyed silk fibers

The fibers dyed with *Phyllanthus emblica* dye showed good colour fastness. In pre-mordanting gray scale ratings ranged between 5 – 3. All mordants except potassium dichromate showed excellent colour fastness. Potassium dichromate showed moderate colour fastness (Table 19).

Table 19. Gray scale ratings of colour fastness of silk fibers dyed with *Phyllanthus emblica* dye (Pre-mordanting).

Dyes and mordants used	Rub fastness (5 minutes)	Light fastness (6 hours)	Wash fastness
Phyllanthus emblica	4	4	4
Dye + $FeSO_4$	5	4/5	4/5
Dye + $CuSO_4$	5	5	5
Dye + $FeCl_2$	5	5	5
Dye + $SnCl_2$	5	4/5	4/5
Dye + Alum	5	4/5	4/5
Dye + $K_2Cr_2O_7$	3/4	3/4	3

In post-mordanting ferrous sulphate, copper sulphate and ferric chloride showed good colour fastness with ratings between 5 – 4. Stannous chloride, alum and potassium

dichromate showed moderate colour fastness with gray scale ratings of 4/5 – 2/3 (Table 20).

Table 20. Gray scale ratings of colour fastness of silk fibers dyed with *Phyllanthus emblica* dye (Post-mordanting).

Dyes and mordants used	Rub fastness (5 minutes)	Light fastness (6 hours)	Wash fastness
Dye + $FeSO_4$	5	4/5	4
Dye + $CuSO_4$	4/5	5	4/5
Dye + $FeCl_2$	5	4	4/5
Dye + $SnCl_2$	3	3	3
Dye + Alum	4/5	3/4	3/4
Dye + $K_2Cr_2O_7$	4/5	3	2/3

In simultaneous mordanting copper sulphate and ferric chloride showed good colour fastness. Potassium dichromate showed very poor gray scale ratings (3 – 1) indicating very poor fastness to colour. Ferrous sulphate showed good fastness to rubbing and washing but poor fastness to light exposure. Stannous chloride and alum showed moderate to poor colour fastness (Table 21).

Table 21. Gray scale ratings of colour fastness of silk fibers dyed with *Phyllanthus emblica* dye (Simultaneous mordanting).

Dyes and mordants used	Rub fastness (5 minutes)	Light fastness (6 hours)	Wash fastness
Dye + $FeSO_4$	4/5	1/2	4/5
Dye + $CuSO_4$	4/5	4/5	5
Dye + $FeCl_2$	4/5	4/5	4/5

Dye + $SnCl_2$	3	3	4
Dye + Alum	2	3	2
Dye + $K_2Cr_2O_7$	3	1/2	1

4.5 Staining of plant sections

4.51 Optimum staining time for sections

The monocot and dicot stem sections were stained for 5, 10, 15 and 20 minutes. *Artocarpus lakoocha* and *Calliandra surinamensis* dyes showed optimum staining after 10 minutes. *Heliotropium indicum* and *Piper betle* dyes showed proper staining after 15 minutes. *Phyllanthus emblica* dye showed optimum staining after 5 minutes. Staining the sections for 20 minutes led to over staining.

4. 52 Effects of natural dyes on monocot stem sections

The unstained monocot sections showed auto fluorescence under different excitation filters of the fluorescence microscope. Under UV excitation filter the epidermis and the vascular tissues showed white fluorescence, chlorophyll fluoresced red and ground tissue fluoresced light blue; under violet excitation filter the vascular tissues and bundle sheath fluoresced blue and the chlorophyll fluoresced light brown; under blue excitation filter the vascular tissues fluoresced green (Plate 27).

The section with *Artocarpus lakoocha* dye stained the vascular tissues in light brown colour under bright field microscope. Under UV excitation filter the epidermis, vascular tissue and the ground tissue fluoresced blue, chlorophyll fluoresced bright red. The violet excitation filter fluoresced the vascular tissues light blue and chlorophyll dark brown. The blue filter showed the vascular tissues green.

The dye extracted from the bark of *Calliandra sumariensis* stained the phloem dark orange brown, xylem and bundle sheath was stained light brown. The vascular tissues and bundle sheath fluoresced bluish white under UV and violet excitation filter. The blue filter green fluorescence of vascular tissues and bundle sheath.

The extracts of *Heliotropium indicum* leaf stained the epidermis yellowish brown while the vascular tissues were stained light brown under the bright field microscope. The UV excitation filter fluoresced the vascular tissues and bundle sheath bluish white. The vascular tissues, epidermis and ground tissues showed prominent blue fluorescence under violet excitation filter. Under blue excitation filter the vascular tissues fluoresced green.

The *Piper betle* dye stained the vascular tissues light brown. The vascular tissues and bundle sheath fluoresced bluish white under UV excitation filter, blue under violet filter and green under blue filter. The ground tissues did not show fluorescence.

The leaf extracts of *Phyllanthus emblica* stained the vascular tissues and bundle sheath brown under bright field microscope. The UV filter fluoresced the chlorophyll red, the vascular tissues, ground tissues and bundle sheath fluoresced bluish white. The bundle sheath showed blue fluorescence and chlorophyll showed red fluorescence under violet filter. The blue filter showed the green fluorescence of vascular tissues.

4. 5.3 Effects of natural dyes on dicot stem sections

The unstained sections of the dicot stem showed auto fluorescence under the excitation filters. The UV filter showed bluish white fluorescence of periderm, xylem, phloem and the pith, the chlorophyll fluoresced red. The periderm, xylem and phloem exhibited blue fluorescence under violet filter. Blue filter did not show fluorescence (Plate 28).

The stem section with *Artocarpus lakoocha* dye stained the vascular tissues light brown under bright field microscope. The UV filter fluoresced periderm, xylem and phloem white and the chlorophyll fluoresced red. The violet excitation filter showed blue fluorescence of periderm, xylem and phloem. The blue filter fluoresced the phloem greenish white.

The *Calliandra surinamensis* dye stained the cambium dark brown and the xylem and phloem appeared white under bright field microscope. The UV excitation filter showed the cuticle, xylem and phloem in blue fluorescence while the chlorophyll fluoresced red. Under violet filter the cuticle, xylem and phloem fluoresced blue and the chlorophyll fluoresced red. The vascular tissues fluoresced light green under blue excitation filter.

The *Heliotropium indicum* leaf extracts stained the cambium light brown and xylem and phloem appeared white under the bright field microscope. Under UV excitation filter the cuticle, xylem and phloem showed bluish white fluorescence while the chlorophyll fluoresced red. The violet filter fluoresced the cuticle, xylem and phloem in blue fluorescence blue and the chlorophyll fluoresced red. The blue excitation filter showed light green fluorescence of xylem and phloem.

The section with *Piper betle* dye stained the pith yellow and phloem appeared white under the bright field microscope. The cuticle, xylem and phloem fluoresced blue and chlorophyll fluoresced red under UV filter. Under violet filter the cuticle, xylem and phloem in blue fluorescence blue while the chlorophyll fluoresced red. The blue excitation filter fluoresced the vascular tissues light green.

The leaf extracts of *Phyllanthus emblica* dye stained the vascular tissues brown under bright field microscope. The cuticle, xylem and phloem fluoresced blue under UV

filter. The violet excitation filter fluoresced the cuticle, xylem and phloem blue and the chlorophyll fluoresced red. Under blue excitation filter the vascular tissues fluoresced green.

4.6 Phytochemical analysis of extracted dye samples

The dyes extracted in methanol showed the presence of carbohydrates, proteins, saponins and terpenoids in all the three plant extracts. Alkaloids were present only in the dye extract of *Artocarpus lakoocha*. Anthraquinones and coumarins were absent in all the three dye extracts. Dye extracts of *Artocarpus lakoocha* and *Phyllanthus emblica* showed the presence of flavonoids and tannins while steroids were present in the extracts of *Heliotropium indicum* and *Phyllanthus emblica* (Table 22) (Plate 29).

Table 22. Qualitative phytochemical analysis of methanol dye extracts.

Phytochemical Tests	***Artocarpus lakoocha***	***Heliotropium indicum***	***Phyllanthus emblica***
1. Test for Alkaloids Wagner's test	+	-	-
2. Test for Anthraquinones	-	-	-
3. Test for Carbohydrates Benedict's test	+	+	+
4. Test for Coumarins NaOH test	-	-	-
5. Test for Flavonoids Lead acetate test	+	-	+
6. Test for Proteins Biuret test	+	+	+

7. Test for Saponins Foam test	+	+	+
8.Test for Steroids Liebermann Burchard reagent test	-	+	+
9.Test for Tannins Ferric chloride test	+	-	+
10. Test for Terpenoids	+	+	+

The ethyl acetate extracts of dye showed the presence of proteins and steroids in all the plant extracts. Coumarins were absent in all the dye extracts. Alkaloids, anthraquinones, carbohydrates and tannins were present only in the extracts of *Artocarpus lakoocha*. The dye extracts of *Artocarpus lakoocha* and *Phyllanthus emblica* showed the presence of flavonoids and terpenoids while they were absent in *Heliotropium indicum* dye extract. Saponins were present only in the extracts of *Heliotropium indicum* (Table 23) (Plate 30).

Table 23. Qualitative phytochemical analysis of ethyl acetate extracts

Phytochemical Tests	***Artocarpus lakoocha***	***Heliotropium indicum***	***Phyllanthus emblica***
1. Test for Alkaloids Wagner's test	+	-	-
2. Test for Anthraquinones	+	-	-
3. Test for Carbohydrates Benedict's test	+	-	-
4. Test for Coumarins NaOH test	-	-	-

5. Test for Flavonoids Lead acetate test	+	-	+
6. Test for Proteins Biuret test	+	+	+
7. Test for Saponins Foam test	-	+	-
8.Test for Steroids Liebermann Burchard reagent test	+	+	+
9.Test for Tannins Ferric chloride test	+	-	-
10. Test for Terpenoids	+	-	+

The dyes extracted in water showed the presence of carbohydrates, flavonoids, proteins and terpenoids in all the extracts. Alkaloids, anthraquinones, coumarins and steroids were absent in all. Saponins were present in the extracts of *Artocarpus lakoocha* and *Heliotropium indicum* and absent in *Phyllanthus emblica*. *Artocarpus lakoocha* and *Phyllanthus emblica* showed the presence of tannins which were absent in *Heliotropium indicum* (Table 24) (Plate 31).

Table 24. Qualitative phytochemical analysis of aqueous dye extracts

Phytochemical Tests	***Artocarpus lakoocha***	***Heliotropium indicum***	***Phyllanthus emblica***
1. Test for Alkaloids Wagner's test	-	-	-
2. Test for Anthraquinones	-	-	-

3. Test for Carbohydrates Benedict's test	+	+	+
4. Test for Coumarins NaOH test	-	-	-
5. Test for Flavonoids Lead acetate test	+	+	+
6. Test for Proteins Biuret test	+	+	+
7. Test for Saponins Foam test	+	+	-
8.Test for Steroids Liebermann Burchard reagent test	-	-	-
9.Test for Tannins Ferric chloride test	+	-	+
10. Test for Terpenoids	+	+	+

4.7 Characterization of natural dyes

4.7.1 UV-Visible Spectroscopic Analysis

The extracts of the selected dyes were subjected to UV-Visible spectroscopy at 200-800 nm. The methanolic extracts of *Phyllanthus emblica* dye showed seven absorption peaks at 269, 372, 407, 467, 534, 605 and 662 nm. The ethyl acetate extract showed six absorption peaks at 266, 409, 472, 532, 605 and 664 nm. The aqueous extract showed two absorption peaks at 217 and 268 nm (Fig. 1-3).

The methanolic extracts of *Artocarpus lakoocha* dye showed three absorption peaks at 277, 317 and 771 nm. The ethyl acetate extract showed two absorption peaks at 277 and 663 nm. The aqueous extract showed two absorption peaks at 276 and 339 nm (Fig. 4-6).

The methanolic extracts of *Heliotropium indicum* dye showed three absorption peaks at 309, 420 and 659 nm. The ethyl acetate extract showed nine absorption peaks at 209, 270, 330, 408, 445, 532, 556, 606 and 665 nm. The aqueous extract showed a single absorption peak at 740 nm (Fig. 7-9).

4.7.2 Separation of compounds by Thin Layer Chromatography (TLC)

The extracts of selected dyes were subjected to TLC for separation of flavonoids and phenols. For separation of phenols the solvent system toluene:acetone;formic acid (4.5:4.5:1) (v/v/v/) was used. The plates were visualized after exposing to ammonia in the UV transilluminator. For separation of flavonoids the solvent system methanol: chloroform:hexane (7:2:1) (v/v/v) was used and the plate was visualized after exposing to ammonia in the UV trans-illuminator.

4.7.2.1 Separation of Phenols

The methanolic extract of *Artocarpus lakoocha* did not show any spots. *Heliotropium indicum* extract showed a single spot under visible light and six spots under long UV. The extract of *Phyllanthus emblica* showed four spots under visible light and three spots under long UV (Table 25) (Plate 32).

Table 25. TLC of methanolic plant extracts

Plant	Rf value	Colour of spot	
		Under visible light	Under long UV (365 nm)
Artocarpus lakoocha	-	-	-
Heliotropium indicum	0.59	-	Red
	0.64	-	Red
	0.73	-	Blue
	0.78	-	Blue
	0.84	-	Light red
	0.98	-	Red

	0.99	Dark green	-
Phyllanthus emblica	0.66 0.72 0.95 0.98	Light gray Gray Olive green Dark green	Red Reddish brown Brownish red -

The ethyl acetate extract of *Artocarpus lakoocha* showed two spots under long UV. *Heliotropium indicum* extract showed a six spots under visible light and seven spots under long UV. The extract of *Phyllanthus emblica* showed six spots under visible light and five spots under long UV (Table 26) (Plate 32).

Table 26. TLC for ethyl acetate plant extracts

Plant	**Rf value**	**Colour of spot**	
		Under visible light	**Under long UV (365 nm)**
Artocarpus lakoocha	0.83 0.99	- -	Greenish blue Blue
Heliotropium indicum	0.60 0.67 0.72 0.76 0.81 0.83 0.89 0.91 0.98 0.99	Gray - Green Brown Blue Yellow Green - -	- Light blue Red - Red Green Red - Orange Blue
Phyllanthus emblica	0.54 0.60 0.71 0.75 0.79 0.83	Gray Blue Brownish green Dull green - Yellowish green	- - Red - Brownish red Green

	0.92 0.98	Olive green -	Reddish brown Blue

The aqueous extract of *Artocarpus lakoocha* and *Heliotropium indicum* di not show any spots while the extract of *Phyllanthus emblica* showed a single spot under long UV (Table 27) (Plate 32).

Table 27. TLC for aqueous plant extracts

Plant	**Rf value**	**Colour of spot**	
		Under visible light	**Under long UV (365 nm)**
Artocarpus lakoocha	-	-	-
Heliotropium indicum	-	-	-
Phyllanthus emblica	0.27	-	Purple

4.7.2.2 Separation of Flavonoids

The methanol extract of *Artocarpus lakoocha* did not show any spots. *Heliotropium indicum* extract showed six spots under visible light and two spots under long UV. The extract of *Phyllanthus emblica* showed seven spots under visible light and four spots under long UV (Table 28) (Plate 33).

Table 28. TLC for methanolic plant extracts

Plant	**Rf value**	**Colour of spot**	
		Under visible light	**Under long UV (365 nm)**
Artocarpus lakoocha	-	-	-

Heliotropium indicum	0.84 0.88 0.90 0.92 0.97 0.99	Yellow Bluish green Dark green Yellow Light green Gray	- - Green - White -
Phyllanthus emblica	0.81 0.83 0.86 0.88 0.90 0.95 0.97	Orange Gray Green Brown Green Gray Brown	- - Green Brown Red - Green

The ethyl acetate extract of *Artocarpus lakoocha* showed a single spot under long UV. *Heliotropium indicum* extract showed seven spots under visible light and two spots under long UV. The extract of *Phyllanthus emblica* showed seven spots under visible light and three spots under long UV (Table 29) (Plate 33).

Table 29. TLC for ethyl acetate plant extracts

Plant	**Rf value**	**Colour of spot**	
		Under visible light	**Under long UV (365 nm)**
Artocarpus lakoocha	0.94	-	Blue
Heliotropium indicum	0.79 0.85 0.88 0.90 0.91 0.93 0.97	Gray Orange Green Yellow Blue Gray Green	- - Green - - Red -

Phyllanthus emblica	0.79 0.82 0.86 0.88 0.90 0.94 0.97	Gray Yellowish green Green Brown Yellow Gray Dark green	- - Brown - Red Blue -

The aqueous extract of *Artocarpus lakoocha* showed a single spot under visible light, while the extracts of *Heliotropium indicum* and *Phyllanthus emblica* did not show any spots (Table 30) (Plate 33).

Table 29. TLC for aqueous plant extracts

Plant	**Rf value**	**Colour of spot**	
		Under visible light	**Under long UV (365 nm)**
Artocarpus lakoocha	0.97	Orange	-
Heliotropium indicum	-	-	-
Phyllanthus emblica	-	-	-

5. DISCUSSION

5.1 Selection of dye yielding plants

In the present study, six plants were selected for the extraction of dye, namely, *Artocarpus lakoocha, Calliandra surinamensis, Cymbopogon citrates, Heliotropium indicum, Piper betle* and *Phyllanthus emblica*. Plant parts like bark and leaves were used for the dye extraction. Different plant parts can be used for the extraction of dye. Uddin (2015) extracted the dyes from mango leaves and studied its application on silk fabric. Sinha *et al.* (2012) extracted the dye from the petals of *Butea monosperma* flowers.

5.2 Optimization of dye extraction parameters

Artocarpus lakoocha bark took the maximum time to give the dye of maximum colour intensity, while *Cymbopogon citratus, Piper betle* and *Phyllanthus emblica* showed maximum extraction at moderate time duration. *Calliandra surinamensis* and *Heliotropium indicum* showed maximum absorption at minimum time duration. Usually the intensity of colour increases with the increase in extraction time because the dye molecules get more time to get dissolved with the solvent (Farooq *et al.,* 2013). This trend was observed in case of *Artocarpus lakoocha* dye however it was not the same with other plant parts may be because those dyes were more readily soluble with the solvent.

The optimum extraction temperature was found to be 60°C for most plants except for *Artocarpus lakoocha* (40°C) and *Phyllanthus emblica* (80°C). Usually the colour intensity increases with increase in temperature because the cell wall ruptures with rise in temperature and more dye molecules dissolve in the solvent (Farooq *et al*., 2013). Similar pattern was observed for all the plant parts except for *Artocarpus lakoocha*.

5.3 Dyeing of cotton and silk fibers with natural dyes

The different shades of colour ranging from brownish orange, moderate orange, moderate yellow, moderate brown, moderate orange yellow and brown were observed on cotton fibers while moderate orange yellow, yellowish white, light greenish yellow, grayish yellow and light olive brown were observed on silk fabrics dyed with *Artocarpus lakoocha, Calliandra surinamesis, Cymbopogon citratus, Heliotropium indicum, Pipe betle* and *Phyllanthus emblica.* The barks are usually rich in tannins and thus dyes obtained from the bark produce brown colour on the fabrics. Dyeing with the mordants produce different shades on the fabrics, so mordants can be used to obtain different shades with the single dye.

The method of mordanting, that is, pre-post- and simultaneous also has some effect on the development of the shade. The strength of the shade depends on the type of mordant used. Iron enhances the dye uptake by the fibers (Kulkarni *et al.,* 2011). Alum, potassium dichromate and stannous chloride are called as brightening mordants and copper sulphate and ferrous sulphate are called as dulling mordants based on the final colour they produce on the fabrics. Cotton fibers lack the affinity for natural dyes and thus mordants are necessary for their dyeing while silk is amphoteric in nature and show better dye absorption (Prabhu *et al.,* 2012).

5.3.1 Fastness tests of the dyed cotton and silk fibers

The cotton fibers dyed with *Artocarpus lakoocha* dye showed better wash fastness compared to the rub and light fastness. Most of the mordants improved the fastness properties of the dyed fibers. Similar observations were recorded in the cotton fibers dyed with *Heliotropium indicum* and *Phyllanthus emblica* dyes. Silk fibers dyed with *Artocarpus lakoocha* dye showed good rub and light fastness but showed poor wash

fastness. In the case of silk fibers also the mordants improved the rub, light and wash fastness of the fibers. Similar observations were noted for silk fibers dyed with *Heliotropium indicum* dye. The fibers with *Phyllanthus emblica* dye showed poor fastness properties. In such cases mordanting is essential. The mordants improves the colour fastness because they can insolubilize the dye (Jothi, 2008).

The method of mordanting employed also has the effect on the colour fastness properties of the dyed fibers. In the case of *Artocarpus lakoocha* dye pre-mordanting of cotton fibers showed better colour fastness compared to other mordanting methods and for silk fibers post-mordanting method showed better colour fastness. For *Heliotropium indicum* dye cotton fibers dyed by pre-mordanting showed better colour fastness and for silk fibers simultaneous mordanting showed good colour fastness. Cotton and silk fibers dyed using *Phyllanthus emblica* dye and pre-mordanting method showed better colour fastness compared to other mordanting methods.

5.4 Natural dyes as biological stain for plant tissues

The dyes obtained from *Artocarpus lakoocha, Calliandra surinamensis, Heliotropium indicum* and *Phyllanthus emblica* stained the vascular tissues of the monocot and dicot plant sections. The studies on natural dyes as biological stains indicate that, mostly the lignified areas show better staining. Akinloye *et al*. (2012) stained the wood sections of *Cola gigantea* with the dye extracts of *Bixa orellana, Curcuma domestica, Lonchocarpus cyanescens* and *Pterocarpus osun* and observed that most of the dyes stained the fibers and vessels which contained lignified cells. *Piper betle* dye showed the staining of the parenchymatous pith along with the vascular tissues in the case of dicot stem. The dyes also showed characteristic fluorescence of the vascular tissues under different filters of fluorescence microscopy.

5.5 Phytochemical analysis of natural dyes

The phytochemical analysis revealed the presence of flavonoids, steroids and terpenoids in most of the plant extracts. Tannins and alkaloids were mostly abundant in the bark extracts of *Artocarpus lakoocha*. Different solvents are used for the extraction of phyto-constituents because polar constituents like flavonoids and tannins show better extraction in polar solvents compared to other solvents. It is essential to use different solvents for extraction in order to determine most of the phytoconstituents. Gupta & Gupta (2013) found the presence of steroids, alkaloids, flavonoids, carbohydrates, saponins and tannins in the methanolic extracts of *Phyllanthus emblica* leaves, similar results were obtained by Elangovan *et al.* (2015). The phytochemical analysis of *Artocarpus lakoocha* extracts showed the presence of flavonoids, phenols, tannins, saponins, triterpenoids and steroids (Pandey & Bhatnagar, 2009) which are consistent with the current observations.

5.6 UV-Visible Spectroscopic Analysis

The different extracts of plants were analyzed by UV-Visible spectroscopy. The flavonoids and their derivatives show the absorption maxima at two wavelength ranges, Band-I at 230-290 nm and Band-II at 300-350 nm (Saxena & Saxena, 2012). Tannins show the maximum absorbance between 350-500 nm and carotenoids between 400-450 nm (Patle *et al.,* 2020). Terpenoids have maximum absorbance between 400-550 nm while the peaks obtained between 600-700 nm correspond to chlorophyll (Alara *et al.*, 2018).

All three plant extracts showed the absorbance peaks in the range of 230 – 290 nm and 300 – 350 nm which might be due to the presence of the presence of flavonoids in these extracts. Absorption in the range of 600 – 700 nm might be due to chlorophyll. The peaks in the range of 350 - 500 nm were obtained in all the three samples; these may indicate the presence of tannins in the samples. *Phyllanthus emblica* and *Heliotropium*

indicum extracts showed the peaks in the range of 400 - 450 nm which may be due to the presence of carotenoids. The peaks in the range of 400 - 550 nm may also indicate the presence of terpenoids in these two plants.

5.7 Thin Layer Chromatography analysis

TLC analysis was carried out for the separation of phenols and flavonoids. The Rf value of 0.78 in *Heliotropium indicum* methanolic extract corresponds to syringic acid and Rf value of 0.64 corresponds to chlorogenic acid (Marimuthu *et al.,* 2020). The presence of syringic acid in the extracts of *Heliotropium indicum* was recorded by Ghosh *et al*. (2020). The Rf value of 0.81 in ethyl acetate extract of *Heliotropium indicum* corresponds to flavon (Owoade *et al*., 2016). The Rf value 0.66 corresponds to quercetin and 0.81 corresponds to naringenin (Saric *et al.,* 2004) in *Phyllanthus emblica* methanolic extract. The Rf value of 0.75 in ethyl acetate extract of *Phyllanthus emblica* corresponds to kaempferol (Marimuthu *et al.,* 2020; Puravankara & Gopal, 2014). El Amir *et al*. (2016) recorded a high concentration of kaempferol and quercetin from the leaves and fruits of *Phyllanthus emblica*.

CONCLUSION

Dyes were extracted from six selected plants. The optimum conditions for extraction of dye with respect to time and temperature varied for all the six plants. The extracts were used for the dyeing of cotton and silk fibers. *Artocarpus lakoocha*, *Heliotropium indicum* and *Phyllanthus emblica* dyes gave better shades of colour on the fibers. The colour fastness tests indicated that mordanting improved the fastness properties of the dyes. For dyeing of cotton fibers pre-mordanting method showed better colour fastness compared to post- and simultaneous mordanting. The suitable method of mordanting varied for the silk fibers dyed with different extracts.

Monocot and dicot stem sections were stained with the extracted natural dyes. Dyes exhibited good staining of the lignified cells. The sections also showed differential staining when observed under bright field and fluorescence microscopy.

Phytochemical, UV-Visible spectroscopic and Thin Layer Chromatographic analysis indicated the presence of flavonoids and tannins in the extracted dyes.

The extracted dyes have good potential to be used as histological stains for plant tissues. The different shades obtained on the cotton and silk fibers and the colour fastness properties of the dyes indicate their potential use for dyeing textiles.

SUMMARY

The plants *Artocarpus lakoocha, Cymbopogon citratus*, *Calliandra surinamensis, Heliotropium indicum, Piper betle* and *Phyllanthus emblica* were used for the extraction of the dyes. For the dye extraction, leaves and bark of the plants were used. The plant parts were shade dried, ground into powder and was used for the extraction. The dye was extracted by boiling the powdered sample in water in a hot water bath. The colour of the extracted dye was determined by comparing with the pantone colour matching charts. The optimum conditions for extraction of dye with respect to time and temperature were determined. The optimum extraction time and temperature varied for different plants.

Dyeing of cotton and silk fibers was carried out using the extracted dyes. Different mordants were also used to obtain different shades of colour on the fibers. The effect of different mordanting methods on the colour fastness of the dyed samples was also evaluated. The dyes without mordant developed good shades on the fibers but the colour fastness to rubbing, light and washing was low of these fibers compared to the fibers dyed by using mordants. Pre-mordanting gave better results for cotton fibers while for silk fibers the suitable mordanting method varied with the dyes used. The colour developed on the fibers was determined by comparing with ISSC-NBS colour system and the colour fastness was determined by comparing with standard gray scales.

Monocot and dicot stem sections were stained with the extracted dyes. The optimum time for staining was determined. The optimum staining period varied for different dyes between 5 to 15 minutes, staining the sections beyond 20 minutes led to over staining. The dyes showed good staining of the lignified cells of the tissues. The different tissues also exhibited characteristic fluorescence under different excitation filters.

The phytochemical analysis revealed the presence of flavonoids, tannins and terpenoids in most of the samples. UV-Visible spectroscopic analysis showed the peaks which might represent the presence of flavonoids and tannins in the samples. Thin layer chromatography was carried out for the separation of phenols and flavonoids from the sample and some of the compounds were tentatively identified based on their Rf values.

REFERENCES

Affat, S. (2021). *Classifications, Advantages, Disadvantages, Toxicity Effects of Natural and Synthetic Dyes: A review*. *8*, 130–135.

Ahlström, L.-H., Sparr Eskilsson, C., & Björklund, E. (2005). Determination of banned azo dyes in consumer goods. *TrAC Trends in Analytical Chemistry*, *24*(1), 49–56. https://doi.org/https://doi.org/10.1016/j.trac.2004.09.004

Akinloye, A., Illoh, H., & Olagoke, A. (2012). Screening of some indigenous herbal dyes for use in plant histological staining. *Journal of Forestry Research*, *21*, 81–84. https://doi.org/10.1007/s11676-010-0014-2

Alara, O. R., Abdurahman, N. H., Mudalip, S. K. A., & Olalere, O. A. (2018). Characterization and effect of extraction solvents on the yield and total phenolic content from Vernonia amygdalina leaves. *Journal of Food Measurement and Characterization*, *12*(1), 311–316.

Algarni, H. M., Awwad, N. S., & Samanta, A.K. (2020). *Fundamentals of Natural Dyes and Its Application on Textile Substrates* (p. Ch. 2). IntechOpen. https://doi.org/10.5772/intechopen.89964

Andriamanantena, M., Razafimbelo, F. F., Raonizafinimanana, B., Cardon, D., Danthu, P., Lebeau, J., Petit, T., & Caro, Y. (2021). Alternative sources of red dyes with high stability and antimicrobial properties: Towards an ecological and sustainable approach for five plant species from Madagascar. *Journal of Cleaner Production*, *303*, 126979. https://doi.org/10.1016/J.JCLEPRO.2021.126979

Arora, J., Agarwal, P., & Gupta, G. (2017). Rainbow of Natural Dyes on Textiles Using Plants Extracts: Sustainable and Eco-Friendly Processes. *Green and Sustainable*

Chemistry, *07*(01), 35–47. https://doi.org/10.4236/gsc.2017.71003

Auwal, M. S., Saka, S., Mairiga, I. A., Sanda, K. A., Shuaibu, A., & Ibrahim, A. (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (Thorn mimosa). *Veterinary Research Forum : An International Quarterly Journal*, *5*(2), 95–100. https://pubmed.ncbi.nlm.nih.gov/25568701

Bechtold, T, Turcanu, A., Ganglberger, E., & Geissler, S. (2003). Natural Dyes in Modern Textile Dyehouses – How to Combine Experiences of Two Centuries to Meet the Demands of the Future? *Journal of Cleaner Production*, *11*, 499–509. https://doi.org/10.1016/S0959-6526(02)00077-X

Bechtold, Thomas, Mussak, R., Mahmud-Ali, A., Ganglberger, E., & Geissler, S. (2006). Extraction of natural dyes for textile dyeing from coloured plant wastes released from the food and beverage industry. *Journal of the Science of Food and Agriculture*, *86*(2), 233–242. https://doi.org/10.1002/jsfa.2360

Britannica, T. (2019). Silk. In *Encyclopedia Britannica*. https://www.britannica.com/topic/silk

Clark, G., Kasten, F. (1983). History of Staining. In *History of Staining* (3rd ed., p. 35). Williams & Wilkins.

Conn, H. J. (Harold J. (1953). *Biological stains; a handbook on the nature and uses of the dyes employed in the biological laboratory* (6th ed., r). Biotech Publications. https://www.biodiversitylibrary.org/item/27041

Cook, R. P. (1961). Reactions of steroids with acetic anhydride and sulphuric acid (the Liebermann-Burchard test). *Analyst*, *86*(1023), 373–381.

https://doi.org/10.1039/AN9618600373

Cs, V., Vb, H., Suja, Vilash, Krishnakumar, N. M., & Latha, P. G. (2014). Comparative preliminary phytochemical analysis of ethanolic extracts of leaves of Olea dioica Roxb., infected with the rust fungus Zaghouania oleae (E.J. Butler) Cummins and non-infected plants. *Journal of Pharmacognosy and Phytochemistry*, *3*, 69–72.

Deepali, K., Lalita, S., Deepika, M., Stem, M., & Hibiscus, F. O. (2014). Application of aqueous plant extracts as Biological stains. *Int J Eng Sci Res*, *5*(2).

El Amir, D., AbouZid, S., Mawad, A., Amin, E., Hetta, M., Shahat, A., & El-Shanawany, M. (2016). HPLC Profiling Of Phyllanthus Emblica L. Extracts. *Planta Medica*, *82*. https://doi.org/10.1055/s-0036-1578625

Farooq, A. Ali, S. Abbas, N. Zahoor, N. Ashraf, M. (2013). Optimization of Extraction and Dyeing Parameters for Natural Dyeing of Cotton Fabric using Marigold (Tagetes erecta). *Asian Journal of Chemistry*, *25*(11), 5955–5959.

Ghosh, P., Das, C., Biswas, S., Nag, S. K., Dutta, A., Biswas, M., Sil, S., Hazra, L., Ghosh, C., Das, S., Saha, M., Mondal, N., Mandal, S., Ghosh, A., Karmakar, S., & Chatterjee, S. (2020). Phytochemical composition analysis and evaluation of in vitro medicinal properties and cytotoxicity of five wild weeds: A comparative study. *F1000Research*, *9*, 493. https://doi.org/10.12688/f1000research.22966.1

Gray, D. (2014). *The history of making cotton fabric*. https://www.cottonmill.com/blog/the-history-of-making-cotton-fabric/

Grifoni, D., Bacci, L., Zipoli, G., Albanese, L., & Sabatini, F. (2011). The role of natural dyes in the UV protection of fabrics made of vegetable fibres. *Dyes and Pigments*, *91*(3), 279–285. https://doi.org/10.1016/J.DYEPIG.2011.04.006

Gul, R., Jan, S. U., Faridullah, S., Sherani, S., & Jahan, N. (2017). Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from *Ephedra intermedia* Indigenous to Balochistan. *The Scientific World Journal*, *2017*, 5873648. https://doi.org/10.1155/2017/5873648

Gupta, J., & Gupta, A. (2013). Phytochemical screening of Phyllanthus emblica (L.) using different solvents. *Journal Chemtracks*, *15*, 117–120.

Haji, A. (2019). Dyeing of Cotton Fabric with Natural Dyes Improved by Mordants and Plasma Treatment. *Review of Progress in Coloration and Related Topics*, *12*, 191–201.

Hosseini Ravandi, S. A., & Valizadeh, M. (2011). Properties of fibers and fabrics that contribute to human comfort. *Improving Comfort in Clothing*, 61–78. https://doi.org/10.1533/9780857090645.1.61

Hussein, SAM. Barakat, HH. Merfort, I. Nawwar, M. (1997). Tannins from the leaves of Punica granatum. *Photochemistry*, *45*, 197–205.

Jagessar, R.C. Cox, M. (2010). Phytochemical screening of the CHCl3 and CH3CH2OH ectracts of stems, twigs, roots and barks of Conocarpus erectus L. *International Journal of Academic Research*, *2*, 36–45.

Jan, H. U., Shinwari, Z. K., Marwat, K. B., & others. (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.

Jiang, H. Y., Hu, X. D., Zhu, J. J., Wan, J., & Yao, J. B. (2021). Studies on the photofading of alizarin, the main component of madder. *Dyes and Pigments*, *185*, 108940. https://doi.org/10.1016/j.dyepig.2020.108940

Jothi, D. (2008). Extraction of natural dyes from African marigold flower (Tagetes ereectal) for textile coloration. *Autex Research Journal*, *8*(2), 49–53.

Kandasamy, N., Kaliappan, K., & Palanisamy, T. (2021). Upcycling sawdust into colorant: Ecofriendly natural dyeing of fabrics with ultrasound assisted dye extract of Pterocarpus indicus Willd. *Industrial Crops and Products*, *171*, 113969. https://doi.org/10.1016/J.INDCROP.2021.113969

Kasiri, M. B., & Safapour, S. (2014). Natural dyes and antimicrobials for green treatment of textiles. *Environmental Chemistry Letters*, *12*(1), 1–13. https://doi.org/10.1007/s10311-013-0426-2

Kelly, K. L., 1910-, Judd, D. B., & 1900-1972. (n.d.). *ISCC-NBS color-name charts illustrated with centroid colors* (F. A. O. of the UN (ed.); p. 1 volume (unpaged) color illustrations 27 cm). [U.S. National Bureau of Standards].

Kiumarsi, A., Abomahboub, R., Rashedi, S. M., & Parvinzadeh, M. (2009). *Achillea millefolium, a new source of natural dye for wool dyeing*.

Krishnamurthy, K. V. (1999). *Methods in Cell Wall Cytochemistry*. CRC Press.

Kulkarni, S. S., Gokhale, A. V, Bodake, U. M., & Pathade, G. R. (2011). Cotton Dyeing with Natural Dye Extracted from Pomegranate (Punica granatum) Peel. *Universal Journal of Environmental Research \& Technology*, *1*(2).

Lee, J., Kang, M. H., Lee, K.-B., & Lee, Y. (2013). Characterization of Natural Dyes and Traditional Korean Silk Fabric by Surface Analytical Techniques. In *Materials* (Vol. 6, Issue 5). https://doi.org/10.3390/ma6052007

Liu, J., Mouri, C., Laursen, R., Zhao, F., Zhou, Y., & Li, W. (2013). Characterization of

dyes in ancient textiles from Yingpan, Xinjiang. *Journal of Archaeological Science*, *40*(12), 4444–4449. https://doi.org/10.1016/J.JAS.2013.06.034

Mansour, R., Dhouib, S., & Sakli, F. (2022). UV Protection and Dyeing Properties of Wool Fabrics Dyed with Aqueous Extracts of Madder Roots, Chamomiles, Pomegranate Peels, and Apple Tree Branches Barks. *Journal of Natural Fibers*, *19*(2), 610–620. https://doi.org/10.1080/15440478.2020.1758280

Owoade, O., Adetutu, A., & Olorunnisola, O. (2016). Influence of Polyphenolic Contents on the Antioxidant Properties of Hibiscus sabdariffa Extract (HSE), Aged Garlic Extract (AGE) and Garlic Tablet (GT) in Vitro. *Food and Nutrition Sciences*, *07*, 417–428. https://doi.org/10.4236/fns.2016.76043

Pandey, A., & Bhatnagar, S. (2009). Preliminary Phytochemical screening and antimicrobial studies on Artocarpus lakoocha Roxb. *Ancient Science of Life*, *28*, 21–24.

Patle, T. K., Shrivas, K., Kurrey, R., Upadhyay, S., Jangde, R., & Chauhan, R. (2020). Phytochemical screening and determination of phenolics and flavonoids in Dillenia pentagyna using UV--vis and FTIR spectroscopy. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *242*, 118717.

Prabhu, K. H., Bhute, A. S., & others. (2012). Plant based natural dyes and mordants: A Review. *J. Nat. Prod. Plant Resour*, *2*(6), 649–664.

Puravankara, D., & Gopal, G. (2014). GC-MS analysis of ethyl acetate extract of Phyllantus emblica L. bark. *British Biomedical Bulletin*, *2*, 285–292.

Rai, H. S., Bhattacharyya, M. S., Singh, J., Bansal, T. K., Vats, P., & Banerjee, U. C. (2005). Removal of Dyes from the Effluent of Textile and Dyestuff Manufacturing

Industry: A Review of Emerging Techniques With Reference to Biological Treatment. *Critical Reviews in Environmental Science and Technology*, *35*(3), 219–238. https://doi.org/10.1080/10643380590917932

Rajendran, T. P., Venugopalan, M., & Praharaj, C. (2005). Cotton research towards sufficiency to Indian textile industry. *Indian Journal of Agricultural Sciences*, *75*, 699–708.

Reyes-Trujeque, J., Hermosin, B., & Azamar-Barrios, J. (2012). Characterization of a Natural Dye by Spectroscopic and Chromatographic Techniques. *MRS Proceedings*, *1374*, 49–59. https://doi.org/10.1557/opl.2012.1377

Rodiah, M. H., Noor Hafizah, S., Noor Asiah, H., Nurhafizah, I., Norakma, M. N., & Norazlina, I. (2022). Extraction of natural dye from the mesocarp and exocarp of Cocos nucifera, textile dyeing, and colour fastness properties. *Materials Today: Proceedings*, *48*, 790–795. https://doi.org/10.1016/J.MATPR.2021.02.315

Roy Choudhury, A. K. (2017). Wool and silk finishing processes. *Principles of Textile Finishing*, 417–466. https://doi.org/10.1016/B978-0-08-100646-7.00013-8

Saiki, P., Romratanapun, S., & Thitipramote, N. (2011). *Extraction of Natural Histological Dye from Black Plum Fruit (Syzygium cumini)*.

Samanta, A. K., & Agarwal, P. (2009). *Application of natural dyes on textiles*.

Saxena, M., & Saxena, J. (2012). Evalution of phytoconstituents of Acorus calamus by FTIR and UV-VIS spectroscopic analysis. *International Journal of Biological \& Pharmaceutical Research*, *3*(3), 498–501.

Selvam, R. M., Athinarayanan, G., Nanthini, A. U. R., Singh, A. J. A. R., Kalirajan, K., & Selvakumar, P. M. (2015). Extraction of natural dyes from Curcuma longa, Trigonella foenum graecum and Nerium oleander, plants and their application in antimicrobial fabric. *Industrial Crops and Products*, *70*, 84–90. https://doi.org/https://doi.org/10.1016/j.indcrop.2015.03.008

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. https://doi.org/https://doi.org/10.1002/jemt.23650

Singh, R. (2019). *Extraction of Natural Dyes from Plant Sources* [University of Allahabad]. http://hdl.handle.net/10603/311618

Singh, R., & Srivastava, S. (2017). A critical review on extraction of natural dyes from leaves. *International Journal of Home Science*, *100*(2), 100–103. www.homesciencejournal.com

Sinha, K., Saha, P. Das, & Datta, S. (2012). Extraction of natural dye from petals of Flame of forest (Butea monosperma) flower: Process optimization using response surface methodology (RSM). *Dyes and Pigments*, *94*(2), 212–216. https://doi.org/10.1016/J.DYEPIG.2012.01.008

Siva, R. (2007). Status of natural dyes and dye-yielding plants in India. *Current Science*, *92*(7), 916–925. http://www.jstor.org/stable/24097672

Sivakumar, V., Vijaeeswarri, J., & Anna, J. L. (2011). Effective natural dye extraction from different plant materials using ultrasound. *Industrial Crops and Products*, *33*(1), 116–122. https://doi.org/10.1016/J.INDCROP.2010.09.007

Sofowora, A. (1993). *Medicinal plants and traditional medicine in Africa* (2nd ed.). Spectrum books ltd., sunshine house.

Stothers, J., Edward, N., A. (2019). Dye. In *Britannica*. https://www.britannica.com/technology/dye

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*, 340. https://doi.org/10.4103/jomfp.JOMFP_62_17

Taghizadeh borujeni, R., Akbari, A., Gharehbaii, A., & Yunessnia lehi, A. (2021). Extraction and preparation of dye powders from Reseda luteola L. using membrane processes and its dyeing properties. *Environmental Technology & Innovation*, *21*, 101249. https://doi.org/10.1016/J.ETI.2020.101249

Tousson, E., & Al-Behbehani, B. (2011). Black mulberries (Morus nigra) as a natural dye for animal tissues staining. *Animal Biology*, *61*(1), 49–56. https://doi.org/https://doi.org/10.1163/157075511X554419

Trigoso, C. I., & Stockert, J. C. (2005). Fluorescence of the natural dye saffron: Selective reaction with eosinophil leucocyte granules. *Histochemistry and Cell Biology*, *104*, 75–77.

Uddin, M. G. (2015). Extraction of eco-friendly natural dyes from mango leaves and their application on silk fabric. *Textiles and Clothing Sustainability*, *1*(1), 7. https://doi.org/10.1186/s40689-015-0007-9

Vankar, P.S. (2000). Chemistry of natural dyes. *Resonance*, *5*(10), 73–80.

Vankar, Padma S. (2000). Chemistry of natural dyes. *Resonance*, *5*(10), 73–80.

https://doi.org/10.1007/bf02836844

Wagner, H. (1993). *Pharmazeutische Biologic* (5th ed.). Gustav fisher Vwelag.

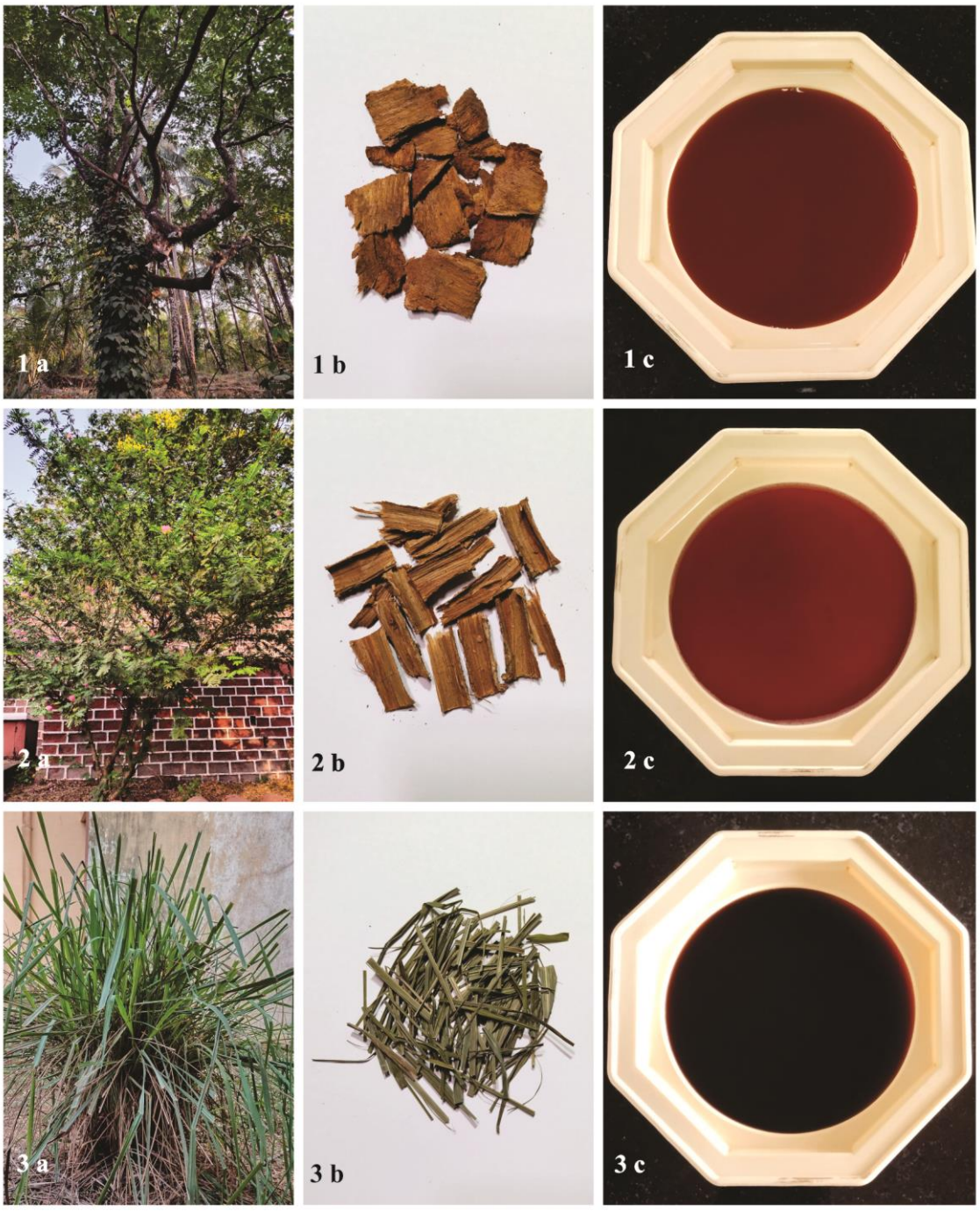

Plate 1. Selected dye yielding plants.
1. *Artocarpus lakoocha* a. Habit b. Bark c. Dye extracted
2. *Calliandra surinamensis* a. Habit b. Bark c. Dye extracted
3. *Cymbopogon citratus* a. Habit b. Bark c. Dye extracted

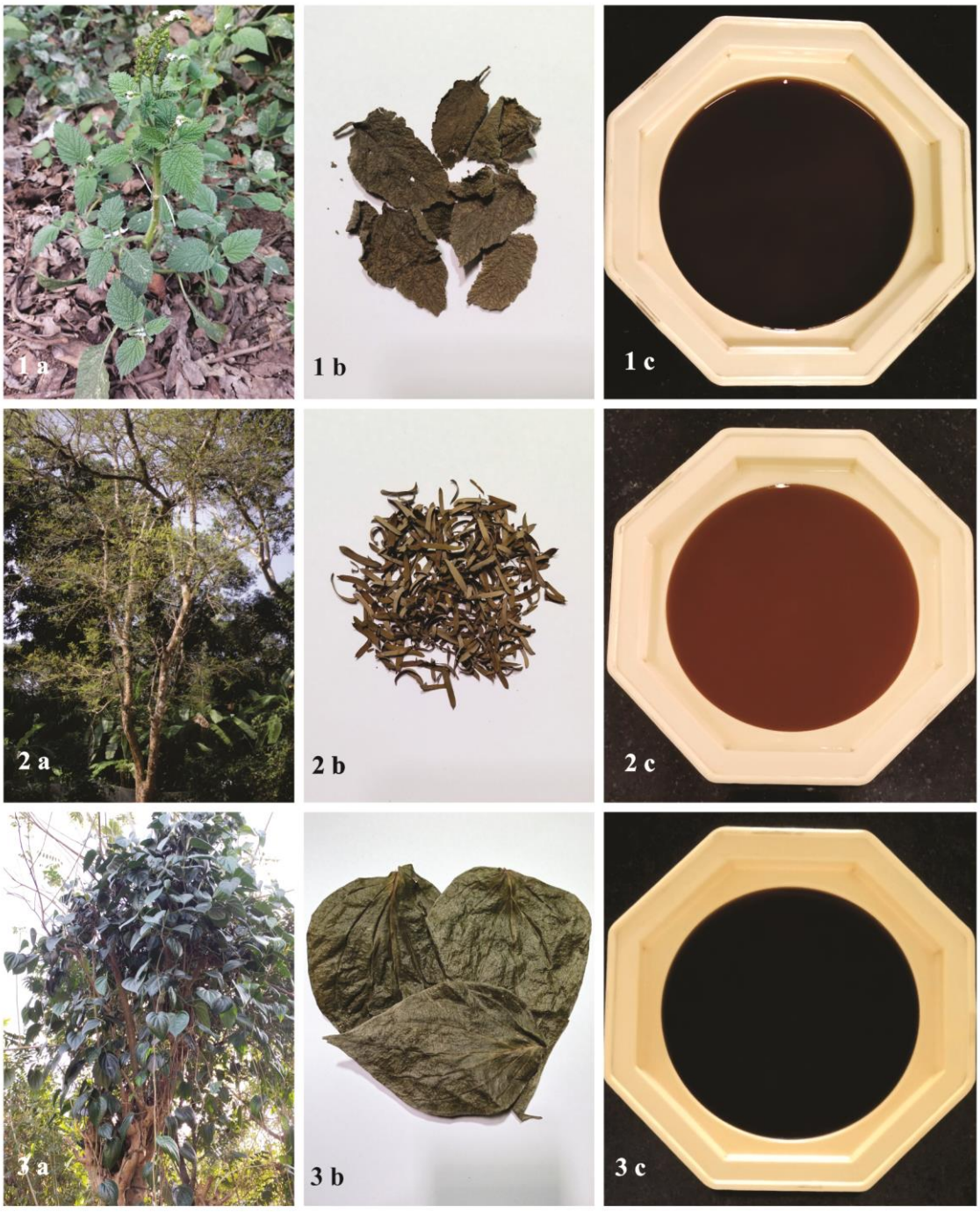

Plate 2. Selected dye yielding plants.
1. *Heliotropium indicum* a. Habit b. Bark c. Dye extracted
2. *Phyllanthus emblica* a. Habit b. Bark c. Dye extracted
3. *Piper betle* a. Habit b. Bark c. Dye extracted

Plate 3. Dyeing of cotton fibers with *Calliandra surinamensis* dye.
1. Dyed with *C. surinamensis* dye
2-4. Dyed with *C. surinamensis* dye and $FeSO_4$
5-7. Dyed with *C. surinamensis* dye and $CuSO_4$
8-10. Dyed with *C. surinamensis* dye and $FeCl_2$
11-13. Dyed with *C. surinamensis* dye and $SnCl_2$
14-16. Dyed with *C. surinamensis* dye and Alum
17-19. Dyed with *C. surinamensis* dye and $K_2Cr_2O_7$

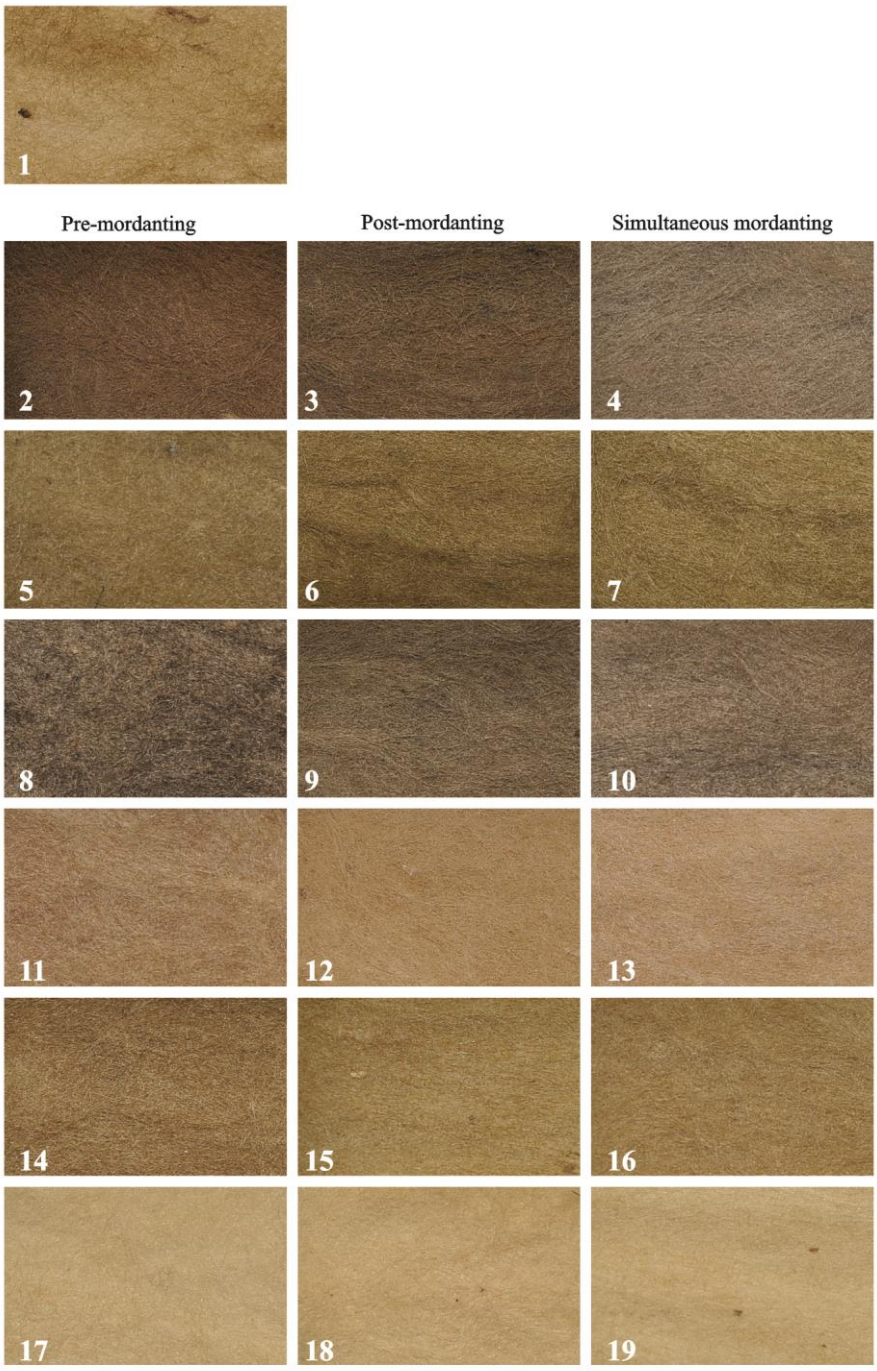

Plate 4. Dyeing of cotton fibers with *Cymbopogon citratus* dye.
1. Dyed with *C. citratus* dye
2-4. Dyed with *C. citratus* dye and $FeSO_4$
5-7. Dyed with *C. citratus* dye and $CuSO_4$
8-10. Dyed with *C. citratus* dye and $FeCl_2$
11-13. Dyed with *C. citratus* dye and $SnCl_2$
14-16. Dyed with *C. citratus* dye and Alum
17-19. Dyed with *C. citratus* dye and $K_2Cr_2O_7$

Plate 5. Dyeing of cotton fibers with *Piper betle* dye.
1. Dyed with *P. betle* dye
2-4. Dyed with *P. betle* dye and $FeSO_4$
5-7. Dyed with *P. betle* dye and $CuSO_4$
8-10. Dyed with *P. betle* dye and $FeCl_2$
11-13. Dyed with *P. betle* dye and $SnCl_2$
14-16. Dyed with *P. betle* dye and Alum
17-19. Dyed with *P. betle* dye and $K_2Cr_2O_7$

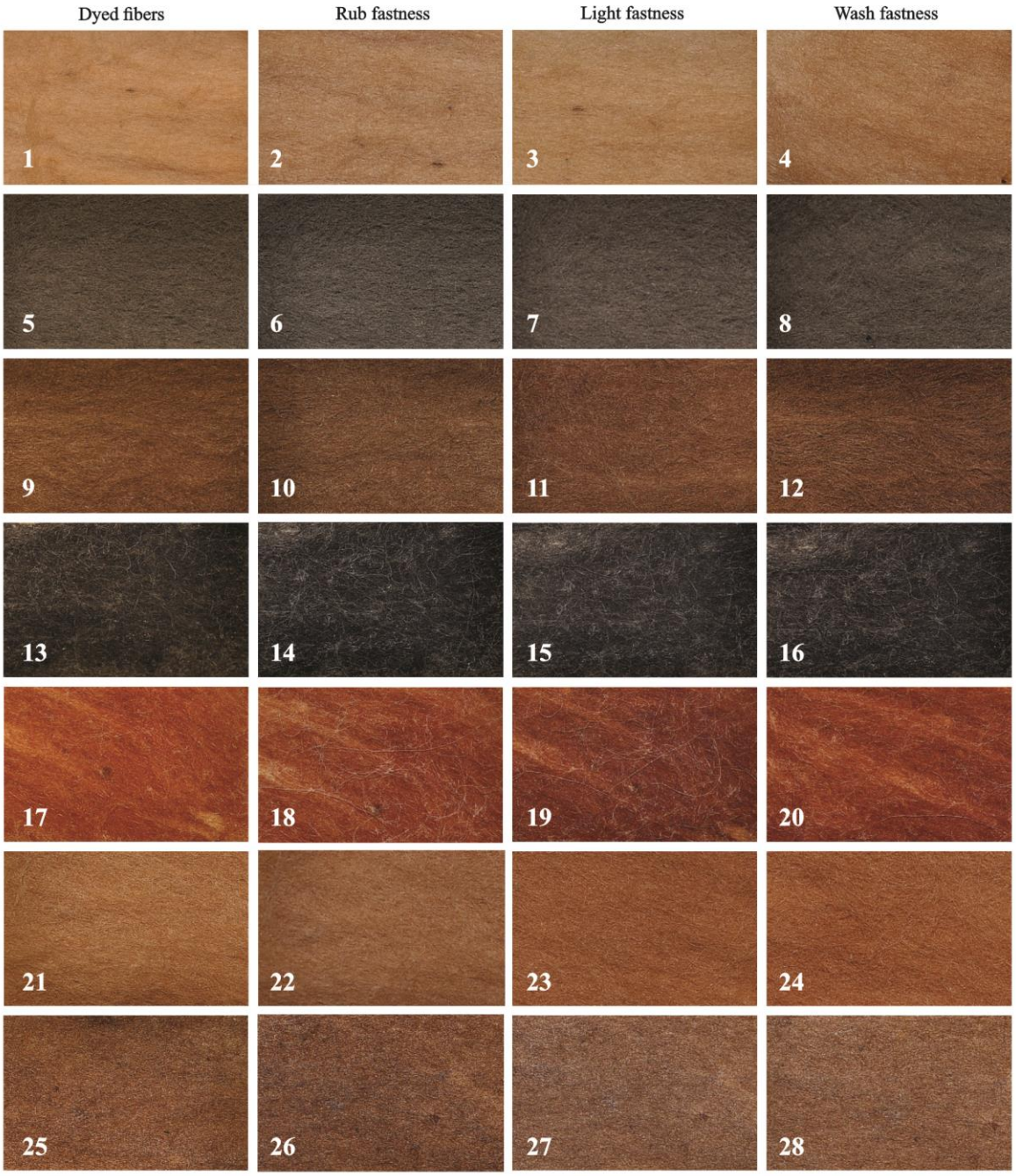

Plate 6. Dyeing of cotton fibers with *Artocarpus lakoocha* dye (Pre-mordanting).
1-4. Dyed with *A. lakoocha* dye
5-8. Dyed with *A. lakoocha* dye and $FeSO_4$
9-12. Dyed with *A. lakoocha* dye and $CuSO_4$
13-16. Dyed with *A. lakoocha* dye and $FeCl_2$
17-20. Dyed with *A. lakoocha* dye and $SnCl_2$
21-24. Dyed with *A. lakoocha* dye and Alum
25-28. Dyed with *A. lakoocha* dye and $K_2Cr_2O_7$

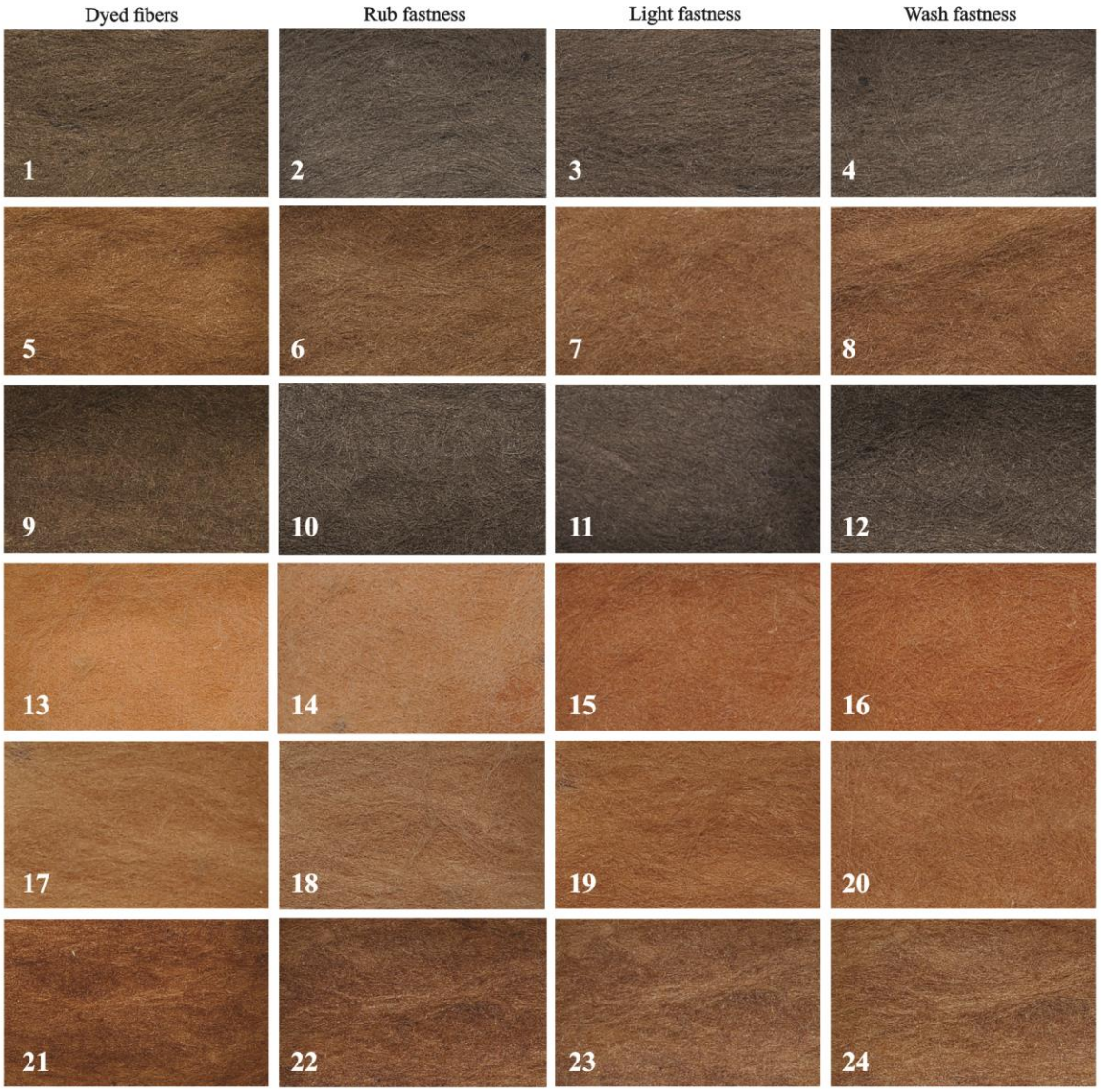

Plate 7. Dyeing of cotton fibers with *Artocarpus lakoocha* dye (Post-mordanting).
1-4. Dyed with *A. lakoocha* dye and $FeSO_4$
5-8. Dyed with *A. lakoocha* dye and $CuSO_4$
9-12. Dyed with *A. lakoocha* dye and $FeCl_2$
13-16. Dyed with *A. lakoocha* dye and $SnCl_2$
17-20. Dyed with *A. lakoocha* dye and Alum
21-24. Dyed with *A. lakoocha* dye and $K_2Cr_2O_7$

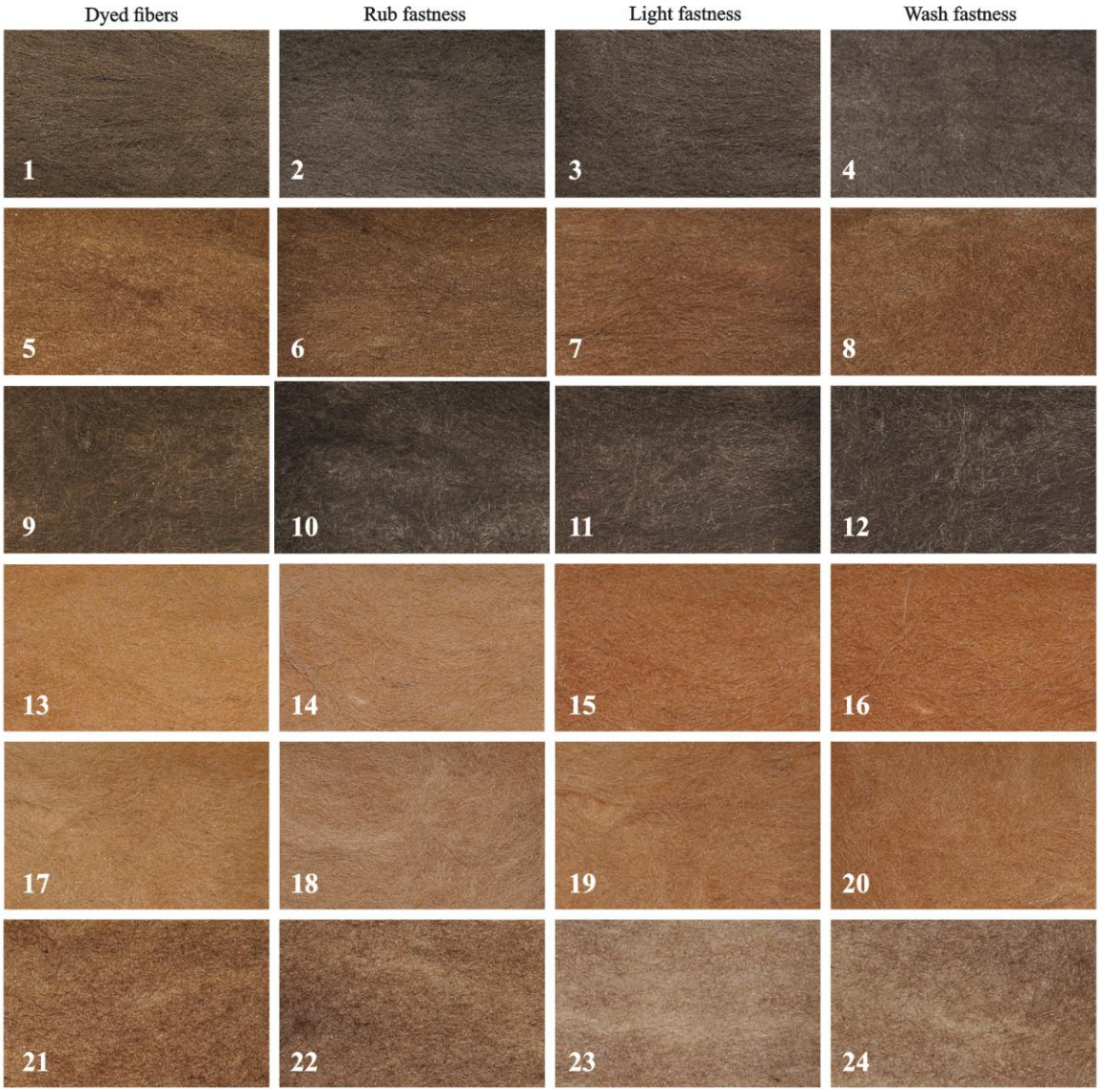

Plate 8. Dyeing of cotton fibers with *Artocarpus lakoocha* dye (Simultaneous mordanting).
1-4. Dyed with *A. lakoocha* dye and $FeSO_4$
5-8. Dyed with *A. lakoocha* dye and $CuSO_4$
9-12. Dyed with *A. lakoocha* dye and $FeCl_2$
13-16. Dyed with *A. lakoocha* dye and $SnCl_2$
17-20. Dyed with *A. lakoocha* dye and Alum
21-24. Dyed with *A. lakoocha* dye and $K_2Cr_2O_7$

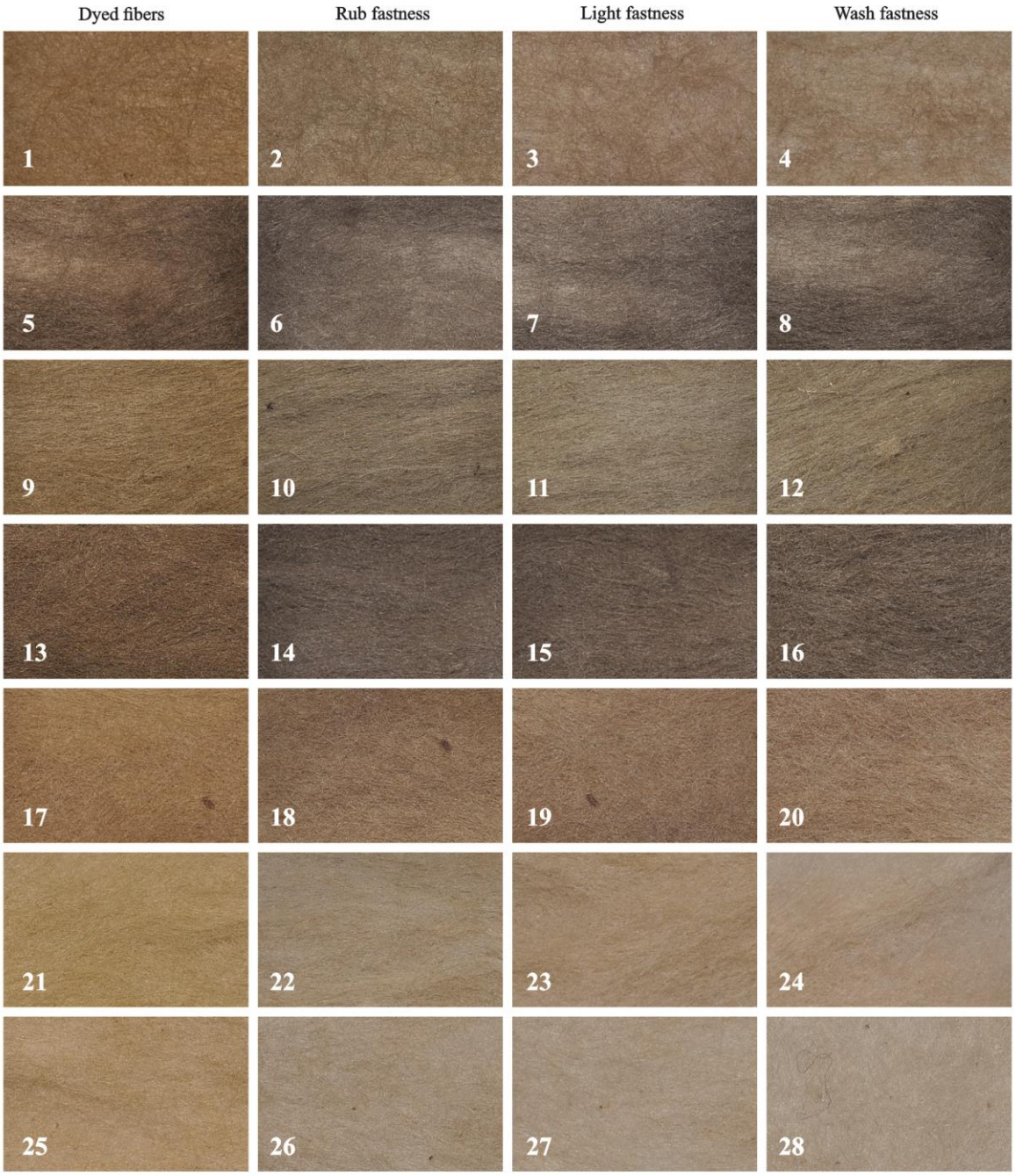

Plate 9. Dyeing of cotton fibers with *Heliotropium indicum* dye (Pre-mordanting).
1-4. Dyed with *H. indicum* dye
5-8. Dyed with *H. indicum* dye and $FeSO_4$
9-12. Dyed with *H. indicum* dye and $CuSO_4$
13-16. Dyed with *H. indicum* dye and $FeCl_2$
17-20. Dyed with *H. indicum* dye and $SnCl_2$
21-24. Dyed with *H. indicum* dye and Alum
25-28. Dyed with *H. indicum* dye and $K_2Cr_2O_7$

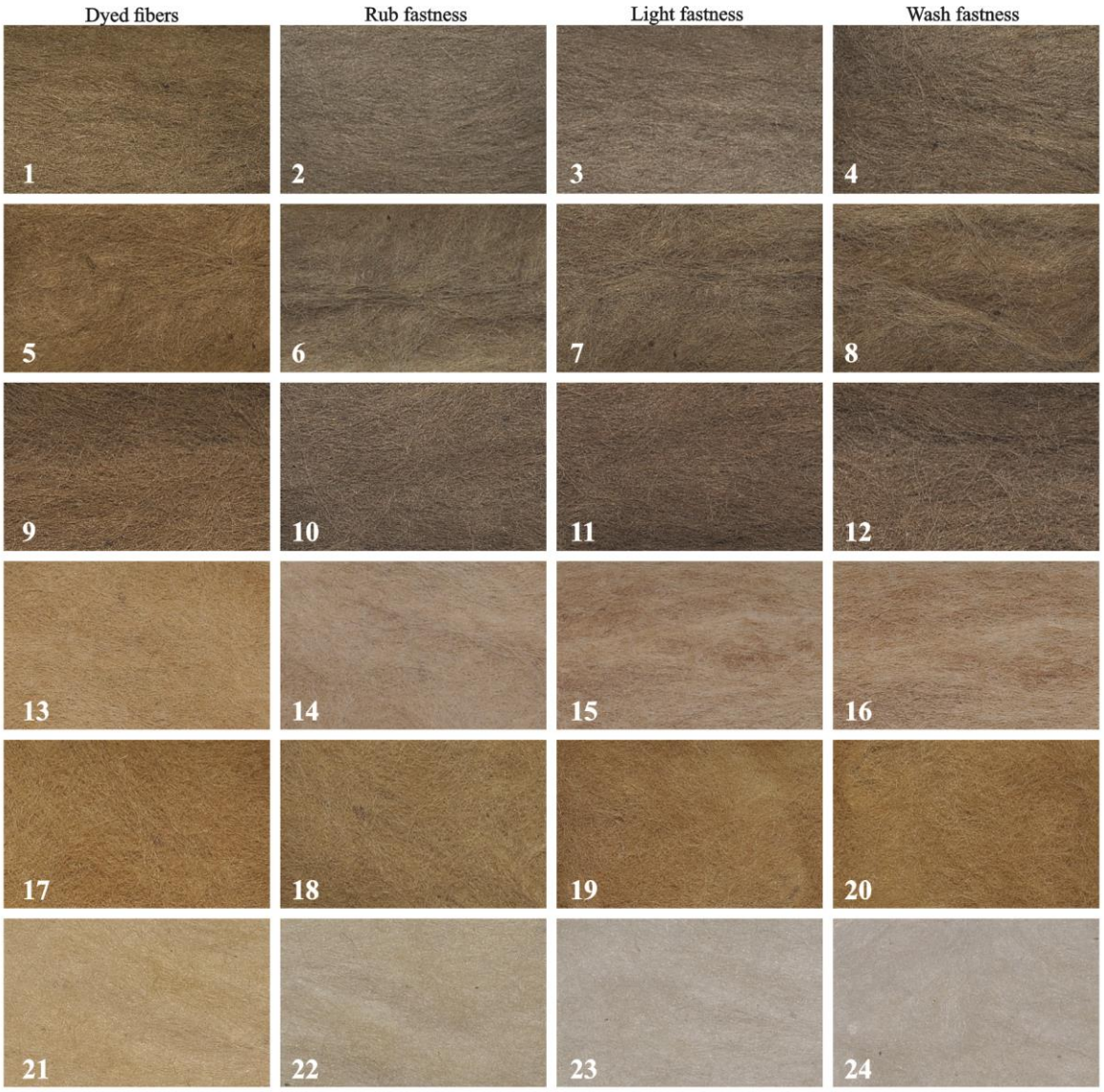

Plate 10. Dyeing of cotton fibers with *Heliotropium indicum* dye (Post-mordanting).
1-4. Dyed with *H. indicum* dye and $FeSO_4$
5-8. Dyed with *H. indicum* dye and $CuSO_4$
9-12. Dyed with *H. indicum* dye and $FeCl_2$
13-16. Dyed with *H. indicum* dye and $SnCl_2$
17-20. Dyed with *H. indicum* dye and Alum
21-24. Dyed with *H. indicum* dye and $K_2Cr_2O_7$

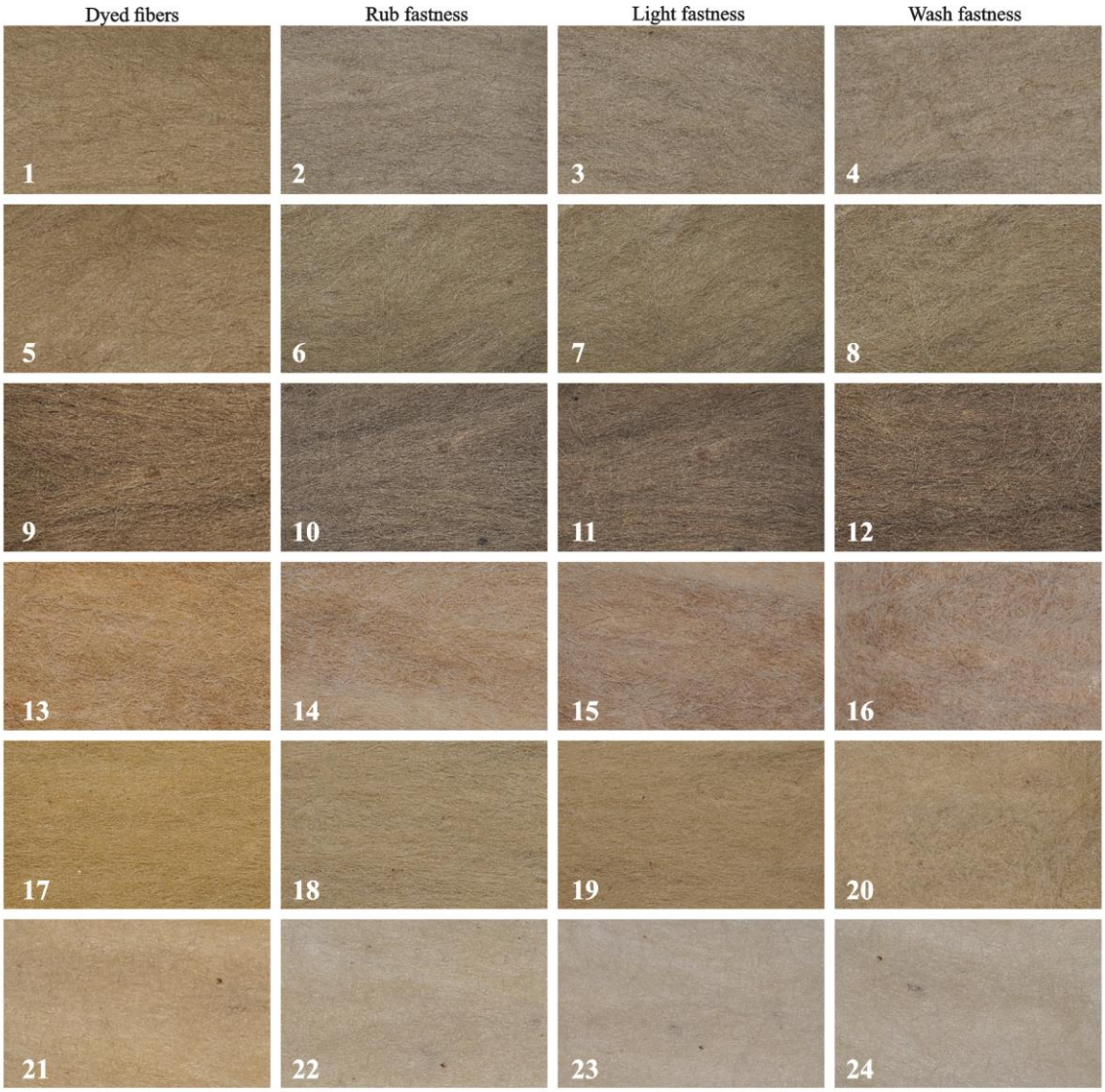

Plate 11. Dyeing of cotton fibers with *Heliotropium indicum* dye (Simultaneous mordanting).
1-4. Dyed with *H. indicum* dye and $FeSO_4$
5-8. Dyed with *H. indicum* dye and $CuSO_4$
9-12. Dyed with *H. indicum* dye and $FeCl_2$
13-16. Dyed with *H. indicum* dye and $SnCl_2$
17-20. Dyed with *H. indicum* dye and Alum
21-24. Dyed with *H. indicum* dye and $K_2Cr_2O_7$

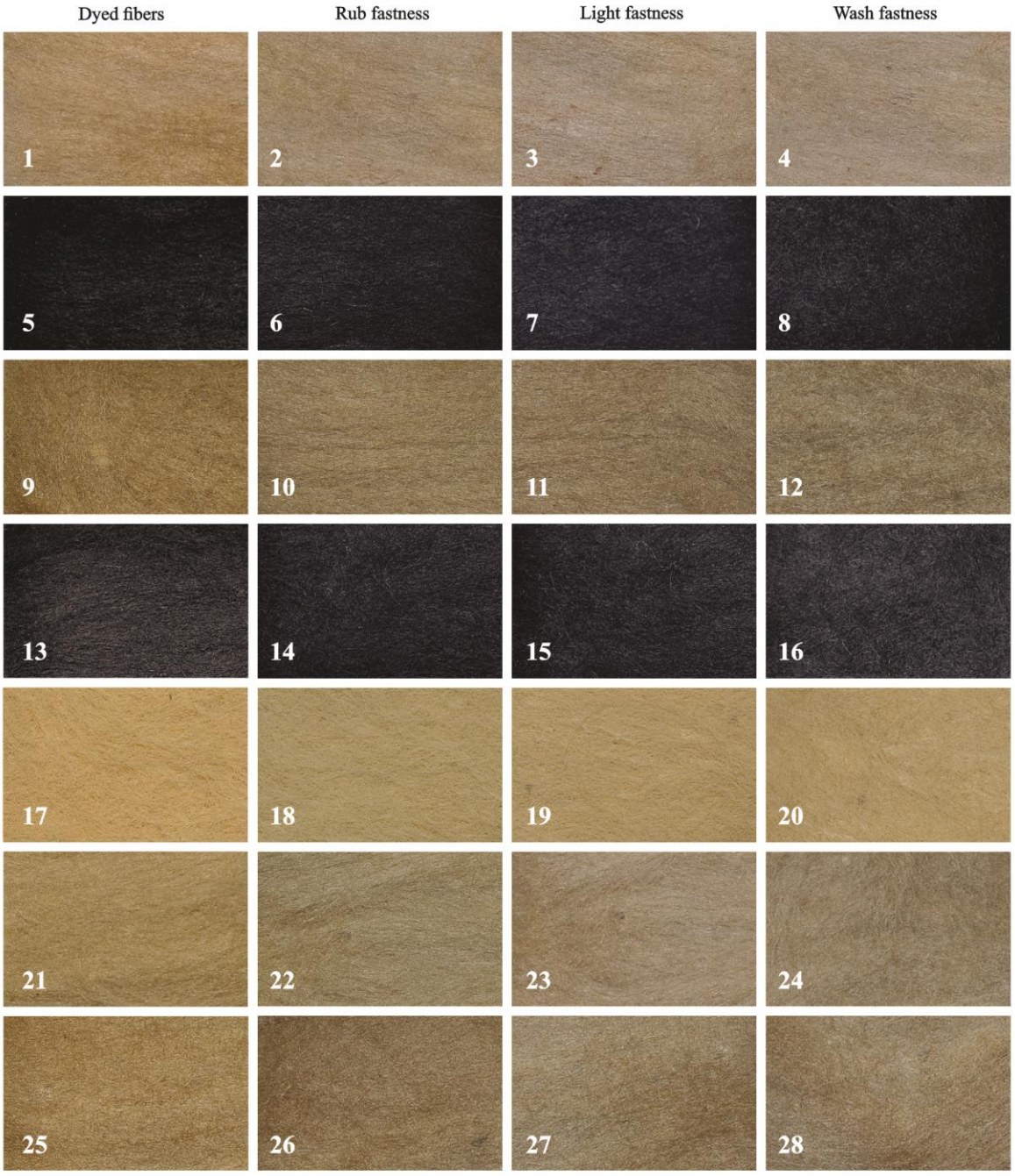

Plate 12. Dyeing of cotton fibers with *Phyllanthus emblica* dye (Pre-mordanting).
1-4. Dyed with *P. emblica* dye
5-8. Dyed with *P. emblica* dye and $FeSO_4$
9-12. Dyed with *P. emblica* dye and $CuSO_4$
13-16. Dyed with *P. emblica* dye and $FeCl_2$
17-20. Dyed with *P. emblica* dye and $SnCl_2$
21-24. Dyed with *P. emblica* dye and Alum
25-28. Dyed with *P. emblica* dye and $K_2Cr_2O_7$

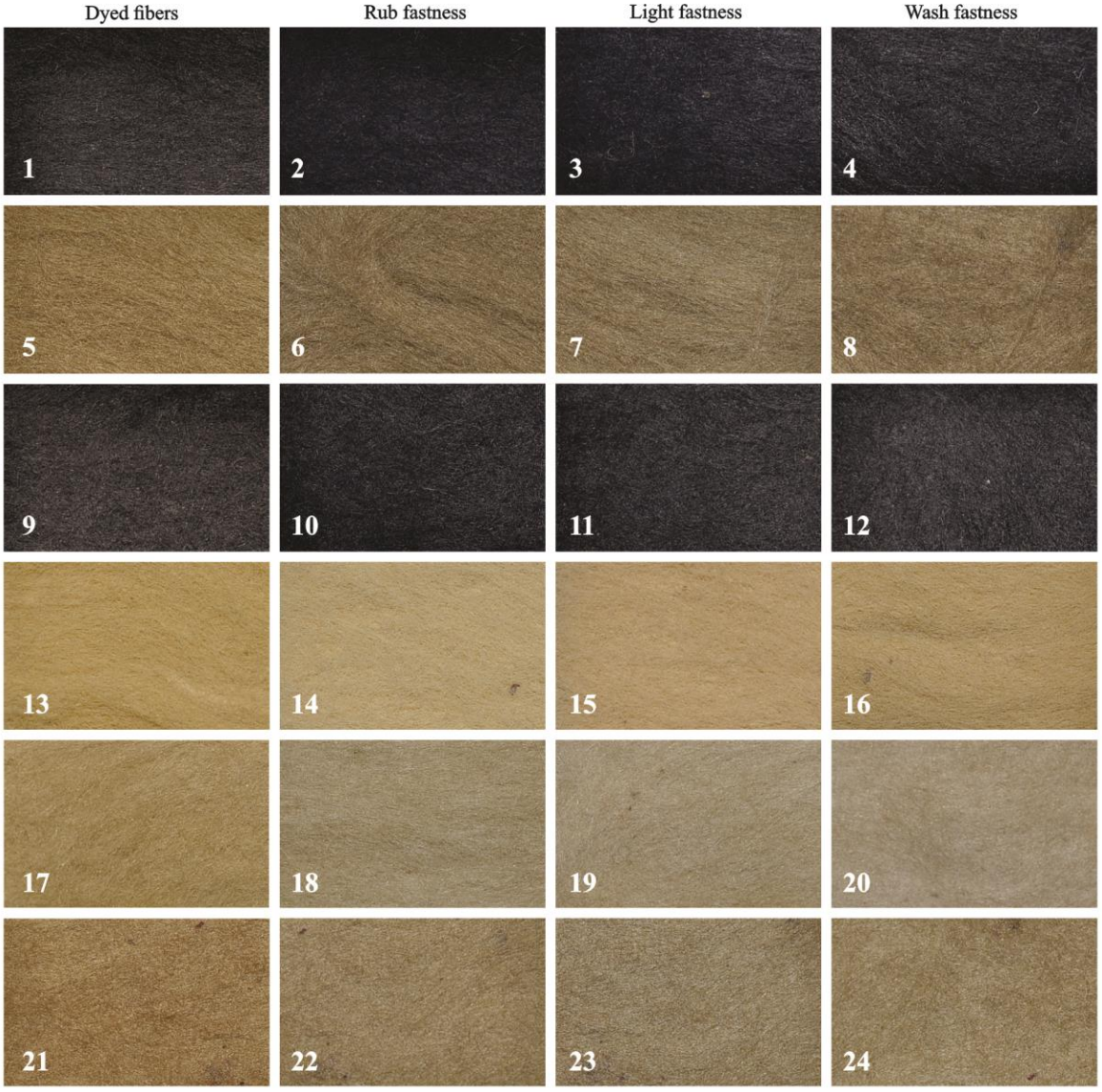

Plate 13. Dyeing of cotton fibers with *Phyllanthus emblica* dye (Post-mordanting).
1-4. Dyed with *P. emblica* dye and $FeSO_4$
5-8. Dyed with *P. emblica* dye and $CuSO_4$
9-12. Dyed with *P. emblica* dye and $FeCl_2$
13-16. Dyed with *P. emblica* dye and $SnCl_2$
17-20. Dyed with *P. emblica* dye and Alum
21-24. Dyed with *P. emblica* dye and $K_2Cr_2O_7$

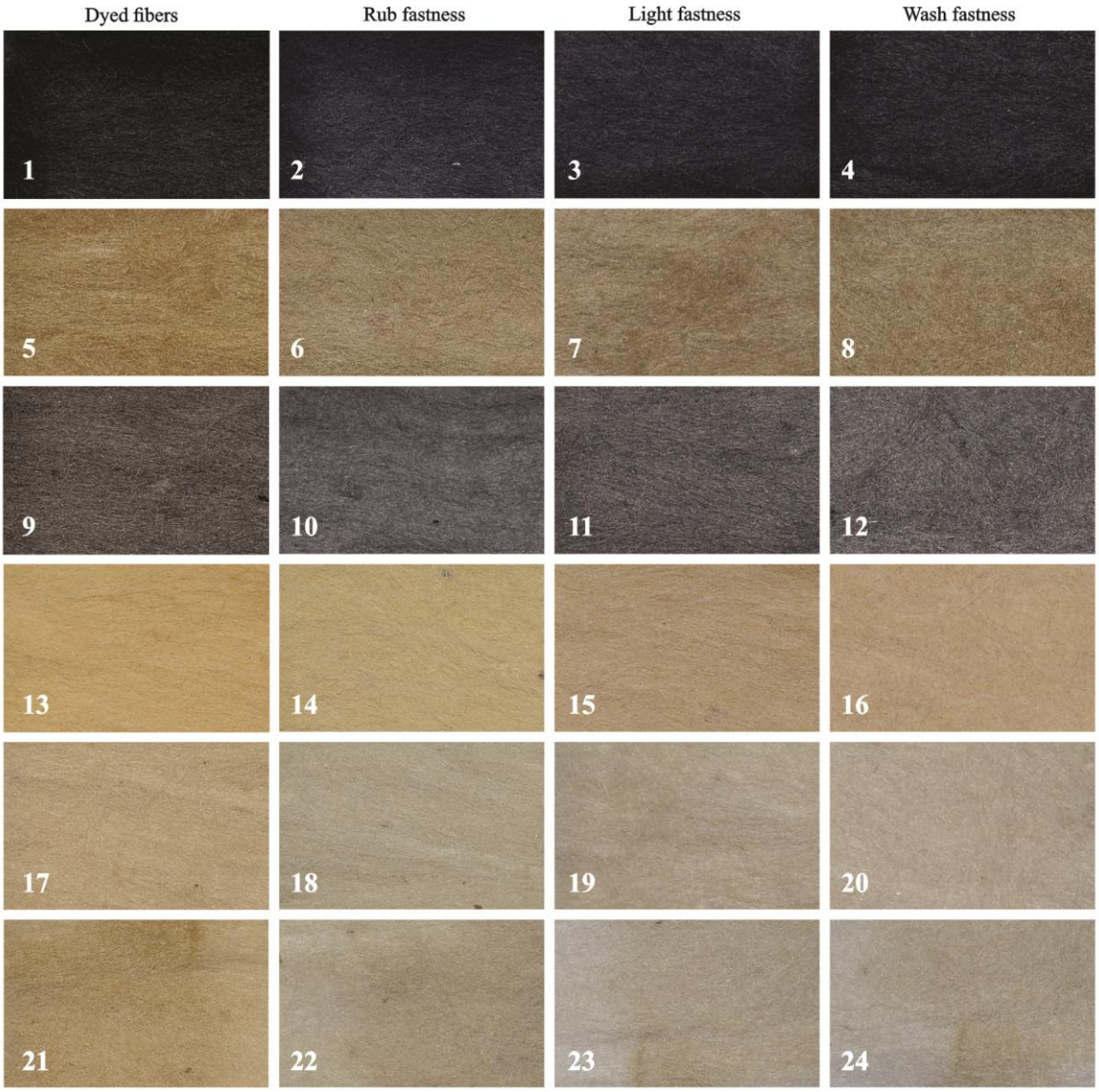

Plate 14. Dyeing of cotton fibers with *Phyllanthus emblica* dye (Simultaneous mordanting).
1-4. Dyed with *P. emblica* dye and $FeSO_4$
5-8. Dyed with *P. emblica* dye and $CuSO_4$
9-12. Dyed with *P. emblica* dye and $FeCl_2$
13-16. Dyed with *P. emblica* dye and $SnCl_2$
17-20. Dyed with *P. emblica* dye and Alum
21-24. Dyed with *P. emblica* dye and $K_2Cr_2O_7$

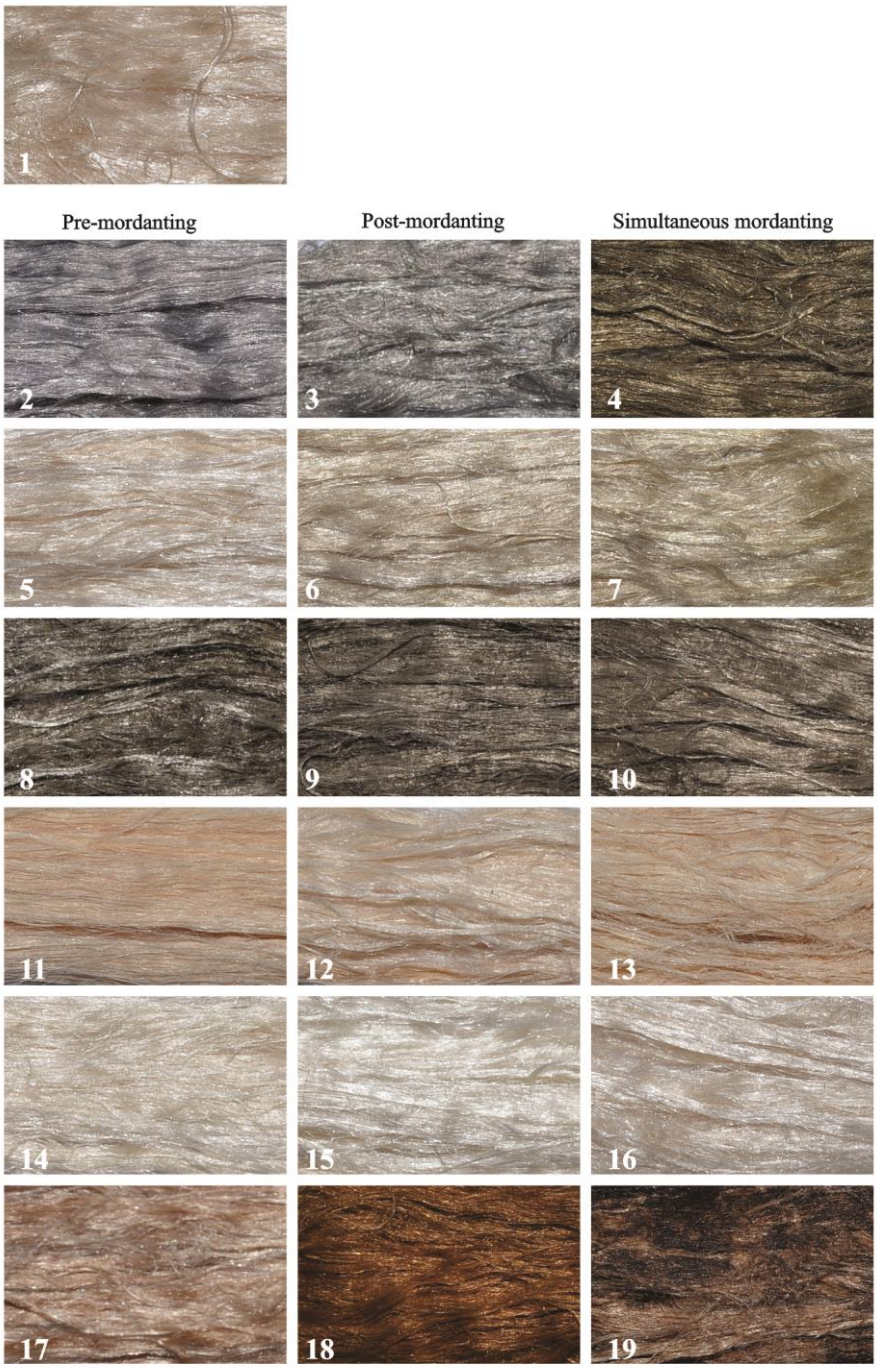

Plate 15. Dyeing of silk fibers with *Calliandra surinamensis* dye.
1. Dyed with *C. surinamensis* dye
2-4. Dyed with *C. surinamensis* dye and $FeSO_4$
5-7. Dyed with *C. surinamensis* dye and $CuSO_4$
8-10. Dyed with *C. surinamensis* dye and $FeCl_2$
11-13. Dyed with *C. surinamensis* dye and $SnCl_2$
14-16. Dyed with *C. surinamensis* dye and Alum
17-19. Dyed with *C. surinamensis* dye and $K_2Cr_2O_7$

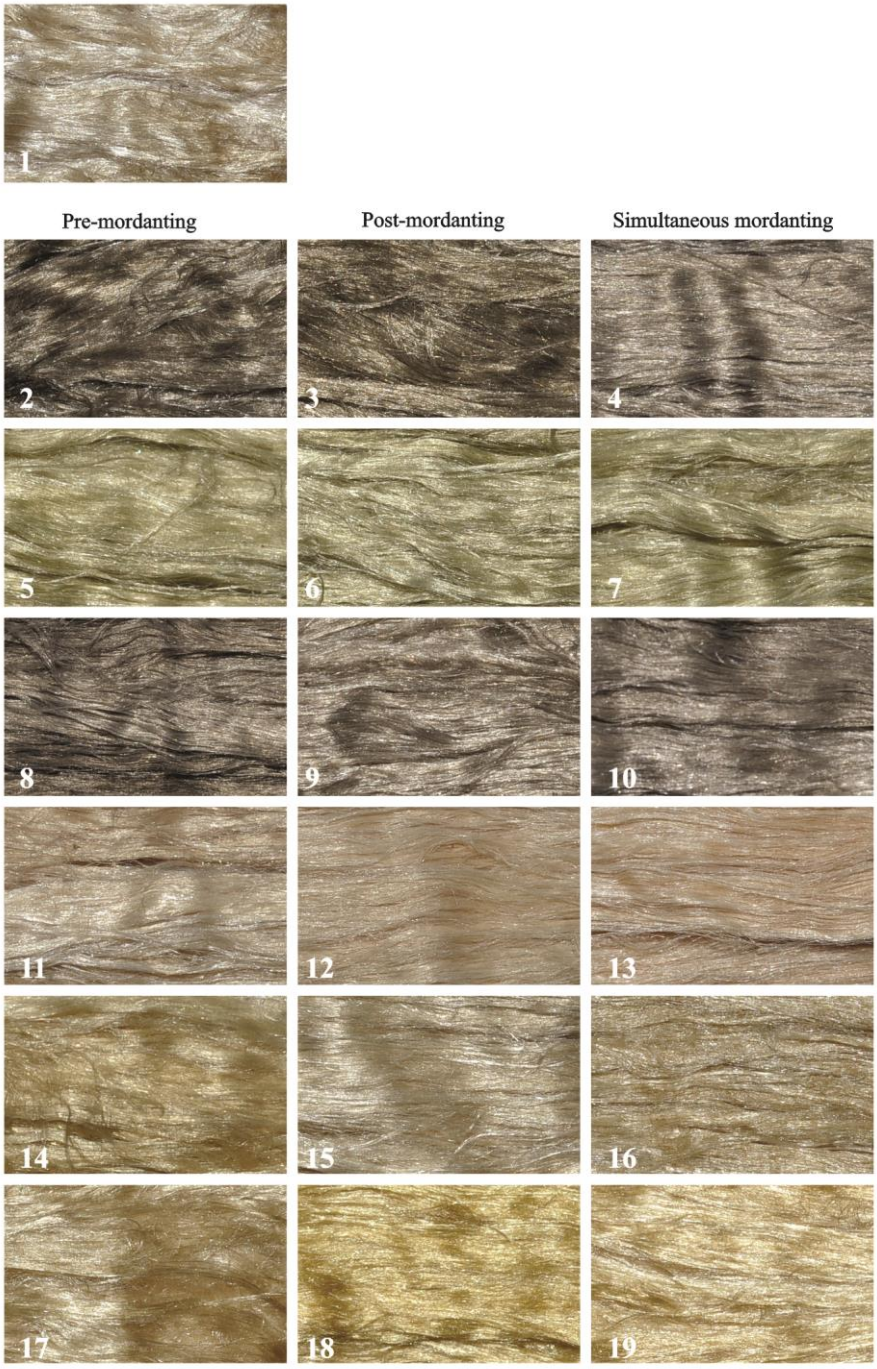

Plate 16. Dyeing of silk fibers with *Cymbopogon citratus* dye.
1. Dyed with *C. citratus* dye
2-4. Dyed with *C. citratus* dye and $FeSO_4$
5-7. Dyed with *C. citratus* dye and $CuSO_4$
8-10. Dyed with *C. citratus* dye and $FeCl_2$
11-13. Dyed with *C. citratus* dye and $SnCl_2$
14-16. Dyed with *C. citratus* dye and Alum
17-19. Dyed with *C. citratus* dye and $K_2Cr_2O_7$

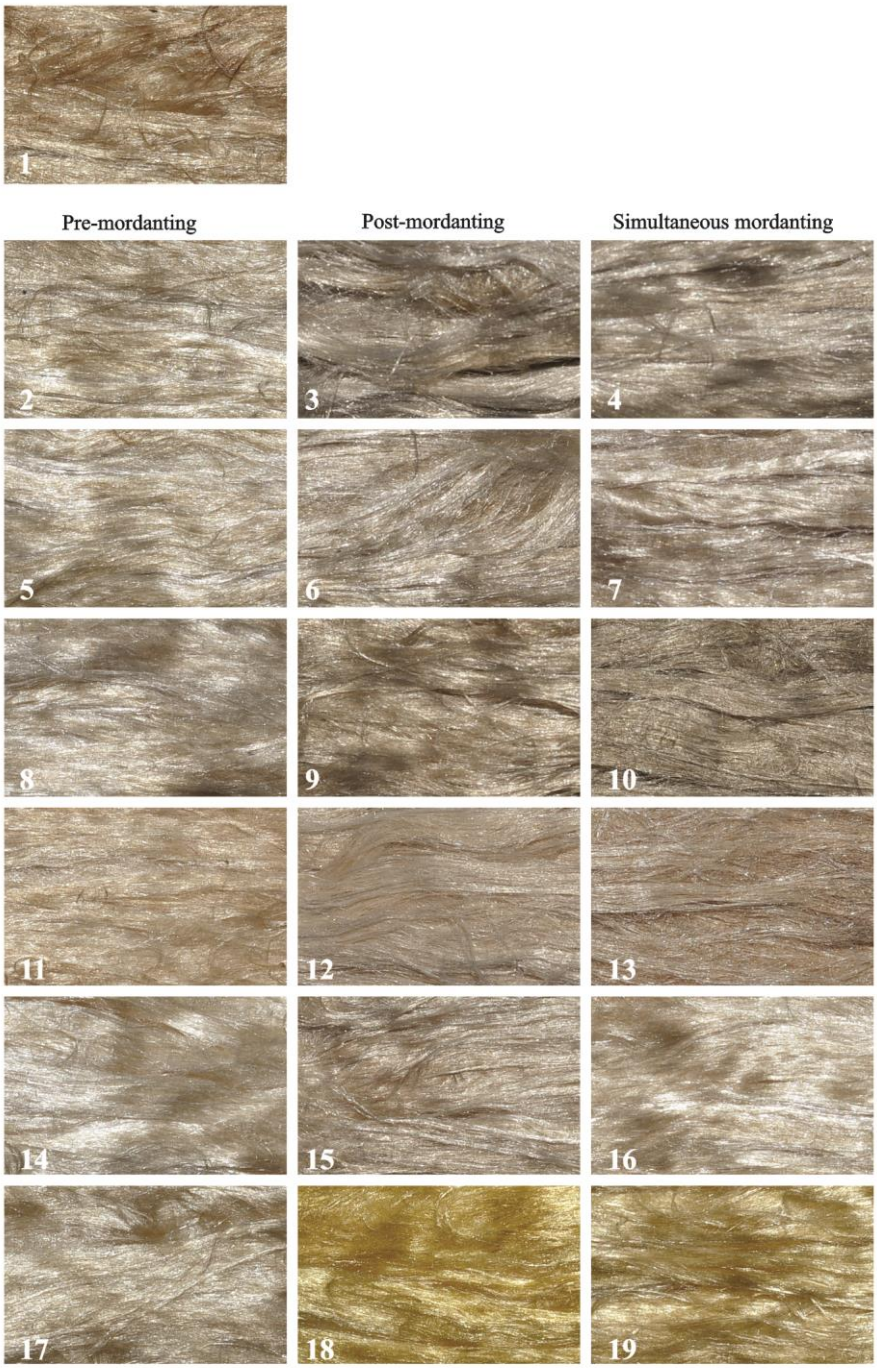

Plate 17. Dyeing of silk fibers with *Piper betle* dye.
1. Dyed with *P. betle* dye
2-4. Dyed with *P. betle* dye and $FeSO_4$
5-7. Dyed with *P. betle* dye and $CuSO_4$
8-10. Dyed with *P. betle* dye and $FeCl_2$
11-13. Dyed with *P. betle* dye and $SnCl_2$
14-16. Dyed with *P. betle* dye and Alum
17-19. Dyed with *P. betle* dye and $K_2Cr_2O_7$

Plate 18. Dyeing of silk fibers with *Artocarpus lakoocha* dye (Pre-mordanting).
1-4. Dyed with *A. lakoocha* dye
5-8. Dyed with *A. lakoocha* dye and $FeSO_4$
9-12. Dyed with *A. lakoocha* dye and $CuSO_4$
13-16. Dyed with *A. lakoocha* dye and $FeCl_2$
17-20. Dyed with *A. lakoocha* dye and $SnCl_2$
21-24. Dyed with *A. lakoocha* dye and Alum
25-28. Dyed with *A. lakoocha* dye and $K_2Cr_2O_7$

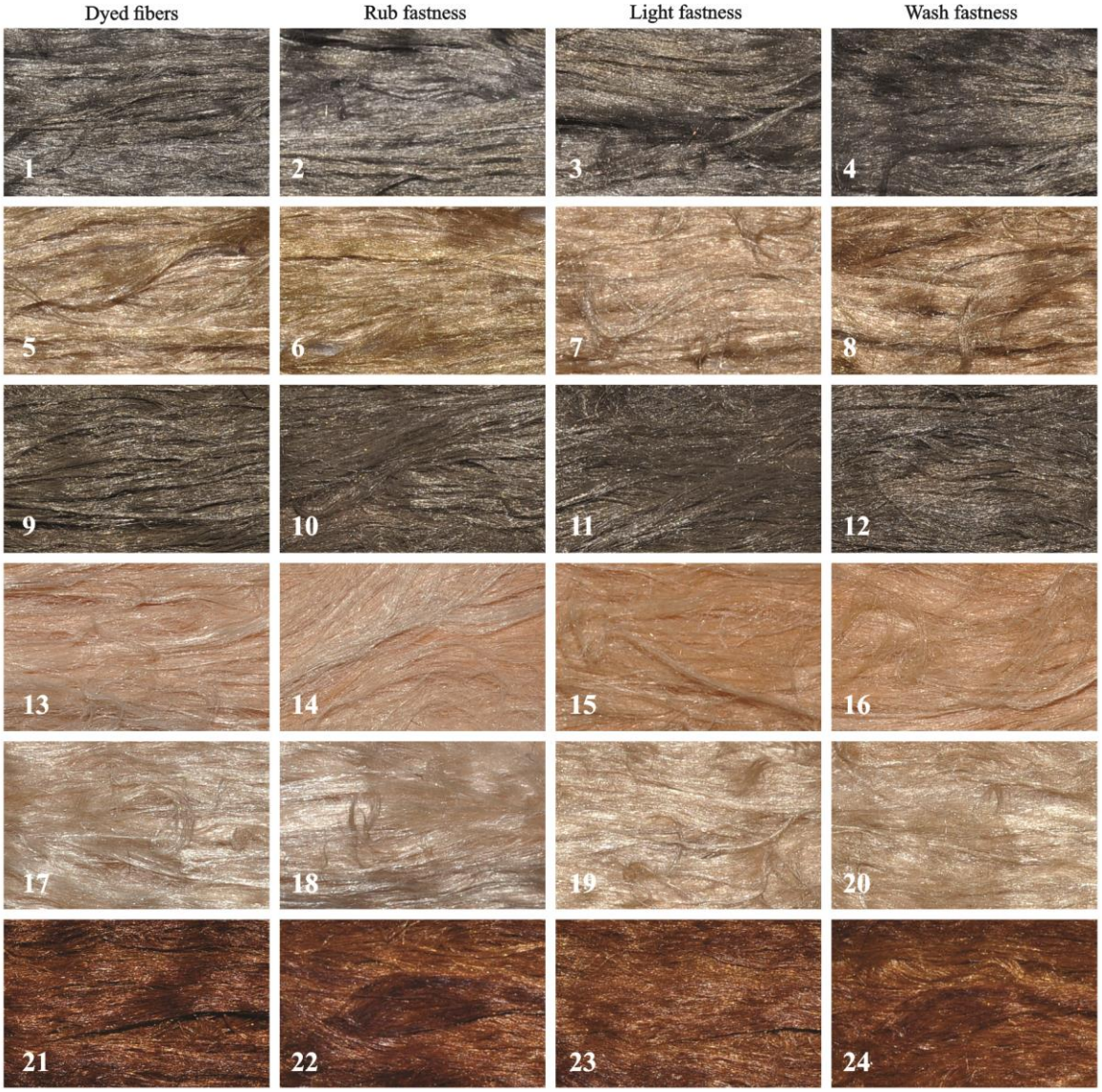

Plate 19. Dyeing of silk fibers with *Artocarpus lakoocha* dye (Post-mordanting).
1-4. Dyed with *A. lakoocha* dye and $FeSO_4$
5-8. Dyed with *A. lakoocha* dye and $CuSO_4$
9-12. Dyed with *A. lakoocha* dye and $FeCl_2$
13-16. Dyed with *A. lakoocha* dye and $SnCl_2$
17-20. Dyed with *A. lakoocha* dye and Alum
21-24. Dyed with *A. lakoocha* dye and $K_2Cr_2O_7$

Plate 20. Dyeing of cotton fibers with *Artocarpus lakoocha* dye (Simultaneous mordanting).
1-4. Dyed with *A. lakoocha* dye and $FeSO_4$
5-8. Dyed with *A. lakoocha* dye and $CuSO_4$
9-12. Dyed with *A. lakoocha* dye and $FeCl_2$
13-16. Dyed with *A. lakoocha* dye and $SnCl_2$
17-20. Dyed with *A. lakoocha* dye and Alum
21-24. Dyed with *A. lakoocha* dye and $K_2Cr_2O_7$

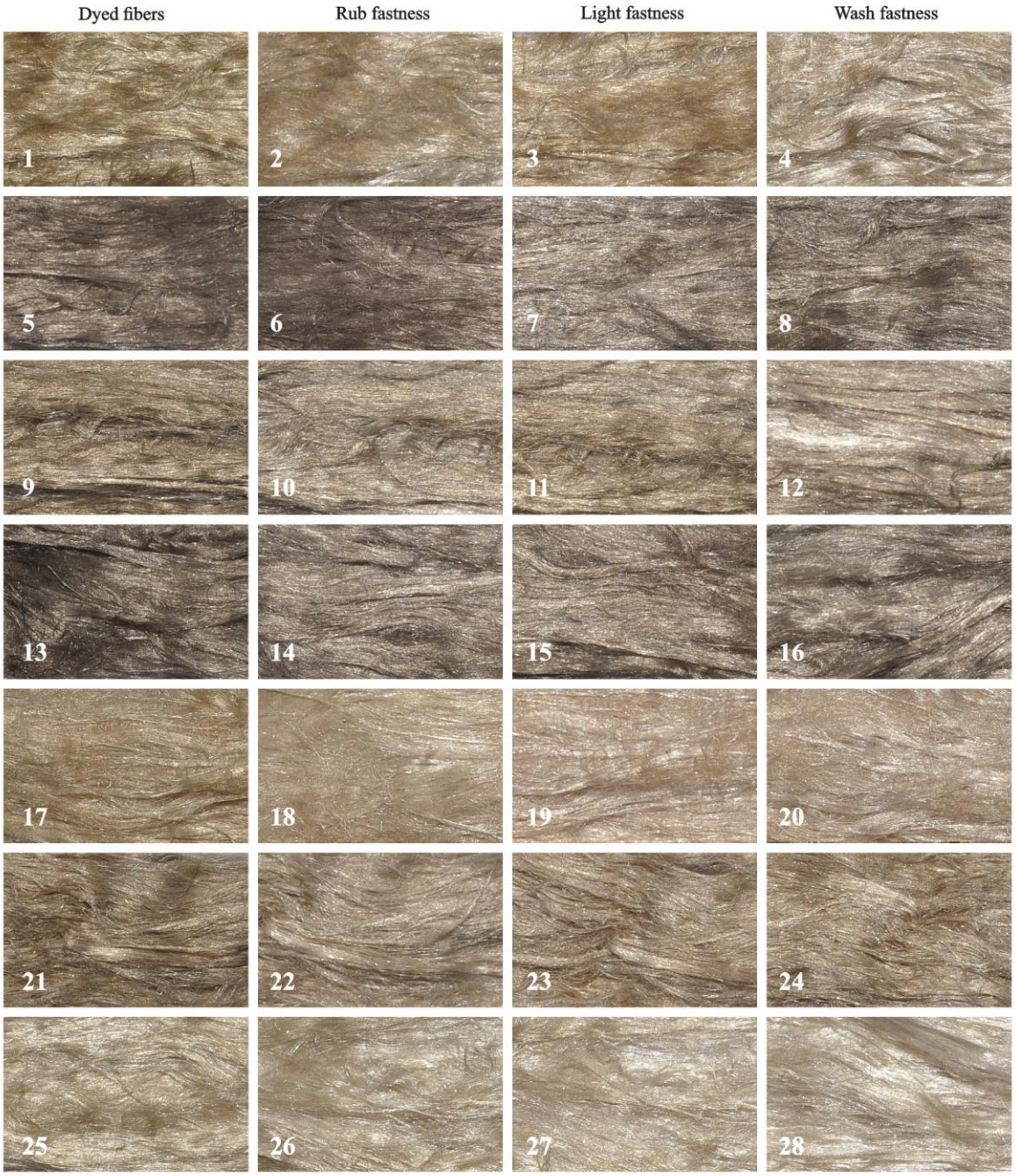

Plate 21. Dyeing of silk fibers with *Heliotropium indicum* dye (Pre-mordanting).
1-4. Dyed with *H. indicum* dye
5-8. Dyed with *H. indicum* dye and $FeSO_4$
9-12. Dyed with *H. indicum* dye and $CuSO_4$
13-16. Dyed with *H. indicum* dye and $FeCl_2$
17-20. Dyed with *H. indicum* dye and $SnCl_2$
21-24. Dyed with *H. indicum* dye and Alum
25-28. Dyed with *H. indicum* dye and $K_2Cr_2O_7$

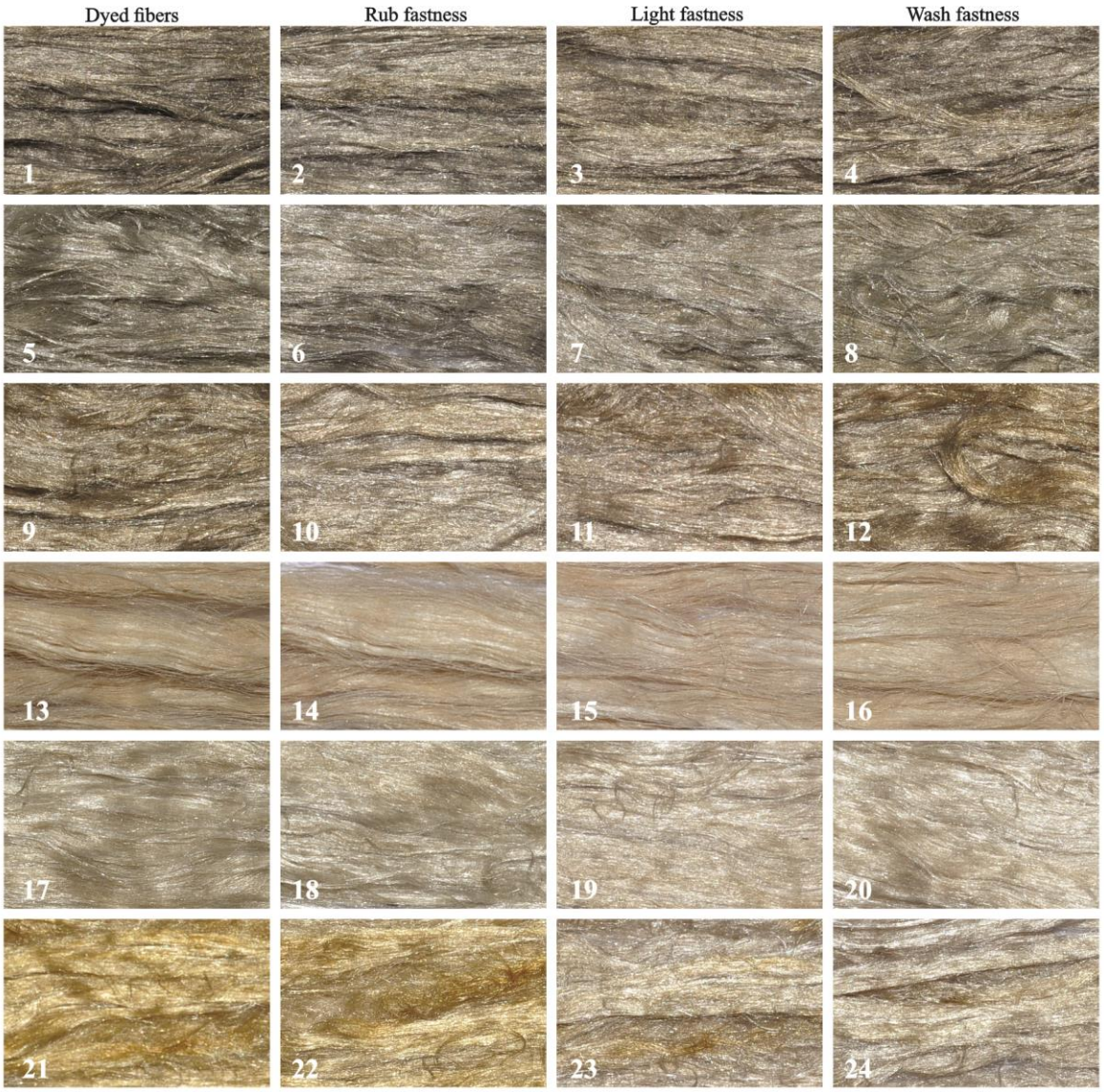

Plate 22. Dyeing of silk fibers with *Heliotropium indicum* dye (Post-mordanting).
1-4. Dyed with *H. indicum* dye and $FeSO_4$
5-8. Dyed with *H. indicum* dye and $CuSO_4$
9-12. Dyed with *H. indicum* dye and $FeCl_2$
13-16. Dyed with *H. indicum* dye and $SnCl_2$
17-20. Dyed with *H. indicum* dye and Alum
21-24. Dyed with *H. indicum* dye and $K_2Cr_2O_7$

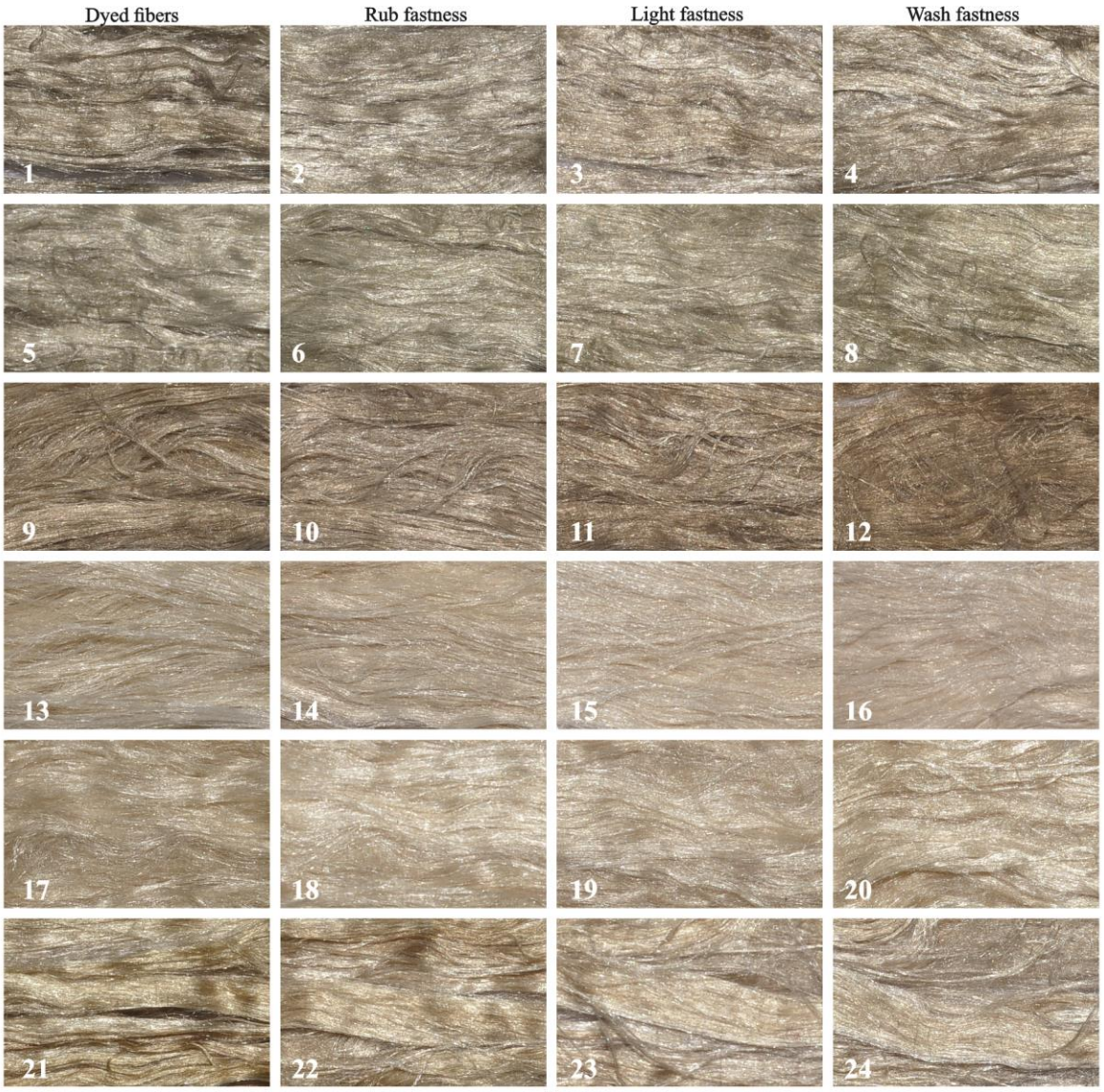

Plate 23. Dyeing of silk fibers with *Heliotropium indicum* dye (Simultaneous mordanting).
1-4. Dyed with *H. indicum* dye and $FeSO_4$
5-8. Dyed with *H. indicum* dye and $CuSO_4$
9-12. Dyed with *H. indicum* dye and $FeCl_2$
13-16. Dyed with *H. indicum* dye and $SnCl_2$
17-20. Dyed with *H. indicum* dye and Alum
21-24. Dyed with *H. indicum* dye and $K_2Cr_2O_7$

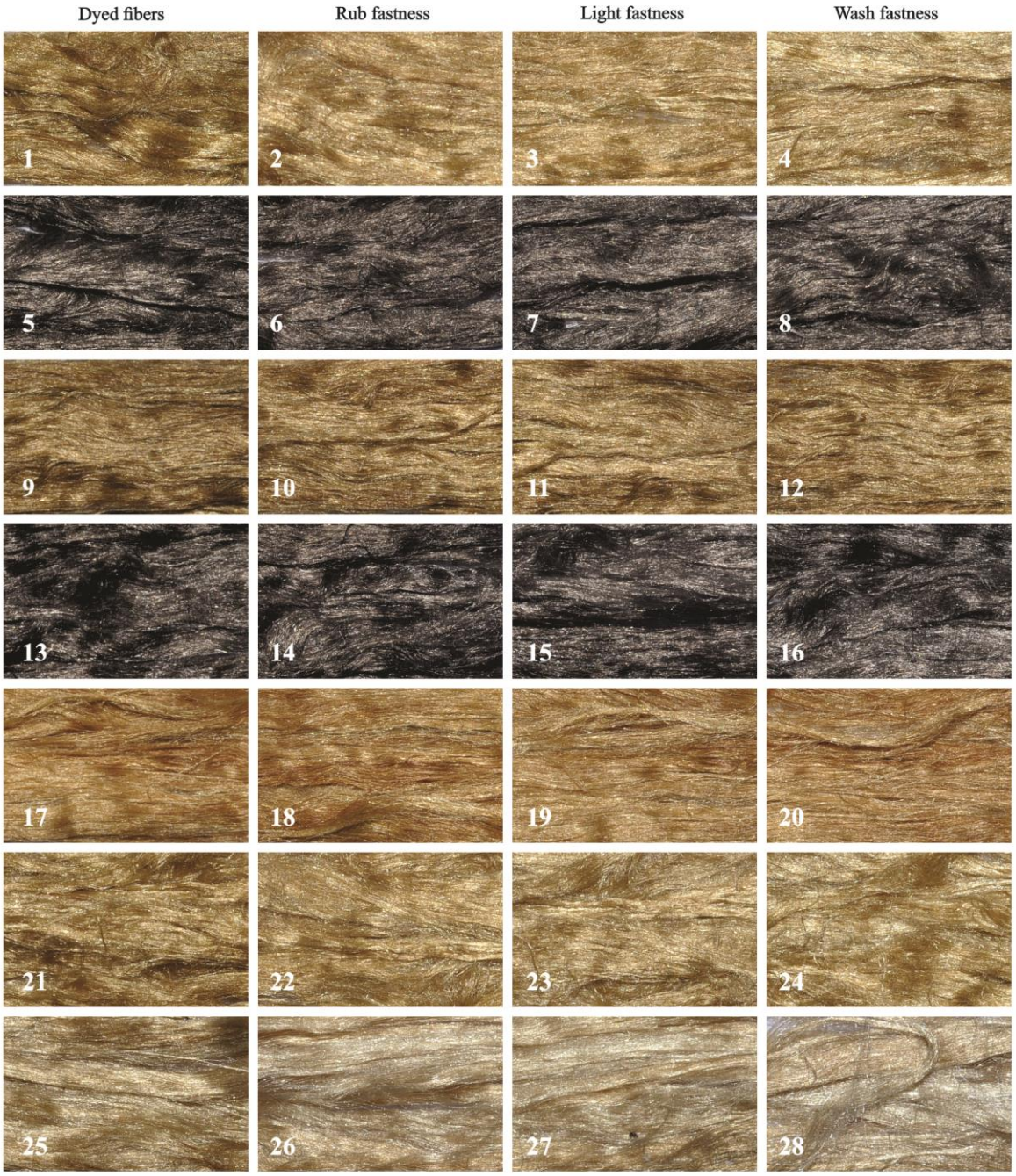

Plate 24. Dyeing of silk fibers with *Phyllanthus emblica* dye (Pre-mordanting).
1-4. Dyed with *P. emblica* dye
5-8. Dyed with *P. emblica* dye and $FeSO_4$
9-12. Dyed with *P. emblica* dye and $CuSO_4$
13-16. Dyed with *P. emblica* dye and $FeCl_2$
17-20. Dyed with *P. emblica* dye and $SnCl_2$
21-24. Dyed with *P. emblica* dye and Alum
25-28. Dyed with *P. emblica* dye and $K_2Cr_2O_7$

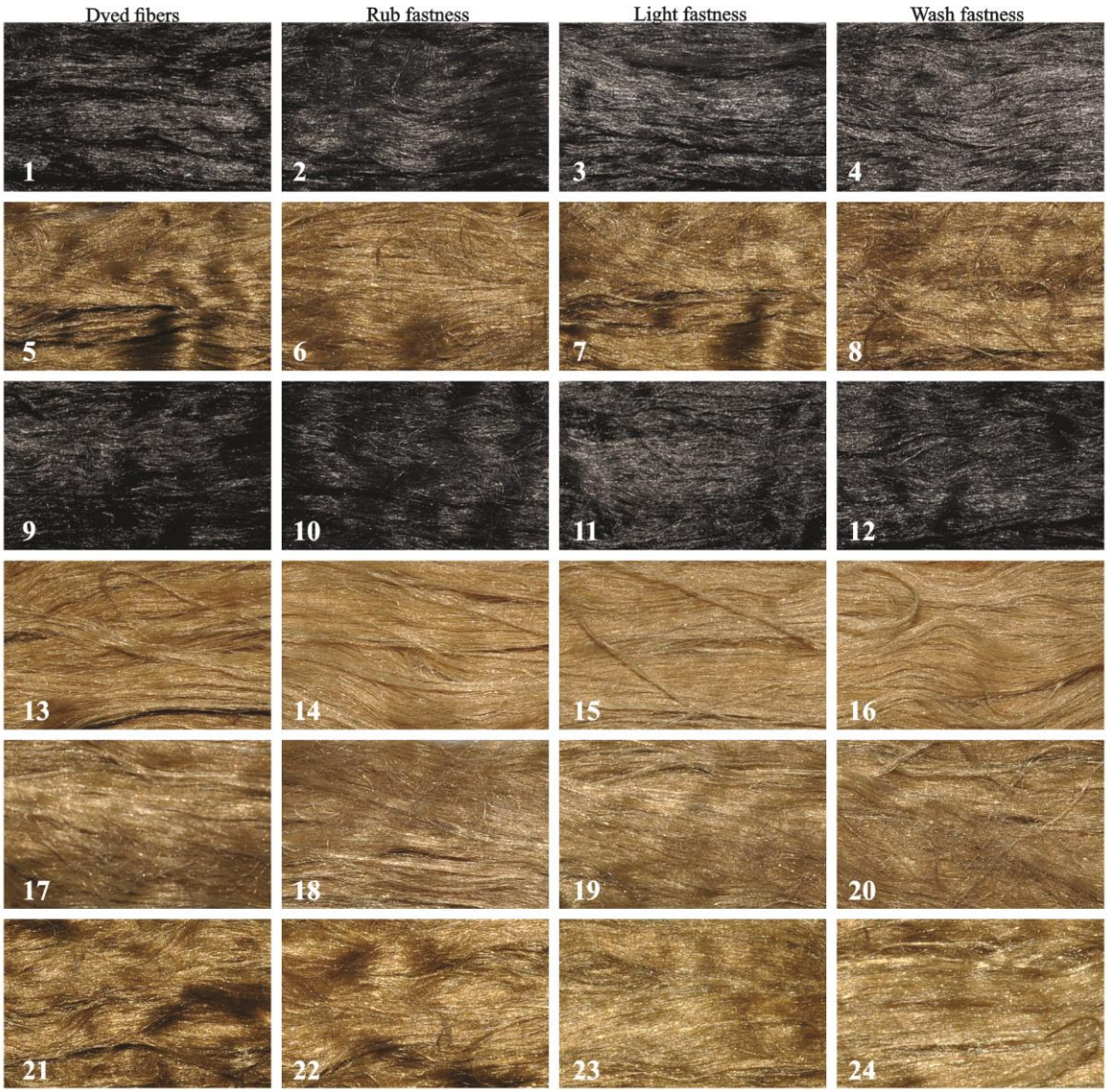

Plate 25. Dyeing of silk fibers with *Phyllanthus emblica* dye (Post-mordanting).
1-4. Dyed with *P. emblica* dye and $FeSO_4$
5-8. Dyed with *P. emblica* dye and $CuSO_4$
9-12. Dyed with *P. emblica* dye and $FeCl_2$
13-16. Dyed with *P. emblica* dye and $SnCl_2$
17-20. Dyed with *P. emblica* dye and Alum
21-24. Dyed with *P. emblica* dye and $K_2Cr_2O_7$

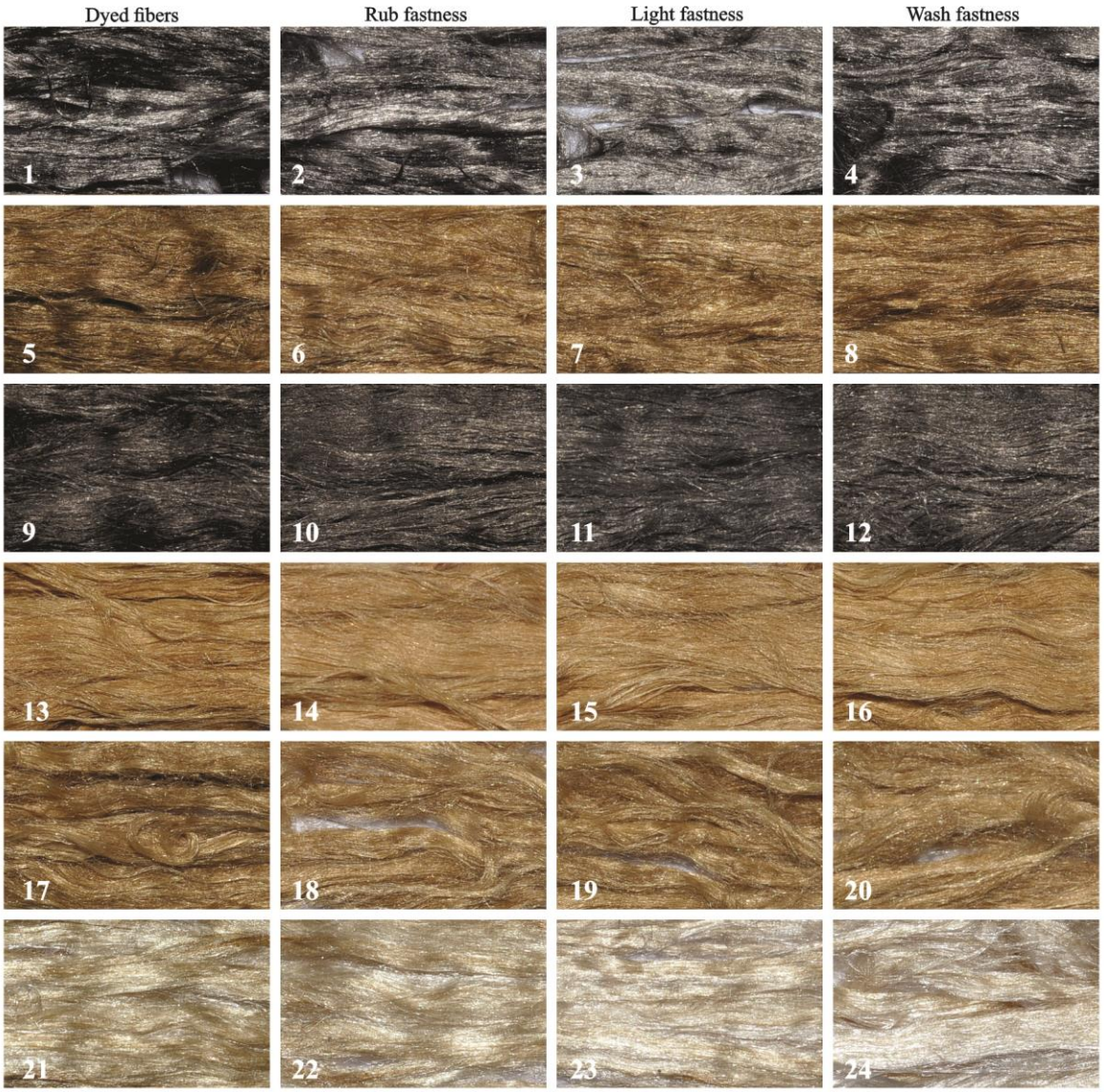

Plate 26. Dyeing of silk fibers with *Phyllanthus emblica* dye (Simultaneous mordanting).
1-4. Dyed with *P. emblica* dye and $FeSO_4$
5-8. Dyed with *P. emblica* dye and $CuSO_4$
9-12. Dyed with *P. emblica* dye and $FeCl_2$
13-16. Dyed with *P. emblica* dye and $SnCl_2$
17-20. Dyed with *P. emblica* dye and Alum
21-24. Dyed with *P. emblica* dye and $K_2Cr_2O_7$

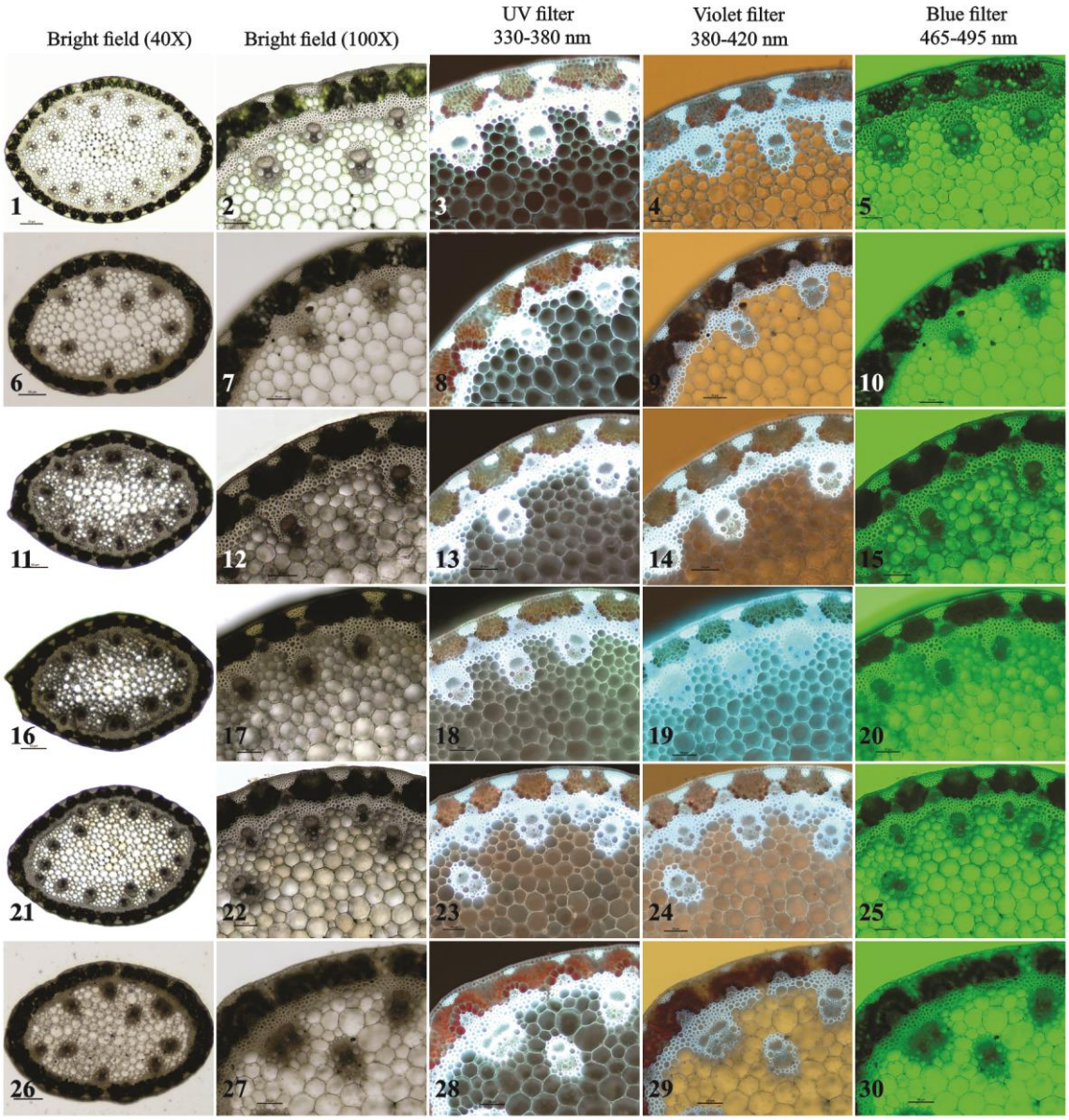

Plate 27. Bright field and fluorescence images of monocot stem.
1-2. Unstained section; 3-5. Auto - fluorescence
11-15. Section stained with *Artocarpus lakoocha* dye
16-20. Section stained with *Calliandra surinamensis* dye
21-25. Section stained with *Heliotropium indicum* dye
26-30. Section stained with *Piper betle* dye
31-35. Section stained with *Phyllanthus emblca* dye

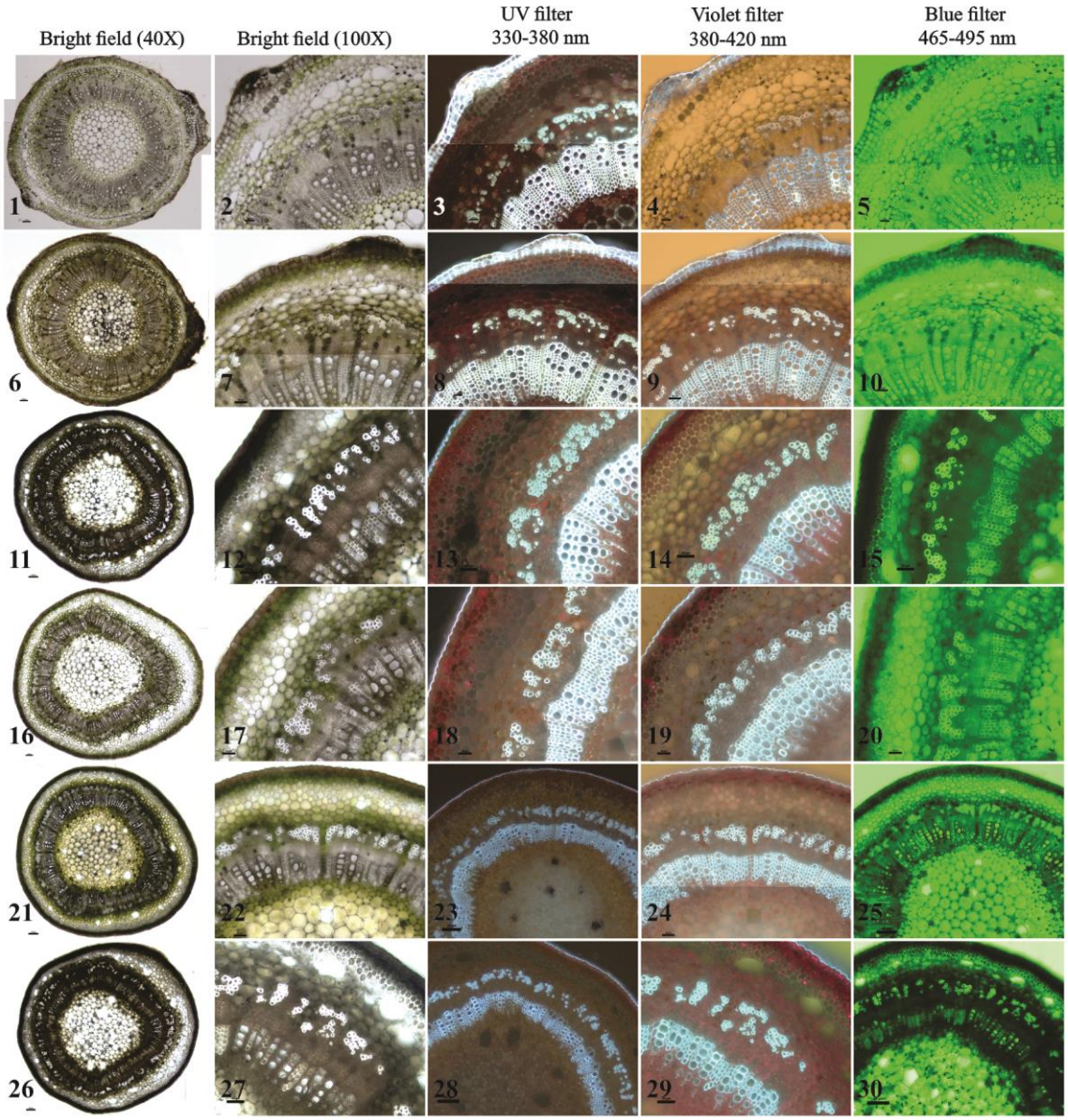

Plate 28. Bright field and fluorescence images of dicot stem.
1-2. Unstained section; 3-5. Auto - fluorescence
11-15. Section stained with *Artocarpus lakoocha* dye
16-20. Section stained with *Calliandra surinamensis* dye
21-25. Section stained with *Heliotropium indicum* dye
26-30. Section stained with *Piper betle* dye
31-35. Section stained with *Phyllanthus emblca* dye

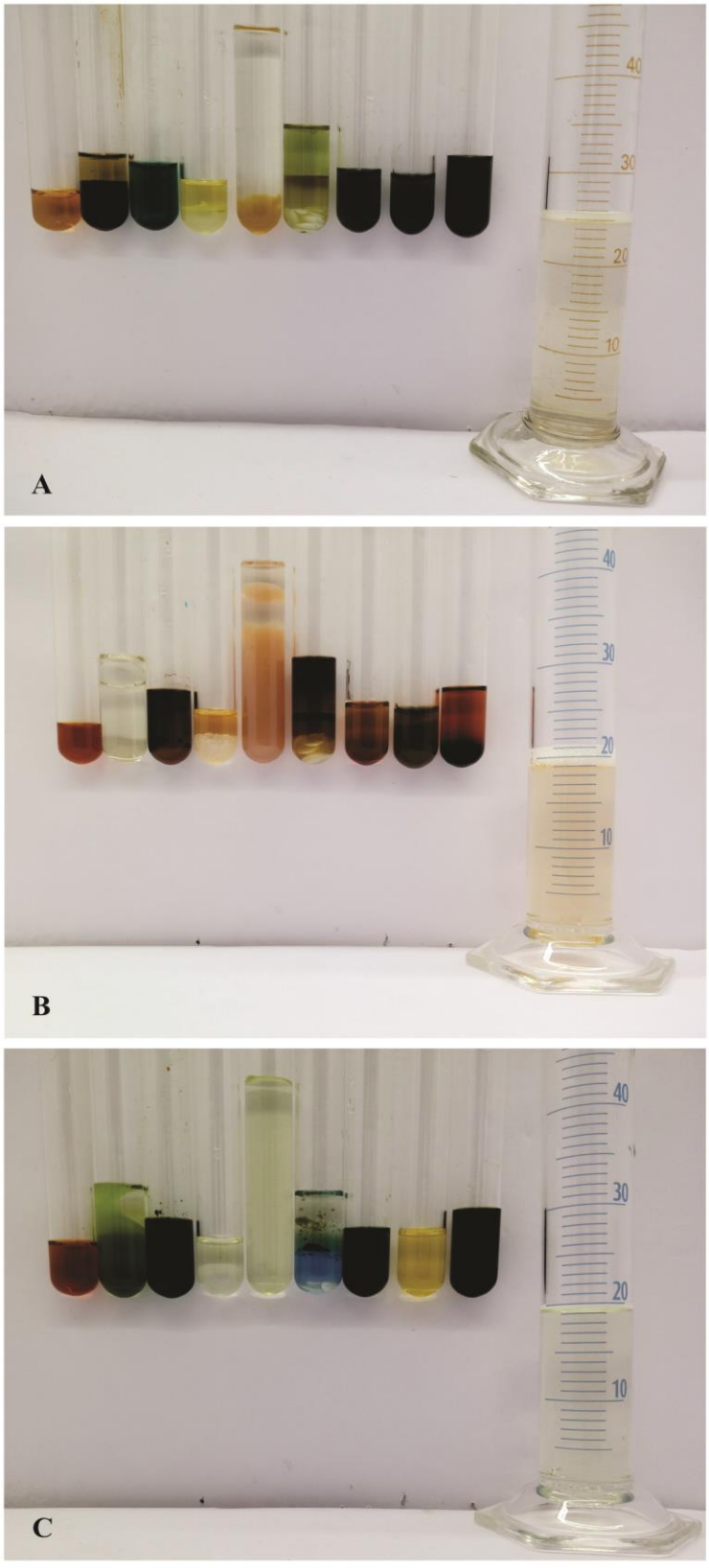

Plate 29. Qualitative phytochemical analysis of methanolic plant extracts.
A. *Phyllanthus emblica*
B. *Artocarpus lakoocha*
C. *Heliotropium indicum*

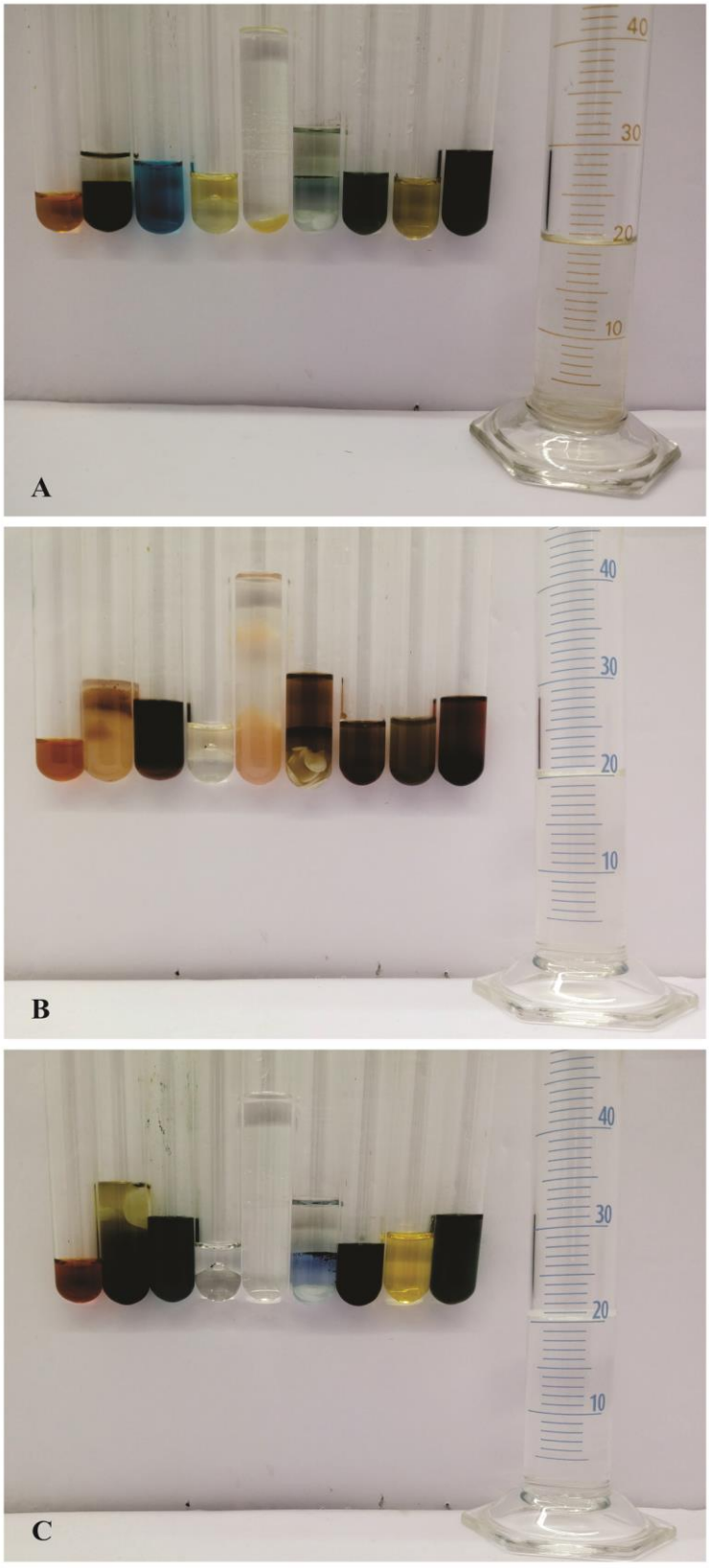

Plate 30. Qualitative phytochemical analysis of ethyl acetate plant extracts.
A. *Phyllanthus emblica*
B. *Artocarpus lakoocha*
C. *Heliotropium indicum*

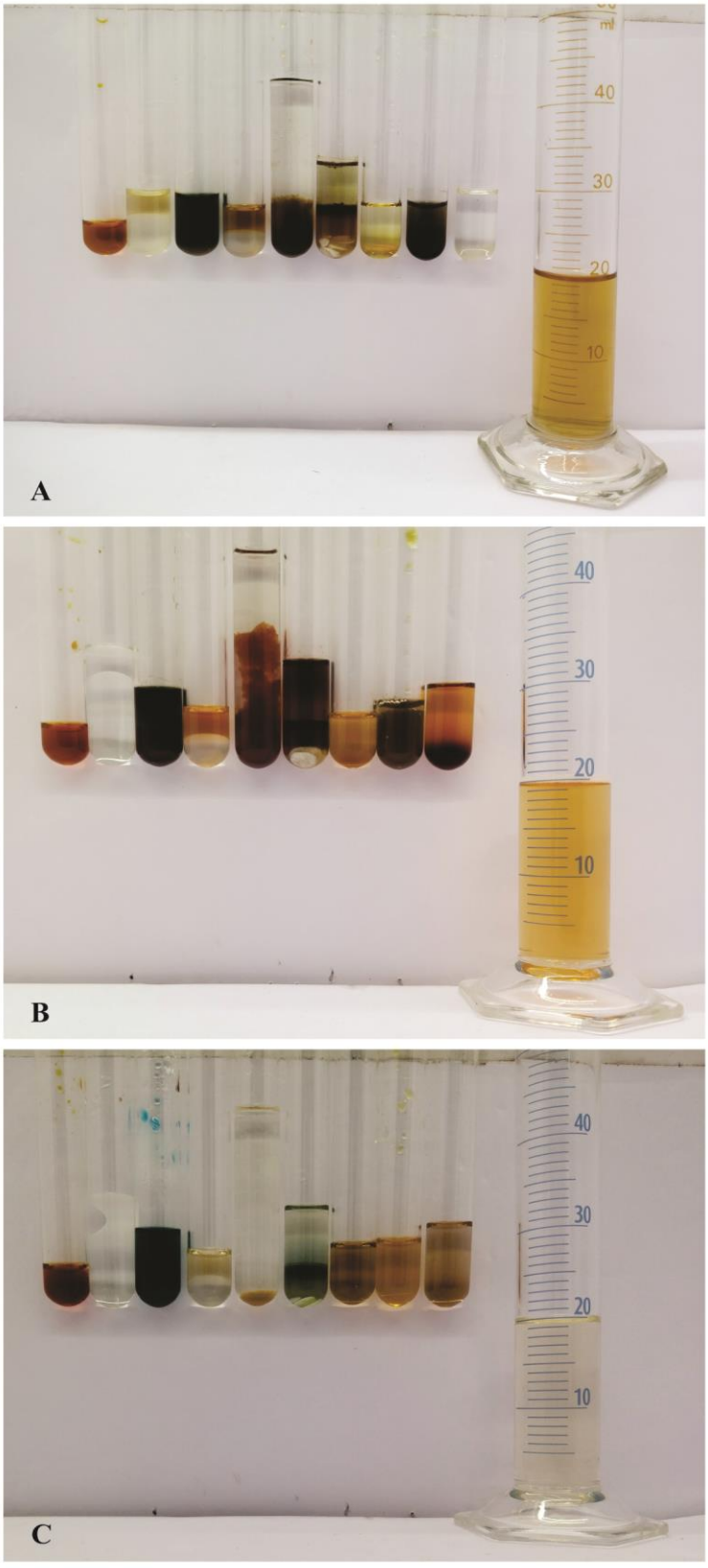

Plate 31. Qualitative phytochemical analysis of aqueous plant extracts.
A. *Phyllanthus emblica*
B. *Artocarpus lakoocha*
C. *Heliotropium indicum*

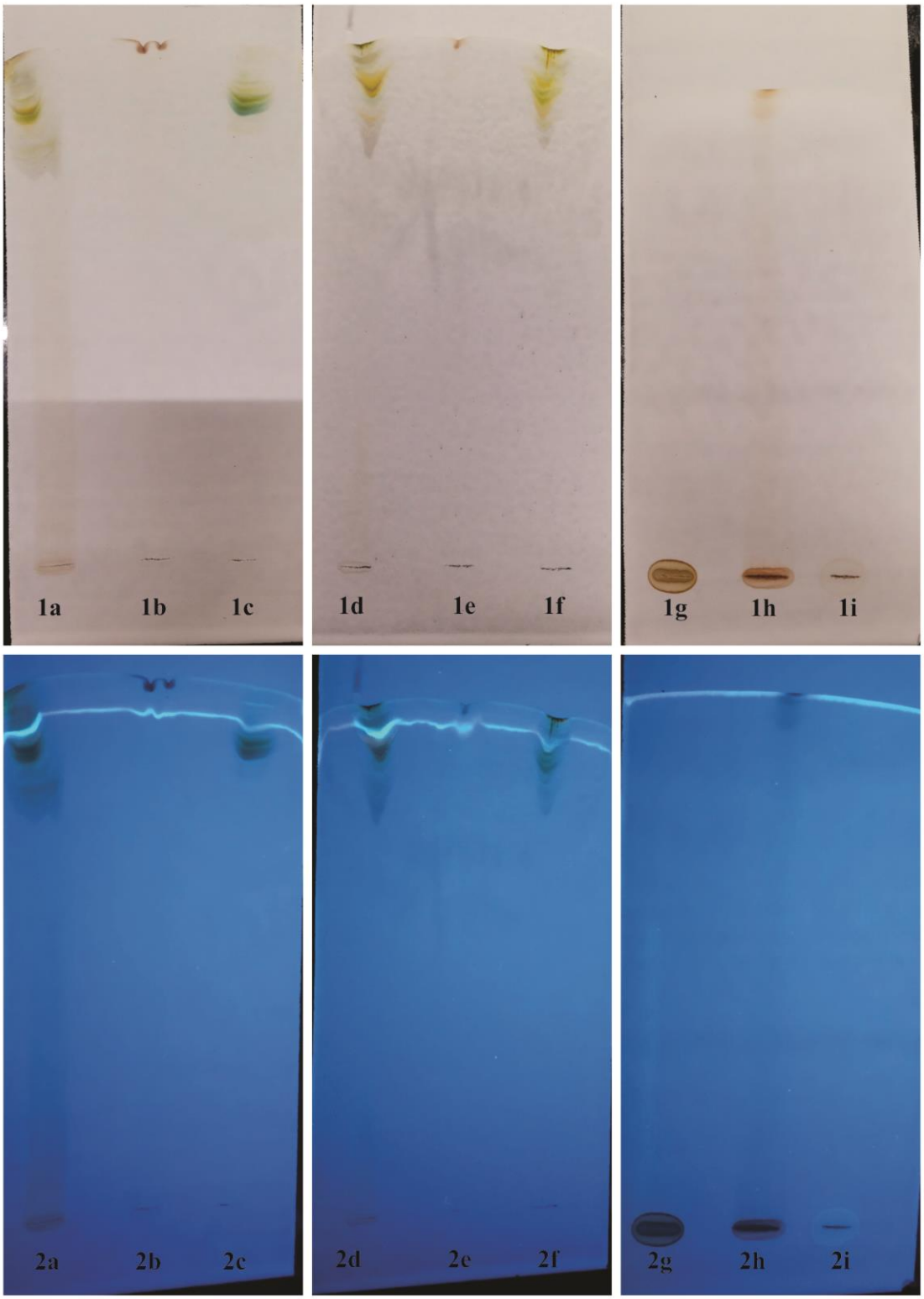

Plate 32. TLC plates showing separation of flavonoids.
1. Under visible light; 2. Under long UV
a. Methanol extract of *Phyllanthus emblica,* b. Methanol extract of *Artocarpus lakoocha,* c. Methanol extract of *Heliotropium indicum;* d. Ethyl acetate extract of *Phyllanthus emblica, e.* Ethyl acetate extract of *Artocarpus lakoocha,* f. Ethyl acetate extract of *Heliotropium indicum;* g. Aqueous extract of *Phyllanthus emblica,* h. Aqueous extract of *Artocarpus lakoocha,* i. Aqueous extract of *Heliotropium indicum.*

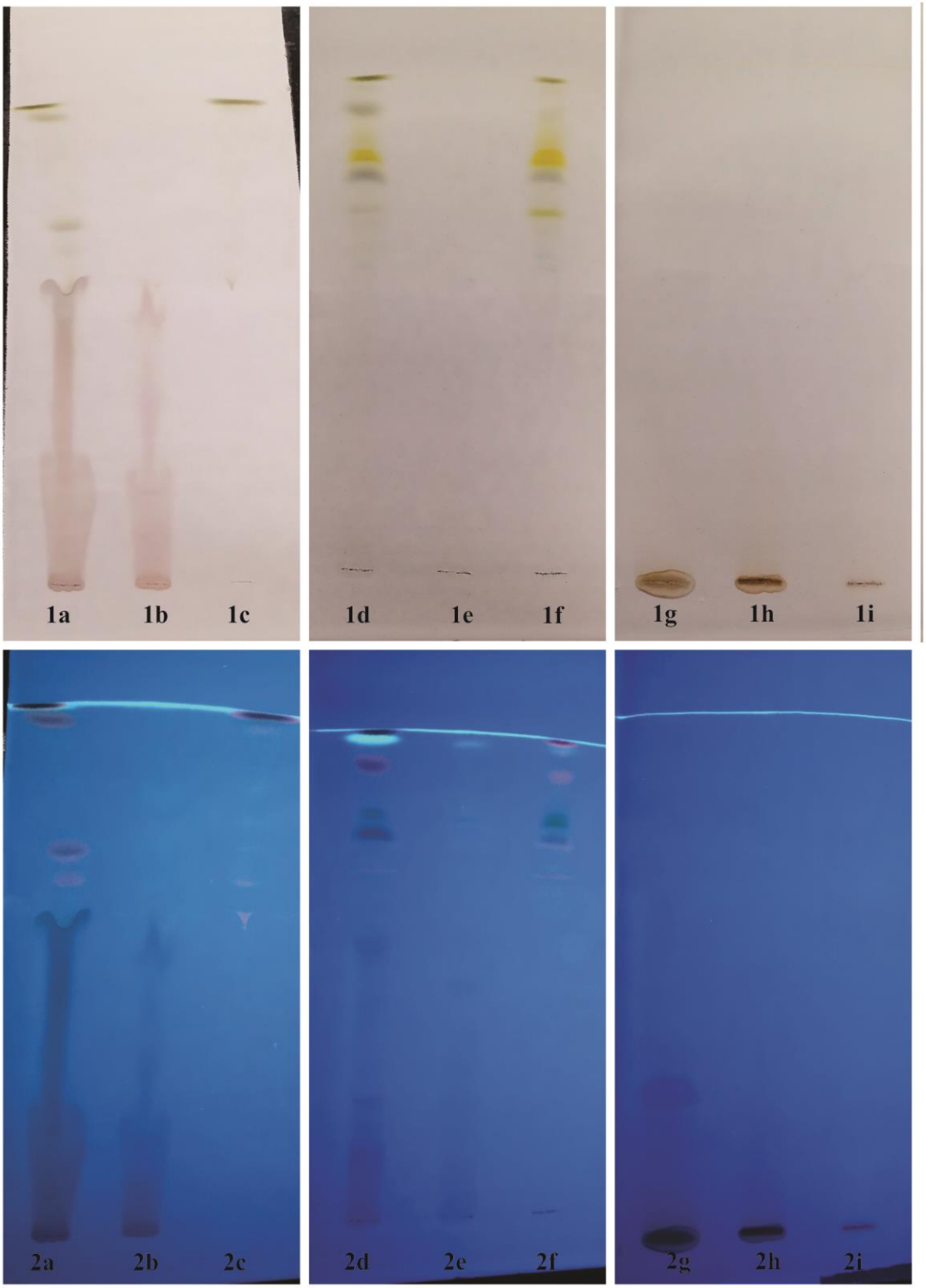

Plate 33. TLC plates showing separation of phenols.
1. Under visible light; 2. Under long UV
a. Methanol extract of *Phyllanthus emblica,* b. Methanol extract of *Artocarpus lakoocha,* c. Methanol extract of *Heliotropium indicum;* d. Ethyl acetate extract of *Phyllanthus emblica, e.* Ethyl acetate extract of *Artocarpus lakoocha,* f. Ethyl acetate extract of *Heliotropium indicum;* g. Aqueous extract of *Phyllanthus emblica,* h. Aqueous extract of *Artocarpus lakoocha,* i. Aqueous extract of *Heliotropium indicum.*

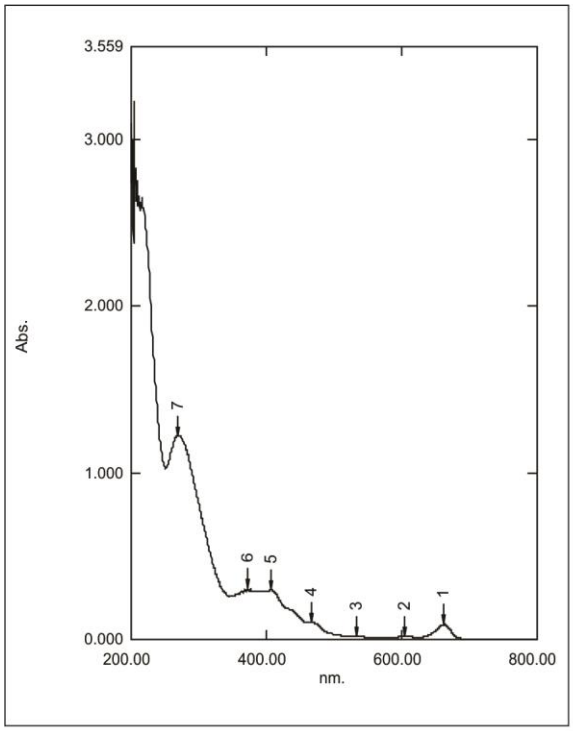

Figure 1. UV-Vis spectrum of *Phyllanthus emblica* methanol extract.

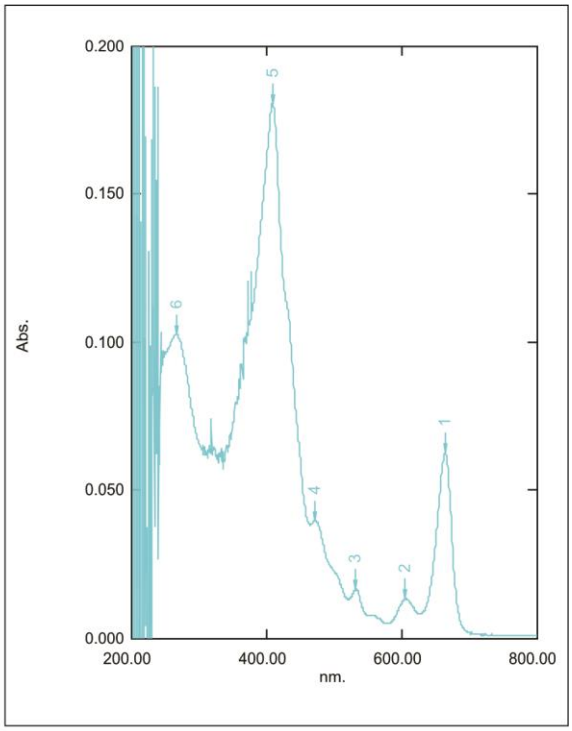

Figure 2. UV-Vis spectrum of *Phyllanthus emblica* ethyl acetate extract.

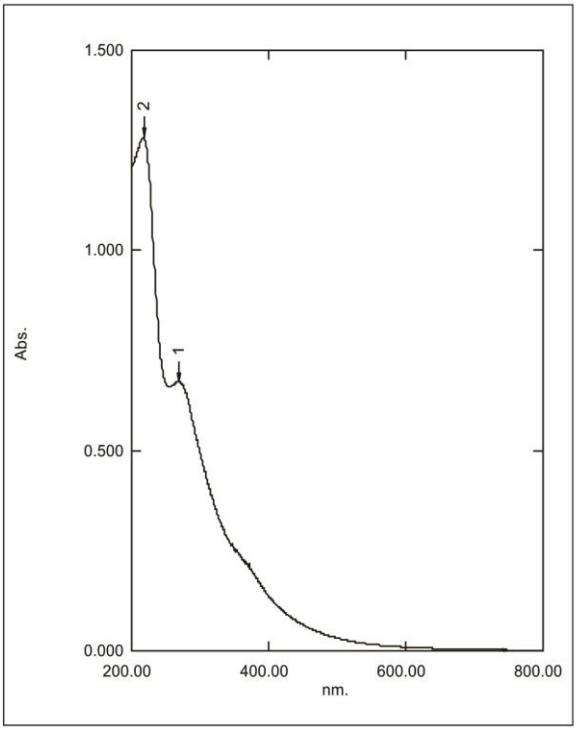

Figure 3. UV-Vis spectrum of *Phyllanthus emblica* aqueous extract.

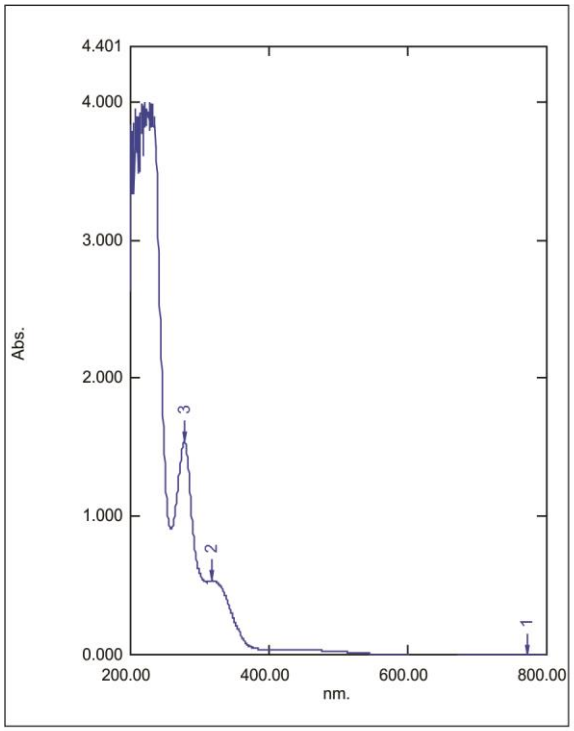

Figure 4. UV-Vis spectrum of *Artocarpus lakoocha* methanol extract.

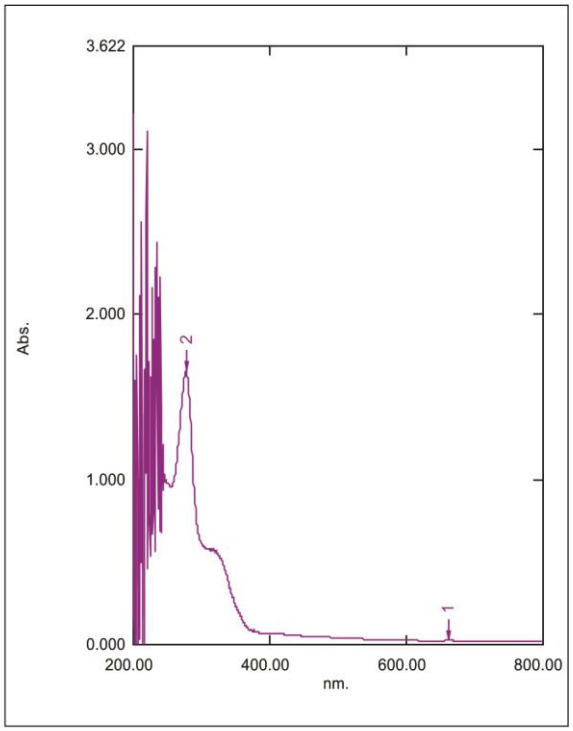

Figure 5. UV-Vis spectrum of *Artocarpus lakoocha* ethyl acetate extract.

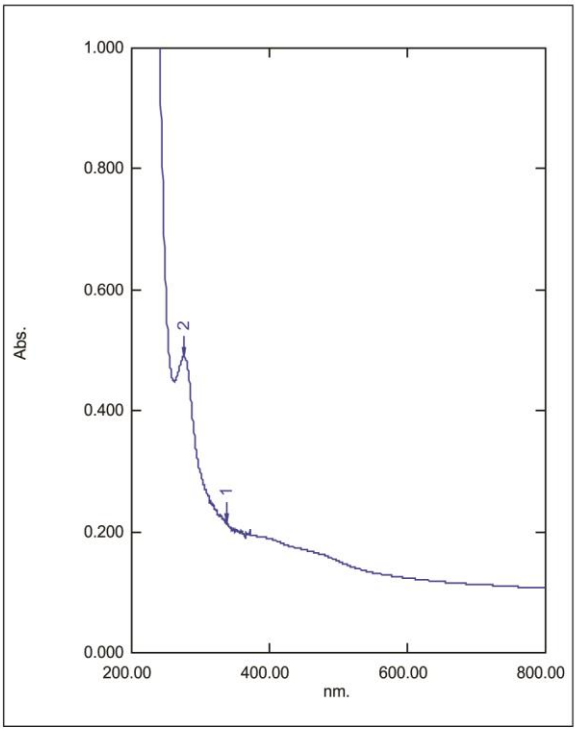

Figure 6. UV-Vis spectrum of *Artocarpus lakoocha* aqueous extract.

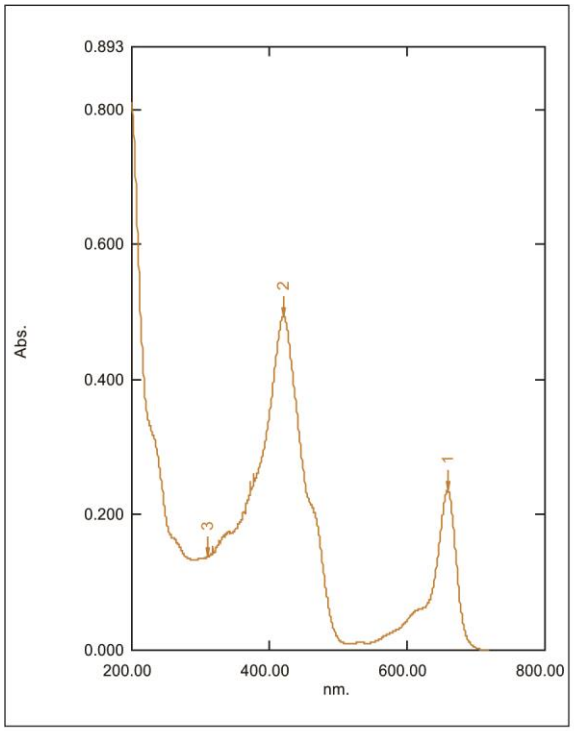

Figure 7. UV-Vis spectrum of *Heliotropium indicum* methanol extract.

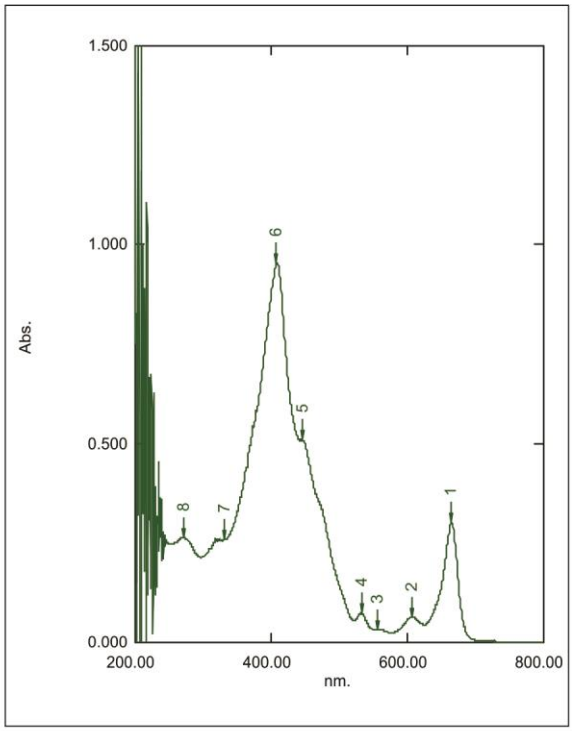

Figure 8. UV-Vis spectrum of *Heliotropium indicum* ethyl acetate extract.

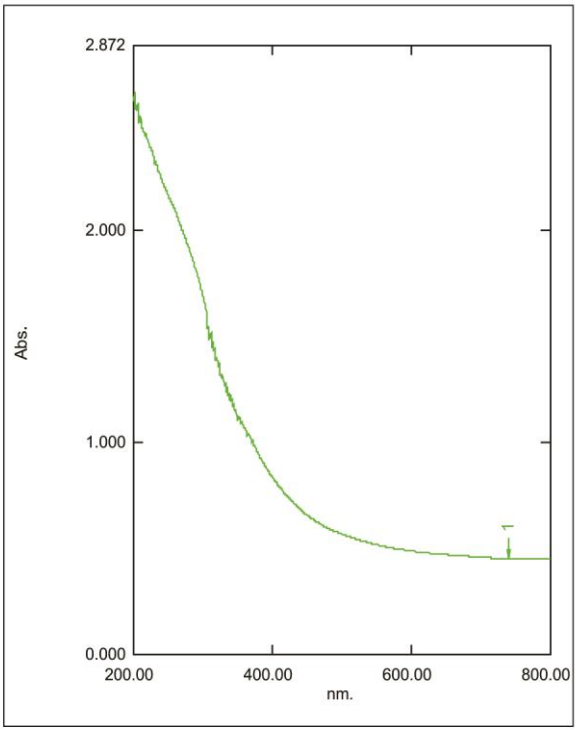

Figure 9. UV-Vis spectrum of *Heliotropium indicum* aqueous extract.