EXTRACTION OF NATURAL DYES FROM PLANT SOURCES AND THEIR POTENTIAL USE AS TEXTILE DYES AND BIOLOGICAL STAINS

A Dissertation for

BOT-651 Discipline specific Dissertation

16 Credits

Submitted in partial fulfilment of

The degree of Master of Science in Botany

By

Ms. SIMRAN SANTOSH CHARI

22PO480034

ABC ID – 983255551193

PR.NO- 201905646

Under the supervision of

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

School of Biological Science and Biotechnology
Department of Botany

Goa University

APRIL 2024

Seal of the School

Examined by

DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "**Extraction of Natural dyes from plant sources and their potential use as textile dyes and biological stains**" is based on the results of investigations carried out by me in the M.Sc. Botany, at the Botany Discipline, School of Biological Sciences and Biotechnology, Goa University, under the Supervision of **Prof. S. Krishnan** and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations /experimental or other findings given the dissertation.

I hereby authorize the University authorities to upload this dissertation on the dissertation repository or anywhere else as the UGC regulations demand and make it available to any one as needed.

Schari.

Signature and Name of Student
(Ms. Simran Santosh Chari)
Seat No: 22PO480034

Date: 08 April 2024
Goa University

COMPLETION CERTIFICATE

This is to certify that the dissertation report **"Extraction of Natural dyes from plant sources and their potential use as textile dyes and biological stains"** is a bonafide work carried out by **Ms. Simran Santosh Chari** under my supervision in partial fulfilment of the requirements for the award of the degree of **M.Sc. Botany** in the Botany Discipline, at the School of Biological Science and Biotechnology, Goa University.

Signature and Name of Supervising Teacher

(Prof. S. Krishnan)

Date: 08 April 2024

Signature of Dean of the School

Date: 8 April 2024

Place: Goa University

School Stamp

GOA UNIVERSITY
SBSB
TALEIGAO
GOA
BOTANY DISCIPLINE

CONTENTS

DETAILED CONTENTS

PREFACE

The dissertation work conducted on the extraction of natural dyes and their potential use as a textile dyes and biological stains, during the M.Sc. Botany, represents the result of an exploration into the world of natural dyes, specifically focusing on their applications in the cotton and wool dyeing and plant tissue staining. As I immersed myself in this research journey, I found myself captivated by the potential of natural dyes which can be used as a substitute for synthetic dyes in textile dyeing and in biological staining. The prospects of enhancing sustainability in textile dyeing through the use of eco-friendly colourants became a driving force behind this study. This dissertation aims to contribute to the growing knowledge of natural dyes by presenting comprehensive findings and analysis of their efficiency in cotton and wool dyeing applications and plant tissue staining.

ACKNOWLEDGMENTS

I express my deep sense of gratitude to the Almighty God who has given me strength and patience during the course of M.Sc. Botany, without whom this work would have not been possible.

My sincere Gratitude to my guide Prof. S. Krishnan for his valuable time and guidance throughout the course of my dissertation work which helped me in successful completion of my work.

I am also thankful to all my teachers Prof. B. F. Rodrigues, Prof. Vijaya U. Kerkar, Dr. Rupali Bhandari Dr. Aditi Naik and Dr. Siddhi Jalmi, Botany Discipline, Goa University, for extending necessary facilities for the smooth functioning of my research work.

I specially thank Ms. Arti Dabolkar for her constant support, help and guidance in making this project successful.

I owe my sincere thanks to all research scholars of phytochemistry lab for their kind cooperation, help, support, encouragement and valuable suggestions. I would also like to thank the non-teaching staff of the Botany Discipline, for their help and support. I also thank all my classmates, friends for their help in completing my work.

I express my gratitude to my parents, family and friends for their love, constant help, support and encouragement during the course of my study.

Ms. Simran Santosh Chari

TABLES

FIGURES

PLATES

Plate No.	Description
1	Selected dye yielding plants: 1. *Clitoria ternatea* & 2. *Amaranthus cruentus.*
2	Dyeing of cotton fibres with *Clitoria ternatea* dye with pre-mordanting, post-mordanting and simultaneous mordanting.
3	Dyeing of cotton fibres with *Amaranthus cruentus* dye with pre-mordanting, post-mordanting and simultaneous mordanting.
4	Dyeing of wool fibres with *Clitoria ternatea* dye with pre-mordanting, post-mordanting and simultaneous mordanting.
5	Dyeing of Wool fibres with *Amaranthus cruentus* dye with pre-mordanting, post-mordanting and simultaneous mordanting.
6	Fastness result of the cotton fibres dyed with *Clitoria ternatea* dye in pre-mordanting.
7	Fastness result of the cotton fibres dyed with *Clitoria ternatea* dye in post-mordanting.
8	Fastness result of the cotton fibres dyed with *Clitoria ternatea* dye in simultaneous-mordanting.
9	Fastness result of the cotton fibres dyed with *Amaranthus cruentus* dye in in pre-mordanting.
10	Fastness result of the cotton fibres dyed with *Amaranthus cruentus* dye in post-mordanting.
11	Fastness result of the cotton fibres dyed with *Amaranthus cruentus* dye in simultaneous-mordanting.
12	Fastness result of the wool fibres dyed with *Clitoria ternatea* dye in Pre-mordanting.
13	Fastness result of the wool fibres dyed with *Clitoria ternatea* dye in Post-mordanting.
14	Fastness result of the wool fibres dyed with *Clitoria ternatea* dye in simultaneous-mordanting.
15	Fastness result of the cotton fibres dyed with *Amaranthus cruentus* dye in in pre-mordanting.

ABBREVIATIONS USED

Entity	Abbreviation
Alkaloid	Alk
Amaranthus	A.
Carbohydrate	Carb
Clitoria	C.
Copper sulphate	$CuSO_4$
Coumarin	Cou
Degree centigrade	˚ C
Ferrous sulphate	$FeSO_4$
Fourier Transform-Infra red	FT-IR
Grams	G
Hydrochloric acid	HCL
High Performance Liquid Chromatography	HPLC
Indian Society Colour Council-National Bureau of Standards	ISCC-NBS
Litre	L
Liquid Chromatography-Mass Spectrometry	LC-MS
Material to liquor ratio	MLR
Microlitre	Ml
Millilitres	Ml
Minutes	Min
Nanometre	Nm

Pantone Matching System	PMS
Percent	%
Phenol	Phe
Potential of hydrogen	pH
Protein	Pro
Pyrolysis-gas Chromatography Mass Spectrometry	PY-GC-MS
Retention factor	Rf
Revolutions per minute	Rpm
Saponin	Sap
Scanning Electron Microscope	SEM
Tannin	Tan
Time of Flight Secondary Ion Mass Spectrometry	TOF-SIMS
Thin Layer Chromatography	TLC
Ultra Violet	UV
Visible	Vis
X-ray Photoelectron Spectroscopy	XPS

ABSTRACT

Natural dyes were extracted from *Clitoria ternatea* L. and *Amaranthus cruentus* L. for potential application in textile dyeing and biological staining. The extracted dyes, obtained using boiling method and concentrated with rotatory evaporator, were utilized for plant tissue staining, which exhibited differential staining for monocot and dicot stem under fluorescence and bright field microscopy. Cotton and wool fabrics dyed with extracted natural dyes, using various chemical and natural mordants, demonstrated good wash, light and rub fastness properties with pre-mordanting method. Chemical mordant exhibited poor fastness ratings with post and simultaneous mordanting, whereas natural mordants showed relatively better fastness properties in pre and simultaneous mordanting. Phytochemical analysis and UV-Vis spectroscopy characterization revealed the presence of alkaloids, flavonoids, phenols, and tannins, with specific absorption peaks for anthocyanin and betalains. The extracted dye displayed promising potential for use in textile dyeing and histological staining applications.

1. INTRODUCTION

1.1 Dyes

Dyes play a crucial role in various industries, ranging from biological sciences to fashion and textiles, for centuries. In the field of biological sciences, dyes are essential for staining cells, tissues, and organs to enhance contrast and facilitate microscopic examination. Techniques such as immunohistochemistry and fluorescent labelling rely on dyes to visualize cellular structures, identify specific molecules, and study biological processes. Similarly, in the textile industry, dyes are fundamental for imparting color and aesthetics to fabrics, garments, and other textile products (Yadav *et al.*, 2023).

1.2 Natural dyes and Synthetic dyes

Natural dyes are colourants derived from organic sources such as plants, animals, and minerals in contrast to synthetic dyes created chemically in laboratory. Natural dyes are obtained through extraction processes that harness the pigments present in these organic materials (Singh, 2017). The extraction methods vary depending on the type of natural dye source and the desired colourant. Natural dyes can also be obtained from the waste products of agricultural activities, food and beverage industry (Bechtold *et al*., 2003).

Natural dyes were the primary textile dyes until the 19th century. However, their usage decreased once synthetic dyes were discovered, which offered greater affordability and versatility (Yadav *et al*., 2023).

Synthetic dyes have numerous advantages over natural dyes. They are simpler to manufacture, easy to apply and provide superior wash fastness and light fastness properties to fabrics. Synthetic dyes offer a wide array of shades, making them more

versatile. These advantages ultimately led to the replacement of natural dyes by synthetic alternatives. However, they come with significant environmental concerns, including water pollution, chemical toxicity and environmental contamination due to non-biodegradable nature of synthetic dye. Presence of hazardous chemicals such as carcinogens or heavy metals poses harm upon prolonged exposure or ingestion and causes potential allergic responses such as skin irritation (Kulkarni *et al*., 2003).

Studies on synthetic dyes have revealed the presence of carcinogenic compounds, including pentachlorophenol, formaldehyde, lead, zinc, benzidine, cadmium, aryl amine, mercury, and halogen carriers (Singh & Srivastava, 2017). Azo dyes are stable in oxygen-rich environments but can generate dangerous aromatic amines when oxygen is scarce, leading to health problems such as cancer and birth defects. They are prohibited in several regions including China, Japan, India, Vietnam, and the European Union due to their environmental and health risks (Rai *et al.*, 2005).

1.3 Difference between dyes and pigments

Dyes and pigments are essential colourant widely used in textiles, pharmaceuticals, food, cosmetics, paint, ink and paper industries for their ability to impart colour to wide range of materials. Dyes are organic compounds which dissolves or reacts with substrate, are less stable and prone to fading. Pigments are inorganic substances which are insoluble, more stable and resistant to fading (Yadav *et al*., 2023).

1.4 History

The history of natural dye usage extends to the Neolithic era, where they were employed for adorning skin, jewellery, clothing, and cave paintings, often conveying specific information. Prior to the advent of synthetic dyes, natural sources such as plants

(fruits, flowers, stems, roots, barks, leaves, and wood) and animal-derived materials (like molluscs) were predominantly utilized for dye extraction (Kumarmath *et al*., 2022).

One of the earliest known natural dyes, henna traces back to 2500 BC (Gulrajani *et al*., 2000). By the 4th century A.D. dyes like Madder, Brazilwood, and Indigo were well-established. The era of natural dyes and pigments persisted until the mid-19th century, when the synthesis of the first synthetic dye, mauve or aniline purple, by William Henry Perkin in 1856, marked a transformative moment in fashion, medicine, and chemical industries, catalysing a significant transition from natural to synthetic dyes (Yadav *et al*., 2023).

Presently, with growing concerns about health and environmental safety, there has been an increase of interest in natural dyes, prompting the utilization of these dyes even in dyeing synthetic fibres (Salauddin *et al*., 2021).

1.5 Classification of natural dyes

Various types of dyes can be categorized based on their chemical structures as follows:

- Anthocyanins: Offering blue and pink shades, these dyes are a class of water-soluble flavonoids widely present in fruits and vegetables including berries, (strawberries, blueberries and raspberries) grapes, apples, plumps and cabbage.
- Anthraquinone class: These dyes, found in plants and insects like madder and cochineal, are red in color and offer good light and wash fastness.
- Betalains class: The dye is found in plants like beetroots, cacti, and amaranth species. These pigments are responsible for vibrant red, purple, and yellow colors.
- Carotenoids: Giving an orange color, these dyes are sourced from Saffron and Annatto

- Flavones: Yielding a yellow colour, these dyes are derivatives of flavones and isoflavones, example: *Reseda luteola*.
- Indigoid class: Including dyes like Indigo blue and Tyrian purple, giving blue and purple colours.
- Quinoid class: These dyes are characterized by their quinoid structures. They exhibit diverse properties and can be sourced from various natural materials. Example of a dye in this class is Lawsone, which is obtained from the henna plant.
- Tannins class: Contains polyphenolic compounds found in various plant tissues. Tannins are commonly found in plant parts such as tea leaves, and oak bark. They contribute to the astringent taste of certain foods and have been used traditionally in tanning leather and in the production of inks and dyes (Yadav *et al*., 2023).

1.6 Mordants

Mordants are substances used in dyeing to improve the binding of the dye to the fabric. They form chemical complexes with both the fibres of the fabric and the dye molecules, enhancing the color fastness (Geetha & Sumathi, 2013).

The chemical groups within the dye molecules interact with metal ions present in the mordants and enhance dye adherence to fabric by creating complexes with both the fibers and dye molecules. Mordanting improves the dyeing procedure and also provides a range of shades for the fabrics with improved dye fastness properties (Singh & Srivastava, 2015).

Historically, dyeing involved boiling plant materials in water to extract dye, followed by application to textiles. However, this method lacked durability. Subsequently, it was found that the addition of salt, vinegar derived from fermented fruits, and natural alum

acted as mordants, enhancing dye fastness. Salt aids dye fixation, while vinegar counteracts carbonates in hard water, particularly benefiting red and purple dyes (Singh, 2019).

Traditionally, natural mordants like Myrobalan and Chitosan are utilized in dyeing processes. Myrobalan functions as a natural tannin, while chitosan is derived from deacetylation of chitin, forming a linear polysaccharide (Kasiri & Safapour, 2014). *Aloe vera* and banana sap is investigated as alternative natural mordants, demonstrating promising fastness results. Historically, tea and coffee were commonly employed for dyeing fabrics due to their tannin content, doubling as mordants (Rafi *et al*., 2021).

Metallic mordants, such as potassium dichromate, copper sulphate, ferrous sulphate, ferric chloride, and stannous chloride, are commonly employed in textile dyeing, along with oil mordants. Combining metallic and oil mordants can enhance wash fastness by forming a strong complex with dye molecules, although it may result in excessive color variations due to chemical reactions between the dye and mordant. However, this practice raises concerns about environmental impact, such as water and soil pollution (Vankar, 2000).

1.7 Dyeing cotton and wool yarn

Cotton cultivation for textiles likely began during the Harappa civilization, employing Asiatic cotton (Rajendran *et al*., 2005), with evidence of cotton clothing production dating back over 7,000 years (Gray, 2014). Its widespread use in the textile industry is attributed to its exceptional absorbency, softness, strength, and ability to withstand abrasion and high temperatures (Ravandi &Valizadeh, 2011). Cotton satisfies approximately half of the world’s fiber demand (Ali & Sarwar, 2010).

Wool is obtained from the fleece of sheep through a process called shearing. Once harvested, the wool is cleaned, processed, and spun into yarn, which can then be used for various textile applications. Wool yarn comes in various thicknesses and textures, making it suitable for a wide range of knitting, crocheting, and weaving. Wool is renewable, biodegradable, and has inherent moisture-wicking and insulating properties, that make them excellent for various applications (Arora *et al*., 2017).

1.8 Biological staining

Natural dyes offer a promising alternative to chemically synthesized stains commonly used in biological staining, as they are not harmful to both humans and the environment. Before the advent of synthetic stains, natural dyes like Haematoxylin, extracted from the *Haematoxylon campechianum*, were employed for biological staining purpose. Dyes including Carmine and Saffron, possess the capability to selectively stain various biological specimens, showcasing their potential for differential staining of plant and animal tissues (Suryawanshi *et al*., 2017). For instance, madder-derived stain can effectively highlight nuclear material, facilitating the study of different cell cycle. Natural dyes offer a promising alternative to synthetic stains for various biological applications, including staining microbes, spores, fungal, plant, and animal tissues (Manimekalai *et al*., 2018).

1.9 Characterisation of natural dye

Natural dyes possess a molecular structure comprising two distinct components: chromophores and auxochromes. Chromophores are responsible for the dye coloration, primarily constituted of aromatic rings, and auxochromes facilitate the attachment of the dye molecule onto the substrate. The presence and quantity of unsaturated double bonds

within chromophores dictate the intensity of the dye coloration, with a higher number of these bonds result a range of bright colours (Krishnamurthy, 1999).

Primary characterization of natural dyes involves utilizing phytochemical analysis, UV-Visible spectroscopy, and Thin layer chromatography (TLC) more comprehensive characterization techniques such as High-Performance Liquid Chromatography (HPLC), Fourier Transform Infrared Spectroscopy (FTIR), and Gas Chromatography and Mass Spectrometry (GC-MS) are utilized for detailed analysis. UV-Vis spectroscopy aids in identifying dyes based on absorption peaks, while TLC enables the identification of color components. Whereas, methods like FTIR, GC-MS gives detailed information about the components of the dye (Algarni, 2020).

1.10 Advantages of natural dyes

- Natural dyes offer numerous benefits, including being non-toxic, non-allergenic, and non-carcinogenic, as they are derived from animals or plants without chemical processing.
- Their effluents are biodegradable and renewable, with no disposal issues. Colors from natural dyes are typically gentle and soothing to the eyes, some even having therapeutic effects.
- Extracted from minerals, plants, and animals, they provide harmonious, soft tones and can give protection against UV light.
- Moreover, they possess functional properties like antimicrobial and antioxidant effects, with some having insect repellent qualities. Natural dyes find application in various industries including textiles, food, paper, and cosmetics (Chungkrang *et al*., 2021, Shahidi *et al*., 2022).

1.11 Limitations of natural dye

- The availability of natural dyes sources is seasonal. Natural dyes are applicable to natural fibres only (cotton, wool and silk)
- Non-standardised method, low dyeing efficiency and only few have good fastness to light and washing.
- Chemical mordants may act harmful and pose water and soil pollution due to leaching of heavy metals in the soil (Yadav *et al*., 2023).

1.12 OBJECTIVES

The present work was carried out with the following objectives:

1. Extraction of natural dyes from *Clitoria ternatea* flowers and *Amaranthus cruentus* leaves.
2. Dyeing of cotton and wool fibres with extracted natural dyes with chemical and natural mordants and to study the colour fastness properties of dyed fibres.
3. Use of dyes as biological stains for monocot and dicot plant stem tissue staining.
4. Phytochemical analysis and characterisation of dyes by UV-Visible spectroscopy and Thin Layer Chromatography.

1.13. HYPOTHESIS

Chemical dyes are widely used in paper, food, cosmetics, paints and textile industry, but these dyes have negative impacts on environment and living beings. The use of natural dyes over synthetic dyes in textile production and biological staining will have a positive impact on environmental sustainability, due to their biodegradability and reduced ecological footprint. Natural dyes along with bio-mordants can also be utilised to reduce the negative impact of chemical mordants. Natural dyes along with bio-mordants may demonstrate the comparable colourfastness property. Additionally, survey for new plants may provide natural dye sources which can yield natural dyes easily, are fast-growing and can be cultivated in all seasons. This will contribute to abundance of source and easy extraction; further natural dyes can be used as sustainable alternative to synthetic counterparts.

1.13 SCOPE

The study on the extraction of natural dyes and its application in textile and biological staining will help in identifying new plant species or plant parts that yields rich pigment content suitable for dye extraction. Investigating dye-yielding capacity of selected plants using suitable extraction methods will reveal the potential applications of these obtained natural dyes on plant tissue staining and textile material dyeing by using natural and chemical mordants. Study will also reveal the chemical composition and pigments present in the extracted dyes and the colour fastness property of these dyes. Dyes extracted from selected plant species can be used to minimize the usage of synthetic dyes and hence can be used as a substitute to artificial colors**.**

2. REVIEW OF LITERATURE

2.1 Natural dye

Singh and Srivastava, (2017) documented 15 dye-yielding plants belonging to 13 different families found in India and highlighted the importance of leaves as a natural dye source in textile dyeing as they serve as a promising resource.

Vernekar and Krishnan, (2017) documented 62 dye-producing plants from 37 distinct families found in the Indian state of Goa. They extracted dyes from various plant species and recommended their utilization for biological and textile staining purposes and also highlighted on the significance of creating employment by planting dye-yielding plants.

Bruckner *et al*., (1997) extracted dyes from plants such as henna, logwood, madder, saffron, turmeric, beetroot, and tea. They utilized different mordants, including alum and copper sulphate, for dyeing polyester and wool fabrics. The colour fastness ratings for the dyed fabrics ranged from moderate to low.

Vankar *et al*., (2008) extracted reddish-orange dye from *Rubia cordifolia*. The extracted dye was used to dye cotton fabric using *Eurya acuminate* as a bio-mordant which resulted in very good fastness properties.

Geetha and Sumathi, (2013) extracted natural dyes from *Caesalpinia pulcherima, Bougainvillea glabra, Beta vulgaris, Brassica oleracea,* and *Allium cepa*. Various natural mordants such as alum, vinegar and salt were used to dye cotton fabric which exhibited favourable wash fastness properties.

Mokashi *et al*., (2023) extracted dyes from onion peel, pomegranate rind and marigold petals using water and methanol, concentrated the dyes using a rotary evaporator and then used for dyeing cotton. Marigold dye showed good results for wash fastness test.

2.2 Methods of extraction

Different techniques are utilized to enhance the extraction of dye from various plant parts, aiming to improve both the efficiency of extraction and the quality of the resulting dyes. Methods of extraction are given below:

- Aqueous extraction: Plant material is powdered, soaked in water and then boiled to extract the dye. It is then filtered to remove the debris.
- Acid and alkali extraction: Dyes are extracted in an acidic or alkaline medium using acid or base. Dyes with phenolic groups are extracted by alkaline extraction method.
- Ultrasonic microwave extraction: Ultrasonic waves and microwave radiations are used to accelerate the extraction process. Ultrasonic waves create cavitation bubbles in the solvent, leading to the disruption of plant cell walls and enhancing the release of dye molecules.
- Enzymatic extraction involves using enzymes to break down cell walls and release dyes from plant materials. Enzymes such as cellulase, pectinase, and protease are commonly used to degrade specific components plant cell walls. This method is particularly effective for dyes and can enhance extraction efficiency while preserving dye quality (Salauddin *et al*., 2021).

Micheal *et al*., (2019) studied optimum extraction methods of *Clitoria ternatea* (butterfly pea flower) and its use as a pH-dependent natural colourant. Water, ethanol and methanol were used as solvents for extraction with time interval of 30 to 120 minutes and temperatures ranging from 30℃ to 80℃. Study revealed that ethanol and water were the best solvents, giving greater colour yield at 70˚C with 60-minute extraction time.

Sankaranarayanan *et al*., (2022) extracted dye from leaves and flowers of *Amaranthus cruentus* for the devising of dye-sensitized solar cells using acetone, ethanol and water as solvents. Extraction showed that solvent is responsible for domination of pigments in the dye. Acetone and ethanol extracted more chlorophyll into the dye and water extracted more betalains into the dye.

Marpaung and paramputri, (2023) studied on the extraction of anthocyanin from *Clitoria ternatea* flowers through spectrophotometry to observe the quantity of anthocyanin content. Study revealed that the highest yield of anthocyanin was obtained by extraction at 60˚C to 70˚C for 60 minutes.

Kumarmath *et al*., (2022) extracted the dye from the hull of *Terminalia catappa* using various extraction techniques such as microwave-assisted extraction and an acidic extraction method, employing different concentrations viz. 0.2%, 0.3% and 1%. Dye was analysed using UV-Vis spectroscopy which showed the presence of flavonoid.

Sivakumar *et al*., (2011) investigated the use of ultrasound for extracting dye from various sources such as green wattle bark, marigold flowers, pomegranate rinds, 4’o clock plant flowers, and cocks comb flowers, with magnetic stirring employed as a control method. They conducted UV-Vis spectrophotometry and gravimetric analysis to assess the dye extraction process, revealing a 13% enhancement in extraction efficiency compared to the control method.

2.3 Natural dyes for textile dyeing

Singh *et al*., (1993) extracted natural dye from the madder plant for silk dyeing and studied the fastness property of the fabric produced a wide range of colours from bright reddish brown, dark brownish red, and peach to greenish brown. Post mordanting method gave the best result with ferrous sulphate and copper sulphate mordant. Fastness property was fair to good for light fastness and wash fastness exhibited fair to poor ratings.

Yoo and Kim, (2005) studied the effect of natural dyeing using tea and coffee waste on silk cloth. Silk dyed with coffee extract gave bright golden-yellow colour with 0.5% concentration of mordants like copper sulphate and alum. Dyeing with coffee and tea showed good wash fastness property for silk.

Kulkarni *et al*., (2011) extracted dye from green chilli (*Capsicum annum*) and used it for cotton dyeing using chemical mordants such as $CuSO_4$, $FeSO_4$ with different ratios viz. 1:1, 1:3, & 3:1. Different shades of yellow were obtained from the dye. Dyed fabric showed moderate wash fastness and good rub and light fastness properties.

Singh and Srivastava, (2016) dyed mulberry silk using a natural dye from black cardamom using alum as a mordant. Results showed successful dyeing of silk with excellent wash and light fastness properties.

Arora *et al*., (2017) extracted natural dyes from various plants like turmeric rhizomes, safflower petals, and barberry roots. They evaluated these dyes on silk, cotton, and wool fabrics through pre-mordanting, post-mordanting and simultaneous-mordanting. Results showed diverse spectrum of colour including yellow, red, brown and orange shades.

Vernekar and Krishnan, (2017) studied dyeing of cotton and silk fabrics using natural dyes derived from mangrove plants such as *Rhizophora mucronata* and *Ceriops tagal* using chemical mordants such as copper sulphate, ferrous sulphate and potassium dichromate and natural mordants like Cowdung, baking soda and lemon juice. Fabrics showed different shades of brown and red by employing both chemical and natural mordants in the dyeing process. Both the dyes were bearing good to excellent fastness properties.

Teklemedhi and Gopalkrishnan, (2018) conducted research on dyeing silk fabrics using *Cassia singueana* bark dye along with *Aloe vera* as a natural mordant. They found that the dyed fabrics showed excellent resistance to washing, light exposure, and rubbing. Kumar and Prabha, (2018) extracted dyes from various plants such as *Ixora coccinea*, *Tagata erecta* and *Impatiens balsamina* to dye cotton, jute, and woollen yarns. They utilized different mordants including vinegar, sodium chloride as a natural mordant and potassium dichromate as a chemical mordant. Yarns treated with sodium chloride mordant exhibited notably strong resistance to washing.

Verenkar and Krishnan, (2020) dyed cotton and silk fabrics with *Mammea suriga* dye extracted from the bark. The results produced good fastness properties in pre-mordanting, post-mordanting and simultaneous-mordanting methods.

Rafi *et al*., (2021) examined the use of *Aloe vera* and banana sap as bio mordant with teak dye extract. Evaluated staining with different textile materials including cotton, polyacrylic and wool. Dyed fabric with natural mordant showed good fastness properties against dry and wet rub fastness test.

Yadav *et al*., (2023) paper reviews on natural dyes, pigments and recent advances. Highlighted on some fundamental aspects of colorants their classification, chemical

constituents responsible for producing various colours, newer methods of production extraction and mechanism of dyeing.

2.4 Natural dyes as biological stain

Sikhruadong *et al*., (2009) studied the chromosome staining of Crinum lily (*Crinum asiaticum*) using 12 natural dyes. Results indicate that dye extracted from white mulberry fruit (*Morus alba*) and black glutinous rice (*Oryza sativa*) are capable for plant chromosome staining.

Chukwu *et al*., (2011) extracted natural dye from dried leaves of *Lawsonia inermis* using ethyl alcohol as solvent. Dye was used for staining angiospermic plant stem. Plant tissue showed yellow colour staining.

Deepak *et al*., (2013) extracted pink dye from the fruits of *Melastoma malabathricum* for staining plant anatomical sections. Using natural mordants like *Garcinia cambogia* and *Averrhoa carambola L.* in the staining procedure. Results showed red colour staining of plant tissue after mordanting.

Deepali *et al*., (2014) aqueous extracts from Henna leaves, Madder stem, and flowers of Hibiscus, Rose, Fire flame bush, and Madder were used for histological and fungal staining. Rose exhibited optimal outcomes for fungal and plant tissue staining and a combination of Rose, Hibiscus, and Henna showed promising results for animal tissue staining.

Kamel and Najamaddin, (2016) used natural dyes from plants such as Myrtle, Rosella and Walnut fruits to stain Gram-negative and Gram-positive bacteria. The results showed well stained bacterial wall. The staining showed good result well comparable to Gram stain with respect to clarity, differentiation, and economic cost.

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 chemical mordants such as copper sulphate and ferrous sulphate, 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.

2.5 Benefits of natural dyes

Plants capable of yielding dye are mostly medicinal and they exhibit antimicrobial activities. Singh *et al*., (2005) investigated the antimicrobial properties of natural dyes extracted from five plants: *Acacia catechu*, *Kerria lacca*, *Quercus infectoria*, *Rubia cordifolia*, and *Rumer maritimes*, against various bacteria including *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*. The research revealed that *Quercus infectoria* dye exhibited the highest level of antimicrobial activity against all bacterial strain.

Napoleon *et al*., (2013) extracted betacyanin pigment from the bracts of *Bougainvillea glabra*. Betacyanins were screened for antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* by Agar well-diffusion method using Ampicillin as standard. In-vitro antibacterial studies showed that betacyanins possess better antibacterial activity against *Bacillus subtilis* and *Pseudomonas aeroginosa*.

Shahidi *et al*., (2022) examined the use of natural dyes like Turmeric, Cinnamon, and Saffron to color cotton fabrics using $CuSO_4$ as a mordant. The dyes were evaluated for the UV protection and antibacterial properties. Saffron dye gave the highest UV protection, while turmeric showed effective antimicrobial property.

2.6 Characterisation of natural dyes

Morales, (2012) extracted purple dye from *Justicia spicigera*. Chemical analysis using UV-Vis, FT-IR, and PY-GC/MS (Pyrolysis-gas chromatography-mass spectrometry) techniques revealed the presence of anthocyanins, indicated by a peak at 581nm in UV-Vis spectra, and characteristic polar compounds like hydroxybenzoic acids and phenolics. Geetha and Sumathi, (2013) performed IR Spectroscopy analysis for *Caesalpinia pulchirima* which showed the presence of alkanes and phenolic compounds and beetroot showed the presence of alkanes and alkenes compounds.

Lee *et al*., (2013) conducted an analysis of natural dyes on Korean silk fabric using surface analytical techniques. They utilized methods such as Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS), X-ray Photoelectron Spectroscopy (XPS), and Fourier Transform Infrared Spectroscopy (FTIR) to examine the constituents of the dye present on the surface of the silk fabric. The TOF-SIMS spectra revealed molecular ions from the plant dyes, element ions from metallic mordants, and specific fragment ions. These techniques offer a means of characterizing dyes without the need for dye extraction, as is typically necessary with UV-Visible and chromatography techniques.

Analysing dyes in ancient textiles can aid in identifying the plants used for dyeing. Liu *et al*., (2013) employed high-performance liquid chromatography with diode array and mass spectrometry to study textiles from Yingpan, a Silk Road archaeological site. By employing a soft extraction method, they isolated dyes and identified *Rubia tinctorum* and *Rubia cordifolia* as sources for red and brown hues, and *Phellodendron spp*. for yellow and green colors in silk threads. Their findings suggest a blend of Eastern and Western dyeing practices during the early Silk Road era.

3. MATERIALS AND METHODS

3.1 Collection of plants

The plants selected for extraction of dyes were *Clitoria ternatea* and *Amaranthus cruentus.* The plants were collected from different parts of Bardez and Tiswadi taluka from the State of Goa, India. Flowers of *Clitoria ternatea* and leaves of *Amaranthus cruentus* were used for dye extraction.

3.2 Extraction of dye

The collected plant parts were washed thoroughly and dried in shade. The dried materials were then ground into fine powder. The dye was extracted by aqueous extraction method using distilled water as a solvent, by boiling the powdered sample.

3.2.1 Extraction of dye from *Clitoria ternatea* flower

The dye was extracted by adding 10g of powdered plant sample in 100ml of distilled water and subjecting to water bath at 70˚C for 60 minutes. The extract was then filtered using Whatman filter paper Grade-A. The filtered extract was used for dyeing cotton and wool fibres.

3.2.2 Extraction of dye from *Amaranthus cruentus* leaves

The dye was extracted by adding 10g of powdered plant sample in 100ml of distilled water and kept in water bath at 60˚C for 60 minutes. The extract was then filtered using Whatman filter paper Grade-A. The filtered extract was used for dyeing cotton and wool fibres.

3.3 Pantone Matching System (PMS) chart

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

3.4 Dyeing of cotton and wool fibres with natural dyes

3.4.1 Pre-treatment of cotton fibres and wool fibres

Prior to dyeing the cotton and wool fibres were treated with Sodium carbonate solution (1g/l) for 30 minutes at 60˚C in hot water bath. The fibres were then washed with distilled water to remove the traces of sodium carbonate. The pre-treated fibres were then dried at room temperature and used for dyeing.

3.4.2 Dyeing of cotton and wool fibres

The cotton and wool fibres were dyed with natural extracts of *Clitoria ternatea* and *Amaranthus cruentus.* Six mordants were used for dyeing. Chemical mordants used were alum (Potassium alum dodecahydrate), copper sulphate ($CuSO_4$) and ferrous sulphate ($FeSO_4$). Natural mordants used were *Aloe vera*, coffee and tea.

Dyeing was carried out by pre-, post- and simultaneous-mordanting methods. In pre-mordanting the fibres were first treated with the mordant for 60 minutes and then placed in the dye solution for 60 minutes. In post-mordanting the fibres were first placed in the dye solution for 60 minutes and then treated with the mordant solution for 60 minutes. In simultaneous- mordanting fibres were dyed in the solution containing both mordant and dye.

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

3.5 Preparation of chemical and natural mordants

3.5.1. Alum

Mordant solution was prepared by adding 0.1 g of Potassium alum dodecahydrate into 10ml of distilled water. 1% of mordant solution was used for mordanting the cotton and wool fibres.

3.5.2. Copper sulphate

Mordant solution was prepared by adding 0.1 g of Copper sulphate into 10ml of distilled water. 1% of mordant solution was used for mordanting the cotton and wool fibres.

3.5.3. Ferrous sulphate

Mordant solution prepared by adding 0.1 g of ferrous sulphate into 10ml of distilled water. 1% of mordant solution was used for mordanting the cotton and wool fibres.

3.5.4. *Aloe vera*

Aloe vera gel was used as mordant. Gel was removed from leaves and 10 ml of gel was used for mordanting cotton and wool fibres.

3.5.5. Coffee

Coffee mordant was prepared by adding 0.1g of coffee powder (*Coffea arabica* seeds powder) into 10ml of distilled water. 1% of mordant coffee solution was used for mordanting the cotton and wool fibres.

3.5.6. Tea

Tea mordant was prepared by adding 0.1g of tea powdered (*Camellia sinensis* leaves powder) into 10ml of distilled water.1% of mordant solution was used for mordanting cotton and wool.

3.6 Colourfastness tests of the dyed fibres

The dyed fibres were subjected to colour fastness to rubbing, light and washing. The colour change after the fastness test was determined by comparing with the standard gray scale (ISO 105 – A02) **(Fig. 1)**

Gray scales helps to specify a rating from 1-5 with 5 (no change) and 1 (severe change). The scale has 9 possible values: 5, 4/5, 4, 3/4, 3, 2/3, 2, 1/2, 1.

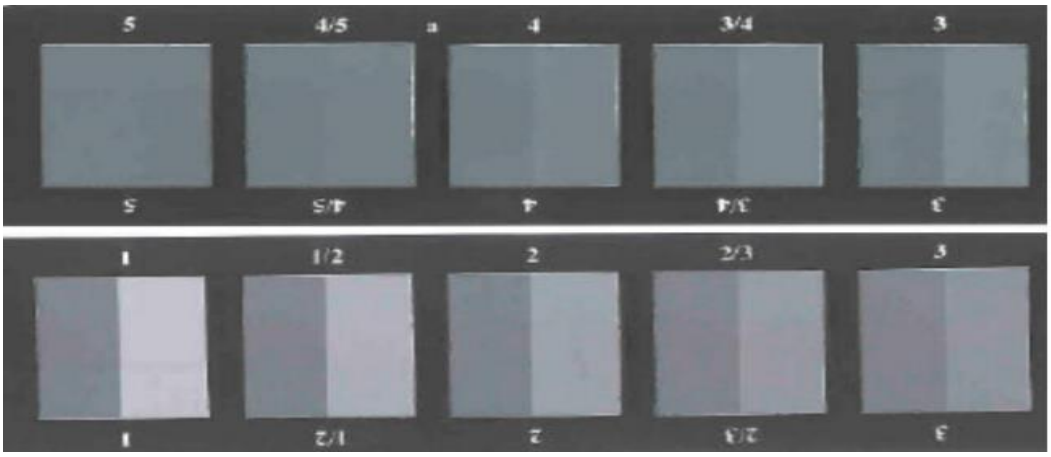


Figure 1. Gray scales for assessing change in colour.

Colourfastness to washing: The dyed cotton and wool fibres were washed with soap water for 5 minutes and allowed to dry.

Colourfastness to light: The dyed fibres were kept in the direct light for 6 hours.

Colourfastness to rubbing: The dyed cotton and wool fibres were placed between the undyed cloths and rubbed manually.

3.6.1 Photography of the fabrics

The photographs of the dyed cotton and wool fabric with dyeing and colour fastness tests were taken on Nikon digital camera.

3.7 Extraction of dye for biological staining

The dye was extracted by adding 5g of powdered sample in 50ml of distilled water and subjected to boiling in water bath for 60 min. The extract was then filtered through Whatman filter paper Grade-A. The filtered extract was then concentrated and dried with rotary evaporator at 40˚C with 50 rpm for 60 min. The dried powder was scraped and weighed. 0.1g of powder was dissolved in 1ml of distilled water and was used as stain.

3.7.1 Sectioning of plant material

The stem of monocot and dicot plant was selected for sectioning. For monocot plant *Cynodon dactylon* stem was choosen and for dicot *Chromolaena odorata* stem was selected. Free hand thin sections were taken using a sharp blade and placed in water. The thin sections were selected and used for staining.

3.7.2. Staining of plant sections

The sections were stained for 5, 10, 15 and 20 minutes to find the optimum staining time. Excess stained was removed by washing the section slightly in distilled water. The sections were mounted on a clean glass slide using a drop of diluted glycerine (10%).

The sections were observed under bright field microscope for their staining effect. The sections were also observed under fluorescence microscope in two filters viz. Ultraviolet excitation filter (UV-23) (330-380nm) and Violet excitation filter (V-2A) (380-420nm) The photographs of the sections were taken with the photographic unit attached with the microscope.

3.8 Qualitative phytochemical screening of natural dye

The plant samples were screened for the presence or absence of different phytochemicals. Extraction was carried out by using three solvents, methanol, ethanol and water. 10g of powdered sample was added to 50ml of the solvent and kept on water bath at 60˚C for 30 minutes. The extract was then filtered using Whatman filter paper Grade-A. The filtered extract was then dried on water bath and dried powdered extract was used for phytochemical tests.

3.8.1 Test for Alkaloids

Hager's test: To 2ml of extract, 1-2 drops of Hager's reagent was added. The appearance of yellow precipitate indicates the presence of alkaloids (Wagner *et al*., 1996).

Wagner's test: To 2ml of extract, few drops of Wagner's reagent were added. The appearance reddish-brown precipitate indicates the presence of alkaloids (Wagner, 1993).

3.8.2. Test for Tannins

Ferric chloride test: To 2ml of filtrate, 2 ml of $FeCl_3$ was added. Presence of blue, black and green precipitate indicates presence of tannins (Mace, 1963).

3.8.3. Test for Saponins

Foam test: 5ml of extract was continuously shaken with 5 ml of distilled water in a test tube. The stable foam indicates the presence of saponins (Kokate, 1999).

3.8.4 Test for Phenolics and Flavonoids

Ferric chloride test: To 2ml of extract, some drops of 10% ferric chloride solution was added. Occurrence of blue or violet colour indicates flavonoids (Mace, 1963).

Alkaline reagent test: The solution of extract was treated with 10% ammonium hydroxide solution. Appearance of yellow to orange fluorescence indicates the presence of flavonoids (Singh and Bag, 2013).

Lead acetate test: To 5ml of extract and 3ml of lead acetate solution was added. Presence of white precipitate indicates flavonoids (Singh and Bag, 2013).

3.8.5 Test for Carbohydrates

Benedict test: To 2ml of filtrate few drops of Benedict's reagent was added. The solution is then heated on a water bath for 3 minutes. Appearance of green precipitate indicates the presence of carbohydrates (Sofowora, 1993).

3.8.6 Test for Proteins

Biuret test: 1ml of 4% sodium hydroxide and 1ml of 1% copper sulphate was added to 3ml of extract. The change of the solution to violet or pink indicates the presence of proteins (Shriner *et al*., 1964).

Ninhydrin test: To 3ml of extract few drops of 5 % lead acetate solution was added and boiled on water bath for 10 minutes. The change in colour of solution to purple or blue indicates the presence of amino acids (Shriner *et al*., 1964).

3.8.7 Test for Coumarins

NaOH test: To 1ml of filtrate 10% NaOH and chloroform was added. Formation of yellow colour shows the presence of coumarins (Jagessar and Cox, 2010).

3.9 Characterisation of natural dyes

The methanolic and aqueous extracts of the selected plants, *Clitoria ternatea* and *Amaranthus cruentus* were used for the characterization.

3.9.1 Thin layer Chromatographic analysis

Thin layer chromatography (TLC) was used for the separation of the pigments. 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 an oven at 80˚C for 1 hour. 10µl of each

extract was loaded above 2cm from the edge of the plate and allowed to dry. The plate was then placed in the chromatography chamber containing the particular solvent system. The solvent system used for the separation of pigments from *Clitoria ternatea* flower extract was, hexane: ethyl acetate (3:1) and for separation of pigments from *Amaranthus cruentus* leaves extract, petroleum ether: acetone (7:3) was used.

TLC plate was visualised under visible light and long UV using UV trans-illuminator. The bands visualized on each plate were photographed and their Rf values were calculated.

3.9.2 UV-Visible Spectroscopy

The methanol and water extract of each plant sample was tested for the presence of phytochemicals and pigments by using UV-Vis spectroscopy. Each plant extract was taken in quartz cuvette and blank was set by using the respective solvent. The spectrum of the extract was taken in the 200-800nm range using UV -1800 Shimadzu UV spectrophotometer. The peaks obtained in the spectrum at the particular wavelength indicate the presence of specific pigments or phytochemicals in the extract.

4. RESULTS

4.1 Selection of dye yielding plants

The plants selected for dye extraction were *Clitoria ternatea* and *Amaranthus cruentus*. The plant part used for dye extraction, the Pantone Matching System (PMS) and the colour code of dye obtained is provided in **Table 1, Plate 1.**

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

Plant name	Plant part used	Colour code of the dye
Clitoria ternatea L.	Flowers	Pantone 655
Amaranthus cruentus L.	Leaves	Pantone 1810

4.2 Dye extraction

Clitoria ternatea dye was extracted at 70˚C for 60 minutes and *Amaranthus cruentus* dye was extracted at 60˚C for 60 minutes in water bath. The extracted dye was filtered and subjected to rotary evaporator for drying. 10% of dye was used for staining plant tissue and dyeing each cloth sample.

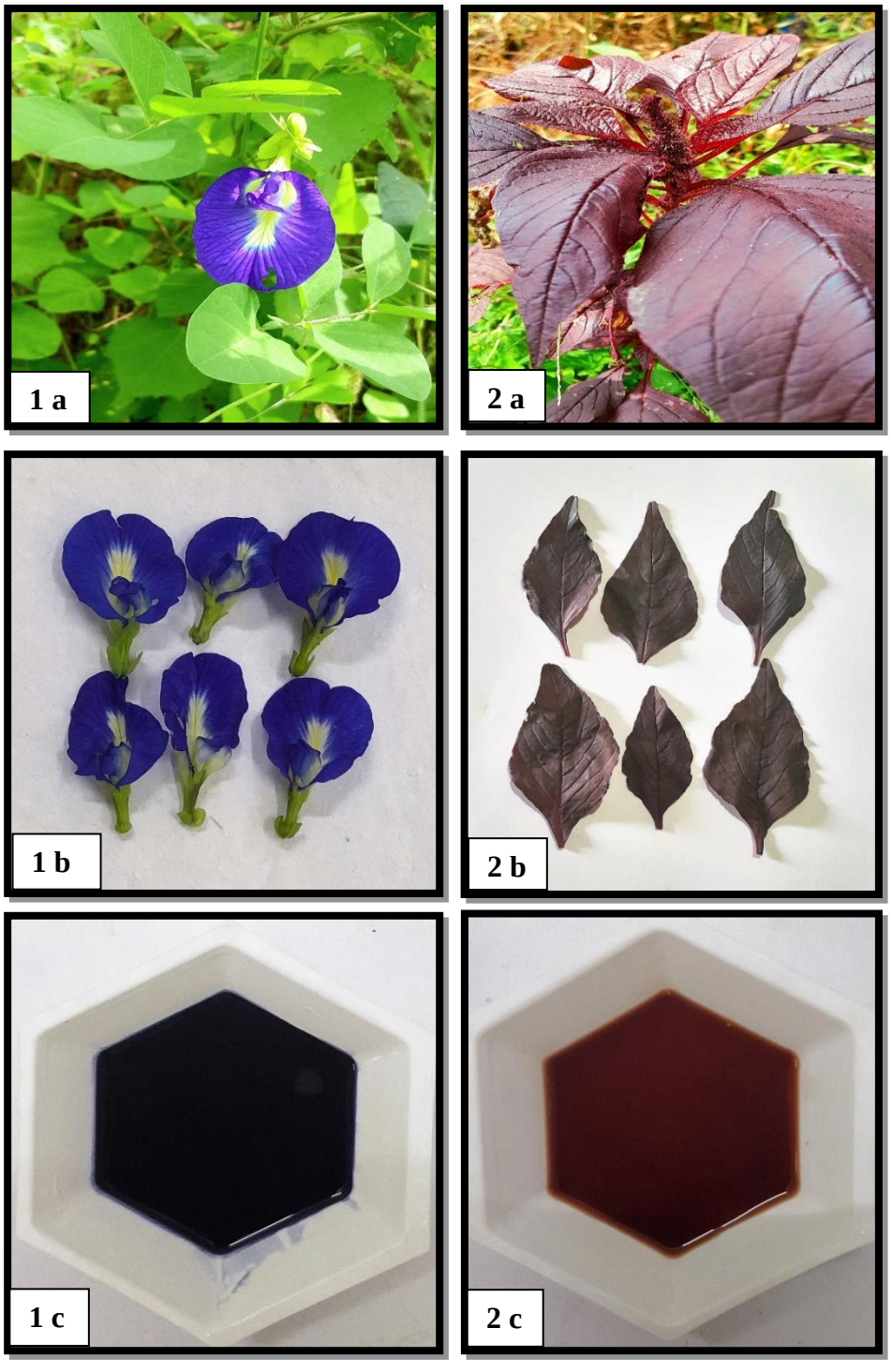


Plate 1. Selected dye-yielding plants: 1. *Clitoria ternatea*: (a) Habit, (b) Flowers, (c) Dye extracted 2. *Amaranthus cruentus* (a) Habit, (b) Leaves, (c) Dye extracted.

4.3 Dyeing of cotton fibres

4.3.1 Dyeing of cotton fibres with *Clitoria ternatea* dye

Dyeing with *Clitoria ternatea* dye on cotton fibre without any mordant produced strong blue colour. In pre-mordanting, alum and copper sulphate mordant produced moderate violet colour and ferrous sulphate gave dark brown colour. *Aloe vera*, coffee and tea produced shades of purplish-blue colours. In post-mordanting, alum gave purplish red colour, copper sulphate and ferrous sulphate produced shades of pink, whereas *Aloe vera* produced purplish-blue shades and coffee and tea produced yellowish brown and brownish grey shades. In simultaneous-mordanting alum developed purplish pink colour, copper sulphate formed reddish-pink and ferrous sulphate showed dark greyish brown colour. While, *Aloe vera* coffee and tea produced shades of purplish blue and bluish grey respectively**. Table 2, Plate 2.**

Table 2. Shades of colour obtained on cotton fibres dyed with *Clitoria ternatea* dye with different mordanting methods described according to the ISCC-NBS colour system.

Dyes and mordant used	**Pre-mordanting**	**Post-mordanting**	**Simultaneous-mordanting**
Clitoria ternatea dye	Strong blue		
Dye + Alum	Moderate violet	Moderate purplish red	Strong purplish pink
Dye + $CuSO_4$	Moderate violet	Dark pink	Dark reddish pink
Dye + $FeSO_4$	Dark brown	Moderate pink	Dark greyish brown
Dye + *Aloe vera*	Moderate purplish blue	Moderate purplish blue	Moderate purplish blue
Dye + Coffee	Light purplish blue	Moderate yellowish brown	Bluish grey
Dye + Tea	Light purplish blue	Brownish grey	Bluish grey

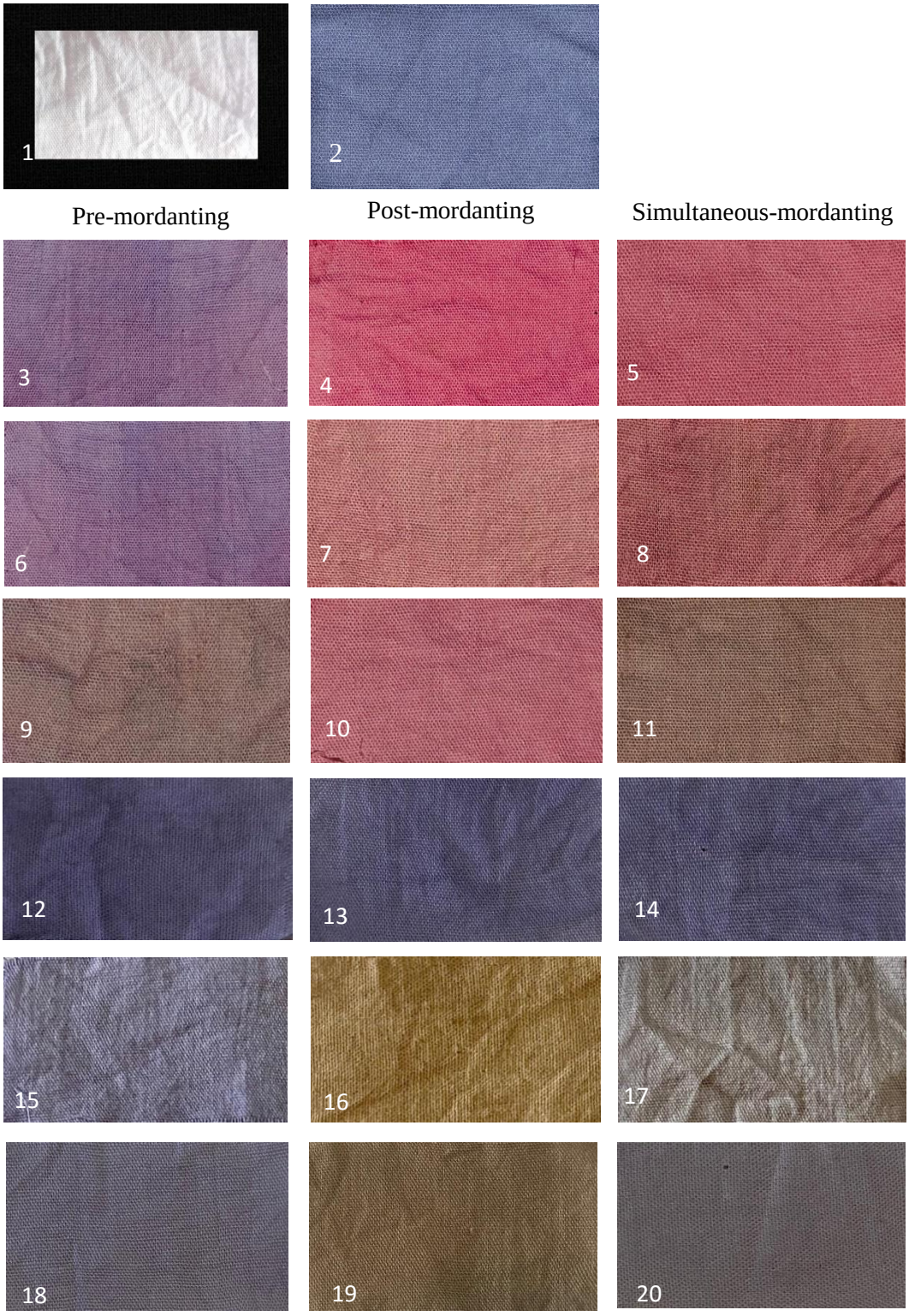


Plate 2. Dyeing of cotton fibres with *Clitoria ternatea* dye:
(1) Undyed cotton cloth.
(2) Dyed with *C. ternatea* dye.
(3-5) Dyed with *C. ternatea* dye and Alum.
(6-8) Dyed with *C. ternatea* dye and $CuSO_4$.
(9-11**)** Dyed with *C. ternatea* dye and $FeSO_4$.
(12-14) Dyed with *C. ternatea* dye and *Aloe vera*.
(15-17) Dyed with *C. ternatea* dye and Coffee.
(18-20) Dyed with *C. ternatea* dye and Tea.

4.3.2 Dyeing of cotton fibres with *Amaranthus cruentus* dye

Dyeing with *Amaranthus cruentus* dye on cotton fibre without any mordant produced a pinkish-red colour. However, pre-mordanting with alum and copper sulphate produced olive-brown and olive-yellow colours respectively, whereas ferrous sulphate gave a purplish-pink colour. *Aloe vera,* coffee and tea produced shades of reddish-purple when used for pre-mordanting. In post-mordanting, alum gave a greyish brown colour, copper sulphate and ferrous sulphate produced greyish yellow and olive green colors respectively, whereas *Aloe vera* produced a light-pink shade and coffee and tea produced reddish brown colours. In simultaneous-mordanting alum developed greyish yellow colour, copper sulphate formed olive-green and ferrous sulphate showed greyish-olive colour while, *Aloe vera*, coffee and tea produced shades of purplish pink, dark pink and reddish pink respectively. **Table 3, Plate 3.**

Table 3. Shades of colour obtained on cotton fibres dyed with *Amaranthus cruentus* dye with different mordanting methods described according to the ISCC-NBS colour syst

Dyes and mordant used	**Pre-mordanting**	**Post-mordanting**	**Simultaneous-mordanting**
Amaranthus cruentus dye	Pinkish red		
Dye + Alum	Olive brown	Greyish brown	Greyish yellow
Dye + $CuSO_4$	Olive yellow	Dark greyish yellow	Dark olive green
Dye + $FeSO_4$	Purplish pink	Olive green	Greyish olive
Dye + *Aloe vera*	Reddish purple	light pink	Moderate purplish pink
Dye + Coffee	Reddish purple	Reddish brown	Dark pink
Dye + Tea	Reddish purple	Reddish brown	Reddish pink

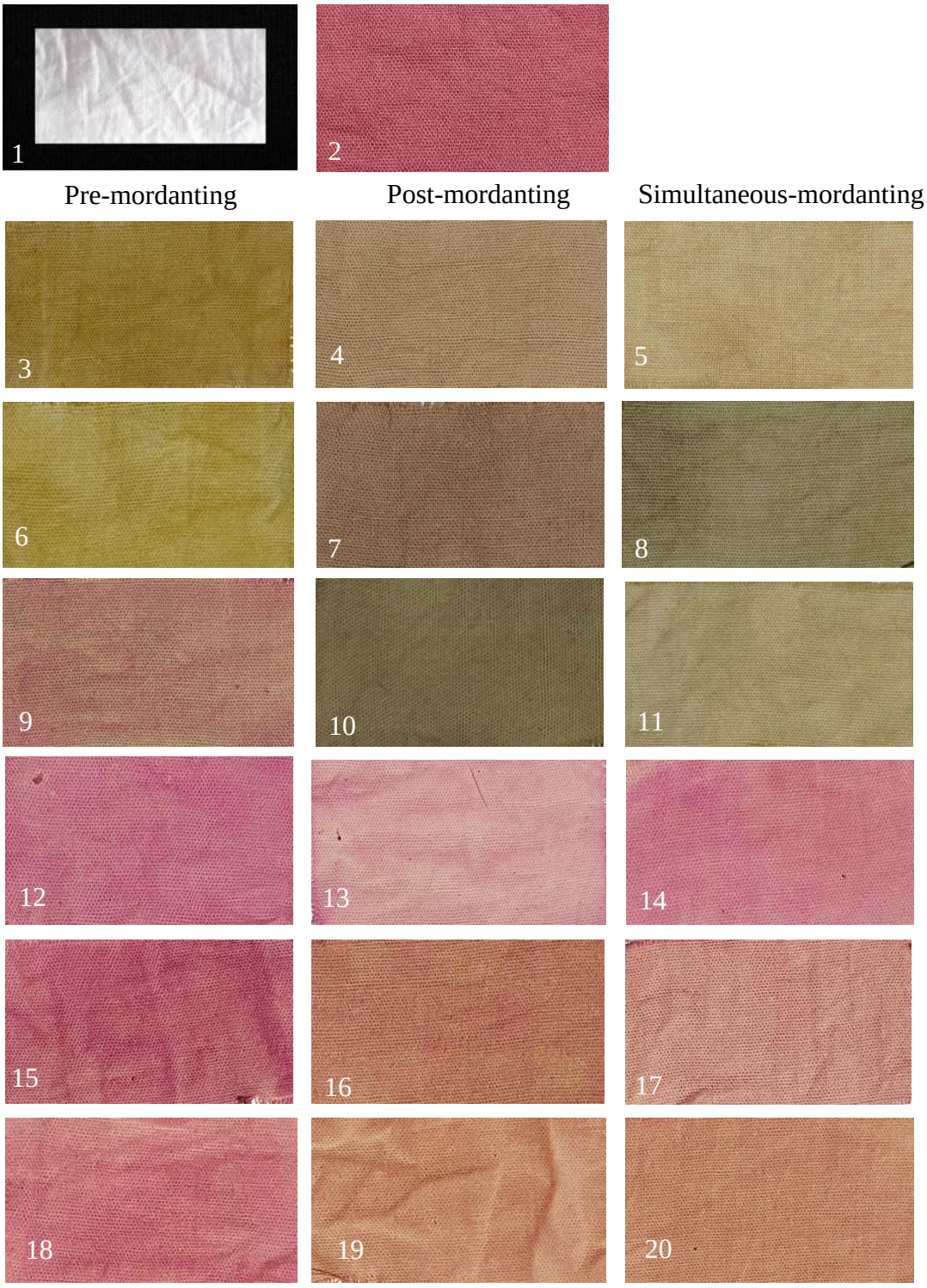


Plate 3. Dyeing of cotton fibres with *Amaranthus cruentus* dye:
(1) Undyed cotton cloth.
(2) Dyed with *A. cruentus* dye.
(3-5) Dyed with *A. cruentus* dye and Alum.
(6-8) Dyed with *A. cruentus* dye and $CuSO_4$.
(9-11**)** Dyed with *A. cruentus* dye and $FeSO_4$.
(12.-14) Dyed with *A. cruentus* dye and *Aloe vera*.
(15-17) Dyed with *A. cruentus* dye and Coffee.
(18-20) Dyed with *A. cruentus* dye and Tea.

4.4 Dyeing of wool fibres

4.4.1 Dyeing of wool fibres with *Clitoria ternatea* dye

Dyeing with *Clitoria ternatea* dye produced a deep blue colour without any mordant. In pre-mordanting alum and copper sulphate produced shades of purplish-blue and ferrous sulphate produced violet colour. Light blue shades were produced by *Aloe vera* and coffee while tea produced moderate blue colour. In post-mordanting alum, copper sulphate and ferrous sulphate showed pink shades ranging from light to pale pink. Coffee and tea developed a brown colour while *Aloe vera* gave a greyish-blue shade. In simultaneous- mordanting alum, copper sulphate and ferrous sulphate developed reddish-purple, reddish-pink and purplish pink colours respectively. Natural mordants i.e. tea and coffee developed bluish-grey colour while *Aloe vera* showed bluish-black colour. **Table 4, Plate 4.**

Table 4. Shades of colour obtained on Wool fibres dyed with *Clitoria ternatea* dye with different mordanting methods, described according to the ISCC-NBS colour system.

Dyes and mordant used	**Pre-mordanting**	**Post-mordanting**	**Simultaneous-mordanting**
Clitoria ternatea dye	Deep blue		
Dye + Alum	Strong purplish blue	Pinkish white	Moderate reddish purple
Dye + $CuSO_4$	Purplish blue	Light pink	Deep reddish pink
Dye + $FeSO_4$	Moderate violet	Pale pink	Purplish pink
Dye + *Aloe vera*	Light blue	Greyish blue	Bluish black
Dye + Coffee	Light blue	Brown	Bluish grey
Dye + Tea	Moderate blue	Dark brown	Bluish grey

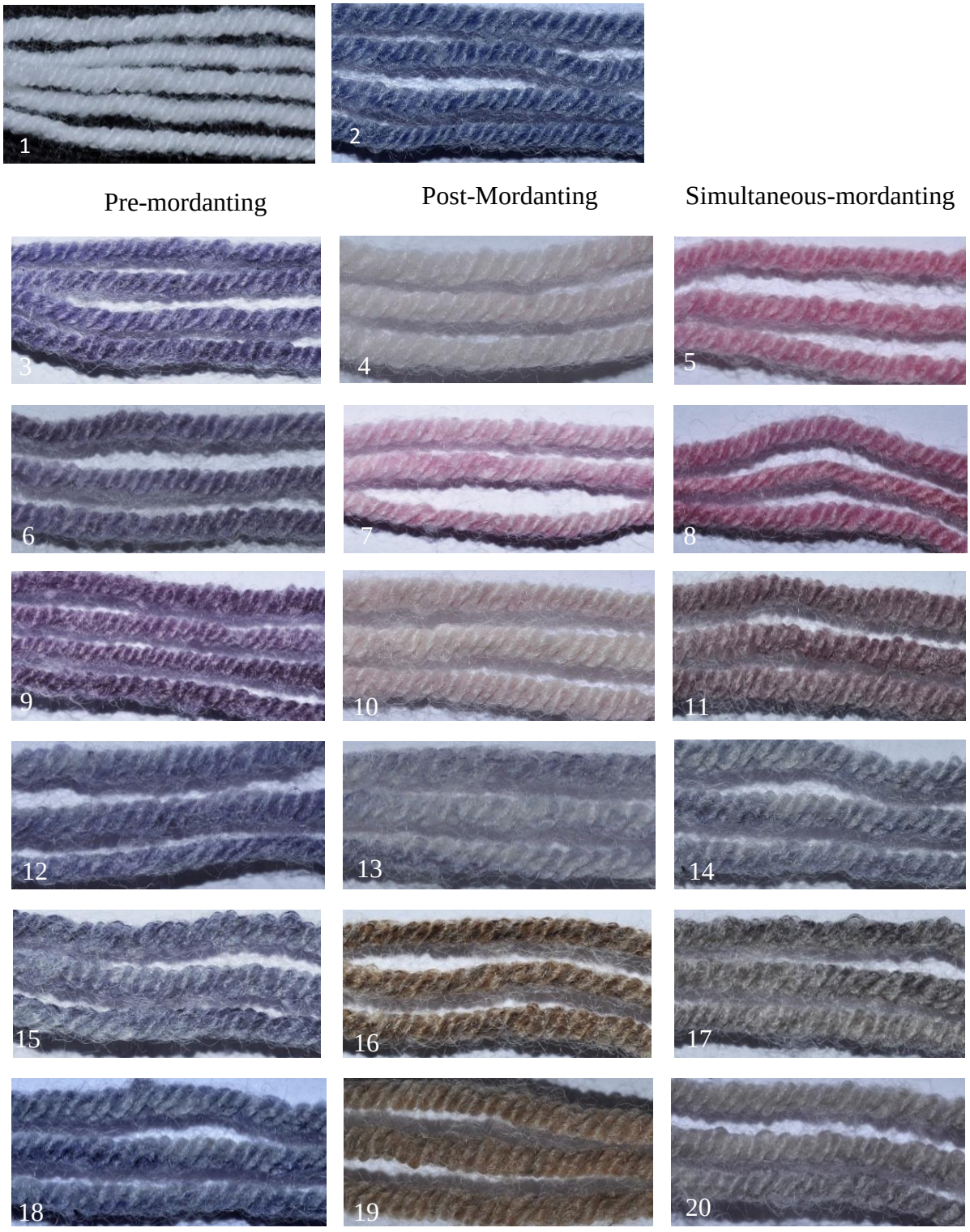


Plate 4. Dyeing of wool fibres with *Clitoria ternatea* dye.
(1) Undyed wool fibre.
(2) Dyed with *C. ternatea* dye.
(3-5) Dyed with *C. ternatea* dye and Alum.
(6-8) Dyed with *C. ternatea* dye and $CuSO_4$.
(9-11**)** Dyed with *C. ternatea* dye and $FeSO_4$.
(12.-14) Dyed with *C. ternatea* dye and *Aloe vera.*
(15-17) Dyed with *C. ternatea* dye and Coffee.
(18-20) Dyed with *C. ternatea* dye and Tea.

4.4.2 Dyeing of wool fibres with *Amaranthus cruentus* dye

Dyeing with *Amaranthus cruentus* dye produced purplish pink colour on wool without any mordant. In pre-mordanting, alum and copper sulphate mordant produced yellowish brown and greyish olive colour whereas, ferrous sulphate gave dark olive colour. *Aloe vera*, coffee and tea produced shades of pinkish-purple, deep pink and moderate red colours. In post-mordanting, alum gave light brown colour, copper sulphate and ferrous sulphate produced light olive and olive black colour, whereas *Aloe vera* produced strong-pinks and coffee and tea produced dark brown and reddish-brown shades. In simultaneous-mordanting alum developed moderate brown colour, copper sulphate formed moderate olive and ferrous sulphate showed olive grey colour while, *Aloe vera* coffee and tea produced shades of strong pink, reddish brown and reddish purple respectively. **Table 5, Plate 5.**

Table 5. Shades of colour obtained on wool fibres dyed with dye with *Amaranthus cruentus* different mordanting methods described according to the ISCC-NBS colour system.

Dyes and mordant used	**Pre-mordanting**	**Post-mordanting**	**Simultaneous-mordanting**
Clitoria ternatea dye	Purplish pink		
Dye + Alum	Yellowish brown	Light brown	Moderate brown
Dye + $CuSO_4$	Greyish olive	Light olive	Moderate olive
Dye + $FeSO_4$	Dark olive	Olive black	Olive grey
Dye + *Aloe vera*	Pinkish purple	Strong pink	Strong pink
Dye + Coffee	Deep pink	Dark brown	Reddish brown
Dye + Tea	Moderate red	Reddish brown	Reddish purple

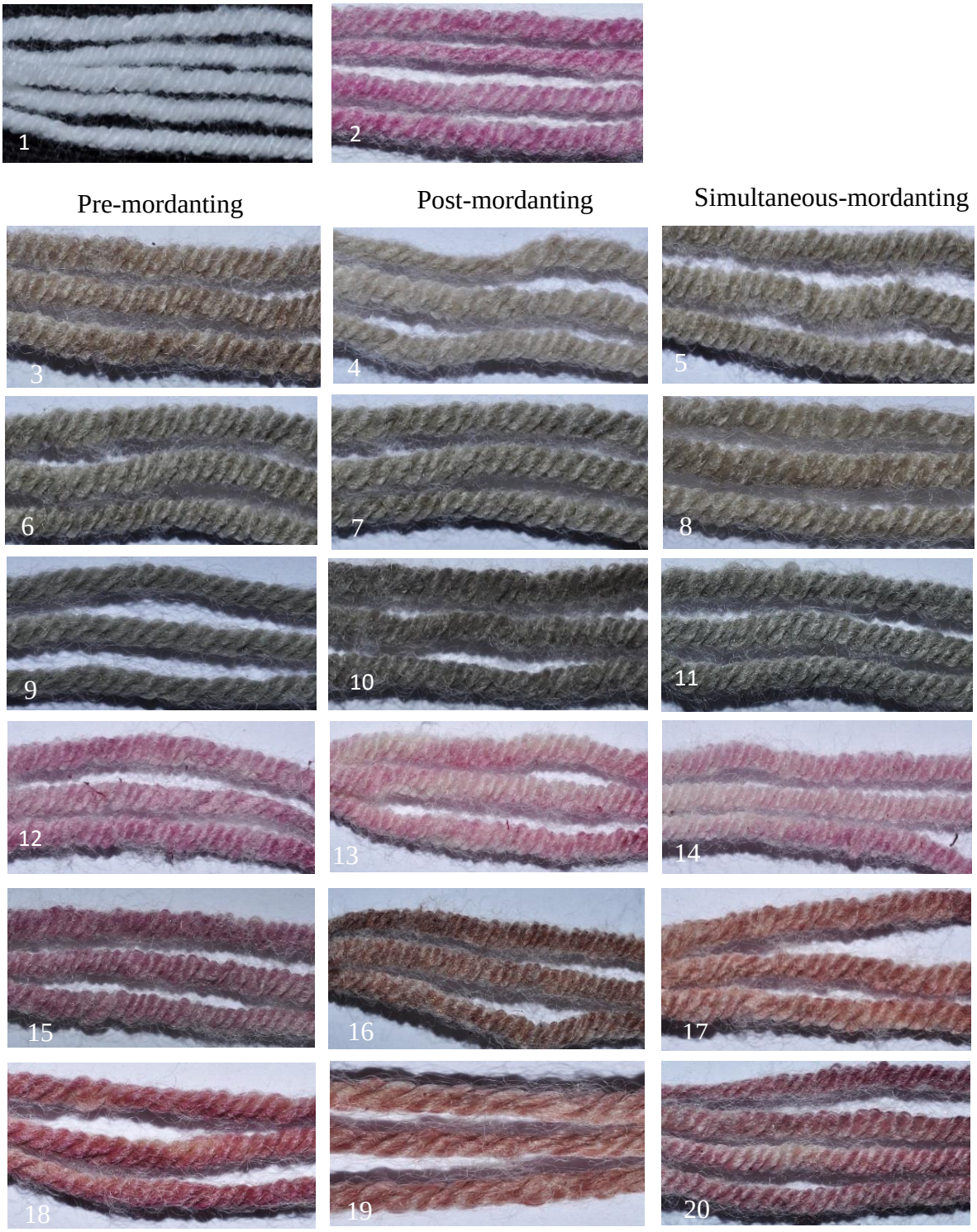


Plate 5. Dyeing of wool fibres with *Amaranthus cruentus* dye.
1 Undyed wool fibre
2.Dyed with *A. cruentus* dye
3-5 Dyed with *A. cruentus* dye and Alum
6-8. Dyed with *A. cruentus* dye and $CuSO_4$
9-11**.** Dyed with *A. cruentus* dye and $FeSO_4$
12.-14. Dyed with *A. cruentus* dye and *Aloe vera*
15-17. Dyed with *A. cruentus* dye and Coffee
18-20. Dyed with *A. cruentus* dye and Tea

4.5 Colour fastness result of the dyed fibres

4.5.1 Colour fastness result of the cotton fibre dyed with *Clitoria ternatea* dye

Dyeing with *Clitoria ternatea* dye without any mordant developed strong blue colour which changed to moderate blue after the wash, rub and light fastness tests. In pre-mordanting, dyed with alum and copper sulphate, moderate violet colour was obtained which faded to light purple after wash and light fastness and remained unchanged after rub fastness. Ferrous sulphate produced dark brown colour which changed into moderate brown after wash fastness, light brown and brown after light and rub fastness. *Aloe vera,* coffee and tea produced purplish blue shade in pre-mordanting which after wash fastness faded slightly to lighter shade of blue for *Aloe vera* and coffee and grey shade was remnant for tea. **(Plate 6)**

In post-mordanting, moderate purplish red colour was obtained with alum, which changed to purplish pink after the fastness tests. Copper sulphate mordant produced dark pink colour, which faded to light pink colour after wash, light and rub fastness test. Applications of ferric chloride mordant developed moderate pink colour which changed to strong pink after rub, light and wash fastness test. *Aloe vera* mordant with dye gave moderate purplish-blue colour, which changed to lighter shade of purplish-blue after wash fastness. However, after light and rub fastness colour change was moderate. Brownish shades of colour were developed with coffee and tea mordant which changed to light brown after wash test and remained unchanged after light and rub fastness test. **(Plate 7)**

In simultaneous mordanting, alum and copper sulphate gave strong purplish-pink colour which after fastness tests faded to light-moderate pink. Ferrous sulphate produced dark reddish pink colour which remained unchanged after rub fastness and changed into slight

Plate 6-: Colour fastness results of the cotton fibres dyed with *Clitoria ternatea* dye in pre-mordanting:
(1-4) Dyed with *C. ternatea* dye.
(5-8) Dyed with *C. ternatea* dye and alum.
(9-12) Dyed with *C. ternatea* dye and $CuSO_4$
(13-16) Dyed with *C. ternatea* dye and $FeSO_4$
(17-20) Dyed with *C. ternatea* dye and *Aloe vera*
(21-24) Dyed with *C. ternatea* dye and coffee
(25-28) Dyed with *C. ternatea* dye and tea

Plate 7. Colour fastness results of the cotton fibres dyed with *Clitoria ternatea* dye post-mordanting.
(1-4) Dyed with *C. ternatea* dye
(5-8) Dyed with *C. ternatea* dye and Alum
(9-12) Dyed with *C. ternatea* dye and $CuSO_4$
(13-16) Dyed with *C. ternatea* dye and $FeSO_4$
(17-20) Dyed with *C. ternatea* dye and *Aloe vera*
(21-24) Dyed with *C. ternatea* dye and coffee
(25-28) Dyed with *C. ternatea* dye and Tea

Plate 8. Colour fastness results of the cotton fibres dyed with *Clitoria ternatea* dye Simultaneous-mordanting.
1-4. Dyed with *C. ternatea* dye.
5-8. Dyed with *C. ternatea* dye and Alum.
9-12. Dyed with *C. ternatea* dye and $CuSO_4$.
13-16. Dyed with *C. ternatea* dye and $FeSO_4$.
17-20. Dyed with *C. ternatea* dye and *Aloe vera*.
21-24. Dyed with *C. ternatea* dye and coffee.
25-28. Dyed with *C. ternatea* dye and Tea.

brown shade after wash and light fastness. Moderate purplish-blue shade was produced by *Aloe vera* which faded to whitish blue after and tea and coffee colour faded to greyish blue after fastness tests. **(Plate 8)**

4.5.2 Colour fastness result of the cotton fibre dyed with *Amaranthus cruentus* dye

Dyeing with only *Amaranthus cruentus* dye without any mordant developed pinkish red colour which changed to moderate pink after wash and light fastness and light pinkish red after rub test. In pre-mordanting, dyed with alum and copper sulphate produced olive brown and yellow shades, which changed to light olive yellow and whitish yellow after all 3 fastness tests. Ferrous sulphate produced purplish pink which changed into moderate purplish pink after wash, light and rub fastness. Reddish-purple colour was obtained after mordanting with *Aloe vera*, coffee and tea which changed to light pink after wash fastness in case of *Aloe vera*, coffee and tea. **(Plate 9)**

In post-mordanting alum, produced greyish brown colour which changed to light brown after wash and light fastness. Greyish yellow and olive-green shades were produced by copper sulphate and ferrous sulphate which changed to light brown and light olive-green colour. Very slight colour change was observed for rub fastness with chemical mordants. *Aloe vera* produced light-pink colour which changed into pale pink after wash, rub and light fastness tests. Coffee and tea developed reddish brown colour which changed to pale brown after wash fastness and light to moderate brown after light and rub fastness respectively. **(Plate 10)**

In simultaneous mordanting alum, copper sulphate and ferrous sulphate developed greyish yellow, dark olive green and greyish olive colour which faded to pale colour shades for alum and light yellow to light olive shades after wash fastness for copper sulphate and ferrous sulphate. Moderate pink shade produced by *Aloe vera* mordant

Plate 9. Colour fastness results of the cotton fibres dyed with *Amaranthus cruentus* dye in pre-mordanting.
(1-4) Dyed with *A. cruentus* dye
(5-8) Dyed with *A. cruentus* Dye and Alum
(9-12) Dyed with *A. cruentus* Dye and $CuSO_4$
(13-16) Dyed with *A. cruentus* dye and $FeSO_4$
(17-20) Dyed with *A. cruentus* dye and *Aloe vera*
(21-24) Dyed with *A. cruentus* dye and Coffee
(25-28) Dyed with *A. cruentus* dye and Tea

Plate 10. Colour fastness results of the cotton fibres dyed with *Amaranthus cruentus* dye in post-mordanting.
(1-4) Dyed *A. cruentus* with dye
(5-8) Dyed with *A. cruentus* Dye and Alum
(9-12) Dyed with *A. cruentus* Dye and $CuSO_4$
(13-16) Dyed with *A. cruentus* dye and $FeSO_4$
(17-20) Dyed with *A. cruentus* dye and *Aloe vera*
(21-24) Dyed with *A. cruentus* dye and Coffee
(25-28) Dyed with *A. cruentus* dye and Tea

Plate 11. Colour fastness results of the cotton fibers with *Amaranthus cruentus* dye in simultaneous-mordanting.
1-4. Dyed with *A. cruentus* dye.
5-8. Dyed with *A. cruentus* Dye and Alum.
9-12. Dyed with *A. cruentus* Dye and $CuSO_4$.
13-16. Dyed with *A. cruentus* dye and $FeSO_4$.
17-20. Dyed with *A. cruentus* dye and *Aloe vera.*
21-24. Dyed with *A. cruentus* dye and Coffee.
25-28. Dyed with *A. cruentus* dye and Tea.

changed to pale pink after wash, light and rub fastness. Tea and coffee mordant gave light moderate pink and light brownish pink after wash and light fastness. **(Plate 11)**

4.5.3 Colour fastness result of the wool fibre dyed with *Clitoria ternatea* dye

Dyeing with *Clitoria ternatea* dye without any mordant produced deep blue colour on wool fibres which changed to light blue and moderate blue after wash and light fastness and strong blue after rub fastness. In pre-mordanting, alum and copper sulphate produced purplish blue which turned to light purplish blue after wash and light fastness in alum and pale purplish after wash and light fastness in copper sulphate. Moderate violet colour was developed by ferrous sulphate mordant which changed to bluish violet after wash fastness test and moderate violet after light and rub fastness. *Aloe vera*, coffee and tea produced blue shades which changed into greyish blue after wash and light fastness tests. All mordants showed slight colour change after rub fastness. **(Plate 12)**

In post-mordanting, alum mordant gave pinkish white colour which changed to pale pink after performing all 3 fastness tests. Copper sulphate and ferrous sulphate also gave pink shades which changed into very dull pink after wash fastness and moderate pink colour shade after light fastness, and light pink after rub fastness. *Aloe vera* developed greyish blue colour which faded the colour to dull grey after wash fastness and light grey colour was observed after light and rub fastness. Coffee and tea gave brown colour shades which showed moderate colour change after wash, light and rub fastness test. **(Plate 13)**

In-simultaneous mordanting, dye with alum, copper sulphate and ferrous sulphate produced pink shades. Wash and light fastness changed the colour to light and moderate pink and rub fastness gave moderate colour shades of pink. *Aloe vera* produced greyish blue which faded to pale greyish after fastness tests. Tea and coffee produced light

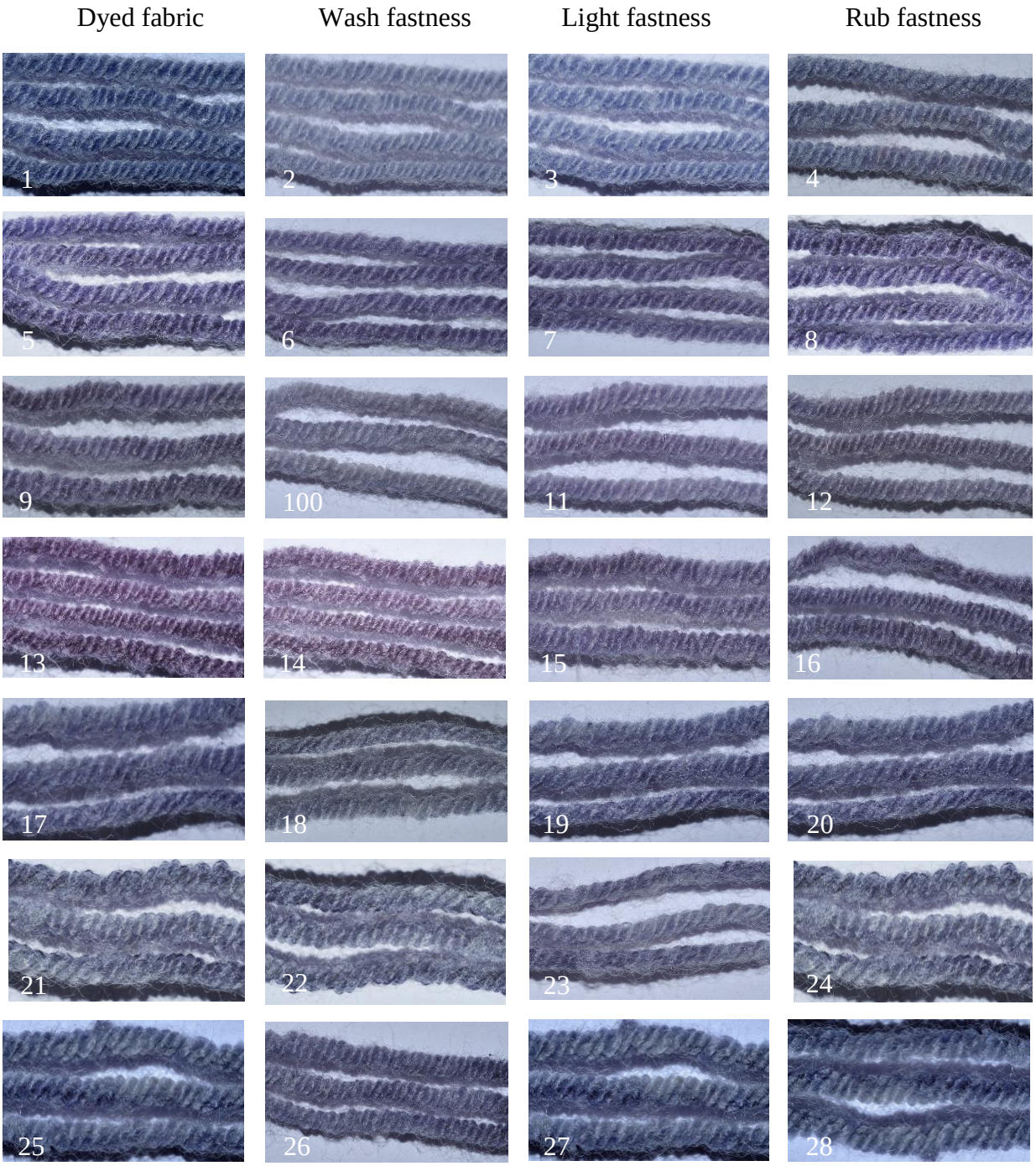


Plate 12. Colour fastness results of the wool fibres dyed with *Clitoria ternatea* dye in pre-mordanting.
(1-4) Dyed with *C. ternatea* dye.
(5-8) Dyed with *C. ternatea* dye and alum.
(9-12) Dyed with *C. ternatea* dye and $CuSO_4$.
(13-16) Dyed with *C. ternatea* dye and $FeSO_4$.
(17-20) Dyed with *C. ternatea* dye and *Aloe vera*.
(21-24) Dyed with *C. ternatea* dye and Coffee.
(25-28) Dyed with *C. ternatea* dye and Tea.

Plate 13. Colour fastness results of the wool fibres dyed with *Clitoria ternatea* dye in post-mordanting.
(1-4) Dyed with *C. ternatea* dye.
(5-8) Dyed with *C. ternatea* Dye and Alum.
(9-12) Dyed with *C. ternatea* Dye and $CuSO_4$.
(13-16) Dyed with *C. ternatea* dye and $FeSO_4$.
(17-20) Dyed with *C. ternatea* dye and *Aloe vera*.
(21-24) Dyed with *C. ternatea* dye and Coffee.
(25-28) Dyed with *C. ternatea* dye and Tea.

Plate 14. Colour fastness results of the wool fibers dyed with *Clitoria ternatea* dye in simultaneous-mordanting.
(1-4) Dyed with *C. ternatea* dye.
(5-8) Dyed with *C. ternatea* dye and Alum.
(9-12) Dyed with *C. ternatea* dye and $CuSO_4$.
(13-16) Dyed with *C. ternatea* dye and $FeSO_4$.
(17-20) Dyed with *C. ternatea* dye and *Aloe vera*.
(21-24) Dyed with *C. ternatea* dye and Coffee.
(25-28) Dyed with *C. ternatea* dye and Tea.

brownish shades which after wash, light and rub fastness gave light to moderate shade for the same colour. **(Plate 14)**

4.5.4 Colour fastness result of the wool fibre dyed with *Amaranthus cruentus* dye

Wool fibres dyed with *Amaranthus cruentus* dye without any mordant produced purplish pink colour. The colour of the fibre changed to pale pink after wash fastness test and light to moderate pink after light and rub fastness tests. Alum, copper sulphate and ferrous sulphate produced yellowish brown, greyish olive and dark olive colour which changed to light brown for alum and copper sulphate and greyish green for ferrous sulphate after wash fastness respectively. Moderate colour change was observed for rub and light fastness. Strong pink, reddish brown and reddish-purple colour were obtained with *Aloe vera*, tea and coffee which changed to moderate colour shades of same colour giving good fastness result for wash fastness, light fastness and rub fastness. **(Plate 15)**

In post-mordanting light brown, light olive and olive black colour developed by alum, copper sulphate and ferrous sulphate respectively, which changed to lighter colour shades of the same colour after wash fastness and gave moderate change in colour for light and rub fastness. *Aloe vera*, coffee and tea gave pink, brown and reddish-brown colour which changed to light pink and moderate brown shade after wash, rub and light fastness. **(Plate 16)**

In simultaneous mordanting, wool fibres dyed with alum, copper sulphate and ferrous sulphate developed moderate brown, olive and olive grey colour which changed to light brown, olive and grey colour respectively, after wash fastness and gave moderate shades of brown and olive colour after rub fastness and light fastness. Light pink colour obtained by *Aloe vera* changed to pale pink after wash, rub and light fastness. Reddish-

Plate 15. Colour fastness results of the wool fibres dyed with *Amaranthus cruentus* dye in Pre-mordanting.
(1-4) Dyed with *A. cruentus* dye.
(5-8) Dyed with *A. cruentus* dye and Alum.
(9-12) Dyed with *A. cruentus* dye and $CuSO_4$.
(13-16) Dyed with *A. cruentus* dye and $FeSO_4$.
(17-20) Dyed with *A. cruentus* dye and *Aloe vera.*
(21-24) Dyed with *A. cruentus* dye and Coffee.
(25-28) Dyed with *A. cruentus* dye and Tea.

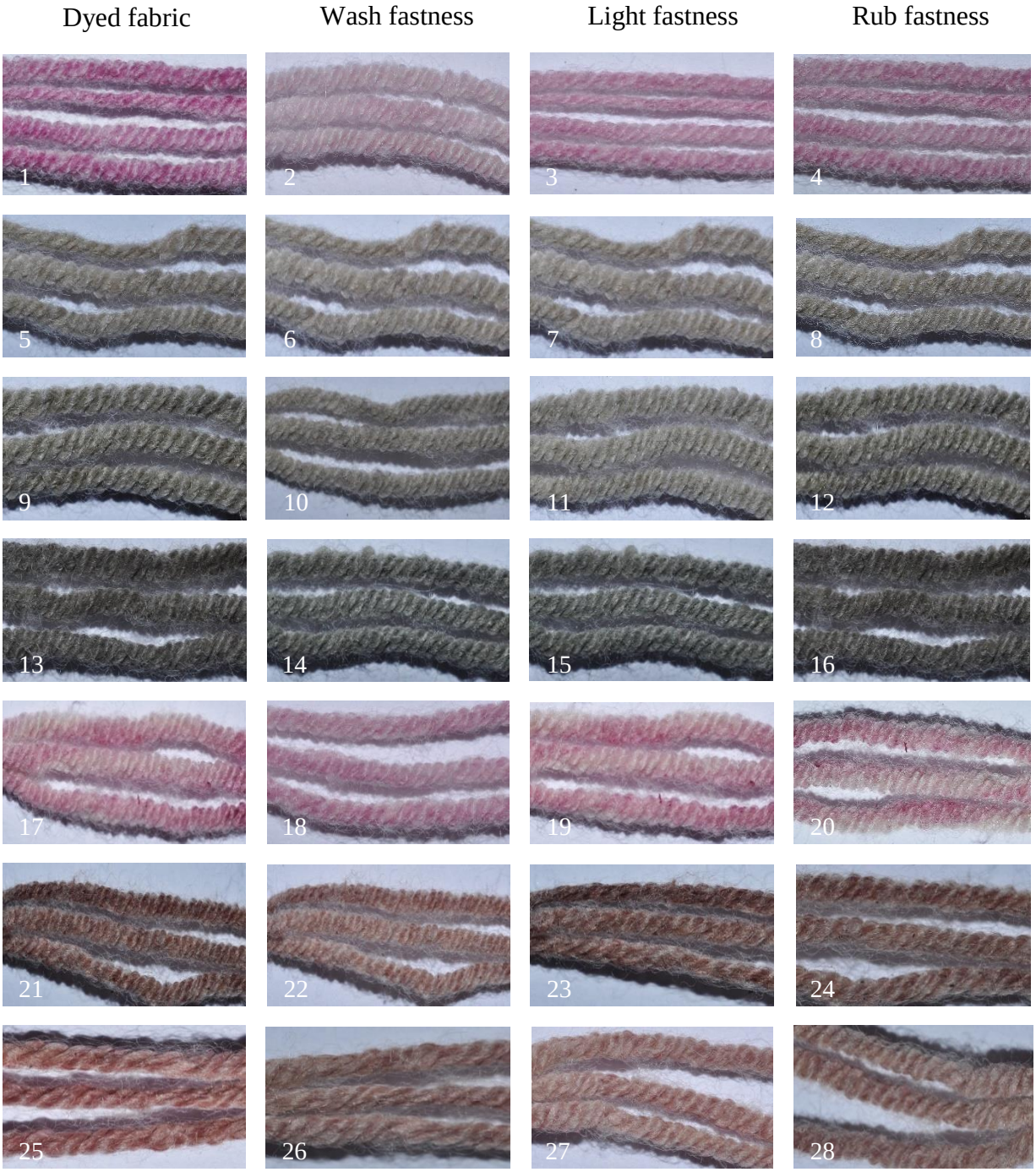


Plate 16. Colour fastness results of the wool fibres dyed with *Amaranthus cruentus* dye in post-mordanting.
(1-4) Dyed with *A. cruentus* dye.
(5-8) Dyed with *A. cruentus* Dye and Alum.
(9-12) Dyed with *A. cruentus* Dye and $CuSO_4$.
(13-16) Dyed with *A. cruentus* dye and $FeSO_4$.
(17-20) Dyed with *A. cruentus* dye and *Aloe vera.*
(21-24) Dyed with *A. cruentus* dye and Coffee.
(25-28) Dyed with *A. cruentus* dye and Tea.

Plate 17. Colour fastness results of the wool fibres dyed with *Amaranthus cruentus* dye in simultaneous-mordanting.
(1-4) Dyed with *A. cruentus* dye.
(5-8) Dyed with *A. cruentus* Dye and Alum.
(9-12) Dyed with *A. cruentus* Dye and $CuSO_4$.
(13-16) Dyed with *A. cruentus* dye and $FeSO_4$.
(17-20) Dyed with *A. cruentus* dye and *Aloe vera.*
(21-24) Dyed with *A. cruentus* dye and Coffee.
(25-28) Dyed with *A. cruentus* dye and Tea.

brown and reddish-purple colour was obtained by coffee and tea mordant which changed to light blue colour shades after wash, rub and light fastness. **(Plate 17)**

4.5.5 Gray scale ratings of coloured fibres

Table 6. Gray scale ratings of colour fastness of cotton fibres dyed with *Clitoria ternatea* dye for Pre-mordanting.

Dyes and mordants used	Wash fastness	Light fastness (6 hours)	Rub fastness (5 minutes)
Clitoria ternatea	3/4	4	4/5
Dye + Alum	4	4	5
Dye + $CuSO_4$	3/4	3/4	5
Dye + $FeSO_4$	3	3/4	4
Dye + *Aloe vera*	3/4	3	4
Dye + Coffee	3	3/5	4/5
Dye + Tea	4	4/5	5

Cotton dyed with *Clitoria ternatea* extract showed good colour fastness ratings. In pre-mordanting, rating varied from 5- 3/4 for the fibres dyed along with the chemical mordants. Alum showed very good wash, rub and light fastness ratings. Copper sulphate and ferrous sulphate showed moderate wash fastness rating for wash and light fastness and good rating for rub fastness test. For natural mordants, fastness ratings ranged between 3 to 4/5. Coffee and tea showed good results for all 3 fastness tests. Pre-mordanting with all 6 mordants exhibited excellent ratings for rub fastness and moderate to good ratings for wash and light fastness **(Fig. 2)**

Table 7. Gray scale ratings of colour fastness of cotton fibres dyed with *Clitoria ternatea* dye for post-mordanting.

Dyes and mordant used	Wash fastness	Light fastness (6 hours)	Rub fastness (5 minutes)
Dye + Alum	3	3/4	4/5
Dye + $CuSO_4$	¾	3	4/5
Dye + $FeSO_4$	3	2/3	4/5
Dye + *Aloe vera*	2/3	3	4
Dye + Coffee	3	3/ 4	4
Dye + Tea	2	2/3	4

In post-mordanting, the gray scale ratings for chemical mordant ranged between 2/3-4/5, showing moderate results for wash and light fastness and very good results for rub fastness. For natural mordants ratings ranged between 2 – 4, with *Aloe vera* and tea showing poor results for wash fastness and moderate results for light fastness and good resistance to rub fastness test. **(Fig.3)**

Table 8. Gray scale ratings of colour fastness of cotton fibres dyed with *Clitoria ternatea* dye for simultaneous-mordanting.

Dyes and mordant used	Wash fastness	Light fastness (6 hours)	Rub fastness (5 minutes)
Dye + Alum	2/3	3/4	4
Dye + $CuSO_4$	3	2/3	4
Dye + $FeSO_4$	3	3/4	4
Dye + *Aloe vera*	3	3/4	4

Dye + Coffee	3	3/4	4/5
Dye + Tea	¾	3/4	4

In simultaneous-mordanting, fastness ratings varied from 3 to 4 for chemical mordants. Poor wash and light fastness rating were observed for alum compared to copper sulphate and ferrous sulphate which showed moderate ratings. Natural mordants: tea, coffee and *Aloe vera* showed moderate wash and light fastness ratings. All the mordants showed good ratings for rub fastness. **(Fig.4)**

Table 9. Gray scale ratings of colour fastness of cotton fibres dyed with *Amaranthus cruentus* dye for pre-mordanting.

Dyes and mordant used	**Wash fastness**	**Light fastness (6 hours)**	**Rub fastness (5 minutes)**
Amaranthus cruentus	3/4	4	4/5
Dye + Alum	4/5	4	4/5
Dye + $CuSO_4$	4	4	4/5
Dye + $FeSO_4$	4	4	4/5
Dye + *Aloe vera*	3/4	3/4	4
Dye + Coffee	3/4	3/4	4/5
Dye + Tea	3	4	4/5

Cotton dyed with *Amaranthus cruentus* dye extract without mordant showed moderate rating for wash fastness and good results for light and rub fastness. Fastness ratings ranged between 3/4 to 4/5 with chemical mordants showing very good results for

wash, light and rub fastness. Natural mordants showed moderate to good fastness ratings for wash and light fastness and very good rub fastness property**. (Fig.5)**

Table 10. Gray scale ratings of colour fastness of cotton fibres dyed with *Amaranthus cruentus* dye for post-mordanting.

Dyes and mordant used	**Wash fastness**	**Light fastness (6 hours)**	**Rub fastness (5 minutes)**
Dye + Alum	3	3/4	4
Dye + $CuSO_4$	2/3	3	¾
Dye + $FeSO_4$	2	3/4	4
Dye + *Aloe vera*	3/4	3	3 /4
Dye + Coffee	3	4	4/5
Dye + Tea	3/4	3/4	4

In post-mordanting, the ratings for colour fastness varied from 2 – 4 for chemical mordants and 3 to 4/5 for natural mordants. All 6 mordants showed good fastness for rub and light fastness tests giving moderate to good ratings ranging from 3/4 to 4/5 and 3 to 4 respectively. Wash fastness rating was moderate for alum, *Aloe vera*, coffee and tea. Ferrous sulphate and copper sulphate mordant showed poor ratings for wash fastness test. **(Fig.7)**

Table 11. Gray scale ratings of colour fastness of cotton fibres dyed with *Amaranthus cruentus* dye for simultaneous-mordanting

Dyes and mordant Used	Wash fastness	Light fastness (6 hours)	Rub fastness (5 minutes)
Dye + Alum	3 /4	4	4
Dye + $CuSO_4$	3	3/4	4
Dye + $FeSO_4$	2	4	4
Dye + *Aloe vera*	2/3	3	4
Dye + Coffee	3	3	4
Dye + Tea	3	3	4

In simultaneous-mordanting fastness rating for chemical mordants ranged from 2 to 4 and for natural mordant 2/3 to 4. All chemical mordants showed moderate wash fastness results, expect for $FeSO_4$ and *Aloe vera,* which showed poor wash fastness ratings. Light and rub fastness result for chemical and natural mordants were moderate to good respectively. **(Fig 6)**

Table 12. Gray scale ratings of colour fastness of Wool fibres dyed with *Clitoria ternatea* dye for Pre-mordanting.

Dyes and mordants used	Wash fastness	Light fastness (6 hours)	Rub fastness (5 minutes)
Clitoria ternatea	3	3/4	4
Dye + Alum	4	4/5	5

Dye + $CuSO_4$	3	3/4	4
Dye + FeSO4	4	3/4	4
Dye + *Aloe vera*	3/4	4	4
Dye + Coffee	4	4	4/5
Dye + Tea	4	4	4/5

Wool fibres dyed with *Clitoria ternatea* dye showed good colour fastness. In pre-mordanting, gray scale ratings ranged between 3 to 4/5 for chemical mordants and 3/5 to 4/5 for natural mordants. All mordants showed excellent ratings for rub fastness and good ratings for light fastness. Colour fastness ratings for washing showed good results for Alum, coffee and tea and moderate ratings for *Aloe vera* and copper sulphate**. (Fig.8)**

Table 13. Gray scale ratings of colour fastness of Wool fibres dyed with *Clitoria ternatea* dye for post-mordanting.

Dyes and mordant used	**Wash fastness**	**Light fastness (6 hours)**	**Rub fastness (5 minutes)**
Dye + Alum	3	3	3
Dye + $CuSO_4$	3	3	4
Dye + $FeSO_4$	3	3/ 4	4
Dye + *Aloe vera*	3	3/ 4	4
Dye + Coffee	3/4	4	4
Dye + Tea	3	3/ 4	4

In post-mordanting, chemical mordants showed moderate gray scale ratings for wash fastness and light fastness ranging from 3 to 3/4 and good ratings for rub fastness test ranging to 4. Natural mordants rating varied from 3 to 4 showing moderate colour fastness to washing and light fastness. All 6 mordants showed good ratings for rub fastness. **(Fig.9)**

Table 14. Gray scale ratings of colour fastness of Wool fibres dyed with *Clitoria ternatea* dye for simultaneous-mordanting.

Dyes and mordant Used	**Wash fastness**	**Light fastness (6 hours)**	**Rub fastness (5 minutes)**
Dye + Alum	2/3	3	3
Dye + $CuSO_4$	2/3	3	3
Dye + $FeSO_4$	2/3	3	3
Dye + *Aloe vera*	2/3	3	3
Dye + Coffee	3	3	3/4
Dye + Tea	3	3 /4	3/4

In simultaneous-mordanting, colour range varied from 2/3 to 3/4 for both the mordants. Chemical mordants showed poor ratings for wash fastness and moderate ratings for rub and light fastness. Tea and coffee showed moderate ratings for wash, rub and light fastness whereas *Aloe vera* showed poor ratings for wash fastness and moderate ratings for rub and light fastness. **(Fig.10)**

Table 15. Gray scale ratings of colour fastness of Wool fibres dyed with *Amaranthus cruentus* dye for pre-mordanting.

Dyes and mordant used	Wash fastness	Light fastness (6 hours)	Rub fastness (5 minutes)
Amaranthus cruentus	3	3/4	4
Dye + Alum	3	3/4	4
Dye + $CuSO_4$	3	3/4	4
Dye + $FeSO_4$	3	3/4	4
Dye + *Aloe vera*	3	3/4	4
Dye + Coffee	3/5	3/4	4
Dye + Tea	3	3	4

Wool fibres dyed with *Amaranthus cruentus* dye showed moderate ratings for wash and light fastness and good rating for rub fastness tests. In pre-mordanting, fibres dyed with chemical and natural mordant showed moderate colour fastness for washing and light fastness test and good ratings for rub fastness, with all 6 mordants. **(Fig.11)**

Table 16. Gray scale ratings of colour fastness of Wool fibres dyed with *Amaranthus cruentus* dye (Post-mordanting).

Dye and mordant used	Wash fastness	Light fastness (6 hours)	Rub fastness (5 minutes)
Dye + Alum	3	3	3/4
Dye + $CuSO_4$	3	3/4	3/4
Dye + $FeSO_4$	3/ 4	3/4	4
Dye + *Aloe vera*	3	3/4	3/4

Dye + Coffee	3	3/4	4/5
Dye + Tea	3	3	4

In post-mordanting, fastness ratings for all the mordants ranged from 3 to 4. Chemical and natural mordants showed moderate ratings for wash rub and light fastness with $FeSO_4$ and tea showing good ratings for rub fastness. **(Fig 12)**

Table 17. Gray scale ratings of colour fastness of Wool fibres dyed with *Amaranthus cruentus* dye (Simultaneous-mordanting).

Dyes and Mordant Used	**Wash fastness**	**Light fastness (6 hours)**	**Rub fastness (5 minutes)**
Dye + Alum	3	3	3/4
Dye + $CuSO_4$	3	3/4	3/4
Dye + $FeSO_4$	2/3	3/4	3/4
Dye + *Aloe vera*	2	2/3	3
Dye + Coffee	3	3 /4	3
Dye + Tea	3 /4	3	3

In simultaneous-mordanting, colour fastness ratings varied from 2 to 3/4 for chemical and natural mordants. Alum and $CuSO_4$ showed moderate rating for wash, rub and light fastness whereas, $FeSO_4$ showed poor ratings for wash fastness and moderate ratings for rub and light fastness. Tea and coffee showed good results for wash, rub and light fastness whereas, *Aloe vera* showed very poor colour fastness ratings for wash and light fastness and moderate rating for light fastness test. **(Fig 13)**

Figure 2. Bar graph showing Pre-mordanting colour fastness ratings of cotton fibres dyed with *Clitoria ternatea* dye

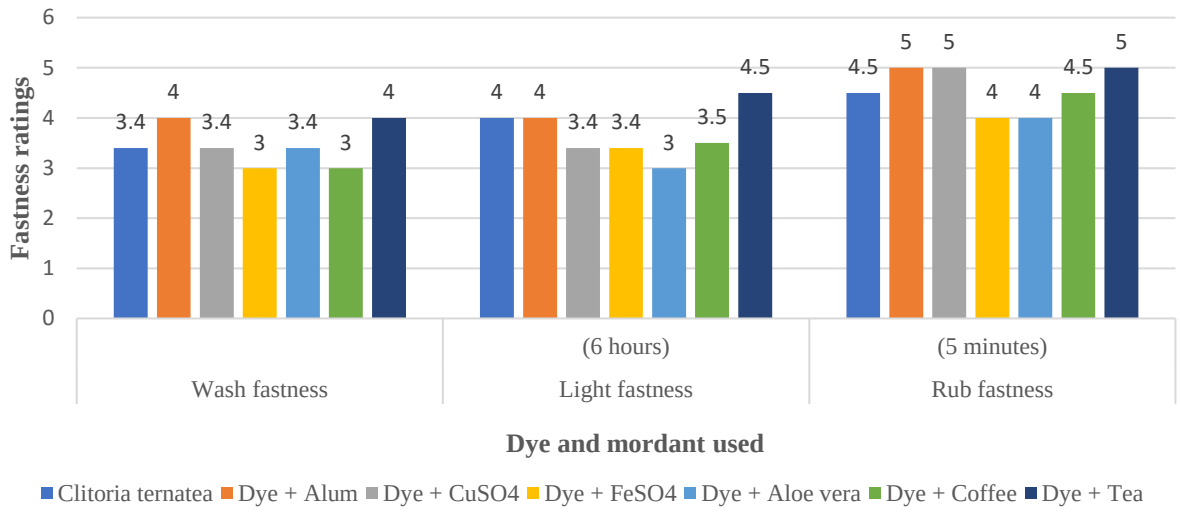


Figure 3. Bar graph showing Post-mordanting colour fastness ratings of cotton fibres dyed with *Clitoria ternatea* dye

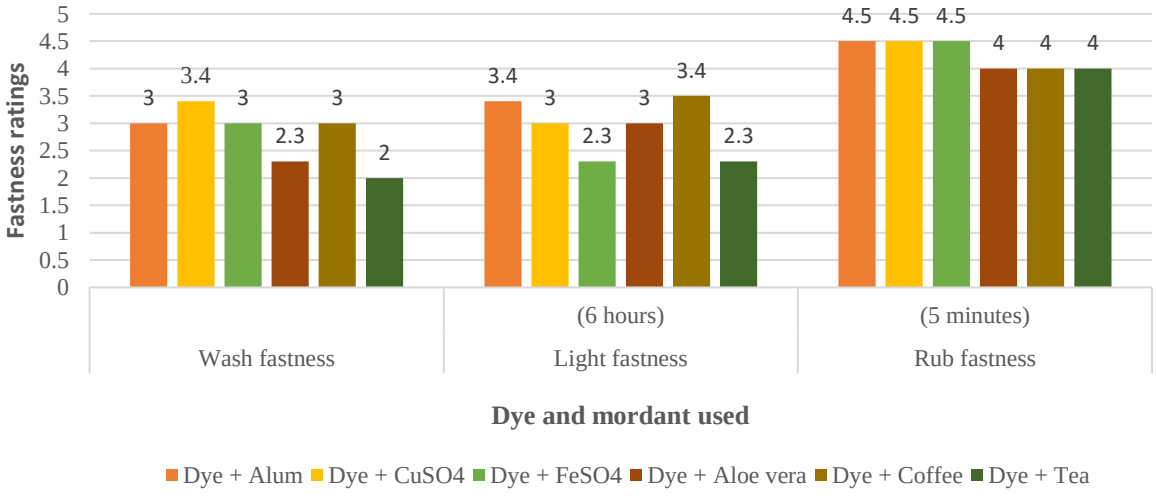


Figure 4. Bar graph showing Simultaneous-mordanting colour fastness rating of cotton fibres dyed with *clitoria ternatea* dye

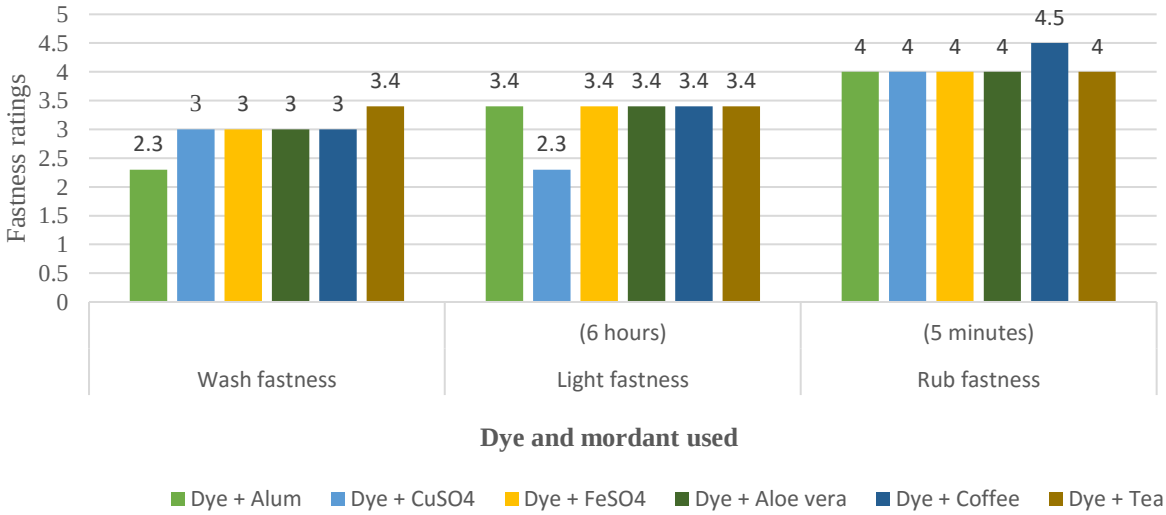


Figure 5. Bar graph showing Pre-mordanting colour fastness ratings of cotton fibres dyed with *Amaranthus cruentus* dye

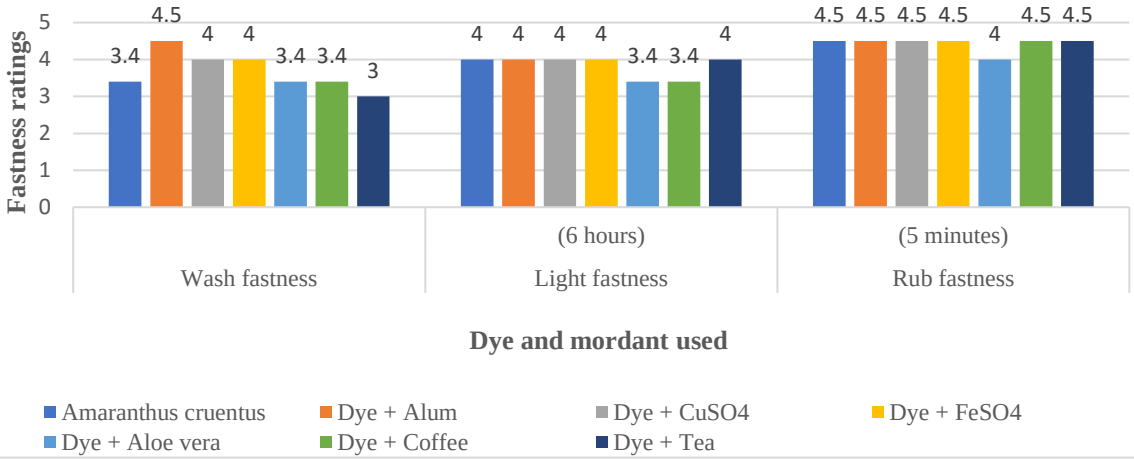


Figure 7. Post mordanting colour fastness ratings of cotton fibres dyed with Amaranthus cruentus dye

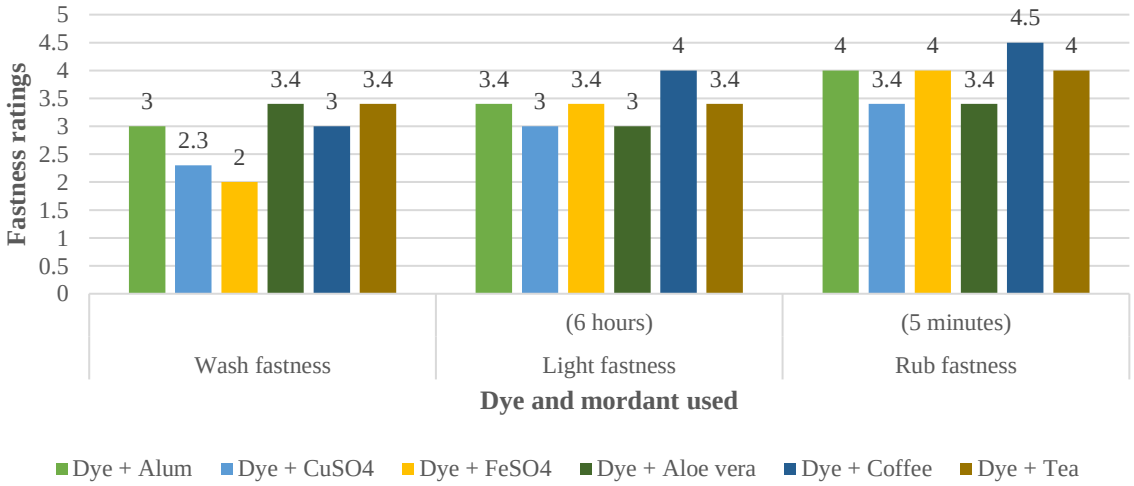


Figure 6. Bar graph showing Simultaneous mordanting colour fastness ratings of cotton fibres dyed with Amaranthus cruentus dye

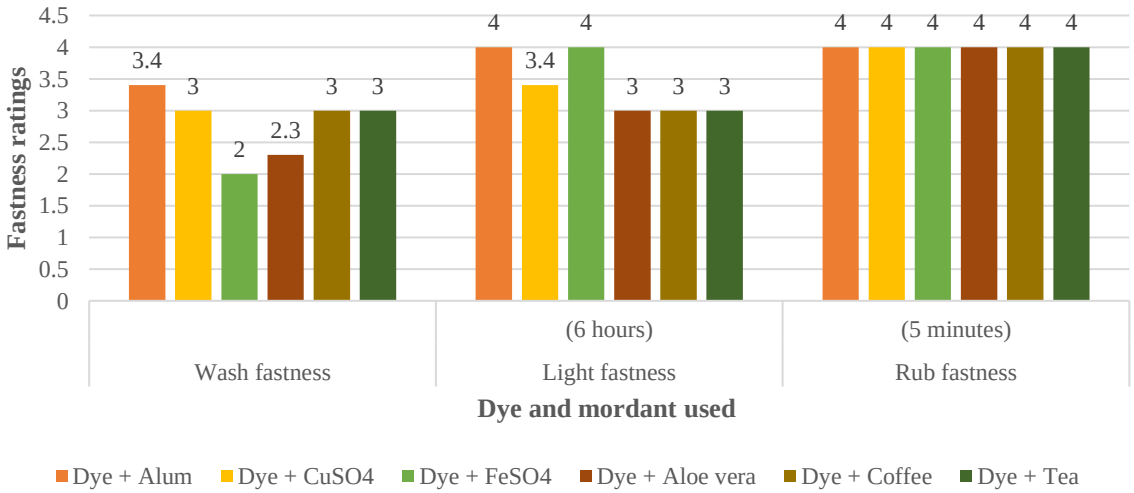


Figure 8. Bar graph showing Pre-mordanting colour fastness ratings of wool fibres dyed with Clitoria ternatea dye

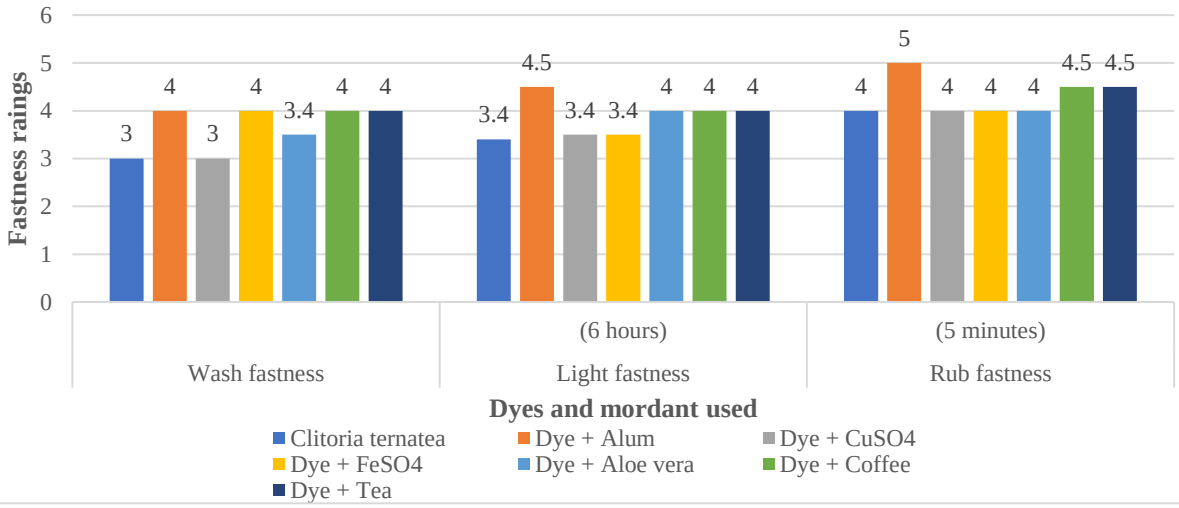


Figure 9. Bar graph Post-mordanting colour fastness ratings of wool fibres dyed with Clitoria ternatea dye

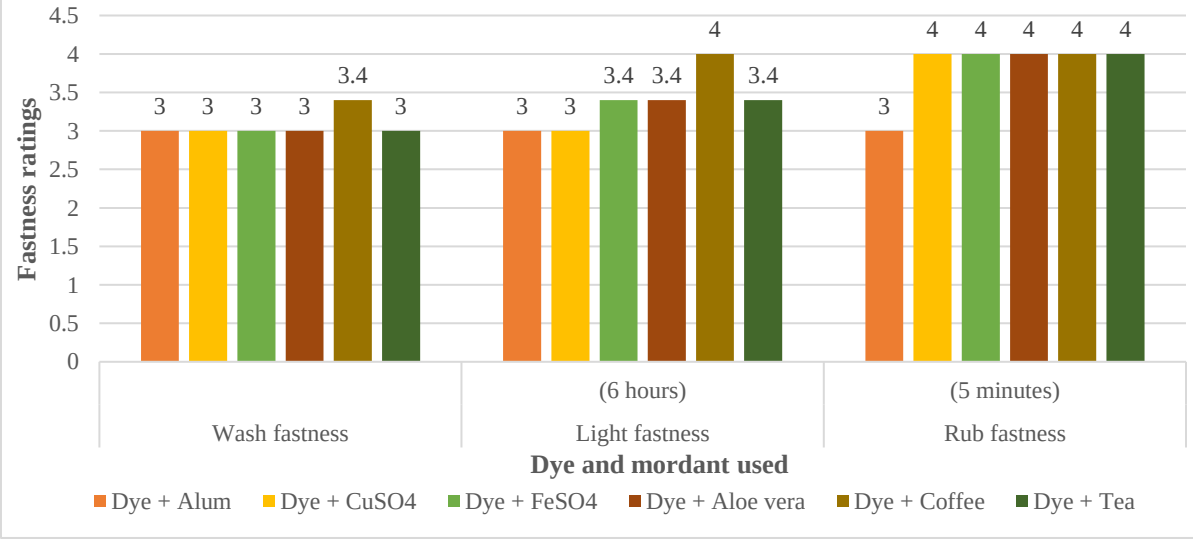


Figure 10.Bar graph showing simultaneous-mordanting colour fastness ratings of wool fibres dyed with Clitoria ternatea dye

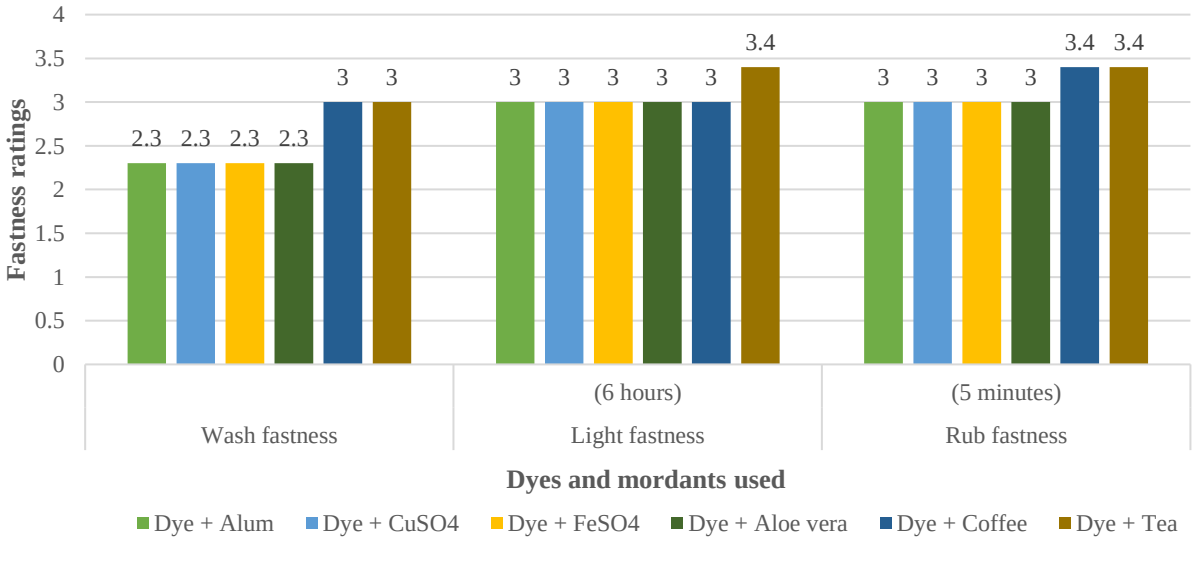


Figure 11. Bar graph showing Pre-mordanting colour fastness ratings of wool fibres dyed with Amaranthus cruentus dye

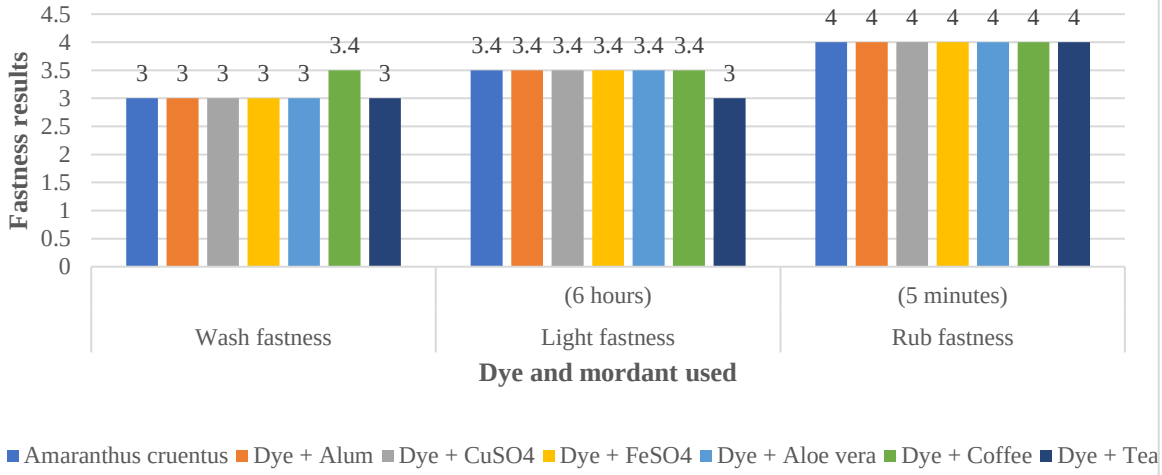


Figure 12. Bar graph showing Post-mordanting colour fastness ratings of wool fibres dyed with Amaranthus cruentus dye

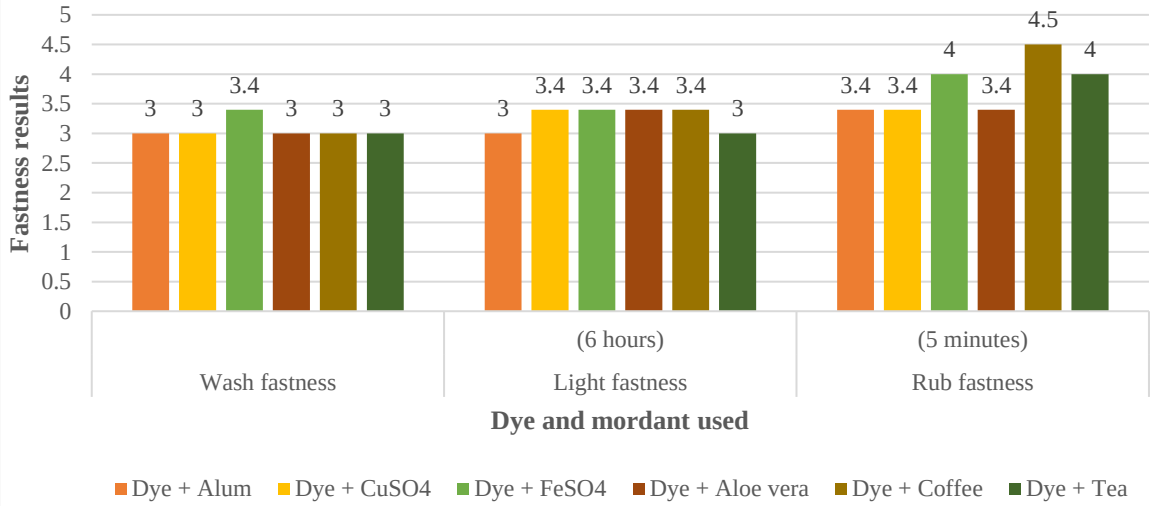
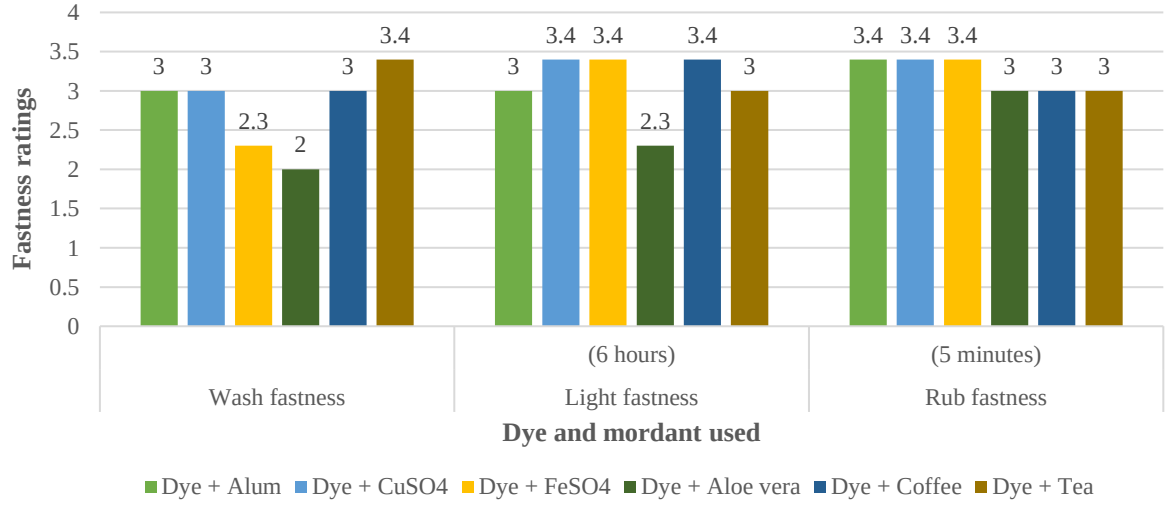


Figure 13. Bar graph showing Simultaneous-mordanting colour fastness ratings of wool fibres dyed with Amaranthus cruentus dye



4.6 Staining of plant sections

4.6.1 Optimum staining time for sections

The monocot and dicot stem were stained for 5, 10, 15 and 20 minutes. *Clitoria ternatea* dye showed optimum staining after 15 minutes. *Amaranthus cruentus* dye showed proper staining after 10 minutes. Staining the sections for 20 minutes led to overstaining.

4.6 2 Effect of natural dyes on monocot stem sections

The monocot stem section stained with *Clitoria ternatea* flower dye showed pinkish-blue staining for whole section under bright field microscope. Epidermis tissue showed light pinkish staining whereas hypodermis, vascular bundles, and ground tissue showed dark blue colour. Sclerenchyma tissue did not show any stain uptake. The monocot stem section was also stained with toluidine blue dye as a control stain which showed bright blue staining for epidermis, vascular bundles and sclerenchyma tissue.

The unstained monocot sections showed autofluorescence of white and light blue colour under UV and Violet excitation filter. Under Violet excitation filter hypodermis, vascular tissues and ground tissues showed dark pink fluorescence, whereas epidermis, bundle sheath and sclerenchyma showed greenish blue fluorescence. Under UV excitation filter epidermis, vascular tissues, and ground tissue fluoresced in bright red and sclerenchyma fluoresced in light blue colour**. (Plate 18)**

The stem section stained with *Amaranthus cruentus* leaf dye stained the epidermis, hypodermis and vascular tissue in red and sclerenchyma tissue in light pink under bright field microscope. The monocot stem section was also stained with safranin dye as a control stain which showed bright pink staining for epidermis, hypodermis, vascular bundles, sclerenchyma and ground tissue.

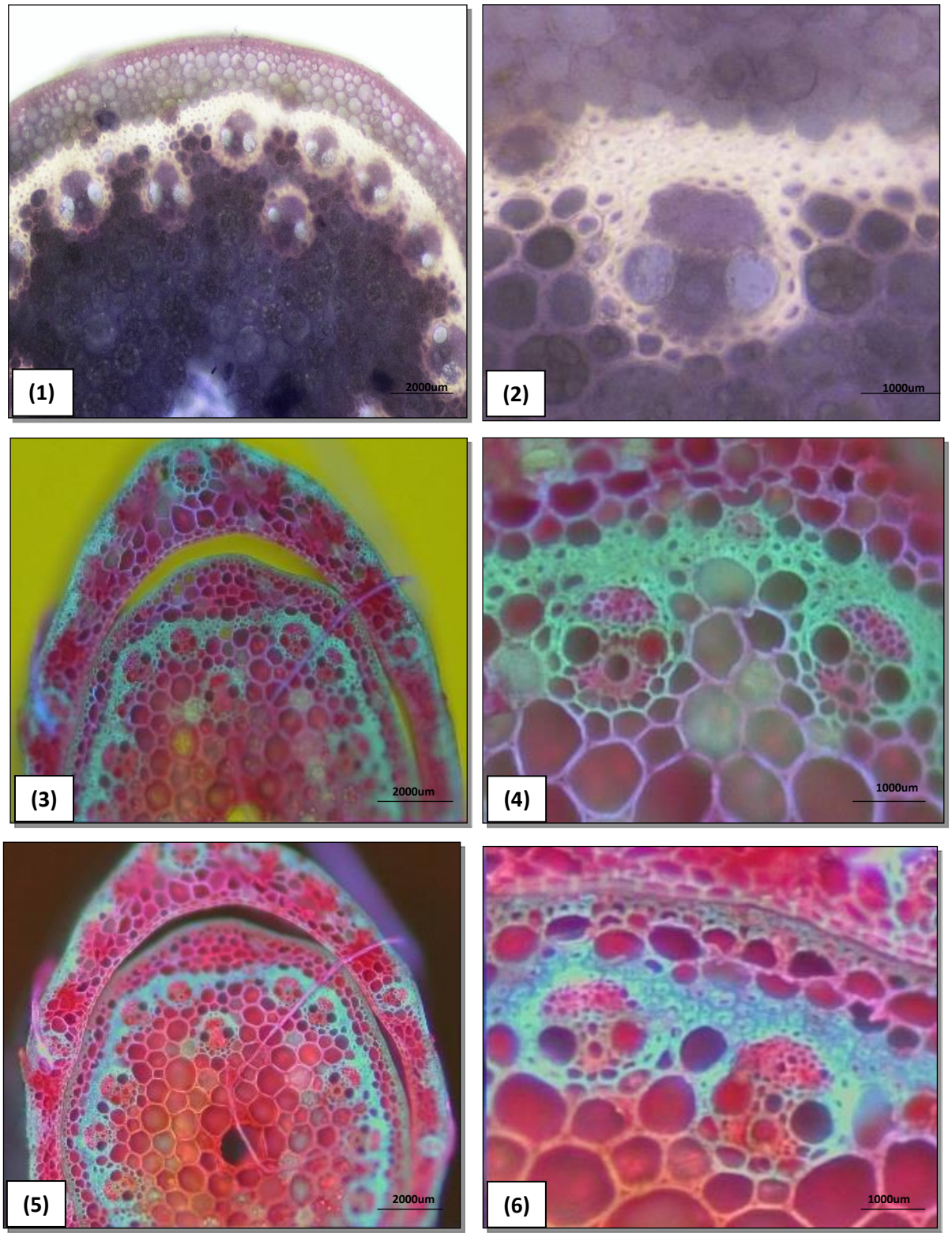


Plate 18. Bright field and fluorescence images of Monocot stem stained with *Clitoria ternatea* dye: **(1)**. Monocot stem stained with *Clitoria ternatea* dye, **(2)** Vascular bundle, **(3)** Section stained with *Clitoria ternatea* dye under Violet filter 380-420nm**, (4)** Vascular bundle, **(5)** Section stained with *Clitoria ternatea* dye under UV filter 330-380nm, **(6)** Vascular bundle.

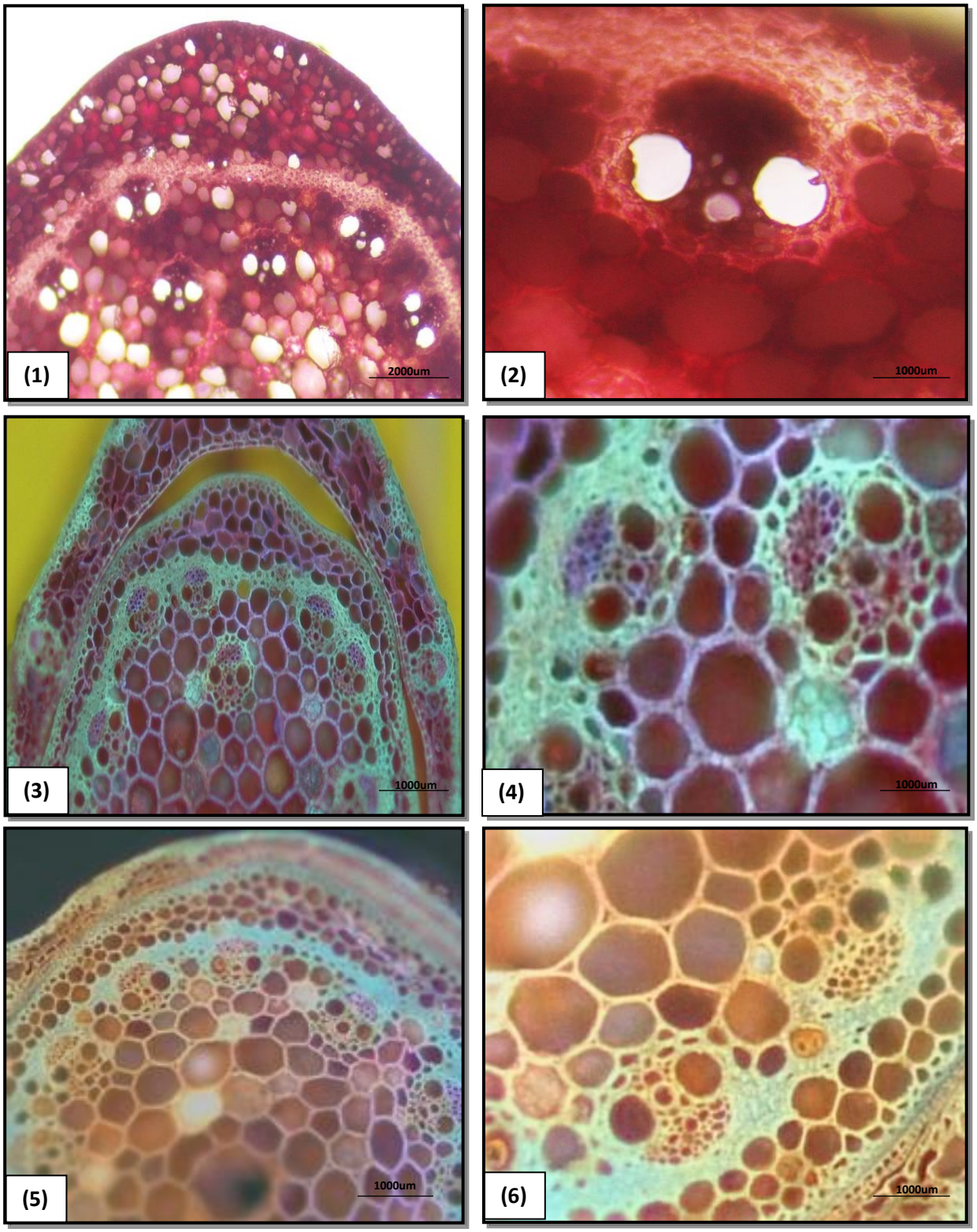


Plate 19. Bright field and fluorescence images of monocot stem stained with *Amaranthus cruentus* dye: **(1)** Monocot stem stained with *Amaranthus cruentus* dye, **(2)** vascular bundle, **(3)** Section stained with *Amaranthus cruentus* dye under Violet filter 380-420nm, **(4)** Vascular bundles**, (5)** Section stained with *Amaranthus cruentus* dye under UV filter 330-380nm, **(6)** Vascular bundles.

Under fluorescence microscope stain was showing pinkish-red colour fluorescence for epidermis, vascular tissue and ground tissue with violet excitation filter. UV-excitation filter showed yellowish fluorescence for epidermis, vascular tissue and ground tissue. Sclerenchyma cells surrounding vascular bundles showed bluish green fluorescence under both the excitation filter.

4.6 3 Effect of natural dyes on dicot stem sections

The dicot stem section stained with *Clitoria ternatea* flower dye showed purple colour staining under bright field microscope. Epidermis, trichome, chlorenchyma, vascular bundles and ground tissues showed purplish-violet colour staining. Xylem showed slight yellow colour staining. The dicot stem section was also stained with toluidine blue dye as a control stain which showed bright blue staining for epidermis, trichome, vascular bundles, sclerenchyma and ground tissues.

Under fluorescence microscope with violet excitation filter epidermis fluoresced bluish-white in colour whereas vascular bundles and ground tissue fluoresced pink. Under UV excitation filter it showed bright pink fluorescence. **(Plate 20)**

The *Amaranthus cruentus* dye showed yellowish-orange and red color staining for dicot stem section under bright field microscope. Epidermis stained in dark red colour. Xylem, phloem, vascular bundles, and trichome stained yellow orange in colour and ground tissue showed bright yellow colour. The dicot stem section was also stained with safranin dye as a control stain which showed bright pink staining for epidermis, hypodermis, vascular bundles and ground tissue.

Under violet excitation filter epidermis, ground tissue and vascular bundles showed blue and brownish fluorescence. Vascular bundles and epidermis fluoresced pinkish blue in colour under UV excitation filter. **(Plate 21)**

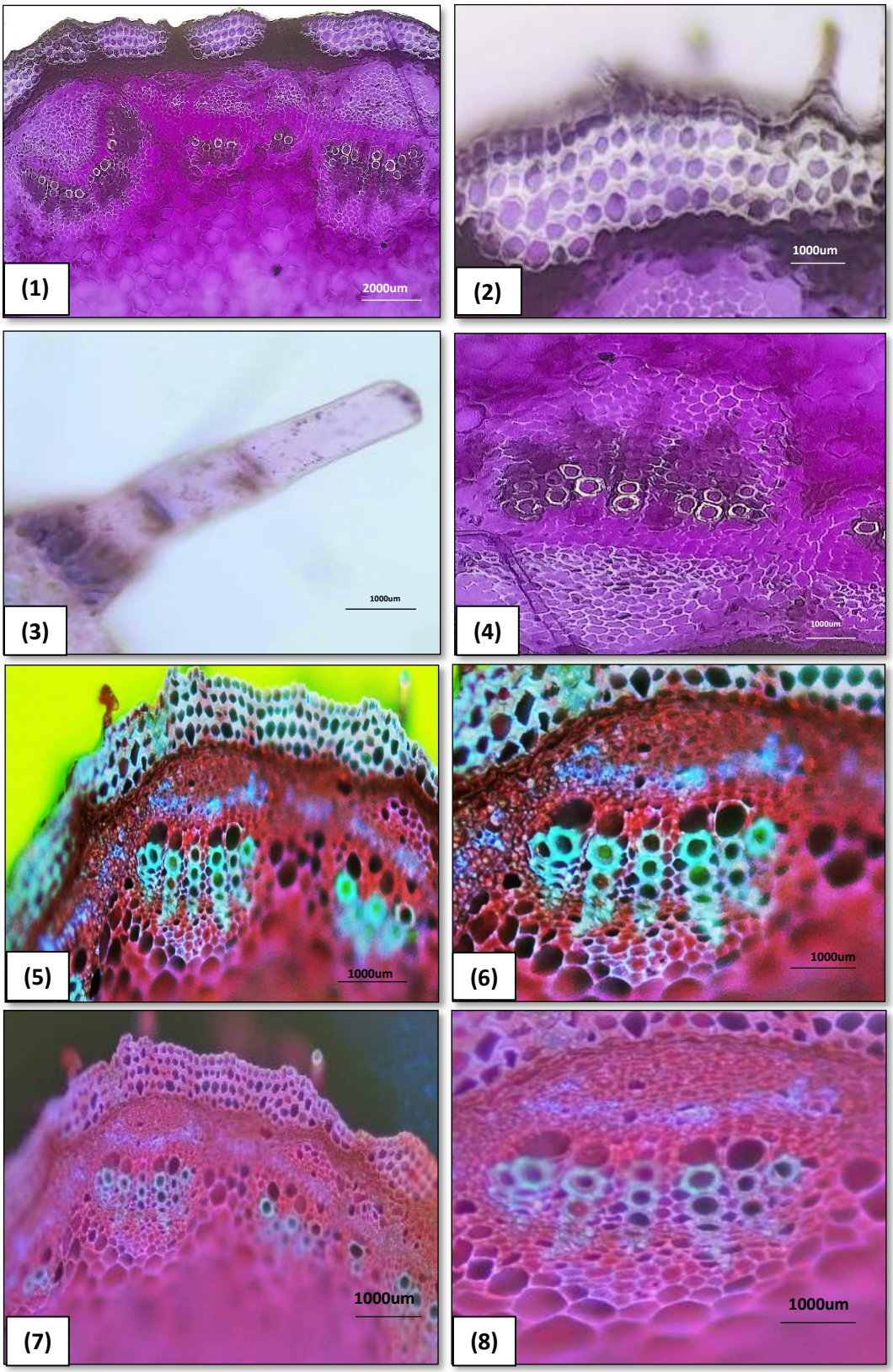


Plate 20. Bright field and fluorescence images of dicot stem stained with *Clitoria ternatea* dye: **(1)**. Dicot stem stained with *Clitoria ternatea* dye, **(2)** Epidermis layer, **(3)** Trichome, **(4)** vascular bundle, **(5-6)** Section stained with *Clitoria ternatea* dye under Violet filter 380-420nm, **(7-8)** Section stained with *Clitoria ternatea* dye under UV filter 330-380nm.

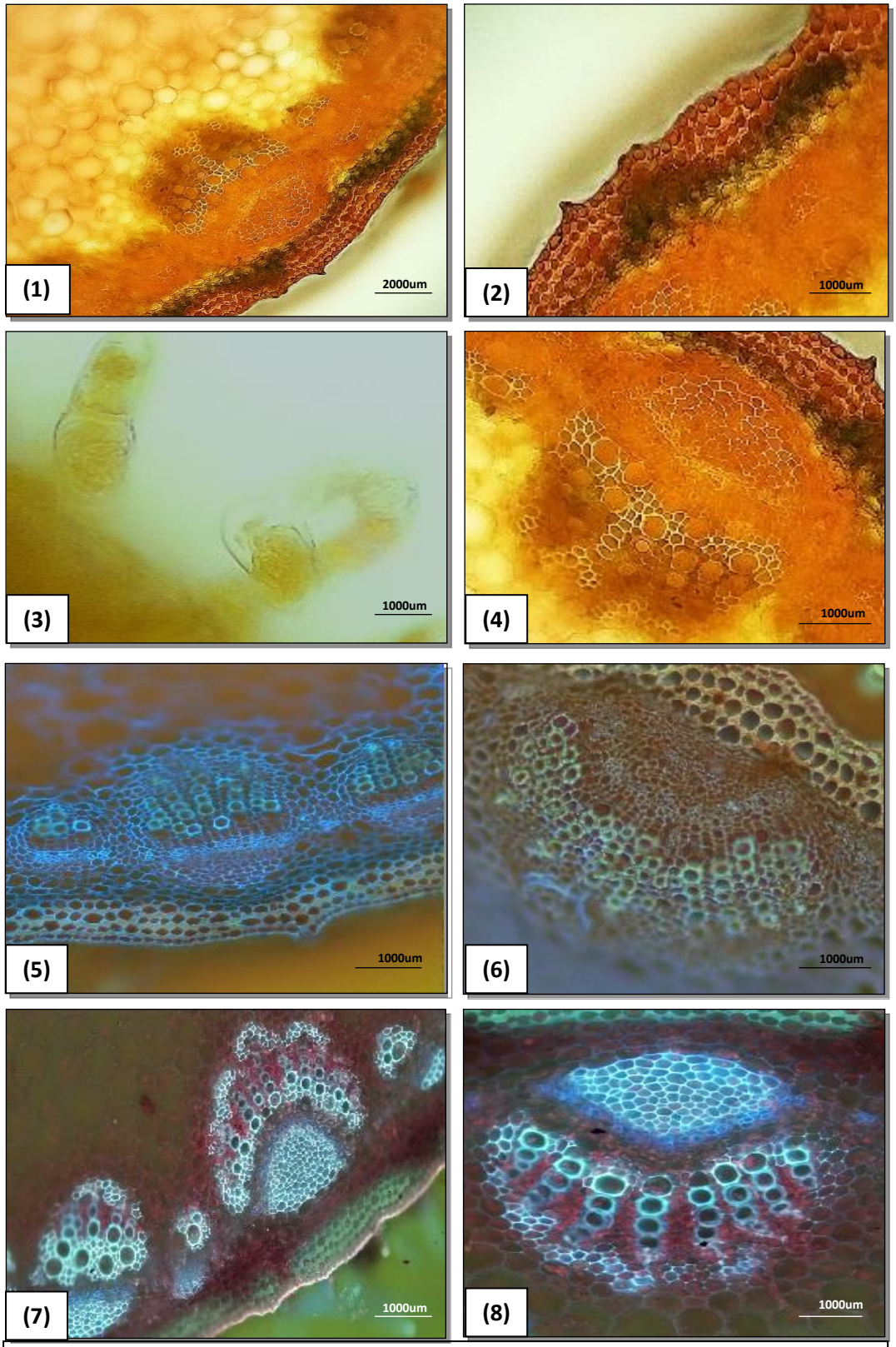


Plate 21. Bright field and fluorescence images of dicot stem stained with *Amaranthus cruentus* dye: **(1)** Dicot stem stained with *Amaranthus cruentus* dye, **(2)** Epidermis layer, **(3)** Trichome, **(4)** vascular bundle, **(5-6)** Section stained with *Amaranthus cruentus* dye under Violet filter 380-420nm, **(7-8)** Section stained with *Amaranthus cruentus* dye under UV filter 330-380nm.

4.7 Phytochemical analysis of extracted dye samples

The *Clitoria ternatea* dye extracted in water, ethanol and methanol showed presence of alkaloids, saponins, phenols, flavonoids, carbohydrate, protein and coumarins. Dye extract showed absence of tannins in all 3 solvent extracts. Alkaline reagent test showed absence of phenols in water and methanol extract while they showed presence in ethanol extract. Similarly, Biuret test showed absence of protein in all 3 solvent extracts. [**(+)** = Present; **(-)** = Absent] **(Plate 22)**

Table 18. Qualitative phytochemical analysis of *Clitoria ternatea* dye extract.

Phytochemical tests	**Water extract**	**Ethanol extract**	**Methanol extract**
1. Test for Alkaloids			
Hager's test	+	+	+
Wagner's test	+	+	+
2. Test for Tannins			
Ferric chloride test	–	–	–
3. Test for Saponin			
Foam test	+	+	+
4. Test for phenol and flavonoids			
Ferric chloride test	+	+	+
Alkaline reagent test	–	+	–
Lead acetate test	+	+	+
5.Test for Carbohydrates Benedict's test	+	+	+
6. Test for protein			
Biuret test	–	–	–
Ninhydrin test	+	+	+
7. Test for Coumarin			
NaOH test	+	+	+

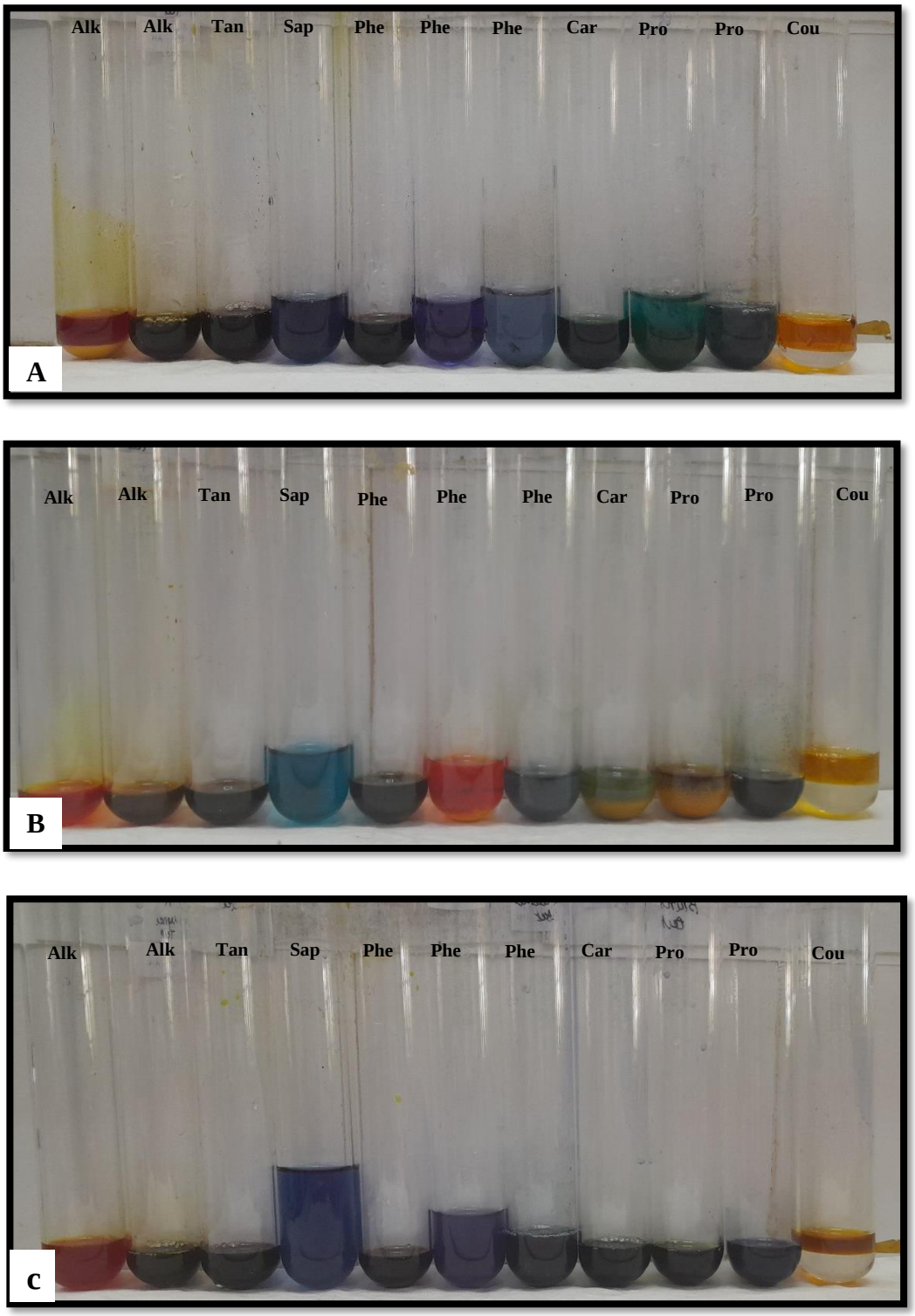


Plate 22. Qualitative phytochemical analysis of *Clitoria ternatea* flower Extract: (A)Water extract, (B) Ethanol extract, (C) Methanol extract.

The *Amaranthus cruentus* dye extract showed presence of alkaloids, tannins, saponins, phenols, flavonoids, carbohydrates, protein and coumarin in all 3 dye extracts. Ferric chloride test and biuret test showed absence of phenols and proteins in all 3 dye extracts; alkaline reagents test showed presence in all 3 extracts; and lead acetate showed absence of phenols in only methanol extract. Ninhydrin test showed presence of protein in water and methanol extracts. [**(+)** = Present; **(-)** = Absent] **(Plate 22)**

Table 19 Qualitative phytochemical analysis of *Amaranthus cruentus* dye extract

Phytochemical tests	**Water extract**	**Ethanol extract**	**Methanol extract**
1. Test for Alkaloids			
Hager's test	+	+	+
Wagner's test	+	+	+
2. Test for Tannins			
Ferric chloride test	+	+	+
3. Test for Saponin			
Foam test	+	+	+
4. Test for phenol and flavonoids			
Ferric chloride test	–	–	–
Alkaline reagent test	+	+	+
Lead acetate test	+	+	–
5. Test for Carbohydrates			
Benedict's test	+	+	+
6. Test for protein			
Biuret test	–	–	–
Ninhydrin test	+	–	+
7. Test for Coumarin			
NaOH test	+	+	+

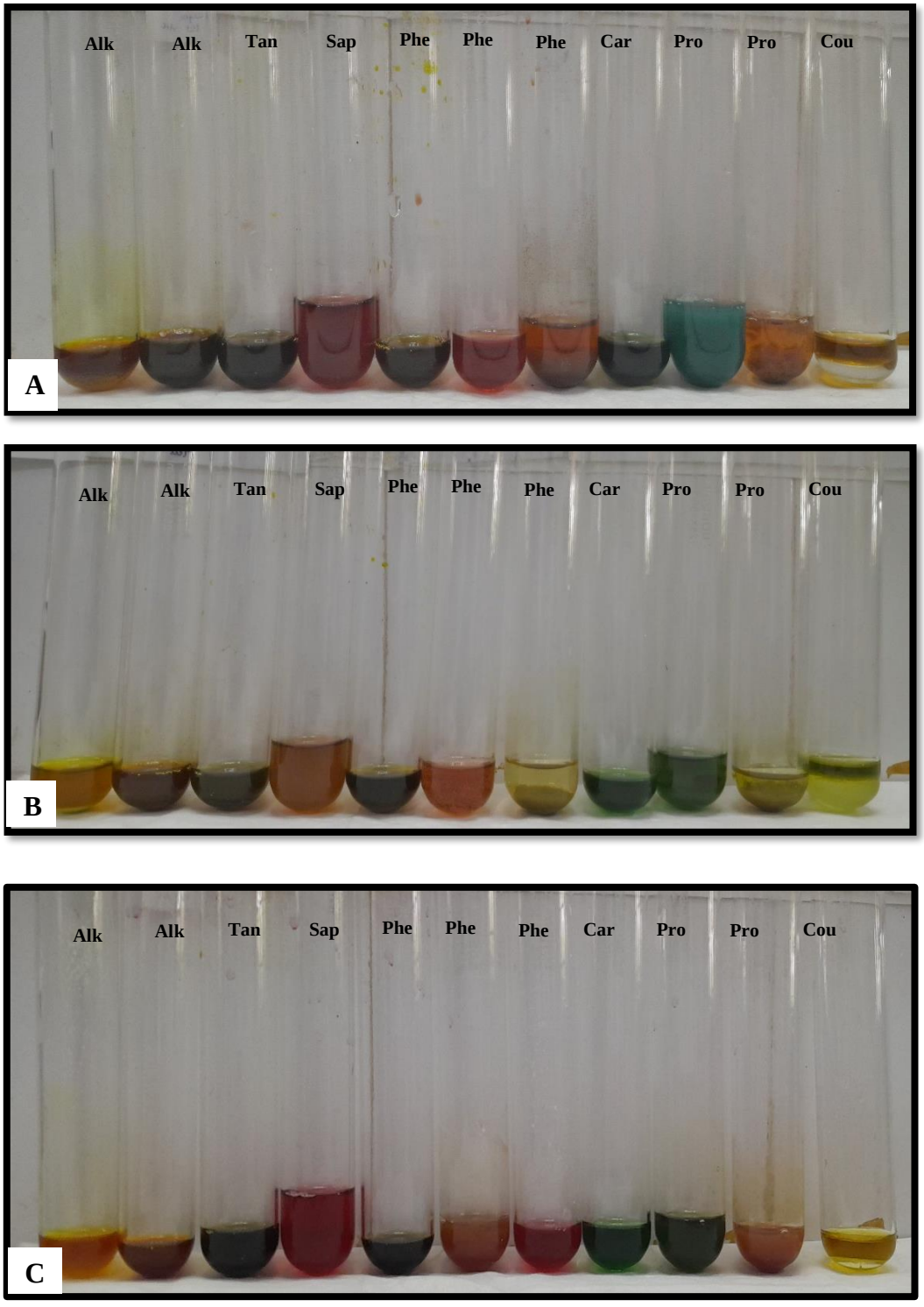


Plate 23. Qualitative phytochemical analysis of *Amaranthus cruentus* leaves Extract: (A)Water extract, (B)Ethanol extract, (C)Methanol extract.

4.8 Characterisation of natural dyes

4.8 1 Separation of pigments by Thin Layer Chromatography (TLC)

The extract of selected dye was subjected to TLC for separation of pigments. For separation of anthocyanin from *Clitoria ternatea* leaf extract the solvent system used was: - hexane: ethyl acetate (3:1). For separation of pigments from *Amaranthus cruentus* leaves extract, petroleum ether: acetone (7:3) was used as the solvent system and the plates were visualised under visible light and long UV using UV trans-illuminator.

4.8.2 Separation of pigments

The aqueous extract of *Clitoria ternatea* dye showed single blue colour spot under UV and visible light with Rf value 0.53. The *Amaranthus cruentus* aqueous extract showed 2 spots: pink and yellow, with Rf value 0.66 and 0.46 respectively.

Table 20. TLC results of aqueous plant extracts for selected plant species

Plant Name	No. of spot	Rf values	Colour of spot	
			Under Visible light	Under UV light
Clitoria ternatea	1	0.53	Blue	Blue
Amaranthus cruentus	2	0.66	Pink	-
		0.46	Yellow	-

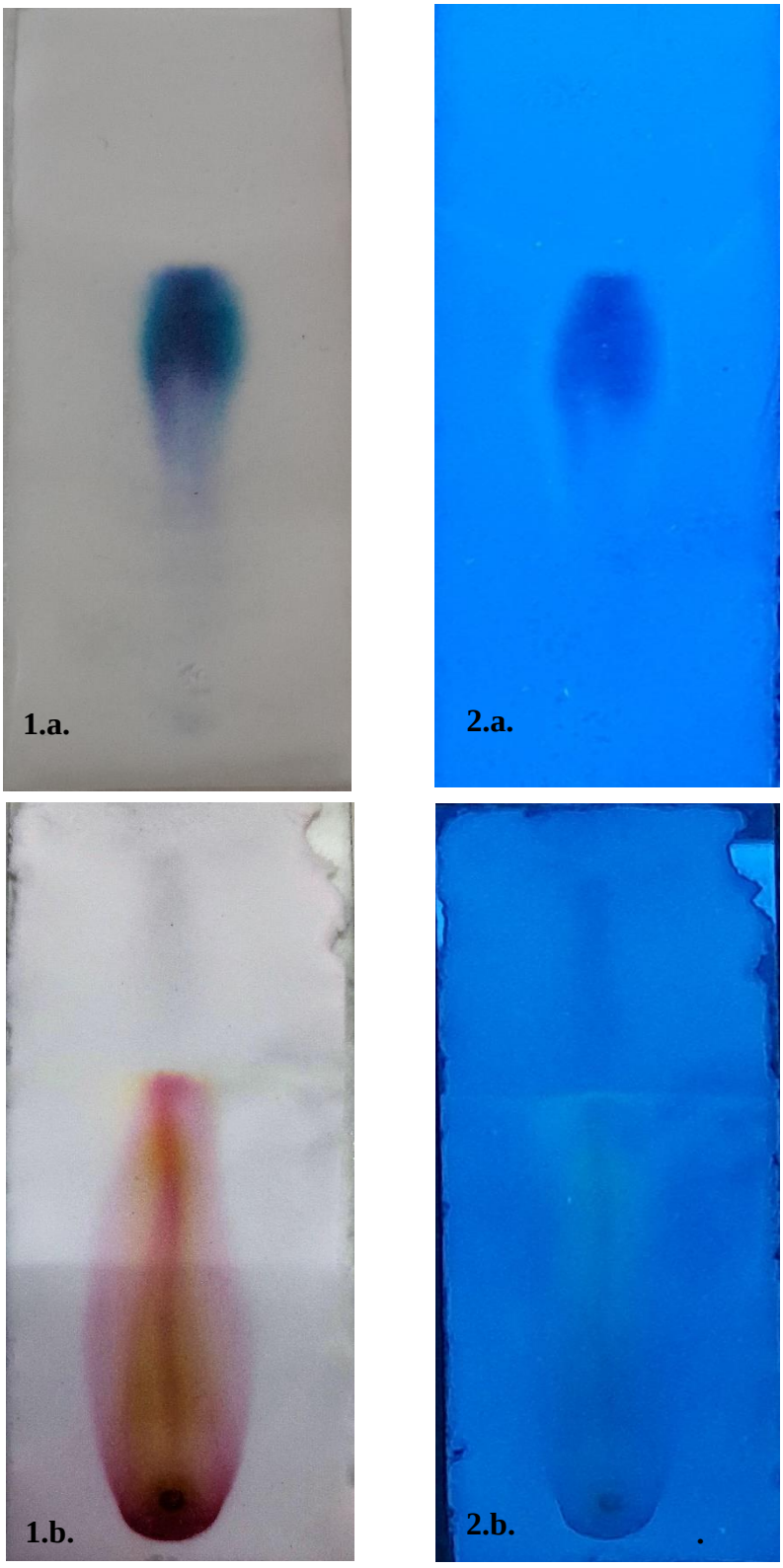


Plate 24. TLC Plate showing separation of pigments:

(1) Under visible light, (2) Under long UV
(1.a). Aqueous extract of *Clitoria ternatea*.
(1.b.) Aqueous extract of *Amaranthus cruentus*

4.8.2 UV-Visible spectroscopic analysis

The extracts of selected plant dyes were subjected to UV-Visible spectroscopy analysis at 200-800nm range. The water extract of *Clitoria ternatea* dye showed 4 absorption peaks at 266, 290, 573 and 618nm. **(Fig.14)** The methanolic extract of the dye also showed similar peaks at 265, 294, 574 and 619. **(Fig.15)**

The water extract of *Amaranthus cruentus* dye showed only 1 peak at 533nm and methanolic extract showed 8 absorption peaks at 231, 239, 247, 268, 393, 533, 604 and 633nm (Fig. 16-17)

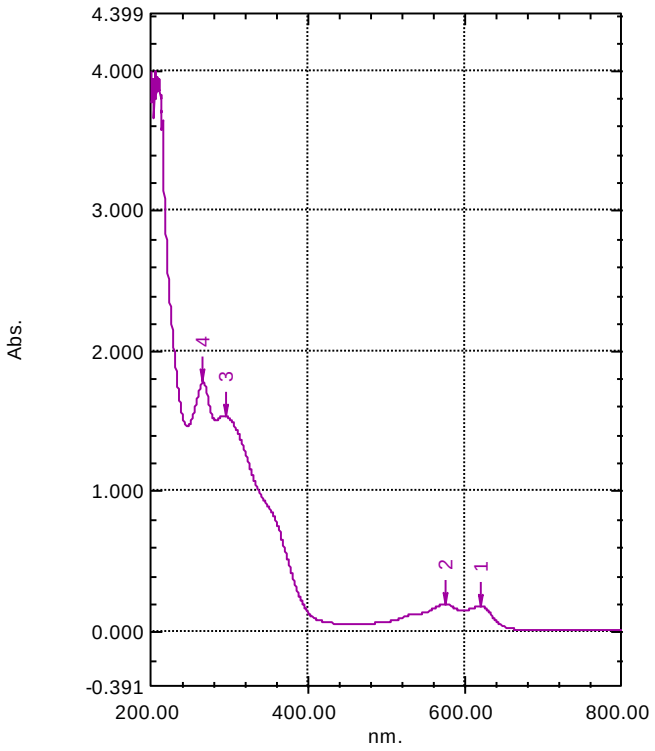


Figure 14. UV-Vis spectrum of *Clitoria ternatea* aqueous extract

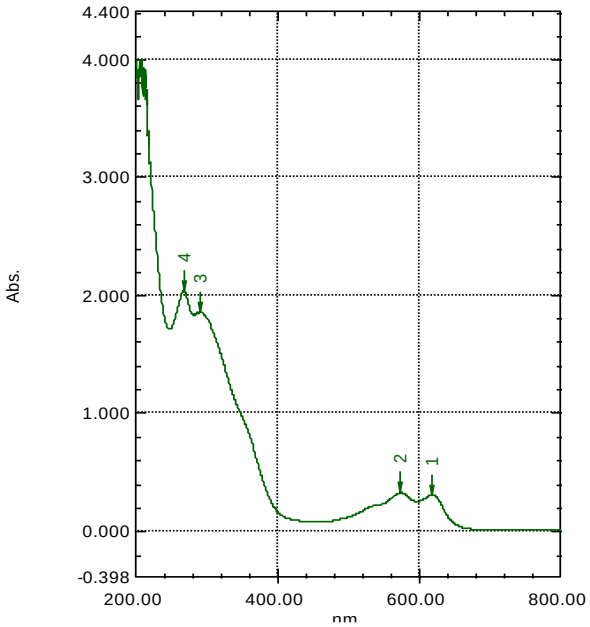


Figure 15. UV-Vis spectrum of *Clitoria ternatea* methanol extract

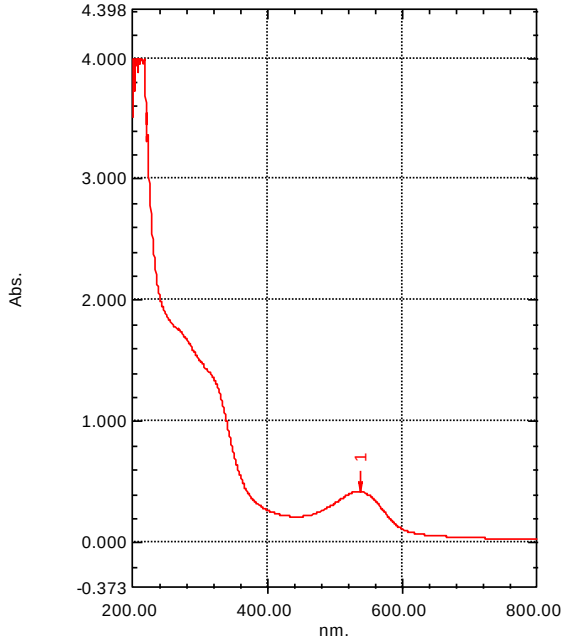


Figure 16 UV-Vis spectrum of *Amaranthus cruentus* aqueous extract

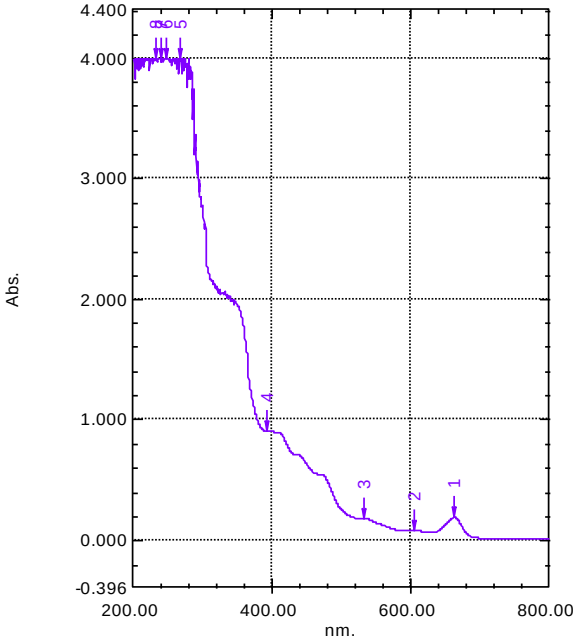


Figure 17. UV-Vis spectrum of methanol extract

DISCUSSIONS

Natural dye extraction

In the present study two plants were selected for extraction of dyes namely *Clitoria ternatea* and *Amaranthus cruentus*. Plants parts like flowers and leaves were used for dye extraction. Selected plants are available in all season and have potential to be used as a good source for yielding natural dyes. Natural dyes can be extracted from different parts of plants. Leaves are considered as a promising source for yielding natural dye (Singh and Srivastava, 2017).

Clitoria ternatea dye was extracted at 70˚C and *Amaranthus cruentus* dye was extracted at 60˚C for 60 min. Extraction temperature was selected based on the stability of primary colour component of selected plants species. Anthocyanin is a pigment which gives a blue colour dye from *Clitoria ternatea* (Marpaung and paramputri, 2023) and red colour dye obtained from *Amaranthus cruentus* is due to the presence of betalains (Bartosz and Bartosz, 2021). Extraction temperature less than 70˚C and 60˚C for *Clitoria ternatea a*nd *Amaranthus cruentus* respectively might results in low colour component. Similarly, high extraction temperature will degrade the colour component of the dyes. Both the pigments are highly susceptible to temperature (Michael *et al*., 2019, Delgado *et al*., 2000).

Dyeing of cotton and wool fibres with natural dyes

The different shades of colour ranging from moderate to strong and mixed shades of blue, purple, violet, pink, red, yellow, brown, green, olive, grey and blackish grey colour were observed on cotton and wool fibres dyed with *Clitoria ternatea* flower dye and *Amaranthus cruentus* leaf dye. The flowers and leaves are rich in pigments and

phytochemicals which were responsible for imparting colour. Anthocyanins gave blue, violet and purple colour shades which are produced by *Clitoria* ternatea flower dye (Hock *et al*., 2017) *Amaranthus cruentus* gave shades of pink, red, yellow and green on fabrics, which was due to the presence of betalains, betaxanthins and chlorophyll pigments in leaves (Maria, 2018).

Dyeing with mordant produced different shades on the fabrics, so mordant can be used to obtain different shades with a single dye. The method of mordanting also has effect on the shade produced and fastness property. Alum is considered as brightening mordant and copper sulphate and ferrous sulphate are dulling mordants (Kulkarni *et al*.,2012). Natural fibres like cotton lack affinity for natural dyes uptake and thus mordants are necessary for their dyeing (Arora *et al*., 2017). Coffee and tea were used as mordant as they contain tannin, which is utilised as mordanting substance since ancient time (Yoo and Kim, 2005). *Aloe vera* was also used as natural mordant as it acts as a fixative and also creates a strong bond between the plant dye and fibre (Ibrahim *et al*., 2017).

Fastness test of the dyed cotton and wool fibres

The cotton and wool fibres dyed with *Clitoria ternatea* dye and *Amaranthus cruentus* dye showed better wash, rub and light fastness property when dyed with pre-mordanting technique. Mordants improved the fastness property of the dyed fabrics. Alum and copper sulphate gave good wash fastness result over ferrous sulphate. Chemical mordants showed good fastness results only with pre-mordanting technique, whereas bio-mordants exhibited good fastness properties in pre- and simultaneous-mordanting methods. Tea and coffee gave better wash fastness property compared to *Aloe vera* which showed poor fastness on both cotton and wool fibres. Rub and light fastness results were good to moderate, for all the mordants in all 3 mordanting methods.

Cotton and wool dyed with *Amaranthus cruentus* dye with all 3 chemical mordants showed good wash fastness property only in pre-mordanting; and moderate to good light and rub fastness properties, whereas, dye with tea and coffee mordant showed moderate to good wash, rub and light fastness properties in pre- and simultaneous-mordanting.

Cotton and wool dyed with *Clitoria ternatea* dye with all 3 chemical mordants showed good wash fastness rating with pre-mordanting, but very poor fastness when dyed with post- and simultaneous-mordanting. Tea and coffee showed good mordanting properties resisting all 3-fastness test with slight colour change.

. **Natural dye as biological stain for plant tissues**

The dyes obtained from *Amaranthus cruentus* and *Clitoria ternatea* were used to stain monocot and dicot plant sections. This study indicates that these dyes can be effectively used as stain for plant tissues staining. Deepali *et al*., (2014) used aqueous extracts from Henna leaves, Madder stem, and flowers of hibiscus for histological and fungal staining. *Clitoria ternatea* dye showed purplish violet-blue staining for monocot and dicot stem and showed prominent staining on vascular bundles, epidermis, and ground tissues. Bright pink and red fluorescence were observed under Violet and UV excitation filter. *Amaranthus cruentus dye* showed red staining for monocot and bright yellow-orange staining for dicot stem with epidermis staining in red and vascular bundle and ground tissue showed yellow staining. Reddish pink and yellow fluorescence were obtained under fluorescence microscope with Violet and UV excitation filter. Different colour fluorescence depends on the different chemical groups present in the dye which emits fluorescence when get excited at certain wavelength and give prominent fluorescence (Lima *et al*., 2019).

Phytochemical analysis of Natural dyes

The phytochemical analysis revealed the presence of alkaloids, tannins, saponins, phenols, flavonoids, carbohydrates, proteins and coumarins. Tannins were absent in *Clitoria ternatea* flower extract. Phenols, flavonoids and alkaloids are mostly abundant in the flower and leaf extracts of selected dyes. Different extraction solvents were used for extraction of phytoconstituents, because polar constituents like phenols and flavonoids show better extraction in polar solvents compared to other solvents. Pratibha *et al*., (2023) found the presence of alkaloids, saponins, phenols, flavonoids, carbohydrates and proteins in water and methanolic extract of *Clitoria ternatea* flower, similar result was obtained by Manjula *et al*., (2013). The phytochemical analysis of *Amaranthus cruentus* extract revealed the presence of alkaloids, tannins, saponins and flavonoids (Nana *et al*.,2012) which are consistent with the current observations.

Thin Layer Chromatography Analysis

TLC analysis was carried out for separation of pigments from the dye extract. The Rf value of 0.53 in aqueous extract of *Clitoria ternatea* was obtained with a single blue spot, which corresponds to anthocyanin. The presence of anthocyanin in blueberries was recorded by Filip *et al*., (2011). The Rf value of 0.66 and 0.46 was obtained with pink and yellow spot in *Amaranthus cruentus* which corresponds with betacyanin and betaxanthin pigments. Similar results were obtained by Matos *et al*., (2001) who separated betacyanin pigments from *Opuntia boldinghii* fruits.

UV-Visible Spectroscopic Analysis

The aqueous and methanolic extract of the plants were analysed by UV-Visible spectroscopy. Flavonoids and their derivatives showed absorption maxima at two

wavelength ranges, Band-I at 230-290nm and Band-II at 300-350nm (Saxena & Saxena, 2012). Tannins showed maximum absorbance between 350-500nm and carotenoids between 400-550nm, while peaks obtained 600-700nm corresponds to chlorophyll (Alara *et al*., 2018). Anthocyanin show different absorption bands based on structure. Bands between 550-580nm corresponds to purple quinoidal base and 600-620nm corresponds to blue anionic quinoidal base (Marpaung *et al*., 2019). Betalains show maximum absorption in the wavelength interval from 535nm to 540nm (Bartosz and Bartosz, 2021).

Both plant extracts showed absorbance peaks in the range of 230-290nm and 300-350nm which might be due to the presence of phenols and flavonoids derivates in the extract. Absorption in the range of 350 to 500nm indicates the presence of tannins. *Amaranthus cruentus* aqueous and methanolic extracts showed absorption peaks at 535nm indicating presence of betalains. Methanolic extract show absorption peaks at 268, 247 and 293nm which supports the presence of flavonoids; peaks at 393nm and 604nm indicate presence of tannins and chlorophyll. *Clitoria ternatea* methanol and aqueous extract show absorption peaks at 265nm and 295nm supports the presence of phenolic compounds and benzene derivatives; and peak at 574nm is due to the presence of anthocyanin.

CONCLUSIONS

Dyes were extracted from *Clitoria ternatea* flower and *Amaranthus cruentus* leaves with temperature 70˚C and 60˚C for 60 minutes using boiling method and were dried using rotary evaporator. The extracts were used for staining plant tissues as well as for dyeing cotton and wool fabrics. Dyeing was performed using chemical mordants viz. alum, copper sulphate and ferrous sulphate and natural mordants viz. *Aloe vera*, coffee and tea, by pre-mordanting, post-mordanting and simultaneous mordanting methods. *Clitoria ternatea* gave shades of blue, violet, purple and pink colours on cotton and wool fabrics whereas *Amaranthus cruentus* gave green, olive red pink and brown shades. The colour fastness test indicated that mordanting improved the fastness properties of dyed clothes. Dyeing cotton and wool fibres in pre-mordanting method showed better colour fastness with chemical mordant compared to post- and simultaneous-mordanting. Natural mordants showed good fastness property in pre- and simultaneous-mordanting techniques and moderate fastness property in post-mordanting, with tea and coffee demonstrating better results than *Aloe vera.* Alum mordant gave good fastness results over other chemical mordants used. Monocot and dicot stem sections were stained with the extracted natural dyes. Under bright field microscope, *Clitoria ternatea* dye stained monocot and dicot stem in purplish-violet colour and *Amaranthus cruentus* showed red and yellow staining for monocot and dicot stem, respectively. Dyes exhibited good staining in epidermal, vascular and ground tissues, when observed under bright field and fluorescence microscopy. Phytochemical, UV-Visible spectroscopy and Thin Layer Chromatographic analysis indicated the presence of alkaloids, flavonoids, phenols, tannins, anthocyanin, betacyanin and chlorophyll in the extracted dyes. The different shades obtained on cotton and silk fibres and the colour fastness properties of these dyes

indicate their potential use for dyeing textiles. The extracted dyes also show a good potential to be used as histological stains for plant tissue staining.

REFERENCES

1. Algarni, H. M., Awwad, N. S., & Samantha, A.K. (2020). Fundamentals of natural dyes and its application on textile. *Intech Open*, 4(3) https:// doi.org /10.5772 /inte chopen.89964.
2. 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*, 7(1), 35-47. http://dx.doi.org/10.4236/gsc.2017.71003.
3. Aztatzi-Rugerio, L., Granados-Balbuena, S. Y., Zainos-Cuapio, Y., Ocaranza-Sánchez, E., & Rojas-López, M. (2019). Analysis of the degradation of betanin obtained from beetroot using Fourier transform infrared spectroscopy. *Food Science and Technology*, 56(8), 3677-3686. https://doi.org/10.1007413197-019-03826-2.
4. Bechtold, Thomas, Mussak, R., Mahmud-Ali, A., Gangiberger Bech, 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.
5. Chukwu, O. C., Odu, C. E., Chukwu, D. I., Hafiz, N., Chidozie, V. N. And Onyimba, I. A. (2011). Application of extracts of henna (*Lawsonia inermis*) leaves as a counter Stain. *African journal of microbiology research*,5(21),3351-3356. http://dx.doi.org/10.5897/JMR10.418.
6. 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*, 6 (2),456-460.
7. Deepak, M. S. And Philpose, O. (2013). Use of dye extract of *Melastoma malabathricum* Linn. For plant anatomical staining. *Acta Biologica Indica*, 2(2),454-456.
8. Filip, M., Vlassa, M., Copaclu, F., & Coman, V. (2012). Identification of anthocyanins and anthocyanidins from berry fruits by chromatographic and spectroscopic techniques to establish the juice authenticity from market. *Journal of Planar Chromatography*, 25(6), 534-541.https://doi.org/10.1558/UPC.25. 2012.8.8

9. Gaude, A. A. (2022). Extraction of Natural Dyes from Selected Plants and its Potential as Textile Dye and Biological stains. *M.Sc., dissertation,* Department of Botany, Goa University.

10. Geetha. B and V. Judia (2013). Extraction of Natural Dyes from Plants. *International Journal of Chemistry and Pharmaceutical Sciences,*1(8), 502-509. www.pharmaresea rchlibrary.com/ijcps.

11. Gita, H. Zulharmita Z. And Ridho A. (2021). Utilization of Natural Dyes Substances for Histological Staining: A Review. *Asian Journal of Pharmaceutical Research and Development*, 9(1), 149-158. http://dx.doi.org/10. 22270/ajprd.v9i1.925.

12. Gulrajani, M. L. (2000). Present status of natural dyes. *Indian Journal of Fibre and Textile Research*, 2(6), 191-201.

13. Gupta, J., & Gupta, A. (2013). Phytochemical screening of *Phyllanthus emblica* (L.) using different solvents. *Journal Chemtracks*, 1(5), 117-120. http://dx.doi.org/10.13140/RG.2.2.34406.91207.

14. Harborne, J. B. (1958). Spectral methods of characterizing anthocyanins. *Biochemical Journal*, 70(1), 22-28.

15. Hock Eng Khoo, Azrina Azlan, Sou Teng Tang & See Meng Lim (2017). Anthocyanidins and anthocyanins: coloured pigments as food, pharmaceutical Ingredients, and the potential health benefits, *Food & Nutrition Research*, 61(1), 1361779-1361785. http://dx.doi.org/10.1080/16546628.2017.1361779.

16. Hong, K. H. (2018). Effects of tannin mordanting on coloring and functionalities of wool fabrics dyed with spent coffee grounds. *Fashion and Textiles*, 5(1), 33. https://doi.org/10.1186/s40691-018-0138-x.

17. Hosseinnezhad, M., Gharanjig, K., Jafari, R., & Imani, H. (2021). Green dyeing of woolen yarns with weld and madder natural dyes in the presence of biomordant. *Progress in Color, Colorants and Coatings*, 1(4), 35-45. https://doi.org/10.22034/PCCC.2021.131303

18. Korade. D., Sonawane. L. and Mahale.D., (2014). Application of aqueous plant extracts as Biological stains. *International Journal of Scientific & Engineering Research*. 5(2), 1586-1589. https://www.researchgate.net/publication/281546 702.

19. 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*, 3(2). 456-459.

20. Kumar, V. And Prabha, R. (2018). Extraction and analysis of natural dye. *Journal of Natural Products and Plant Resources*, 8(2), 32- 38, www.scholarsresearchlibrary.com

21. L. Chungkrang., Smita. B.and Aava.R, P. (2021) Natural Dyes: Extraction and Applications. *International Journal of Current Microbiology and Applied Sciences*, 10(1), 1669-1677. http://dx.doi.org/10.20546/ijcmas.2021.1001.195.

22. Lee, J., Kang, M. H., Lee, K.-B., & Lee, Y. (2013). Characterization of Natural Dyes and Traditional Korean Silk Fabric by Surface Analytical Techniques. *Materials*, 6(5), 2007-2025. https://doi.org/10.3390/ma6052007

23. Liu, J., Mouri, C., Laursen, R., Zhao, F., Zhou, Y., & Li, W. (2013). Characterization of dyes in textiles from Yingpan, Xinjiang. *Journal of Archaeological Science*, 40 (12),4444-4449.https://doi.org/10.1016/J.JAS.2013.06.034.

24. Manjula, P., Mohan, C., Sreekanth, D., Keerthi, B., & Prathibha Devi, B. (2013). Phytochemical analysis of *Clitoria ternatea* Linn., a valuable medicinal plant. *Indian Botanical Society Journal*, 92 (344),173-178. https://doi.org/10.3390/xxxxx

25. Marpaung, A. M., Andarwulan, N., Hariyadi, P., & Faridah, D. N. (2019). The difference in colour shifting of *Clitoria ternatea* L. flower extract at pH 1, 4, and 7 during storage. *Current Nutrition and Food Science*, 15(7), 694-699. https://doi.org/10.2174/1573401315666190325143827.

26. Marpaung, A. M., Andarwulan, N. And Prangdimurti, E. (2013). The optimization of anthocyanin pigment extraction from butterfly pea (*Clitoria ternatea* L.) petal using response surface methodology. *Acta Horticulturae*, 10(11), 205-211. https://doi.org/10.17660/ActaHortic.2013.1011.24

27. Mukherjee, S. And Kanakarajan, S. (2017). Extraction, optimisation and dyeing of silk yarn Using natural dye from cosmos sp. *International Journal of Development Research*, 7(7), 13865-13871. http://www.journalijdr.com.

28. Nam, C., & Xiang, C. (2019). Natural dyeing application of used coffee grounds as a potential resource. *International Journal of Fashion Design, Technology and Education*, 12(3), 335-345. https://doi.org /10.1080/17543266.2 018.153909

29. Nkechi. A, O., Ifeoma. B, E., Ifeanyi. O., Evelyn. U. And Clinton. C. (2018) Use of Extracts from Nigerian Indigenous Plants as Staining Techniques in

Bacteriology, Mycology and Histopathology. *International Journal of Plant Research*, 8(1),15-24. https://doi.org/10.5923/j.plant.20180801.03.

30. Nsangou, A., Sibiescu, D., Nkemaja, D. E., Noah, P. M. A., Betene, F. E., & Gomdjé, V. H. (2022). *Aloe vera* gel as a biomordant in the finishing of vegetable textiles: A review. *Buletinul Institutului Politehnic din Iaşi*, 68(72), 2-7. http://dx.doi.org/10.5281/zenodo.7539811.

31. Paramaputri, A. M. A. (2023). UV-visible light spectra of *Clitoria ternatea* L. Flower extract during aqueous extraction and storage. *International Food Research Journal*, 30(3), 224-230. https://doi.org/10.31491/IFRJ-2023-03.

32. Paul, R., Jayesh, M. and Naik, S. R. (1996). Natural dye: Classification, extraction and fastness, properties. *Textile Dyer and Printer*, 29(2), 16-23.

33. Paul.T 2020, Staining Cells with Extracts Prepared from Flowers of Bougainvillea X Buttiana. *Journal of Histochemistry and Cell Biology*, 29(8), 1-14. https://doi.org/10.51168/sjhrafrica.v1i12.8.

34. Renu S. And Srivastava. S. (2017). Critical review on extraction of natural dyes from leaves. *International Journal of Home Science,* 3(2), 100-103. http:// dx. doi.org/ 10.31881/TLR.2021.09.

35. Sadowska-Bartosz, I., & Bartosz, G. (2021). Biological properties and applications of betalains. *Molecules*, 26(9), 337- 340. https://doi.org/10.3390/molecules26092520

36. Salauddin M, Mia R, Haque MA, Shamim AM. Review on Extraction and Appl cation of Natural Dyes. *Textile and Leather Review*, 4(4), 218-233. http:// dx.doi.org/10.5923/j.textile.20190802.02

37. Sankaranarayanan, S., Kathiravan, I., Balasundaram, J., Shkir, M., & AlFaify, S. (2022). An analysis of the dye-sensitized solar cells fabricated with the dyes extracted from the leaves and flowers of Amaranthus cruentus. *Environmental Science and Pollution Research*, 29(44271-44281). https://doi. org/10.1007/s 11356- 022-19004-5

38. 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.

39. Shahidi, S., Khoshechin, E., Sharifi, S. D., & Mongkholrattanasit. (2022). Investigation of the effect of various natural dyes on UV protection properties and

antibacterial activity of cotton fabrics. *Journal of Natural Fibers*, 19(13), 7213-7228. https://doi.org/10.1080/15440478.2021.1944433

40. Shet Verenkar, N. G. & Sellappan, K. (2021). Evaluation of natural dyes Curcuma and Nyctanthes arbor-tristis with different mordants on plants under fluorescence microscopy. *Microscopy Research and Technique*, (8415), 902-911 https://doi.org/https://doi.org/10.1002/jem. 2.3650

41. Siddham, P., Sonali, R., & Jagtap, M. N. (2023). Phytochemical analysis and antimicrobial screening of *Clitoria ternatea* L. *Acta Scientific Microbiology*, 6(4), 4-8. https://doi.org/10.31080/ASM12023.06.1223

42. Singh, R., & Srivastava, S. (2017). A critical review on extraction of natural dyes from leaves. *International Journal of Home Science*, (1002), 100-103 www.homesciencejournal.com

43. Srivastava, A. And Singh, T. G. (2011). Utilization of *Aloe vera* for dyeing natural fabrics. *Asian Journal of Home Science*, 6(1), 1-4. https://www.scinapse .io/papers/76693247

44. Teklemedhin, T. B. And Gopalakrishnan, H. L. (2018). Environmentally friendly dyeing of silk Fabric with natural dye extracted from Cassia singueana plant. *Journal of Textile Science and Engineering*, 2-6. http://dx.doi.org/10.4172/2165-8064.S3-001

45. Tiwari. S.k., (2020). Extraction of natural dye and preparation of herbal gulal from beetroot (Beta vulgaris). *Journal of Pharmacognosy and Phytochemistry*, 9(4), 3206-3211.

46. Vankar, P. S., Shanker, R., Mahanta, D. And Tiwari, S. C. (2008). Eco-friendly sonicator Dyeing of cotton with Rubia cordifolia Linn. Using biomordant. *Dyes and Pigments*, 76(1), 207-212. http://dx.doi.org/10.1016/j.dyepig.2006.08.023.

47. Verenkar, N. G. S. And Krishnan, S. (2017). Some potential dye yielding plants from the State of Goa, *India. Indian Journal of Natural Products and Resources*, 8(4), 306-315.

48. Verenkar.N.G.S. (2021). *Screening, evaluation, characterisation of potential biological stain and textile dyes of plant origin.* Doctoral thesis in Botany, Goa University.

49. Verenkar, N.G.S. and Krishnan, S. (2017). Dyeing of cotton and silk with eco-friendly dyes Extracted from bark of mangrove species *Rhizophora mucronata*

and *Ceriops tagal*. *International Journal of ChemTech Research*, 10(12), 102-110. http://dx.doi.org/10.1016/j.indcrop.2013.04.041

50. Verenkar, N.G.S. and Krishnan, S. (2020.) Extraction of natural dye from *Mammea suriga* and evaluation of dyeing potential on silk and cotton fabrics. *Indian Journal of Fibre and Textile Research,* 45, 402-410.

51. Verenkar, N.G.S. and Krishnan, S. (2020). Evaluation of natural dyes *Curcuma longa* and *Nyctanthes arbor- tristis* with different mordants on plant tissues under Fluorescence microscopy. *Microscopy Research and Technique*, 1-10. http://dx.doi.org/10.1002/jemt.23650.

52. Viloria-Matos, A., Moreno-Alvarez, M. J., & Hidalgo-Báez, D. (2001). Isolation and identification of betacyanin from fruits of *Opuntia boldinghii*. CYTA – *Journal of Food*, 3(3), 140-143. https://doi.org/10.1080/11358120109487720

53. Yadav, S., Tiwari, K. S., Gupta, C., Kumar, M. T., Khan, A. And Sonkar, S. P., (2023). A brief Review on natural dyes, pigments: Recent advances and future perspectives. *Results in Chemistry,* 5(100733),1-17. http://dx.doi. org/10.1016/ j. rechem.2022.100733.

54. Y. Fu., Jia Shi.Si-Yi Xie., Ting-Yi Zhang., Olugbenga P. and Rotimi E. (2020). Beetroot Betalains: Perspectives on Extraction, Processing. *Journal of Agriculture and Food Chemistry and Potential Health Benefits*,6(8),11955-11669. https://doi.org/10.1021/acs.jafc.0c04241.

55. Yoo, H.-G., & Kim, S.-H. (2005). Natural dyeing using tea extract I: The effect of mordants on dyeing characteristics of coffee extract dyed silk fabric. *Journal of Fashion Business*, 9(6), 29-38. https://doi.org/10.12939/JFB.2005.9.6.029.