SCREENING, EVALUATION, CHARACTERIZATION OF POTENTIAL BIOLOGICAL STAINS AND TEXTILE DYES OF PLANT ORIGIN

A Thesis Submitted to
Goa University for the Award of the Degree of
DOCTOR OF PHILOSOPHY
In
BOTANY

By
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CERTIFICATE

As required by the University Ordinance OA-19.3 (ix), this is to certify that the thesis entitled **"Screening, Evaluation, Characterization of Potential Biological Stains and Textile Dyes of Plant Origin"** submitted by **Miss Nikita Gokuldas Shet Verenkar** for the award of degree of Doctor of Philosophy in Botany, is based on her original work carried out by her during the period of study, under my supervision.

The thesis or any part thereof has not been previously submitted for any other degree or diploma in any other University or Institute.

Place: Goa **(S. Krishnan)**
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As required by the University Ordinance OA-19.3 (ix), I state that the present thesis **"Screening, Evaluation, Characterization of Potential Biological Stains and Textile Dyes of Plant Origin"** is my contribution and the same has not been submitted on any occasion for any other degree or diploma of this University or any other University/ Institute.

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ABBREVIATIONS

BR	Biosphere reserve
cm/cm^{-1}	Centimeter
CIELAB	Colour space specified by the international commission on illumination
$CuSO_4$	Copper sulphate
°C	Degree centigrade
DNA	Deoxyribonucleic acid
E	East
$FeSO_4$	Ferrous sulphate
FA	Ferulic acid
FT-IR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography/Mass spectrometry
g	Grams
g/l	Grams per litre
g/ml	Grams per millilitre
h	Hours
HCl	Hydrochloric acid
HTHP	High temperature high pressure
HPLC	High Performance Liquid Chromatography
ISO	Indian Standard Organisation
ISCC-NBS	Indian Society Colour Council-National Bureau of Standards
kg	Kilogram
Km	Kilometer
KGy	KiloGrays
l	Litre

LC-MS	Liquid Chromatography-Mass Spectrometry
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
≤	Less than or equal to
MLR/M:L	Material to liquor ratio
µl	Microlitre
mL	Milliliter
mm	Millimeter
min	Minutes
nm	Nanometer
HNO_3	Nitric acid
N	Normal
NIST	National Institute of Standards and Technology
OD	Optical density
PMS	Pantone matching system
%	Percent
PVA	Polyvinyl alcohol
KBr	Potassium bromide
$K_2Cr_2O_7$	Potassium dichromate
pH	Potential of hydrogen
Rf	Retention factor
rpm	Revolutions per minute
SEM	Scanning Electron Microscope
Na_2CO_3	Sodium carbonate
NaOH	Sodium hydroxide
SD	Standard deviation
H_2SO_4	Sulphuric acid

TLC	Thin Layer Chromatography
UV	Ultra violet
w/v	Weight by volume

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1. INTRODUCTION

1.1. Natural Dye - An Introduction

Colours have always fascinated the human race since ancient times. Our nature has gifted us with wealth of plants which harbours several economically useful plant resources, which yields their colour for dyeing purpose. The term dye is derived from English word “daeg” meaning “colour” (Forbes, 1965). The dyes are coloured substances which consist of organic matter which is soluble in water whereas pigments are composed of inorganic matter which is insoluble in water. The dyes are obtained from different sources like plants, animals, insects, minerals, lichens etc.

Plants are considered as potential source of dye. Natural colour is produced from various plant parts like leaves, stems, twigs, flowers, barks, roots, outer skins, husks, heartwoods, berries, seeds etc. However, not all the colours can be used as dyes. A dye molecule contains auxochromes and chromophores. The chromophores are unsaturated groups and it makes the compound coloured (Vanker, 2000). The auxochrome when attached to chromophore helps in imparting brighter colour to the substrate (Krishnamurthy, 1999).

1.2. History of natural dyes

The natural colourants were available to people during Greco-Roman periods. Henna, one of the oldest dyes was used even before 2500 B.C. (Gulrajani, 2000). By the 4th century A.D., dyes such as Madder, Brazilwood and Indigo were known. Indigo dye has been mentioned in Sanskrit writings more than 4000 years ago (Siva, 2007). Until the nineteenth century, all dyes available to man came from natural sources, mainly plant extracts and few from animal products (Chukwu, 2011). But the invention of synthetic dyes in 1856 by the English chemist William Henry Perkin (Travis, 2007) replaced the natural dyes with synthetic dyes to a greater extent. Perkin was considered to be the father of the synthetic dye and was famed for his creation of "mauveine" in 1856, the world's first synthetic dye.

These synthetic dyes produced vibrant colours and showed good fastness property (Singh and Singh, 2002). 10,000,000 tons per annum was the estimated use of synthetic dyes (Saravanan and Chandramohan, 2011). The extensive use of dyes gave rise to large amounts of waste thereby creating health hazards (Lekshmi and Ravi, 2013). However, the research also proved that these synthetic dyes release harmful chemicals which are allergic to human health. Hence, the natural dyes slowly started gaining its lost importance. Germany was the first country to ban synthetic dyes in 1996 (Narayanayyar and Sivasubramanian, 2016).

1.3. Natural dyes used in histochemistry

In anatomical sections, cellular structures are not significantly different to one another and hence use of dyes is necessary to display the intra or extracellular elements. The first use of natural dye “saffron” from *Crocus sativus* to stain histological structures was credited to Antonia Leeuwenhoek (1714-1719). H F link (1807) used iron sulphate for determining tannic acid in leaves. M Raspail (1825) used iodine for microchemical detection of starch. *Haematoxylin* is a natural substance from logwood tree with useful properties and a long history in histo-pathology. It was first used by Wilhelm Von Waldeyer in 1863. It is especially used in Erhlich’s haemotoxylin and eosin technique (Avwioro, 2010) for the demonstration of general tissue structures where it stains tissue in shades of blue, pink and red enabling recognition of malignant and non-malignant cells as well as several intracellular and extracellular substances necessary for diagnosis of disease in tissues and cells. It is also used in the demonstration of certain parasites, lipids, carbohydrates, nucleic acids, metals, connective tissue fibres and in immune histochemistry (Avwioro, 2011). *Haematoxylin* is a weak dye, and the staining solutions are based on hematin, which is its oxidized form.

Before the era of *Haematoxylin*, the natural dyes like carmine and saffron was used for staining tissues (Kuhlmann, 1849). Carmine is obtained from the female insect *Dactylopius coccus* cacti in which active colouring agent is carminic acid. Pelletier and Caventou obtained carminic acid from cochineal which dissolved in water producing crimson red tint (Wisniak, 2013). The production of trinito cocussic acid from carmic acid by Warren de la Rue (1874) has gained attention among the workers (Perkin and Everest,

1918). But the real credit for discovering the process of tissue staining with carmine was given to T Hartig (1854).

In 1814 Chevreul isolated morin from old fustic, quercitrin from quercitron bark (1831), luteolin from weld (1833), fustin from fustic and ellagic acid from gall-nuts.

In 1826 Robiquet and Colin isolated alizarin from madder. In 1832 Neesvan Esenbeck obtained catechin from catechu. Among all these dyes other colouring matters were also discovered like berberin (1826), gentisin (1827) and rutin (1842) (Perkin and Everest, 1918).

Schunk (1851) isolated rubian from madder plant, the amorphous-transparent hard mass which was converted into glucose, alizarin. His study also revealed that in addition to alizarin the root of madder also contains purpurin which is an anthraquinone derivative (Farrar, 1977).

In 1924 the scientist R Feulgen, formulated the stain for detecting the DNA in the nucleus. In the course of further developments of microscopical staining, a significant step forward was obtained with the introduction of enzyme histochemistry, when G Gomori (1939) and H Takamatsu (1939) described independently the methods for the demonstration of phosphatases in tissue sections (Kuhlmann, 2008).

1.4. Dyeing with natural dyes

The exact period of the commencement of the art of dyeing in the world could not be ascertain but the archaeological evidence however shows that dyeing was a wide-spread industrial enterprises in Egypt and India around third millenium B.C. (Crockett, 1964). Indians have been considered as forerunners in the art of natural dyeing. Dyeing is the art of imparting colours to the yarns, fabric and other materials by using colouring matter. The colouring matter can be obtained from both natural and synthetic sources. The technique of dyeing was practiced by ancient people before thousands of years (Geelani *et al*., 2015; Prabhu and Bhute, 2012). Dyeing of textile in the fibre form provides a deeper penetration of the dye into the fibre, thereby giving a more even colour and excellent colour fastness than the textile in other forms. The property of colour fastness depends on the textile materials to be dyed and the selection of the method for dyeing the fibre yarn or fabric.

Naturally dyed textiles represent the good opportunity for value added manufacturing to the eco-friendly process and as well as generate more employment for rural people (Deo and Paul, 2000). These products are comfortable for the wearer because of its soothing colour and non-allergic properties (Samanta *et al*., 2003).

1.5. Mordants used in dyeing

For a dye to impart colour to the substance it should be completely bound to the object, else the colour quickly fades or washes away when brought in contact with water. Natural dyes mostly have lighter tones and are not very colourfast therefore must be applied to the textiles with the aid of mordants (metallic salt). The mordant have affinity for both the colouring matter and the fibre. When applied individually or with combination they are known to produce varying shades, improve the uptake and enhance the colour fastness behaviour of the natural dye (Rungruangkitkrai and Mongkholrattansit, 2012). Common metal mordants are alum, ferrous sulphate, copper sulphate etc. (Geelani *et al*., 2016).

1.6. Silk fibres

Silk (queen of textiles) is a natural protein fibre and its discovery goes back to 2640 B.C. It is produced from silk worms. Fibre consists of 97% protein-fibroin, a filamentous protein and sericin (gum) a non-filamentous protein and also other impurities such as pigments, wax, carbohydrates and inorganic salts. It contains 75% fibroin and 25% sericin by weight (Ebtsam, 2016). Sericin is a gummy substance that cements the two filaments together and is removed by dilute solution of sodium carbonate. Removing this gum improves the sheen, colour, texture of the silk (Pervin *et al*., 2016). The silk fibres have general chemical formula $NH_2CHRCOOH$. The most important ones for dyeing are COOH and NH_2 (Viney, 2000). The fibre is well known for its water absorbency, dyeing affinity, thermal tolerances, insulation properties and luster. Apart from this it also possess high tensile strength and excellent draping property which is the unique characteristics of silk fibre that are lacking in any other fibre.

1.7. Cotton fibres

Cotton is a soft, fluffy staple fibre that grows on the cotton plants. It is cellulose fibre. The use of cotton for dyeing is known from prehistoric times and the fragments of cotton fabric dated to the fifth millennium B.C. have been found in the Indus valley civilization (Pervin *et al*., 2016). Cotton swells in a high humidity environment, in water and also in the concentrated solutions of acids, bases and salts. This swelling effect of cotton is mainly because of the absorption of highly hydrated ions. The moisture regain for cotton is 7.1-8.5% and the moisture absorption is 7-8%. The immersion of cotton fibres in 18-25% of sodium hydroxide leads in removal of natural waxes, fats, pectins and non-cellulosic material thereby increasing the absorption property. It is one of the easiest fabrics to dye due to its natural whiteness and high absorbency rate. Cotton keeps the body cool in summer and warm in winter as it is good conductor of heat making it a perfect fibre for clothing.

1.8. Status of natural dye yielding plants in India

India has a rich Biodiversity and harbours a wealth of useful germplasm resources. The plant kingdom is a treasure house of large range of natural products. About 450-500 dye yielding plants have been recorded in India (Siva, 2007).

1.9. Natural dyes in Goa

Goa being a small state, on the western coast of India is gifted with more than 3,000 different species of flowering plants. The tribal population of Goa constitutes Kunbis, Gowdas and Velips etc. Kunbis are aboriginal people and exists mostly in the state of Goa, Karnataka, Gujarat and Kerala. Their population is about 75,000. They are known for keeping their tradition and culture alive. Their songs and dance belonging to the pre-portuguese era are exclusively social. They are engaged in dyeing and weaving of kunbi sarees. Traditionally the sarees were red and white and mostly used during their work in the field. The natural dye from roots of *Rubia cordifolia* (Manjeestha) plant was used for dyeing by soaking the roots for many days in water. This traditional saree was worn by Goan kunbi tribal women before the advent of Portuguese in 16th century (Gawas and

Velip, 2015). Recently (Verenkar and Krishnan, 2017) have recorded 62 dye yielding plants from State of Goa belonging to 37 different families.

1.10. Studies on natural dyes

The natural dyes have application in various fields. They are used in staining of tissue (Akinloye *et al*., 2010; Ullah *et al*., 2010; Jan *et al*., 2011; Yong *et al*., 2011; Toussan and Behbehani, 2011; Shehua *et al*., 2012; Okpidu *et al*., 2012; Mohanad *et al*., 2012; Jadhao and Rathod, 2012; Deepak and Philpose, 2013). They are used in the large scale in textile industry for dyeing silk (Kongkachvichay *et al*., 2002; Young and Han, 2004; Subhashini *et al*., 2009; Kumaresan *et al*., 2010; Nainwal and Sati, 2011; Lokesh and Swamy, 2013; Akhter *et al*., 2014) for dyeing cotton (Young and Han, 2004; Tarafder, 2009; Kulkarni *et al*., 2011; Ratnapandian *et al*., 2012; Kanchana *et al*., 2013; Devi *et al*., 2014) for dyeing wool (Montazer and Parvinzadeh, 2007; Mongkholrattanasit *et al*., 2010) for dyeing nylon (Lokhande and Dorugade, 1999; Tarafder, 2009).

1.11. Advantages of natural dyes

The natural dyes have following advantages over synthetic dyes:

1. Potential of natural dyes in producing wide range of shades of colours.
2. Eco-friendly nature of natural dyes over allergic and harmful synthetic dyes.
3. The naturally dyed shades are soft, lustrous and soothing to the human eye (Patel, 2002).
4. It helps in protecting the traditional dyeing technology and using the waste lands for growing dye yielding plants thus generating livelihood for poor dyers/artisan which in turn generate employment facility (John and Margaret, 1994).
5. Availability of some archaeological study for conservation and restoration of heritage of old textiles of India for natural dyed textiles.
6. They produce vibrant colours and thus encourage the people to celebrate an eco-friendly Holi (Patel *et al*., 2014).

1.12. Disadvantages of natural dyes

1. Low dyeing efficiency and only few have good fastness to light and washing.
2. Some of the mordants used while dyeing could be harmful (Jihad, 2014).
3. Dyeing with natural dyes is more expensive than synthetic dyes (Shanmathi and Soundri, 2016).

1.13. Research needs

The use of natural dyes is limited, in-spite of their many advantages over synthetic dyes. This may be due to non-availability of standard ways of their production, application, standardization and characterization of natural dyes. These drawbacks can be solved only through scientific research study. The great challenge is to increase the yield of the natural dye, to explore more dye plant sources and to standardize the techniques for dyeing purpose. Hence, there is a need to standardize the best possible method of dye extraction from various plants and to evaluate their dyeing potential on various types of textiles.

1.14. Objectives

The present work focuses on, the optimization of extraction time, temperature for the target plant species, studying the phytochemical and physicochemical properties of dye yielding plants, anatomical staining of plant tissues using natural dyes, dyeing of silk and cotton fibres with natural dyes independently and along with the use of various chemical mordants, colour fastness of dyed fibres and characterization of chemical compounds responsible for staining properties. The specific objectives of study were as under:

1. Survey, collection and documentation of natural dye yielding plants from the State of Goa and adjoining regions.
2. Extraction of dyes from different plant species and plant parts using various solvents.
3. Histochemical screening and evaluation of natural dyes independently and along with different mordants on various biological tissues and textile fibres.
4. Identification and characterization of chemical compounds responsible for staining properties by using UV-Visible spectrophotometer /TLC /GC-MS.

2. REVIEW OF LITERATURE

2.1. Natural dyes

The colourants derived from natural sources of plants, invertebrates and minerals is called as natural dye. The dyes from plants are mainly extracted by either aqueous, acid or basic solvent method. Dyes from fruits are extracted by squeezing the fruits, dyes from leaves and flowers are extracted by grinding leaves/flowers, whereas dyes from bark is extracted by boiling method (Ponmozhi *et al*., 2011, Antima *et al*., 2012). These natural dyes produce soothing and soft shades and hence they are preferred over synthetic dyes.

2.2. Classification of natural dyes

Natural dyes are classified on basis of origin (source), method of application and chemical nature.

2.2.1. Classification based on source

The natural dyes can be classified as plant dyes, animal dyes, insect dyes and mineral dyes. Almost every part of plant viz. leaves, flowers, stems, barks, roots, fruit seeds, rinds can be used as a dye. The widely known insect/animal dyes are cochineal and kermes. The mineral dye commonly used is Geru (red ochre).

2.2.2. Classification based on method of application

The natural dyes are categorized into two classes i.e substantive and non-substantive based on their method of application. Substantive dyes (eg. indigo, turmeric) do not require any mordant to bind to the fabric. Whereas, non-substantive dyes requires a mordant to bind the fabric. Examples of the non-substantive natural dyes are madder, cochineal, beetroot and marigold (Singh, 2000).

The substantive dyes are classified as basic and acid dyes. Acid dyes are applied to the substrate from acidic medium. Basic dyes are cationic dyes, which on ionization gives coloured cations.

The dyes are also classified as monogenetic and polygenetic dyes. Monogenetic dyes produce only single colour irrespective of the mordant employed, whereas polygenetic dyes produce many colours according to the mordants employed (Gulrajani, 1992).

Further dyes are also categorized as vat dyes and disperse dyes. The indigo is the most common example of vat dye. The dye holds affinity for natural fibres, and can be oxidized back on the fibre when exposed to air. Whereas, the disperse dyes do not have solubalizing groups and are sparingly soluble in water. These dyes are applied on hydrophobic fibres like polyester and nylon.

2.2.3. Classification based on chemical nature

According to the nature of chromophores, the natural dyes are further classified into different types such as carotenoids, anthocyanins, flavonoids, anthraquinones, naphthoquinones and indigoids (Zhang, 2010).

2.2.3.1. Carotenoids

Carotenoids are produced by plants, algae and several bacteria and fungi. They are classified as xanthophylls (contains oxygen) and carotenes (do not contain oxygen). They are lipid soluble pigments known to found in carrots, and is responsible for pale yellow to deep red colouration. The crocetin (yellow pigment) found in stigma of saffron and bixin and norbixin in seeds of Annatto (*Bixa orellana*) are common examples of carotenoids.

2.2.3.2. Anthocyanins

Anthocyanins are water soluble pigments that appear or blue, red or purple based on the pH of the extract. These are derived from anthocyanidins by adding sugars (Andersen, 2001). They are known to present in black raspberry, blueberry, cranberry, red cabbage etc.

2.2.3.3. Flavonoids

Flavonoids are responsible for yellow colouration in flower petals. Their molecular structure contains two phenyl rings joined by pyrone ring forming 2-phenyl-1,4-benzopyrone as a molecular backbone.

2.2.3.4. Anthraquinones

Anthraquinones are red coloured dyes with anthraquinone molecular structure. They are known for good light and wash fastness property and have capability to form complexes with metallic mordants.

2.2.3.5. Naphthoquinones

The Lawsone molecule derived from *Lawsonia inermis* (leaves) is a well known member of naphthoquinones. Its structure contains two isomers.

2.2.3.6. Indigoids

The Indigo dye obtained from plant of *Indigofera tinctoria* is a common example of this group.

2.3. Research overview on natural dyes

Giles and McKay (1963) provided a review on the light fastness of natural dyes. Giles (1965) explained the causes and nature of fading of organic colouring matters. According to him the fading of colours occurs almost in all the dyes and it is caused mainly due to nitrogen oxides in air. The hue of many soluble dyes becomes reddish on fading. The fading is rapid at first and later it slows to a constant rate. He described five types of fading rate curves which are typical of synthetic dyes. These curves were constructed by plotting dye concentration versus time.

Padfield and Landi (1966) reported that the natural dyes have poor light fastness property and their preservation in the presence of light is not possible. Turmeric fades in air within 7 days. Annatto initially produces reddish colour but fades in application on cotton and silk after 18 days. Safflower and Orchil completely fades in air after 20 days. Indigo fades after 15 days on its application on cotton, but fades slowly on silk and woolen fabrics.

Egerton and Morgan (1970) discussed the effect of various factors like oxygen, temperature, humidity, atmospheric contaminants and dye concentration on fading of dyes. They stated structure of the chromophore of dye is the major influencing factor which

influences the light fastness results. The increase of dye concentration also promotes the possibility of energy transmission between adjacent molecules. This is the reason for dark shades having good light fastness property than the lighter shades.

Adrosko (1971) studied the dye producing plants of America which was used during 18th and 19th centuries. The wide ranges of shades were obtained using the mordants. The dyed samples showed good fastness to alkali agents or sunlight.

Anonymous (1972) dyed woolen fabrics with flowers of *Toona ciliata* (toon). These flowers contain a red colouring-matter, nyctanthin, which is similar to the colouring matter from *Nyctanthes arbor-tristis.* The fabrics were dyed dull yellow in colour with the extract of these flowers.

Kashiwagi (1973) studied colour characteristics of traditional vegetable dyeing. His study revealed tannins decrease the chroma of dyed colour. Jeanne (1974) featured colour photographs of dye yielding plants. He included the dye recipes for marigold and lichens.

Dyer (1976) discussed all the aspects of natural dyeing viz. mordants used, dyeing process/methods, gathering and storing of all types of dye plants and the art of tie-dye technique.

Duff *et al*. (1977) studied the colour change of naturally dyed woolen samples when exposed to microscal fading lamp. The fading of colour was observed visually and instrumentally and were characterized in terms of light fastness grades, grey scale differences, colour differences in CIELAB colour space and in Munsell colour co-ordinates.

Schweppe (1980) established a simple method for the identification of natural dyes on old textiles. The identification was done by preparing lakhs of dyes and comparing them with lakhs of known dyes using Thin Layer Chromatography.

Crews (1982) dyed woolen samples with 18 yellow natural dyes and tested its light fastness with application of mordants like alum, chrome, copper, iron and tin. The dyed specimens were exposed to xenon-arc lamp and the change in colour were rated using CIE L* a* b* units. The results revealed turmeric, fustic and marigold dyes faded more

significantly, also the dyes applied with tin and alum mordants faded more than the dye applied with chrome, copper and iron mordants.

Ethanobotanical study by Corbman (1983) revealed that the primitive people obtain dye from berries, nuts, flowers, minerals and animal sources. These sources of natural dyes have been obtained throughout civilization.

The interesting study was carried by Gaffar (1984) wherein the vegetable dye from beetroot was utilized in detecting plaque on surface of the teeth which becomes visible to the naked eye.

Paksoy (1985) examined use of *Glycyrrhiza glabra* L. extract as a source of textile dyeing. Various methods were used for dyeing and the colour fastness tests were performed which gave appreciable results. This dyed wool was found to be suitable for use in hand-crafted carpets.

Taylor (1986) studied fastness properties of natural dyes. He re-constructed the traditional dyeing techniques as well as the original colours of old faded textiles.

The fading rate curves of some selected natural dyes were constructed by difference in colour measurements using a tri-stimulus colorimeter by Crews (1987). In her study, most of the dyes exhibited two types of fading rate curves i.e type II (i.e initial rapid fading followed by fading at a constant rate) and type III (i.e constant rate of fading). The dyes like cochineal, indigo and fustic mordanted with chrome exhibited type III fading rate curves.

Udayini and Jacob (1988) introduced kalamkari painting on Indian textiles by using various colours. Orange, blue, lavender were used on desized, bleached and cleaned grey cotton materials. Washing, sunlight, perspiration and pressing were employed in order to test colour fastness. Blue was rated the most efficient colour.

Kay and Mayolo (1989) listed about fifty-six dye yielding plants from Peruvian sources. A literature and field survey was carried out to collect the information about the traditional use of dye plants in Peru, from pre-historic to recent times.

Vednere *et al.* (1990) studied dyeing of wool using two natural dyes viz. *Tectona grandis* and *Pinus roxburghii*. *Tectona grandis* gave purplish-pink shades and exhibited good light and wash fastness whereas *Pinus roxburghii* showed good to excellent light and wash fastness. Sayeed (1990) reported raw and unbleached jute fibres dyed with catechu tannic acid and catechin using mordants like chromium sulphate, alum, copper sulphate, zinc sulphate, aluminium sulphate and ferrous sulphate at various concentrations. The absorption of dye was increased with increase in mordant concentrations and reaches maximum at 8-10% mordant concentration. However, the presence of catechol group in the dye nucleus makes it difficult to determine the absorption of catechin dye. Shades produced on the fibres were even and attractive.

Rama and Gupta (1990) extracted dye from flowers of *Nycthanthes arbor-tristis*. In their study the post-mordanting method gave excellent results with all the mordants employed. But the stannous chloride gave good results in simultaneous mordanting method. Different hues of yellow, yellow orange, yellow green and yellow ochre were obtained.

Agarwal *et al*. (1992) dyed wool with natural dye obtained from turmeric plant. The simultaneous mordanting method was considered best for dyeing wool. The brilliant colours were obtained using chemical mordants i.e 25% alum, 7% chrome, 7% ferrous sulphate and 6% stannous chloride. Gulrajani *et al*. (1992) reported some local plant extracts which were used in various parts of the country for dyeing silk, wool and cotton. The fabrics were dyed using different mordants. Colours obtained on fabrics were yellow with alum, orange with tin, brown with iron and olive with chromium.

Singh *et al*. (1993) studied dyeing of silk with madder dye and reported that all the mordanting methods produced a wide range of colours from bright reddish brown, dark brownish red, peach to greenish brown. The post-mordanting method gave the best colour with ferrous sulphate, copper sulphate and chrome mordant. The fastness property was fair to good for light fastness and wash fastness exhibited fair to poor ratings for all the dyed samples.

Tsatsaroni and Eleftheriadis (1994) dyed the cotton and woolen fabrics with the aqueous extract of natural dye *Crocus sativus* (saffron). Wash fastness on woolen fabrics

was good whereas it was poor for cotton fabrics. But the fastness on cotton could be improved by using metal salts before dyeing.

Tsatsaroni and Kyriakides (1995) used natural dyes from chlorophyll and carmine after cellulase treatment on dyeing the cotton and woolen fabrics. Wash and light fastness of the dyed fabrics were evaluated. Goodwin (1995) reported the major processes involved in dyeing wool throughout UK. A method involved in obtaining a dye and using it for dyeing woolen fabrics was provided.

Paul *et al*. (1996) classified the natural dyes based on their chemical classes such as indigoid, anthraquinone, alpha-naphthoquinones, flavones, dihydropyrans, anthocyanidions and carotenoids. On the basis of colour, they were classified into red dyes, yellow dyes, blue dyes, black dyes, brown dyes, green dyes and orange dyes. They also studied the chemistry, extraction procedures and the fastness properties of some natural dyes.

Bruckner *et al.* (1997) studied fastness of several plants like henna, logwood, madder, saffron and turmeric along with other plant extracts like beetrol, bilberry, parsley, stinging nettle and tea. Various mordants like alum, cupric sulphate etc. were used for dyeing polyester, nylon, acrylic and woolen fabrics. The fastness ratings were moderate to poor for the dyed fabrics.

Prabhu and Senthilkumar (1998) studied dyeing potential of natural colours from *Rosa indica* on cotton and silk fabrics. The dye exhibited dull yellow colour on cotton and bright yellow colour on silk fabrics. Eco-friendly dyeing of silk samples using the extract from *Eucalyptus* leaves was carried (Vineeth and Bharthi, 1998). The results showed fair to good light fastness and poor to very good wash fastness in mordanted woolen yarns.

Lokhande and Dorugade (1999) dyed the nylon fabric with three natural dyes i.e. onion, lac and turmeric by two different techniques HTHP (High temperature high pressure) and open bath. HTHP dyeing gave better results compared to open bath dyeing. All the three dyes showed good results for wash fastness tests. Singh *et al*. (1999) worked on dyeing wool with bark of *Berberis* species, *Acacia nilotica* L. and *Juglans regia* L., roots of *Berberis aristata* DC., leaves of *Eupatorium odoratum* L. and *Mentha pulengium*

L., flowers of *Woodfordia fruticosa* (L.) Kurz and *Butea monosperma* (Lam.) Taub. The dyeing conditions were optimized and fastness properties were evaluated.

Vankar (2000) observed that the natural fibres like cotton, linen, wool, silk, jute, ramie and sisal can easily take up natural stains. However, wool takes up dye more easily than any other fibre.

Kapoor and Pushpangadan (2001) developed the process for the preparation of Herbal Gulal (dry colour powder) by using natural dyes in order to play safe holi.

Senthilkumar *et al*. (2002) used ultrasonic technique for dyeing cotton fabrics with waste cabbage leaves. The mordants produced brilliant yellow hues on the fabrics. This ultrasonic method proved to be faster thereby increasing the wash and light fastness property of the fabrics. Kapoor and Pushpangadan (2002) prepared the herbal gulal from *Curcuma longa* L., *Butea monosperma* (Lam.) Taub., *Dactylopius coccus* Costa, *Beta vulgaris* L. and *Indigofera tinctoria* L. to celebrate eco-friendly holi.

Pan *et al*. (2003) dyed jute with aqueous extracts of Deodara leaf, Jackfruit leaf and *Eucalyptus* leaf. His study revealed light brown to light mustard shades with good wash fastness on jute fabrics. The use of ferrous sulphate mordant resulted in deeper shades to the fabrics.

Gokhale *et al*. (2004) provided the checklist of about 500 dye yielding plants from India. Macfoy (2004) carried ethanobotanical survey of dye yielding plants in Sierra Leone. Along with the dyes used for dyeing the fabrics, the technique to produce various patterns on fabrics were also investigated.

Avwioro *et al*. (2005) stained the sections of normal and post mortem tissues of rat intestine, skin, kidney, heart, liver and spleen by using the alcoholic extract of *Pterocarpus osun* in acidic, neutral and alkaline medium. No significant difference was observed in the staining reactions by using the fixatives like 10% formalin saline, Carnoy's fluid, Zenker's fluid, Helly's fluid and Bouin's fluid therefore it was concluded that any of these fixatives could be used along with *P. osun* for histology purpose. Grover *et al*. (2005) dyed the silk yarns with flowers of *Jatropha integrimma*. They studied the mordant wise colour change and fastness property to light, wash, crocking and perspiration on dyed silk. The flower

dye gave satisfactory results with good fastness to light, wash, crocking and perspiration. Paul *et al*. (2005) extracted pink colour dye from the leaves of *Hamelia patens* which served as a valuable dye for dyeing silk fabrics. The dye produced diverse colours ranging from pink to grey on silk yarns. Their study also involved optimizing the various dyeing variables for dyeing silk with *Hamelia* dye. Aqueous tea extract was used by Das *et al*. (2005) for dyeing silk and woolen fabrics in the presence and absence of mordants such as magnesium sulphate, aluminium sulphate and ferrous sulphate. Both the protein fibres showed highest affinity at pH 2 to 4 in the presence and absence of all the mordants.

Cristea and Vilarem (2006) evaluated the light fastness of natural dyes such as madder, woad and weld on cotton. All the three dyes have poor light fastness property and they suggested the use of some additives can improve the fastness of these natural dyes.

Tiwari and Bharat (2007) recorded 33 dye yielding plants belonging to 18 families with dominant species in Fabaceae family in Achanakmar-Amarkantak Biosphere Reserve (BR) of Chhattisgarh State. The list of plants was provided with the indigenous method of dye extraction along with their ethnic uses. Montazer *et al.,* (2007) studied dyeing of woolen fabrics with natural dye. The wool yarn was pre-treated with alum mordant and then dyed with marigold followed by treating with different percentages of ammonia solutions. The result was appreciable, but on treating with ammonia solution the fabric exhibited poor light fastness. Siva (2007) listed 2000 pigments from various parts of plants. He recorded nearly 450 dye yielding plants from India. Kar and Borthakur (2007) listed 47 dye yielding plants from Assam which is used for dyeing cotton, silk and woolen fabrics along with their local names. Nagia and El-Mohamedy (2007) dyed the woolen fabrics with natural anthraquinone compounds from liquid cultures of *Fusarium oxysporium*. Two new anthraquinones were detected i.e 2-acetyl-3, 8 dihydroxy-6-methoxy anthraquinone and 3-acetyl-2, 8 dihydroxy-6-methoxy anthraquinone. The colour strength values, dye uptake and fastness of dyed fabrics were good in all mordanting methods for wool fabrics.

Vankar *et al*. (2008) extracted the reddish orange dye from the roots of *Rubia cordifolia* for dyeing cotton fabrics. The bio-mordant (*Eurya acuminate*) was found to enhance the dye uptake upto 23.5%. The dye showed good fastness results and hence can

be used as an alternative to synthetic dye. Egbujo *et al*., (2008) stained the sections of rabbit tissue by using red dye from *Hibiscus sabdariffa*. Jothi (2008) extracted natural dyes from African marigold flowers (*Tagetes erecta* L.) for textile colouration. Yellow dye was extracted from flower and potential of dye was evaluated by dyeing on cotton and silk fabrics under normal dyeing conditions. Studies on dye ability, wash fastness, light fastness and colour were undertaken. Dessai (2008) studied the natural dyes for biological staining. Many natural dyes were extracted from various parts of the plants and their staining property on angiosperm sections were observed under bright-field and fluorescence microscopy. Some mordants like potassium dichromate, stannous chloride, ferrous sulphate, potash alum and cupric sulphate were known to enhance the staining property during the study. Zhang (2008) extracted dye from woolen threads of ancient textiles from Xinjiang and characterized the dye using HPLC analysis.

Rekaby *et al*. (2009) used dye from alkanet and rhubarb for printing wool, silk, cotton and flax fabrics. Mazeyar (2009) dyed wool fibres with the outer skin of egg plant (*Solanum melongena*). The fibres were also tested for light and wash fastness. The mordanted samples showed good light, wash fastness due to the strong complex formation between dye and the fibre.

Mati and Boer (2010) provided traditional knowledge of dyeing textiles, with the plants used by the nomadic tribes of Iraqi Kurdistan. Zarkogianni *et al*. (2010) investigated dyeing potential of natural dyes on cotton and woolen fabrics using brazilwood, red sandalwood, cochineal, madder, safflower, indigo, alkanna, henna and logwood. The mordants like potassium dichromate, copper sulphate, zinc chloride, iron (III) chloride, iron (II) sulphate and tin chloride was used to improve the dye absorption on the fabrics. Dyeing was evaluated through colour measurements and by standard wash, rub and light fastness tests. Good to excellent results was shown for woolen samples in all mordanting methods. Karolia and Joshi (2010) lead the concept of using tie and dye technique with walnut dye on silk and wool fabrics. They also visualized product line of fashion accessories which was thought to be a novel idea. Akinloye *et al*., (2010) used dye extracts from *Bixa orellana, Curcuma domestica, Lonchocarpus cyanescens* and *Pterocarpus osun* to stain wood sections. All the extracts had an affinity for fibre and vessel elements except the extract from *L. cyanescens*. The extract of *C. domestica* and *B. orellana* had higher

selectivity for fibre than that of *P. osun*. This study showed dye extracts from *B. orellana* and *C. domestica* could be solitary used for plant histological staining. Samuel *et al*. (2010) studied extraction of dye from seeds of *Nyctanthes arbor-tristis, Hydnocarpus wightiana, Wrigtia tinctoria* etc. using various solvents like methanol, ethanol, acetone and aqueous. The extracts were screened for antimicrobial activity and even phytochemical studies were carried out. Among the solvents used, methanol showed highest antimicrobial activity compared to other extracts. Ullah *et al*., (2010) extracted yellow dye by using water and ethanol from wood of *Berberis vulgaris* L. Its potential for staining stem tissues was studied. Both the dye extracts stained the lignified tissues of stem sections. However, the ethanol extract was found more effective in staining the tissues.

Nainwal and Sati (2011) were successful in dyeing the silk with flowers of *Calendula officinalis*. They obtained vivid colours ranging from soft to light using mordants in combinations. The fabrics showed good to excellent fastness to rub and perspiration in both acidic and alkaline medium. Jan *et al.*, (2011) isolated natural dye lawsone from dry leaves of *L. inermis* using clove oil, ethyl alcohol and water as solvent and tested its effectiveness as staining agent for angiospermic stem tissue. Chukwu *et al.* (2011) obtained the extract from leaves of *Lawsonia inermis* for the first time as a counter stain in gram staining reaction wherein different extracts of *L. inermis* leaves were formulated into various staining solutions of different concentration and modified with hydrogen peroxide, ferric chloride etc. The aqueous extracts of the henna plant (cold and hot) oxidised with potassium permanganate gave a better staining reaction with gram negative bacteria, while the ethanol extract oxidized with potassium permanganate had no staining reaction with gram negative bacteria. Hence, aqueous henna leaves extracts when oxidised with potassium permanganate could be a substitute to the usual counter stains used in gram staining procedure. Kulkarni *et al*. (2011) extracted the natural dye from green chilli (*Capsicum annum*) and used it for textile colouration. Two mordants such as copper sulphate and ferrous sulphate were used in different ratios viz. 1:1, 1:3, 3:1. 100% scoured cotton cloth was used for dyeing by three different mordanting techniques such as pre-mordanting, simultaneous mordanting and post-mordanting. Colour fastness studies on the dyed cloth were undertaken. Different shades of yellow were obtained from the dye. Good rub and light fastness and moderate wash fastness was observed in fabrics dyed with

the dye extracted from green chilli. Saravanan *et al*. (2011) reported art of dyeing bleached silk fabrics with natural dye obtained from the barks of *Ficus religiosa* L. using chemical and natural mordants. The colour changes were measured by computer colour matching software. The dye proved to have good scope in textile industry. Jan *et al.* (2011) extracted yellow dye from dried stem of *Berberis pachyacantha* Kochne using different solvents and studied its staining effect on sections of monocot (*Zea mays* L.) and dicot (*Helianthus annus* L.) stem. Yong *et al.* (2011) extracted natural dye from (*Syzygium cumini*) using various solvents and its staining potential on hepatic tissue of rat was studied. The results showed that the natural dye from black plums could stain the cytoplasm as well as nucleus of hepatic cells with violet colour. This finding suggests that the dye from *Syzygium cumini* could be used as an alternative dye for histological staining. Wan *et al*., (2011) extracted dye from wood of *Gluta aptera* and dyed silk and cotton fabrics. The ability to withstand washing, light and perspiration was compared between silk and cotton fabrics. Srivastava and Singh (2011) obtained the 'Chrysammic acid' from *Aloe vera* to dye silk and woolen fabrics. The dye produced different colours varying from yellow, pink, khaki to brown. Washing the fabrics deepened the colour which was of great importance to the consumer.

Das and Mondal (2012) identified dye yielding plants like *Woodfordia fruticosa* (L.) Kurz., *Morinda citrifolia* L., *Clitoria ternatea* (L.), *Butea monosperma* (Lam.) Taub., *Butea superb* Roxburgh, *Carthamus tinctorius* L., *Ventilago denticulate* Willd., *Cordia dichotoma* G. Forst., *Nyctanthes arbor-tristis* L., *Tectona grandis* L. f. from the lateritic zone of West Bengal. The paper revealed the traditional use of these plants and along with its medicinal importance. Ahmad *et al*. (2012) dyed silk fabrics using natural dye from *Melastoma malabathricum* with several synthetic and natural mordants. It showed good wash fastness, which served its use in printing industries mainly in batik dyeing. Suebkhampet and Sotthibandhu (2012) extracted the bluish dye from the butterfly pea flower (*Clitoria ternatea*) and tested its staining effectiveness on animal blood smear. They found the extract could be successfully used in differentiating blood cells in animal peripheral blood smears as compared to the other stains. Sharma *et al*. (2012) analyzed dye plants of Garhwal Himalaya. Their extensive field survey from 2008 to 2011 reported 46 dye yielding plants, belonging to 33 different families. The list of plants with their

vernacular names, habit, parts used nature of dye and distribution was provided. The investigation by Singh and Mathur (2012) revealed 19 dye plants species belonging to 17 families from Firozabad, Uttar Pradesh. From which 14 species were known to be used as main dye whereas others were used as adhesive for the main dye. Teparin *et al*. (2012) used two types of tamarind seeds sour and sweet for dyeing cotton, *Bombyx mori* silk and Eri silk fabrics. High degree of dye exhaustion was observed on *Bombyx mori* silk fabric than the Eri silk fabrics. Wash fastness for dyed fabrics was rated from good to excellent. Thus, the walnut dye was considered as an excellent source of dyeing in textile industry. Petroviciu *et al*. (2012) developed the protocol for the identification of natural dyes in historic textiles by LC-MS and LC-MS/MS. The study discussed the application of this approach in characterization of dyes. Ismal and Yildirim (2012) dyed woolen fibres with almonds shells. Various colours were obtained using different mordants and satisfactory colour change values were exhibited. Iron sulphate mordant ensured the best fastness to light. Avinc *et al*. (2012) used extract of waste barks of Turkish Red pine (*Pinus brutia*) to dye cotton, flax, wool, silk, tencel, polyamide and acrylic fibres. Alum mordanted samples exhibited better colour fastness properties. Semwal *et al*. (2012) worked on dyeing properties of roots of *Berberis aristata* using natural and synthetic mordants. Their study revealed that the dye could be useful in dyeing industry. The colour can be enhanced using natural mordants which is safe and eco-friendly in nature. Okpidu *et al*. (2012) stained human tissues with aqueous extract of *Ceratonia siliqua* bark and it was adapted for the first time as a counter stain in Hematoxylin and Eosin staining reaction. Baltac *et al*. (2012) discussed synthesis of four dyes namely Methylene Blue, Neutral Red, Eozine and Orange G, belonging to four different classes (*triazinic*, *phenazinic*, *xanthenic* and *monoazo*) from both chemical and biological point of view. Swami *et al*. (2012) dyed the silk and cotton polyester blend with the aqueous extract of *Sesbania aculeata* (green manure plant). The dye gave brilliant colour in combination with metal mordants on silk fabrics compared to the polyester cotton blend. It also gave excellent results for wash and rub fastness. Natural dye was obtained from the flower of *Callistemon citrinus* by Baishya *et al*. (2012). The extract was separated in two parts autoclaved and non-autoclaved which was later used for dyeing cotton fabrics. Autoclaving and using the extract for dyeing was reported first time and the study revealed that this led to good colour yield and fastness of the natural dye compared to the non-autoclaved extract.

Alawa *et al*. (2013) recorded 18 dye yielding plants used by tribals for making specific colour combinations in Dhar district of Madhya Pradesh. These dyes play an important role in the social and religious life of these tribal people. Kanchana *et al*., (2013) extracted dye from flowers of *Clitoria ternatea*, *Tagetes erecta* and outer peel of *Punica granatum*. The extracted dyes were tested on various fabrics using different mordants and colour fastness to wash, rub and light was evaluated. The antimicrobial properties of these dyes were also evaluated against four bacteria and diameter of zone of inhibition was noted down. Deepak *et al.* (2013) extracted pink colour dye from fruits of *Melastoma malabathricum* for staining plant anatomical sections. Also the effectiveness of natural mordants such as *Garcinia cambogia* L., *Citrus aurantifolia* (Christm.) Swingle and *Averrhoa carambola* L. in the dyeing process was investigated. Gandhi *et al*. (2013) used the colourant from *Nyctanthes arbor-tristis* to dye the enzyme treated organic cotton fabric. The ultrasonic power dyed fabric was compared with the conventional dyeing method. Results was found that ultrasonic assisted dyeing process gave better dye absorption and fastness properties than that of the conventional methods. Ozturk *et al*. (2013) provided the list of 143 dye plants of Turkey which are of medicinal importance. The plants involved in dyeing process belong to the Asteraceae, Rosaceae, Lamiaceae, Polygonaceae and Euphorbiaceae families. They also listed highly medicinal dye yielding plants which can be used for producing antihelmintic, antiseptic, appetizer, carminative, diuretic, exudative, laxative, sedative and stomachic preparations. A new source of natural dye from *Telanthera ficoidea* was used by Duarah (2013) to dye silk fabrics under varying conditions. The dye produced various shades of colour ranging from cream to dark brown with good colour fastness to sunlight, washing, pressing, crocking and perspiration. The natural colourant from *Casuarina equisetifolia* bark was used to dye silk, which was considered as a waste material. The extracted dye was verified for eco-friendliness by analyzing its metal content. The dye showed excellent colour fastness values thereby indicating its potential for use in textile industry Narayanaswamy *et al*. (2013).

Rahman *et al*. (2013) dyed silk fabrics with extract of *Xylocarpus moluccensis*. The dye exhibited good wash, rub and light fastness property. Geelani *et al*. (2013) studied comparative dyeing quality from fruit cups of *Quercus robur* on cotton and wool fabrics using *Punica granatum* peel as a natural mordant. The cotton fabric did not show much

affinity for dye whereas attractive and bright colour shades were obtained on the woolen fabrics using different combination of mordants. Rashid (2013) explored the dye yielding plants from Rajouri district of Jammu and Kashmir. Around 48 dye yielding plant species belonging to 27 families were documented with their traditional use by Gujjar Bakeral tribe. Janani and Hillary (2013) used fresh leaves from *Vernonia amygdalina* for dyeing woven cotton along with mordants like potassium dichromate, alum, ferrous sulphate, iron water and ash water. Multiple colours were produced by the dye and the post-mordanting method was found to produce best colour characteristics with most brilliant colours on the fabric.

Liu *et al*. (2014) in Tongdao Dong Autonomous country China, reported 13 dye plant species, of which 7 species were used for colouring food items, 3 species were used for dyeing clothes and 3 species were known to colour both food and clothing. Gupta and Rastogi (2014) studied effect of chemical washing on the woolen samples dyed with natural dyes. A wide variety of colours were produced using different mordants. After chemical washing all the samples dyed with combination of dyes became darker and brighter. Also the colour remained unchanged after subsequent washing. Hence, the dyes from *Punica granatum* L., *Quercus infectoria* Oliv., *Acacia catechu* (L.) Willd etc. can be used for dyeing wool yarns. Teli *et al*. (2014) used natural dyes from madder, sappanwood and onion peels for dyeing cotton using Alum mordant. The light fastness property of madder and onion was good to excellent whereas sappanwood showed poor fastness to light. Papadaki *et al*. (2014) used waste dyestuff from plants like *Eucalyptus globules*, *Allium cepa*, *Inula graveolens* and *Cydonia oblonga* for dyeing chemical pulp. Pulp dyeing is of great importance due to the ample range of products made from dyed pulp, such as packaging materials, printing papers, household care, personal hygiene papers etc. Thus, this study added one unique application of natural dyes apart from its known uses. Hasan *et al*. (2014) studied the dyeing potential of *Curcuma longa* dye on cotton and polyester fabrics. The dye contained curcumin which is responsible for yellow colour on the fabrics. Dyeing of wool yarns (Karaboyaci and Ugur, 2014) with pulps of lavender, broom and red wine was achieved using non-toxic and ecological mordants. Thus, this study was of great importance as the pulps that would create environmental pollution could be used as a source of natural dye. The dye extract from *Caesalpinia sappan* was used for dyeing

cotton and silk yarns (Ohama and Tumpat, 2014). Brazilein was the major dye component in the *Caesalpinia sappan* extract which was responsible for dyeing. Aluminium potassium sulphate, ferrous sulphate and copper sulphate were used as mordants for producing various shades to the fabrics. Khatib *et al*. (2014) dyed the silk fabrics with the natural colouring matter saffron (*Crocus sativus*) using traditional and microwave heating methods. The microwave extraction method was more effective for dyeing fabrics than the traditional extraction method. The results showed good fastness properties. Alemayehu and Teklemariam (2014) provided the review on application of natural dyes on textiles, wherein he discussed the chemical and biochemical analysis of natural dyes. Effect of different mordants on natural dyes and characterization of natural dyes using UV-visible spectrophotometer and chromatographic analysis was studied. Prabhavati *et al*. (2014) improved colour fastness of natural dye *Eucalyptus* bark on cotton fabrics. Different dye fixings agents were used like alum, stannous chloride, ferrous sulphate, vinegar, ammonia, lime juice and calcium chloride to obtain various shades from a single dye. The fabrics post-mordanted with vinegar deepened the shade of the dye compared to alum and ferrous sulphate mordants.

Lawarence *et al*. (2015) provided the diversity of dye yielding plants from Ponmudi hills of Western Ghats. This area is being utilized by the Kani tribes including its local inhabitants. They reported 49 species belonging to 27 families with its utility values. Sutradhar *et al*. (2015) elucidated 39 dye yielding plants of Tripura, Northeast India belonging to 26 families which were traditionally used by the tribes of Tripura. The study also provided the active colouring agents in each plant species with their immense significances in the socio-economic and socio-cultural life of ethnic people. Ado *et al*. (2015) dyed cotton and polyster fabrics with three varieties of *Kola* nuts (red, white and bitter). The fastness properties of dyed fabrics were evaluated and simultaneous mordanting produced best results. Kikim *et al*. (2015) undertook the survey to document dye-yielding plants and the indigenous knowledge of preparation of natural dyes by the tribal communities of Senapati District of Manipur State. During the survey, 30 dye-yielding plant species belonging to 23 families have been identified. Choudhury and Mitra (2015) provided domestic method of silk dyeing with raw plant components (flowers, leaves, bark and root) and by natural mordant. Good wash and light fastness were observed

on silk for some of the natural dyes. Ali *et al*. (2015) studied anti-microbial activity of silk fabrics pre-treated with *Moringa olefera*, dyed with natural colouring matter (madder) using microwave heating methods. The samples treated with *Moringa olefera* exhibited highest inhibition percent than the untreated samples. Wangatia *et al*. (2015) used extract of mango bark as a natural mordant for dyeing cotton fibres. Bitter leaves (*Vernonia amygdalina* Del.) extract was used as a natural dye. The amount of dye in the sample after dyeing and after washing was tested using CIELAB equipment. Different mordanting techniques were used from which post-mordanting gave better wash fastness results than pre-mordanting. Srivastava *et al*. (2015) dyed the silk fabric with natural colourant *Rheum emodi* in the presence of five natural mordants. A wide range of shades ranging from yellow to olive green were obtained. Jahan and Datta (2015) studied comparative account of dyeing of cotton and silk fabrics with madder as a natural dye. The penetration of colours on silk samples was deeper compared to the cotton samples. The dye showed good fastness to light, wash, rub and perspiration. Uddin (2015) compared the colour fastness of silk fabrics on mordanted and un-mordanted dyed samples. The dyeing was carried out using aqueous extracts of mango, guava and heena leaves. It was found that mordanted dyed samples have better colour fastness than the un-mordanted ones. The stems of *Combretum latifolium* containing flavonoids were used as dyestuffs for dyeing cotton fabrics by Chairat *et al*. (2015). The sodium chloride was used as additives and it was found that addition of sodium chloride increased the amount of dye uptake, whereas the absence of sodium chloride decreased the dye uptake property. Singh and Srivastava (2016) made an attempt to dye pure mulberry silk with natural spice i.e. Black cardamom. Alum was used as a mordant and the fabric was dyed using three different techniques in acidic medium. The attempt was successful in dyeing silk with good fastness property and hence proved to be an eco-friendly dye.

Preliminary study on dye yielding plants in Bhadravathi taluka, Karnataka was carried out by Parisara and Kiran (2016), which revealed 29 dye yielding plants belonging to 26 genera and 19 families.

Khan and Sangi (2016) explored the ethnobotanical information from Rajgarh district of Madhya Pradesh and reported 15 dye plants belonging to 13 families. Apart

from this the medicinal uses and the preparation of dyeing stuff of each plant is also been mentioned.

Ramasubramanian *et al*. (2016) studied the dyeing effect of *Lawsonia inermis* dye on silk fabrics. Different hues were produced on the silk fabrics using the same dye and potash alum mordant. Fastness property of the dye revealed simultaneous mordanted samples have better fastness property than the pre and post mordanting samples.

Kashyap *et al*. (2016) used a natural dye from coconut husk for dyeing cotton fabrics. The fabrics were dyed with two eco-friendly mordants i.e alum and vinegar. The dye showed good results for wash and light fastness tests.

Thiyagarajan *et al*. (2016) prepared dye from flower of *Acacia eburnea* to dye silk samples. They dyed bleached silk fabrics with different chemical mordants by three different dyeing methods such as pre-mordanting, simultaneous mordanting and post-mordanting. The dye showed good wash, light, rub and perspiration fastness property. The dyeing of silk was done with eco-friendly natural dye from the flower of *Plumeria* species (Kumaresan and Senthilkumar, 2016) using different mordants. The fastness property and colour strength of dyed silk samples were determined. The results revealed simultaneous mordanting method gave excellent results with 3% mordant.

Das *et al*. (2016) made an attempt to utilize the petals of Chrysanthemum (*Dendranthema grandiflora*) flower and peel of badam fruit (*Prunus dulcis*) to dye the cotton and silk fabrics. Copper sulphate was used as a mordant and the dyes were treated with and without mordant to examine their effects on the dyeing. The performance was better with all the natural dyes which can serve as an alternative to synthetic dyes. Ketut *et al*. (2016) made an attempt to dye the silk and cotton fabrics with red pigment of *Penicillium purpurogenum*. This pigment was produced in potato dextrose broth at 30°C in 14 days under static condition. The pigment showed high affinity to silk and cotton fabric and is found to have good colour fastness property to wash and iron.

Gogoi and Gogoi (2017) documented 175 traditional dye yielding plants of Assam. The dyeing method existing during the period of Sri Sri Sankardeva as he used various

colours for different occasions such as writing manuscript in sanchipat, depicting the picture of Lord Krishna etc.

Arora *et al*. (2017) made an attempt to extract natural dyes from a variety of plant sources such as rhizomes of turmeric, fruits of harda, petals of safflower and roots of barberry using specific techniques. Dyes were tested on silk, cotton and woolen fabrics by using three different mordanting methods. A rainbow of natural dyes was obtained with wide-ranging shades of each colour.

Manimozhi and Kanakarajan (2017) used flower of *Torenia* species for colouring silk yarns. Tannins from *Peltophorum pterocarpum* bark and *Tamarindus indica* seed coat were used as bio-mordant while dyeing. These bio-mordants showed good fastness property.

Kiumarsi *et al*. (2017) dyed silk yarns with flavonoids of *Delphinium zalil* flowers. Quercitin was identified as main aglycon responsible for colour in this plant. Pre-mordanting method proved best for dyeing.

Sarkar *et al*. (2017) worked on dyeing of textile with micro-organisms. Pigment producing isolates were identified and its dyeing capability on cotton and silk were evaluated. Pigments were also tested for antimicrobial activity and allergic test to human skin.

Masae *et al*. (2017) used stink bean pod (*Parkia speciosa*) extract for dyeing silk fabrics. Various mordants like aluminium potassium sulphate, iron sulphate, iron chloride, sodium hydroxide and mud were employed for dyeing. Silk fabrics mordanted with iron chloride along with the dye showed good UV-protection and thus the dye proved to be used as a natural colourant for dyeing silk. Dye from waste leaves of *Terminalia catappa* was used for dyeing nylon (Vadwala and Kola, 2017). Both eco-friendly and non-ecofriendly mordants were used for dyeing. Different shades with excellent to good fastness property were obtained.

Baaka *et al*. (2017) used orange peel as a natural dye for dyeing cotton fabrics at a very low cost. Mixture of different solvents was used to extract the carotenoids from the

orange waste peels. The copper sulphate mordant proved excellent mordant to improve the colour yield and light fastness of the fabric.

Mukherjee and Kanakarajan (2017) extracted the natural dye from *Cosmos* species flower to dye the silk yarns. The mordants alum and ferrous sulphate were used to obtain different shades of colour with good fastness property. The presence of flavonoids in the dye extract was responsible for deep yellow colouration.

Seema (2017) explored the use of unconventional natural substance i.e. onion peel as a dyeing material in acidic and neutral medium. Two types of mordants viz. natural and chemical and two types of techniques such as pre-mordanting and post-mordanting were adopted. Dyeing in acidic medium gave better results in terms of dye absorption, brightness, colour variation compared to the samples dyed in neutral medium.

Failisnur (2017) used the fresh solution of gambier, first waste of dyed gambier solution and second waste of dyed gambier solution for dyeing purpose. The fresh gambier solution was used as a control. Compared to fresh gambier solution, first and the second waste of dyed gambier solution dyed the cotton yarns better with 52.14% tannin content.

Verenkar and Krishnan (2017) dyed cotton and silk fabrics with natural dyes from mangrove species viz. *Rhizophora mucronata* and *Ceriops tagal*. Different shades were obtained by using various chemical and natural mordants.

Verenkar and Krishnan (2017) recorded 62 dye-yielding plants belonging to 37 different families in State of Goa, India. Dyes from various plant species have been extracted and suggested their use as biological and textile staining and further emphasized on the importance of generating employment opportunities for rural people.

Yan *et al*. (2017) dyed the silk fabrics with the extract of *Sophora* flower bud. The yellow colour of the flower was mainly due to the presence of rutin and quercetin in the dye. Good wash, rub and perspiration fastness was observed on the fabric which revealed the *Sophora* extract as an effective natural dye.

Zerin *et al*. (2017) made an attempt to use seawater in dyeing cotton fabrics and comparing the differences in shades of dyed samples with ground water. The effects of

colour fastness to wash, rub and perspiration fastness of dyed fabrics were also studied. The results showed sea water dyed samples showed lighter shades than the ground water dyed fabric samples.

Kumari *et al*. (2018) documented 51 dye yielding woody plants belonging to 39 genera and 30 families along the National Highway from Parwanoo to Kaurik in Himachal Pradesh. The genera represented by higher number of species were *Berberis* and *Rubus* (4 species), *Prunus* (3 species), *Acacia* and *Vitis* (2 species) and rest of the genera with one species.

Kumaresan *et al*. (2018) studied fastness property of *Thespesia populnea* flower on cotton fabrics using different combinations of mordants. They evaluated the wash, rub, light and perspiration fastness of dyed samples. Out of all the mordanting methods simultaneous method gave better results with 1:3 mordant combinations.

Akinloye and Illoh (2018) examined the potential of phytodyes from *Enantia cholarantha*, *Harungana madagascariensis*, *Hibiscus sabdariffa*, *Sarcocephalus latifolius*, *Spenocentrum jollyanum* and *Sorghum bicolour* in staining the wood sections and macerates. All the dyes imparted their colours indiscriminately on all the cells but intense staining was observed on fibre and lignified tissues. Natural colourant from leaves of *Azadirachta indica* (Abdel-Zaher, 2018) was used as eco-friendly dye for colouring silk fabrics. The fabric was dyed under different conditions such as pH values, temperature and time to obtain various ranges of shades.

Baneerjee *et al*. (2018) used four natural dyes ie. *Camellia sinensis* (tea leaves), *Allium cepa* (onion peel), *Laccifer lacca* (lac insect) and iron ore to dye Eri-silk yarns. The widely available natural mordants were used like *Terminalia chebula*, *Tsuga Canadensis*, *Snep sohmylleng*, *Oroxylum indicum* and *Baccaurea ramiflora* to achieve different colours on silk fabrics.

Teklemedhi and Gopalkrishnan (2018) dyed the silk fabrics with *Cassia singueana* bark dye in the presence of *Aloe vera* as a natural mordant. The wash, light and rub fastness of all the dyed fabrics were quite good.

Rosyida and Suranto (2018) used anthocyanins from teak leaves to colour the silk cloth based on variations in pH and mordants. The purple to red colour was seen at pH 5, when no additional mordant was added. Grey to black colour was produced after addition of ferrous sulphate mordant. Good results were found for wash fastness.

Dogan and Akan (2018) used the natural dyes from *Rubia tinctorium* (madder), *Juglans regia* (walnut), *Reseda luteola* (weld) and indigo to dye wool yarn, cotton fabric and paper. Red tones were obtained from the materials dyed with madder, soil colour was obtained from materials dyed with walnut, yellow colour from the materials dyed with weld and blue tones from the materials dyed with indigo.

Kumar and Prabha (2018) extracted dyes from different plants for dyeing cotton, jute and woolen yarns. The mordants used were vinegar, sodium chloride and potassium dichromate. Good wash fastness property was observed with the yarns treated with sodium chloride mordant.

Sharma *et al*. (2019) dyed the linen using waste water of heena dyebath. The colour measurements, UV protection and anti-oxidant property of dye were analyzed.

Mir *et al*. (2019) made an attempt to dye cotton fabrics using green algal species *Cladophora glomerata*. Hues of colours from light green, yellow to brown were obtained using different mordants.

Vujcic *et al*. (2019) studied the effect of gamma radiation on both natural dyed and synthetic dyed wool, linen, silk and cotton fabrics. The samples were expressed at ambient temperature to gamma radiation doses from 0.5 to 25 kGy. The results revealed change in colour was pronounced more in the fabrics dyed with natural colours.

Phan *et al*. (2020) tested the potential of using anthocyanins from blueberry waste for dyeing cotton fabrics using biomordants. The study developed green routes towards the use of anthocyanins as a natural dye for cellulose based materials.

Verenkar and Krishnan (2020) studied the effect of natural dyes *Curcuma longa* and *Nyctanthes arbor-tristis* on various plant tissues under fluorescence microscopy. The monocot and dicot stem sections showed auto-fluorescence under fluorescence

microscopy. Among the dyes evaluated *Nyctanthes arbor-tristis* showed better fluorescence compared to *Curcuma longa* dye and hence can be used as potential biological stain for staining plant tissues.

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

Aggarwal (2021) presented the review article dealing with the information on dye-yielding plants of India. The paper discussed the classification of dyes, their extraction techniques and their medicinal importance. Thus, this review serves as an aid to collective inquiry into Indian dye plants.

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted in the state of Goa, which is located in the South western region of India. It encompasses an area of 3,702 km^2. It lies between the latitudes 14°53′54″ N and 15°40′00″ N and longitudes 73°40′33″ E and 74°20′13″ E. It is a former Portuguese province and is renowned for its beaches, places of worship and world heritage architecture. It has a rich flora and fauna, owing to its location on Western Ghats range, it is considered as one of the biodiversity hotspots.

Regular field trips and survey were conducted in different talukas of Goa (Figure 1) viz. Salcete, Sattari, Ponda, Tiswadi, Quepem etc. during the year 2013-2016 for the collection of dye yielding plants.

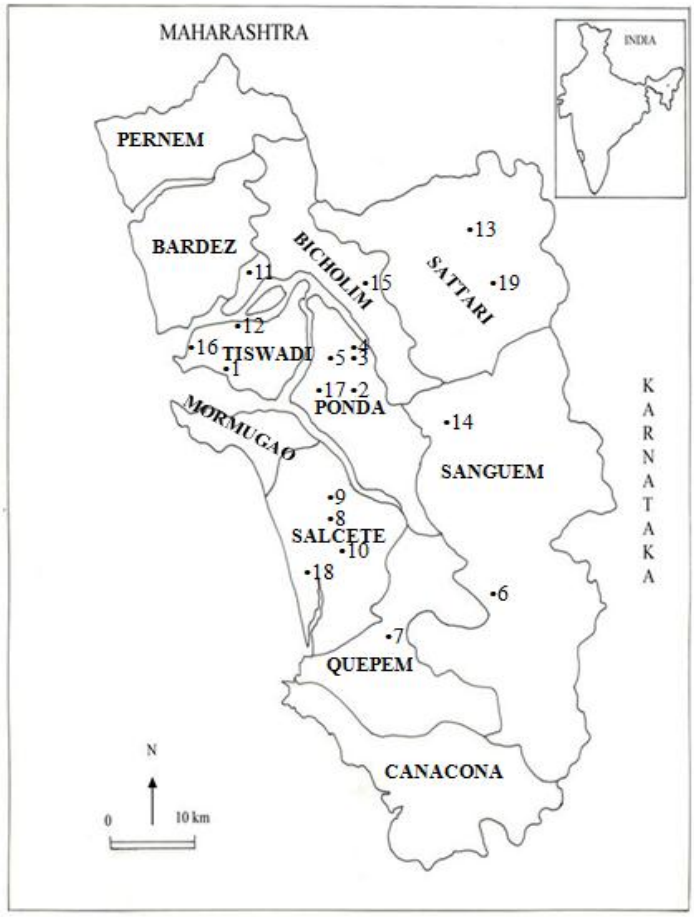

Figure 1. Map of Goa showing collection sites of dye yielding plant species.

1. Goa University campus; 2. Farmagudi; 3. Savoi Verem; 4. Betki; 5. Velkashi; 6. Selaulim dam; 7. Shantadurga temple; 8. Chowgule college campus; 9. Pajifond; 10. Margao; 11. Chorao island; 12. Ribandar; 13. Dhavo; 14. Darbandora; 15. Sanquelim; 16. Caranzalem; 17. Shiroda; 18. Borda; 19. Valpoi

3.2. Collection of dye yielding plants

The various plant parts such as colourful flowers, fruits, berries, leaves, roots, barks, seeds etc. were collected for their dyeing potential from the state of Goa. The fresh dye yielding plant parts were explored for the purpose of extraction of dye. The genus and species of each plant was identified using Flora of Goa and Flora of Karnataka. Later, the part yielding the dye was used for study purpose.

3.3. Extraction of dye from the plant materials

The plant part yielding the dye was collected, washed thoroughly with water and then used for extraction purpose. The dyes from flowers, leaves were extracted by grinding the fresh parts in mortar and pestle using the distilled water. The dyes from berries and fruits were extracted by crushing the material in distilled water, whereas the barks of trees were ground to a fine powder (50 meshes) and then boiled in distilled water for easy extraction of dye.

3.4. Optimization of extraction of dye parameters

3.4.1. Optimization of material to liquor ratio (MLR) of selected natural dyes

The selected plant parts yielding dye were taken in varying ratios of 1:10, 1:20, 1:30, 2:10 and 2:20 (g/ml) and were subjected for extraction by varying one parameter and keeping another parameter constant. The extracts were filtered in Whatman filter paper Grade-A. From each extract 1 mL of aliquot was taken separately and subjected to their optical density measurement. The optimum material to liquor ratio was determined in UV-visible spectrophotometer (UV-1800 Shimadzu) based on the highest value of optical density. The experiments were carried out in replicates of three.

3.4.2. Optimization of extraction time of selected natural dyes

The optimized quantity of plant parts yielding dye were boiled for varying time period viz. 30 min, 60 min, 180 min, 300 min and 480 mins in distilled water. The extracts were filtered in Whatman filter paper Grade-A. 1 mL of aliquot from each extract was taken separately and subjected to their optical density measurement. The optimum dye extraction time was determined in UV-visible spectrophotometer (UV-1800 Shimadzu) based on highest value of optical density. The experiments were carried out in replicates of three.

3.4.3. Optimization of extraction temperature of selected natural dyes

Based on optimum MLR and extraction time the selected dyes were subjected to extraction by varying temperature range from 10°C, 30°C, 50°C, 60°C and 80°C. The extract were filtered in Whatman filter paper Grade-A. 1 mL of aliquot from each extracts was taken separately and subjected to their optical density measurement. The optimum dye extraction temperature was determined in UV-visible spectrophotometer (UV-1800 Shimadzu) based on highest value of optical density. The experiments were carried out in replicates of three.

3.5. Pantone Matching System (PMS) chart

The extracted natural dyes were matched with the colour chart ie. Pantone Matching System (PMS) and the corresponding colour code i.e PMS number of each dye was determined (www.cal-print.com/InkColourChart.htm).

3.6. Physicochemical properties of dyes

Physicochemical value is one of the most simple and reliable tools to obtain complete information of crude drugs. Hence, the selected natural dyes were evaluated for their physicochemical characteristics using standard methods (Khandelwal, 2003). Physicochemical properties such as dye appearance, pH of 1% solution, water-soluble extractive, methanol-soluble extractive, ether soluble extractive, total ash, water soluble ash, acid insoluble ash and fluorescence analysis for natural dyes were determined.

3.6.1. Determination of appearance

The powdered dye was observed for their colour appearance. The observations of each dye were recorded.

3.6.2. Determination of pH of 1% solution

1 g of powdered natural dyes was separately extracted in 100 mL of distilled water by vigorous shaking using shaker. The solutions were then filtered using Whatman filter paper Grade-A. The pH value of each filtrate was examined using Eutech Instruments pH tutor. The observations were replicated three times.

3.6.3. Determination of water-soluble extractive

4 g of powdered dye sample was weighed and transferred into the conical flask. To this 100 mL of distilled water was added. The flask was continuously shaken for 24 h in the shaker. The contents were filtered using Whatman filter paper Grade-A into a separate conical flask. The 25 mL filtrate was then transferred into weighed porcelain dish and evaporated to dryness on a water bath and completely dried in oven at 100°C. The dish was cooled in desiccator and weighed. The soluble matter percentage was calculated with reference to the initial weight of the dye material. The observations were replicated three times.

Calculation Weight of plant material = X g

Weight of empty crucible = Y g

Weight of dried material + crucible = Z g

Weight of dried extract = (Z-Y) g

25 mL of water extract = (Z-Y) g of residue

100 mL of water extract = [(Z-Y)/25] × 100

= P g of dried extract

4 g of air-dried drug gives = P g of water-soluble extract

Therefore, 100 g of air-dried drug gives = [P/4] × 100 g of water-soluble extract

3.6.4. Determination of methanol soluble extractive

4 g of powdered dye sample was weighed and transferred into the conical flask. To this, 100 mL of methanol was added. The flask was continuously shaken for 24 h in the shaker. The contents were filtered using Whatman filter paper Grade-A into a separate conical flask. The 25 mL of filtrate was then transferred into the weighed porcelain dish and evaporated to dryness on a water bath and completely dried in oven at 100°C. The dish was cooled in the desiccator and weighed. The soluble matter percentage was calculated with reference to the initial weight of the dye material. The observations were replicated three times.

Calculation Weight of plant material = X g

Weight of empty crucible = Y g

Weight of dried material + crucible = Z g

Weight of dried extract = (Z-Y) g

25 mL of methanol extract = (Z-Y) g of residue

100 mL of methanol extract = [(Z-Y)/25] × 100

= P g of dried extract

4 g of air-dried drug gives = P g of methanol soluble extract

Therefore 100 g of air-dried drug gives = [P/4] × 100 g of methanol soluble extract

3.6.5. Determination of ether soluble extractive

4 g of powdered dye sample was weighed and transferred into the conical flask. To this 100 mL of petroleum ether was added. The flask was continuously shaken for 24 h in the shaker. The contents were filtered using Whatman filter paper Grade-A into a separate conical flask. The 25 mL of filtrate was then transferred into the weighed porcelain dish and evaporated to dryness on a water bath and completely dried in oven at 100°C. The dish

was cooled in the desiccator and weighed. The soluble matter percentage was calculated with reference to the initial weight of the dye material. The observations were replicated three times.

Calculation Weight of plant material = X g

Weight of empty crucible = Y g

Weight of dried material + crucible = Z g

Weight of dried extract = (Z-Y) g

25 mL of ether extract = (Z-Y) g of residue

100 mL of ether extract = [(Z-Y)/25] × 100

= P g of dried extract

4 g of air-dried drug gives = P g of ether soluble extract

Therefore 100 g of air-dried drug gives = [P/4] × 100 g of ether soluble extract

3.6.6. Determination of total ash

2 g of powdered dye samples were taken separately in a silica crucible previously ignited and weighed. The crucible with the powdered material was incinerated using Laboratory Muffle Furnace (MIC, India) by gradually increasing the heat, not exceeding (450-500°C) for 5 h, until the white ash free from carbon was obtained. The ash obtained was cooled in desiccator and weighed till constant weight. The observations were replicated three times and ash percentage was calculated with reference to the initial quantity of dye.

Calculation Weight of empty crucible = X g

Weight of plant material taken = Y g

Weight of crucible + ash (after complete incineration) = Z g

Weight of ash = (Z-X) g

Y g of plant material gives = (Z-X) g of ash

Therefore 100 g of plant material gives = [(Z-X)/Y] × 100 g of ash

Total ash value of sample = [(Z-X)/Y] × 100 %

3.6.7. Determination of water-soluble ash

The ash obtained was boiled with 25 mL of distilled water for five minutes and the residue was collected in an ashless filter paper (Grade 42). The filter paper was folded into the crucible and incinerated in the Laboratory Muffle Furnace (MIC, India) until all the carbon has been removed. The residue was weighed. The water-soluble ash percentage was calculated with reference to the initial quantity of sample. The observations were replicated three times.

Calculation Weight of the residue = X g

Y g of plant material gives = X g of water-soluble ash

Therefore 100 g of plant material gives = [X×Y]/100

Water-soluble ash value of the sample = [X×Y]/100 %

3.6.8. Determination of acid insoluble ash

The ash obtained was boiled with 25 mL of dilute HCl for five minutes and the residue was collected in an ashless filter paper (Grade 42). The filter paper was folded into the crucible and incinerated in the Laboratory Muffle Furnace (MIC, India) until all the carbon has been removed. The acid insoluble ash percentage was calculated with reference to the initial quantity of sample. The observations were replicated three times.

Calculation Weight of the residue = X g

Y g of plant material gives = X g of ash insoluble ash

Therefore 100 g of plant material gives = [X×Y]/100

Acid insoluble ash value of the sample = [X×Y]/100 %

3.6.9. Fluorescence analysis of dye samples

The small pinch of powdered plant material was taken in the test tubes. To this 5 mL of chemicals-reagents such as ethanol, methanol, NaOH, HCl, H_2SO_4, acetic acid, ferric chloride, ammonia, acetone, benzene, petroleum ether, chloroform, ethyl acetate, acetonitrile, picric acid, 2-propanol and di-ethyl ether were added. All the tubes were shaken well and after 30 minutes, the drop was taken on the slide and was observed for their characteristic colour reaction under the visible light, long UV of 365 nm and short UV of 254 nm under UV-Visualizer. The change in colour was noted.

3.7. Qualitative phytochemical screening of natural dyes

The powdered natural dyes were subjected to the evaluation of presence/absence of different groups of phytochemicals. The plant samples selected were extracted separately in water, ethanol, methanol, petroleum ether, chloroform and ethyl acetate solvents (10g/50 mL). The extracts so obtained were filtered and used for qualitative studies. All the tests were performed as per the standard methods (Raaman, 2006).

3.7.1. Test for Alkaloids

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

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

Mayer's test: To few mL of extract, 1-2 drops of Mayer's reagent was added. The creamish or white precipitate indicates the presence of alkaloids (Evans, 1997).

Dragendorff's test: To few mL of extract, 1-2 drops of Dragendorff's reagent was added. The yellow precipitate indicates the presence of alkaloids (Waldi, 1965).

3.7.2. Test for Tannins

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

3.7.3. Test for Saponins

Foam test: 5 mL 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).

3.7.4. Test for Phenolics and Flavonoids

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

Magnesium and HCl reduction test (Shinoda test): 5 g of each dye sample was dissolved in ethanol and warmed for few minutes, small pieces of magnesium chips were added to the filtrate followed by few drops of concentrated HCl. Formation of pink, orange, red to purple colouration indicates flavonoids (Harborne, 1998).

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

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

3.7.5. Test for Steroids

Salkowski test: To 2 mL of extract, 2 mL of chloroform was added; 2 mL of concentrated H_2SO_4 was added along the sides of the test tube. The chloroform layer showed red and the acid layer showed greenish yellow fluorescence, which indicates the presence of steroids (Sheel *et al*., 2014).

Liebermann Burchard reagent test: The dye sample (50 mg) was dissolved in 2 mL of acetic anhydride. 2 drops of concentrated H_2SO_4 was added slowly along the sides of the test tube. The colour changes from (first red, blue to green) shows the presence of phytosterols (Finar, 1986).

3.7.6. Test for Terpenoids

The powdered dyes (5 g) were dissolved in ethanol, 1 mL of acetic acid was added followed by concentrated H_2SO_4. The colour change from pink to violet indicates the presence of terpenoids (Sofowora, 1993).

3.7.7. Test for Anthraquinones

To 2 mL of filtrate, benzene (2 mL) and ammonia solution (1 mL) was added. Appearance of pink/red/ violet colour indicates anthraquinones (Gul *et al*., 2016).

3.7.8. Test for Carbohydrates

Molisch test: To 1 mL filtatre, few drops of Molisch's reagent was added followed by 1 mL of concentrated H_2SO_4 along the side of the test tube. The mixture was allowed to stand for two minutes and then diluted with 5 mL of distilled water. Formation of red or dull violet colour at the interface of the two layers was a positive test (Rasch and Swift, 1960).

Barfoed test: To the filtrate 1 mL of Barfoed's reagent was added and then heated on water bath for a period of 2 minutes. Reddish precipitate of cuprous oxide was considered as a positive test (Sofowora, 1993).

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

Fehling test: 1 mL of filtrate was heated with 5 mL of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of positive test (Sofowora, 1993).

3.7.9. Test for Proteins

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

Xanthoprotein test: To 3 mL of extract 1 mL concentrated H_2SO_4 was added. The appearance of white precipitate which turns to yellow on boiling and orange on addition of ammonium hydroxide (1 mL) indicates the presence of proteins containing tyrosine tryptophan (Shriner *et al*., 1964).

Ninhydrin test: To 3 mL 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).

3.7.10. Test for Coumarins

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

3.8. Sectioning of plant materials

The stem of monocot (*Cyanodon dactylon*) and dicot (*Chromolaena odorata*) were chosen for sectioning. Several thin-free hand sections of stems were taken with single stoke using sharp blade. The sections were allowed to float in water and thin sections were chosen for staining purpose.

3.9. Preparation of synthetic stain

Acridine orange stain and Safranin stain were prepared by dissolving 0.1 g of powder in 20 mL of distilled water. From this 1 mL of the solution were used for staining purpose. These stains were used for comparing the staining of natural dyes with the synthetic dyes.

3.10. Preparation of mordant solution

The three chemical mordants like potassium dichromate, copper sulphate and ferrous sulphate were used for staining purpose. 0.1 g of each mordants were weighed and dissolved in 20 mL of distilled water and 1 mL of the mordant solution was used for staining purpose.

3.11. Preparation of natural dye extracts for staining purpose

3.11.1. Extraction of dye from flowers

The water extract from the fresh flowers of *Averrhoa bilimbi* and corolla tube of *Nyctanthes arbor-tristis* were used for staining. 1 g of flowers/corolla tube was grinded in 20 mL of distilled water using mortar and pestle. The extract was filtered using Whatman filter paper (Grade-A) and 2 mL of extract was used for staining purpose.

3.11.2. Extraction of dye from fruits

The fruits of *Melastoma malabathricum*, *Garcinia indica* and *Phyllanthus reticulates* were used for staining. The fruits were crushed in water using mortar and pestle (1g/20mL). The reddish colour dye was filtered in Whatman filter paper (Grade-A) and 2 mL of extract was used for staining purpose.

3.11.3. Extraction of dye from bark

The bark was scraped and the heartwood was collected, shade-dried and milled using laboratory mixer. The powdered bark material of *Cassia fistula*, *Ceriops tagal*, *Mammea suriga*, *Peltophorum pterocarpum* and *Rhizophora mucronata* was weighed and the dye was extracted in distilled water (1g/20mL) in waterbath at 60°C for about 4-5 hours. The extracted dye was filtered using Whatman filter paper (Grade-A) and 2 mL of extract was used for staining purpose.

3.12. Staining of plant sections

The sections were stained for about 5, 15, 20 and 30 minutes. The optimum staining time with good contrast was determined for synthetic dyes ie. Safranin and Acridine orange, natural dyes individually and along with the chemical mordants. According to the optimum time, the staining of the section was carried out by following the staining protocol chart (Figure 2). After staining the sections were washed in distilled water to remove excess stain and mounted on the clean slide using glycerine.

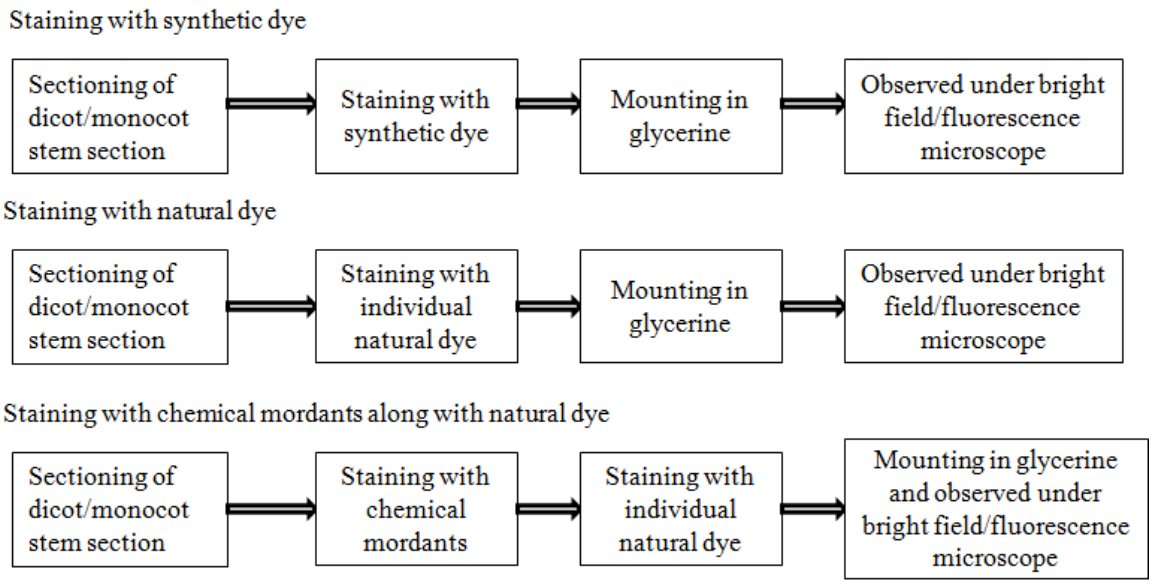

Figure 2. Staining protocols followed for monocot and dicot stem sections.

3.13. Photography of sections

The slides were observed under bright field compound microscope and the observation of all the selected slides were made and evaluated microscopically. The stained slides were studied under Nikon Eclipse E200 microscope and their staining intensity was identified and the photographs of selected preparations were taken using photographic unit attached with the microscope.

The slides were also observed under fluorescence microscope in five different filters of wavelength 400-450 nm (Violet excitation filter), 450-500 nm (Blue filter), 500-570 nm (Green filter), 570-610 nm (Yellow filter) and 610-750 nm (Red filter) under OLYMPUS BX53 microscope in X40 and X20 magnification. The staining intensity of each section were identified and studied.

3.14. Pre-treatment of the fabrics

3.14.1. Cotton fabrics

The commonly available *Gossypium* species was used for dyeing purpose. The white cotton was treated in sodium carbonate solution (2g/l) for 30 minutes at 60°C in hot water

bath and then washed with water and dried at room temperature. This cotton was used for dyeing purpose.

3.14.2. Silk fabrics

The hank of raw silk, non-twisted, containing 28% of sericin was purchased from Reshmnagar, Bangalore, India. The raw silk fabric contains sericin (gummy substance) which makes the dyeing difficult. Hence, this gum should be removed before dyeing. The hank of silk was dipped in water containing sodium carbonate (2g/l) for 30 minutes in water bath at 60°C. The fabric was later thoroughly washed with water, air dried and used for dyeing purpose.

3.15. Optimization of dyeing conditions

Optimization of dyeing parameters is very important in gaining optimum consuming condition of dye and mordants. For dyeing of cotton and silk fabrics, parameters like dye concentration and dyeing time were optimized based on the highest values of optical density.

3.15.1. Optimization of dye concentration

Different concentration of dyes (1 to 5 g) was extracted in 50 mL of distilled water. The optical density of each solution was noted down by UV-visible spectrophotometer. Around 0.1 g of silk and cotton fabrics were dipped separately in all the solutions prepared for each dye sample for about 30 minutes. After 30 minutes the dyed samples were removed from the dye bath and the optical density of each solution was measured spectrophotometrically. Finally, the percentage of dye absorption was calculated by using the following formula (Bhuyan and Saikia, 2004).

$$\% \text{ dye absorbance} = \frac{\text{Absorbance before dyeing - Absorbance after dyeing}}{\text{Absorbance before dyeing}} \times 100$$

3.15.2. Optimization of dyeing time

The optimum amount of dye material for each dye was taken in five different beakers and the dye was extracted at different periods. The optical density of each solution was noted down by UV-visible spectrophotometer (UV-1800 Shimadzu). Around 0.1g of silk and cotton fabrics were dipped separately in all the solutions prepared for each dye sample for about 30, 45, 60, 75 and 90 minutes and again the optical density of each solution was measured spectrophotometrically.

3.16. Dyeing and mordanting of the fabrics

The cotton and silk fabrics were dyed by using the natural extracts of *Mammea suriga* (bark) and *Ceriops tagal* (bark). Three chemical mordants were used for dyeing viz. potassium dichromate ($K_2Cr_2O_7$), ferrous sulphate ($FeSO_4$) and copper sulphate ($CuSO_4$) (Table 1). The dyeing was also done with the combination of these mordants. Potassium dichromate is a red-orange crystalline solid, which is highly soluble in water, it has many industrial applications and also used for dyeing purpose as a brightening mordant. Copper sulphate is readily soluble in water. It is used for dyeing purpose to give special effects in shades. It is known to produce green shades to the fabrics. Ferrous sulphate is one of the oldest mordant, which is easily soluble in water. It is used to get grey to black colour shades. The various shades obtained on using these natural dyes and mordants were described according to the ISCC-NBS (Inter-Society Colour Council) and (National Bureau of Standards) colour system (Kenneth and Deane, 1976). Around 267 colour categories with non-technical names were provided by ISCC-NBS colour system (Figure 3).

	ISCC-NBS Level 3 Name	sRGB
1	Vivid pink	253,121,146
2	Strong pink	244,143,160
3	Deep pink	230,105,128
4	Light pink	248,195,206
5	Moderate pink	226,163,174
6	Dark pink	197,128,138
7	Pale pink	239,209,220
8	Grayish pink	203,173,183
9	Pinkish white	239,221,229
10	Pinkish gray	199,182,189
11	Vivid red	213,28,60
12	Strong red	191,52,75
13	Deep red	135,18,45
14	Very deep red	92,6,37
15	Moderate red	177,73,85
16	Dark red	116,36,52
17	Very dark red	72,17,39
18	Light grayish red	180,136,141
19	Grayish red	152,93,98
20	Dark grayish red	83,56,62
21	Blackish red	51,33,39
22	Reddish gray	146,129,134
23	Dark reddish gray	93,78,83
24	Reddish black	48,38,43
25	Vivid yellowish pink	253,126,93
26	Strong yellowish pink	245,144,128
27	Deep yellowish pink	239,99,102
28	Light yellowish pink	248,196,182
29	Moderate yellowish pink	226,166,152
30	Dark yellowish pink	201,128,126
31	Pale yellowish pink	241,211,209
32	Grayish yellowish pink	203,172,172
33	Brownish pink	203,175,167
34	Vivid reddish orange	232,59,27
35	Strong reddish orange	219,93,59
36	Deep reddish orange	175,51,24
37	Moderate reddish orange	205,105,82
38	Dark reddish orange	162,64,43
39	Grayish reddish orange	185,117,101
40	Strong reddish brown	139,28,14
41	Deep reddish brown	97,15,18
42	Light reddish brown	172,122,115
43	Moderate reddish brown	125,66,59
44	Dark reddish brown	70,29,30
45	Light grayish reddish brown	158,127,122
46	Grayish reddish brown	108,77,75
47	Dark grayish reddish brown	67,41,42
48	Vivid orange	247,118,11
49	Brilliant orange	NaN,NaN,NaN
50	Strong orange	234,129,39

	ISCC-NBS Level 3 Name	sRGB
51	Deep orange	194,96,18
52	Light orange	251,175,130
53	Moderate orange	222,141,92
54	Brownish orange	178,102,51
55	Strong brown	138,68,22
56	Deep brown	87,26,7
57	Light brown	173,124,99
58	Moderate brown	114,74,56
59	Dark brown	68,33,18
60	Light grayish brown	153,127,117
61	Grayish brown	103,79,72
62	Dark grayish brown	62,44,40
63	Light brownish gray	146,130,129
64	Brownish gray	96,82,81
65	Brownish black	43,33,30
66	Vivid orange yellow	NaN,NaN,NaN
67	Brilliant orange yellow	255,190,80
68	Strong orange yellow	240,161,33
69	Deep orange yellow	208,133,17
70	Light orange yellow	252,194,124
71	Moderate orange yellow	231,167,93
72	Dark orange yellow	195,134,57
73	Pale orange yellow	238,198,166
74	Strong yellowish brown	158,103,29
75	Deep yellowish brown	103,63,11
76	Light yellowish brown	196,154,116
77	Moderate yellowish brown	136,102,72
78	Dark yellowish brown	80,52,26
79	Light grayish yellowish brown	180,155,141
80	Grayish yellowish brown	126,105,93
81	Dark grayish yellowish brown	77,61,51
82	Vivid yellow	241,191,21
83	Brilliant yellow	247,206,80
84	Strong yellow	217,174,47
85	Deep yellow	184,143,22
86	Light yellow	244,210,132
87	Moderate yellow	210,175,99
88	Dark yellow	176,143,66
89	Pale yellow	239,215,178
90	Grayish yellow	200,177,139
91	Dark grayish yellow	169,144,102
92	Yellowish white	238,223,218
93	Yellowish gray	198,185,177
94	Light olive brown	153,119,54
95	Moderate olive brown	112,84,32
96	Dark olive brown	63,44,16
97	Vivid greenish yellow	235,221,33
98	Brilliant greenish yellow	233,220,85
99	Strong greenish yellow	196,184,39
100	Deep greenish yellow	162,152,18

	ISCC-NBS Level 3 Name	sRGB
101	Light greenish yellow	233,221,138
102	Moderate greenish yellow	192,181,94
103	Dark greenish yellow	158,149,60
104	Pale greenish yellow	230,220,171
105	Grayish greenish yellow	190,181,132
106	Light olive	139,125,46
107	Moderate olive	100,89,26
108	Dark olive	53,46,10
109	Light grayish olive	142,133,111
110	Grayish olive	93,85,63
111	Dark grayish olive	53,48,28
112	Light olive gray	143,135,127
113	Olive gray	88,81,74
114	Olive black	35,33,28
115	Vivid yellow green	167,220,38
116	Brilliant yellow green	195,223,105
117	Strong yellow green	130,161,43
118	Deep yellow green	72,108,14
119	Light yellow green	206,219,159
120	Moderate yellow green	139,154,95
121	Pale yellow green	215,215,193
122	Grayish yellow green	151,154,133
123	Strong olive green	44,85,6
124	Deep olive green	NaN,NaN,NaN
125	Moderate olive green	73,91,34
126	Dark olive green	32,52,11
127	Grayish olive green	84,89,71
128	Dark grayish olive green	47,51,38
129	Vivid yellowish green	63,215,64
130	Brilliant yellowish green	135,217,137
131	Strong yellowish green	57,150,74
132	Deep yellowish green	23,106,30
133	Very deep yellowish green	5,66,8
134	Very light yellowish green	197,237,196
135	Light yellowish green	156,198,156
136	Moderate yellowish green	102,144,105
137	Dark yellowish green	47,93,58
138	Very dark yellowish green	16,54,26
139	Vivid green	35,234,165
140	Brilliant green	73,208,163
141	Strong green	21,138,102
142	Deep green	NaN,NaN,NaN
143	Very light green	166,226,202
144	Light green	111,172,149
145	Moderate green	51,119,98
146	Dark green	22,78,61
147	Very dark green	12,46,36
148	Very pale green	199,217,214
149	Pale green	148,166,163
150	Grayish green	97,113,110

	ISCC-NBS Level 3 Name	sRGB
151	Dark grayish green	57,71,70
152	Blackish green	31,42,42
153	Greenish white	224,226,229
154	Light greenish gray	186,190,193
155	Greenish gray	132,136,136
156	Dark greenish gray	84,88,88
157	Greenish black	33,38,38
158	Vivid bluish green	19,252,213
159	Brilliant bluish green	53,215,206
160	Strong bluish green	13,143,130
161	Deep bluish green	NaN,NaN,NaN
162	Very light bluish green	152,225,224
163	Light bluish green	95,171,171
164	Moderate bluish green	41,122,123
165	Dark bluish green	21,75,77
166	Very dark bluish green	10,45,46
167	Vivid greenish blue	NaN,NaN,NaN
168	Brilliant greenish blue	45,188,226
169	Strong greenish blue	19,133,175
170	Deep greenish blue	NaN,NaN,NaN
171	Very light greenish blue	148,214,239
172	Light greenish blue	101,168,195
173	Moderate greenish blue	42,118,145
174	Dark greenish blue	19,74,96
175	Very dark greenish blue	11,44,59
176	Vivid blue	27,92,215
177	Brilliant blue	65,157,237
178	Strong blue	39,108,189
179	Deep blue	17,48,116
180	Very light blue	153,198,249
181	Light blue	115,164,220
182	Moderate blue	52,104,158
183	Dark blue	23,52,89
184	Very pale blue	194,210,236
185	Pale blue	145,162,187
186	Grayish blue	84,104,127
187	Dark grayish blue	50,63,78
188	Blackish blue	30,37,49
189	Bluish white	225,225,241
190	Light bluish gray	183,184,198
191	Bluish gray	131,135,147
192	Dark bluish gray	80,84,95
193	Bluish black	36,39,46
194	Vivid purplish blue	68,54,209
195	Brilliant purplish blue	128,136,226
196	Strong purplish blue	83,89,181
197	Deep purplish blue	42,40,111
198	Very light purplish blue	183,192,248
199	Light purplish blue	137,145,203
200	Moderate purplish blue	77,78,135

	ISCC-NBS Level 3 Name	sRGB
201	Dark purplish blue	34,34,72
202	Very pale purplish blue	197,201,240
203	Pale purplish blue	142,146,183
204	Grayish purplish blue	73,77,113
205	Vivid violet	121,49,211
206	Brilliant violet	152,127,220
207	Strong violet	97,65,156
208	Deep violet	60,22,104
209	Very light violet	201,186,248
210	Light violet	155,140,202
211	Moderate violet	92,73,133
212	Dark violet	52,37,77
213	Very pale violet	208,198,239
214	Pale violet	154,144,181
215	Grayish violet	88,78,114
216	Vivid purple	185,53,213
217	Brilliant purple	206,140,227
218	Strong purple	147,82,168
219	Deep purple	101,34,119
220	Very deep purple	70,10,85
221	Very light purple	228,185,243
222	Light purple	188,147,204
223	Moderate purple	135,94,150
224	Dark purple	86,55,98
225	Very dark purple	55,27,65
226	Very pale purple	224,203,235
227	Pale purple	173,151,179
228	Grayish purple	123,102,126
229	Dark grayish purple	81,63,81
230	Blackish purple	47,34,49
231	Purplish white	235,223,239
232	Light purplish gray	195,183,198
233	Purplish gray	143,132,144
234	Dark purplish gray	92,82,94
235	Purplish black	43,38,48
236	Vivid reddish purple	212,41,185
237	Strong reddish purple	167,73,148
238	Deep reddish purple	118,26,106
239	Very deep reddish purple	79,9,74
240	Light reddish purple	189,128,174
241	Moderate reddish purple	150,88,136
242	Dark reddish purple	95,52,88
243	Very dark reddish purple	63,24,60
244	Pale reddish purple	173,137,165
245	Grayish reddish purple	134,98,126
246	Brilliant purplish pink	252,161,231
247	Strong purplish pink	244,131,205
248	Deep purplish pink	223,106,172
249	Light purplish pink	245,178,219
250	Moderate purplish pink	222,152,191

	ISCC-NBS Level 3 Name	sRGB
251	Dark purplish pink	198,125,157
252	Pale purplish pink	235,200,223
253	Grayish purplish pink	199,163,185
254	Vivid purplish red	221,35,136
255	Strong purplish red	184,55,115
256	Deep purplish red	136,16,85
257	Very deep purplish red	84,6,60
258	Moderate purplish red	171,75,116
259	Dark purplish red	110,41,76
260	Very dark purplish red	67,20,50
261	Light grayish purplish red	178,135,155
262	Grayish purplish red	148,92,115
263	White	231,225,233
264	Light gray	189,183,191
265	Medium gray	138,132,137
266	Dark gray	88,84,88
267	Black	43,41,43

Figure 3. Chart representing names of colours by ISCC-NBS colour system.

Table 1. Preparation and mordanting time of mordants on cotton and silk fabrics.

Sr. No.	Mordants	Concentration (g)	Water added (mL)	Mordanting time (min)
1	Potassium dichromate	0.1	10	30
2	Copper sulphate	0.1	10	30
3	Ferrous sulphate	0.1	10	30

3.16.1. Methods used for dyeing

In pre-mordanting, the fabric was treated with respective mordant for 30 min and then in the dye solution as per the optimum time of dyeing. In simultaneous mordanting the dye and the mordant solution was mixed together and used for dyeing purpose. In post-mordanting the fabric was treated with the solution of dye prior to the mordant solution (Bhuyan and Saikia, 2004).

3.16. 2. pH test for dyed fabrics

The pH test for both the cotton and silk fabrics dyed with and without mordants was done using the standard method (ISO: 3071). 0.1 g of dyed fabric was weighed and cut into pieces and added in 50 mL of distilled water. The conical flask was kept on shaker for about 2 h at 30 rpm. After 2 h the pH of each dye was determined using Eutech Instruments pH tutor. All the readings were taken in replicates of three.

3.17. Determination of colourfastness of dyed cotton and silk fabrics

Colour fastness is the property of the colourant which allows the fabric to retain its characteristics despite of degradation conditions like washing, rubbing, exposure to light, human perspiration rate etc. The fastness property mainly depends on the dye and the process involved in dyeing. The silk and cotton fabrics were undergone following different fastness tests to understand the best method and best dye for dyeing purpose.

- Colourfastness to washing
- Colourfastness to rubbing
- Colourfastness to sunlight

3.17.1. Colourfastness to washing

The dyed silk and cotton fabrics were tested for their colourfastness to washing without following any laundering procedure. The dyed cotton and silk specimens were placed in the solution containing (2g/l) sodium carbonate. The specimens were washed for about 15 minutes and allowed to dry. The change in colour of the washed specimen with that of the unwashed specimen was evaluated with the help of the gray scale. The washing of the same specimen was done three times, and the sample evaluation was done with the gray scale after every wash.

3.17.2. Colourfastness to rubbing

The dyed silk and cotton fabrics to be tested for rub fastness was sandwiched between 10×10 cm size tissue paper and was rubbed manually to and fro in a straight line for about 5 minutes and 15 minutes. After rubbing, the fabric was allowed to dry and evaluated with the gray scale.

3.17.3. Colourfastness to sunlight

The dyed silk and cotton fabrics were fixed on the paper and the sample was exposed to direct sunlight for about 6 h and 12 h. After 6 h and 12 h the sample was rated with the gray scale. The fastness was assessed by change in colour of the exposed fabric to that of the unexposed one.

All the colourfastness tests of dyed cotton and silk fabrics were assessed for change of colour and staining using the standard gray scales (ISO 105-A02).

Gray scales helps to specify a rating from 1-5 with 5-good (no change) and 1-poor (severe change) (Figure 4).

The scale has 9 possible values: 5, 4/5, 4, 3/4, 3, 2/3, 2, 1/2, 1.

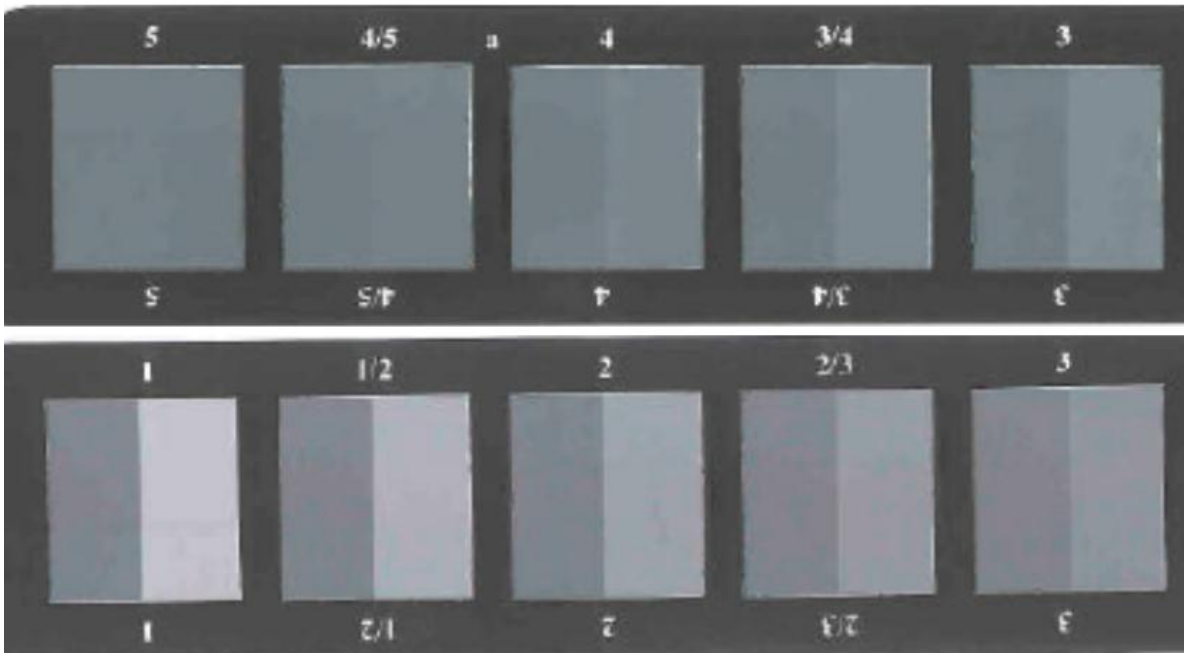

Figure 4. Gray scales for assessing change in colour.

3.18. Photography of fabrics

The photographs of fabric before dyeing, after dyeing and after colour fastness tests were taken on digital camera fitted with macro lens (Nikon D-90).

3.19. Bright field microscopy and Scanning Electron Microscopy (SEM) of dyed fabrics

0.1 g of silk and cotton fabrics were dyed in 10 mL of natural dye extracts of *Cassia fistula*, *Ceriops tagal*, *Garcinia indica*, *Mammea suriga*, *Nyctanthes arbor-tristis*, *Peltophorum pterocarpum* and *Rhizophora mucronata* for 1 h. The dyed fabrics were dried and the single fibre of each dyed and undyed fabric was observed under bright field microscope under X40. The SEM was used to investigate the surface morphology of the fabrics. The single fibre was coated with Gold and Palladium and observed under 1 KX magnification using EVO18 Zeiss Special edition SEM. The SEM was done in USIC (University Science Instrumentation Centre), Goa University.

3.20. Characterization of natural dyes

3.20.1. Extraction of plant sample

The powdered plant material (40 g) was subjected to extraction in soxhlet apparatus in (250 mL) methanol as a solvent. Continuous extraction was performed for about 24 h at 60°C till the extraction process was completed. The obtained extract was filtered in Whatman filter paper Grade- A and concentrated in rotary evaporator. The concentrated extract obtained was used for experimental purpose.

3.20.2. UV-Visible spectroscopic analysis

UV-Visible spectroscopy is used to identify the species according to their absorption band in the spectra (Donner *et al*., 1997). The spectrum of the dye shows its peak at predominating wavelength, thereby indicating the main hue. Peaks in the UV and visible region usually at (200-800 nm or higher) indicates the main colour and absorption. For analysis, UV visible spectrophotometer (model: UV-1800 Shimadzu UV spectrophotometer) was used. A solution of each dye sample of known concentration was prepared using methanol as a solvent. Methanol was served as blank since the extracts were prepared in methanol. The optic glass cuvettes were used for analysis. The dyes were scanned through a range of wavelength 200 to 800 nm and the absorbance spectrum of each dye was recorded in UV-visible spectrophotometer. The wavelength that gave the highest absorption was taken as the maximum absorption wavelength for each dye.

3.20.3. Separation of compounds by TLC (Thin Layer Chromatography)

A silica glass plate was placed on a clear surface and a faint pencil line parallel to one of the edges at a distance of 2 cm was drawn from the edge. The solution of dye sample was loaded using capillary tube at the marked point which serves as an origin. After continuously loading the spots, the spots were allowed to dry and then placed in the chromatography chamber. The different solvent systems in various ratios were prepared and the loaded plates were placed in chromatography chamber. The care was taken to ensure that the developing solvent does not touch the spotted sample. The separation was carried out till the solvent reach $3/4^{th}$ of the plate. The solvent front was marked with the

pencil and the plate was allowed to dry. The bands visualized on each plate were photographed and finally the Rf values of each were calculated.

3.20.4. FT-IR analysis (Fourier Transform Infrared Spectroscopy)

The spectrometer (model: Shimadzu FT-IR 8900) was used to record the IR spectra of samples. The wave number (IR) range was 5000 to 400 cm^{-1} and far IR range was 700 to 50 cm^{-1} with resolution 0.1 cm^{-1}. Before the sample measurement, the instrument was calibrated using KBr pellet. The IR spectrum of each sample was recorded by mixing a small amount of sample with KBr. The powdered sample and KBr was ground to fine homogeneous mixture (particle size ≤ 5 mm) as the large particles scatter the infrared beam and cause a slope baseline of spectrum. The amount of powder sample used was about 0.1-2%.

3.20.5. GC-MS analysis (Gas Chromatography- Mass Spectrometry)

The GC-MS analysis of plant extracts was carried out using Gas chromatography-mass spectrophotometer model Trace 1300 GC coupled with THERMO TSQ8000 Triple Quadrupole MS. 1 µl of each sample was used for GC-MS analysis. The GC-MS analysis was carried out using Agilent HP5MS with column length 30 metre, 0.25 mm ID, and 0.32 thicknesses. Helium gas was used as carrier gas at constant flow rate of 1mL/ minute. Injector temperature was set at 260°C. The oven temperature was programmed from 50°C to 280°C at 10°C/ minute to 200°C then 100°C/3 minutes to 250°C ending with a 5 minutes isothermal at 270°C. The sample was injected in split mode as 10:1. The chemical components were identified by comparing their mass fragmentation patterns with those on the stored NIST library (National Institute of Standards and Technology).

4. RESULTS

4.1. Checklist of dye yielding plants

The present investigation revealed 62 dye yielding plants belonging to 37 different families. Maximum number of eleven dye yielding plant species were documented from the family Fabaceae, followed by Myrtaceae, Phyllanthaceae, Rhizophoraceae and Rubiaceae with three species each. Remaining families namely Amaranthaceae, Bignoniaceae, Clusiaceae, Combretaceae, Euphorbiaceae, Lythraceae, Meliaceae and Nyctanginaceae contained two species and Acanthaceae, Alliaceae, Anacardiaceae, Apiaceae, Apocynaceae, Arecaceae, Asterceae, Bixaceae, Bombacaceae, Cactaceae, Caricaceae, Casurinaceae, Chenopodiaceae, Lecythidaceae, Lamiaceae, Malvaceae, Melastomataceae, Moraceae, Oleaceae, Oxalidaceae, Rhamnaceae, Rutaceae, Solanaceae and Zingiberaceae with one species each.

All the dye yielding plants were identified at species level and listed alphabetically along with their botanical name, family, plant parts used and the place of collection (Table 2).

Table 2. Botanical name, family and collection sites of extracted natural dyes.

Botanical Name	Family	Season of Flowering/ Fruiting	Application of Dye	Place of Collection
***Acacia auriculiformis* A.Cunn. ex Benth.**	Fabaceae	Throughout the year	Used for dyeing textiles	Goa University (GU) campus, Tiswadi
***Acacia nilotica* (L.) Delile**	Fabaceae	Throughout the year	Used for dyeing textiles, used in preparation of ink	Dharbandora, Sanguem
***Aegle marmelos* (L.) Correa**	Rutaceae	May-June	Used for dyeing textiles	Shiroda, Ponda
***Allium cepa* L.**	Alliaceae	May-July	Used for dyeing textiles	Borda, Salcete

***Amaranthus tricolour* L.**	Amaranthaceae	April-May	Edible dye	Margao, Salcete
***Areca catechu* L.**	Arecaceae	April-May	Edible dye	Savoi Verem, Ponda
***Artocarpus heterophyllus* Lam.**	Moraceae	Throughout the year	Used for dyeing textiles	Savoi Verem, Ponda
***Averrhoa bilimbi* L.**	Oxalidaceae	October-December	Used for colouring foodstuffs	Betki, Ponda
***Azadirachta indica* A. Juss.**	Meliaceae	Throughout the year	Used for dyeing textiles	Pajifond, Salcete
***Bauhinia purpurea* L.**	Fabaceae	September-November	Used for dyeing textiles	GU campus, Tiswadi
***Beta vulgaris* L.**	Chenopodiaceae	Throughout the year	Used for dyeing textiles, edible	Pajifond, Salcete
***Bixa orellana* L.**	Bixaceae	Throughout the year	Dye is used in food colouring and cosmetics	Selaulim dam, Sanguem
***Bombax ceiba* L.**	Bombacaceae	November-december	Used for dyeing silk, cotton	GU campus, Tiswadi
***Bougainvillea glabra* Choisy**	Nyctaginaceae	Throughout the year	Used for dyeing textiles	GU campus, Tiswadi
***Bridelia retusa* (L.) A. Juss.**	Phyllanthaceae	Throughout the year	Used for dyeing textiles	Chowgule college campus, Salcete
***Butea monosperma* (Lam.) Taub.**	Fabaceae	January-march	Used for dyeing textiles, food colouring	Selaulim dam, Sanguem
***Caesalpinia pulcherrima* (L.) Sw.**	Fabaceae	Throughout the year	Used in dyeing textiles	Dhavo, Sattari
***Capsicum annuum* L.**	Solanaceae	Throughout the year	Edible dye	Pajifond, Salcete
***Carica papaya* L.**	Caricaceae	Throughout the year	Used for dyeing textiles	GU campus, Tiswadi
***Cassia fistula* L.**	Fabaceae	March-May	Used for dyeing textiles	GU campus, Tiswadi
***Casuarina equisetifolia* L.**	Casuarinaceae	Throughout the year	Used in dyeing textiles	GU campus, Tiswadi
Catunaregam	Rubiaceae	Throughout	Used in dyeing	Mollem,

***spinosa* (Thunb.) Tirveng.**		the year	textiles	Dharbandora
***Ceriops tagal* (Pers.) C. B. Rob.**	Rhizophoraceae	Throughout the year	Used in dyeing textiles	Chorao island, Tiswadi
***Clitoria ternatea* L.**	Fabaceae	Throughout the year	Used for dyeing textiles	Ribandar, Tiswadi
***Couroupita guianensis* Aubl.**	Lecythidaceae	Throughout the year	Used as ink	Chowgule college campus, Salcete
***Curcuma longa* L.**	Zingiberaceae	Throughout the year	Dye is used as antiseptic	Valpoi, Sattari
***Dalbergia sissoo* Roxb.**	Fabaceae	March-May	Colouring textiles	Savoi Verem, Ponda
***Daucus carota* L.**	Apiaceae	June-September	Orange dye is used as a food dye	Pajifond, Salcete
***Delonix regia* (Bojer.) Raf.**	Fabaceae	April-May	Textile dyeing	GU campus, Tiswadi
***Eucalyptus globulus* Labill.**	Myrtaceae	January-August	Dye from bark is used as a food dye	Chowgule college campus, Salcete
***Garcinia indica* Choisy**	Clusiaceae	April-june	Used in cosmetics and textile dyeing	Dhavo, Sattari
***Hibiscus rosa-sinensis* L.**	Malvaceae	Throughout the year	Rich in anthocyanins, textile dyeing	GU campus, Tiswadi
***Ixora coccinea* L.**	Rubiaceae	Throughout the year	Used as acid-base indicator	GU campus, Tiswadi
***Justicia adhatoda* L.**	Acanthaceae	Throughout the year	Used for dyeing textiles	Pajifond, Salcete
***Lannea coromandelica* (Houtt.) Merr.**	Anacardiaceae	Throughout the year	Used in dyeing textiles	GU campus, Tiswadi
***Lawsonia inermis* L.**	Lythraceae	Throughout the year	Used in hair dyeing	Savoi Verem, Ponda
Mallotus philippensis	Euphorbiaceae	April-May	Used for dyeing textiles	Velkashi, Ponda

(Lam.) Muell. Arg.				
***Mammea suriga* (Buch.- Ham. ex Roxb.) Kosterm.**	Clusiaceae	April-May	Used in dyeing textiles	GU campus, Tiswadi
***Melastoma malabathricum* L.**	Melastomataceae	Throughout the year	Dye is used as natural stain	Farmagudi, Ponda
***Mirabilis jalapa* L.**	Nyctanginaceae	Throughout the year	Used to colour cakes and jellies	Pajifond, Salcete
***Nerium oleander* L.**	Apocynaceae	Throughout the year	Used in textiles	GU campus, Tiswadi
***Nyctanthes arbor-tristis* L.**	Oleaceae	October-December	Yellow dye is used for colouring foodstuffs	Shantadurga temple, Quepem
***Opuntia ficus-indica* (L.) Mill.**	Cactaceae	Throughout the year	Dyeing wool, also as food colouring	Sanquelim
***Peltophorum pterocarpum* (DC.) Backer ex K. Heyne**	Fabaceae	March-May	Used to colour batik	GU campus, Tiswadi
***Phyllanthus acidus* (L.) Skeels**	Phyllanthaceae	April-June	Used in textile dyeing	GU campus, Tiswadi
***Phyllanthus reticulates* Poir.**	Phyllanthaceae	Throughout the year	Used as an ink	Caranzalem, Tiswadi
***Psidium guajava* L.**	Myrtaceae	Throughout the year	Used in dyeing textiles	GU campus, Tiswadi
***Punica granatum* L.**	Lythraceae	Throughout the year	Dyeing cotton fabrics	Pajifond, Salcete
***Rhizophora apiculata* Blume**	Rhizophoraceae	August-December	Used in dyeing textiles	Chorao island, Tiswadi
***Rhizophora mucronata* Lam.**	Rhizophoraceae	August-December	Used in dyeing textiles	Chorao island, Tiswadi
***Rubia cordifolia* L.**	Rubiaceae	August-September	Used for dyeing textiles	Shiroda, Ponda
***Spathodea campanulata* P.**	Bignoniaceae	Throughout the year	Used for dyeing textiles	GU campus, Tiswadi

Beauv.				
***Spinacia oleracea* L.**	Amaranthaceae	Throughout the year	Edible green dye	Pajifond, Salcete
***Switenia mahogany* (L.) Jacq.**	Meliaceae	Throughout the year	Used to flavor puddings	Selaulim dam, Sanguem
***Syzygium cumini* (L.) Skeels.**	Myrtaceae	July-September	Used for histological staining	Chowgule college campus, Salcete
***Tagetes erecta* L.**	Asteraceae	Throughout the year	Used in flavouring cacao	Pajifond, Margao
***Tamarindus indica* L.**	Fabaceae	December-January	Used for dyeing textiles	GU campus, Tiswadi
***Tecoma stans* (L.) Juss. ex Kunth.**	Bignoniaceae	Throughout the year	Used as substitutes for synthetic dyes	GU campus, Tiswadi
***Tectona grandis* L. f.**	Lamiaceae	June-September	Used for dyeing cotton and wool	Savoi Verem, Ponda
***Terminalia catappa* L.**	Combretaceae	November-March	Used in dyeing textiles	GU campus, Tiswadi
***Terminalia paniculata* Roth.**	Combretaceae	April-July	Used in dyeing textiles	GU campus, Tiswadi
***Ziziphus mauritiana* Lam.**	Rhamnaceae	February-March	Used in dyeing silk, cotton	GU campus, Tiswadi

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4.1.2. Selected dye yielding plants

Out of 62 dye yielding plants, 10 potential dye plants such as flowers of *Averrhoa bilimbi*, bark of *Cassia fistula*, bark of *Ceriops tagal*, fruits of *Garcinia indica*, bark of *Mammea suriga*, fruits of *Melastoma malabathricum*, corolla tube of *Nyctanthes arbor-tristis*, bark of *Peltophorum pterocarpum*, fruits of *Phyllanthus reticulates* and bark of *Rhizophora mucronata* were selected for elaborate study (Plate 1).

Plate 1. Selected dye yielding plants: 1. flowers of *Averrhoa bilimbi*; 2. bark of *Cassia fistula*; 3. bark of *Ceriops tagal*; 4. fruits of *Garcinia indica*; 5. bark of *Mammea suriga*; 6. fruits of *Melastoma malabathricum*; 7. corolla tube of *Nyctanthes arbor-tristis;* 8. bark of *Peltophorum pterocarpum*; 9. fruits of *Phyllanthus reticulates;* 10. bark of *Rhizophora mucronata*

4.2. Optimization of extraction parameters of natural dyes

Various parameters for dye extraction were optimized for all the selected dye species by varying one parameter and keeping the other parameter constant. The parameters considered during dye extraction process were material to liquor ratio (MLR), dye extraction time and dye extraction temperature. Based on the highest value of optical density for each particular set of the experiment the extraction was carried out for each selected dye.

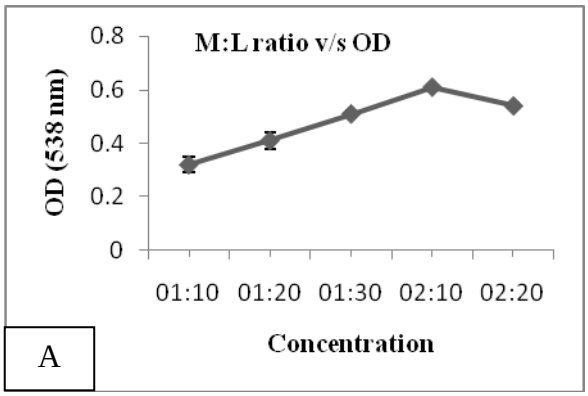
4.2.1. Optimization of Material to Liquor Ratio (MLR)

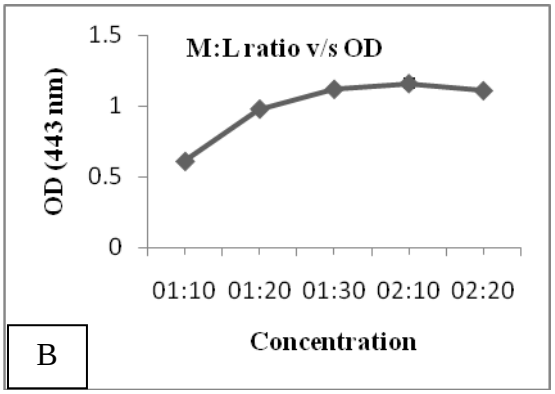
The highest value of optical density for each dye was found at (2g/10 mL). Hence, (2g/10 mL) MLR was used for extraction of dye from selected plants. Results of MLR optimization is tabulated (Table 3) and (Figure 5).

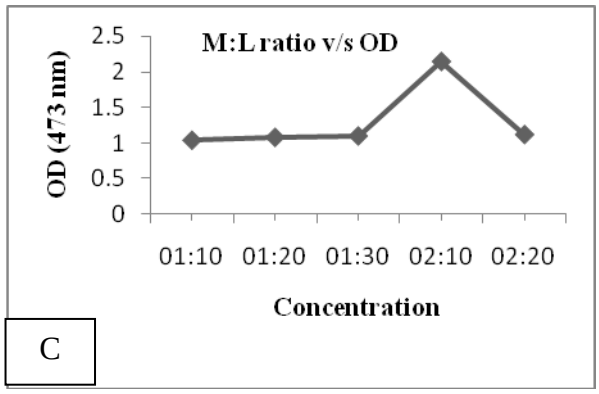
Table 3. Optimization of MLR for selected dye species.

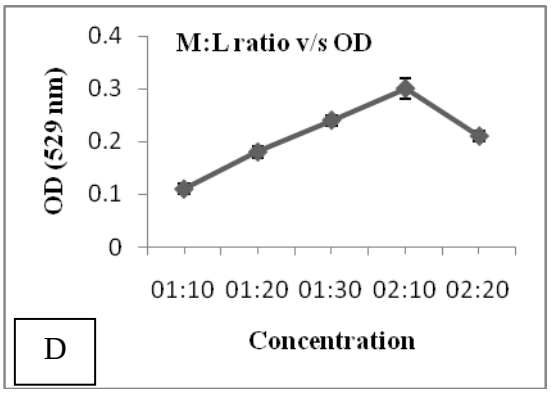
Plant species	**Wavelength λ_{max} (nm)**	**Absorbance at different (M:L) material to liquor ratio (g/ml)**				
		1:10	**1:20**	**1:30**	**2:10**	**2:20**
Averrhoa bilimbi	**538**	0.32±0.03	0.41±0.03	0.51±0.01	**0.61±0.01**	0.54±0.01
Cassia fistula	**443**	0.61±0.01	0.98±0.01	1.12±0.01	**1.16±0.03**	1.11±0.01
Ceriops tagal	**473**	1.04±0.02	1.08±0.02	1.10±0.01	**2.14±0.01**	1.12±0.02
Garcinia indica	**529**	0.11±0.01	0.18±0.01	0.24±0.01	**0.3±0.12**	0.21±0.01
Melastoma malabathricum	**531**	0.43±0.01	0.46±0.04	0.49±0.03	**0.54±0.01**	0.47±0.03
Mammea suriga	**568**	0.25±0.02	0.29±0.01	0.31±0.02	**0.42±0.01**	0.32±0.04
Nyctanthes arbor-tristis	**440**	1.12±0.01	1.14±0.01	1.16±0.01	**1.70±0.01**	1.12±0.01
Peltophorum pterocarpum	**529**	1.08±0.03	1.14±0.01	1.25±0.03	**1.42±0.02**	1.40±0.01
Phyllanthus reticulates	**437**	0.94±0.04	0.98±0.05	1.12±0.01	**1.24±0.03**	1.13±0.01
Rhizophora mucronata	**474**	0.67±0.01	0.74±0.01	0.84±0.04	**0.91±0.01**	0.30±0.02

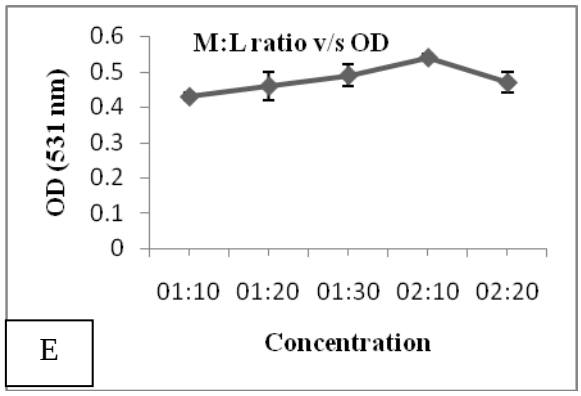
Values are mean of 3 readings and ± is standard deviation

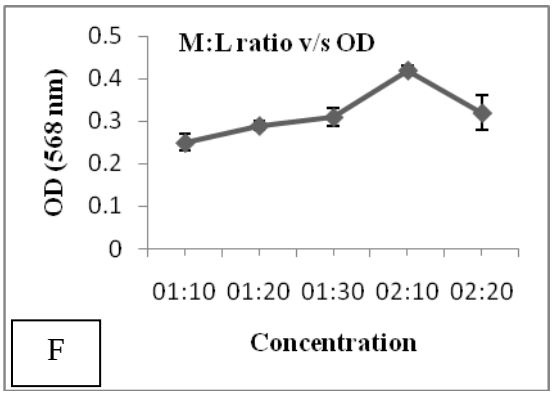


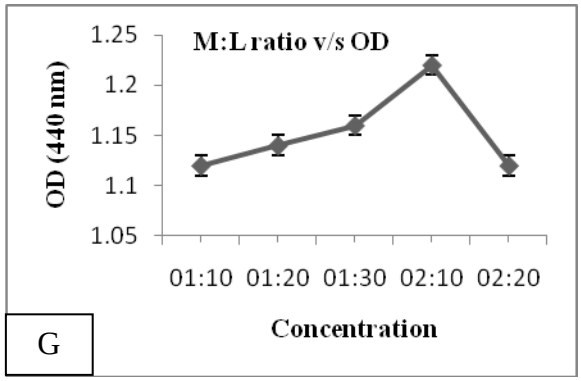


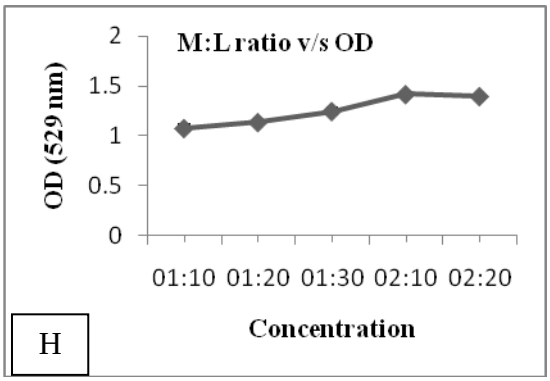












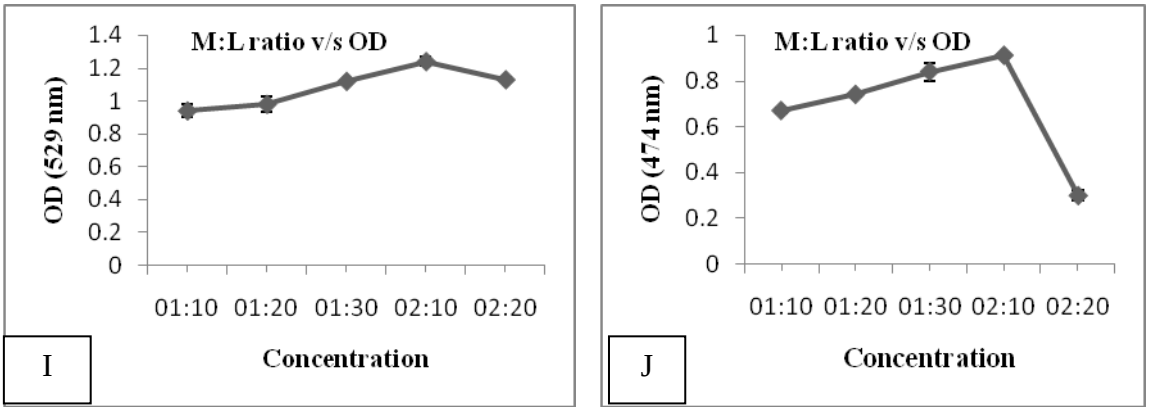


Figure 5. Optimization of Material to liquor ratio (MLR) A- *Averrhoa bilimbi*; B- *Cassia fistula*; C- *Ceriops tagal*; D- *Garcinia indica*; E- *Melastoma malabathricum*; F- *Mammea suriga*; G- *Nyctanthes arbor-tristis*; H- *Peltophorum pterocarpum*; I- *Phyllanthus reticulates*; J- *Rhizophora mucronata.*

4.2.2. Optimization of extraction time

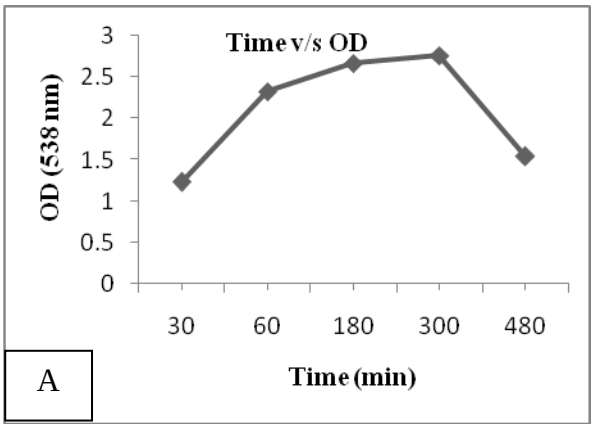
The extraction time for each dye was determined based on the highest value of optical density measured spectroscopically under the optimized MLR ratio. The optical density increased as the dye extraction time was increased, and reached maximum at 300 mins. At 480 mins the optical density was lowered. Hence, 300 mins was determined as the optimum dye extraction time for all the plant species (Table 4) and (Figure 6).

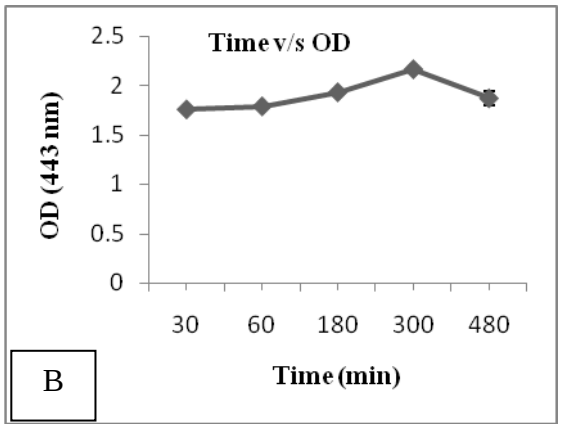
Table 4. Optimization of extraction time for selected dye species.

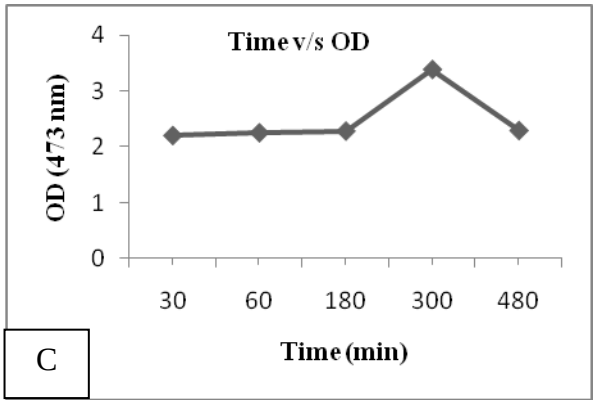
Plant species	Wavelength λ_{max} (nm)	Absorbance at different time (min)				
		30	60	180	300	480
Averrhoa bilimbi	**538**	1.23±0.01	2.33±0.01	2.67±0.01	**2.76±0.01**	1.54±0.01
Cassia fistula	**443**	1.76±0.01	1.79±0.01	1.93±0.02	**2.16±0.01**	1.87±0.07
Ceriops tagal	**473**	2.20±0.01	2.25±0.02	2.28±0.01	**3.38±0.01**	2.29±0.01
Garcinia indica	**529**	2.11±0.01	2.16±0.01	2.18±0.01	**3.29±0.01**	1.12±0.01
Melastoma malabathricum	**531**	2.12±0.04	2.23±0.02	3.51±0.02	**3.81±0.03**	1.60±0.09
Mammea suriga	**568**	1.13±0.01	2.19±0.01	2.23±0.04	**2.38±0.01**	1.25±0.02

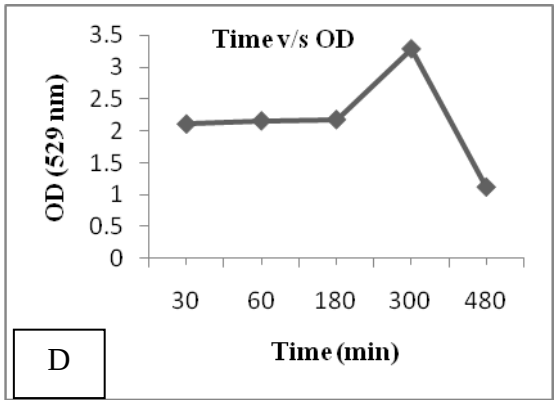
Nyctanthes arbor-tristis	**440**	1.97±0.09	2.27±0.07	2.40±0.11	**2.78±0.19**	2.29±0.11
Peltophorum pterocarpum	**449**	1.59±0.13	2.45±0.23	2.69±0.02	**3.63±0.33**	2.79±0.06
Phyllanthus reticulates	**529**	1.37±0.05	1.46±0.04	1.57±0.01	**1.63±0.01**	0.53±0.02
Rhizophora mucronata	**474**	1.50±0.04	2.14±0.05	2.40±0.05	**2.67±0.18**	1.95±0.16

Values are mean of 3 readings and ± is standard deviation









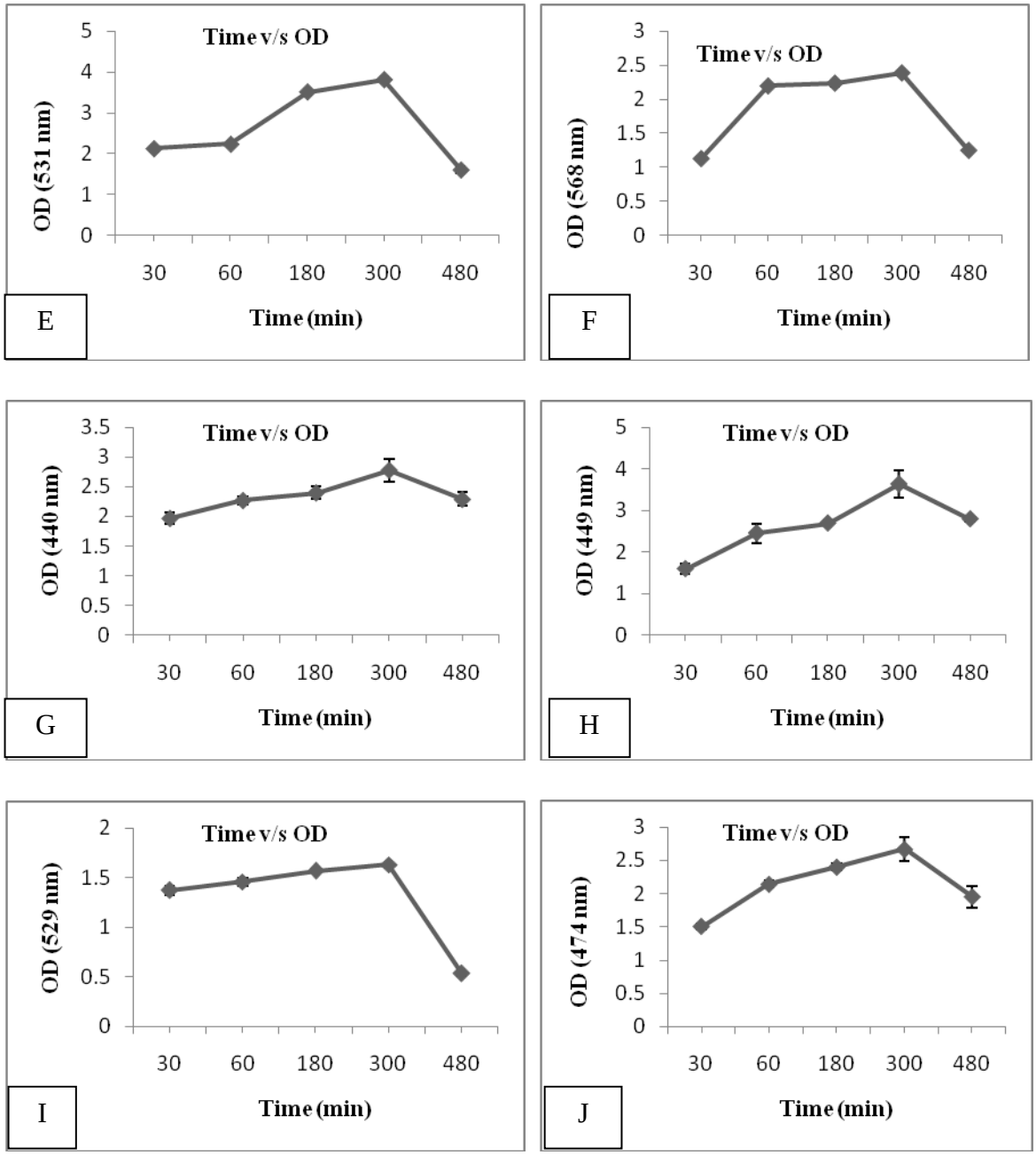


Figure 6. Optimization of Extraction Time A- *Averrhoa bilimbi*; B- *Cassia fistula*; C- *Ceriops tagal*; D- *Garcinia indica*; E- *Melastoma malabathricum*; F- *Mammea suriga*; G- *Nyctanthes arbor-tristis*; H- *Peltophorum pterocarpum*; I- *Phyllanthus reticulates*; J- *Rhizophora mucronata.*

4.2.3. Optimization of extraction temperature

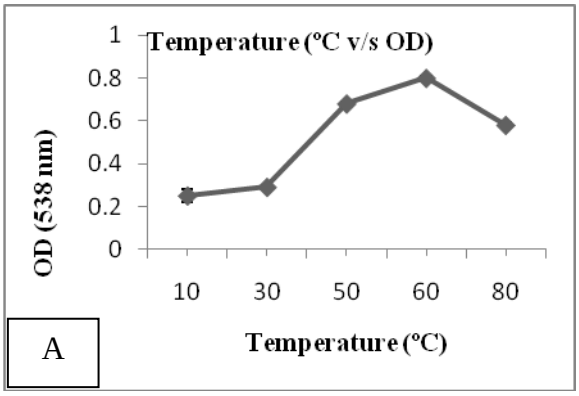
The extraction temperature for each dye was determined based on the highest value of optical density measured spectroscopically under the optimized MLR ratio and

optimized extraction time. The optical density increased as the dye extraction temperature was increased, but it was highest at 60°C. At 80°C the optical density was lowered. Hence, 60°C was determined as the optimum dye extraction temperature for all the plant species (Table 5) and (Figure 7).

Table 5. Optimization of extraction temperature for selected dye species.

Plant species	Wavelength λ_{max} (nm)	Absorbance at different temperature (°C)				
		10	**30**	**50**	**60**	**80**
Averrhoa bilimbi	**538**	0.25±0.03	0.29±0.01	0.68±0.01	**0.80±0.01**	0.58±0.01
Cassia fistula	**443**	0.74±0.08	0.91±0.01	0.98±0.01	**1.61±0.08**	0.83±0.04
Ceriops tagal	**473**	0.12±0.01	0.24±0.01	0.34±0.02	**0.41±0.01**	0.33±0.02
Garcinia indica	**529**	0.09±0.01	0.11±0.01	0.14±0.02	**0.27±0.01**	0.14±0.01
Melastoma malabathricum	**531**	0.14±0.01	0.25±0.02	0.38±0.04	**0.61±0.04**	0.41±0.04
Mammea suriga	**568**	0.13±0.01	0.15±0.01	0.30±0.06	**0.75±0.05**	0.24±0.01
Nyctanthes arbor-tristis	**440**	0.82±0.01	0.94±0.01	1.45±0.04	**2.11±0.07**	1.52±0.05
Peltophorum pterocarpum	**449**	0.56±0.01	0.82±0.01	0.93±0.02	**1.14±0.01**	0.94±0.01
Phyllanthus reticulates	**529**	0.74±0.01	0.99±0.01	1.01±0.01	**1.04±0.01**	1.00±0.01
Rhizophora mucronata	**474**	0.44±0.01	0.58±0.06	1.31±0.05	**1.45±0.02**	1.34±0.07

Values are mean of 3 readings and ± is standard deviation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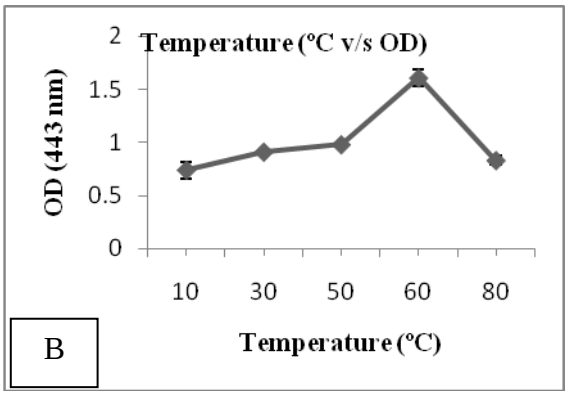
0.2
0
10
30
50
60
80
Temperature (°C)
A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OD (443 nm)
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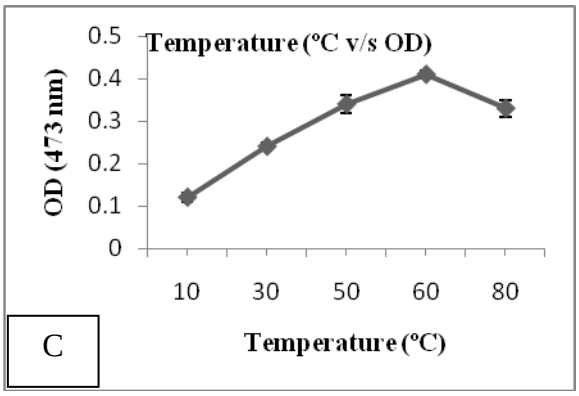
60
80
Temperature (°C)
B


Temperature (°C v/s OD)
OD (473 nm)
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0
10
30
50
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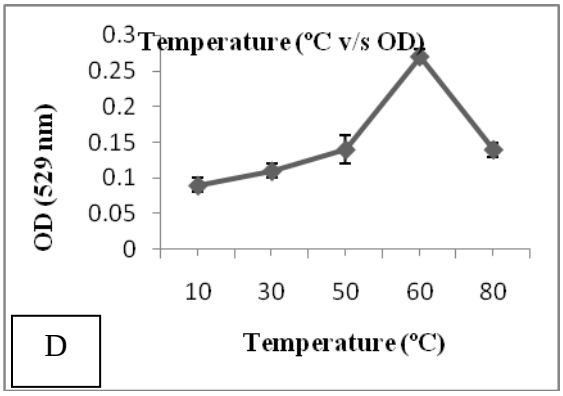
Temperature (°C)
C


Temperature (°C v/s OD)
OD (529 nm)
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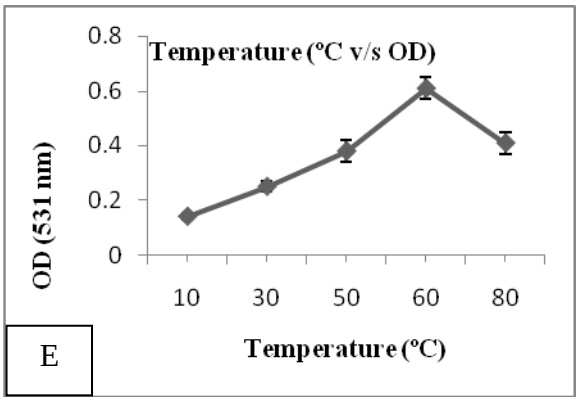
0.1
0.05
0
10
30
50
60
80
Temperature (°C)
D


Temperature (°C v/s OD)
OD (531 nm)
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10
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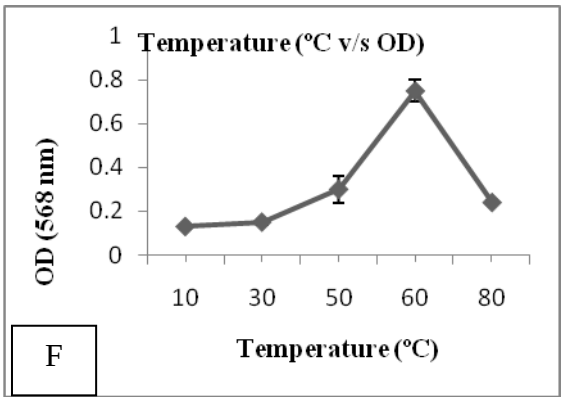
50
60
80
Temperature (°C)
E


Temperature (°C v/s OD)
OD (568 nm)
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Temperature (°C)
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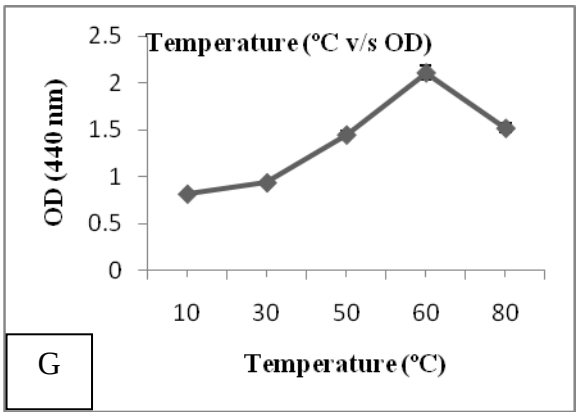
Temperature (°C v/s OD)
OD (440 nm)
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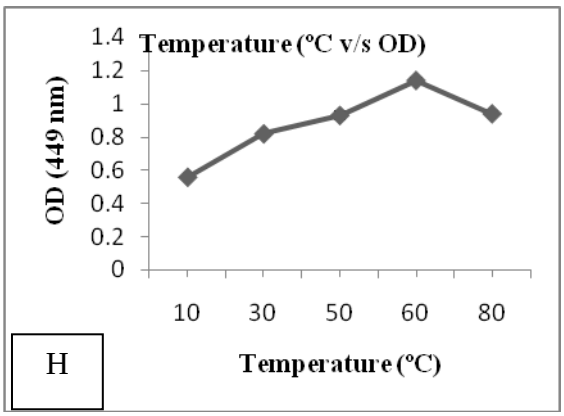
10
30
50
60
80
Temperature (°C)
G


Temperature (°C v/s OD)
OD (449 nm)
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0.6
0.4
0.2
0
10
30
50
60
80
Temperature (°C)
H

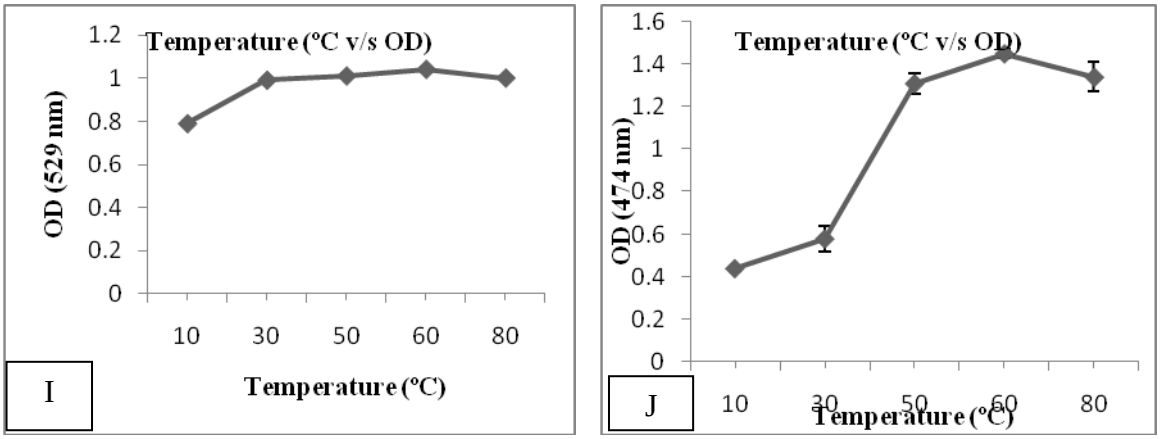

Figure 7. Optimization of Extraction Temperature A- *Averrhoa bilimbi*; B- *Cassia fistula*; C- *Ceriops tagal*; D- *Garcinia indica*; E- *Melastoma malabathricum*; F- *Mammea* suriga; G- *Nyctanthes arbor-tristis*; H- *Peltophorum pterocarpum*; I- *Phyllanthus reticulates*; J- *Rhizophora mucronata.*

Thus, from the above experiments the optimum values for all parameters were finalized. The MLR ratio of each dye was (2g/l), extraction time was 300 min and extraction temperature were found to be 60°C for all the plant species.

Dyes were extracted from all sixty- two plant species and the colour of the dye obtained from each species is provided (Table 6) (Plate 2 and Plate 3).

Table 6. Plant species, parts used, methods of extraction and PMS number of extracted dye species.

Plant Name	Plant part used	Quantity of material (g)	Quantity of water (mL)	Methods of extraction of dyes	PMS (Pantone Matching System) number of extracted natural dyes
Acacia auriculiformis	Bark	2	10	Powdered bark is dissolved in water, and heated in the water bath at	PMS151

				60°C for 4-5 hours.	
Acacia nilotica	Pods	2	10	Powder of pods is dissolved in water and boiled in the water bath at 60°C for 4-5 hours.	PMS141
Aegle marmelos	Fruit rinds	2	10	The rind of the fruits is dried and grounded to fine powder and boiled in water at 60°C for 4-5 hours.	PMS143
Allium cepa	Outer skin of bulb	2	10	The outer skin of onion bulb is boiled in water at 60°C for 4-5 hours.	PMS4755
Amaranthus tricolour	Leaves	2	10	The leaves are washed thoroughly and then cut into fine pieces and boiled in water for 4-5 hours.	PMS412
Areca catechu	Nuts	2	10	The nuts are grounded into fine powder and then boiled in water for 4-5 hours.	PMS4665
Artocarpus	Bark	2	10	Powdered	PMS1505

heterophyllus				bark is dissolved in water, and heated in water bath at 60°C for 4-5 hours.	
Averrhoa bilimbi	Flowers	2	10	The flowers are cut into fine pieces and grinded in mortar and pestle to extract the dye.	PMS412
Azadirachta indica	Leaves	2	10	The leaves are washed thoroughly and then cut into fine pieces and boiled in water for 4-5 hours.	PMS384
Bauhinia purpurea	Bark	2	10	Powdered bark is dissolved in water, and heated in water bath at 60°C for 4-5 hours.	PMS143
Beta vulgaris	Roots	2	10	Roots are chopped into fine pieces and soaked in water to extract the dye.	PMS1797
Bixa orellana	Seeds	2	10	Seeds are boiled in	PMS173

				water at 60°C for 4-5 hours to extract the dye.	
Bombax ceiba	Bark	2	10	Powdered bark is dissolved in water, and heated in the water bath at 60°C for 4-5 hours.	PMS150
Bougainvillea glabra	Bracts of flowers	2	10	The bracts of flowers are cut into pieces and grinded in mortar and pestle to extract the dye.	PMS199
Bridelia retusa	Bark	2	10	Powdered bark is dissolved in water, and heated in the water bath at 60°C for 4-5 hours.	PMS154
Butea monosperma	Flowers	2	10	Flowers are cut into fine pieces and grinded in mortar and pestle to extract the dye.	PMS4695
Caesalpinia pulcherrima	Bark	2	10	Powdered bark is dissolved in water, and	PMS136

				heated in the water bath at 60°C for 4-5 hours.	
Capsicum annuum	Fruits	2	10	Fruits are crushed and soaked in water and grinded in mortar and pestle to extract the dye.	PMS172
Carica papaya	Leaves	2	10	Leaves are washed thoroughly in water and then cut into fine pieces and boiled in water for 4-5 hours.	PMS370
Cassia fistula	Bark	2	10	Powdered bark is dissolved in water, and heated in water bath at 60°C for 4-5 hours.	PMS484
Casuarina equisetifolia	Bark	2	10	Powdered bark is dissolved in water, and heated in water bath at 60°C for 4-5 hours.	PMS1365
Catunaregam spinosa	Bark	2	10	Powdered bark is dissolved in	PMS103

				water, and heated in water bath at 60°C for 4-5 hours.	
Ceriops tagal	Bark	2	10	Powdered bark is dissolved in water, and heated in the water bath at 60°C for 4-5 hours.	PMS158
Clitoria ternatea	Flowers	2	10	Flowers are cut into fine pieces and grinded in mortar and pestle to extract the dye.	PMS2757
Couroupita guianensis	Fruits	2	10	Fruits are crushed and soaked in water and grinded in mortar and pestle to extract the dye.	PMS124
Curcuma longa	Rhizome	2	10	Fresh rhizomes are chopped into pieces and soaked in water to extract the dye.	PMS137
Dalbergia sissoo	Bark	2	10	Powdered bark is dissolved in	PMS153

				water, and heated in the water bath at 60°C for 4-5 hours.	
Daucus carota	Roots	2	10	The roots of the bark is chopped into fine pieces and then dissolved in water until the maximum dye is extracted.	PMS137
Delonix regia	Bark	2	10	Powdered bark is dissolved in water, and heated in water bath at 60°C for 4-5 hours.	PMS139
Eucalyptus globulus	Bark	2	10	Powdered bark is dissolved in water, and heated in the water bath at 60°C for 4-5 hours.	PMS1395
Garcinia indica	Fruit rinds	2	10	The rind of the fruits is dried and grounded to fine powder and then boiled in water bath at 60°C for 4-5 hours.	PMS1797

Hibiscus rosa-sinensis	Flowers	2	10	Flowers are cut into pieces and grinded in mortar and pestle to extract the dye.	PMS5025
Ixora coccinea	Flowers	2	10	Flowers are cut into pieces and grinded in mortar and pestle to extract the dye.	PMS136
Justicia adhatoda	Leaves	2	10	Leaves were thoroughly washed with water and then cut into fine pieces and boiled in water for 4-5 hours to extract the dye.	PMS583
Lannea coromandelica	Bark	2	10	Powdered bark is dissolved in water, and heated in water bath at 60°C for 4-5 hours.	PMS173
Lawsonia inermis	Leaves	2	10	Leaves are washed thoroughly and then cut into fine pieces and	PMS399

				grinded in water to extract the dye.	
Mallotus philippensis	Fruit capsules	2	10	Fruit capsules are collected and soaked and boiled in water to extract the dye.	PMS478
Mammea suriga	bark	2	10	Powdered bark is dissolved in water, and heated in water bath at 60°C for 4-5 hours.	PMS1375
Melastoma malabathricum	Fruits	2	10	Fruits are crushed in water and grinded in mortar and pestle to extract the dye.	PMS5115
Mirabilis jalapa	Flowers	2	10	Flowers are cut into fine pieces and grinded in mortar and pestle to extract the dye.	PMS483
Nerium oleander	Flowers	2	10	Flowers are cut into fine pieces and grinded in mortar and pestle to	PMS434

				extract the dye.	
Nyctanthes arbor-tristis	Flower stalk	2	10	Flower stalk are cut into fine pieces and grinded in mortar and pestle to extract the dye.	PMS130 2X
Opuntia ficus-indica	Cladodes	2	10	The cladodes of the plants are chopped into fine pieces and grinded in water to extract the dye.	PMS1805
Peltophorum pterocarpum	Bark	2	10	Powdered bark is dissolved in water, and heated in water bath at 60°C for 4-5 hours.	PMS130 2X
Phyllanthus acidus	Bark	2	10	Powdered bark is dissolved in water, and heated in water bath at 60°C for 4-5 hours.	PMS144
Phyllanthus reticulates	fruits	2	10	Fruits are crushed and soaked in water and grinded in mortar and	PMS209

				pestle to extract the dye.	
Psidium guajava	Bark	2	10	Powdered bark is dissolved in water, and heated in water bath at 60°C for 4-5 hours.	PMS718
Punica granatum	Fruit rinds	2	10	The rinds of the fruits are soaked in water and grinded in mortar and pestle for extraction of dye.	PMS110
Rhizophora apiculata	Bark	2	10	Powdered bark is dissolved in water, and heated in water bath at 60°C for 4-5 hours.	PMS717
Rhizophora mucronata	Bark	2	10	Powdered bark is dissolved in water, and heated in water bath at 60°C for 4-5 hours.	PMS717
Rubia cordifolia	Roots	2	10	Roots are chopped into fine pieces and then soaked in	PMS711

				water until the maximum dye is extracted.	
Spathodea campanulata	Bark	2	10	Powdered bark is dissolved in water and heated in water bath at 60°C for 4-5 hours.	PMS145
Spinacia oleracea	Leaves	2	10	Leaves are washed thoroughly in water and then cut into fine pieces and boiled in water to extract the dye.	PMS364
Switenia mahogany	Bark	2	10	Powdered bark is dissolved in water and heated in water bath at 60°C for 4-5 hours.	PMS1235
Syzygium cumini	Fruits	2	10	Fruits are crushed and soaked in water and grinded in mortar and pestle to extract the dye.	PMS2385
Tagetes erecta	Flowers	2	10	Flowers are cut into fine	PMS121

				pieces and grinded in mortar and pestle to extract the dye.	
Tamarindus indica	Leaves	2	10	Leaves are washed thoroughly with water and then cut into fine pieces and boiled in water to extract the dye.	PMS383
Tecoma stans	Flowers	2	10	Flowers are cut into pieces and grinded in mortar and pestle to extract the dye.	PMS106
Tectona grandis	Leaves	2	10	Leaves are washed thoroughly in water and then cut into fine pieces and grinded in water to extract the dye.	PMS1805
Terminalia catappa	Bark	2	10	Powdered bark is dissolved in water and heated in water bath at	PMS1788 2X

				60°C for 4-5 hours.	
Terminalia paniculata	Bark	2	10	Powdered bark is dissolved in water and heated in water bath at 60°C for 4-5 hours.	PMS145
Ziziphus mauritiana	Bark	2	10	Powdered bark is dissolved in water and heated in water bath at 60°C for 4-5 hours.	PMS717

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Plate 2. Different colours obtained from dye yielding plants: 1. *Acacia auriculiformis*; 2. *Acacia nilotica*; 3. *Aegle marmelos*; 4. *Allium cepa*; 5. *Amaranthus tricolor*; 6. *Areca catechu*; 7. *Artocarpus heterophyllus*; 8. *Averrhoa bilimbi*; 9. *Azadirachta indica*; 10. *Bauhinia purpurea*; 11. *Beta vulgaris*; 12. *Bixa orellana*; 13. *Bombax ceiba*; 14. *Bougainvillea glabra*; 15. *Bridelia retusa*; 16. *Butea monosperma*; 17. *Caesalpinia pulcherrima*; 18. *Capsicum annuum*; 19. *Carica papaya*; 20. *Cassia fistula*; 21. *Casuarina equisetifolia*; 22. *Catunaregam spinosa*; 23. *Ceriops tagal*; 24. *Clitoria ternatea*; 25. *Couroupita guianensis*; 26. *Curcuma longa*; 27. *Dalbergia sissoo;* 28. *Daucus carota;* 29. *Delonix regia;* 30. *Eucalyptus globulus.*

Plate 3. Different colours obtained from dye yielding plants: 1. *Garcinia indica*; 2. *Hibiscus rosa-sinensis*; 3. *Ixora coccinea*; 4. *Justicia adhatoda*; 5. *Lannea coromandelica*; 6. *Lawsonia inermis*; 7. *Mallotus phillipensis*; 8. *Mammea suriga* 9. *Melastoma malabathricum*; 10. *Mirabilis jalapa*; 11. *Nerium oleander*; 12. *Nyctanthes arbor-tristis*; 13. *Opuntia ficus-indica*; 14. *Peltophorum pterocarpum*; 15. *Phyllanthus acidus*; 16. *Phyllanthus reticulates* 17. *Psidium guajava*; 18. *Punica granatum*; 19. *Rhizophora apiculata*; 20. *Rhizophora mucronata*; 21. *Rubia cordifolia*; 22. *Spathodea campanulata*; 23. *Spinacia oleracea*; 24. *Switenia mahogany*; 25. *Syzygium cumini*; 26. *Tagetes erecta*; 27. *Tamarindus indica*; 28. *Tecoma stans*; 29. *Tectona grandis*; 30. *Terminalia catappa*; 31. *Terminalia paniculata*; 32. *Ziziphus mauritiana.*

4.3. Physicochemical properties of dyes

Physicochemical properties are used for identification and standardization of plant material for pharmaceutical purpose. Physicochemical properties such as colour, pH of 1% solution, water soluble extractive, methanol soluble extractive, ether soluble extractive, total ash, water soluble ash, acid insoluble ash and fluorescence analysis for selected natural dyes are summarized (Table 7-20).

4.3.1. Colour of dye yielding plants

The powders of *Averrhoa bilimbi*, *Melastoma malabathricum* were pink in colour, the bark powders of *Cassia fistula*, *Ceriops tagal*, *Mammea suriga*, *Peltophorum pterocarpum* and *Rhizophora mucronata* were brown in colour, *Garcinia indica* powder appeared red in colour, orange colour was exhibited by *Nyctanthes arbor-tristis* whereas powder from *Phyllanthus reticulates* was purple in colour (Table 7).

Table 7. Physicochemical parameters (colour) for dye yielding plants.

Dye yielding plants	Colour
Averrhoa bilimbi	Pink
Cassia fistula	Brown
Ceriops tagal	Brown
Garcinia indica	Red
Mammea suriga	Brown
Melastoma malabathricum	Dark pink
Nyctanthes arbor-tristis	Orange
Peltophorum pterocarpum	Brown
Phyllanthus reticulates	Purple
Rhizophora mucronata	Brown

4.3.2. Determination of pH of 1% solution of dye extracts

All the dye extracts showed pH as acidic in nature ranging from 2-6 pH. The fruit extract of *Garcinia indica* is more acidic in nature with (2.57 pH), followed by fruit extract of *Phyllanthus reticulates* (4.09 pH) and *Nyctanthes arbor-tristis* (4.32 pH). The other dye extracts such as *Averrhoa bilimbi*, *Cassia fistula*, *Mammea suriga*, *Melastoma malabathricum*, *Peltophorum pterocarpum* and *Rhizophora mucronata* exhibited the pH in the range of (5-6 pH). The pH of each dye extract is provided (Table 8).

Table 8. pH of 1% solution of dye yielding plants.

Dye yielding plants	pH	Inference
Averrhoa bilimbi	5.06±0.02	acidic
Cassia fistula	5.53±0.02	acidic
Ceriops tagal	5.21±0.01	acidic
Garcinia indica	2.57±0.02	acidic
Mammea suriga	5.71±0.01	acidic
Melastoma malabathricum	5.03±0.02	acidic
Nyctanthes arbor-tristis	4.32±0.01	acidic
Peltophorum pterocarpum	5.04±0.02	acidic
Phyllanthus reticulates	4.09±0.02	acidic
Rhizophora mucronata	6.10±0.01	acidic

pH 1-6 denotes acidic, pH 7 denotes neutral and pH 8-14 denotes basic

All readings are mean of three values and ± SD (standard deviation)

4.3.3. Determination of water-soluble, methanol soluble and ether soluble extractive

The water soluble, methanol soluble and ether soluble extractive was determined for each plant species and it was found that the methanol soluble extractive was higher compared to that of water soluble extractive and ether soluble extractive. The methanol soluble extractive was found to be in the range of (24.9-3.9), water soluble extractive was found to be in the range of (9.6-3.1) and ether soluble extractive was in the range of (2.5-1.2). The percentage of extractive yield of dye in different solvents is tabulated (Table 9) (Figure 8).

Table 9. Percentage extractive yield of dye plants using different solvents.

Dye yielding plants	Water-soluble extractive	Methanol soluble extractive	Ether soluble extractive
Averrhoa bilimbi	4.1±0.1	12.0±0.21	2.1±0.13
Cassia fistula	3.1±0.23	6.0±0.1	2.0±0.12
Ceriops tagal	7.2±0.12	24.9±0.03	2.1±0.08
Garcinia indica	5.1±0.14	8.3±0.13	2.0±0.07
Mammea suriga	9.6±0.03	21.1±0.22	2.5±0.13
Melastoma malabathricum	8.1±0.07	14.1±0.21	2.5±0.14
Nyctanthes arbor-tristis	6.2±0.2	8.4±0.23	2.3±0.23
Peltophorum pterocarpum	3.1±0.2	3.9±0.07	1.2±0.13
Phyllanthus reticulates	5.2±0.12	10.1±0.09	2.1±0.13
Rhizophora mucronata	4.3±0.13	15.5±0.12	2.4±0.12

All readings are mean of three values ± SD (standard deviation)

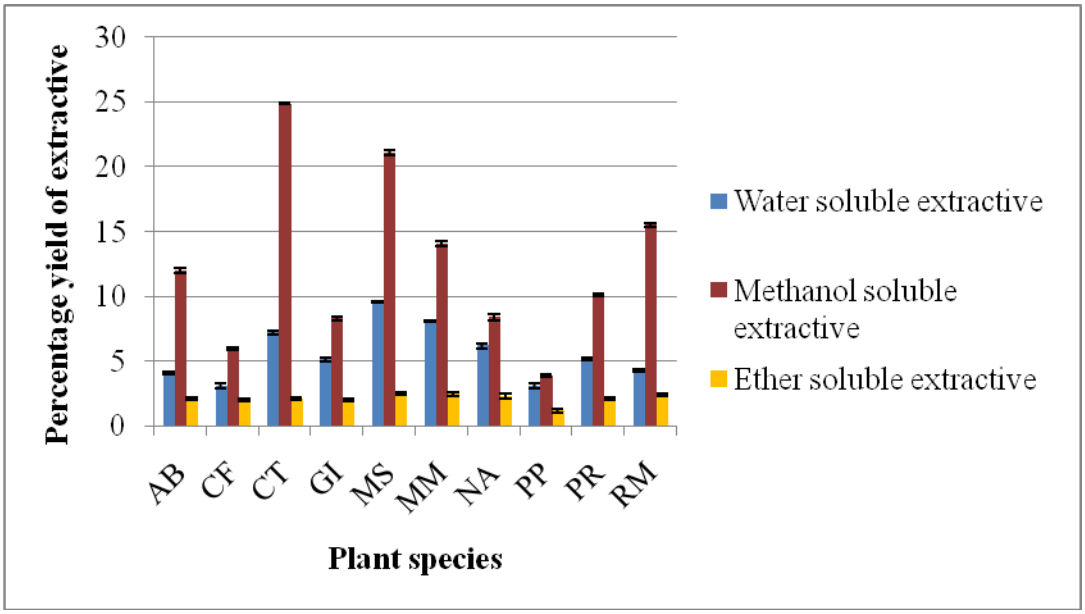


Figure 8. Percentage extractive yields of dye plants.

4.3.4. Determination of total ash, water-soluble and acid insoluble ash

Total ash values was found to be highest in all the bark extracts ie. (13.5%) for *R. mucronata* followed by *P. pterocarpum* (13.2 %), *M. suriga* (10.67 %), *C. fistula* (10.26 %) and *C. tagal* (10.1%). The dye extracts with higher ash values indicates the presence of inorganic matter in higher amount. The total ash values was lowest for fruits of *M.*

malabathricum (5.5%), followed by *P. reticulates* (7.1%) and *G. indica* (7.17%). The water -soluble ash and acid insoluble ash was found between the range of (3.86-8.12%) and (1.66-6.45%) respectively. The pH of the total ash of all the dye extracts was basic in nature. The total ash content of each dye extract along with the percentage of water soluble and acid insoluble ash is provided (Table 10) (Figure 9).

Table 10. Estimation of total ash, water soluble and acid insoluble ash content in dye yielding plant species.

Dye yielding plants	**Physical state**	**Colour of ash**	**% of Ash Content**			**pH of total ash**
			Total ash	**Water soluble ash**	**Acid insoluble ash**	
Averrhoa bilimbi	fine powder	grey	8.5±0.78	4.55±0.05	3.32±0.10	10.51±0.46
Cassia fistula	fine powder	black	10.26±0.19	7.04±0.03	5.08±0.08	10.64±0.01
Ceriops tagal	fine powder	grey	10.1±1.26	6.34±0.02	5.21±0.33	12.70±0.06
Garcinia indica	fine powder	grey	7.17±0.25	4.08±0.11	3.68±0.12	9.79±0.12
Mammea suriga	coarse powder	dark black	10.67±0.67	8.12±0.06	4.48±0.55	10.66±0.03
Melastoma malabathricum	fine powder	grey	5.5±0.25	3.86±0.06	1.66±0.21	8.60±0.07
Nyctanthes arbor-tristis	fine powder	black	9.3±1.39	7±0.25	3.9±0.11	10.72±0.06
Peltophorum pterocarpum	coarse powder	light grey	13.2±0.26	6.95±0.09	6.45±0.02	13.04±0.03
Phyllanthus reticulates	fine powder	grey	7.1±0.21	6.8±0.15	4.97±0.19	10.66±0.01
Rhizophora	fine powder	black	13.5±0.50	7.5±0.20	3.7±0.15	10.44±0.04

mucronata						

All readings are mean of three values ± SD (standard deviation)

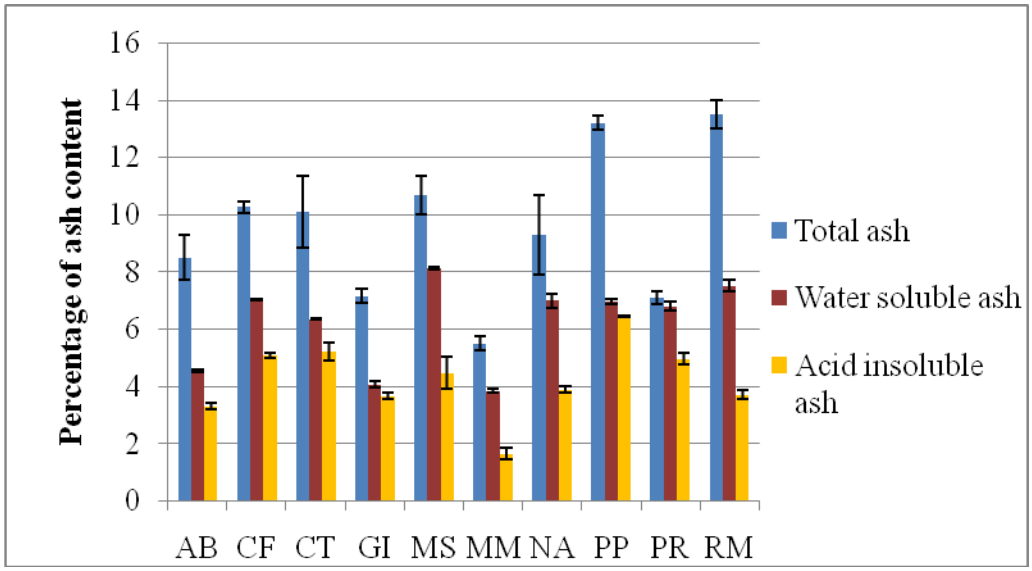


Figure 9. Percentage of ash content in dye plants.

The physicochemical characteristics of the dye extracts are considered as a standard physicochemical marker in authentication and in quality control process. These values help in detection of adulterants and substitutes in natural products.

4.3.5. Fluorescence analysis of dye powders

The UV light produces fluorescence in natural products which do not fluoresce in daylight. The powder analysis helps in identification of crude drugs. Among the chemicals used 1 N HCl, concentrated H_2SO_4, ethanol, methanol, 50% H_2SO_4, ammonia, acetone, chloroform, 2- propanol and picric acid showed fluorescence in some plant powders. The results of powder analysis of all the dye extracts are provided (Table 11-20).

Table 11. Fluorescence analysis of flower powder of *Averrhoa bilimbi.*

Sr. No.	Treatment with chemical reagents	Day light	UV light	
			Short UV (254 nm)	Long UV (365 nm)
1	Powder	Pink	Dark Red	Colourless
2	Powder +Water	Pink	Dark Red	Light Blue
3	Powder +Ethanol	Pink	Light Yellow	Colourless
4	Powder +Methanol	Pink	Colourless	Colourless
5	Powder +1 N Aqueous NaOH	Yellow	Yellow	Yellow
6	Powder +1 N Alcoholic NaOH	Yellow	Yellow	Yellow
7	Powder +1 N HCl	Red	Light Blue	Fluorescent Red
8	Powder +Concentrated HCl	Red	Colourless	Red
9	Powder +50% H_2SO_4	Light Red	Light Yellow	Colourless
10	Powder +Concentrated H_2SO_4	Red	Yellow	Fluorescent Yellow
11	Powder +50% HNO_3	Light Yellow	Light Yellow	Colourless
12	Powder +Acetic acid	Light Pink	Light Blue	Pink
13	Powder +Ferric chloride	Light Yellow	Fluorescent Yellow	Yellow
14	Powder +Ammonia	Light Yellow	Yellow	Brown
15	Powder +Acetone	Colourless	Colourless	Colourless
16	Powder +Benzene	Colourless	Colourless	Colourless
17	Powder +Petroleum ether	Colourless	Colourless	Colourless
18	Powder +Chloroform	Colourless	Colourless	Colourless
19	Powder +Ethyl acetate	Colourless	Light Yellow	Colourless
20	Powder +Acetonitrile	Colourless	Colourless	Colourless
21	Powder +Picric acid	Yellow	Yellow	Yellow
22	Powder +2-propanol	Colourless	Colourless	Colourless
23	Powder +Di ethyl ether	Colourless	Colourless	Colourless

Table 12. Fluorescence analysis of bark powder of *Cassia fistula.*

Sr. No.	Treatment with chemical reagents	Day light	UV light	
			Short UV (254 nm)	Long UV (365 nm)
1	Powder	Brown	Brown	Colourless
2	Powder +Water	Brown	Light Yellow	Colourless
3	Powder +Ethanol	Yellow	Yellow	Fluorescent Yellow
4	Powder +Methanol	Yellow	Yellow	Fluorescent Yellow
5	Powder +1 N Aqueous NaOH	Brown	Brown	Black
6	Powder +1 N Alcoholic NaOH	Light Yellow	Light Yellow	Fluorescent Yellow
7	Powder +1 N HCl	Light Yellow	Light Yellow	Colourless
8	Powder +Concentrated HCl	Light Yellow	Light Yellow	Colourless
9	Powder +50% H_2SO_4	Light Yellow	Light Yellow	Fluorescent Yellow
10	Powder +Concentrated H_2SO_4	Brown	Brown	Yellow
11	Powder +50% HNO_3	Light Yellow	Light Yellow	Colourless
12	Powder +Acetic acid	Light Yellow	Light Yellow	Colourless
13	Powder +Ferric chloride	Yellow	Fluorescent Yellow	Black
14	Powder +Ammonia	Red	Brown	Fluorescent Red
15	Powder +Acetone	Light Brown	Yellow	Fluorescent Yellow
16	Powder +Benzene	Light Yellow	Colourless	Colourless
17	Powder +Petroleum ether	Colourless	Colourless	Colourless
18	Powder +Chloroform	Light Yellow	Light Yellow	Fluorescent Orange
19	Powder +Ethyl acetate	Light Brown	Colourless	Colourless
20	Powder +Acetonitrile	Light Orange	Light Brown	Light Brown
21	Powder +Picric acid	Yellow	Fluorescent Yellow	Fluorescent Yellow
22	Powder +2-propanol	Light Yellow	Light Yellow	Light Yellow
23	Powder +Di ethyl ether	Light Yellow	Light Yellow	Light Yellow

Table 13. Fluorescence analysis of bark powder of *Ceriops tagal.*

Sr. No.	Treatment with chemical reagents	Day light	UV light	
			Short UV (254 nm)	Long UV (365 nm)
1	Powder	Brown	Colourless	Colourless
2	Powder +Water	Brown	Colourless	Colourless
3	Powder +Ethanol	Dark Brown	Yellow	Fluorescent Yellow
4	Powder +Methanol	Brown	Yellow	Fluorescent Yellow
5	Powder +1 N Aqueous NaOH	Brown	Yellow	Black
6	Powder +1 N Alcoholic NaOH	Dark Yellow	Yellow	Fluorescent Yellow
7	Powder +1 N HCl	Light Yellow	Light Yellow	Fluorescent Yellow
8	Powder +Concentrated HCl	Light Yellow	Light Yellow	Fluorescent Blue
9	Powder +50% H_2SO_4	Light Yellow	Light Yellow	Fluorescent Yellow
10	Powder +Concentrated H_2SO_4	Bluish	Blue	Dark Blue
11	Powder +50% HNO_3	Light Yellow	Colourless	Fluorescent Blue
12	Powder +Acetic acid	Yellow	Fluorescent Yellow	Fluorescent Orange
13	Powder +Ferric chloride	Yellow	Fluorescent Yellow	Black
14	Powder +Ammonia	Red	Fluorescent Yellow	Fluorescent Orange
15	Powder +Acetone	Red	Fluorescent Yellow	Fluorescent Orange
16	Powder +Benzene	Yellow	Fluorescent Yellow	Fluorescent Yellow
17	Powder +Petroleum ether	Light Yellow	Light Yellow	Fluorescent Yellow
18	Powder +Chloroform	Orange	Fluorescent Yellow	Fluorescent Yellow
19	Powder +Ethyl acetate	Yellow	Yellow	Fluorescent Yellow
20	Powder +Acetonitrile	Red	Light Brown	Fluorescent Orange
21	Powder +Picric acid	Yellow	Fluorescent Yellow	Yellow
22	Powder +2-propanol	Yellow	Yellow	Fluorescent Yellow
23	Powder +Di ethyl ether	Yellow	Yellow	Fluorescent Red

Table 14. Fluorescence analysis of fruit powder of *Garcinia indica.*

Sr. No.	Treatment with chemical reagents	Day light	UV light	
			Short UV (254 nm)	Long UV (365 nm)
1	Powder	Red	Dark Red	Colourless
2	Powder +Water	Dark Red	Dark Red	Light Blue
3	Powder +Ethanol	Reddish	Red	Red
4	Powder +Methanol	Reddish	Red	Red
5	Powder +1 N Aqueous NaOH	Yellow	Yellow	Yellow
6	Powder +1 N Alcoholic NaOH	Yellow	Yellow	Yellow
7	Powder +1 N HCl	Red	Light Blue	Fluorescent Red
8	Powder +Concentrated HCl	Red	Colourless	Red
9	Powder +50% H_2SO_4	Light Red	Light Yellow	Colourless
10	Powder +Concentrated H_2SO_4	Red	Yellow	Yellow
11	Powder +50% HNO_3	Red	Light Yellow	Colourless
12	Powder +Acetic acid	Light Pink	Light Blue	Pink
13	Powder +Ferric chloride	Light Yellow	Fluorescent Yellow	Yellow
14	Powder +Ammonia	Light Yellow	Yellow	Brown
15	Powder +Acetone	Red	Colourless	Colourless
16	Powder +Benzene	Light Red	Red	Colourless
17	Powder +Petroleum ether	Colourless	Colourless	Colourless
18	Powder +Chloroform	Colourless	Colourless	Colourless
19	Powder +Ethyl acetate	Colourless	Light Yellow	Colourless
20	Powder +Acetonitrile	Colourless	Colourless	Colourless
21	Powder +Picric acid	Yellow	Yellow	Yellow
22	Powder +2-propanol	Colourless	Colourless	Colourless
23	Powder +Di ethyl ether	Colourless	Colourless	Colourless

Table 15. Fluorescence analysis of bark powder of *Mammea suriga.*

Sr. No.	Treatment with chemical reagents	Day light	UV light	
			Short UV (254 nm)	Long UV (365 nm)
1	Powder	Brown	Brown	Black
2	Powder +Water	Brown	Light Yellow	Light Yellow
3	Powder +Ethanol	Orange	Light Brown	Light Yellow
4	Powder +Methanol	Orange	Brown	Black
5	Powder +1 N Aqueous NaOH	Yellow	Yellow	Black
6	Powder +1 N Alcoholic NaOH	Light Yellow	Yellow	Black
7	Powder +1 N HCl	Light Yellow	Light Yellow	Light Yellow
8	Powder +Concentrated HCl	Light Yellow	Light Yellow	Light Yellow
9	Powder +50% H_2SO_4	Light Yellow	Light Yellow	Light Yellow
10	Powder +Concentrated H_2SO_4	Brown	Brown	Brown
11	Powder +50% HNO_3	Light Yellow	Colourless	Colourless
12	Powder +Acetic acid	Colourless	Colourless	Colourless
13	Powder +Ferric chloride	Yellow	Fluorescent Yellow	Black
14	Powder +Ammonia	Red	Brown	Brown
15	Powder +Acetone	Light Brown	Colourless	Black
16	Powder +Benzene	Yellow	Colourless	Light Yellow
17	Powder +Petroleum ether	Light Yellow	Light Yellow	Light Yellow
18	Powder +Chloroform	Light Yellow	Colourless	Black
19	Powder +Ethyl acetate	Light Brown	Light Brown	Black
20	Powder +Acetonitrile	Light Yellow	Light Brown	Light Yellow
21	Powder +Picric acid	Yellow	Fluorescent Yellow	Light Yellow
22	Powder +2-propanol	Light Yellow	Light Yellow	Light Yellow
23	Powder +Di ethyl ether	Colourless	Colourless	Colourless

Table 16. Fluorescence analysis of fruit powder of *Melastoma malabathricum.*

Sr. No.	Treatment with chemical reagents	Day light	UV light	
			Short UV (254 nm)	Long UV (365 nm)
1	Powder	Dark Pink	Black	Black
2	Powder +Water	Pink	Red	Fluorescent Red
3	Powder +Ethanol	Dark Purple	Red	Fluorescent Green
4	Powder +Methanol	pink	Light Yellow	Fluorescent Green
5	Powder +1 N Aqueous NaOH	Light pink	Colourless	Black
6	Powder +1 N Alcoholic NaOH	Light pink	Light Yellow	Black
7	Powder +1 N HCl	Light Yellow	Light Yellow	Colourless
8	Powder +Concentrated HCl	Yellow	Yellow	Black
9	Powder +50% H_2SO_4	Pink	Light Yellow	Black
10	Powder +Concentrated H_2SO_4	Light Yellow	Red	Fluorescent Yellow
11	Powder +50% HNO_3	Red	Colourless	Black
12	Powder +Acetic acid	Light Orange	Light Yellow	Black
13	Powder +Ferric chloride	Light Yellow	Yellow	Fluorescent Yellow
14	Powder +Ammonia	Light Red	Red	Black
15	Powder +Acetone	Orange	Black	Fluorescent Yellow
16	Powder +Benzene	Purple	Purple	Fluorescent Blue
17	Powder +Petroleum ether	Light Red	Colourless	Fluorescent Blue
18	Powder +Chloroform	Red	Brown	Fluorescent Red
19	Powder +Ethyl acetate	Orange	Light Yellow	Fluorescent Green
20	Powder +Acetonitrile	Pink	Light Yellow	Fluorescent Green
21	Powder +Picric acid	Yellow	Yellow	Fluorescent Yellow
22	Powder +2-propanol	Light Red	Black	Fluorescent Green
23	Powder +Di ethyl ether	Light Red	Red	Fluorescent Red

Table 17. Fluorescence analysis of corolla tube powder of *Nyctanthes arbor-tristis.*

Sr. No.	Treatment with chemical reagents	Day light	UV light	
			Short UV (254 nm)	Long UV (365 nm)
1	Powder	Orange	Black	Black
2	Powder +Water	Yellow	Yellow	Fluorescent Red
3	Powder +Ethanol	Yellow	Yellow	Fluorescent Green
4	Powder +Methanol	Yellow	Yellow	Fluorescent Green
5	Powder +1 N Aqueous NaOH	Yellow	Colourless	Black
6	Powder +1 N Alcoholic NaOH	Light Yellow	Light Yellow	Black
7	Powder +1 N HCl	Light Yellow	Light Yellow	Colourless
8	Powder +Concentrated HCl	Yellow	Yellow	Black
9	Powder +50% H_2SO_4	Light Yellow	Light Yellow	Black
10	Powder +Concentrated H_2SO_4	Light Yellow	Light Yellow	Fluorescent Yellow
11	Powder +50% HNO_3	Light Yellow	Colourless	Black
12	Powder +Acetic acid	Light Orange	Light Yellow	Black
13	Powder +Ferric chloride	Light Yellow	Yellow	Fluorescent Yellow
14	Powder +Ammonia	Light Red	Colourless	Black
15	Powder +Acetone	Orange	Black	Fluorescent Yellow
16	Powder +Benzene	Light Red	Colourless	Fluorescent Blue
17	Powder +Petroleum ether	Light Red	Colourless	Fluorescent Blue
18	Powder +Chloroform	Red	Brown	Fluorescent Red
19	Powder +Ethyl acetate	Orange	Light Yellow	Fluorescent Green
20	Powder +Acetonitrile	Yellow	Light Yellow	Fluorescent Green
21	Powder +Picric acid	Yellow	Yellow	Fluorescent Yellow
22	Powder +2-propanol	Light Red	Black	Fluorescent Green
23	Powder +Di ethyl ether	Light Red	Black	Fluorescent Red

Table 18. Fluorescence analysis of bark powder of *Peltophorum pterocarpum*.

Sr. No.	Treatment with chemical reagents	Day light	UV light	
			Short UV (254 nm)	Long UV (365 nm)
1	Powder	Brown	Brown	Black
2	Powder +Water	Brown	Brown	Light Yellow
3	Powder +Ethanol	Orange	Orange	Fluorescent Yellow
4	Powder +Methanol	Orange	Orange	Fluorescent Yellow
5	Powder +1 N Aqueous NaOH	Brown	Brown	Black
6	Powder +1 N Alcoholic NaOH	Light Yellow	Light Yellow	Fluorescent Yellow
7	Powder +1 N HCl	Light Yellow	Light Yellow	Light Yellow
8	Powder +Concentrated HCl	Light Yellow	Light Yellow	Light Yellow
9	Powder +50% H_2SO_4	Light Yellow	Light Yellow	Light Yellow
10	Powder +Concentrated H_2SO_4	Brown	Brown	Brown
11	Powder +50% HNO_3	Light Yellow	Light Yellow	Colourless
12	Powder +Acetic acid	Colourless	Colourless	Colourless
13	Powder +Ferric chloride	Yellow	Yellow	Black
14	Powder +Ammonia	Red	Red	Red
15	Powder +Acetone	Light Brown	Light Brown	Black
16	Powder +Benzene	Colourless	Colourless	Light Yellow
17	Powder +Petroleum ether	Light Yellow	Light Yellow	Light Yellow
18	Powder +Chloroform	Brown	Brown	Black
19	Powder +Ethyl acetate	Light Brown	Light Brown	Black
20	Powder +Acetonitrile	Light Yellow	Light Yellow	Light Yellow
21	Powder +Picric acid	Yellow	Yellow	Fluorescent Yellow
22	Powder +2-propanol	Light Yellow	Light Yellow	Light Yellow
23	Powder +Di ethyl ether	Colourless	Colourless	Black

Table 19. Fluorescence analysis of fruit powder of *Phyllanthus reticulates.*

Sr. No.	Treatment with chemical reagents	Day light	UV light	
			Short UV (254 nm)	Long UV (365 nm)
1	Powder	Purple	Colourless	Colourless
2	Powder +Water	Dark Purple	Dark Blue	Blue
3	Powder +Ethanol	Green	Blue	Fluorescent Red
4	Powder +Methanol	Dark Blue	Blue	Fluorescent Red
5	Powder +1 N Aqueous NaOH	Brown	Yellow	Yellow
6	Powder +1 N Alcoholic NaOH	Yellow	Yellow	Colourless
7	Powder +1 N HCl	Red	Dark Blue	Fluorescent Red
8	Powder +Concentrated HCl	Red	Dark Blue	Red
9	Powder +50% H_2SO_4	Red	Dark Blue	Fluorescent Red
10	Powder +Concentrated H_2SO_4	Dark Red	Dark Blue	Light Red
11	Powder +50% HNO_3	Light Orange	Fluorescent Yellow	Red
12	Powder +Acetic acid	Red	Dark Blue	Fluorescent Red
13	Powder +Ferric chloride	Light Yellow	Fluorescent Yellow	Yellow
14	Powder +Ammonia	Light Green	Fluorescent Yellow	Light Green
15	Powder +Acetone	Dark Blue	Dark Blue	Dark Blue
16	Powder +Benzene	Colourless	Colourless	Colourless
17	Powder +Petroleum ether	Colourless	Colourless	Colourless
18	Powder +Chloroform	Colourless	Colourless	Colourless
19	Powder +Ethyl acetate	Colourless	Colourless	Colourless
20	Powder +Acetonitrile	Purple	Dark Blue	Blue
21	Powder +Picric acid	Red	Yellow	Fluorescent Red
22	Powder +2-propanol	Purple	Dark Blue	Fluorescent Red
23	Powder +Di ethyl ether	Colourless	Colourless	Colourless

Table 20. Fluorescence analysis of bark powder of *Rhizophora mucronata.*

Sr. No.	Treatment with chemical reagents	Day light	UV light	
			Short UV (254 nm)	Long UV (365 nm)
1	Powder	Brown	Brown	Brown
2	Powder +Water	Brown	Brown	Brown
3	Powder +Ethanol	Orange	Yellow	Light Yellow
4	Powder +Methanol	Yellow	Yellow	Light Yellow
5	Powder +1 N Aqueous NaOH	Brown	Brown	Black
6	Powder +1 N Alcoholic NaOH	Light Yellow	Light Yellow	Light Yellow
7	Powder +1 N HCl	Yellow	Yellow	Light Yellow
8	Powder +Concentrated HCl	Light Yellow	Light Yellow	Light Yellow
9	Powder +50% H_2SO_4	Light Yellow	Light Yellow	Light Yellow
10	Powder +Concentrated H_2SO_4	Brown	Brown	Brown
11	Powder +50% HNO_3	Light Yellow	Colourless	Colourless
12	Powder +Acetic acid	Colourless	Colourless	Colourless
13	Powder +Ferric chloride	Yellow	Fluorescent Yellow	Black
14	Powder +Ammonia	Red	Brown	Red
15	Powder +Acetone	Light Brown	Colourless	Black
16	Powder +Benzene	Colourless	Colourless	Light Yellow
17	Powder +Petroleum ether	Light Yellow	Light Yellow	Light Yellow
18	Powder +Chloroform	Brown	Colourless	Light Yellow
19	Powder +Ethyl acetate	Light Brown	Colourless	Black
20	Powder +Acetonitrile	Light Yellow	Light Brown	Light Yellow
21	Powder +Picric acid	Yellow	Fluorescent Yellow	Light Yellow
22	Powder +2-propanol	Light Yellow	Light Yellow	Light Yellow
23	Powder +Di ethyl ether	Colourless	Black	Black

4.4. Phytochemical analysis of natural dyes

Phytochemical tests are used to identify large number of secondary metabolic compounds present in the plants. Phytochemical screening is a simple procedure that helps in identification of phytochemicals in a mixture. The various extracts of natural dyes prepared in water, ethanol, methanol, chloroform, petroleum ether and ethyl acetate (Plate 4) were used to detect the presence or absence of various groups of phytochemicals in the plants. The tests produced the characteristic colour change indicating the presence of various chemical constituents in the dye extracts.

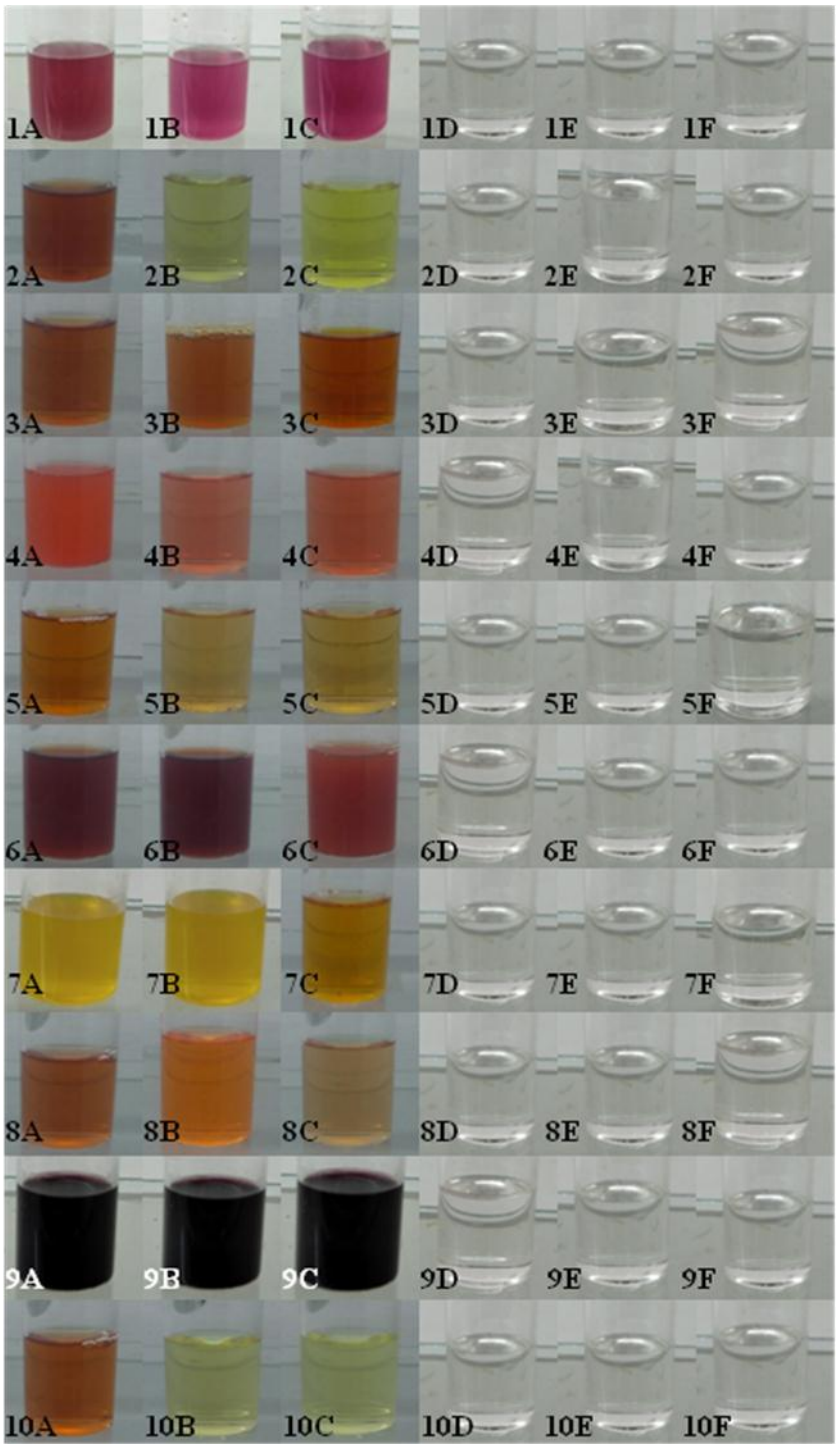


Plate 4. Dye extracts in various solvents A. Water B. Ethanol C. Methanol D. Petroleum ether E. Chloroform F. Ethyl acetate 1. *Averrhoa bilimbi* (flowers); 2. *Cassia fistula* (bark); 3. *Ceriops tagal* (bark); 4. *Garcinia indica* (fruit); 5. *Mammea suriga* (bark); 6. *Melastoma malabathricum* (fruits); 7. *Nyctanthes arbor-tristis* (corolla-tube); 8. *Peltophorum pterocarpum* (bark); 9. *Phyllanthus reticulates* (fruits); 10. *Rhizophora mucronata* (bark).

The dyes extracted in water showed the presence of alkaloids in almost all the dye extracts. The saponin was present in the dye extracts of *Cassia fistula*, *Ceriops tagal*, *Mammea suriga*, *Nyctanthes arbor-tristis*, *Peltophorum pterocarpum* and *Rhizophora mucronata*. High flavonoid content was present in all the dye extracts. Tannins were present in all the bark extracts whereas steroids and terpenoids were present only in some of the bark extracts such as *Cassia fistula*, *Mammea suriga*, *Peltophorum pterocarpum* and *Rhizophora mucronata*. Anthraquinones are highly present in *Averrhoa bilimbi* and *Ceriops tagal* and moderately present in *Garcinia indica*, *Melastoma malabathricum*, *Phyllanthus reticulates* and *Rhizophora mucronata*. The carbohydrates were present only in *Averrhoa bilimbi*, *Cassia fistula*, *Ceriops tagal*, *Garcinia indica* and *Rhizophora mucronata* whereas in rest of the extracts it was absent. The proteins were highly present in *Peltophorum pterocarpum* and *Ceriops tagal*; moderately in *Cassia fistula*, *Garcinia indica*, *Nyctanthes arbor-tristis*, *Phyllanthus reticulates* and *Rhizophora mucronata* and absent in other extracts. The coumarins was absent in all the extracts except *Peltophorum pterocarpum* (Table 21).

Table 21. Qualitative phytochemical analysis of aqueous dye extracts.

Phytochemical tests	**AB**	**CF**	**CT**	**GI**	**MS**	**MM**	**NT**	**PP**	**PR**	**RM**
1.Test for Alkaloids										
Hager's test	-	+	+	-	+	+	-	+	-	+
Wagner's test	-	+	+	+	+	+	+	+	+	+
Mayer's test	+	-	+	-	-	-	-	-	-	-
Dragendorff's test	+	-	-	-	+	-	-	-	-	-
2. Test for Saponins										
Foam test	-	+	+	-	+	-	+	+	-	+
3.Test for Phenolics & Flavonoids										
Ferric chloride test	+	++	++	-	++	++	++	+	++	+
Alkaline reagent test	++	+	-	+	-	+	+	-	+	-

Lead acetate test	+	+	+	+	-	+	+	-	+	-
Shinoda test	++	++	+	++	+	++	++	-	++	+
4. Test for Tannins										
Ferric chloride test	+	++	++	-	++	++	++	+	++	+
5. Test for Steroids										
Salkowski's test	-	-	+	-	+	-	-	+	-	-
Liebermann-Burchard's test	-	-	-	-	+	-	-	-	-	+
6. Test for Terpenoids	-	-	+	-	-	-	+	-	-	-
7. Test for Anthraquinones	++	-	++	+	-	+	-	-	+	+
8. Test for Carbohydrates										
Molisch's test	-	+	+	+	-	-	-	-	-	++
Barfoed's test	-	+	-	-	-	-	-	-	-	+
Benedicts's test	+	+	-	-	-	-	-	-	-	+
Fehling's test	+	+	-	-	-	-	-	-	-	++
9. Test for Proteins and Amino Acids										
Biuret's test	-	+	++	-	-	-	+	+	+	+
Xanthoprotein test	-	+	-	+	-	-	+	++	+	-
Ninhydrin test	-	+	-	-	-	-	-	+	-	-
10. Coumarins	-	-	-	-	-	-	-	+	-	-

Where (++) indicates highly present; (+) indicates moderately present; (-) indicates completely absent. "AB" – *Averrhoa bilimbi*; "CF" – *Cassia fistula*; "CT" – *Ceriops tagal*; "GI" – *Garcinia indica*; "MS" - *Mammea suriga*; "MM"- *Melastoma malabathricum*; "NT"- *Nyctanthes arbor-tristis*; "PP" - *Peltophorum pterocarpum*; "PR" – *Phyllanthus reticulates*; "RM"- *Rhizophora mucronata*.

The ethanol extract of dyes revealed the presence of alkaloids in almost all the dye extracts. However, the saponins was present in the dye extracts of *Cassia fistula*, *Ceriops*

tagal, *Garcinia indica*, *Mammea suriga*, *Nyctanthes arbor-tristis*, *Phyllanthus reticulates* and *Rhizophora mucronata*. The flavonoids and tannins were present in all the dye extracts. The steroids and terpenoids were found only in *Cassia fistula*, *Ceriops tagal*, *Mammea suriga*, *Nyctanthes arbor-tristis*, *Peltophorum pterocarpum* and *Rhizophora mucronata* extracts. The anthraquinones was highly present in *Averrhoa bilimbi* and *Ceriops tagal*. The carbohydrate was present in almost all the dye extracts except in bark extract of *Peltophorum pterocarpum*. The test for proteins gave positive results in all the dye extracts. The coumarins were present in all the dye extracts of ethanol except in *Cassia fistula*, *Ceriops tagal*, *Melastoma malabathricum* and *Rhizophora mucronata* extracts (Table 22).

Table 22. Qualitative phytochemical analysis of ethanol dye extracts.

Phytochemical tests	**AB**	**CF**	**CT**	**GI**	**MS**	**MM**	**NT**	**PP**	**PR**	**RM**
1.Test for Alkaloids										
Hager's test	+	+	+	+	-	+	-	+	-	+
Wagner's test	++	+	++	+	-	+	+	+	+	+
Mayer's test	+	-	-	-	-	+	-	-	-	+
Dragendorff's test	++	+	+	+	+	-	++	+	-	-
2. Test for Saponins										
Foam test	-	+	+	+	+	-	+	-	+	+
3.Test for Phenolics & Flavonoids										
Ferric chloride test	+	++	++	-	++	++	++	+	++	+
Alkaline reagent test	++	+	-	+	-	+	+	-	+	-
Lead acetate test	+	+	+	+	+	+	+	+	+	-
Shinoda test	++	++	+	++	+	++	++	-	++	+
4. Test for Tannins										
Ferric chloride test	+	++	++	+	++	++	++	+	++	+
5. Test for Steroids										
Salkowski's test	-	+	+	-	+	-	-	-	-	+
Liebermann-	-	-	+	-	+	-	-	-	-	+

Burchard's test										
6. Test for Terpenoids	-	-	+	-	-	-	+	+	-	-
7. Test for Anthraquinones	++	-	++	+	+	+	-	-	+	+
8. Test for Carbohydrates										
Molisch's test	+	++	++	++	+	+	-	-	+	++
Barfoed's test	+	++	-	+	-	+	+	-	+	+
Benedicts's test	+	++	++	-	-	-	+	-	-	++
Fehling's test	++	+	++	+	-	-	-	-	-	+
9. Test for Proteins and Amino Acids										
Biuret's test	+	++	++	-	-	+	+	+	+	+
Xanthoprotein test	+	++	-	+	+	-	+	+	+	-
Ninhydrin test	++	++	-	-	-	+	-	+	-	-
10. Coumarins	++	-	-	++	++	-	+	+	+	-

Where (++) indicates highly present; (+) indicates moderately present; (-) indicates completely absent. "AB" – *Averrhoa bilimbi*; "CF" – *Cassia fistula*; "CT" – *Ceriops tagal*; "GI" – *Garcinia indica*; "MS" - *Mammea suriga*; "MM"- *Melastoma malabathricum*; "NT"- *Nyctanthes arbor-tristis*; "PP" - *Peltophorum pterocarpum*; "PR" – *Phyllanthus reticulates*; "RM"- *Rhizophora mucronata*.

The phytochemical tests for methanolic dye extracts showed the presence of high to moderate concentration of alkaloids, flavonoids, tannins, carbohydrates and proteins in all the dye extracts. The saponin was absent only in bark extracts of *Ceriops tagal*. The steroids and terpenoids were moderately present in all the dye extracts except in *Garcinia indica* and *Phyllanthus reticulates*. The anthraquinones were highly present in *Averrhoa bilimbi* and *Ceriops tagal*; moderately present in *Garcinia indica, Mammea suriga, Melastoma malabathricum, Phyllanthus reticulate, Rhizophora mucronata* and absent in *Cassia fistula, Nyctanthes arbor-tristis* and *Peltophorum pterocarpum*. The coumarins

were present in all the dye extracts of methanol except in *Cassia fistula*, *Ceriops tagal*, *Melastoma malabathricum* and *Rhizophora mucronata* extracts (Table 23).

Table 23. Qualitative phytochemical analysis of methanol dye extracts.

Phytochemical tests	**AB**	**CF**	**CT**	**GI**	**MS**	**MM**	**NT**	**PP**	**PR**	**RM**
1.Test for Alkaloids										
Hager's test	+	+	+	+	-	+	-	+	-	+
Wagner's test	++	+	++	+	-	+	+	+	+	+
Mayer's test	+	-	-	-	+	+	-	-	-	+
Dragendorff's test	++	+	+	+	+	-	++	+	-	-
2. Test for Saponins										
Foam test	+	+	-	+	+	+	+	+	+	+
3.Test for Phenolics & Flavonoids										
Ferric chloride test	+	++	++	+	++	++	++	+	++	+
Alkaline reagent test	++	+	-	+	-	+	+	-	+	-
Lead acetate test	+	+	+	+	+	+	+	+	+	-
Shinoda test	++	++	+	++	+	++	++	-	++	+
4. Test for Tannins										
Ferric chloride test	+	++	++	+	++	++	++	+	++	+
5. Test for Steroids										
Salkowski's test	-	+	+	-	+	-	-	-	-	+
Liebermann-Burchard's test	-	-	+	-	+	+	-	-	-	+
6. Test for Terpenoids	+	-	+	-	-	-	+	+	-	-
7. Test for Anthraquinones	++	-	++	+	+	+	-	-	+	+
8. Test for Carbohydrates										
Molisch's test	+	++	++	+	+	+	-	-	+	++

Barfoed's test	+	++	-	-	-	+	+	-	+	+
Benedicts's test	+	++	++	-	+	-	+	-	-	++
Fehling's test	++	+	++	-	-	-	-	-	-	+
9. Test for Proteins and Amino Acids										
Biuret's test	+	++	++	-	+	+	+	+	+	+
Xanthoprotein test	+	++	-	+	-	-	+	+	+	-
Ninhydrin test	++	++	-	-	-	+	-	+	-	-
10. Coumarins	++	-	-	++	++	-	+	+	+	-

Where (++) indicates highly present; (+) indicates moderately present; (-) indicates completely absent. "AB" – *Averrhoa bilimbi*; "CF" – *Cassia fistula*; "CT" – *Ceriops tagal*; "GI" – *Garcinia indica*; "MS" - *Mammea suriga*; "MM"- *Melastoma malabathricum*; "NT"- *Nyctanthes arbor-tristis*; "PP" - *Peltophorum pterocarpum*; "PR"- *Phyllanthus reticulates*; "RM"- *Rhizophora mucronata*.

The phytochemical analysis of chloroform dye extracts revealed moderate presence of alkaloids in all the dye extracts except in *Garcinia indica*, *Melastoma malabathricum*, *Nyctanthes arbor-tristis* and *Phyllanthus reticulates*. The saponins were highly present in *Melastoma malabathricum*; moderately in *Ceriops tagal* and *Peltophorum pterocarpum* and absent in rest of the extracts. The flavonoids were moderately present in all the extracts except the bark extracts of *Ceriops tagal*, *Peltophorum pterocarpum* and *Rhizophora mucronata*. The tannins were present only in *Averrhoa bilimbi*, *Mammea suriga*, *Melastoma malabathricum* and *Phyllanthus reticulates* extracts. No steroids, terpenoids, coumarins, proteins were present in any of the chloroform dye extracts. The presence of moderate carbohydrates was seen only in few dye extracts of *Ceriops tagal*, *Garcinia indica*, *Mammea suriga*, *Melastoma malabathricum* and *Nyctanthes arbor-tristis* (Table 24).

Table 24. Qualitative phytochemical analysis of chloroform dye extracts.

Phytochemical tests	**AB**	**CF**	**CT**	**GI**	**MS**	**MM**	**NT**	**PP**	**PR**	**RM**
1.Test for Alkaloids										

Hager's test	-	-	-	-	-	-	-	-	-	-
Wagner's test	-	-	+	-	-	-	-	-	-	-
Mayer's test	+	-	-	-	+	-	-	-	-	+
Dragendorff's test	-	+	+	-	+	-	-	+	-	+
2. Test for Saponins										
Foam test	-	-	+	-	-	++	-	+	-	-
3.Test for Phenolics & Flavonoids										
Ferric chloride test	+	-	-	-	+	+	-	-	++	-
Alkaline reagent test	++	-	-	-	-	-	+	-	+	-
Lead acetate test	+	-	-	+	-	+	+	-	-	-
Shinoda test	+	+	-	+	-	++	-	-	-	-
4. Test for Tannins										
Ferric chloride test	+	-	-	-	+	+	-	-	++	-
5. Test for Steroids										
Salkowski's test	-	-	-	-	-	-	-	-	-	-
Liebermann-Burchard's test	-	-	-	-	-	-	-	-	-	-
6. Test for Terpenoids	-	-	-	-	-	-	-	-	-	-
7. Test for Anthraquinones	-	-	-	-	-	-	-	-	-	-
8. Test for Carbohydrates										
Molisch's test	-	-	++	+	+	+	+	-	-	-
Barfoed's test	-	-	-	-	-	-	+	-	-	-
Benedicts's test	-	-	-	-	-	-	-	-	-	-
Fehling's test	-	-	-	-	-	-	-	-	-	-
9. Test for Proteins and Amino Acids										
Biuret's test	-	-	-	-	-	-	-	-	-	-

Xanthoprotein test	-	-	-	-	-	-	-	-	-	-
Ninhydrin test	-	-	-	-	-	-	-	-	-	-
10. Coumarins	-	-	-	-	-	-	-	-	-	-

Where (++) indicates highly present; (+) indicates moderately present; (-) indicates completely absent. "AB" – *Averrhoa bilimbi*; "CF" – *Cassia fistula*; "CT" – *Ceriops tagal*; "GI" – *Garcinia indica*; "MS" - *Mammea suriga*; "MM"- *Melastoma malabathricum*; "NT"- *Nyctanthes arbor-tristis*; "PP" - *Peltophorum pterocarpum*; "PR" – *Phyllanthus reticulates*; "RM"- *Rhizophora mucronata*.

The ethyl acetate and petroleum ether extract showed very few phytochemical constituents. The ethyl acetate dye extracts showed the moderate presence of alkaloids only in some of the dye extracts. The moderate presence of tannins was seen only in three ethyl acetate extracts of *Averrhoa bilimbi*, *Nyctanthes arbor-tristis* and *Phyllanthus reticulates*. The ethyl acetate dye extracts showed the absence of steroids, terpenoids, anthraquinones and coumarins.

In petroleum ether extracts, flavonoids were moderately present in *Averrhoa bilimbi*, *Melastoma malabathricum* and *Peltophorum pterocarpum* extracts. However, there was absence of saponins, tannins, steroids, terpenoids, anthraquinones, proteins and coumarins in all the petroleum ether dye extracts.

4.5. Extraction of natural dyes for staining purpose

The water extracts of all the natural dyes prepared were filtered in Whatman filter paper and was used for staining purpose. Dark pink/reddish colour dye was obtained from the flowers of *Averrhoa bilimbi* and yellow colour dye from the corolla tube of *Nyctanthes arbor-tristis*. The reddish colour dye was obtained from the fruits of *Melastoma malabathricum*, *Garcinia indica* and *Phyllanthus reticulates* whereas the bark extracts of *Cassia fistula*, *Ceriops tagal*, *Mammea suriga*, *Peltophorum pterocarpum* and *Rhizophora mucronata* gave dark brown colour dye.

4.5.1. Optimum staining time of plant sections

The transverse sections of both monocot and dicot stem were stained individually with each natural dyes, mordant solution and with the synthetic dyes (Acridine orange and Safranin) to compare the staining effect of natural dyes with the synthetic dyes.

The sections of dicot and monocot stem were stained in the synthetic dyes at various time periods viz. 5, 15, 20, 30 mins to optimize the staining period. The staining quality was good upon staining the section with synthetic dyes for 5 mins. But after 15 mins excellent staining was observed on the sections with good differentiation of vascular tissues. The staining period of 20 mins and 30 mins lead to over-staining of the sections which over-shadowed the differentiation of tissues. Hence, 15 mins was determined as an optimum staining time for staining plant tissues with synthetic dyes. The optimum staining period with synthetic dyes is provided (Table 25).

Table 25. Optimum staining period with synthetic dyes on dicot and monocot stem sections.

Synthetic stain	**Time (minutes)**	**Staining quality**	
		Dicot stem	**Monocot stem**
Safranin	5	good	good
	15	excellent	excellent
	20	over-stained	over-stained
	30	over-stained	over-stained
Acridine orange	5	good	good
	15	excellent	excellent
	20	over-stained	over-stained
	30	over-stained	over-stained

The sections of dicot and monocot stem were stained with various mordant solutions such as potassium dichromate, ferrous sulphate and copper sulphate for 5, 15, 20, 30 mins to optimize the staining period with mordants. The sections were pre-stained with each mordant and then in natural dyes to observe the staining intensity. The time period of 20

mins was optimized for staining the tissues with potassium dichromate mordant. The staining of section for about 5 mins with ferrous sulphate mordant and then in the respective natural dye resulted in excellent differentiation of vascular tissues. Whereas, the staining period of 15, 20, 30 mins lead to the blackening of the section. Thus, the staining period of 5 mins using ferrous sulphate mordant was optimized for the tissues. For copper sulphate mordant, 20 mins lead to excellent staining effect and hence 20 mins was finalized for staining the section with copper sulphate mordant. The optimum staining period with chemical mordants is tabulated in (Table 26).

Table 26. Optimum staining period with chemical mordants on dicot and monocot stem sections.

Mordant used	**Time (minutes)**	**Staining quality**	
		Dicot stem	**Monocot stem**
Potassium dichromate	5	fair	fair
	15	good	good
	20	excellent	excellent
	30	over-stained	over-stained
Ferrous sulphate	5	excellent	excellent
	15	over-stained	over-stained
	20	over-stained	over-stained
	30	over-stained	over-stained
Copper sulphate	5	good	fair
	15	good	good
	20	excellent	excellent
	30	over-stained	over-stained

The sections of monocot and dicot stem were subjected to staining with all the natural dyes for about 5, 15, 20 and 30 mins to note the optimum staining period with these dyes. The dye extracts from *Averrhoa bilimbi*, *Garcinia indica*, *Phyllanthus reticulates* and *Melastoma malabathricum* stained the section excellently after a period of 15 mins which lead to clear differentiation of tissues. But the staining period of 20, 30 mins lead to over-

staining of the tissues thereby making it impossible to differentiate the vascular tissues. Hence, for these dyes 15 mins was determined as an optimum staining time for the plant tissues. The dyes from *Rhizophora mucronata*, *Ceriops tagal*, *Nyctanthes arbor-tristis*, *Mammea suriga*, *Cassia fistula* and *Peltophorum pterocarpum* lead to excellent staining of vascular tissues after 20 mins of staining period, whereas the 30 mins lead to over-staining of the tissues. Hence 20 mins was determined as an optimum staining period for these dyes. The optimum staining period with each natural dye is summarized in (Table 27).

Table 27. Optimum staining period with natural dyes on stems sections.

Mordant used	**Solvent used**	**Time (minutes)**	**Staining quality on stem sections**
Rhizophora mucronata	water	5	fair
		15	good
		20	excellent
		30	over-stained
Ceriops tagal	water	5	good
		15	good
		20	excellent
		30	over-stained
Nyctanthes arbor-tristis	water	5	good
		15	good
		20	excellent
		30	over-stained
Mammea suriga	water	5	fair
		15	good
		20	excellent
		30	over-stained
Cassia fistula	water	5	fair
		15	fair
		20	excellent
		30	over-stained

Peltophorum pterocarpum	water	5	fair
		15	fair
		20	excellent
		30	over-stained
Averrhoa bilimbi	water	5	good
		15	excellent
		20	over-stained
		30	over-stained
Garcinia indica	water	5	good
		15	excellent
		20	over-stained
		30	over-stained
Phyllanthus reticulates	water	5	fair
		15	excellent
		20	over-stained
		30	over-stained
Melastoma malabathricum	water	5	fair
		15	excellent
		20	over-stained
		30	over-stained

Thus, by considering these optimum staining parameters of plant tissues with the synthetic dyes, natural dyes and mordants the staining was carried out accordingly for each natural dye.

4.6. Staining effect of dyes on plant tissues

4.6.1. Staining effect of synthetic dyes on dicot stem section

The unstained section of *Chromolaena odorata* stem (Plate 5A) was stained with the synthetic dye safranin. With the application of synthetic dye (safranin) the vascular tissues viz. xylem and phloem took deep red colour (Plate 5B). Whereas, the application of synthetic dye (Acridine orange) imparted yellow colour to the vascular tissues (Plate 5C).

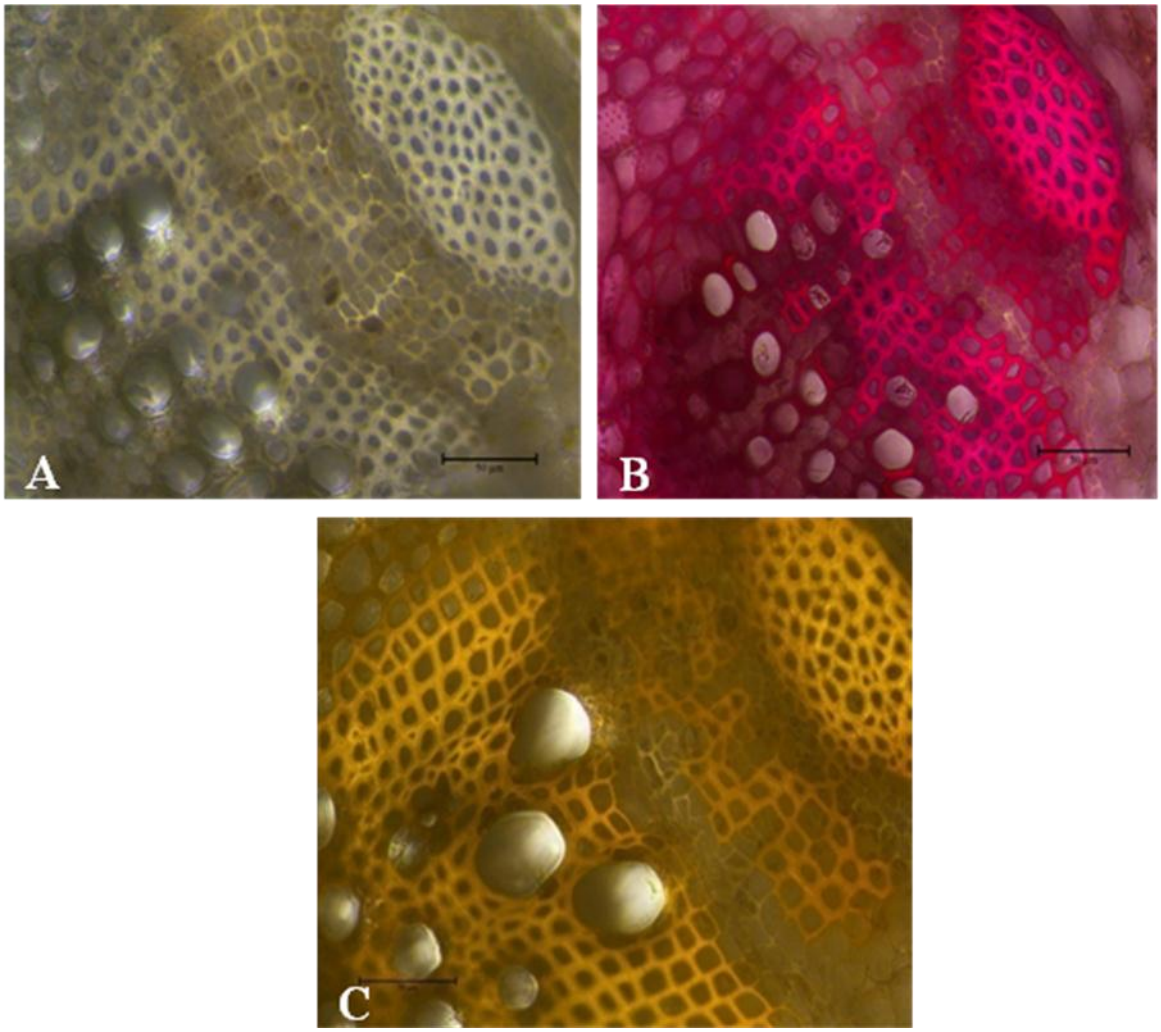


Plate 5. A. unstained section of *Chromolaena odorata* stem; B. section stained with safranin; C. section stained with Acridine orange (X40).

4.6.2. Staining effect of natural dyes on dicot stem section

The reddish-pink extract of *Averrhoa bilimbi* flowers effectively stained the vascular tissues (xylem and phloem) reddish in colour (Plate 6A). The results obtained were comparable to that of synthetic dye safranin. The mordants also played an important role in staining. On application of *A. bilimbi* dye along with the mordants $FeSO_4$ (Plate 6B) and $CuSO_4$ (Plate 6C) purple colouration was developed on vascular bundles, whereas the application of dye with $K_2Cr_2O_7$ mordant (Plate 6D) revealed with reddish-pink colouration. The staining was similar to the safranin dye.

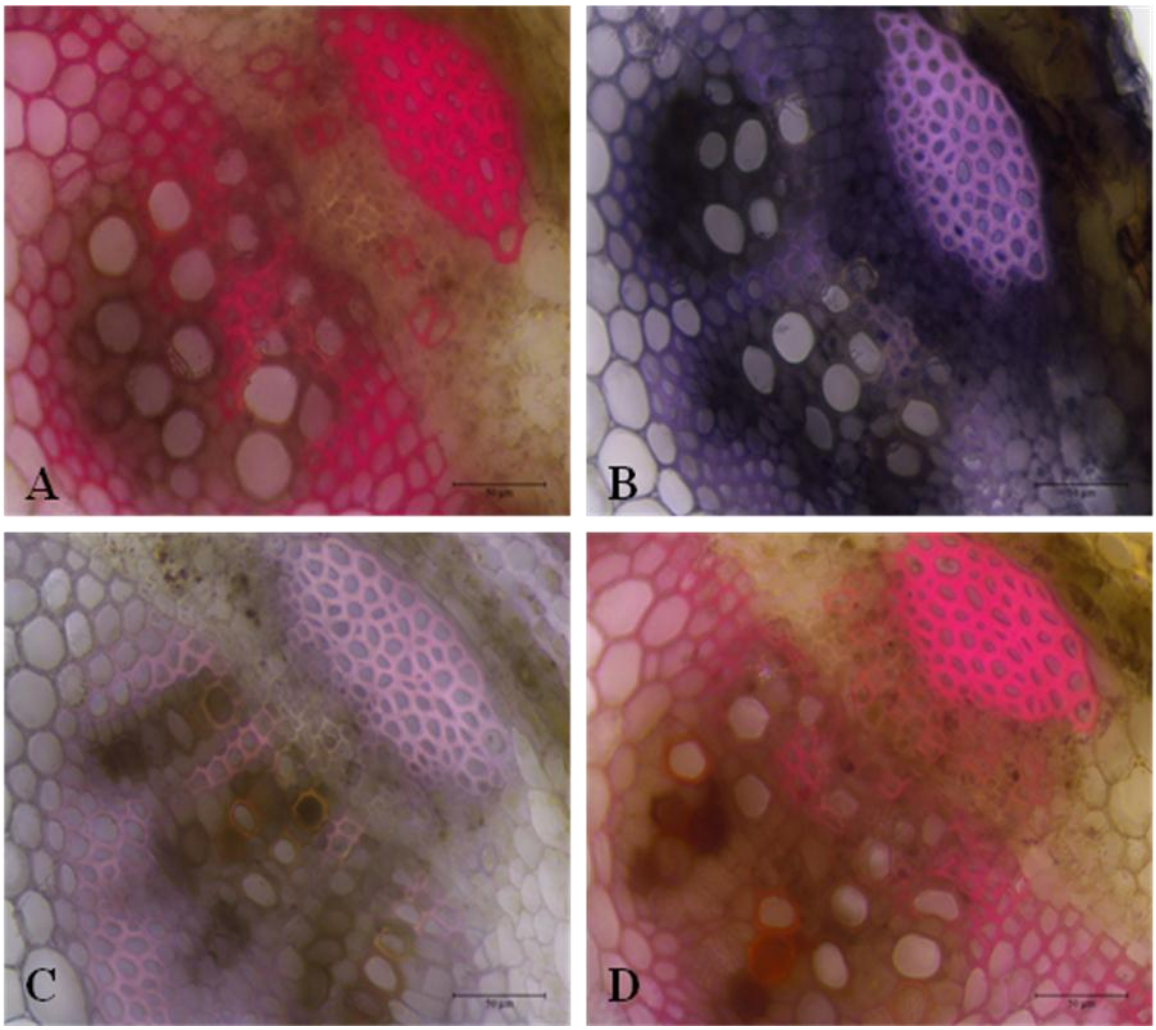


Plate 6. A. Section stained with dye *Averrhoa bilimbi;* B. section stained with dye *A. bilimbi* and $FeSO_4$ mordant; C. section stained with dye *A. bilimbi* and $CuSO_4$ mordant; D. section stained with dye *A. bilimbi* and $K_2Cr_2O_7$ mordant (X40).

The natural dye from fruits of *Garcinia indica* imparted reddish-pink colour to the stem sections (Plate 7A) which was exactly similar to the safranin dye. However, the staining was more pronounced on use of chemical mordants. All mordants examined such as $FeSO_4$ (Plate 7B), $CuSO_4$ (Plate 7C) and $K_2Cr_2O_7$ (Plate 7D) along with the *G. indica* dye showed good mordant effect by distinctly staining the vascular bundles. The staining effect shown by *G. indica* dye individually and along with the mordants was similar to safranin.

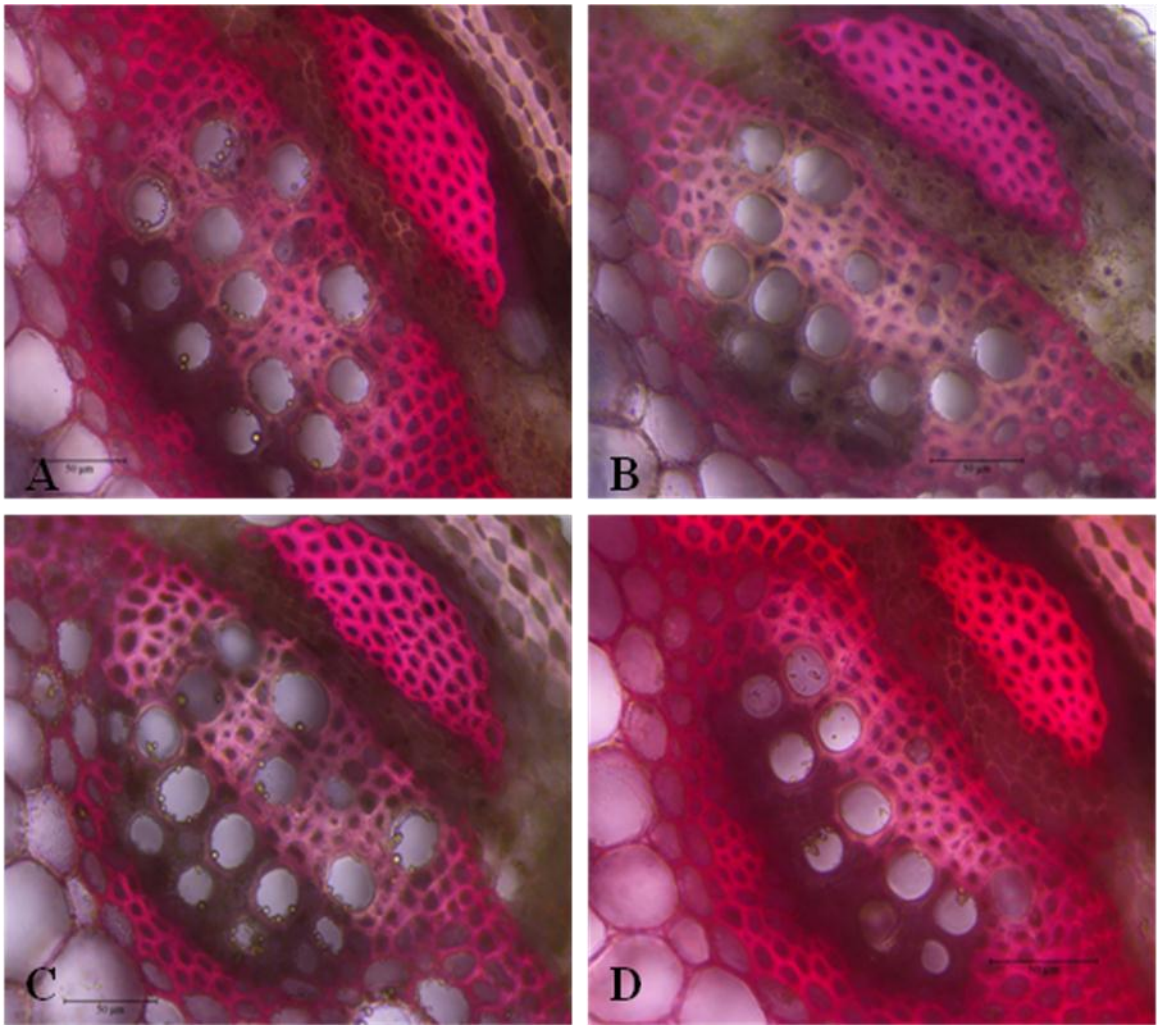


Plate 7. A. Section stained with dye *Garcinia indica*; B. section stained with dye *G. indica* and $FeSO_4$ mordant; C. section stained with dye *G. indica* and $CuSO_4$ mordant; D. section stained with dye *G. indica* and $K_2Cr_2O_7$ mordant (X40).

The section of *Chromolaena odorata* stem stained pink with the dye extracted from *Melastoma malabathricum* fruit extract (Plate 8A). The application of $FeSO_4$ mordant along with the *M. malabathricum* dye stained the xylem and phloem purple in colour (Plate 8B). However, the use of $CuSO_4$ mordant along with the dye did not make any distinct staining on vascular tissues (Plate 8C). The application of $K_2Cr_2O_7$ mordant with the dye gave pinkish shade to the tissue (Plate 8D).

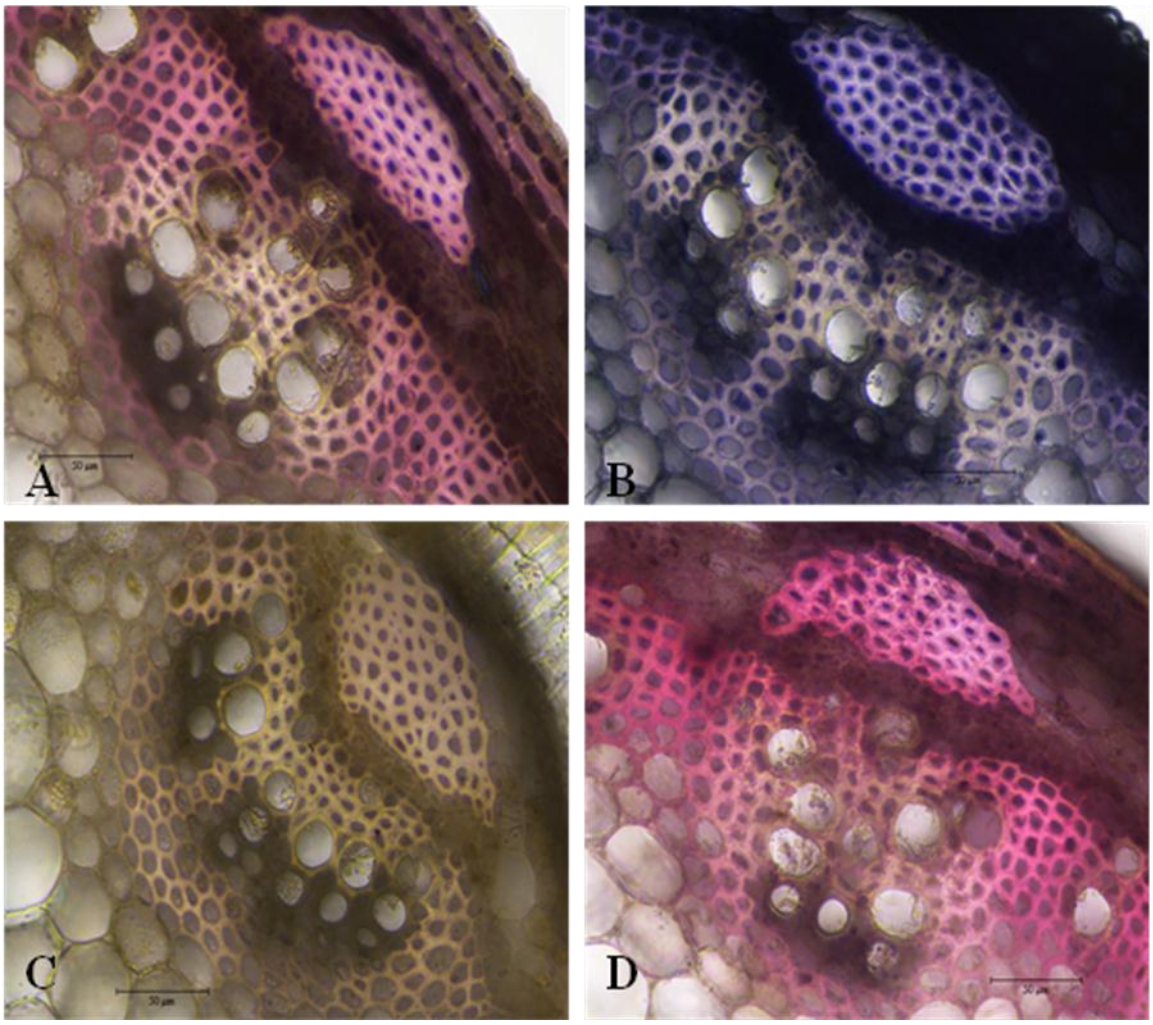


Plate 8. A. Section stained with dye *Melastoma malabathricum*; B. section stained with dye *M. malabathricum* and $FeSO_4$ mordant; C. section stained with dye *M. malabathricum* and $CuSO_4$ mordant; D. section stained with dye *M. malabathricum* and $K_2Cr_2O_7$ mordant (X40).

The differential pink staining was noticed on the vascular tissues stained with the dye extract of fruits of *Phyllanthus reticulates* (Plate 9A) which was similar to safranin. The deep purple colouration was seen on vascular tissues on application of $FeSO_4$ mordant (Plate 9B) and $CuSO_4$ mordant (Plate 9C) along with the dye. The application of $K_2Cr_2O_7$ mordant (Plate 9D) with the dye gave light pink shade to the tissue.

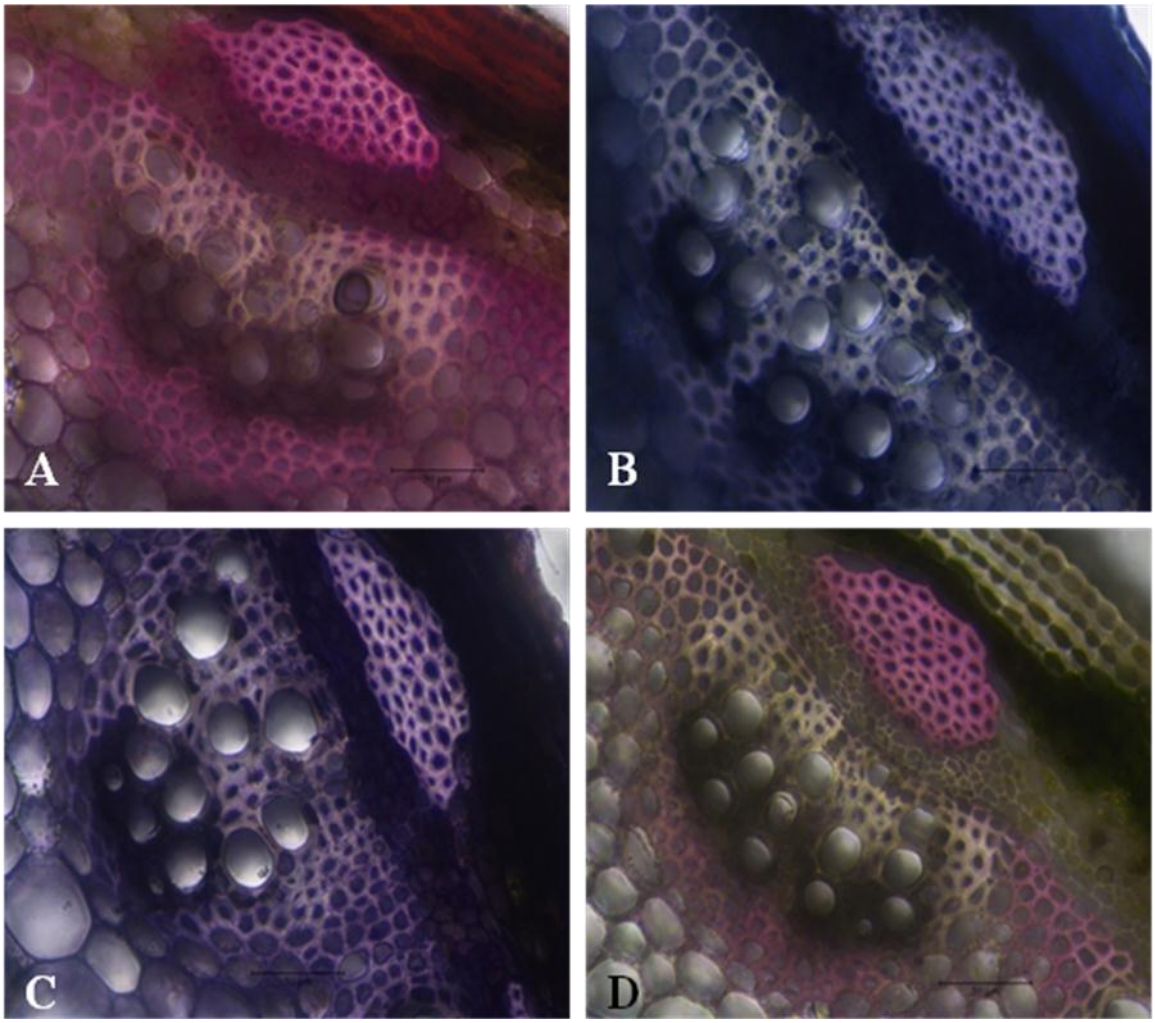


Plate 9. A. Section stained with dye *Phyllanthus reticulates*; B. section stained with dye *P. reticulates* and $FeSO_4$ mordant; C. section stained with dye *P. reticulates* and $CuSO_4$ mordant; D. section stained with dye *P. reticulates* and $K_2Cr_2O_7$ mordant (X40).

The water extract from the bark of *Cassia fistula* stained vascular tissues yellow (Plate 10A). The brown-black staining of vascular tissues was observed on application of $FeSO_4$ mordant (Plate 10B) along with the dye. The use of $K_2Cr_2O_7$ mordant (Plate 10C) and $CuSO_4$ mordant (Plate 10D) with the dye imparted yellow colour to the tissues.

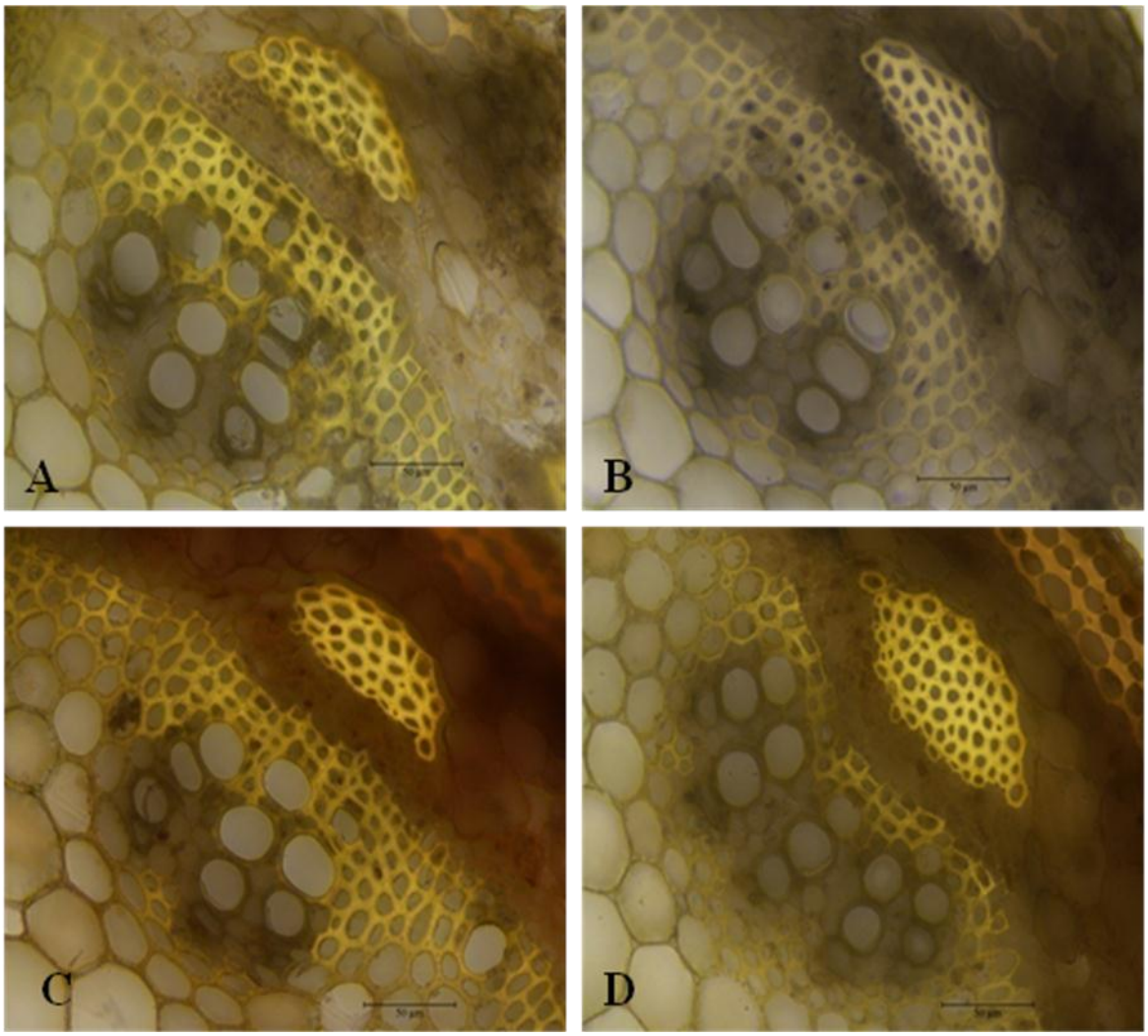


Plate 10. A. Section stained with dye *Cassia fistula*; B. section stained with dye *C. fistula* and $FeSO_4$ mordant; C. section stained with dye *C. fistula* and $CuSO_4$ mordant; D. section stained with dye *C. fistula* and $K_2Cr_2O_7$ mordant (X40).

The section of dicot stem stained yellow with the dye extracted from the bark of *Peltophorum pterocarpum* (Plate 11A). The application of $FeSO_4$ mordant along with the dye stained the xylem and phloem dark black in colour (Plate 11B). However, the use of $CuSO_4$ mordant (Plate 11C) and $K_2Cr_2O_7$ mordant (Plate 11D) along with the dye showed distinct yellow colouration to the vascular tissues.

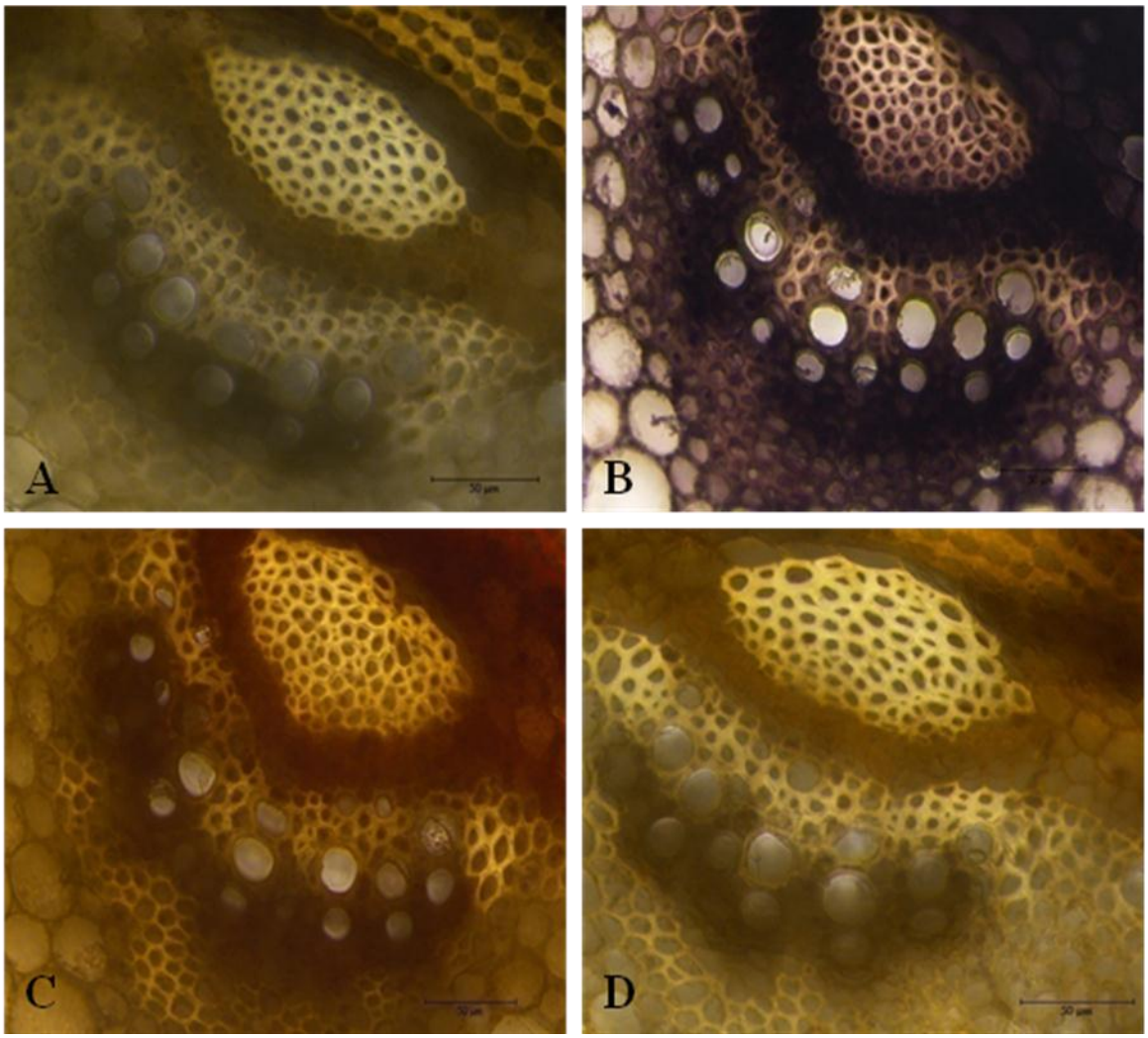


Plate 11. A. Section stained with *Peltophorum pterocarpum* dye; B. section stained with *P. pterocarpum* dye and $FeSO_4$ mordant; C. section stained with *P. pterocarpum* dye and $CuSO_4$ mordant; D. section stained with *P. pterocarpum* dye and $K_2Cr_2O_7$ mordant (X40).

The water extract from bark of *Mammea suriga* stained the vascular tissues yellow in colour which was similar to acridine dye (Plate 12A). All mordants examined such as $FeSO_4$ (Plate 12B), $CuSO_4$ (Plate 12C) and $K_2Cr_2O_7$ (Plate 12D) along with the dye gave yellow colour to the vascular tissues.

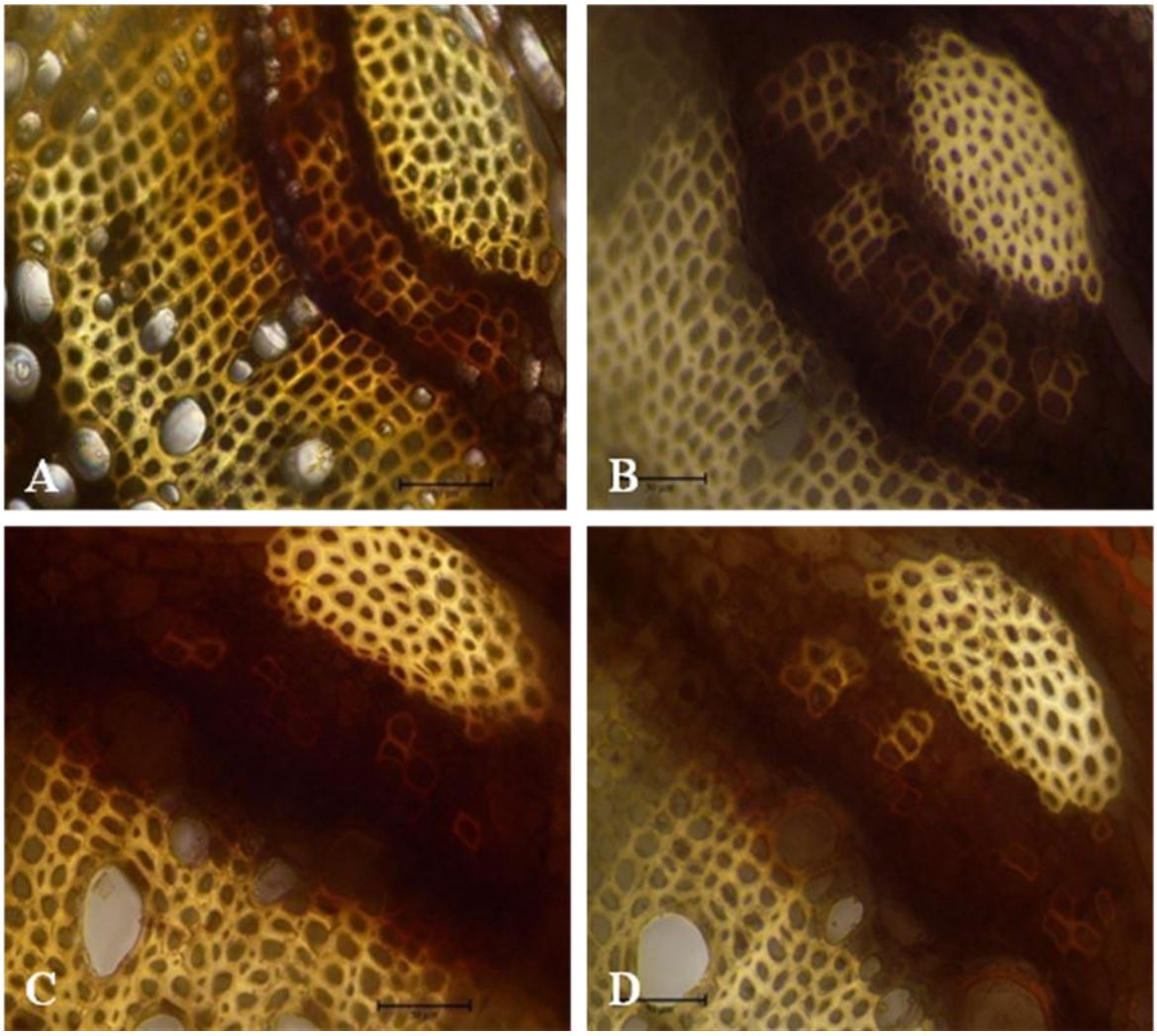


Plate 12. A. Section stained with *Mammea suriga* dye; B. section stained with *M. suriga* dye and $FeSO_4$ mordant; C. section stained with *M. suriga* dye and $CuSO_4$ mordant; D. section stained with *M. suriga* dye and $K_2Cr_2O_7$ mordant (X40).

The section of *Chromolaena odorata* stem was stained yellow with the dye extracted from the corolla tube of *Nyctanthes arbor-tristis* (Plate 13A). The staining was distinct on use of chemical mordants like $FeSO_4$ (Plate 13B), $CuSO_4$ (Plate 13C) and $K_2Cr_2O_7$ (Plate 13D) along with the dye.

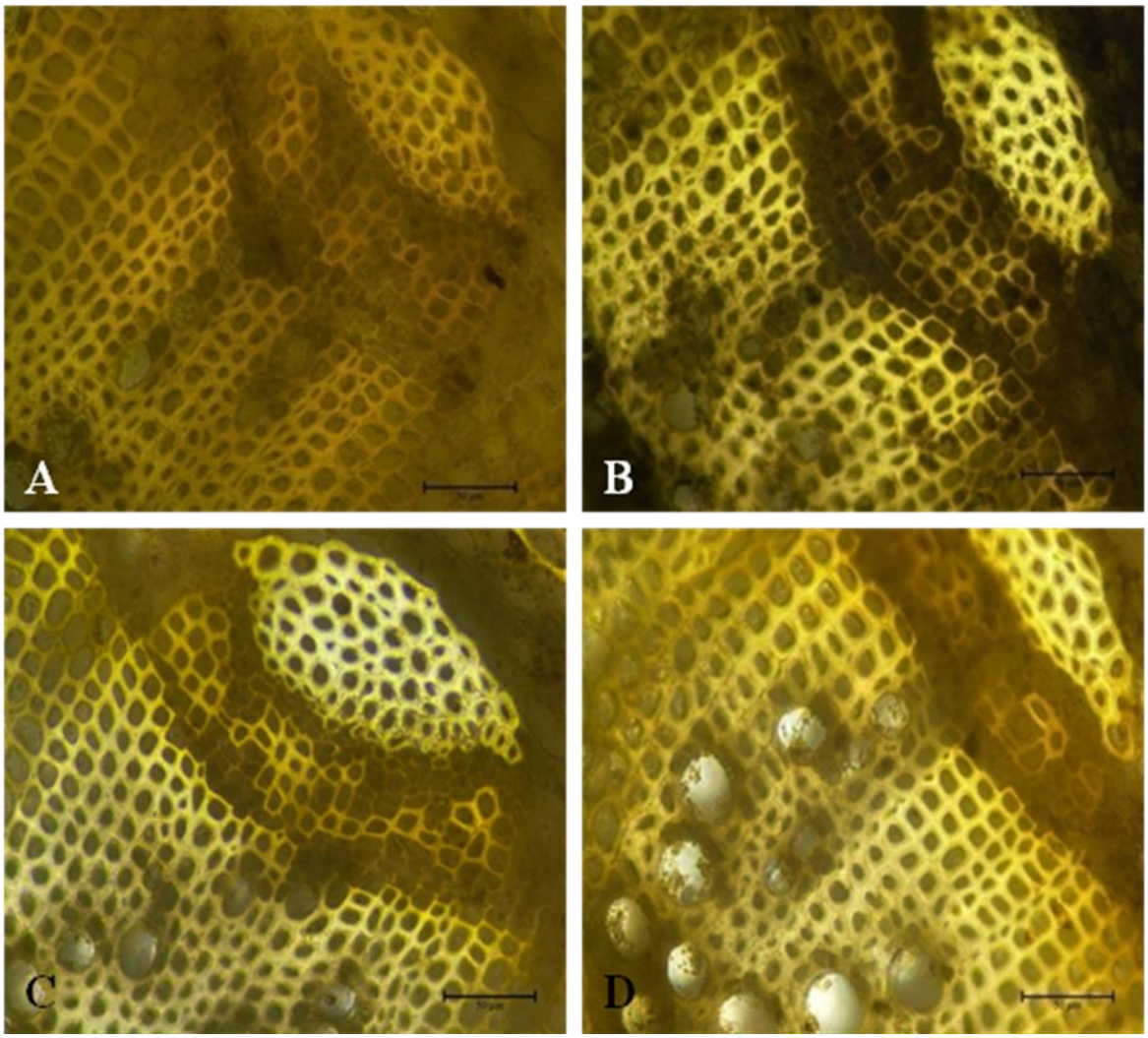


Plate 13. A. Section stained with *Nyctanthes arbor- tristis* dye; B. section stained with *N. arbor-tristis* dye and $FeSO_4$ mordant; C. section stained with *N. arbor-tristis* dye and $CuSO_4$ mordant; D. section stained with *N. arbor-tristis* dye and $K_2Cr_2O_7$ mordant (X40).

The extract of mangrove bark *Ceriops tagal* (Plate 14A) showed dark yellow colouration to the vascular tissues. The yellow colour was pronounced on use of chemical mordants $FeSO_4$ (Plate 14B), $CuSO_4$ (Plate 14C) and $K_2Cr_2O_7$ (Plate 14D) along with the dye. The dye showed the similar staining effect to that of acridine orange dye.

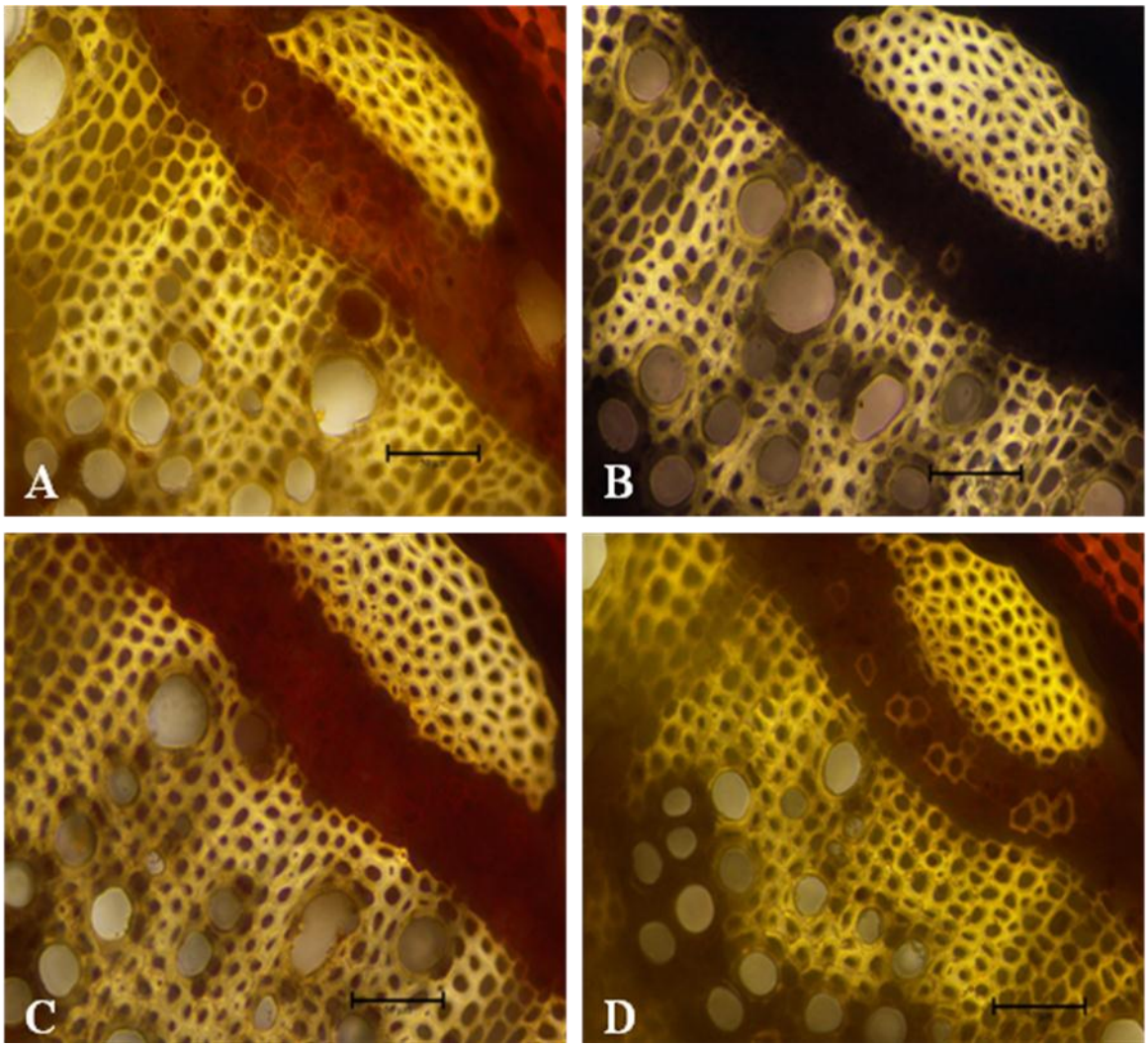


Plate 14. A. section stained with *Ceriops tagal* dye; B. section stained with *C. tagal* dye and $FeSO_4$ mordant; C. section stained with *C. tagal* dye and $CuSO_4$ mordant; D. section stained with *C. tagal* dye and $K_2Cr_2O_7$ mordant (X40).

Similarly, the mangrove *Rhizophora mucronata* bark extract showed dark yellow colour to the tissues (Plate 15A). The light brown colour staining was seen on use of $FeSO_4$ (Plate 15B) mordant along with the dye. Whereas, the use of mordants like $CuSO_4$ (Plate 15C) and $K_2Cr_2O_7$ (Plate 15D) imparted yellow colour to the vascular tissues.

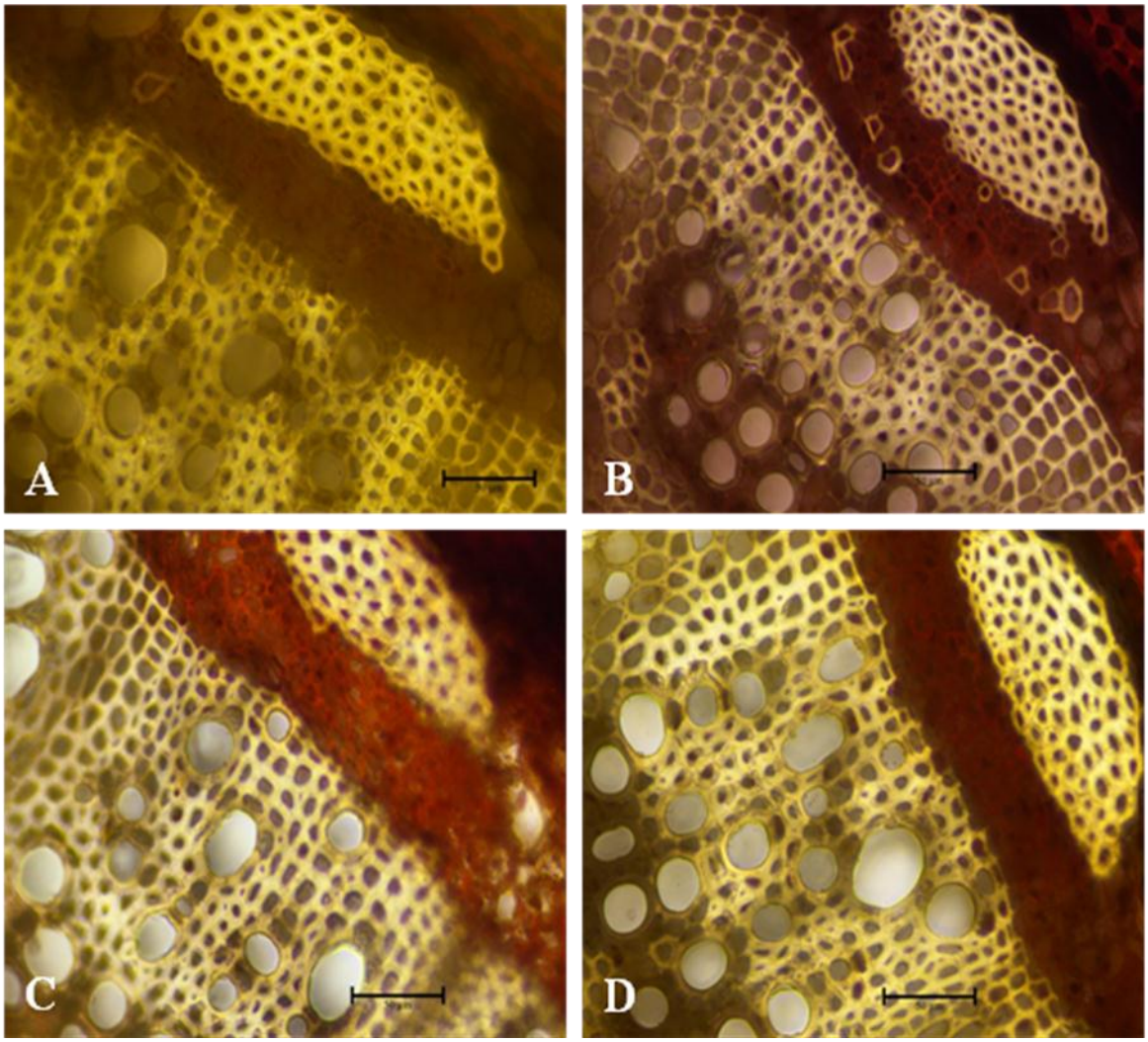


Plate 15. A. section stained with *Rhizophora mucronata* dye; B. section stained with *R. mucronata* dye and $FeSO_4$ mordant; C. section stained with *R. mucronata* dye and $CuSO_4$ mordant; D. section stained with *R. mucronata* dye and $K_2Cr_2O_7$ mordant (X40).

Thus, the natural dyes from *Averrhoa bilimbi*, *Garcinia indica*, *Melastoma malabathricum* and *Phyllanthus reticulates* stained the tissues of dicot stem similar to safranin stain and dyes from *Cassia fistula*, *Peltophorum pterocarpum*, *Mammea suriga*,

Nyctanthes arbor-tristis, *Ceriops tagal* and *Rhizophora mucronata* stained the tissues yellow in colour which was similar to acridine dye.

4.6.3. Staining effect of synthetic dyes on monocot stem section

The unstained monocot stem section of *Cyanodon dactylon* (Plate 16A) was stained pink with the application of synthetic dye safranin (Plate 16B). Whereas, the staining of monocot stem with synthetic dye acridine orange imparted yellowish colour to the phloem but no colour was seen on the xylem tissue (Plate 16C).

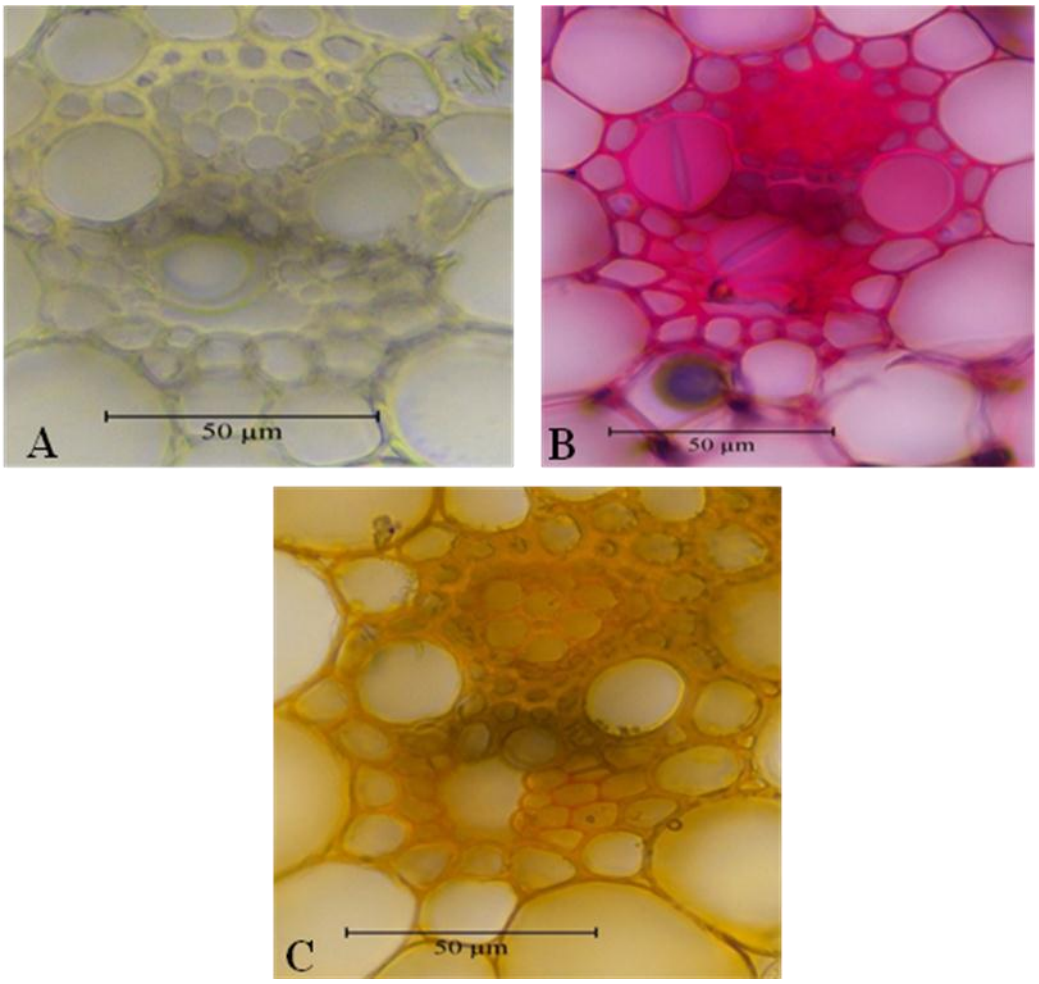


Plate 16. A. unstained section of *Cyanodon dactylon* stem; B. section stained with Safranin; C. section stained with Acridine orange (X40).

4.6.4. Staining effect of natural dyes on monocot stem section

The *Garcinia indica* extract stained the vascular tissue (phloem) pink which was similar to safranin stain. However, no staining was seen in xylem (Plate 17A). The use of mordants $FeSO_4$ (Plate 17B) and $CuSO_4$ (Plate 17C) imparted light pink colour to the phloem, but no colour was taken up by the xylem tissue. The use of mordant $K_2Cr_2O_7$ (Plate 17D) gave very light pink colour to the phloem, with no colour on xylem.

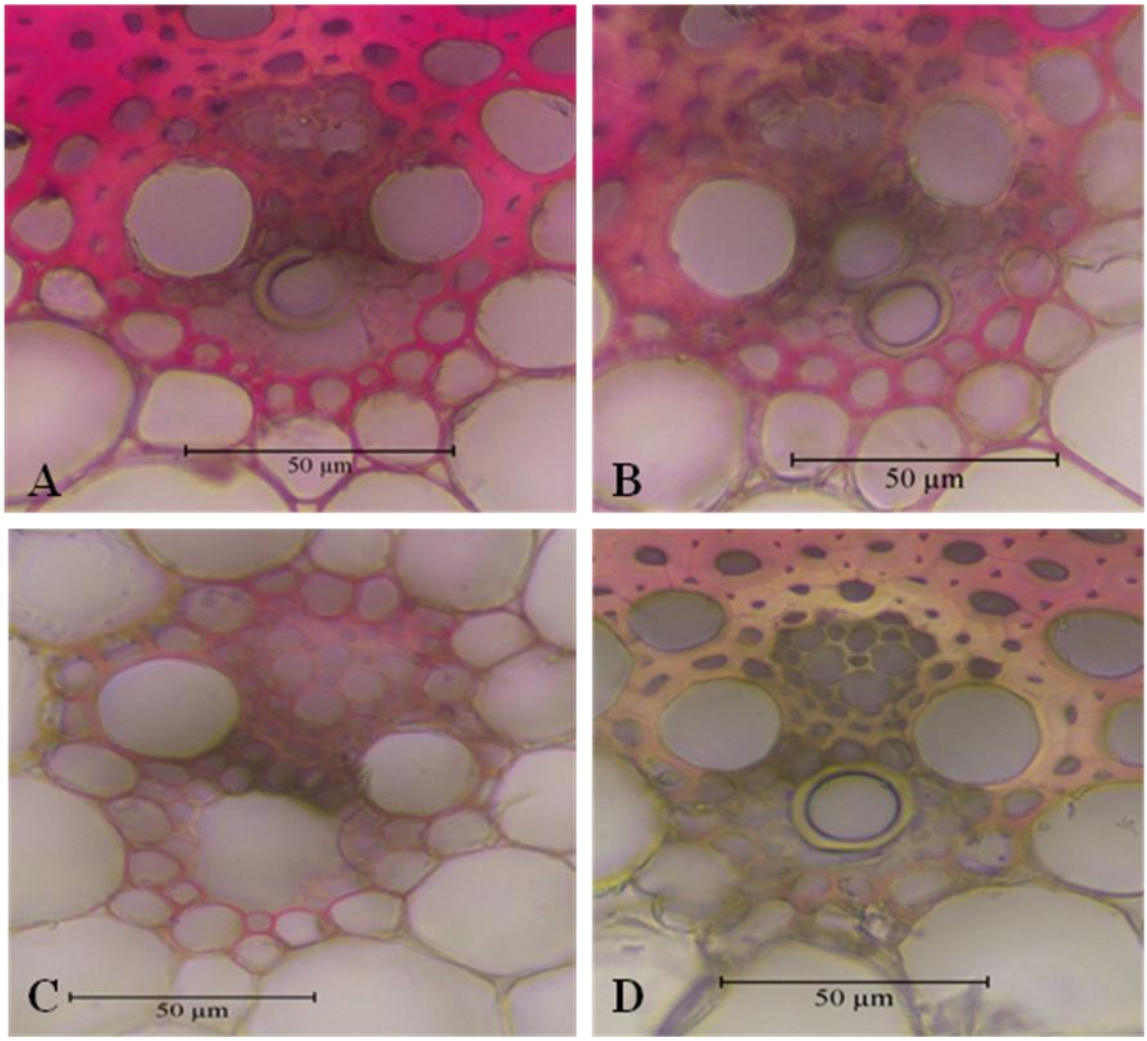


Plate 17. A. Section stained with dye *Garcinia indica*; B. section stained with dye *G. indica* and $FeSO_4$ mordant; C. section stained with dye *G. indica* and $CuSO_4$ mordant; D. section stained with dye *G. indica* and $K_2Cr_2O_7$ mordant (X40).

The water extract of *Averrhoa bilimbi* flowers stained the phloem pink, but no staining was seen on xylem (Plate 18A). The $FeSO_4$ mordant imparted purple colour to the phloem (Plate 18B) and light pink staining of phloem was observed on application of $CuSO_4$ (Plate 18C) and $K_2Cr_2O_7$ (Plate 18D) mordant with no staining on xylem. Thus, the extracts of *Garcinia indica* and *Averrhoa bilimbi* was similar in staining to the safranin stain.

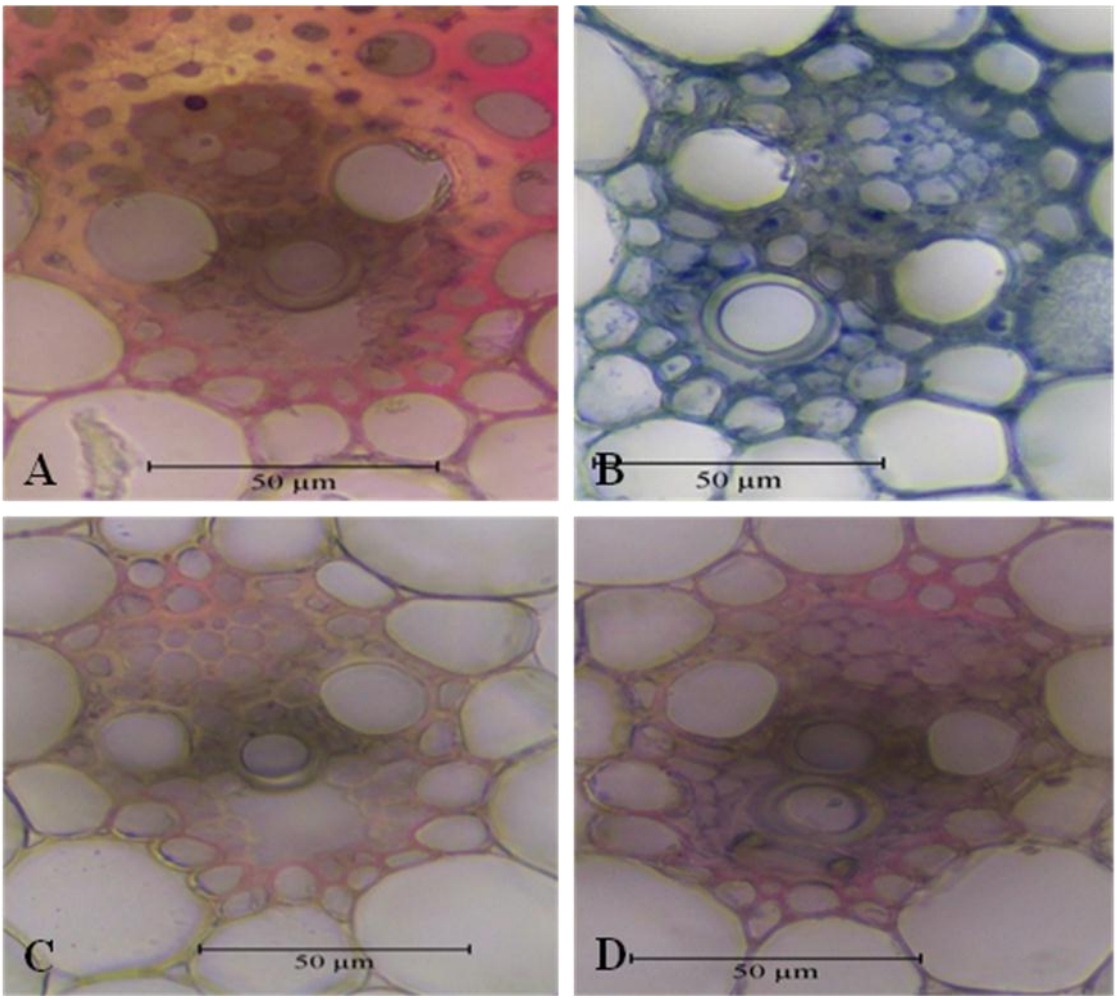


Plate 18. A. Section stained with dye *Averrhoa bilimbi;* B. section stained with dye *A. bilimbi* and $FeSO_4$ mordant; C. section stained with dye *A. bilimbi* and $CuSO_4$ mordant; D. section stained with dye *A. bilimbi* and $K_2Cr_2O_7$ mordant (X40).

The phloem tissue of monocot stem was stained yellow with the dye extract of *Nyctanthes arbor-tristis* (Plate 19A). The light yellow colour was observed on phloem on use of $FeSO_4$ (Plate 19B) and $CuSO_4$ (Plate 19C) mordants. However, the staining was pronounced on use of $K_2Cr_2O_7$ (Plate 19D) mordant along with the dye. No staining was seen on xylem tissue.

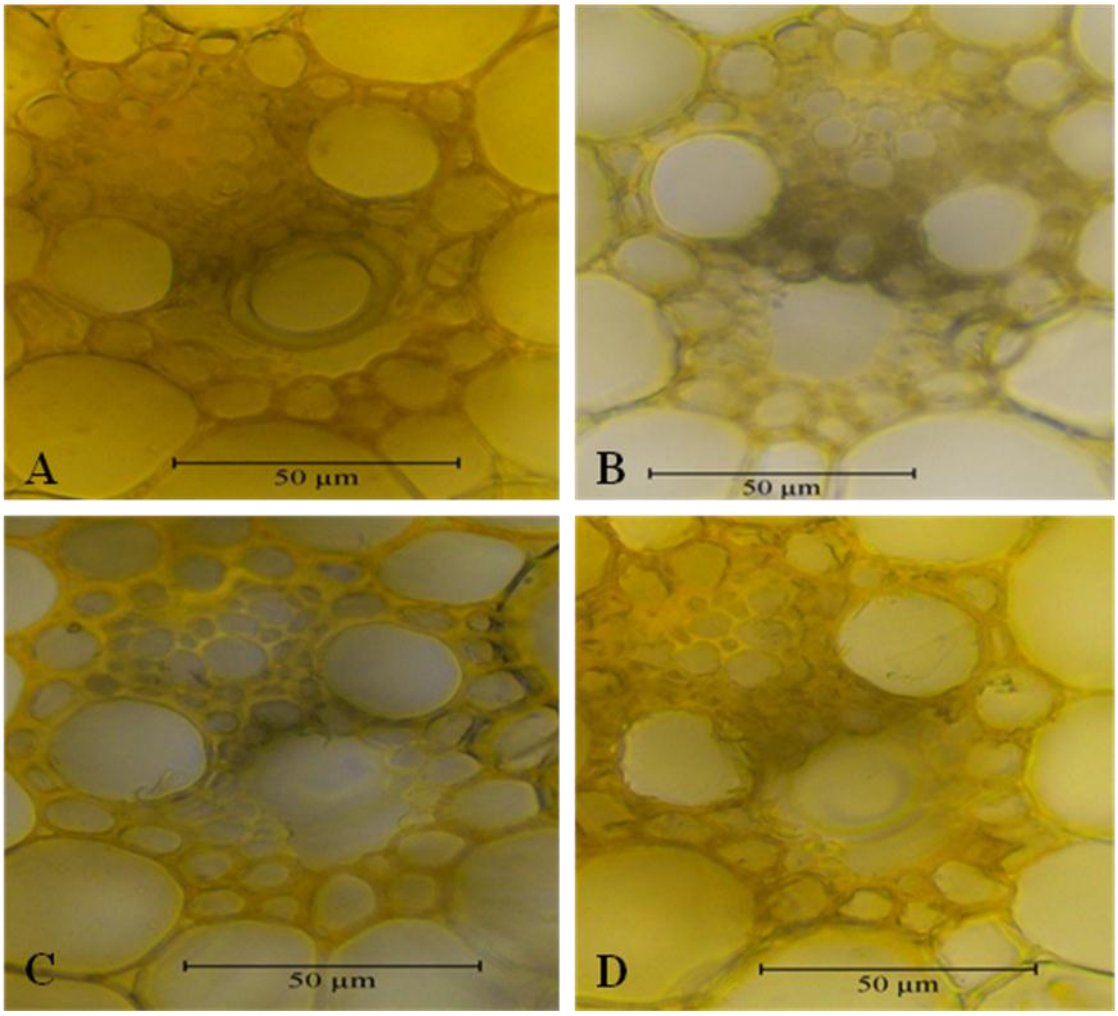


Plate 19. A. Section stained with *Nyctanthes arbor-tristis* dye; B. section stained with *N. arbor-tristis* dye and $FeSO_4$ mordant; C. section stained with *N. arbor-tristis* dye and $CuSO_4$ mordant; D. section stained with *N. arbor-tristis* dye and $K_2Cr_2O_7$ mordant (X40).

The bark extract of *Ceriops tagal* (Plate 20A) showed dark yellow colouration to the vascular tissue i.e. phloem. The yellow colour was pronounced on use of chemical mordants $FeSO_4$ (Plate 20B), $CuSO_4$ (Plate 20C) and $K_2Cr_2O_7$ (Plate 20D) along with the dye on phloem tissue. The dye showed the similar staining effect to that of acridine orange dye. However, no colour was taken up by the xylem tissue.

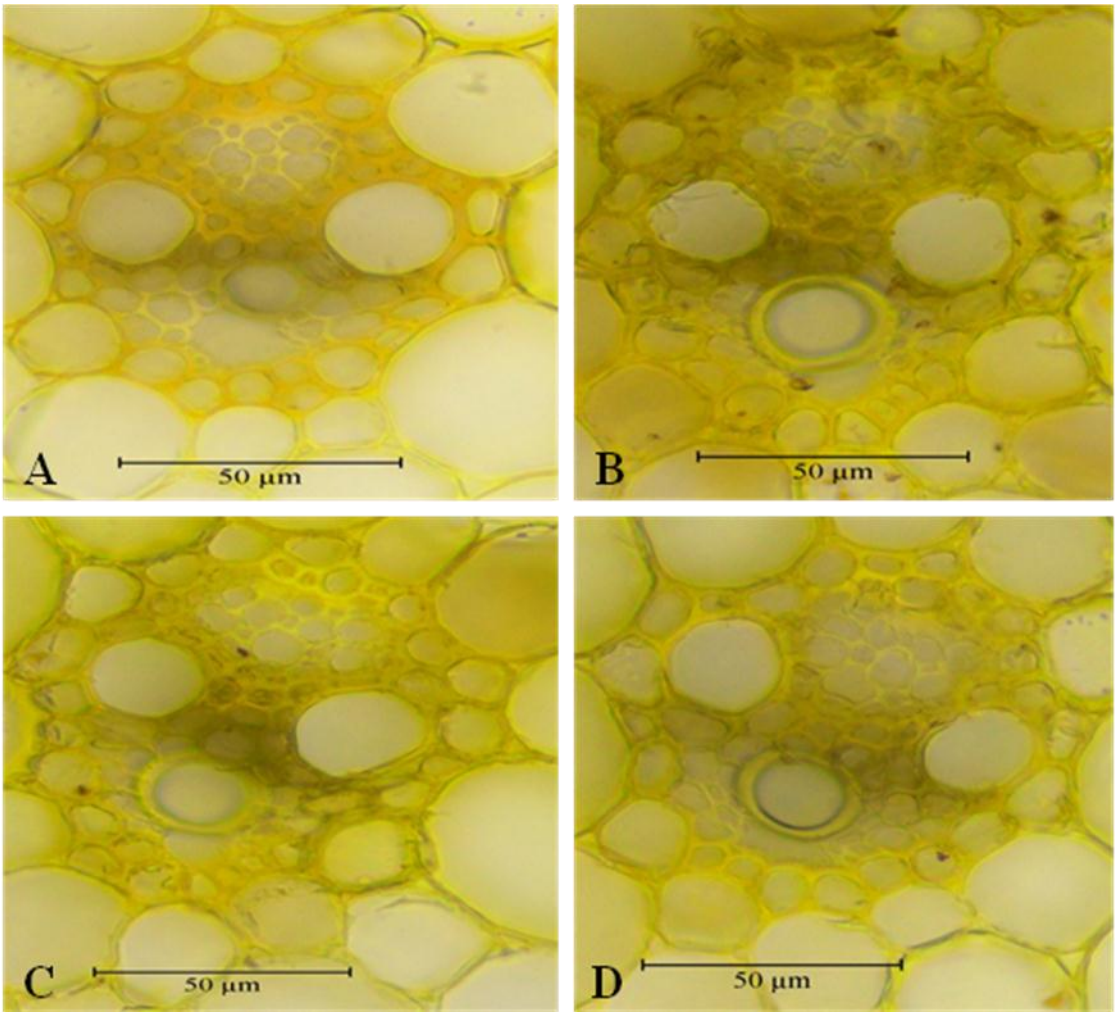


Plate 20. A. Section stained with *Ceriops tagal* dye; B. section stained with *C. tagal* dye and $FeSO_4$ mordant; C. section stained with *C. tagal* dye and $CuSO_4$ mordant; D. section stained with *C. tagal* dye and $K_2Cr_2O_7$ mordant (X40).

Rhizophora mucronata bark extract showed dark yellow colour (Plate 21A) to the phloem tissue. The dark brown staining was seen on phloem on use of $FeSO_4$ (Plate 21B) mordant along with the dye. Whereas, the use of mordants $CuSO_4$ (Plate 21C) and $K_2Cr_2O_7$ (Plate 21D) imparted yellow colour to the phloem. No colouration was seen on xylem tissue.

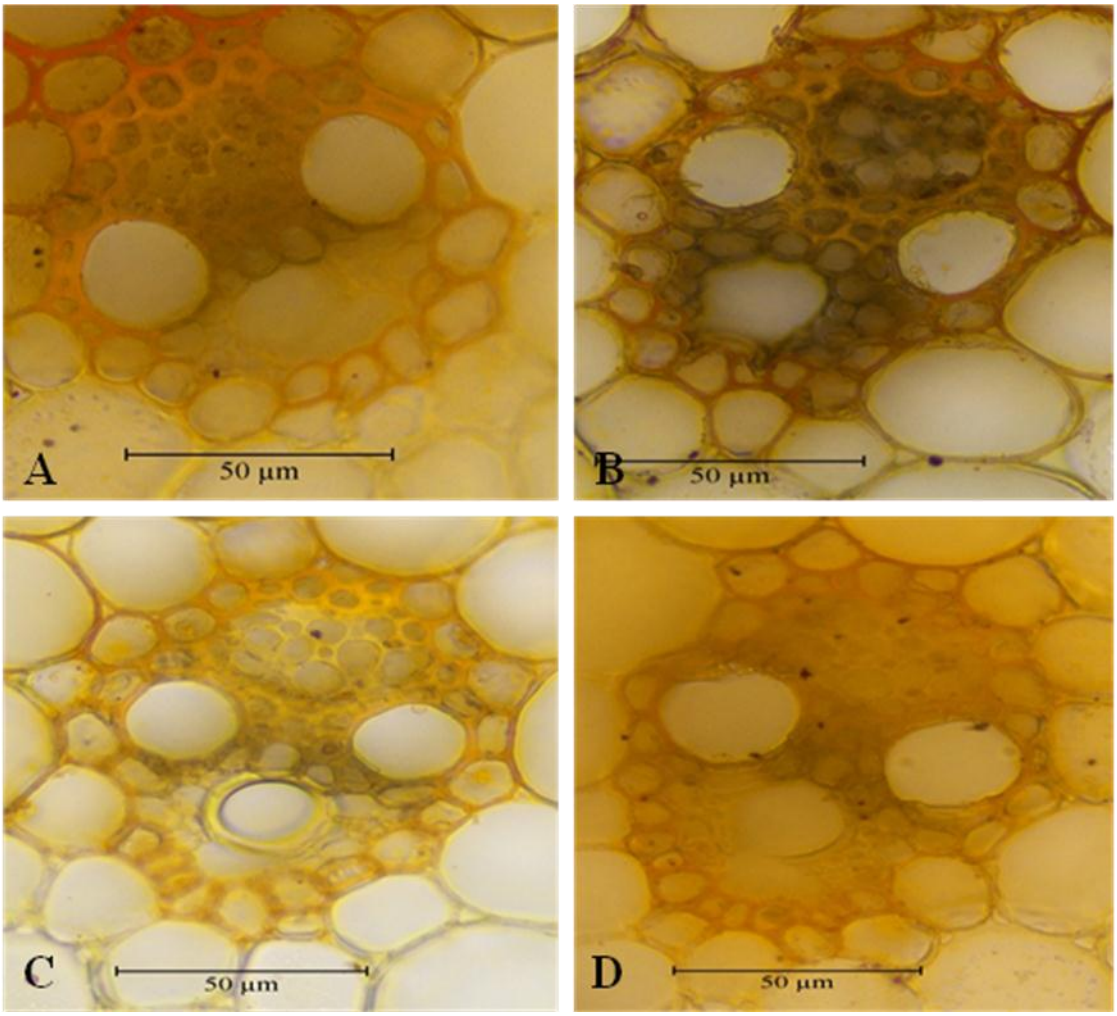


Plate 21. A. section stained with *Rhizophora mucronata* dye; B. section stained with *R. mucronata* dye and $FeSO_4$ mordant; C. section stained with *R. mucronata* dye and $CuSO_4$ mordant; D. section stained with *R. mucronata* dye and $K_2Cr_2O_7$ mordant (X40).

4.7. Fluorescence study of natural dyes

4.7.1. Effect of synthetic and natural dyes on dicot stem section

The unstained dicot stem section when observed under different fluorescence filters lead to auto-fluorescence of the section. In violet filter the vascular tissues showed blue fluorescence, in blue filter vascular tissues showed green fluorescence, in green filter vascular tissues showed red fluorescence, in yellow filter vascular tissues showed yellow fluorescence and in the red filter vascular tissues showed blue fluorescence (Table 28).

Table 28. Auto-fluorescence of dicot stem section under different excitation filters under fluorescence microscopy.

Unstained section	Major plant tissues examined	Bright field microscopy	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm
Dicot stem	Xylem	No colour	Light blue	Light green	Red	Yellow	Light blue
	Phloem	No colour	Light blue	Light green	Red	Yellow	Light blue
	Epidermis	No colour	Light blue	Light green	Red	Yellow	Light blue

The tissues of dicot stem section stained yellow with acridine orange dye in bright field microscope. The yellow fluorescence of vascular tissues was observed in violet and blue filter, whereas no fluorescence and over-fluorescence of tissues was seen in green, yellow and red filter. Likewise, the section stained in safranin dye imparted red colour to the vascular tissues under bright field, which when observed under different fluorescent

filters such as violet, blue, green, yellow showed red fluorescence of vascular tissues. The red filter showed yellow fluorescence of vascular tissues (Table 29) (Plate 22).

Table 29. Effect of different fluorescence filters on dicot stem section stained with synthetic dyes.

Synthetic stain	Major plant tissues examined	Bright field microscopy	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm
Acridine orange	Xylem Phloem Epidermis	yellow yellow yellow	yellow yellow yellow	yellow yellow green	No fluorescence	Over-fluoresced	Over-fluoresced
Safranin	Xylem Phloem Epidermis	red red red	red red red	red red red	red red red	red red red	yellow yellow red

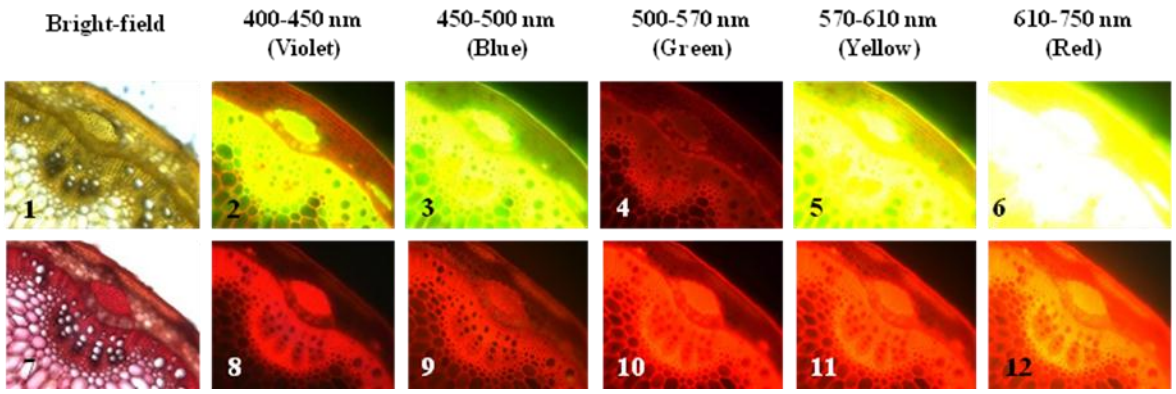


Plate 22. Fluorescence images of transverse section of dicot stem stained with synthetic dye (X200). 1-6. Acridine orange; 7-12. Safranin.

The dye from flowers of *Averrhoa bilimbi* imparted pink colour to the vascular tissues when observed under bright field microscopy. The violet and red filters lead the vascular bundles to fluoresce pink. Whereas the blue filter fluoresced the vascular tissues red in colour. Pink fluorescence of vascular tissues was seen in yellow filter and the epidermis fluoresced brown in colour. Whereas, the green filter did not show any fluorescence. No any special effect of fluorescence was seen on dicot stem using chemical mordants (Table 30) (Plate 23).

Table 30. Effect of *Averrhoa bilimbi* dye and chemical mordants on dicot stem under bright-field microscopy and under different excitation filters in fluorescence microscopy.

Dyes and mordants used	Major plant tissues examined	Bright field microscopy	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm
Averrhoa bilimbi	Xylem Phloem Epidermis	pink pink light pink	light pink light pink brown	red red brown	No fluorescence	pink pink brown	pink pink brown
Averrhoa bilimbi + $CuSO_4$	Xylem Phloem Epidermis	pink pink light pink	light pink light pink brown	red red brown	No fluorescence	pink pink brown	pink pink brown
Averrhoa bilimbi + $FeSO_4$	Xylem Phloem Epidermis	pink pink light pink	light pink light pink brown	red red brown	No fluorescence	pink Pink brown	pink pink brown
Averrhoa bilimbi + $K_2Cr_2O_7$	Xylem Phloem Epidermis	pink pink light pink	light pink light pink brown	red red brown	No fluorescence	pink Pink brown	pink pink brown

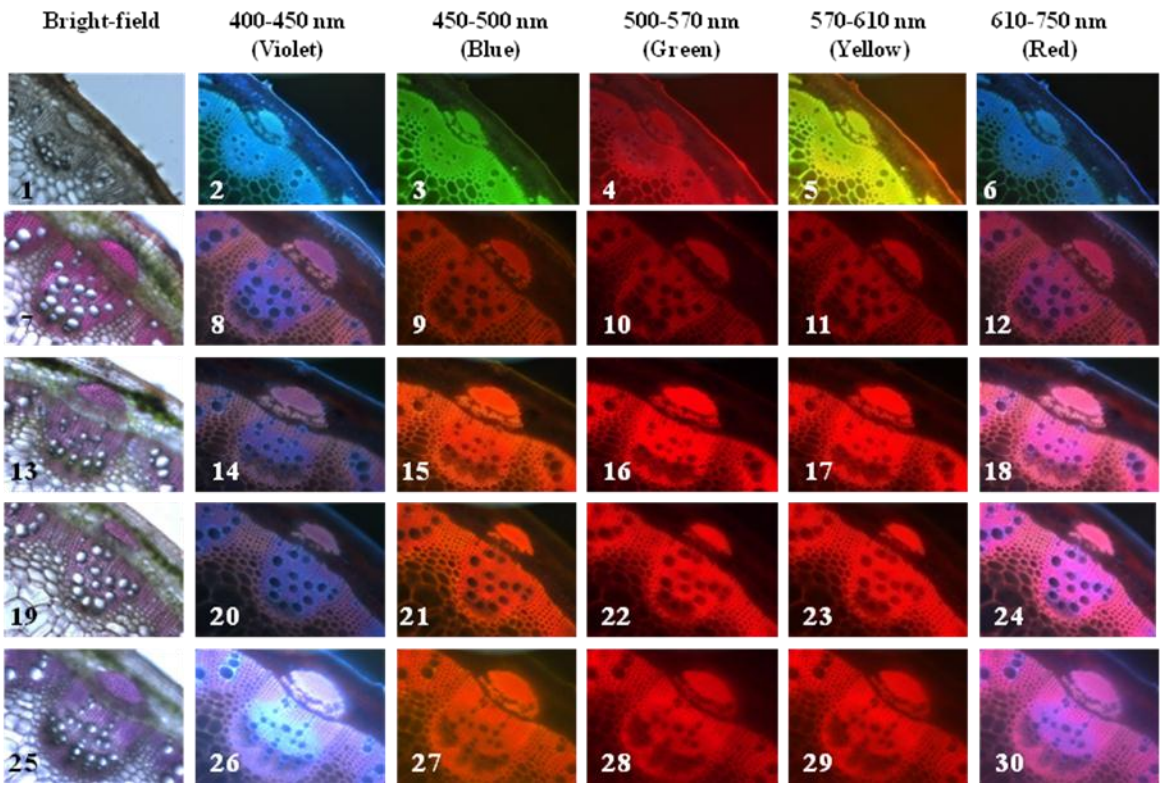


Plate 23. Fluorescence images of transverse section of dicot stem stained with *Averrhoa bilimbi* dye and along with different mordants (X200). 1. Unstained section; 2-6. Auto-fluorescence; 7-12. Stained with *A. bilimbi* dye; 13-18. *A. bilimbi* dye and $CuSO_4$ mordant; 19-24. *A. bilimbi* dye and $FeSO_4$ mordant; 25-30. *A. bilimbi* dye and $K_2Cr_2O_7$ mordant.

The bright red edible dye from fruits of *Garcinia indica* stained the vascular tissues pink when applied individually and with the use of all the chemical mordants. In violet and red filters, the vascular tissues fluoresced pink whereas in blue and yellow filter the vascular tissues (xylem and phloem) fluoresced red in colour. No fluorescence was noticed in green excitation filter (Table 31) (Plate 24).

Table 31. Effect of *Garcinia indica* dye and chemical mordants on dicot stem under bright-field microscopy and under different excitation filters in fluorescence microscopy.

Dyes and mordants used	Major plant tissues examined	Bright field microscopy	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm

Garcinia indica	Xylem Phloem Epidermis	pink pink light pink	pink pink brown	red red brown	No fluorescence	red red brown	pink pink brown
Garcinia indica + $CuSO_4$	Xylem Phloem Epidermis	pink pink light pink	pink pink brown	red red brown	No fluorescence	red red brown	pink pink brown
Garcinia indica + $FeSO_4$	Xylem Phloem Epidermis	pink pink light pink	pink pink brown	red red brown	No fluorescence	red red brown	pink pink brown
Garcinia indica + $K_2Cr_2O_7$	Xylem Phloem Epidermis	pink pink light pink	pink pink brown	red red brown	No fluorescence	red red brown	pink pink brown

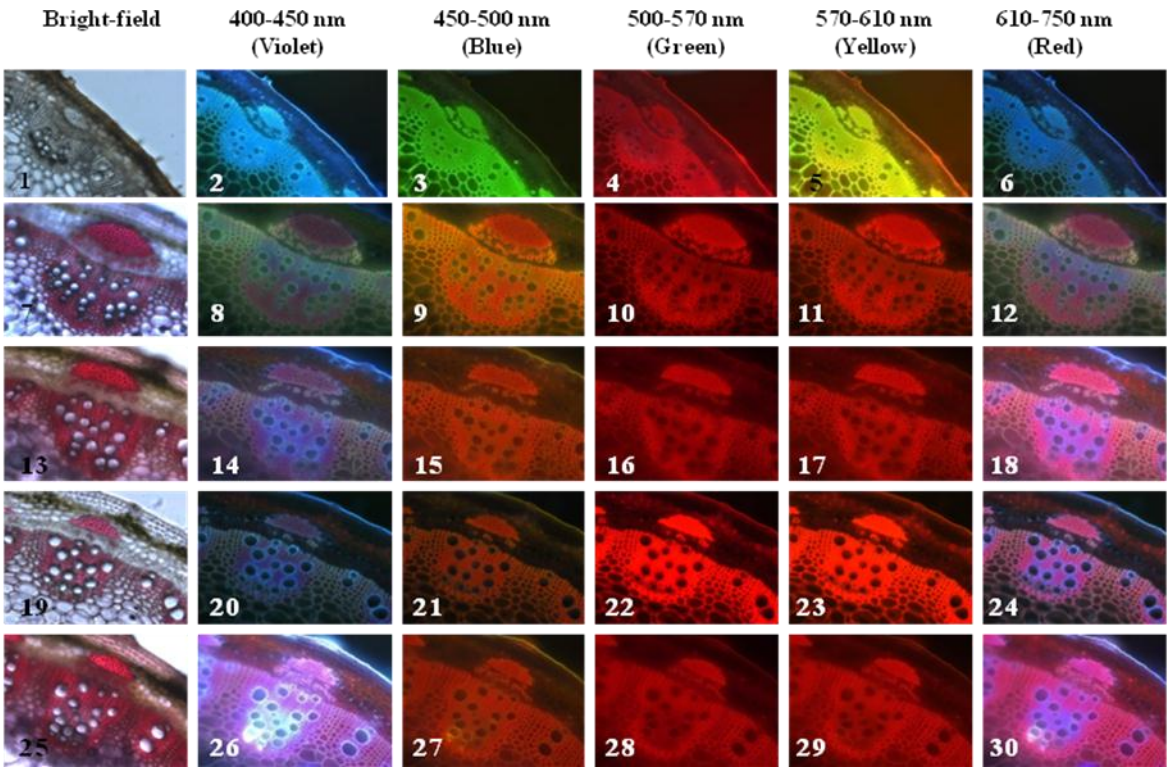


Plate 24. Fluorescence images of transverse section of dicot stem stained with *Garcinia indica* dye and along with different mordants (X200). 1. Unstained section; 2-6. Auto-fluorescence; 7-12. Stained with dye *G. indica*; 13-18. *G. indica* dye and $CuSO_4$ mordant; 19-24. *G. indica* dye and $FeSO_4$ mordant; 25-30. *G. indica* dye and $K_2Cr_2O_7$ mordant.

The *Melastoma malabathricum* dye stained the vascular tissues dark pink under bright field microscopy. The filters blue and yellow imparted red fluorescence to the vascular tissues and brown fluorescence to the epidermis. In violet and red excitation filters vascular tissues fluoresced pink in colour, with blue fluorescence of epidermis in violet filter and brown fluorescence of epidermis in red filter. However, no fluorescence was seen in green excitation filter (Table 32) (Plate 25).

Table 32. Effect of *Melastoma malabathricum* dye and chemical mordants on dicot stem under bright-field microscopy and under different excitation filters in fluorescence microscopy.

Dyes and mordants used	Major plant tissues examined	Bright field microscopy	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm
Melastoma malabathricum	Xylem Phloem Epidermis	dark pink dark pink dark pink	light pink light pink blue	red red brown	No fluorescence	red red brown	pink pink brown
Melastoma malabathricum + $CuSO_4$	Xylem Phloem Epidermis	dark pink dark pink dark pink	light pink light pink blue	red red brown	No fluorescence	red red brown	pink pink brown
Melastoma malabathricum + $FeSO_4$	Xylem Phloem Epidermis	dark pink dark pink dark pink	light pink light pink blue	red red brown	No fluorescence	red red brown	pink pink brown
Melastoma malabathricum + $K_2Cr_2O_7$	Xylem Phloem Epidermis	dark pink dark pink dark pink	light pink light pink blue	red red brown	No fluorescence	red red brown	pink pink brown

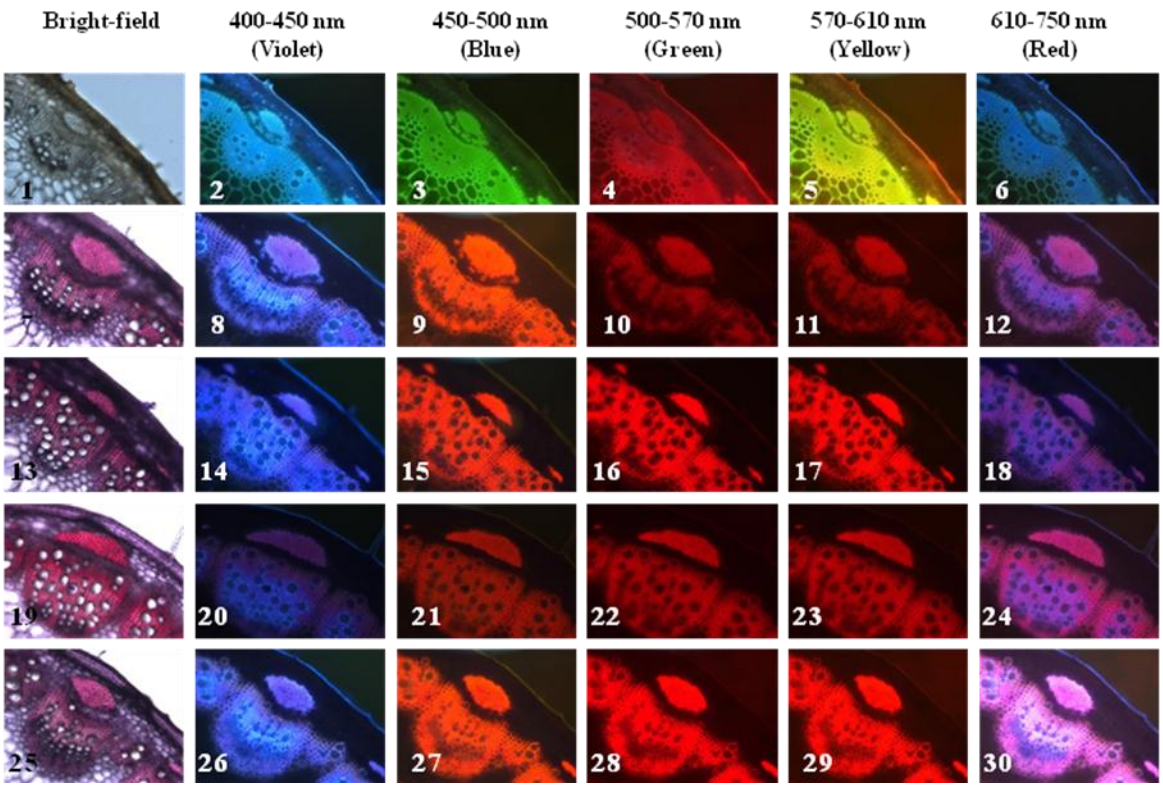


Plate 25. Fluorescence images of transverse section of dicot stem stained with *Melastoma malabathricum* dye and along with different mordants (X200). 1. Unstained section; 2-6. Auto-fluorescence; 7-12. Stained with *M. malabathricum* dye; 13-18. *M. malabathricum* dye and $CuSO_4$ mordant; 19-24. *M. malabathricum* dye and $FeSO_4$ mordant; 25-30. *M. malabathricum* dye and $K_2Cr_2O_7$ mordant.

The dye from *Nyctanthes arbor-tristis* imparted yellow colour to the vascular tissues and epidermis in dicot stem. However, the colour was profound on use of chemical mordants $K_2Cr_2O_7$, $CuSO_4$ and $FeSO_4$ under bright-field microscopy. In violet and red excitation filters the vascular tissues fluoresced bluish-orange with the use of three mordants, except $CuSO_4$ mordant which lead to red fluorescence in red excitation filter. The blue filter showed orange fluorescence whereas no fluorescence was seen in green excitation filter. In yellow filter the vascular tissues and epidermis fluoresced bright red in colour (Table 33) (Plate 26).

Table 33. Effect of *Nyctanthes arbor-tristis* dye and chemical mordants on dicot stem under bright-field microscopy and under different excitation filters in fluorescence microscopy.

Dye and mordants used	Major plant tissues examined	Bright field	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm
Nyctanthes arbor-tristis	Xylem Phloem Epidermis	Yellow Yellow Yellow	Bluish-orange Bluish-orange Bluish-orange	Orange Orange Orange	No fluorescence	Red Red Red	Bluish-orange Bluish-orange Bluish-orange
Nyctanthes arbor-tristis + $CuSO_4$	Xylem Phloem Epidermis	Yellow Yellow Yellow	Bluish-orange Bluish-orange Bluish-orange	Orange Orange Orange	No fluorescence	Red Red Red	Red Red Red
Nyctanthes arbor-tristis + $FeSO_4$	Xylem Phloem Epidermis	Yellow Yellow Yellow	Bluish-orange Bluish-orange Bluish-orange	Orange Orange Orange	No fluorescence	Red Red Red	Bluish-orange Bluish-orange Bluish-orange
Nyctanthes arbor-tristis + $K_2Cr_2O_7$	Xylem Phloem Epidermis	Yellow Yellow Yellow	Bluish-orange Bluish-orange Bluish-orange	Orange Orange Orange	No fluorescence	Red Red Red	Bluish-orange Bluish-orange Bluish-orange

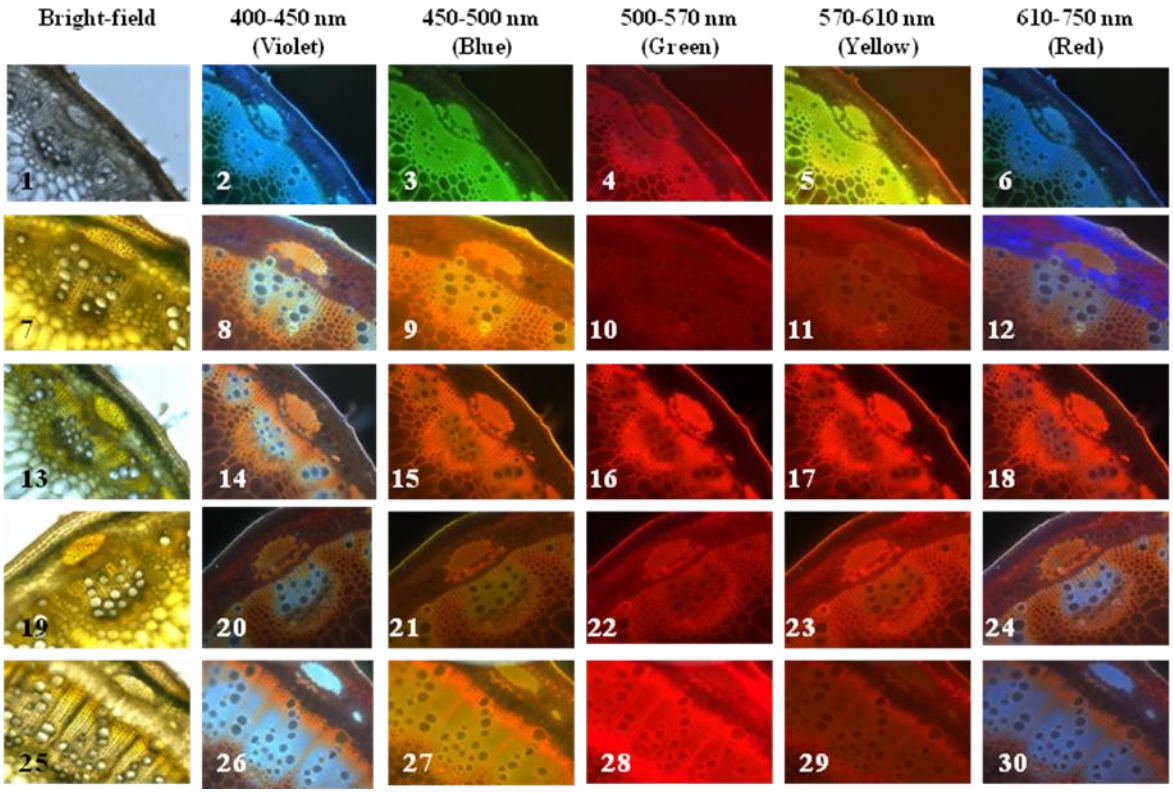


Plate 26. Fluorescence images of transverse section of dicot stem stained with *Nyctanthes arbor-tristis* dye and along with different mordants (X200). 1. Unstained section; 2-6. Auto-fluorescence; 7-12. Stained with dye *N. arbor-tristis*; 13-18. *N. arbor-tristis* dye and $CuSO_4$ mordant; 19-24. *N. arbor-tristis* dye and $FeSO_4$ mordant; 25-30. *N. arbor-tristis* dye and $K_2Cr_2O_7$ mordant.

The dye from *Cassia fistula* bark stained the vascular tissues yellow and epidermis brown in colour. Good fluorescence of vascular tissues was seen using the violet excitation filter. The xylem fluoresced blue and phloem fluoresced yellow on individual staining with *Cassia* extract. Whereas, use of chemical mordants along with the dye lead to distinct blue fluorescence of tissues. The blue filter showed yellow fluorescence of xylem and light green fluorescence of phloem with the application of *Cassia* extract. The use of mordants along with the dye gave light green colour to the vascular tissues. The epidermis was fluorescing brown in colour. In red excitation filter the vascular tissues and epidermis fluoresced red. But with the use of $K_2Cr_2O_7$ mordant, dark pink colour was seen on xylem and light pink colour was noticed on phloem. However, the tissues over-fluoresced in green and yellow filters (Table 34) (Plate 27).

Table 34. Effect of *Cassia fistula* dye and chemical mordants on dicot stem under bright-field microscopy and under different excitation filters in fluorescence microscopy.

Dye and mordants used	Major plant tissues examined	Bright field	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm
Cassia fistula	Xylem Phloem Epidermis	yellow yellow brown	blue yellow brown	yellow light green brown	over-fluorescend	over-fluorescend	red red red
Cassia fistula + $CuSO_4$	Xylem Phloem Epidermis	yellow yellow brown	light blue light blue brown	light green light green light brown	over-fluorescend	over-fluorescend	red red red
Cassia fistula + $FeSO_4$	Xylem Phloem Epidermis	light yellow light yellow light yellow	light blue light blue brown	light green light green light brown	over-fluorescend	over-fluorescend	red red red
Cassia fistula + $K_2Cr_2O_7$	Xylem Phloem Epidermis	light yellow light yellow light yellow	light blue light blue brown	light green light green light brown	over-fluorescend	over-fluorescend	dark pink light pink red

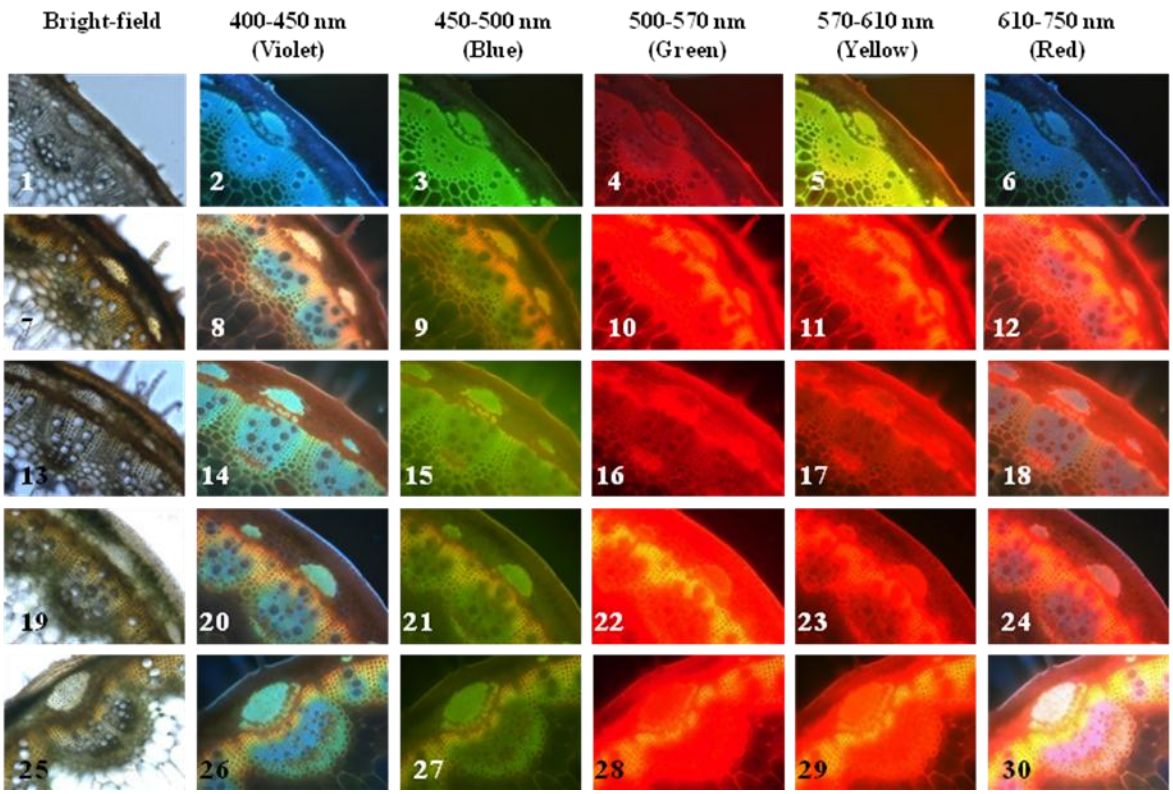


Plate 27. Fluorescence images of transverse section of dicot stem stained with *Cassia fistula* dye and along with different mordants (X200). 1. Unstained section; 2-6. Auto-fluorescence; 7-12. Stained with *C. fistula* dye; 13-18. *C. fistula* dye and $CuSO_4$ mordant; 19-24. *C. fistula* dye and $FeSO_4$ mordant; 25-30. *C. fistula* dye and $K_2Cr_2O_7$ mordant.

The dye from *Peltophorum pterocarpum* stained the vascular bundles yellow on use of chemical mordants. The epidermis was stained brown in colour. In violet filter the vascular tissues and epidermis fluoresced green whereas no fluorescence was seen in blue, green and yellow filter. The red filter leads to over-fluorescence of plant tissues (Table 35) (Plate 28).

Table 35. Effect of *Peltophorum pterocarpum* dye and chemical mordants on dicot stem under bright-field microscopy and under different excitation filters in fluorescence microscopy.

Dye and mordants used	Major plant tissues examined	Bright field	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm
Peltophorum pterocarpum	Xylem Phloem Epidermis	yellow yellow brown	dark green dark green dark green	No fluorescence	No fluorescence	No fluorescence	over-fluoresced
Peltophorum pterocarpum + $CuSO_4$	Xylem Phloem Epidermis	yellow yellow brown	dark green dark green dark green	No fluorescence	No fluorescence	No fluorescence	over-fluoresced
Peltophorum pterocarpum + $FeSO_4$	Xylem Phloem Epidermis	yellow yellow brown	dark green dark green dark green	No fluorescence	No fluorescence	No fluorescence	over-fluoresced
Peltophorum pterocarpum + $K_2Cr_2O_7$	Xylem Phloem Epidermis	yellow yellow brown	dark green dark green dark green	No fluorescence	No fluorescence	No fluorescence	over-fluoresced

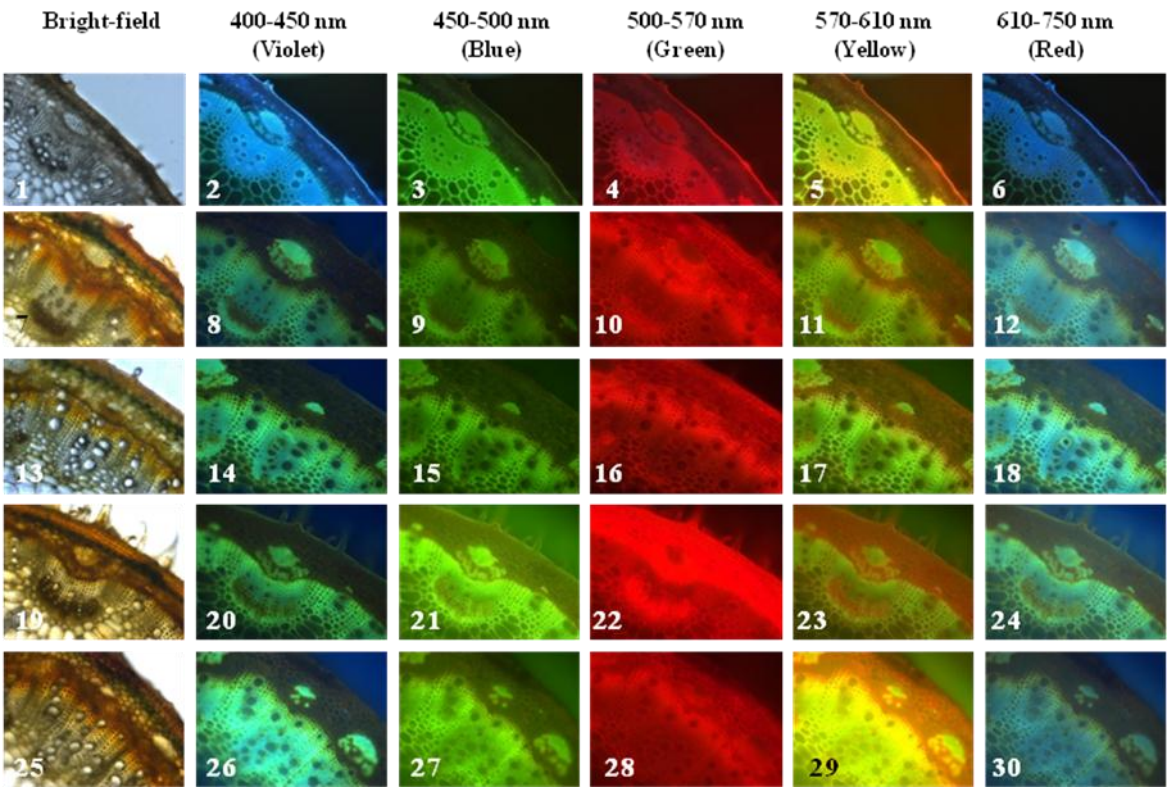


Plate 28. Fluorescence images of transverse section of dicot stem stained with *Peltophorum pterocarpum* dye and along with different mordants (X200). 1. Unstained section; 2-6. Auto-fluorescence; 7-12. Stained with *P. pterocarpum* dye; 13-18. *P. pterocarpum* dye and $CuSO_4$ mordant; 19-24. *P. pterocarpum* dye and $FeSO_4$ mordant; 25-30. *P. pterocarpum* dye and $K_2Cr_2O_7$ mordant.

4.7.2. Effect of synthetic and natural dyes on monocot stem section

The unstained monocot stems section auto-fluoresced in various excitation filters. In violet filter the vascular tissues and epidermis showed blue fluorescence, in blue filter the vascular tissues and epidermis revealed with green fluorescence, in green filter vascular tissues and epidermis showed red fluorescence, in yellow excitation filter vascular tissues fluoresced orange and in red filter vascular tissues showed bluish-pink fluorescence. However, no fluorescence was seen on ground tissue (Table 36).

Table 36. Auto-fluorescence of monocot stem section under different excitation filters in fluorescence microscopy.

Unstained section	Major plant tissues examined	Bright field microscopy	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm
Monocot stem	Vascular tissues Epidermis Ground tissue	no colour no colour no colour	blue blue no colour	green green no colour	red red no colour	orange orange no colour	bluish-pink bluish-pink no colour

The acridine orange imparted yellow colour to the vascular tissues and epidermis under bright field microscopy. In violet, blue, yellow and red filters vascular tissues and epidermis fluoresced green whereas in green filter the tissues and epidermis fluoresced red in colour. Likewise, the vascular tissues and epidermis were stained pink under bright field with the application of safranin. But when observed under various fluorescent filters, red colour was seen on the vascular tissues and epidermis. However, no fluorescence was seen on ground tissue (Table 37) (Plate 29).

Table 37. Effect of different fluorescence filters on monocot stem section stained with synthetic dyes.

Synthetic stain	Major plant tissues examined	Bright field microscopy	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm

Acridine orange	Vascular tissues	yellow	green	green	red	green	green
	Epidermis	yellow	green	green	red	green	green
	Ground tissue	yellow	no colour	no colour	no colour	no colour	no colour
Safranin	Vascular tissues	pink	red	red	red	red	red
	Epidermis	pink	red	red	red	red	red
	Ground tissue	pink	no colour	no colour	no colour	no colour	no colour

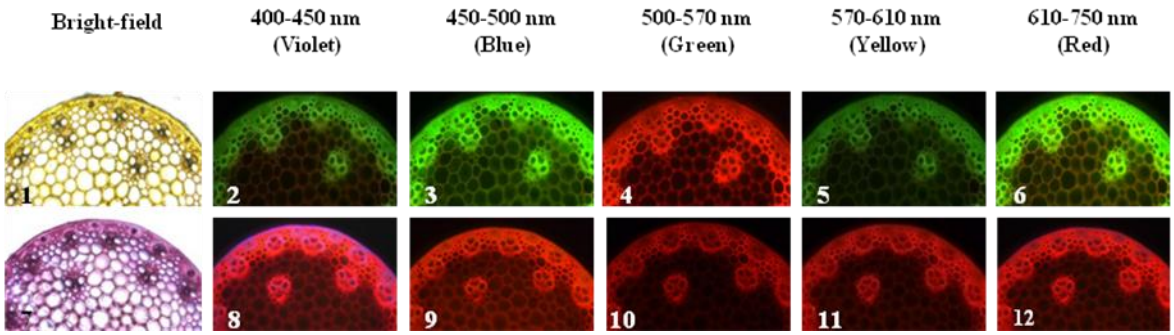


Plate 29. Fluorescence images of transverse section of monocot stem stained with synthetic dye (X400). 1-6. Acridine orange; 7-12. Safranin.

The dye from flower extract of *Averrhoa bilimbi* stained the vascular tissue and epidermis pink under bright field microscope. The violet filter fluoresced the tissue and epidermis blue. No fluorescence was seen on the tissues using $CuSO_4$ mordant. The blue filter fluoresced the tissue and epidermis orange and yellow filter fluoresced the epidermis and tissue red in colour. The green filter did not show any fluorescence. No significant fluorescence was observed on the ground tissue in any of the filters. However, in red filter the pink fluorescence was seen on the tissues and epidermis on use of *Averrhoa bilimbi* dye. The use of mordants along with the dye leads to the blue fluorescence of vascular tissue and pink fluorescence of epidermis in red filter. The ground tissue fluoresced pink using $CuSO_4$ mordant and blue using $FeSO_4$ and $K_2Cr_2O_7$ mordant (Table 38) (Plate 30).

Table 38. Effect of *Averrhoa bilimbi* dye and chemical mordants on monocot stem under bright-field microscopy and under different excitation filters in fluorescence microscopy.

Dyes and mordants used	Major plant tissues examined	Bright field microscopy	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm
Averrhoa bilimbi	Vascular tissues Epidermis Ground tissue	pink pink no colour	blue blue no colour	orange orange no colour	No fluorescence	red red no colour	pink pink no colour
Averrhoa bilimbi + $CuSO_4$	Vascular tissues Epidermis Ground tissue	pink pink no colour	No fluorescence	orange orange no colour	No fluorescence	red red no colour	blue pink pink
Averrhoa bilimbi + $FeSO_4$	Vascular tissues Epidermis Ground tissue	pink pink no colour	blue blue no colour	orange orange no colour	No fluorescence	red red no colour	blue pink blue
Averrhoa bilimbi + $K_2Cr_2O_7$	Vascular tissues Epidermis Ground tissue	pink pink no colour	blue blue no colour	orange orange no colour	No fluorescence	red red no colour	blue pink blue

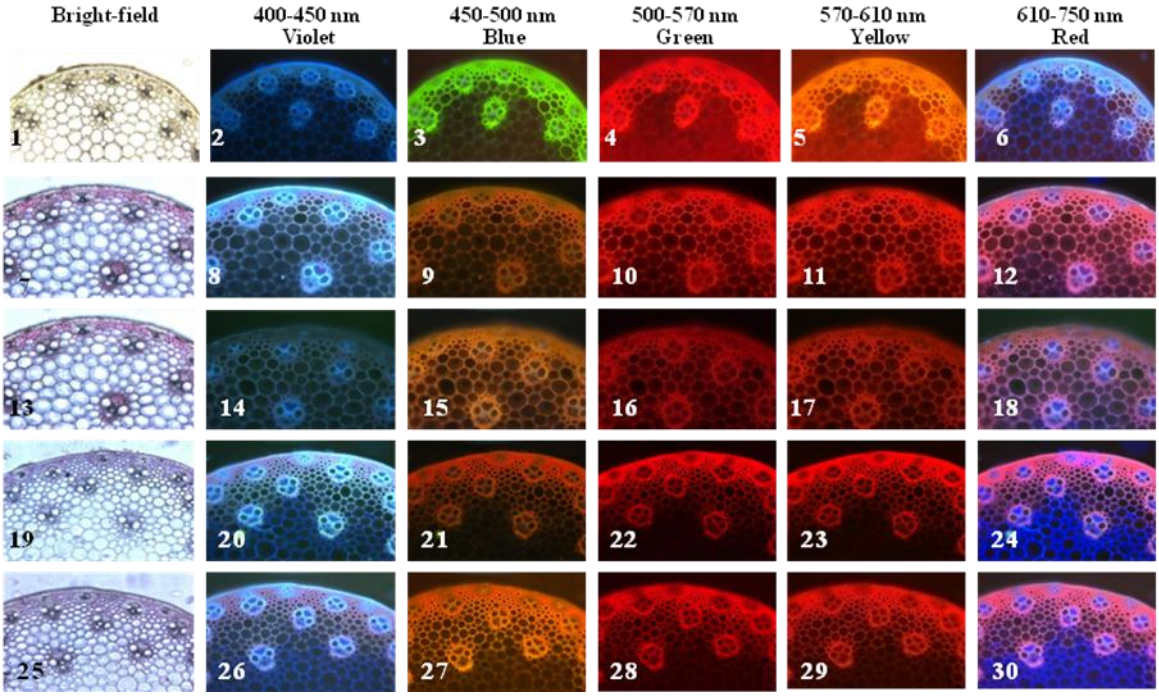


Plate 30. Fluorescence images of transverse section of monocot stem stained with *Averrhoa bilimbi* dye and along with different mordants (X400). 1. Unstained section; 2-6. Auto-fluorescence; 7-12. Stained with *A. bilimbi* dye; 13-18. *A. bilimbi* dye and $CuSO_4$ mordant; 19-24. *A. bilimbi* dye and $FeSO_4$ mordant; 25-30. *A. bilimbi* dye and $K_2Cr_2O_7$ mordant.

The monocot stem section appeared pink in colour when stained with *Garcinia indica* dye. The different fluorescent filters viz. violet and red filter fluoresced the epidermis and vascular tissues pink in colour. The blue filter leads to red fluorescence of vascular tissues and epidermis, whereas no fluorescence was seen on ground tissue. The green and yellow filters did not show any fluorescence (Table 39) (Plate 31).

Table 39. Effect of *Garcinia indica* dye and chemical mordants on monocot stem under bright-field microscopy and under different excitation filters in fluorescence microscopy.

Dyes and mordants used	Major plant tissues examined	Bright field microscopy	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm

Garcinia indica	Vascular tissues Epidermis Ground tissue	pink pink no colour	pink pink no colour	red red no colour	No fluorescence	No fluorescence	pink pink no colour
Garcinia indica + $CuSO_4$	Vascular tissues Epidermis Ground tissue	pink pink no colour	pink pink no colour	red red no colour	No fluorescence	No fluorescence	pink pink no colour
Garcinia indica + $FeSO_4$	Vascular tissues Epidermis Ground tissue	pink pink no colour	pink pink no colour	red red no colour	No fluorescence	No fluorescence	pink pink no colour
Garcinia indica + $K_2Cr_2O_7$	Vascular tissues Epidermis Ground tissue	pink pink no colour	pink pink no colour	red red no colour	No fluorescence	No fluorescence	pink pink no colour

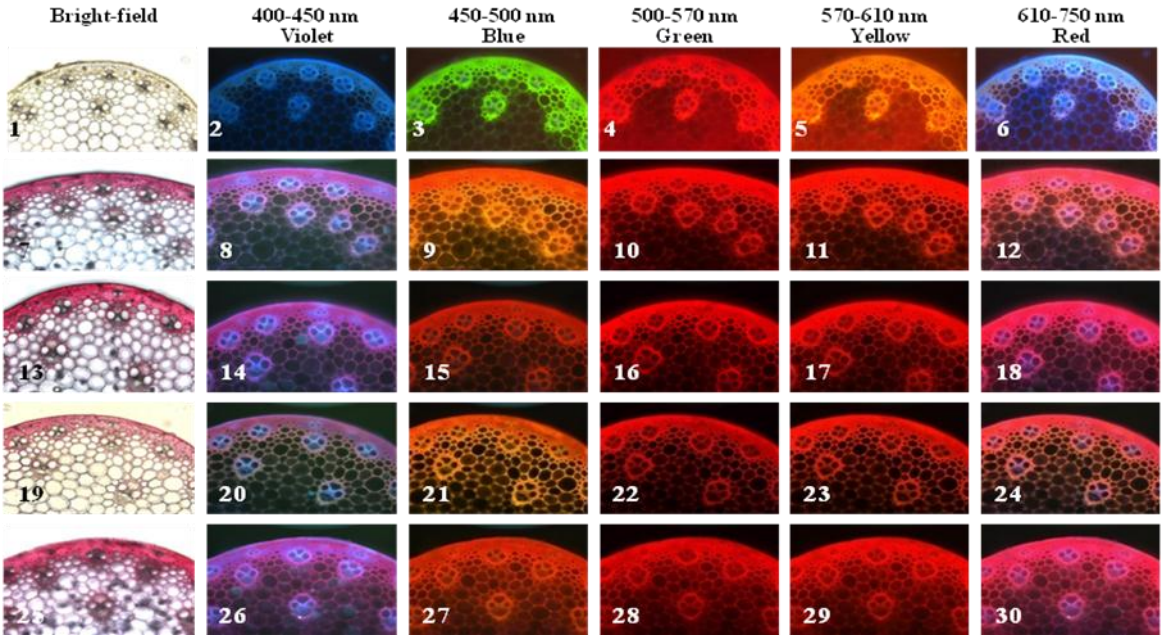


Plate 31. Fluorescence images of transverse section of monocot stem stained with *Garcinia indica* dye and along with different mordants (X400). 1. Unstained section; 2-6. Auto-fluorescence; 7-12. Stained with *G. indica* dye; 13-18. *G. indica* dye and $CuSO_4$ mordant; 19-24. *G. indica* dye and $FeSO_4$ mordant; 25-30. *G. indica* dye and $K_2Cr_2O_7$ mordant.

The fruit extract of *Melastoma malabathricum* stained the vascular tissue purple; however, the colour was more intense using chemical mordants. No fluorescence was seen under violet, green and yellow excitation filters. The red fluorescence of vascular tissue and epidermis was seen in blue filter. The red filter leads to the blue fluorescence of vascular tissue and pink fluorescence of epidermis. Whereas, no fluorescence was seen on the ground tissue (Table 40) (Plate 32).

Table 40. Effect of *Melastoma malabathricum* dye and chemical mordants on monocot stem under bright-field microscopy and under different excitation filters in fluorescence microscopy.

Dyes and mordants used	Major plant tissues examined	Bright field microscopy	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm
Melastoma malabathricum	Vascular tissues Epidermis Ground tissue	purple purple no colour	No fluorescence	red red no colour	No fluorescence	No fluorescence	blue pink no colour
Melastoma malabathricum + $CuSO_4$	Vascular tissues Epidermis Ground tissue	purple purple no colour	No fluorescence	red red no colour	No fluorescence	No fluorescence	blue pink no colour

Melastoma malabathricum + $FeSO_4$	Vascular tissues Epidermis Ground tissue	purple purple no colour	No fluorescence	red red no colour	No fluorescence	No fluorescence	blue pink no colour
Melastoma malabathricum + $K_2Cr_2O_7$	Vascular tissues Epidermis Ground tissue	purple purple no colour	No fluorescence	red red no colour	No fluorescence	No fluorescence	blue pink no colour

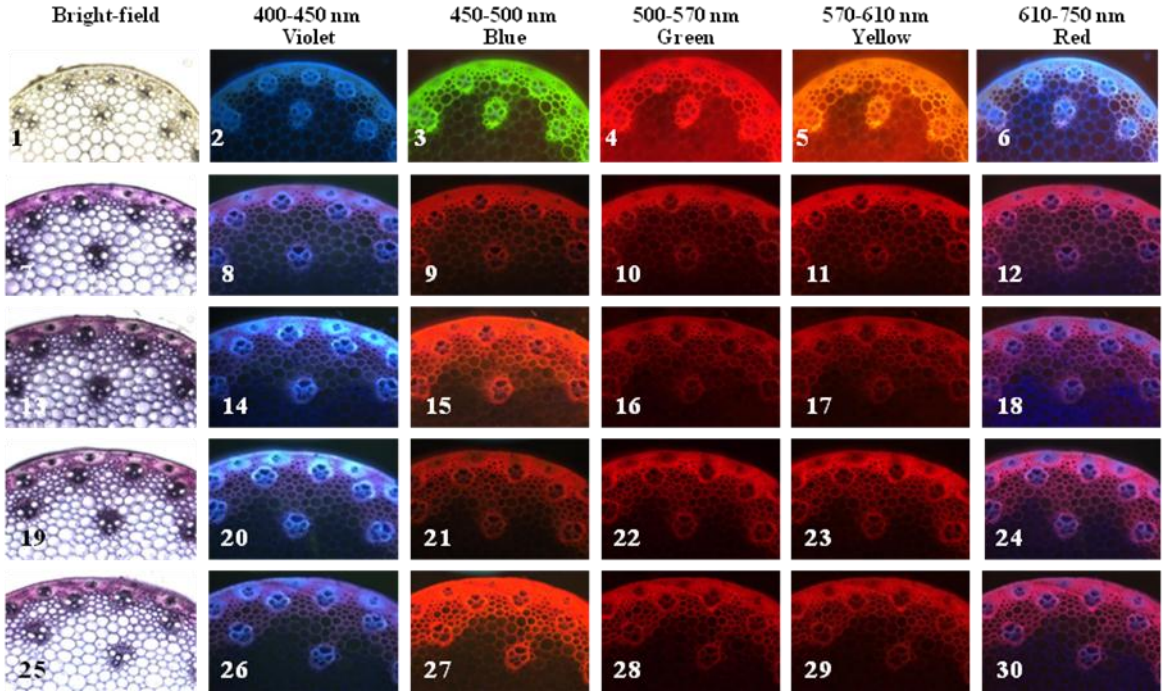


Plate 32. Fluorescence images of transverse section of monocot stem stained with *Melastoma malabathricum* dye and along with different mordants (X400). 1. Unstained section; 2-6. Auto-fluorescence; 7-12. Stained with *M. malabathricum* dye; 13-18. *M. malabathricum* dye and $CuSO_4$ mordant; 19-24. *M. malabathricum* dye and $FeSO_4$ mordant; 25-30. *M. malabathricum* dye and $K_2Cr_2O_7$ mordant.

The yellow colour dye from *Nyctanthes arbor-tristis* (corolla tube) stained the monocot section bright yellow under bright-field microscopy. The violet and red excitation filters lead to bluish-orange fluorescence of the tissues, but no fluorescence was seen on use of $FeSO_4$ mordant in red filter. The blue filter leads to orange fluorescence. No fluorescence was observed in green and yellow filters (Table 41) (Plate 33).

Table 41. Effect of *Nyctanthes arbor-tristis* dye and chemical mordants on monocot stem under bright-field microscopy and under different excitation filters in fluorescence microscopy.

Dye and mordants used	Major plant tissues examined	Bright field	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm
Nyctanthes arbor-tristis	Vascular tissues Epidermis Ground tissue	Yellow Yellow Yellow	Bluish-orange Bluish-orange Bluish-orange	Orange Orange Orange	No fluorescence	No fluorescence	Bluish-orange Bluish-orange Bluish-orange
Nyctanthes arbor-tristis + $CuSO_4$	Vascular tissues Epidermis Ground tissue	Yellow Yellow Yellow	Bluish-orange Bluish-orange Bluish-orange	Orange Orange Orange	No fluorescence	No fluorescence	Bluish-orange Bluish-orange Bluish-orange
Nyctanthes arbor-tristis + $FeSO_4$	Vascular tissues Epidermis Ground tissue	Yellow Yellow Yellow	Bluish-orange Bluish-orange Bluish-orange	Orange Orange Orange	No fluorescence	No fluorescence	No fluorescence
Nyctanthes arbor-tristis + $K_2Cr_2O_7$	Vascular tissues Epidermis Ground tissue	Yellow Yellow Yellow	Bluish-orange Bluish-orange Bluish-orange	Orange Orange Orange	No fluorescence	No fluorescence	Bluish-orange Bluish-orange Bluish-orange

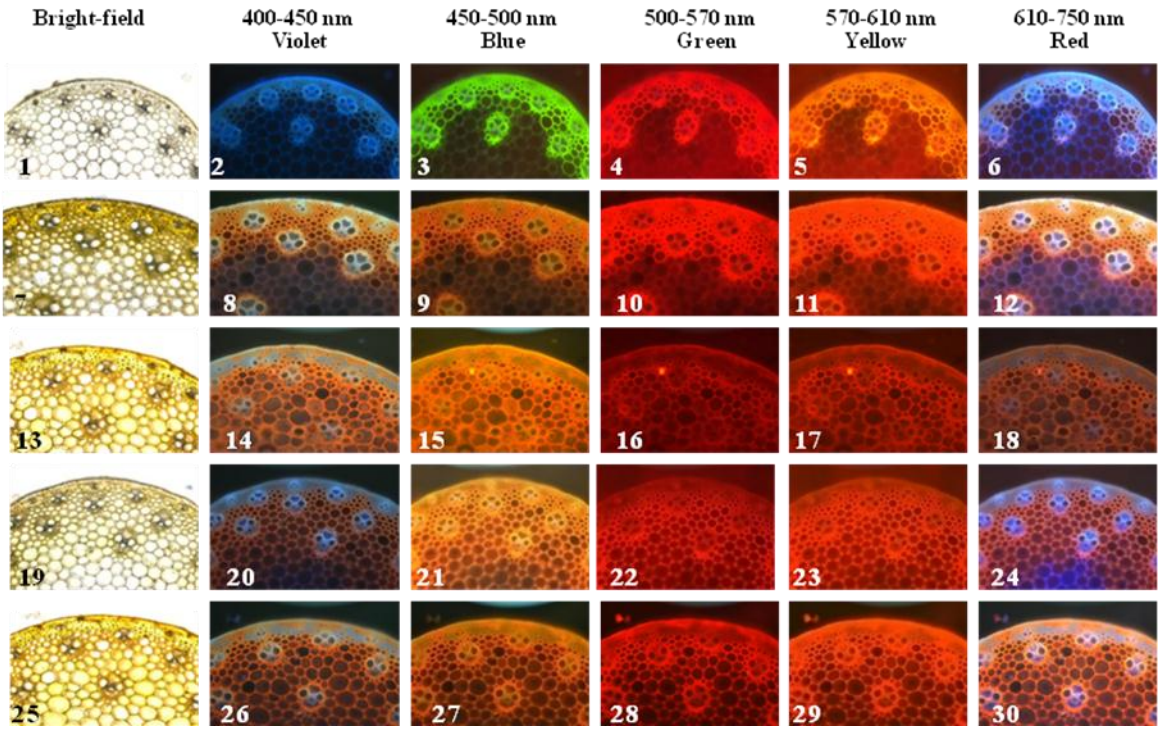


Plate 33. Fluorescence images of transverse section of monocot stem stained with *Nyctanthes arbor-tristis* dye and along with different mordants (X400). 1. Unstained section; 2-6. Auto-fluorescence; 7-12. Stained with *Nyctanthes arbor-tristis* dye; 13-18. *Nyctanthes arbor-tristis* dye and $CuSO_4$ mordant; 19-24. *Nyctanthes arbor-tristis* dye and $FeSO_4$ mordant; 25-30. *Nyctanthes arbor-tristis* dye and $K_2Cr_2O_7$ mordant.

The *Cassia fistula* bark stained the vascular tissue and epidermis brown. In violet filter, the vascular tissues were fluorescing red and epidermis fluoresced blue in colour. The ground tissue fluoresced red on use of dye and orange on use of $CuSO_4$ and $FeSO_4$ mordants. In blue filter the vascular tissues were fluorescing red, epidermis and ground tissue appeared green. In red filter vascular tissue fluoresced pink, but the application of ferrous sulphate and potassium dichromate mordant lead to orange fluorescence of vascular tissues. The epidermis fluoresced blue and pink and orange fluorescence of ground tissue was seen in red filter. However, in green and yellow filter there was no fluorescence (Table 42) (Plate 34).

Table 42. Effect of *Cassia fistula* dye and chemical mordants on monocot stem under bright-field microscopy and under different excitation filters in fluorescence microscopy.

Dye and mordants used	Major plant tissues examined	Bright field	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm
Cassia fistula	Vascular tissues Epidermis Ground tissue	brown brown no colour	red blue red	red green green	No fluorescence	No fluorescence	pink blue pink
Cassia fistula + $CuSO_4$	Vascular tissues Epidermis Ground tissue	brown brown no colour	red blue no colour	red green green	No fluorescence	No fluorescence	pink blue pink
Cassia fistula + $FeSO_4$	Vascular tissues Epidermis Ground tissue	brown brown no colour	red blue orange	red green green	No fluorescence	No fluorescence	orange blue orange
Cassia fistula + $K_2Cr_2O_7$	Vascular tissues Epidermis Ground tissue	brown brown no colour	red blue orange	red green green	No fluorescence	No fluorescence	orange blue orange

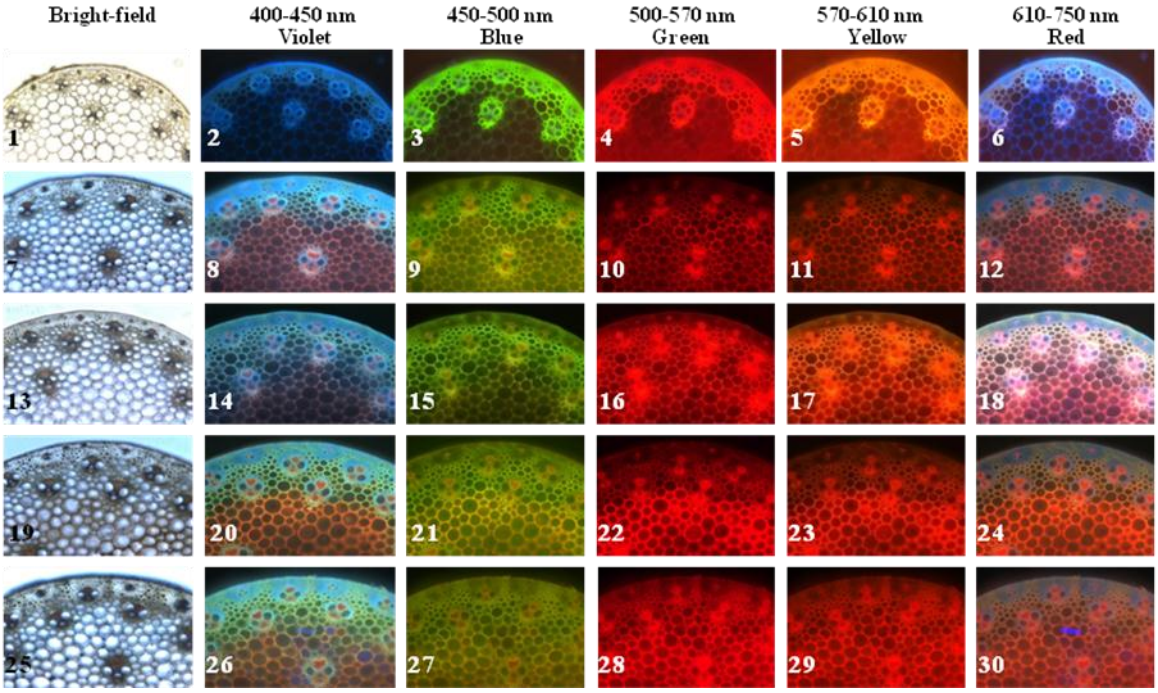


Plate 34. Fluorescence images of transverse section of monocot stem stained with *Cassia fistula* dye and along with different mordants (X400). 1. Unstained section; 2-6. Auto-fluorescence; 7-12. Stained with *C. fistula* dye; 13-18. *C. fistula* dye and $CuSO_4$ mordant; 19-24. *C. fistula* dye and $FeSO_4$ mordant; 25-30. *C. fistula* dye and $K_2Cr_2O_7$ mordant.

In monocot stem section the vascular tissues were stained brown on staining the section with *Peltophorum pterocarpum*. No colour was noticed on epidermis and ground tissue. The violet and blue filters lead the vascular tissues to fluoresce brown, whereas the green fluorescence was noticed on epidermis and ground tissue of monocot stem section. Green filter did not show any fluorescence. In yellow and red filter vascular tissue fluoresced orange and epidermis and ground tissue fluoresced green in colour. But the use of $FeSO_4$ leads to blue fluorescence of epidermis and ground tissue (Table 43) (Plate 35).

Table 43. Effect of *Peltophorum pterocarpum* dye and chemical mordants on monocot stem under bright-field microscopy and under different excitation filters in fluorescence microscopy.

Dye and mordants used	Major plant tissues examined	Bright field	Different excitation filters				
			Violet 400-450 nm	Blue 450-500 nm	Green 500-570 nm	Yellow 570-610 nm	Red 610-750 nm
Peltophorum pterocarpum	Vascular tissues Epidermis Ground tissue	brown no colour no colour	brown green green	brown green green	No fluorescence	orange green green	orange green green
Peltophorum pterocarpum + $CuSO_4$	Vascular tissues Epidermis Ground tissue	brown no colour no colour	brown green green	brown green green	No fluorescence	orange green green	orange green green
Peltophorum pterocarpum + $FeSO_4$	Vascular tissues Epidermis Ground tissue	brown no colour no colour	No fluorescence	brown green green	No fluorescence	orange green green	orange blue blue
Peltophorum pterocarpum + $K_2Cr_2O_7$	Vascular tissues Epidermis Ground tissue	brown no colour no colour	green green blue	brown green green	No fluorescence	orange green green	orange green green

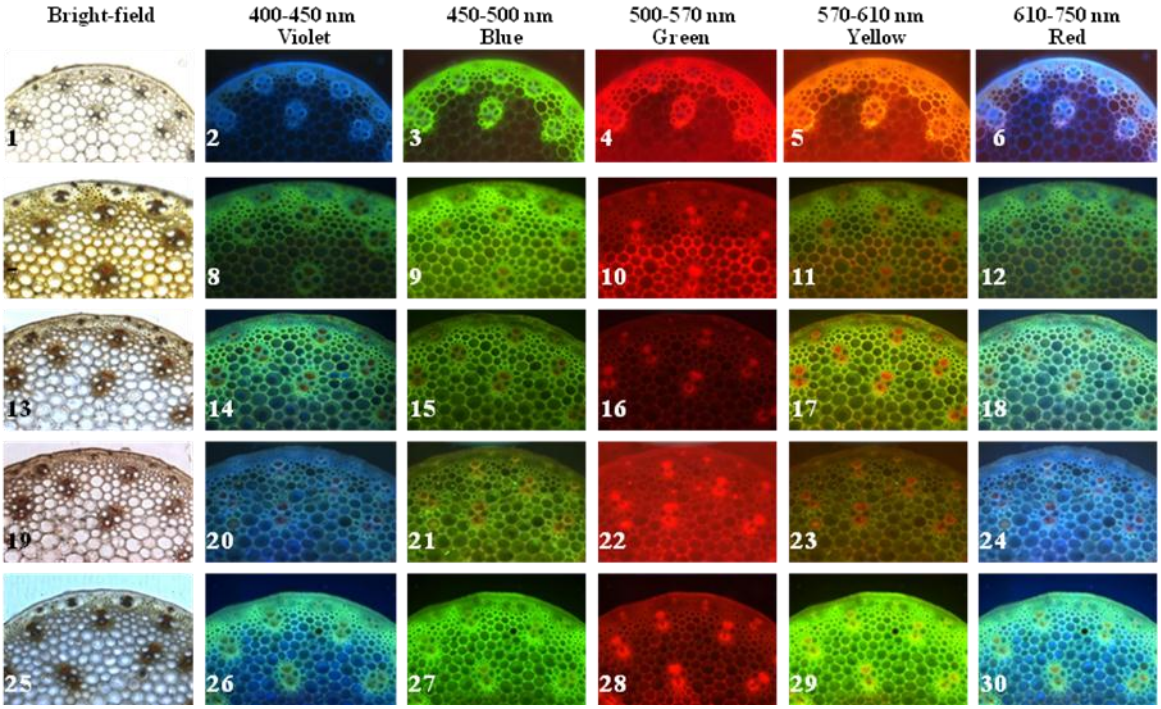


Plate 35. Fluorescence images of transverse section of monocot stem stained with *Peltophorum pterocarpum* dye and along with different mordants (X400). 1. Unstained section; 2-6. Auto-fluorescence; 7-12. Stained with *P. pterocarpum* dye; 13-18. *P. pterocarpum* dye and $CuSO_4$ mordant; 19-24. *P. pterocarpum* dye and $FeSO_4$ mordant; 25-30. *P. pterocarpum* dye and $K_2Cr_2O_7$ mordant.

Among all the filters examined the green filter (500-570 nm) lead no fluorescence or over-fluorescence of plant tissues on staining with all the natural dyes individually and along with the mordants in both the monocot and dicot stem sections. The violet excitation filter (400-450 nm) showed distinct fluorescence of xylem, phloem and epidermis with all the natural dyes and also with the chemical mordants.

4.8. Optimization of dyeing conditions

4.8.1. Optimization of dye concentration

The optical density for each dye solutions and the percentage absorbance of dye on fabrics were calculated based on difference between the absorbance before dyeing and absorbance after dyeing. Considering the highest value of percentage absorption, the optimum dye concentration for dyeing cotton and silk was determined. For *Ceriops tagal*

and *Mammea suriga* the percentage absorption of dye on cotton fabrics was highest at dye concentration (4g/50 mL) and for silk fabrics the percentage absorption of dye was highest at (3g/50 mL) dye concentration. Hence, (4g/50 mL) dye concentration for cotton and (3g/50 mL) for silk was chosen for dyeing purpose for both the dyes. The results of experiments are presented in (Table 44-47).

Table 44. Optimization of dye concentration of *Ceriops tagal* dye for dyeing cotton fabrics.

Dye concentration (g)	Water concentration (mL)	Optical density (473 nm)		Dye uptake (%)
		Before dyeing	After dyeing	
1	50	0.045	0.040	11.11
2	50	0.063	0.054	14.28
3	50	0.125	0.107	14.40
4	50	0.210	0.173	**17.61**
5	50	0.167	0.140	16.16

Table 45. Optimization of dye concentration of *Ceriops tagal* dye for dyeing silk fabrics.

Dye concentration (g)	Water concentration (mL)	Optical density (473 nm)		Dye uptake (%)
		Before dyeing	After dyeing	
1	50	0.219	0.176	19.63
2	50	0.320	0.256	20.00
3	50	0.356	0.268	**24.17**
4	50	0.348	0.264	24.13
5	50	0.336	0.261	22.32

Table 46. Optimization of dye concentration of *Mammea suriga* dye for dyeing cotton fabrics.

Dye concentration (g)	Water concentration (mL)	Optical density (568 nm)		Dye uptake (%)
		Before dyeing	After dyeing	
1	50	0.052	0.051	1.92
2	50	0.070	0.068	2.85
3	50	0.103	0.099	3.88
4	50	0.280	0.267	**4.64**
5	50	0.130	0.124	4.61

Table 47. Optimization of dye concentration of *Mammea suriga* dye for dyeing silk fabrics.

Dye concentration (g)	Water concentration (mL)	Optical density (568 nm)		Dye uptake (%)
		Before dyeing	After dyeing	
1	50	0.440	0.419	4.77
2	50	0.445	0.423	4.94
3	50	0.602	0.569	**5.48**
4	50	0.593	0.562	5.22
5	50	0.491	0.466	5.09

4.8.2. Optimization of dyeing time

The optimum time for dyeing cotton and silk fabrics with *Ceriops tagal* and *Mammea suriga* were determined under optimized conditions of dye concentration. The optimum dyeing time was calculated based on difference between the absorbance before dyeing and absorbance after dyeing using optimized dye concentration for both the dyes under various periods. Considering the highest value of percentage absorption at varying time periods the optimum dyeing time for dyeing cotton and silk was determined. The

optimum dyeing time for cotton fabrics was 60 min and optimized dyeing time for silk fabrics was 45 min. The results of experiments are summarized (Table 48-51).

Table 48. Optimization of dyeing time of *Ceriops tagal* dye for dyeing cotton fabrics.

Dye concentration (g/50 mL)	Dyeing time (min)	Optical density (473 nm)		Dye uptake (%)
		Before dyeing	After dyeing	
4	30	0.340	0.332	2.35
4	45	0.524	0.508	3.05
4	60	1.737	1.623	**6.56**
4	75	1.403	1.345	4.13
4	90	0.968	0.932	3.71

Table 49. Optimization of dyeing time of *Ceriops tagal* dye for dyeing silk fabrics.

Dye concentration (g/50 mL)	Dyeing time (min)	Optical density (473 nm)		Dye uptake (%)
		Before dyeing	After dyeing	
3	30	1.767	1.645	6.90
3	45	2.041	1.841	**9.79**
3	60	1.780	1.655	7.02
3	75	1.787	1.659	7.16
3	90	1.791	1.661	7.25

Table 50. Optimization of dyeing time of *Mammea suriga* dye for dyeing cotton fabrics.

Dye concentration (g/50 mL)	Dyeing time (min)	Optical density (568 nm)		Dye uptake (%)
		Before dyeing	After dyeing	
4	30	0.069	0.067	2.89
4	45	0.095	0.092	3.15

4	60	0.593	0.555	**6.40**
4	75	0.238	0.230	3.36
4	90	0.356	0.335	5.89

Table 51. Optimization of dyeing time of *Mammea suriga* dye for dyeing silk fabrics.

Dye concentration (g/50 mL)	Dyeing time (min)	Optical density (568 nm)		Dye uptake (%)
		Before dyeing	After dyeing	
3	30	0.621	0.576	7.24
3	45	0.795	0.648	**18.49**
3	60	0.712	0.618	13.20
3	75	0.764	0.643	15.83
3	90	0.782	0.645	17.51

4.9. Dyeing of fabrics

Pre-treated silk and cotton fabrics were dyed using the optimum dye concentration, dyeing time and by using the three chemical mordants. Three different mordanting methods such as pre-mordanting, simultaneous mordanting and post-mordanting were followed to get wide range of shades from a single dye. The shades obtained were described according to the ISCC-NBS (Inter-Society Colour Council) and (National Bureau of Standards) colour system.

4.9.1. Dyeing of silk and cotton fabrics with natural dye

Dyeing of silk fabrics with *Ceriops tagal* dye

Various colours were obtained on silk when the fabric was dyed with bark extract of *Ceriops tagal*. The use of mordants resulted in variety of shades from single colour in different mordanting methods. According to ISCC-NBS colour system (level 3) deep brown colour was obtained on silk fabric when dyed with bark extract of *Ceriops tagal*. After washing the fabric with mild detergent, the original colour (deep brown) was

retained on the fabrics. However, after light and rub fastness test the colour of the fabric (deep brown) was slightly changed (strong brown).

The deep brown dye from *Ceriops tagal* when used along with potassium dichromate mordant in pre-mordanting gave moderate orange shade to the fabric, which completely changed its shade to deep reddish orange after first wash. But after second, third wash the shade was changed to moderate reddish orange. The light and rub fastness imparted deep reddish orange shade. The use of copper sulphate mordant along with the dye gave brownish orange shade, which when subjected to fastness tests viz. light, wash and rub imparted strong brown shade to the fabric. The ferrous sulphate mordant gave greenish black colour, but after third wash and rubbing the fabrics gave dark greenish gray colour to the fabrics. The light fastness retained the original colour onto the fabrics i.e. greenish black. The combination of mordants potassium dichromate and copper sulphate along with the dye gave grayish olive shade. The rub fastness did not change the shade but the exposure to sunlight and subsequent washing changed the shade to light grayish olive. The combination of copper sulphate and ferrous sulphate mordant gave dark grayish olive shade which was retained after rub fastness. But the colour was changed to deep brown after first wash which was later turned to brownish orange after third wash. The light fastness gave brownish orange shade. On combination of ferrous sulphate and potassium dichromate mordant the colour obtained on fabric was moderate yellowish brown, which did not change after subsequent wash. Even after light and rub fastness, the colour was retained on the fabric (Table 52) (Plate 36).

Table 52. Colour obtained on silk fabrics with *Ceriops tagal* dye (pre-mordanting).

Dye and mordants used	Colour of dyed fabric	Wash fastness			Light fastness		Rub fastness	
		1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Ceriops tagal	deep brown	deep brown	deep brown	deep brown	strong brown	strong brown	strong brown	strong brown
Dye + $K_2Cr_2O_7$	moderate orange	deep reddish orange	moderate reddish orange	moderate reddish orange	deep reddish orang	deep reddish orange	deep reddish orange	deep reddish orang

					e			e
Dye + $CuSO_4$	brownish orange	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown
Dye + $FeSO_4$	greenish black	greenish black	greenish black	dark greenish gray	greenish black	greenish black	dark greenish gray	dark greenish gray
Dye + $K_2Cr_2O_7$ + $CuSO_4$	grayish olive	light grayish olive	light grayish olive	light grayish olive	light grayish olive	light grayish olive	grayish olive	grayish olive
Dye + $CuSO_4$+ $FeSO_4$	dark grayish olive	deep brown	deep brown	brownish orange	brownish orange	brownish orange	dark grayish olive	dark grayish olive
Dye + $FeSO_4$ + $K_2Cr_2O_7$	moderate yellowish brown	moderate yellowish brown	moderate yellowish brown	moderate yellowish brown	moderate yellowish brown	moderate yellowish brown	moderate yellowish brown	moderate yellowish brown

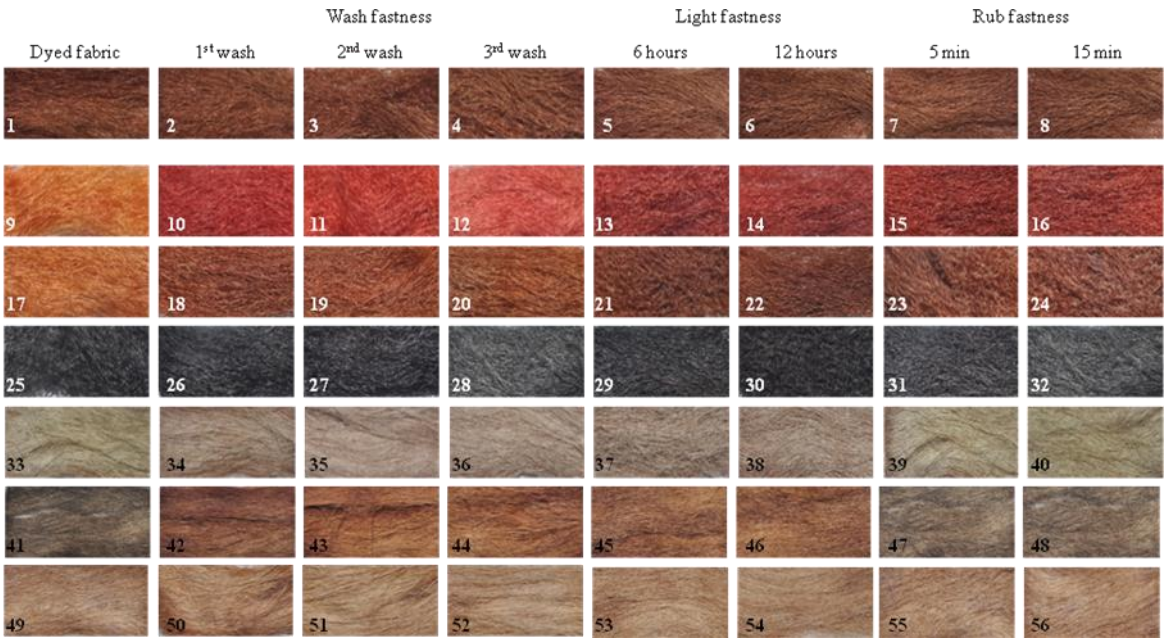


Plate 36. Dyeing of silk fabrics with *Ceriops tagal* dye (Pre-mordanting). 1-8. Dyed with *C. tagal* dye; 9-16. Dyed with *C. tagal* and $K_2Cr_2O_7$; 17-24. Dyed with *C. tagal* and

$CuSO_4$; 25-32. Dyed with *C. tagal* and $FeSO_4$; 33-40. Dyed with *C. tagal* and $K_2Cr_2O_7$ + $CuSO_4$; 41-48. Dyed with *C. tagal* and $CuSO_4$ + $FeSO_4$; 49-56. Dyed with *C. tagal* and $FeSO_4$ + $K_2Cr_2O_7$.

In simultaneous mordanting the potassium dichromate mordant along with the dye gave strong brown colour to the fabric, which did not fade on treatment with light fastness test. However, rubbing the fabric gave brownish orange shade to the fabric. The copper sulphate mordant imparted deep orange colour, which completely changed to deep brown after successive washing. The six hours sun exposure changed the colour to dark brown whereas 12 hours sunlight exposure gave deep brown colour. The 5 min rubbing gave strong brown colour and 15 mins rubbing gave strong orange colour to the fabrics. The ferrous sulphate mordant imparted bluish black shade to the fabric which did not leached out even after subsequent washes. The colour was also retained after rub and light fastness. The combination of potassium dichromate and copper sulphate mordant gave light yellow colour to the fabric, which was unchanged after wash, rub and light fastness test. The combination of copper sulphate and ferrous sulphate mordant gave dark grayish olive to the fabric which turned to moderate brown on first wash and later to moderate orange after second and third wash. It turned brownish orange on light exposure, while the dark grayish olive colour was unchanged on rubbing. The combination of ferrous sulphate and potassium dichromate imparted light grayish brown colour to the fabric which was retained even after successive wash. The colour grayish yellow was obtained after light exposure and rubbing the fabrics (Table 53) (Plate 37).

Table 53. Colour obtained on silk fabrics with *Ceriops tagal* dye (simultaneous mordanting).

Dye and mordants used	Colour of dyed fabric	Wash fastness			Light fastness		Rub fastness	
		1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Dye + $K_2Cr_2O_7$	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown	brownish orange	brownish orange
Dye + $CuSO_4$	deep orange	deep brown	deep brown	moderate brown	dark brown	deep brown	strong brown	strong brown

Dye + $FeSO_4$	bluish black	bluish black	bluish black	bluish black	bluish black	bluish black	bluish black	bluish black
Dye + $K_2Cr_2O_7$ + $CuSO_4$	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown
Dye + $CuSO_4$ + $FeSO_4$	dark grayish olive	moderate brown	moderate orange	moderate orange	brownish orange	brownish orange	dark grayish olive	dark grayish olive
Dye + $FeSO_4$ + $K_2Cr_2O_7$	light grayish brown	light grayish brown	light grayish brown	light grayish brown	grayish yellow	grayish yellow	grayish yellow	grayish yellow

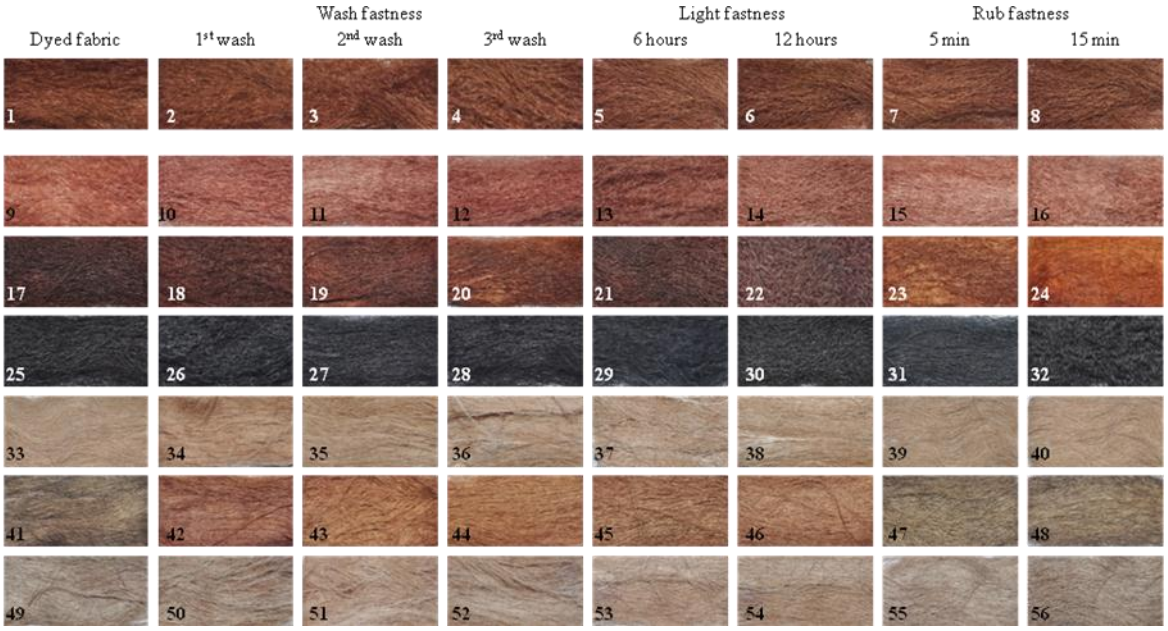


Plate 37. Dyeing of silk fabrics with *Ceriops tagal* dye (Simultaneous mordanting). 1-8. Dyed with *C. tagal* dye; 9-16. Dyed with *C. tagal* and $K_2Cr_2O_7$; 17-24. Dyed with *C. tagal* and $CuSO_4$; 25-32. Dyed with *C. tagal* and $FeSO_4$; 33-40. Dyed with *C. tagal* and $K_2Cr_2O_7$ + $CuSO_4$; 41-48. Dyed with *C. tagal* and $CuSO_4$ + $FeSO_4$; 49-56. Dyed with *C. tagal* and $FeSO_4$ + $K_2Cr_2O_7$.

In post-mordanting, the dye along with potassium dichromate mordant imparted strong red colour to the fabric, which after successive wash changed the colour to deep brown. The light exposure of 6 hours and 12 hours produced deep brown colour to the

fabric. The rub fastness did not change the strong red colour. The copper sulphate mordant gave deep orange colour to the fabric, which after washing completely changed its colour to deep brown. However, the exposure to light did not change the colour of the fabric but the rub fastness resulted in deep orange colour to the fabrics. The ferrous sulphate mordant gave brownish black colour to the fabric which remained unchanged after all the three fastness tests. The combination of potassium dichromate and copper sulphate mordant gave moderate brown colour to the fabric, which remained unchanged even after rub, light and wash fastness tests. The combination of copper sulphate and ferrous sulphate imparted olive gray colour to the silk fabric, which after washing changed to olive black colour. The rubbing of the fabrics and the light exposure did not change the olive gray colour of the fabric. The ferrous sulphate and potassium dichromate mordant gave olive black colour, which was retained after all the three fastness tests (Table 54) (Plate 38).

Table 54. Colour obtained on silk fabrics with *Ceriops tagal* dye (post-mordanting).

Dye and mordants used	Colour of dyed fabric	Wash fastness			Light fastness		Rub fastness	
		1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Dye + $K_2Cr_2O_7$	strong red	deep brown	deep brown	deep brown	deep brown	deep brown	strong red	strong red
Dye + $CuSO_4$	deep orange	deep brown	deep brown	deep brown	deep brown	deep brown	deep orange	deep orange
Dye + $FeSO_4$	brownish black	brownish black	brownish black	brownish black	brownish black	brownish black	brownish black	brownish black
Dye + $K_2Cr_2O_7$ + $CuSO_4$	moderate brown	moderate brown	moderate brown	moderate brown	moderate brown	moderate brown	moderate brown	moderate brown
Dye + $CuSO_4$ + $FeSO_4$	olive gray	olive black	olive black	olive black	olive gray	olive gray	olive gray	olive gray

Dye + $FeSO_4$ + $K_2Cr_2O_7$	olive black	olive black	olive black	olive black	olive black	olive black	olive black	olive black

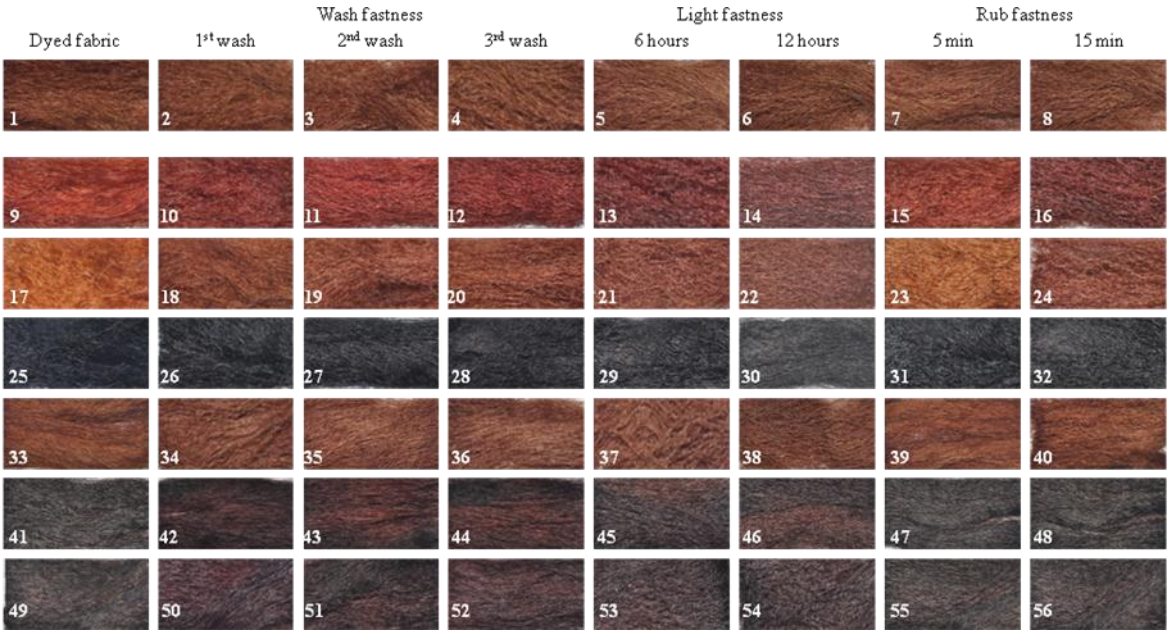


Plate 38. Dyeing of silk fabrics with *Ceriops tagal* dye (Post-mordanting). 1-8. Dyed with *C. tagal* dye; 9-16. Dyed with *C. tagal* and $K_2Cr_2O_7$; 17-24. Dyed with *C. tagal* and $CuSO_4$; 25-32. Dyed with *C. tagal* and $FeSO_4$; 33-40. Dyed with *C. tagal* and $K_2Cr_2O_7$ + $CuSO_4$; 41-48. Dyed with *C. tagal* and $CuSO_4$ + $FeSO_4$; 49-56. Dyed with *C. tagal* and $FeSO_4$ + $K_2Cr_2O_7$.

Dyeing of cotton fabrics with *Ceriops tagal* dye

Dyeing of cotton fabrics with *Ceriops tagal* dye produced moderate orange colour to the fabrics which changed to deep brown colour after first wash and second wash and brownish orange after third wash. The exposure to light gave strong brown colour to the fabrics. Whereas, no colour change was seen after rubbing the fabrics.

The use of potassium dichromate mordant with the dye gave light brown colour to the fabric which was unchanged after fastness tests in pre-mordanting method. The copper sulphate mordant gave strong brown colour to the fabric, which changed to deep brown

after first wash and second wash and later the leaching of dye from the fabric changed its colour to brownish orange after third wash. The light and rub fastness gave moderate orange colour to the fabric. The ferrous sulphate mordant imparted brownish black colour which on washing produced deep brown colour. The exposure to light gave moderate brown colour, whereas rubbing the fabric gave dark grayish brown colour. The combination of copper sulphate and ferrous sulphate mordant gave dark brown colour which further changed to moderate brown after repeated washing. The light fastness produced strong brown colour and rub fastness gave grayish brown colour. The combination of ferrous sulphate and potassium dichromate with the dye gave dark grayish yellow colour which did not change after fastness tests. The combination of potassium dichromate and copper sulphate mordant with dye gave pale yellow colour, which was unchanged after all the three fastness tests (Table 55) (Plate 39).

Table 55. Colour obtained on cotton fabrics with *Ceriops tagal* dye (pre-mordanting).

Dye and mordants used	Colour of dyed fabric	Wash fastness			Light fastness		Rub fastness	
		1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Ceriops tagal	moderate orange	deep brown	deep brown	brownish orange	strong brown	strong brown	moderate orange	moderate orange
Dye + $K_2Cr_2O_7$	light brown	light brown	light brown	light brown	light brown	light brown	light brown	light brown
Dye + $CuSO_4$	strong brown	deep brown	deep brown	brownish orange	moderate orange	moderate orange	moderate orange	moderate orange
Dye + $FeSO_4$	brownish black	deep brown	deep brown	deep brown	moderate brown	moderate brown	dark grayish brown	dark grayish brown
Dye + $K_2Cr_2O_7$ + $CuSO_4$	pale yellow	pale yellow	pale yellow	pale yellow	strong brown	strong brown	grayish brown	grayish brown
Dye +	dark	modera	modera	modera	strong	strong	grayish	grayis

$CuSO_4$ + $FeSO_4$	brown	te brown	te brown	te brown	brown	brown	brown	h brown
Dye + $FeSO_4$ + $K_2Cr_2O_7$	dark grayish yellow	dark grayish yellow	dark grayish yellow	dark grayish yellow	dark grayish yellow	dark grayish yellow	dark grayish yellow	dark grayish yellow

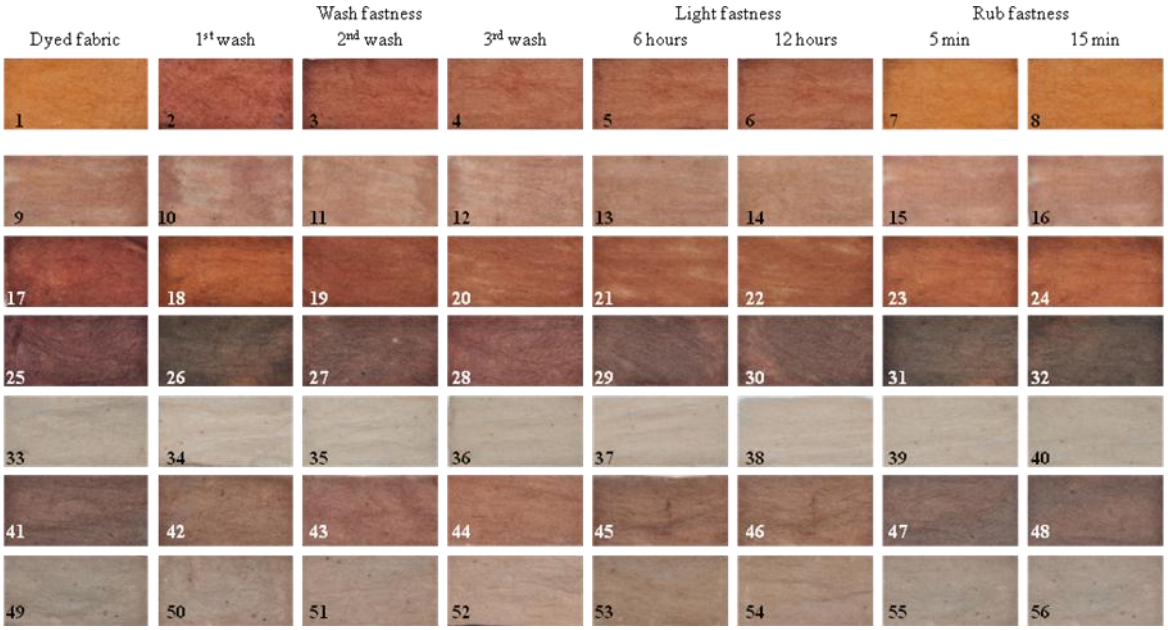


Plate 39. Dyeing of cotton fabrics with *Ceriops tagal* dye (Pre-mordanting). 1-8. Dyed with *C. tagal* dye; 9-16. Dyed with *C. tagal* and $K_2Cr_2O_7$; 17-24. Dyed with *C. tagal* and $CuSO_4$; 25-32. Dyed with *C. tagal* and $FeSO_4$; 33-40. Dyed with *C. tagal* and $K_2Cr_2O_7$ + $CuSO_4$; 41-48. Dyed with *C. tagal* and $CuSO_4$ + $FeSO_4$; 49-56. Dyed with *C. tagal* and $FeSO_4$ + $K_2Cr_2O_7$.

In simultaneous mordanting the potassium dichromate mordant gave light brown colour to the fabric, which did not fade even after fastness tests. The application of copper sulphate mordant gave strong brown colour to the fabric which later turned to deep brown after first and second wash. The third wash lead to moderate orange colour to the fabric. The exposure to light gave moderate orange colour whereas rubbing the fabric produced deep brown colour to the fabrics. The ferrous sulphate mordant dyed the fabric reddish black, which turned dark brown after washing. The rubbing of fabric did not change the colour but exposure to light gave strong brown colour to the fabric. The combination of

ferrous sulphate and copper sulphate imparted strong brown colour to the fabric which did not change after wash, rub as well as on light exposure. The combination of ferrous sulphate and potassium dichromate gave strong yellowish brown colour which did not change much after fastness tests. Likewise, the combination of potassium dichromate and copper sulphate gave light orange yellow colour which was remained unchanged after fastness tests (Table 56) (Plate 40).

Table 56. Colour obtained on cotton fabrics with *Ceriops tagal* dye (simultaneous mordanting).

Dye and mordants used	Colour of dyed fabric	Wash fastness			Light fastness		Rub fastness	
		1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Dye + $K_2Cr_2O_7$	light brown	light brown	light brown	light brown	light brown	light brown	light brown	light brown
Dye + $CuSO_4$	strong brown	deep brown	deep brown	moderate orange	moderate orange	moderate orange	deep brown	deep brown
Dye + $FeSO_4$	reddish black	dark brown	dark brown	dark brown	strong brown	strong brown	reddish black	reddish black
Dye + $K_2Cr_2O_7$ + $CuSO_4$	light orange yellow	light orange yellow	light orange yellow	light orange yellow	light orange yellow	light orange yellow	light orange yellow	light orange yellow
Dye + $CuSO_4$ + $FeSO_4$	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown
Dye + $FeSO_4$ + $K_2Cr_2O_7$	strong yellowish brown	strong yellowish brown	strong yellowish brown	strong yellowish brown	strong yellowish brown	strong yellowish brown	strong yellowish brown	strong yellowish brown

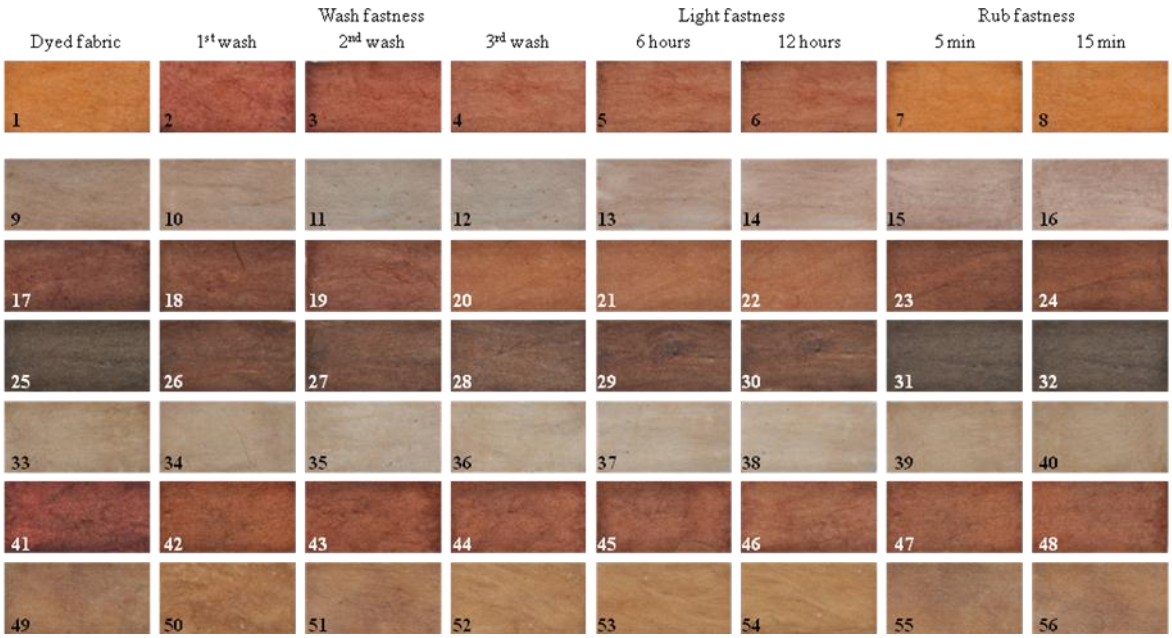


Plate 40. Dyeing of cotton fabrics with *Ceriops tagal* dye (Simultaneous mordanting). 1-8. Dyed with *C. tagal* dye; 9-16. Dyed with *C. tagal* and $K_2Cr_2O_7$; 17-24. Dyed with *C. tagal* and $CuSO_4$; 25-32. Dyed with *C. tagal* and $FeSO_4$; 33-40. Dyed with *C. tagal* and $K_2Cr_2O_7$ + $CuSO_4$; 41-48. Dyed with *C. tagal* and $CuSO_4$ + $FeSO_4$; 49-56. Dyed with *C. tagal* and $FeSO_4$ + $K_2Cr_2O_7$.

In post-mordanting the potassium dichromate mordant gave deep brown colour to the fabric, which turned to strong brown on rub, light and wash fastness tests. The mordant copper sulphate gave deep brown colour, which changed to strong brown on first, second wash and later to deep brown on third wash. The light and rub fastness gave strong brown shade to the fabric. The ferrous sulphate mordant gave dark grayish brown colour which was not changed in all the three fastness tests. The combination of mordant copper sulphate and ferrous sulphate gave moderate yellowish brown colour which on first wash gave moderate brown colour and after second and third wash gave light brown colour. The light fastness leads to light brown colour, whereas the colour was not changed on rubbing. The combination of ferrous sulphate and potassium dichromate gave dark brown colour and first wash changed the colour to moderate yellowish brown. The light fastness gave light brown colour and rub fastness gave moderate yellowish brown colour to the fabric. The combination of potassium dichromate and copper sulphate gave light brown shade which was unchanged after fastness tests (Table 57) (Plate 41).

Table 57. Colour obtained on cotton fabrics with *Ceriops tagal* dye (post-mordanting).

Dye and mordants used	Colour of dyed fabric	Wash fastness			Light fastness		Rub fastness	
		1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Dye + $K_2Cr_2O_7$	deep brown	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown
Dye + $CuSO_4$	deep brown	strong brown	strong brown	deep orange	strong brown	strong brown	strong brown	strong brown
Dye + $FeSO_4$	dark grayish brown	dark grayish brown	dark grayish brown	dark grayish brown	dark grayish brown	dark grayish brown	dark grayish brown	dark grayish brown
Dye + $K_2Cr_2O_7$ + $CuSO_4$	light brown	light brown	light brown	light brown	light brown	light brown	light brown	light brown
Dye + $CuSO_4$ + $FeSO_4$	moderate yellowish brown	moderate brown	light brown	light brown	light brown	light brown	moderate yellowish brown	moderate yellowish brown
Dye + $FeSO_4$ + $K_2Cr_2O_7$	dark brown	moderate yellowish brown	moderate yellowish brown	moderate yellowish brown	light brown	light brown	moderate yellowish brown	moderate yellowish brown

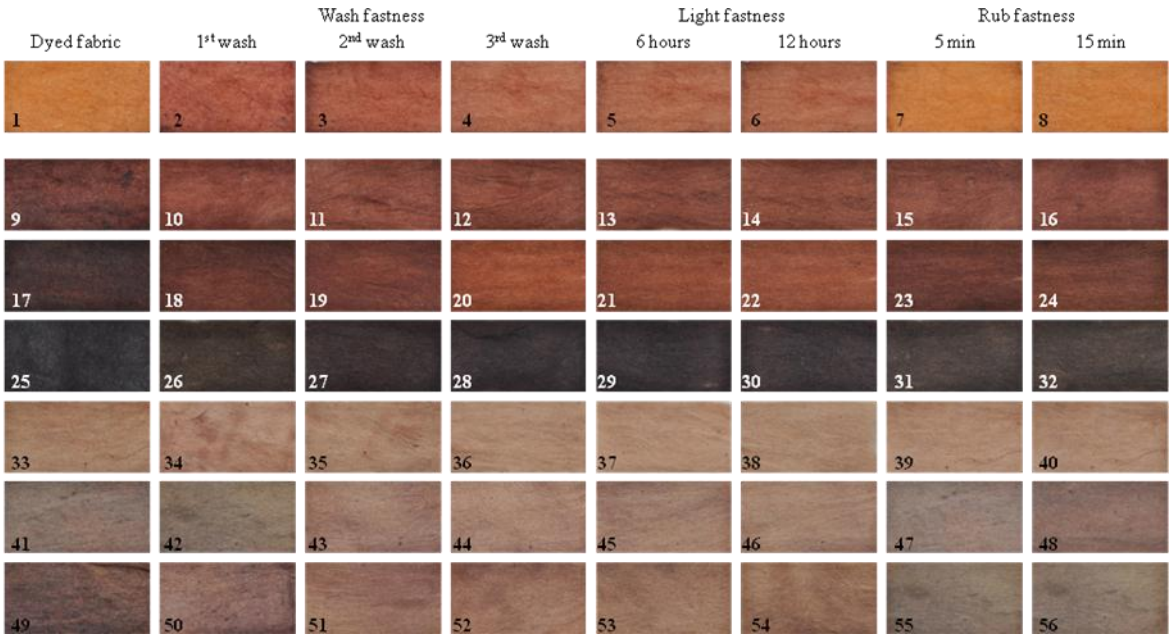


Plate 41. Dyeing of cotton fabrics with *Ceriops tagal* dye (Post-mordanting). 1-8. Dyed with *C. tagal* dye; 9-16. Dyed with *C. tagal* and $K_2Cr_2O_7$; 17-24. Dyed with *C. tagal* and $CuSO_4$; 25-32. Dyed with *C. tagal* and $FeSO_4$; 33-40. Dyed with *C. tagal* and $K_2Cr_2O_7$ + $CuSO_4$; 41-48. Dyed with *C. tagal* and $CuSO_4$ + $FeSO_4$; 49-56. Dyed with *C. tagal* and $FeSO_4$ + $K_2Cr_2O_7$.

Post-mordanting method gave darker shades to the silk as well as on the cotton fabrics after individual dye application as well as on use of mordants. Even the fastness tests were better for the fabric with slight to no colour change to the fabrics. Hence, the post-mordanting method could be adopted for dyeing with *Ceriops tagal* dye.

Dyeing of silk fabrics with *Mammea suriga* dye

Dyeing of silk with *Mammea suriga* bark dye gave strong brown colour to the fabric, which changed to brownish orange after third wash. The exposure to light changed the colour to moderate orange. Rubbing the fabric manually for 15 minutes changed the colour to light brown. In Pre-mordanting the use of potassium dichromate mordant along with the dye gave strong brown colour to the fabric which remained unchanged after wash and rub fastness tests. However, light exposure gave strong brown colour to the fabric. The use of copper sulphate mordant along with the dye gave brownish orange colour to the fabric, which changed to strong brown after subsequent washing and rub fastness tests. The exposure to 6 hours and 12 hours of sunlight lead to deep brown colour to the fabric. The

ferrous sulphate mordant dyed the fabric brownish black which slightly changed to brownish gray after third wash but did not change on light and rub fastness test.

The combination of mordants potassium dichromate and copper sulphate gave strong brown colour which changed to moderate orange after first and second wash and light orange after third wash. The strong brown colour was unchanged after rub fastness, whereas the light exposure leads to light orange colour to the fabric. The combination of copper sulphate and ferrous sulphate gave deep orange colour. Similar colour was obtained with the combination of ferrous sulphate and potassium dichromate mordant. The colour obtained was unchanged after fastness tests in these combinations of mordants along with the dye (Table 58) (Plate 42).

Table 58. Colour obtained on silk fabrics with *Mammea suriga* dye (pre-mordanting).

Dye and mordants used	Colour of dyed fabric	Wash fastness			Light fastness		Rub fastness	
		1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Mammea suriga	strong brown	strong brown	strong brown	brownish orange	moderate orange	moderate orange	brownish orange	light brown
Dye + $K_2Cr_2O_7$	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown
Dye + $CuSO_4$	brownish orange	strong brown	strong brown	strong brown	deep brown	deep brown	strong brown	strong brown
Dye + $FeSO_4$	brownish black	brownish black	brownish black	brownish gray	brownish black	brownish black	brownish black	brownish black
Dye + $K_2Cr_2O_7$	strong brown	moderate orange	moderate orange	light orange	light orange	light orange	strong brown	strong brown

+ $CuSO_4$								
Dye + $CuSO_4$ + $FeSO_4$	deep orange	deep orange	deep orange	deep orange	deep orange	deep orange	deep orange	deep orange
Dye + $FeSO_4$ + $K_2Cr_2O_7$	deep orange	deep orange	deep orange	deep orange	deep orange	deep orange	deep orange	deep orange

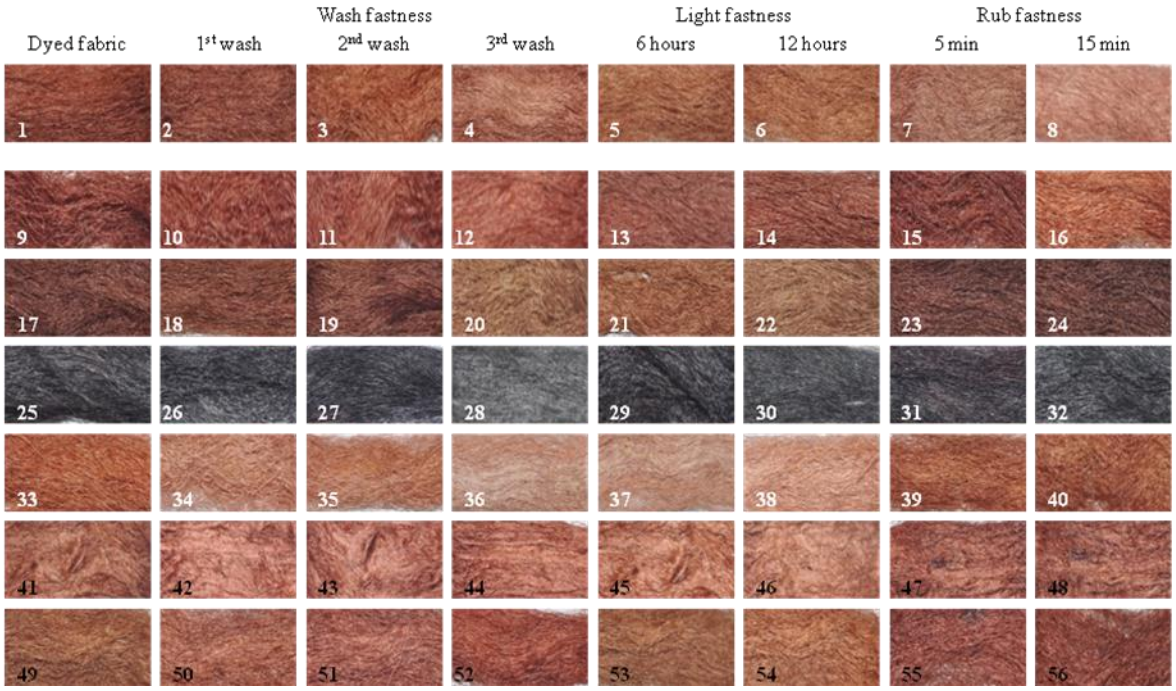


Plate 42. Dyeing of silk fabrics with *Mammea suriga* dye (Pre-mordanting). 1-8. Dyed with *M. suriga* dye; 9-16. Dyed with and $K_2Cr_2O_7$; 17-24. Dyed with *M. suriga* and $CuSO_4$; 25-32. Dyed with *M. suriga* and $FeSO_4$; 33-40. Dyed with *M. suriga* and $K_2Cr_2O_7$ + $CuSO_4$; 41-48. Dyed with *M. suriga* and $CuSO_4$ + $FeSO_4$; 49-56. Dyed with *M. suriga* and $FeSO_4$ + $K_2Cr_2O_7$.

In simultaneous mordanting the potassium dichromate mordant with the dye imparted moderate orange yellow colour to the fabric which was unchanged after wash, rub and light fastness tests. The application of copper sulphate mordant along with the dye gave brownish orange colour to the fabric which changed to deep brown after subsequent washes. The exposure to light changed the colour to strong brown and rubbing the fabric

changed the colour to brownish orange. The use of ferrous sulphate mordant along with the dye produced brownish black colour to the fabric which remained unchanged after fastness test. The combination of mordants potassium dichromate and copper sulphate gave brownish orange colour and combination of copper sulphate and ferrous sulphate mordants gave strong brown colour which changed to moderate orange after third wash. The light fastness leads to moderate orange colour. Whereas, rubbing the fabric did not change the strong brown colour. The ferrous sulphate and potassium dichromate gave strong brown colour which did not show any change in colour after fastness tests (Table 59) (Plate 43).

Table 59. Colour obtained on silk fabrics with *Mammea suriga* dye (simultaneous mordanting).

Dye and mordants used	Colour of dyed fabric	Wash fastness			Light fastness		Rub fastness	
		1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Dye + $K_2Cr_2O_7$	moderate orange yellow	moderate orange yellow	moderate orange yellow	moderate orange yellow	moderate orange yellow	moderate orange yellow	moderate orange yellow	moderate orange yellow
Dye + $CuSO_4$	brownish orange	deep brown	deep brown	deep brown	strong brown	strong brown	brownish orange	brownish orange
Dye + $FeSO_4$	brownish black	brownish black	brownish black	brownish black	brownish black	brownish black	brownish black	brownish black
Dye + $K_2Cr_2O_7$ +	brownish orange	brownish orange	brownish orange	brownish orang	brownish orange	brownish orange	brownish orange	brownish orang

$CuSO_4$				e				e
Dye + $CuSO_4$ + $FeSO_4$	strong brown	strong brown	strong brown	moderate orange	moderate orange	moderate orange	strong brown	strong brown
Dye + $FeSO_4$ + $K_2Cr_2O_7$	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown

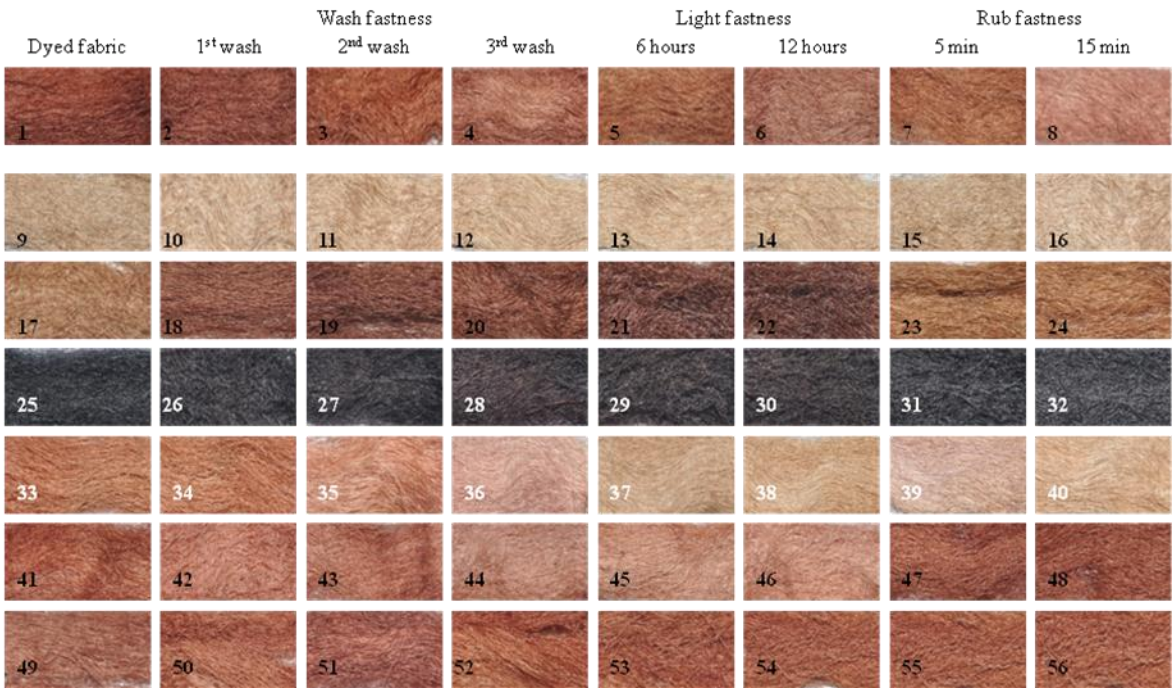


Plate 43. Dyeing of silk fabrics with *Mammea suriga* dye (Simultaneous mordanting). 1-8. Dyed with *M. suriga* dye; 9-16. Dyed with and $K_2Cr_2O_7$; 17-24. Dyed with *M. suriga* and $CuSO_4$; 25-32. Dyed with *M. suriga* and $FeSO_4$; 33-40. Dyed with *M. suriga* and $K_2Cr_2O_7$ + $CuSO_4$; 41-48. Dyed with *M. suriga* and $CuSO_4$ + $FeSO_4$; 49-56. Dyed with *M. suriga* and $FeSO_4$ + $K_2Cr_2O_7$.

In post-mordanting the potassium dichromate mordant with the dye produced deep brown colour to the fabric which was unchanged after rub fastness test. But washing the

fabric and light fastness changed the colour to strong brown. The application of copper sulphate mordant gave deep brown colour to the fabric which turned to brownish orange after second and third wash. The brownish orange colour was observed after 6 hours and 12 hours of sun exposure and rubbing the fabric gave deep brown colour to the fabric. The use of ferrous sulphate gave brownish black colour to the fabric which did not change after fastness tests. The combination of potassium dichromate and copper sulphate gave dark orange yellow colour which was not changed after wash and rub fastness tests but exposure to light changed its colour to moderate orange. The combination of copper sulphate and ferrous sulphate mordant gave strong brown colour which changed to moderate orange after subsequent washing. The strong brown colour did not change on rub fastness but the light fastness gave moderate orange colour to the fabric. The combination of ferrous sulphate and potassium dichromate gave strong brown colour which did not change after fastness tests (Table 60) (Plate 44).

Table 60. Colour obtained on silk fabrics with *Mammea suriga* dye (post-mordanting).

Dye and mordants used	Colour of dyed fabric	Wash fastness			Light fastness		Rub fastness	
		1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Dye + $K_2Cr_2O_7$	deep brown	strong brown	strong brown	strong brown	strong brown	strong brown	deep brown	deep brown
Dye + $CuSO_4$	deep brown	deep brown	brownish orange	brownish orange	brownish orange	brownish orange	deep brown	deep brown
Dye + $FeSO_4$	brownish black	brownish black	brownish black	brownish black	brownish black	brownish black	brownish black	brownish black
Dye + $K_2Cr_2O_7$ + $CuSO_4$	dark orange yellow	dark orange yellow	dark orange yellow	dark orange yello	moderate orange	moderate orange	dark orange yellow	dark orange yello

				w				w
Dye + $CuSO_4$ + $FeSO_4$	strong brown	moderate orange	moderate orange	moderate orange	moderate orange	moderate orange	strong brown	strong brown
Dye + $FeSO_4$ + $K_2Cr_2O_7$	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown

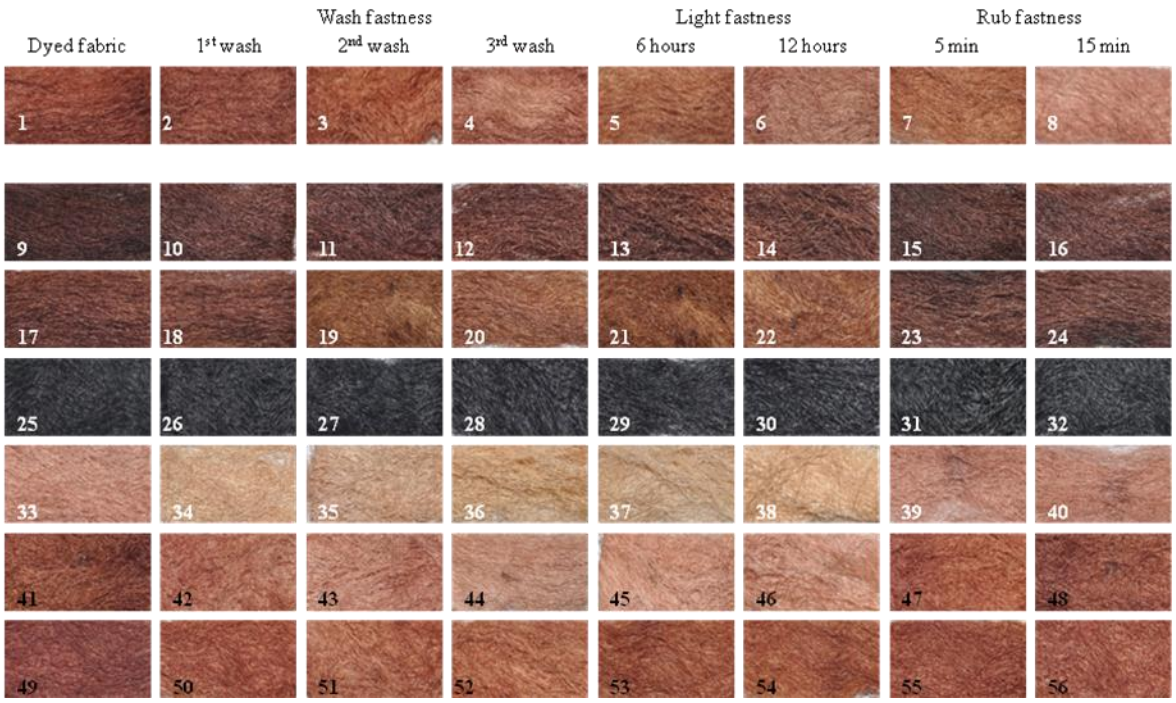


Plate 44. Dyeing of silk fabrics with *Mammea suriga* dye (Post-mordanting). 1-8. Dyed with *M. suriga* dye; 9-16. Dyed with and $K_2Cr_2O_7$; 17-24. Dyed with *M. suriga* and $CuSO_4$; 25-32. Dyed with *M. suriga* and $FeSO_4$; 33-40. Dyed with *M. suriga* and $K_2Cr_2O_7$ + $CuSO_4$; 41-48. Dyed with *M. suriga* and $CuSO_4$ + $FeSO_4$; 49-56. Dyed with *M. suriga* and $FeSO_4$ + $K_2Cr_2O_7$.

Dyeing of cotton fabrics with *Mammea suriga* dye

Dyeing of cotton with *Mammea suriga* extract gave moderate orange colour to the fabric which did not change after fastness tests.

In Pre-mordanting the combination of potassium dichromate mordant with the dye produced dark brown colour to the fabric which turned to strong brown after all the fastness tests. The use of copper sulphate mordant with the dye imparted deep orange colour to the fabric which was later changed to strong brown after wash, rub and light fastness tests. The use of ferrous sulphate mordant along with the dye gave dark grayish colour to the fabric which changed to olive black colour. The dark olive colour was seen after 6 hours and 12 hours of sun exposure. Rubbing the fabric changed the colour to greenish black. The combination of copper sulphate and ferrous sulphate mordant gave light olive gray colour which did not change after wash, rub fastness and light fastness. The combination of ferrous sulphate and potassium dichromate gave grayish brown colour which did not change after all three fastness tests. The combination of potassium dichromate and copper sulphate gave light brown colour which changed to light yellowish brown after fastness tests (Table 61) (Plate 45).

Table 61. Colour obtained on cotton fabrics with *Mammea suriga* dye (pre-mordanting).

Dye and mordants used	Colour of dyed fabric	Wash fastness			Light fastness		Rub fastness	
		1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Mammea suriga	moderate orange	moderate orange	moderate orange	moderate orange	moderate orange	moderate orange	moderate orange	moderate orange
Dye + $K_2Cr_2O_7$	dark brown	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown
Dye +	deep	strong	strong	strong	strong	strong	strong	strong

$CuSO_4$	orange	brown	brown	brown	brown	brown	brown	brown
Dye + $FeSO_4$	dark grayish	olive black	olive black	olive black	dark olive	dark olive	greenish black	greenish black
Dye + $K_2Cr_2O_7$ + $CuSO_4$	light brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown
Dye + $CuSO_4$ + $FeSO_4$	light olive gray	light olive gray	light olive gray	light olive gray	light olive gray	light olive gray	light olive gray	light olive gray
Dye + $FeSO_4$ + $K_2Cr_2O_7$	grayish brown	grayish brown	grayish brown	grayish brown	grayish brown	grayish brown	grayish brown	grayish brown

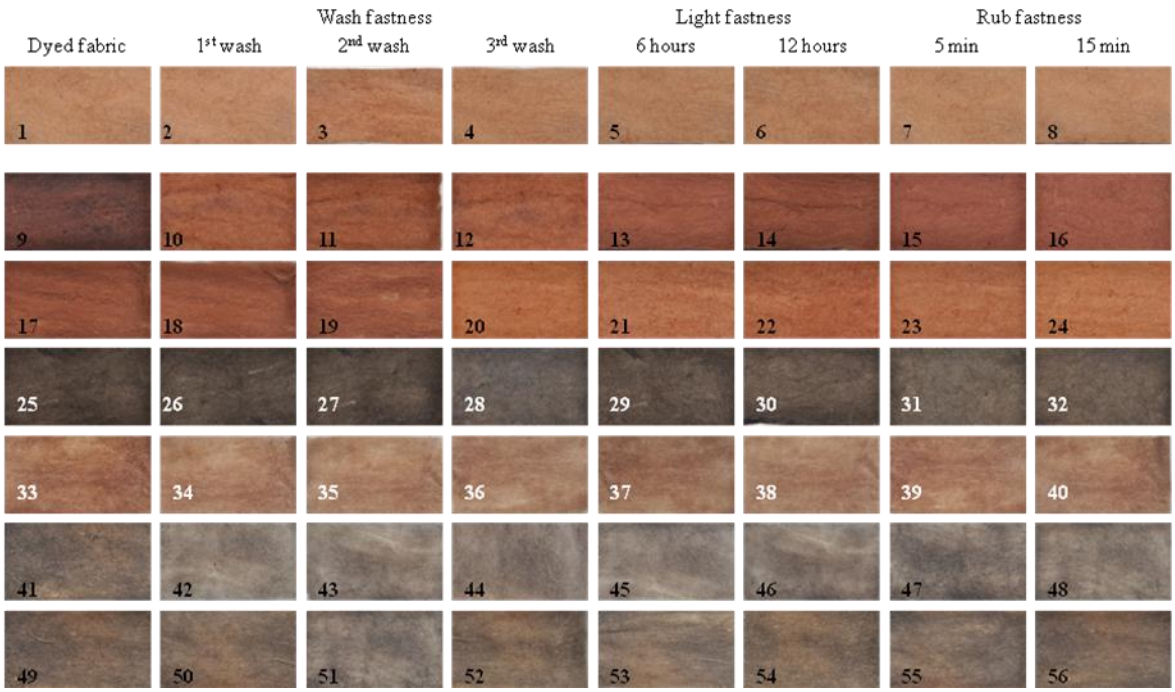


Plate 45. Dyeing of cotton fabrics with *Mammea suriga* dye (Pre-mordanting). 1-8. Dyed with *M. suriga* dye; 9-16. Dyed with and $K_2Cr_2O_7$; 17-24. Dyed with *M. suriga* and $CuSO_4$; 25-32. Dyed with *M. suriga* and $FeSO_4$; 33-40. Dyed with *M. suriga* and $K_2Cr_2O_7$ + $CuSO_4$; 41-48. Dyed with *M. suriga* and $CuSO_4$ + $FeSO_4$; 49-56. Dyed with *M. suriga* and $FeSO_4$ + $K_2Cr_2O_7$.

In simultaneous mordanting the potassium dichromate mordant along with the dye produced light yellowish brown colour to the fabric which remained unchanged after all the three fastness tests. The copper sulphate mordant along with the dye produced deep orange colour to the fabric which turned to strong brown after wash and light fastness. The rub fastness produced deep orange colour. The ferrous sulphate mordant along with the dye gave olive gray colour to the fabric. It was changed to dark grayish brown after first wash and then to brownish gray after second and third wash. However, the olive gray colour remained unchanged after light and rub fastness tests. The combination of copper sulphate and ferrous sulphate mordant along with the dye gave brownish orange colour which was unchanged after washing. The light orange colour was seen after exposing the fabrics to sunlight and rubbing the fabrics. The ferrous sulphate and potassium dichromate mordants gave light brown colour to the cotton which remained same after all the three fastness tests. The combination of potassium dichromate and copper sulphate gave light

yellowish brown colour which was not change even after fastness tests (Table 62) (Plate 46).

Table 62. Colour obtained on cotton fabrics with *Mammea suriga* dye (simultaneous mordanting).

Dye and mordants used	Colour of dyed fabric	Wash fastness			Light fastness		Rub fastness	
		1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Dye + $K_2Cr_2O_7$	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown
Dye + $CuSO_4$	deep orange	strong brown	strong brown	strong brown	strong brown	strong brown	deep orange	deep orange
Dye + $FeSO_4$	olive gray	dark grayish brown	brownish gray	brownish gray	olive gray	olive gray	olive gray	olive gray
Dye + $K_2Cr_2O_7$ + $CuSO_4$	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown
Dye + $CuSO_4$ + $FeSO_4$	brownish orange	brownish orange	brownish orange	brownish orange	light orange	light orange	light orange	light orange
Dye + $FeSO_4$ + $K_2Cr_2O_7$	light brown	light brown	light brown	light brown	light brown	light brown	light brown	light brown

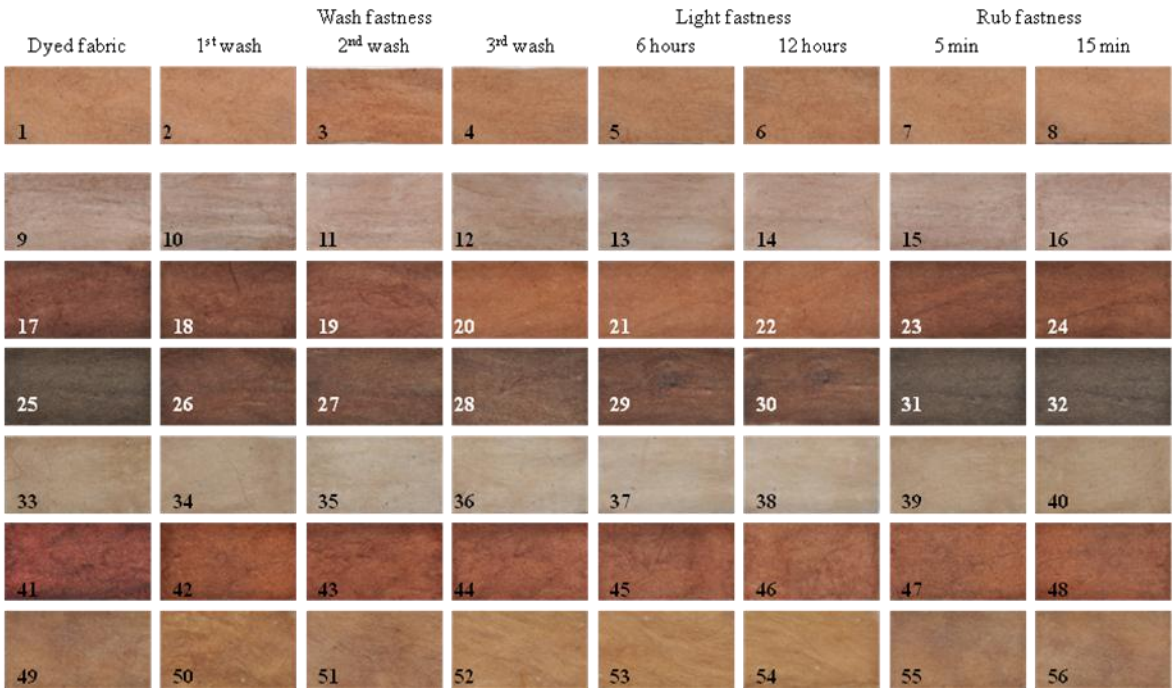


Plate 46. Dyeing of cotton fabrics with *Mammea suriga* dye (Simultaneous mordanting). 1-8. Dyed with *M. suriga* dye; 9-16. Dyed with and $K_2Cr_2O_7$; 17-24. Dyed with *M. suriga* and $CuSO_4$; 25-32. Dyed with *M. suriga* and $FeSO_4$; 33-40. Dyed with *M. suriga* and $K_2Cr_2O_7$ + $CuSO_4$; 41-48. Dyed with *M. suriga* and $CuSO_4$ + $FeSO_4$; 49-56. Dyed with *M. suriga* and $FeSO_4$ + $K_2Cr_2O_7$.

In post-mordanting method the application of potassium dichromate mordant gave deep brown colour which was later changed to strong brown after wash, light and rub fastness tests. The use of copper sulphate mordant imparted deep orange colour to the fabric which turned to strong brown after first wash and second wash. The third wash lead to brownish orange colour. The light exposure lead to brownish orange colour and rubbing the fabric lead to strong brown colour. The use of ferrous sulphate mordant gave olive black colour to the fabric which was not changed after all the three fastness tests. The combination of copper sulphate and ferrous sulphate mordants gave grayish yellowish-brown colour which was changed to light brown after third wash. The light exposure gave light brown colour and rub fastness gave grayish yellowish brown shade. The ferrous sulphate and potassium dichromate mordant gave moderate brown colour which changed to light brown after wash fastness tests. The 6 hours of light exposure lead to light brown colour and 12 hours of exposure lead to light grayish brown colour. The rub fastness resulted in light grayish brown colour. The potassium dichromate and copper sulphate

mordant gave light yellowish brown colour which did not change after fastness tests (Table 63) (Plate 47).

Table 63. Colour obtained on cotton fabrics with *Mammea suriga* dye (post-mordanting).

Dye and mordants used	Colour of dyed fabric	Wash fastness			Light fastness		Rub fastness	
		1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Dye + $K_2Cr_2O_7$	deep brown	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown	strong brown
Dye + $CuSO_4$	deep orange	strong brown	strong brown	brownish orange	brownish orange	brownish orange	strong brown	strong brown
Dye + $FeSO_4$	olive black	olive black	olive black	olive black	olive black	olive black	olive black	olive black
Dye + $K_2Cr_2O_7$ + $CuSO_4$	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown	light yellowish brown
Dye + $CuSO_4$ + $FeSO_4$	grayish yellowish brown	grayish yellowish brown	grayish yellowish brown	light brown	light brown	light brown	grayish yellowish brown	grayish yellowish brown
Dye + $FeSO_4$ + $K_2Cr_2O_7$	moderate brown	light brown	light brown	light brown	light brown	light grayish brown	light grayish brown	light grayish brown

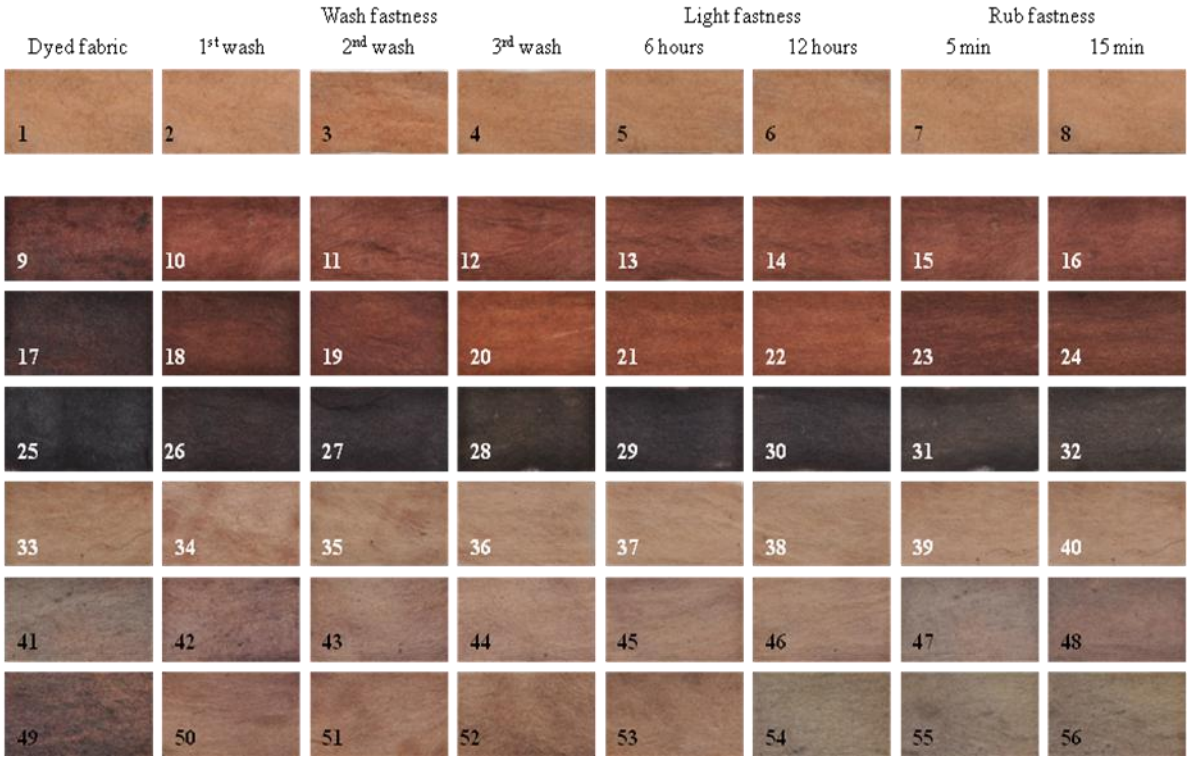


Plate 47. Dyeing of cotton fabrics with *Mammea suriga* dye (Post-mordanting). 1-8. Dyed with *M. suriga* dye; 9-16. Dyed with and $K_2Cr_2O_7$; 17-24. Dyed with *M. suriga* and $CuSO_4$; 25-32. Dyed with *M. suriga* and $FeSO_4$; 33-40. Dyed with *M. suriga* and $K_2Cr_2O_7$ + $CuSO_4$; 41-48. Dyed with *M. suriga* and $CuSO_4$ + $FeSO_4$; 49-56. Dyed with *M. suriga* and $FeSO_4$ + $K_2Cr_2O_7$.

In all the three dyeing methods, the post-mordanting exhibited darker shades to the fabrics in *Mammea suriga* dye extract. The fastness tests were good with slight colour fadedness especially on using ferrous sulphate mordant. Hence, the post-mordanting method could be adopted for this particular dye.

4.9.2. Determination of pH of aqueous extract of dyed cotton and silk fabrics (ISO 3071)

The pH of aqueous extract of dyed cotton and silk fabric was determined by (ISO 3071) method. The silk fibres dyed with *Ceriops tagal* bark extract along with the chemical mordants showed the pH ranging from (7.02-7.41) and cotton fibres dyed with

Ceriops tagal bark extract along with the chemical mordants showed the pH ranging from (7.12-7.64) (Table 64). The silk fibres dyed with *Mammea suriga* bark extract along with the chemical mordants showed the pH ranging from (7.12-7.51) and cotton fibres dyed with *Mammea suriga* bark extract along with the chemical mordants showed the pH ranging from (7.15-7.54) (Table 65).

Table 64. pH of fabrics dyed with *Ceriops tagal* bark extract.

Dye and mordants used	pH of aqueous extract of dyed fabrics	
	Silk	Cotton
Ceriops tagal	7.02±0.01	7.12±0.01
Dye + $K_2Cr_2O_7$	7.41±0.00	7.64±0.01
Dye + $CuSO_4$	7.31±0.00	7.41±0.01
Dye + $FeSO_4$	7.10±0.00	7.13±0.01
Dye + $K_2Cr_2O_7$ + $CuSO_4$	7.24±0.00	7.26±0.01
Dye + $CuSO_4$ + $FeSO_4$	7.12±0.00	7.15±0.00
Dye + $FeSO_4$ + $K_2Cr_2O_7$	7.21±0.00	7.24±0.00

All readings are mean of 3 values ± SD (standard deviation)

Table 65. pH of fabrics dyed with *Mammea suriga* bark extract.

Dye and mordants used	pH of aqueous extract of dyed fabrics	
	Silk	Cotton
Mammea suriga	7.12±0.00	7.15±0.01
Dye + $K_2Cr_2O_7$	7.16±0.01	7.20±0.00
Dye + $CuSO_4$	7.24±0.01	7.31±0.00
Dye + $FeSO_4$	7.17±0.01	7.26±0.00
Dye + $K_2Cr_2O_7$ + $CuSO_4$	7.41±0.00	7.52±0.00
Dye + $CuSO_4$ + $FeSO_4$	7.32±0.00	7.41±0.00
Dye + $FeSO_4$ + $K_2Cr_2O_7$	7.51±0.00	7.54±0.00

All readings are mean of 3 values ± SD (standard deviation)

4.9.3. Determination of colourfastness of dyed fabrics

The dyed fabrics were rated on the basis of change in colour of the samples with the help of gray scale.

Fastness results for silk fabrics dyed with *Ceriops tagal* dye

The unmordanted silk fabrics dyed with *Ceriops tagal* extract in pre-mordanting method showed rating 5 for wash fastness. But the use of mordants and their combinations along with the dye, gave the colour change ratings within 5-2/3. For light fastness test the rating of colour change ranged from 5-2/3. The same was observed in case of rub fastness. The use of potassium dichromate mordant along with the dye gave the colour change ratings between 3/4-3 average. The colour change ratings for copper sulphate mordant was found between 3-2/3 i.e. average and that of ferrous sulphate mordant was found between 5-4/5 i.e. excellent. The dyeing with ferrous sulphate along with the dye showed no to slight colour change of the fabric in all the three fastness tests. The combination of potassium dichromate and copper sulphate mordants along with dye showed very poor wash, rub and light fastness. Whereas the ferrous sulphate mordant, combination of ferrous sulphate and copper sulphate mordant, combination of ferrous sulphate and potassium dichromate mordant resulted in very good fastness property with excellent ratings. Hence, ferrous sulphate mordant proved to be the best mordant in dyeing with *Ceriops tagal* dye extract (Table 66).

Table 66. Ratings of colourfastness of silk fabrics dyed with *Ceriops tagal* (pre-mordanting).

Dye and mordants used	Wash fastness			Light fastness		Rub fastness	
	1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Ceriops tagal	5	5	5	4/5	4/5	4/5	4/5
Dye + $K_2Cr_2O_7$	3/4	3	3	3/4	3/4	3/4	3/4
Dye + $CuSO_4$	3	2/3	2/3	2/3	2/3	2/3	2/3
Dye + $FeSO_4$	5	5	4/5	5	5	4/5	4/5
Dye + $K_2Cr_2O_7$ + $CuSO_4$	4	4	4	4	4	5	4/5
Dye + $CuSO_4$ + $FeSO_4$	4	4	3/4	3/4	3/4	4/5	4/5
Dye + $FeSO_4$ + $K_2Cr_2O_7$	5	5	5	5	5	5	5

In case of simultaneous mordanting the ratings of colour change of fabrics for wash fastness was between 5-3/4, light fastness was between 5-4/5 and rub fastness was between 5-3. The use of potassium dichromate mordant along with the dye showed excellent ratings between 5-4/5. The copper sulphate mordant gave average ratings. But the use of ferrous sulphate mordant showed excellent ratings in all the three fastness tests. The combination of mordant potassium dichromate and copper sulphate showed no colour change with 5 rating in all the fastness tests. The combination of copper sulphate and ferrous sulphate mordant showed the ratings between 5-3/4. Rub and light fastness was excellent for this combination of dye compared to wash fastness. The combination of ferrous sulphate and potassium dichromate mordant along with the dye showed excellent ratings with slight to no colour change in all the three fastness tests (Table 67).

Table 67. Ratings of colourfastness of silk fabrics dyed with *Ceriops tagal* (simultaneous mordanting).

Dye and mordants used	Wash fastness			Light fastness		Rub fastness	
	1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Dye + $K_2Cr_2O_7$	5	5	5	5	5	4/5	4/5
Dye + $CuSO_4$	4/5	4/5	3/4	4	4/5	3	3
Dye + $FeSO_4$	5	5	5	5	5	5	5
Dye + $K_2Cr_2O_7$ + $CuSO_4$	5	5	5	5	5	5	5
Dye + $CuSO_4$ + $FeSO_4$	4/5	3/4	3/4	4/5	4/5	5	5
Dye + $FeSO_4$ + $K_2Cr_2O_7$	5	5	5	4/5	4/5	4/5	4/5

In post-mordanting the excellent to good ratings were found between 5-4 in all the fastness tests viz. light, rub and wash fastness. The use of potassium dichromate mordant along with the dye showed slight colour change after washing but no colour change was seen in rub fastness tests. The use of copper sulphate mordant along with the dye resulted in 4/5-4 ratings. Whereas the use of ferrous sulphate mordant and combination of potassium dichromate and copper sulphate mordant lead to no colour change of the fabric. The combination of copper sulphate and ferrous sulphate mordant lead to slight colour

change after washing the fabrics whereas in case of rub and light fastness no colour change was observed. The combination of mordants ferrous sulphate and potassium dichromate lead to no colour change in all the three fastness tests (Table 68).

Table 68. Ratings of colourfastness of silk fabrics dyed with *Ceriops tagal* (post-mordanting).

Dye and mordants used	Wash fastness			Light fastness		Rub fastness	
	1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Dye + $K_2Cr_2O_7$	4/5	4/5	4/5	4/5	4/5	5	5
Dye + $CuSO_4$	4	4	4	4	4	4/5	4/5
Dye + $FeSO_4$	5	5	5	5	5	5	5
Dye + $K_2Cr_2O_7$ + $CuSO_4$	5	5	5	5	5	5	5
Dye + $CuSO_4$ + $FeSO_4$	4/5	4/5	4/5	5	5	5	5
Dye + $FeSO_4$ + $K_2Cr_2O_7$	5	5	5	5	5	5	5

The use of ferrous sulphate mordant with the *Ceriops tagal* dye resulted in no colour change from the dyed fabric in all the three fastness tests and in all the three dyeing methods. Thus, the ferrous sulphate mordant was considered as the best mordant for dyeing purpose.

Fastness results for cotton fabrics dyed with *Ceriops tagal* dye

The unmordanted cotton fabrics dyed with *Ceriops tagal* dye showed the fastness ratings between 5-3/4. In pre-mordanting method the use of mordants resulted in colour change ratings between 5-3. The wash fastness ratings were between 5-4 which indicated slight leaching of dye from the fabric. The light fastness colour change rating was 5-3/4 and rub fastness was between 5-3. The use of potassium dichromate mordant, combination of copper sulphate and ferrous sulphate mordant, ferrous sulphate and potassium dichromate mordant along with the dye showed no colour change in all the three fastness tests. Whereas the copper sulphate mordant and combination of potassium dichromate and copper sulphate showed the rating between 4/5-3/4 which indicates slight colour change. The colour change ratings were found 4-3 for ferrous sulphate mordant (Table 69).

Table 69. Ratings of colourfastness of cotton fabrics dyed with *Ceriops tagal* (pre-mordanting).

Dye and mordants used	Wash fastness			Light fastness		Rub fastness	
	1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Ceriops tagal	4/5	4/5	4	3/4	3/4	5	5
Dye + $K_2Cr_2O_7$	5	5	5	5	5	5	5
Dye + $CuSO_4$	4/5	4/5	4/5	3/4	3/4	3/4	3/4
Dye + $FeSO_4$	4	4	4	3/4	3/4	3	3
Dye + $K_2Cr_2O_7$ + $CuSO_4$	4/5	4/5	4/5	4	4	3/4	3/4
Dye + $CuSO_4$ + $FeSO_4$	5	5	5	5	5	5	5
Dye + $FeSO_4$ + $K_2Cr_2O_7$	5	5	5	5	5	5	5

In simultaneous mordanting the wash fastness ratings were between 5-3 and light fastness ratings were between 5-3/4 and rub fastness ratings were between 5-4/5. The use of potassium dichromate mordant, combination of potassium dichromate and copper sulphate mordant, combination of copper sulphate and ferrous sulphate and combination of ferrous sulphate and potassium dichromate mordant showed excellent ratings of 5 for all the fastness tests. The use of copper sulphate mordant showed the ratings between 4/5-3/4 and ferrous sulphate mordant showed the ratings between 5-3 for the fastness tests (Table 70).

Table 70. Ratings of colourfastness of cotton fabrics dyed with *Ceriops tagal* (simultaneous mordanting).

Dye and mordants used	Wash fastness			Light fastness		Rub fastness	
	1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Dye + $K_2Cr_2O_7$	5	5	5	5	5	5	5
Dye + $CuSO_4$	4/5	4/5	3/4	3/4	3/4	4/5	4/5
Dye + $FeSO_4$	3	3	3	3/4	3/4	5	5
Dye + $K_2Cr_2O_7$ + $CuSO_4$	5	5	5	5	5	5	5
Dye + $CuSO_4$ + $FeSO_4$	5	5	5	5	5	5	5
Dye + $FeSO_4$ + $K_2Cr_2O_7$	5	5	5	5	5	5	5

In post-mordanting the wash fastness ratings for mordanted fabrics were between 5-3/4. The light fastness was between 5-3 and rub fastness was between 5-3/4. The dyeing of fabric with potassium dichromate mordant along with the dye showed the 4/5 ratings. The use of copper sulphate mordant showed the 4/5 ratings for light and rub fastness except after third wash the colour was changed, which showed the rating 3/4. The ferrous sulphate mordant and combination of potassium dichromate and copper sulphate mordant resulted in excellent ratings i.e. 5. The combination of copper sulphate and ferrous sulphate mordant showed 5-4 and the combination of ferrous sulphate and potassium dichromate mordant showed 3-3/4 fastness values (Table 71).

Table 71. Ratings of colourfastness of cotton fabrics dyed with *Ceriops tagal* (post-mordanting).

Dye and mordants used	Wash fastness			Light fastness		Rub fastness	
	1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Dye + $K_2Cr_2O_7$	4/5	4/5	4/5	4/5	4/5	4/5	4/5
Dye + $CuSO_4$	4/5	4/5	3/4	4/5	4/5	4/5	4/5
Dye + $FeSO_4$	5	5	5	5	5	5	5
Dye + $K_2Cr_2O_7$ + $CuSO_4$	5	5	5	5	5	5	5
Dye + $CuSO_4$ + $FeSO_4$	4/5	4	4	4	4	5	5
Dye + $FeSO_4$ + $K_2Cr_2O_7$	3/4	3/4	3/4	3	3	3/4	3/4

Among all the mordants used potassium dichromate mordant showed excellent ratings in all the fastness tests. The best method of dyeing was post-mordanting with brighter shades of colour with good fastness ratings.

Fastness results for silk fabrics dyed with *Mammea suriga* dye

The ratings between 5-2 for colour change was seen in the silk fabrics dyed with *Mammea suriga* dye extract. In pre-mordanting method the wash fastness showed 5-1 ratings, the light fastness showed 5-1 ratings and rub fastness showed 5-2 ratings with application of mordants and with their combination. The use of potassium dichromate

mordant, combination of copper sulphate and ferrous sulphate mordant and combination of ferrous sulphate and potassium dichromate mordant along with the dye showed excellent ratings with no colour change in all the three fastness tests. The copper sulphate mordant along with dye gave 4-3 ratings, dye with ferrous sulphate mordant gave 5-2 ratings and combination of potassium dichromate and copper sulphate mordant with dye showed 2-1 ratings on silk fabrics (Table 72).

Table 72. Ratings of colourfastness of silk fabrics dyed with *Mammea suriga* (pre-mordanting).

Dye and mordants used	Wash fastness			Light fastness		Rub fastness	
	1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Mammea suriga	5	5	3/4	3	3	3/4	2
Dye + $K_2Cr_2O_7$	5	5	5	5	5	5	5
Dye + $CuSO_4$	4	4	4	3	3	4	4
Dye + $FeSO_4$	5	5	2	5	5	5	5
Dye + $K_2Cr_2O_7$ + $CuSO_4$	1/2	1/2	1	1	1	2	2
Dye + $CuSO_4$ + $FeSO_4$	5	5	5	5	5	5	5
Dye + $FeSO_4$ + $K_2Cr_2O_7$	5	5	5	5	5	5	5

In simultaneous mordanting the wash fastness ratings were between 5-3, the light fastness ratings were between 5-2 and for rub fastness were between 5-4. The use of potassium dichromate mordant, ferrous sulphate mordant, combination of potassium dichromate and copper sulphate mordant, combination of ferrous sulphate and potassium dichromate mordant showed excellent 5 ratings with no colour change. The use of copper sulphate mordant showed 4-2 ratings and the combination of copper sulphate and ferrous sulphate along with the dye showed 4/5-3 ratings (Table 73).

Table 73. Ratings of colourfastness of silk fabrics dyed with *Mammea suriga* (simultaneous mordanting).

Dye and mordants used	Wash fastness			Light fastness		Rub fastness	
	1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min

Dye + $K_2Cr_2O_7$	5	5	5	5	5	5	5
Dye + $CuSO_4$	3/4	3/4	3/4	2	2	4	4
Dye + $FeSO_4$	5	5	5	5	5	5	5
Dye + $K_2Cr_2O_7$ + $CuSO_4$	5	5	5	5	5	5	5
Dye + $CuSO_4$ + $FeSO_4$	4/5	4/5	3	3	3	4/5	4/5
Dye + $FeSO_4$ + $K_2Cr_2O_7$	5	5	5	5	5	5	5

In post-mordanting the use of mordants along with the dye and with the combination of mordants showed the wash fastness ratings between 5-2/3, light fastness ratings between 5-2/3, and rub fastness ratings from 5-3/4. The use of potassium dichromate mordant showed 4/5-4 ratings. The use of copper sulphate mordant showed the colour change range from 5-3/4. The use of ferrous sulphate, combination of potassium dichromate and copper sulphate and combination of ferrous sulphate and potassium dichromate showed excellent result 5 with no loss of colour from the fabric. The use of copper sulphate and ferrous sulphate showed the colour change 3/4-2/3 for fastness tests (Table 74).

Table 74. Ratings of colourfastness of silk fabrics dyed with *Mammea suriga* (post-mordanting).

Dye and mordants used	Wash fastness			Light fastness		Rub fastness	
	1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Dye + $K_2Cr_2O_7$	4	4	4	4	4	4/5	4/5
Dye + $CuSO_4$	5	3/4	3/4	3/4	3/4	5	5
Dye + $FeSO_4$	5	5	5	5	5	5	5
Dye + $K_2Cr_2O_7$ + $CuSO_4$	5	5	5	4/5	4/5	5	5
Dye + $CuSO_4$ + $FeSO_4$	2/3	2/3	2/3	2/3	2/3	3/4	3/4
Dye + $FeSO_4$ + $K_2Cr_2O_7$	5	5	5	5	5	5	5

Among all the mordants, the ferrous sulphate mordant proved to be the good mordant with slight to no colour change in all the fastness tests and in all the mordanting methods. Among the dyeing methods, post-mordanting method showed better results with

brighter shades and good fastness results. Hence, this method of dyeing could be adopted for dyeing of silk with *Mammea suriga* extract.

Fastness results for cotton fabrics dyed with *Mammea suriga* dye

The cotton fabric showed no colour change in all the fastness tests when dyed with *Mammea suriga* dye extract. In pre-mordanting the use of mordant with the dye showed 5-1/2 ratings in all the fastness tests. The use of mordant potassium dichromate along with the dye showed very poor fastness 1/2. Most of the dye was leached out from the fabric after fastness tests. The use of copper sulphate mordant and combination of potassium dichromate and copper sulphate showed 3 ratings. The use of ferrous sulphate showed 4-3 ratings, whereas no colour change was observed on use of combination of mordants copper sulphate and ferrous sulphate and ferrous sulphate and potassium dichromate along with the dye (Table 75).

Table 75. Ratings of colourfastness of cotton fabrics dyed with *Mammea suriga* (pre-mordanting).

Dye and mordants used	Wash fastness			Light fastness		Rub fastness	
	1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Mammea suriga	5	5	5	5	5	5	5
Dye + $K_2Cr_2O_7$	1/2	1/2	1/2	1/2	1/2	1/2	1/2
Dye + $CuSO_4$	3	3	3	3	3	3	3
Dye + $FeSO_4$	4	4	4	3/4	3/4	3	3
Dye + $K_2Cr_2O_7$ + $CuSO_4$	3	3	3	3	3	3	3
Dye + $CuSO_4$ + $FeSO_4$	5	5	5	5	5	5	5
Dye + $FeSO_4$ + $K_2Cr_2O_7$	5	5	5	5	5	5	5

In simultaneous mordanting the use of dye along with the mordants showed the colour change ratings 5-3. The wash fastness ratings were in the range 5-3/4 the light fastness ratings were in the range 5-3/4 and range 5-3 was observed for rub fastness tests. The potassium dichromate mordant, the combination of potassium dichromate and copper

sulphate and the combination of ferrous sulphate and potassium dichromate along with the dye gave excellent rating 5 to the cotton fabric. The use of copper sulphate mordant showed 4/5-3 ratings, ferrous sulphate mordant showed ratings between 4/5-3/4 whereas, the combination of copper sulphate and ferrous sulphate with the dye showed ratings between 5-3/4 (Table 76).

Table 76. Ratings of colourfastness of cotton fabrics dyed with *Mammea suriga* (simultaneous mordanting).

Dye and mordants used	Wash fastness			Light fastness		Rub fastness	
	1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min
Dye + $K_2Cr_2O_7$	5	5	5	5	5	5	5
Dye + $CuSO_4$	4/5	4/5	4/5	4/5	4/5	3	3
Dye + $FeSO_4$	4	3/4	3/4	4/5	4/5	4/5	4/5
Dye + $K_2Cr_2O_7$ + $CuSO_4$	5	5	5	5	5	5	5
Dye + $CuSO_4$ + $FeSO_4$	5	5	5	3/4	3/4	3/4	3/4
Dye + $FeSO_4$ + $K_2Cr_2O_7$	5	5	5	5	5	5	5

In post-mordanting the use of dye along with the mordants revealed the colour change values 5-2/3. The wash fastness values ranged from 5-3, the light fastness ratings were between 5-2/3 and rub fastness ratings were between 5-2/3. The use of potassium dichromate mordant showed fastness values 4. The copper sulphate mordant showed the fastness ratings from 4-3, dyeing with ferrous sulphate mordant and combination of potassium dichromate and copper sulphate mordant lead to no colour change of dyed fabrics. The copper sulphate and ferrous sulphate mordant showed the fastness ratings between 4-3/4 and combination of ferrous sulphate and potassium dichromate mordant resulted in 3-2/3 fastness values (Table 77).

Table 77. Ratings of colourfastness of cotton fabrics dyed with *Mammea suriga* (post-mordanting).

Dye and mordants used	Wash fastness			Light fastness		Rub fastness	
	1st wash	2nd wash	3rd wash	6 hours	12 hours	5 min	15 min

Dye + $K_2Cr_2O_7$	4	4	4	4	4	4	4
Dye + $CuSO_4$	4	4	3	3	3	4	4
Dye + $FeSO_4$	5	5	5	5	5	5	5
Dye + $K_2Cr_2O_7$ + $CuSO_4$	5	5	5	5	5	5	5
Dye + $CuSO_4$ + $FeSO_4$	4	4	3/4	3/4	3/4	4	4
Dye + $FeSO_4$ + $K_2Cr_2O_7$	3	3	3	3	2/3	2/3	2/3

Among all the mordants used potassium dichromate mordant was better for fastness tests as the colour did not leached out from the cotton fabrics. The best mordanting method was pre-mordanting method with good to excellent values.

The silk was dyed better than the cotton because of more intercellular spaces in the silk fabrics. The use of ferrous sulphate mordant showed excellent fastness results for silk dyed fabrics and potassium dichromate mordant was excellent for cotton fabrics followed by ferrous sulphate mordant. Among the dyeing methods the best method follows the order as post-mordanting>pre-mordanting>simultaneous mordanting.

4.10. SEM and bright field microscopy analysis

The single raw undyed silk fibre under microscope appears as solid, thin, long and smooth with lustrous cylinder. The SEM image of the same appears to be thin, long with smooth texture. The single undyed cotton fibre has smooth surface with twisted ribbon like structure. The SEM image of the cotton fibre has smooth texture before dyeing. The fibre dyed with *Nyctanthes arbor-tristis* showed bright yellow colour whereas the fibre dyed with *Garcinia indica* appeared reddish-pink due to the presence of anthraquinones. All other bark extracts of *Ceriops tagal, Cassia fistula, Rhizophora mucronata, Mammea suriga* and *Peltophorum pterocarpum* imparted brown colour to the fibre due to the presence of tannins. Tannins are responsible for imparting brown colour shades to the fabrics and are present in most of the bark extracts (Zin and Moe, 2008). The SEM analysis for all the silk (Plate 48) and cotton (Plate 49) dyed fibres showed that the extracts did not damage the physical appearance of the fibres.

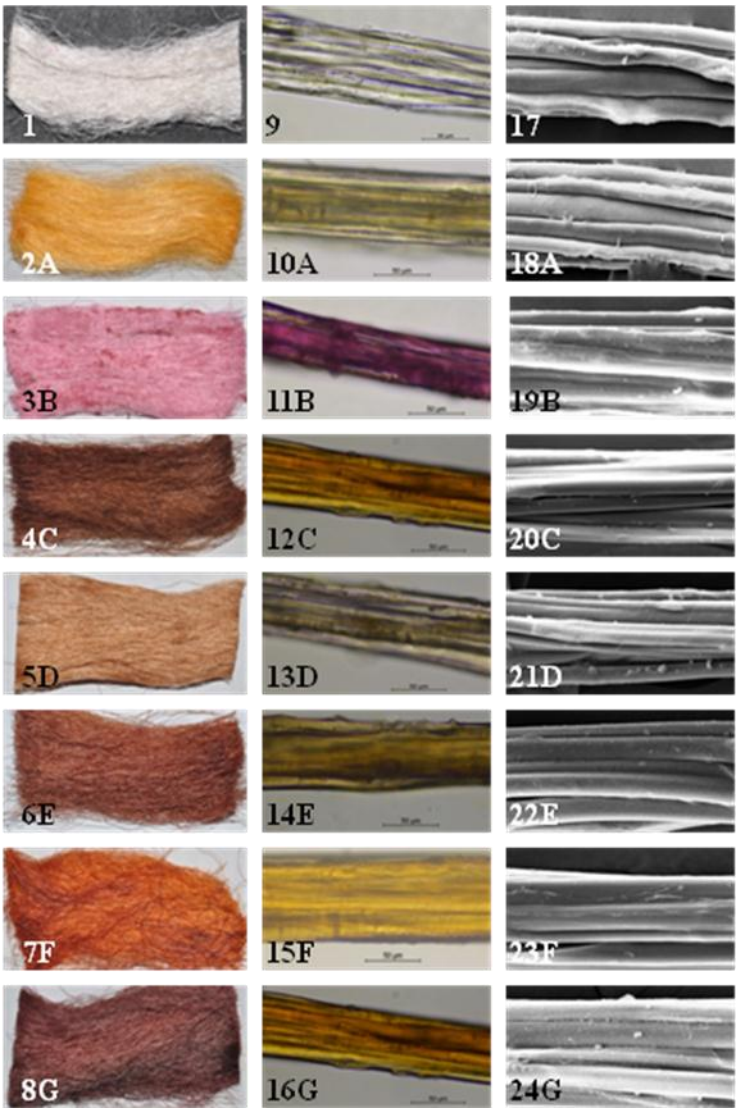


Plate 48. Bright field microscopy and SEM images of silk fibre dyed with natural dyes. 1. undyed silk fabric; 9. undyed silk fabric under bright field microscope; 17. undyed silk fabric under SEM; 2-8. dyed silk fabric; 10-16. dyed silk fabric under bright field microscope (40X); 18-24. dyed silk fabric under SEM (1 KX). A. dyed with *Nyctanthes arbor-tristis* dye; B. *Garcinia indica*; C. *Ceriops tagal*; D. *Rhizophora mucronata*; E. *Mammea suriga*; F. *Peltophorum pterocarpum*; G. *Cassia fistula*.

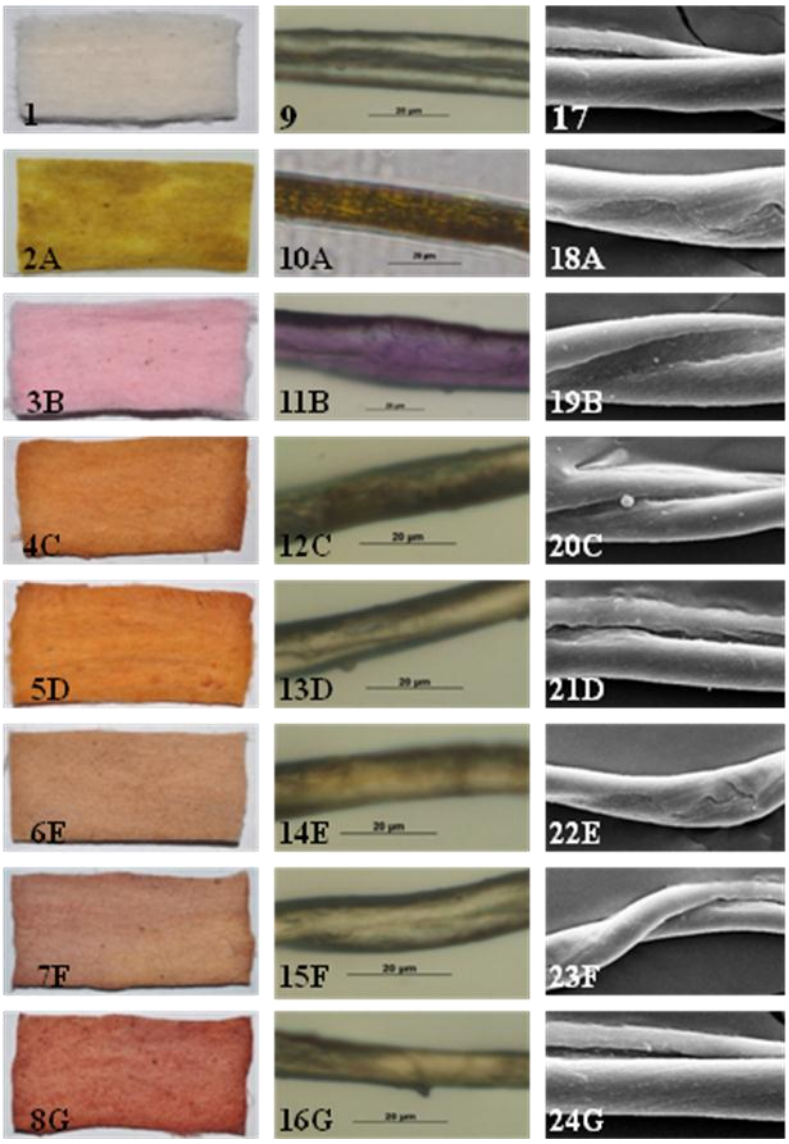


Plate 49. Bright field microscopy and SEM images of cotton fibre dyed with natural dyes. 1. undyed cotton fabric; 9. undyed cotton fabric under bright field microscope; 17. undyed cotton fabric under SEM; 2-8. dyed cotton fabric; 10-16. dyed cotton fabric under bright field microscope (100X); 18-24. dyed cotton fabric under SEM (1 KX). A. dyed with *Nyctanthes arbor-tristis* dye; B. *Garcinia indica*; C. *Ceriops tagal*; D. *Rhizophora mucronata*; E. *Mammea suriga*; F. *Peltophorum pterocarpum*; G. *Cassia fistula*.

4.11. Characterization of natural dyes

The methanol extract of the selected natural dyes (*Averrhoa bilimbi*, *Melastoma malabathricum* and *Phyllanthus reticulates*) obtained from the soxhlet extraction process was filtered in Whatman filter paper (Grade A). The filtered extract was later evaporated

on rotary evaporator to get the concentrated dye extract. This concentrated dye extract was subjected to characterization of dyes.

4.11.1. UV-Visible spectroscopic analysis

The methanolic extracts of the selected dyes were subjected to UV-visible spectroscopy at 200-800 nm. The UV spectra of *Averrhoa bilimbi* dye showed two absorption peaks at 538 nm and 664 nm (Figure 10), the UV spectra of *Melastoma malabathricum* dye showed two absorption peaks at 531 nm and 657 nm (Figure 11) and the UV spectra of *Phyllanthus reticulates* dye showed four absorption peaks at 457, 468, 529 and 662 nm (Figure 12). The peaks in the range of 310-370 nm corresponds to flavones and flavonoles, 460-560 nm corresponds to anthocyanins and 661-654 nm corresponds to chlorophyll (Santos-Buelga *et al*., 2003). The peaks at 538 nm, 531 nm and 529 nm in all the plant extracts of *A. bilimbi*, *M. malabathricum* and *P. reticulates* falls in the range of anthocyanins (460-560 nm) thereby suggesting the presence of anthocyanins in all the three dyes. The anthocyanins are mostly absorbed in visible range. However, the peaks at 664, 657, 662 nm showed the chlorophyll pigment.

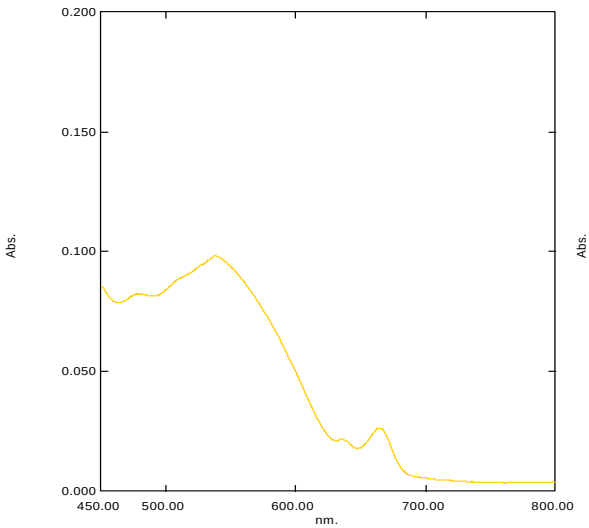


Figure 10. UV spectrum of *A. bilimbi*.

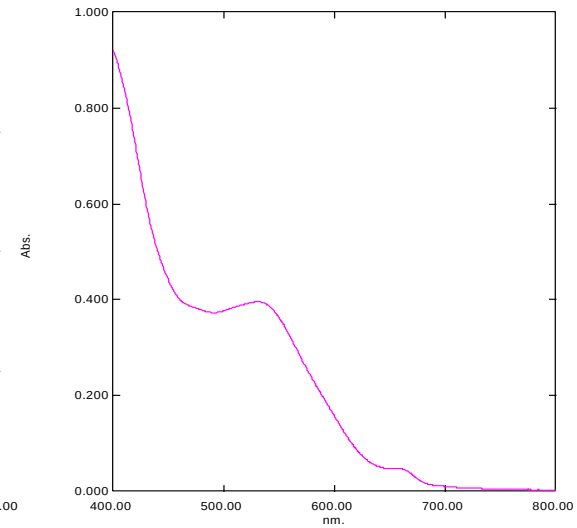


Figure 11. UV spectrum of *M. malabathricum.*

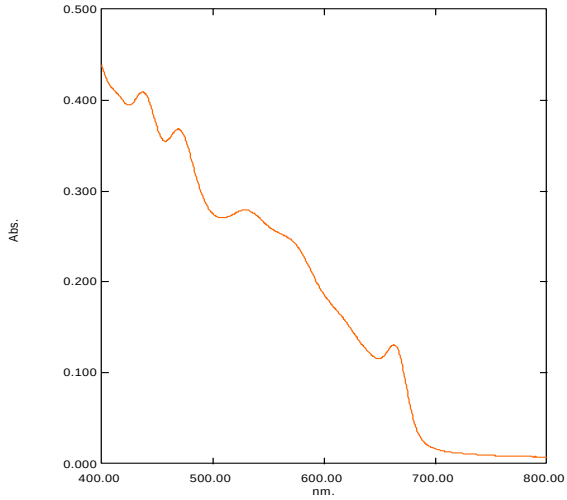


Figure 12. UV spectrum of dye *P. reticulates*.

4.11.2. Separation of compounds by TLC (Thin Layer Chromatography)

Many solvent systems were tried for better separation of anthocyanins from the dye extracts. Finally, the best solvent system chosen was n-butanol: acetic acid: water in (4:1:2) ratio. The methanol extracts of the selected dyes were subjected to TLC for separation of compounds. In the extract of *A. bilimbi* dye, single large red spot was identified with Rf value (0.35) (Plate 50 A), in dye extract of *M. malabathricum* single red spot with Rf value (0.32) (Plate 50 B) and for *P. reticulates* (Plate 50 C) 2 spots were identified with Rf values of 0.37 (purple) 0.57 (red) (Table 78).

Table 78. TLC of selected dye plants.

Plant species	Number of spots	Rf values	Colour of the spot
Averrhoa bilimbi	1	0.35	Red
Melastoma malabathricum	1	0.32	Red
Phyllanthus reticulates	1 2	0.37 0.57	Purple Red

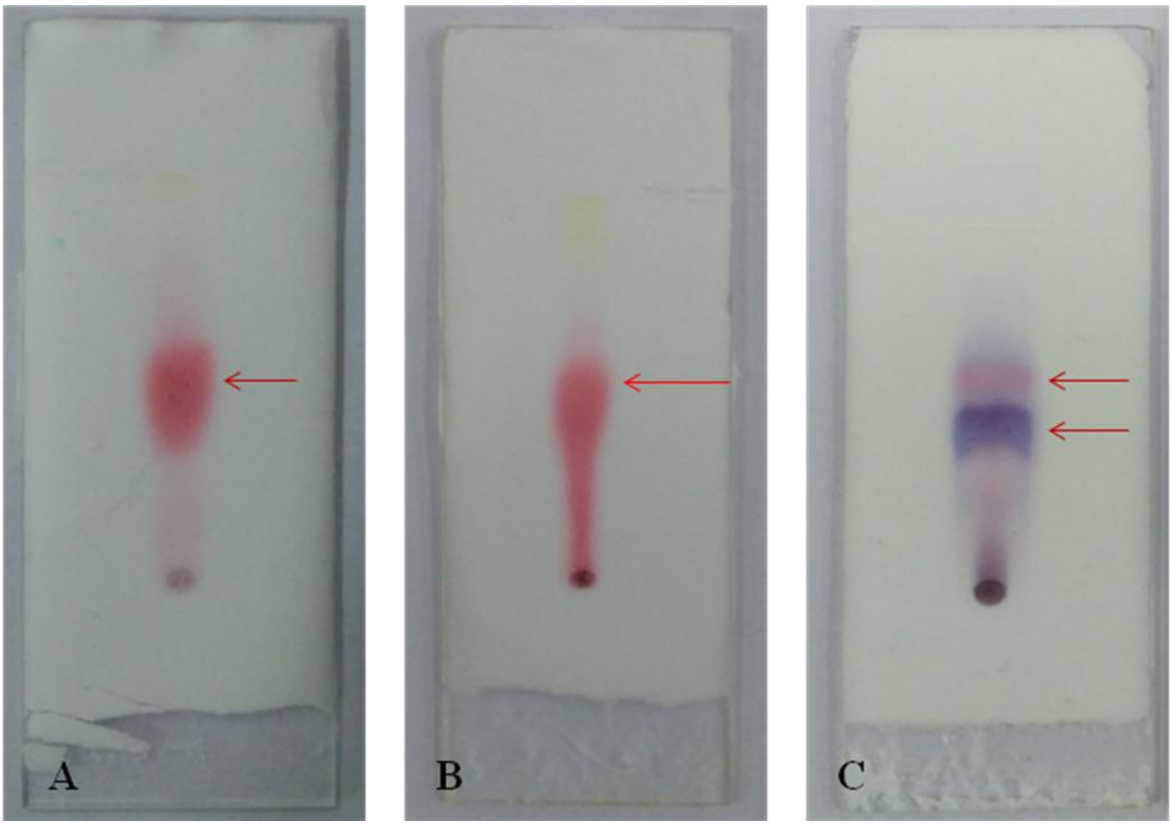


Plate 50 TLC plate showing the number of spots A. *Averrhoa bilimbi*; B. *Melastoma malabathricum*; C. *Phyllanthus reticulates*

4.11.3. FT-IR (Fourier Transform Infrared Spectroscopy) analysis

FT-IR is an important tool which identifies the functional groups. Each functional group absorbs radiation in a characteristic frequency of the infrared spectrum. It is a qualitative analysis which identifies and characterizes the molecular structure and absorption spectra that constitute their characteristic fingerprints.

The broad peak at 3410 cm^{-1} represents O-H (group). The peak at 2914 cm^{-1} is attributed to the CH stretching vibration. The peak at 2100 cm^{-1} represent C=C stretching vibration. The sharp peaks at 1576 cm^{-1} and 1381 cm^{-1} corresponds to C=O stretching vibration and C-H bending vibration. Sharp peak at 742 represent aromatic C-H bond (Table 79). These peaks indicate the presence of flavonoids in the dye. The FT-IR spectrum of *A. bilimbi* dye is provided (Figure 13).

Table 79. FT-IR spectra data (cm $^{-1}$) of *A. bilimbi* dye.

Wave number (cm $^{-1}$)	Band shape	Functional group
3410	Broad peak	O-H alcohol
2914	Broad peak	CH stretching vibration
2100	Broad peak	C=C stretching vibration
1576	Sharp peak	C=O stretching vibration
1381	Sharp peak	C-H bending vibration
742	Sharp peak	Aromatic C-H bond

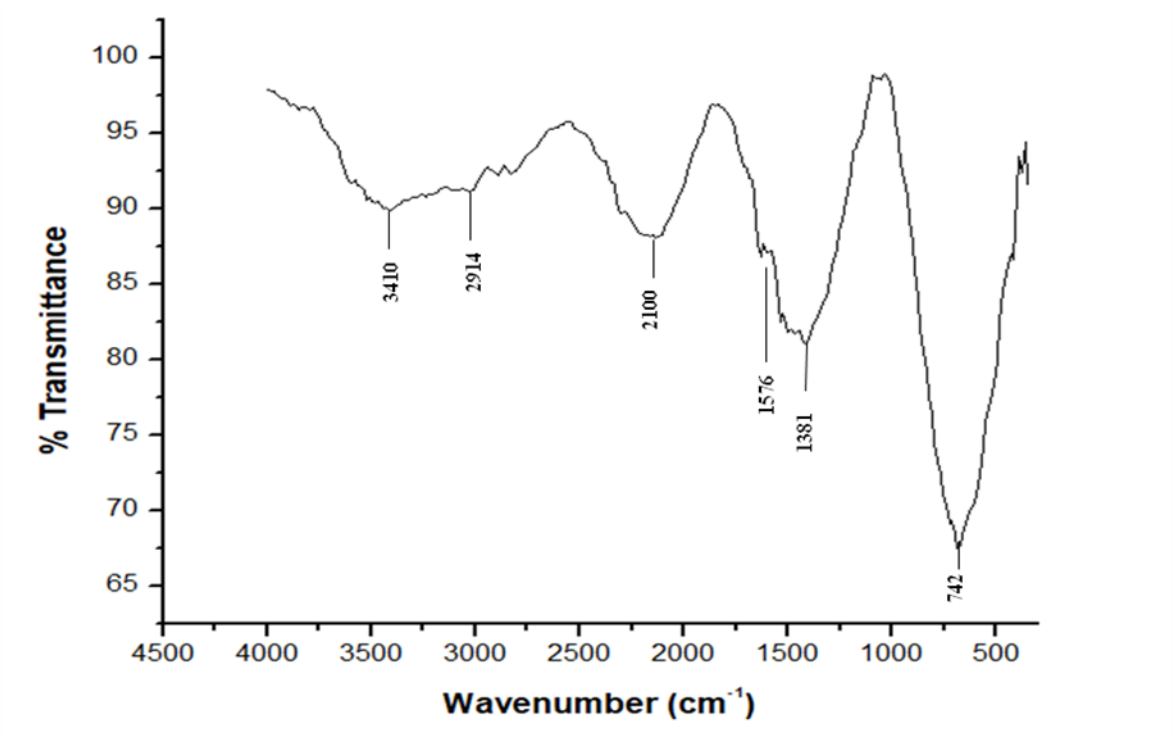


Figure 13. FT-IR spectrum of *A. bilimbi* dye.

The broad peak at 3450 cm^{-1} is attributed to O-H (group). The sharp peaks at 2974 cm^{-1} and 2953 cm^{-1} represents CH stretch. The absorption bands observed at 1641 cm^{-1} is due to C=O stretching vibration. The peak at 1388 cm^{-1} corresponds to C-H bending vibration. The sharp peak at 1064 cm^{-1} is due to C-O stretching vibration and broad peak at 748 cm^{-1} represents aromatic C-H bond (Table 80). These spectral peaks are indicative of

phenolics, especially flavonoids and anthocyanins. The FT-IR spectrum of *M. malabathricum* dye is provided (Figure 14).

Table 80. FT-IR spectra data (cm $^{-1}$) of *M. malabathricum* dye.

Wave number (cm $^{-1}$)	Band shape	Functional group
3450	Broad peak	O-H alcohol
2974	Sharp peak	CH stretch
2953	Sharp peak	CH stretch
1641	Sharp peak	C=O stretching vibration
1388	Sharp peak	C-H bending vibration
1064	Sharp peak	C-O stretching vibration
748	Broad peak	Aromatic C-H bond

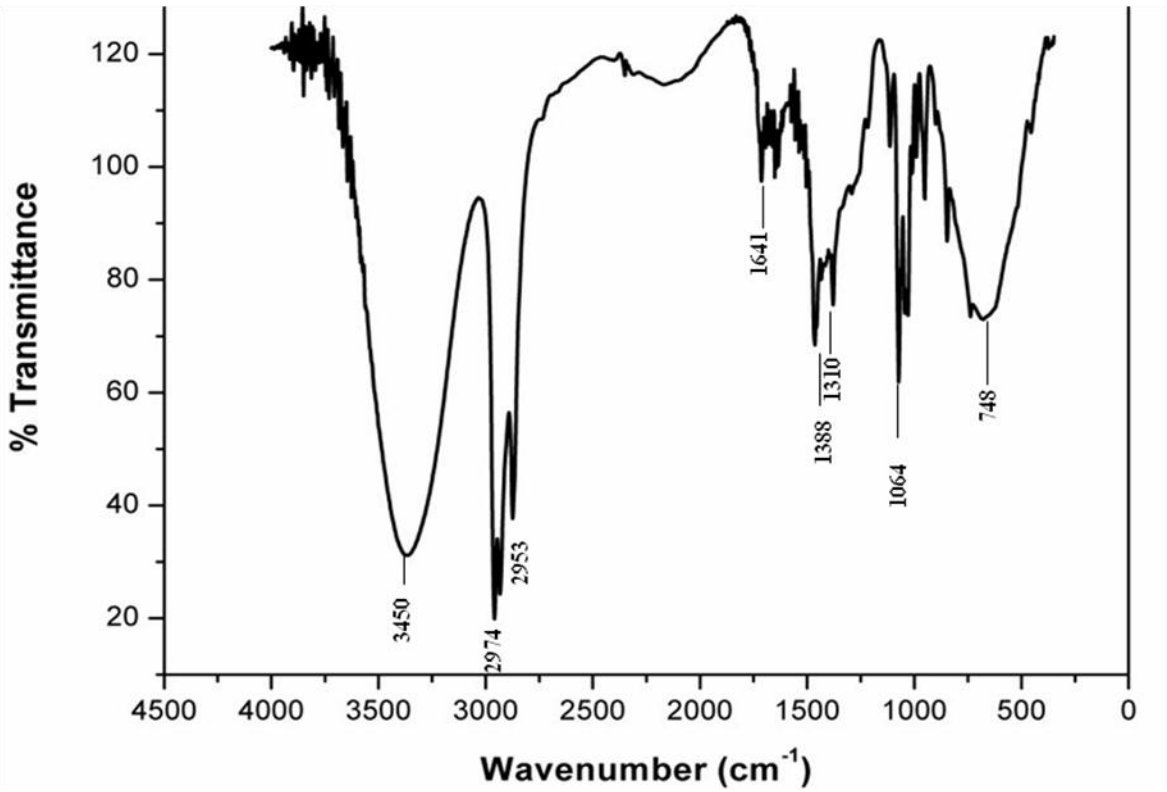


Figure 14. FT-IR spectrum of *M.malabathricum* dye.

The broad peak at 3395.23 cm^{-1} is attributed to O-H (group). The sharp peak at 2923.81 cm^{-1} represents C-H stretch. The absorption bands observed at 1635.32 cm^{-1} is due to C=C stretching vibration. The peak at 1402.73 cm^{-1} corresponds to methylene group CH_2, CH_3. The sharp peak at 1106.98 cm^{-1} is due to the C-O bond (Table 81). These spectral peaks are indicative of phenolics, especially flavonoids and anthocyanins. The FT-IR spectrum of *P. reticulates* dye is provided (Figure 15).

Table 81. FT-IR spectra data (cm^{-1}) of *P. reticulates* dye.

Wave number (cm^{-1})	Band shape	Functional group
3395.23	Broad peak	O-H group
2923.81	Sharp peak	C-H stretching
1635.32	Sharp peak	C=C stretching
1402.73	Sharp peak	Methylene group CH_2, CH_3
1106.98	Sharp peak	C-O bond

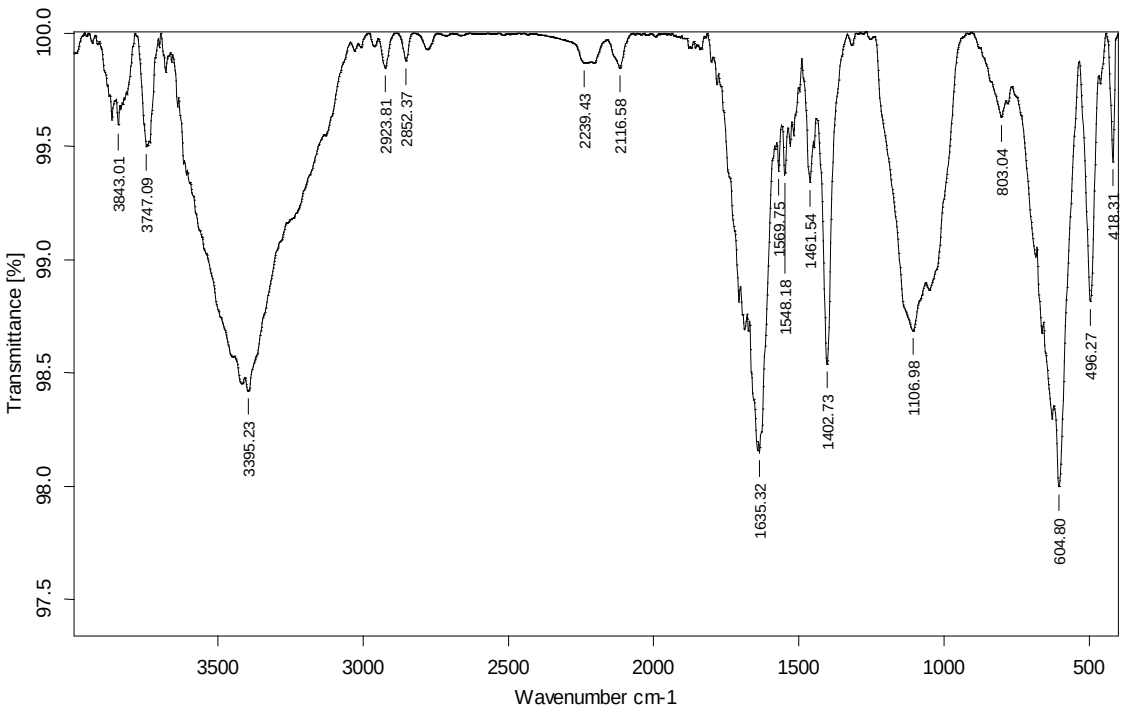


Figure 15. FT-IR spectrum of *P. reticulates* dye.

4.11.4. GC-MS (Gas Chromatography-Mass Spectrometry) analysis

GC-MS analysis helps in identification of volatile compounds. The combination of GC with MS gives characteristic fragments of compounds which makes the identification and quantitative evaluation easier.

Table 82. Compounds identified in the GC-MS analysis of *Melastoma malabathricum* fruit extract.

Sr. No.	RT	Name of the compound	Class of compounds	Molecular formula	Peak area (%)
1	3.92	Acetyl chloride	Acyl chloride	C_2H_3ClO	2.11
2	4.13	2 Pentanone, 4 hydroxy 4 methyl	Ketone	$C_6H_{12}O_2$	23.64
3	6.98	2 Pentanone, 4 hydroxy 4 methyl	Ketone	$C_6H_{12}O_2$	18.49
4	10.13	Octane, 3, 5 dimethyl	Alkane	$C_{10}H_{22}$	7.99
5	12.06	Undecane, 5, 7 dimethyl	Alkane	$C_{13}H_{28}$	1.56
6	18.01	Phenol, 2, 4 bis (1,1 dimethylethyl)	Phenol	$C_{14}H_{22}O$	0.46
7	19.99	Cyclohexanecarboxaldehyde, 3, 3 dimethyl 5 oxo	-	$C_9H_{14}O_2$	0.66
8	22.03	Phthalic acid, cyclobutyl tridecyl ester	-	$C_{25}H_{38}O_4$	0.89
9	22.47	Dodecane, 2, 7,10 trimethyl	Alkane	$C_{15}H_{32}$	2.89
10	22.69	Hexadecanoic acid, 15 methyl, methyl ester	Palmitic acid ester	$C_{18}H_{36}O_2$	4.50
11	23.08	n-Hexadecanoic acid	Palmitic acid ester	$C_{16}H_{32}O_2$	1.97
12	23.46	Dodecane, 2, 6,11trimethyl	alkane	$C_{15}H_{32}$	0.99
13	24.41	Hexadecane	Alkane hydrocarbon	$C_{16}H_{34}$	17.37
14	24.63	Hexadecanoic acid, 15 methyl,	Palmitic acid ester	$C_{18}H_{36}O_2$	0.83

		methyl ester			
15	24.81	6,9,12 Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)	Linolenic acid ester	$C_{25}H_{36}O_2$	0.61
16	25.06	6,9,12 Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)	Linolenic acid ester	$C_{25}H_{36}O_2$	0.92
17	25.19	Eicosanoic acid, phenylmethyl ester	-	$C_{27}H_{46}O_2$	0.50
18	25.34	Eicosane	Alkane	$C_{20}H_{42}$	1.10
19	26.21	1 Decanol, 2 octyl	Fatty alcohol	$C_{18}H_{38}O$	3.60
20	26.37	Heptadecane, 2,6,10,15 tetramethyl	Alkane	$C_{21}H_{44}$	2.67
21	27.11	1,1,1,5,7,7,7 Heptamethy l3,3 bis (trimethylsiloxy) tetrasiloxane	-	$C_{13}H_{40}O_5Si_6$	2.95
22	28.05	Butyl 9 tetradecenoate	Fatty acid	$C_{18}H_{34}O_2$	2.35
23	29.14	Octadecane, 1(ethenyloxy)	Alkane	$C_{20}H_{40}O$	0.40
24	29.63	Phthalic acid, octadecyl 2 propylpentyl ester	-	$C_{34}H_{58}O_4$	0.56

The GC-MS analysis revealed the presence of 24 compounds in the methanolic fruit extract of *Melastoma malabathricum*. The methanolic extract allows the separation of most polar compounds present in the leaves, fruits, flowers like phenolic compounds and its methoxy and carboxylic derivatives (Morales *et al*., 2012). The compounds identified are provided (Table 82). The major compound identified was 2 Pentanone, 4 hydroxy 4 methyl with highest peak area 23.64 % and 18.49 %. It belongs to the class of organic compounds known as beta-hydroxy ketones. This is followed by Hexadecane with peak area of 17.37 %, Octane, 3, 5 dimethyl (7.99 %), Hexadecanoic acid, 15-methyl, methyl ester (4.50 %). This hexadecanoic acid, methyl ester, methyl tetradecanoate is known to contain chromophore cluster, which brings colour to the substance (Qadariyah *et al*., 2018). The Heptadecane, 2,6,10,15 tetramethyl (2.67%), 1,1,1,5,7,7,7 Heptamethy l3,3 bis (trimethylsiloxy) tetrasiloxane (2.95 %), Butyl 9 tetradecenoate (2.35 %) and n-

Hexadecanoic acid (1.97 %). The other minor compounds present are Acetyl chloride (2.11 %), Undecane, 5, 7 dimethyl (1.56 %), Eicosane (1.10 %), Dodecane, 2, 6, 11trimethyl (0.99 %), 6,9,12 Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z) (0.92 %), Phthalic acid, cyclobutyl tridecyl ester (0.89 %), Hexadecanoic acid, 15 methyl, methyl ester (0.83 %), Cyclohexane carboxaldehyde, 3, 3 dimethyl 5 oxo (0.66 %), 6,9,12 Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z) (0.61 %), Phthalic acid, octadecyl 2 propylpentyl ester (0.56 %), Eicosanoic acid, phenylmethyl ester (0.50 %), Phenol, 2, 4 bis (1,1 dimethylethyl) (0.46 %) and Octadecane, 1 (ethenyloxy) (0.40 %) (Figure 16-17). Most of the compounds identified belongs to fatty acids, phenols and alcohols.

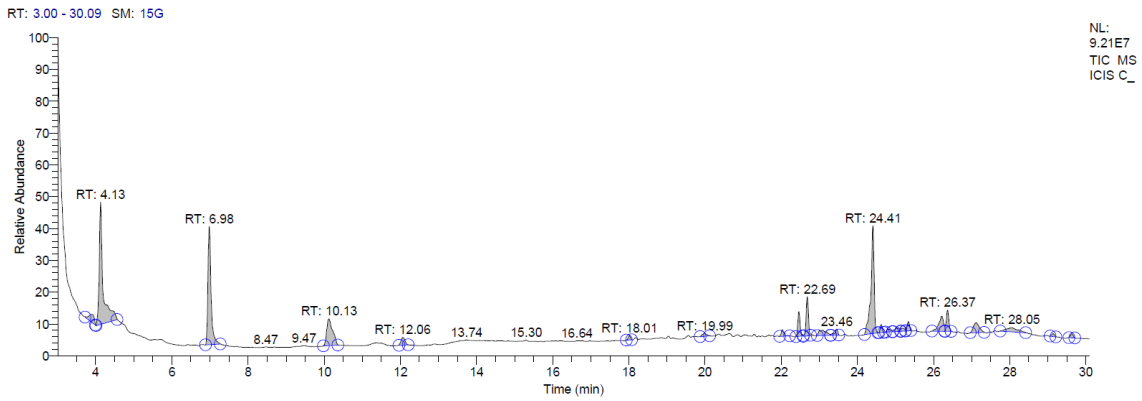
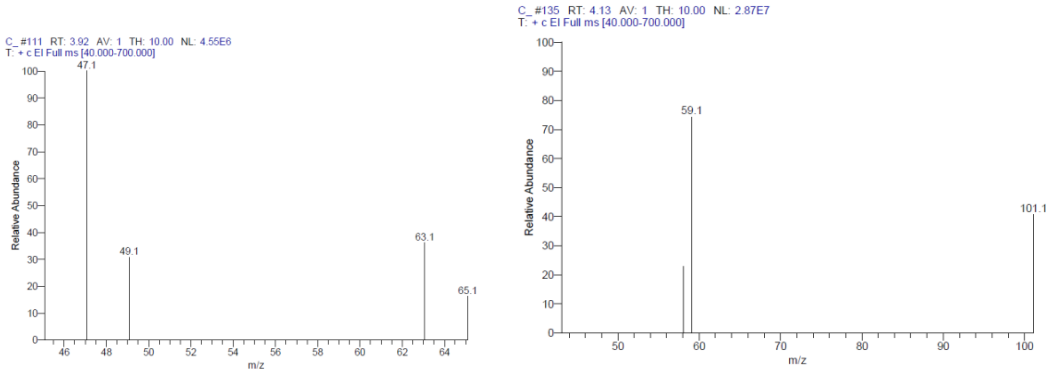
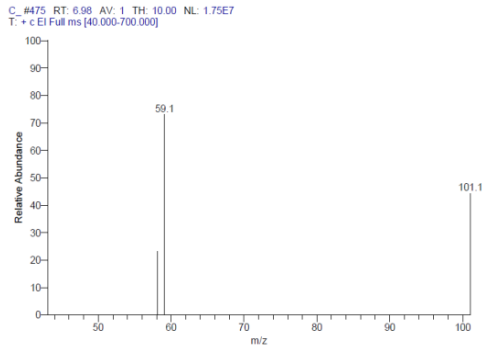
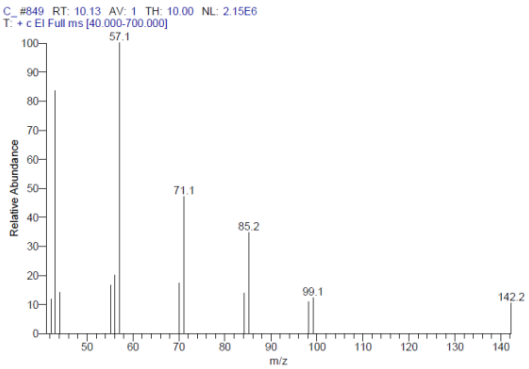
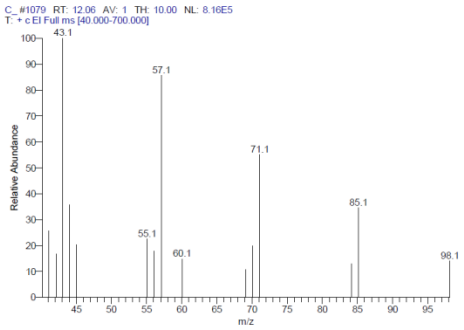


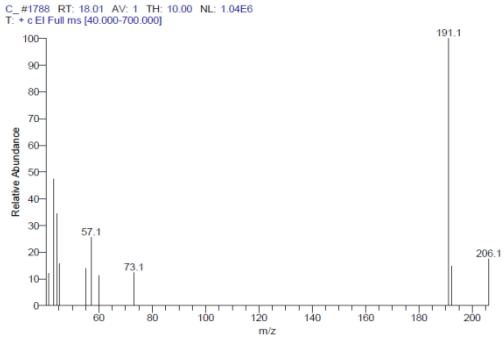
Figure 16. GC chromatogram of *Melastoma malabathricum* fruit extract.

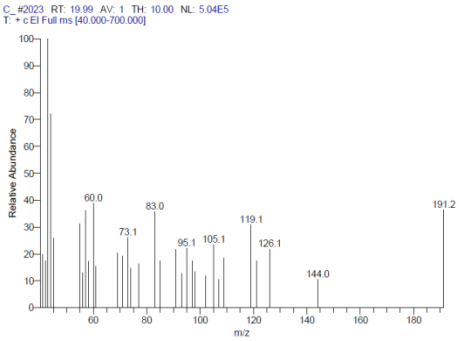


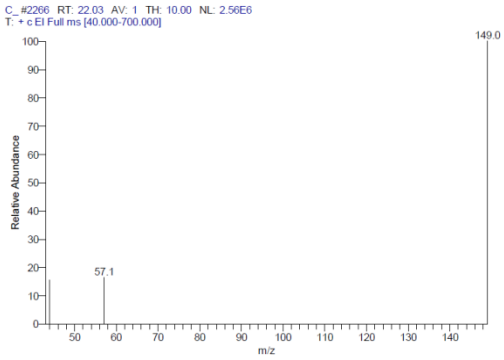

C_ #475 RT: 6.98 AV: 1 TH: 10.00 NL: 1.75E7
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
59.1
101.1

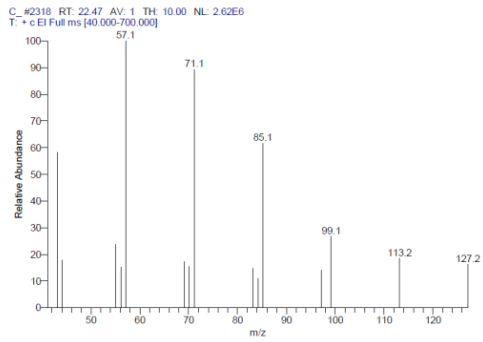

C_ #849 RT: 10.13 AV: 1 TH: 10.00 NL: 2.15E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
57.1
71.1
85.2
99.1
142.2

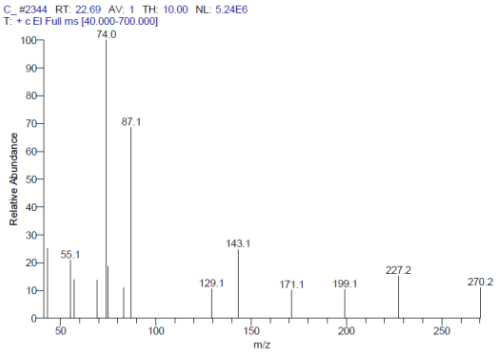

C_ #1079 RT: 12.06 AV: 1 TH: 10.00 NL: 8.16E5
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
43.1
57.1
71.1
85.1
55.1
60.1
98.1


C_ #1788 RT: 18.01 AV: 1 TH: 10.00 NL: 1.04E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
191.1
57.1
73.1
206.1

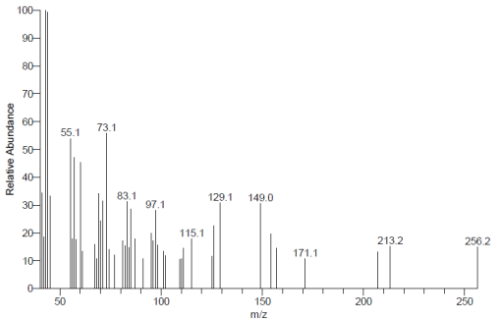

C_ #2023 RT: 19.99 AV: 1 TH: 10.00 NL: 5.04E5
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
60.0
83.0
73.1
95.1
105.1
119.1
126.1
144.0
191.2


C_ #2266 RT: 22.03 AV: 1 TH: 10.00 NL: 2.56E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
149.0
57.1

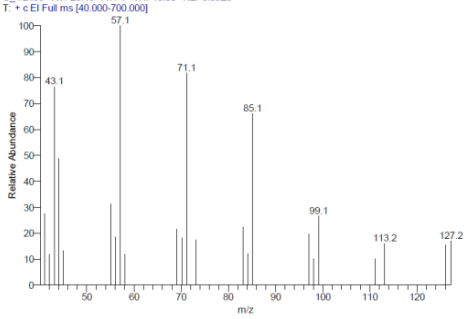

C_ #2318 RT: 22.47 AV: 1 TH: 10.00 NL: 2.62E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
57.1
71.1
85.1
99.1
113.2
127.2


C_ #2344 RT: 22.69 AV: 1 TH: 10.00 NL: 5.24E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
74.0
87.1
55.1
143.1
129.1
171.1
199.1
227.2
270.2

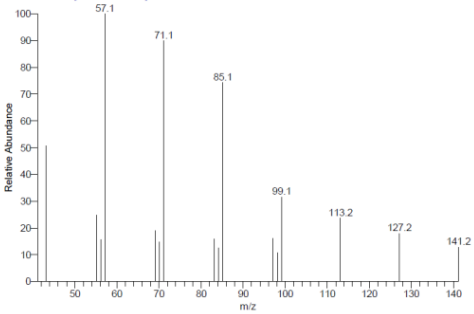
C_ #2391 RT: 23.08 AV: 1 TH: 10.00 NL: 4.15E5
T: + c EI Full ms [40.000-700.000]



C_ #2436 RT: 23.46 AV: 1 TH: 10.00 NL: 8.55E5
T: + c EI Full ms [40.000-700.000]



C_ #2549 RT: 24.41 AV: 1 TH: 10.00 NL: 7.76E6
T: + c EI Full ms [40.000-700.000]



C_ #2575 RT: 24.63 AV: 1 TH: 10.00 NL: 1.05E6
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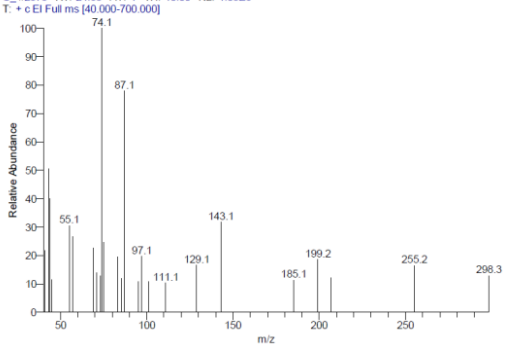

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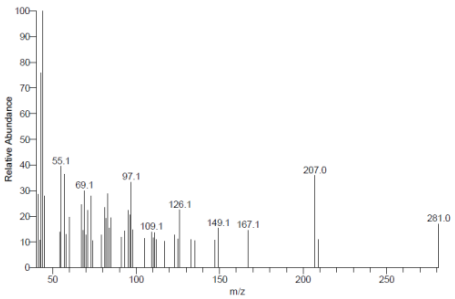

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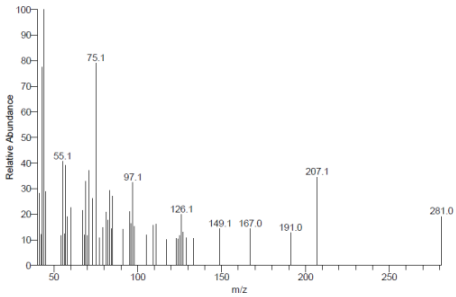
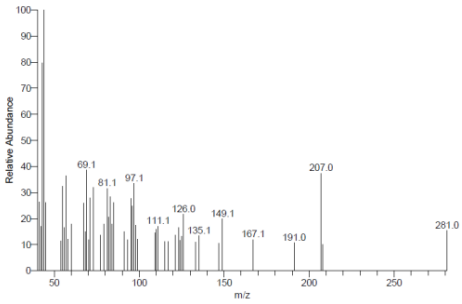
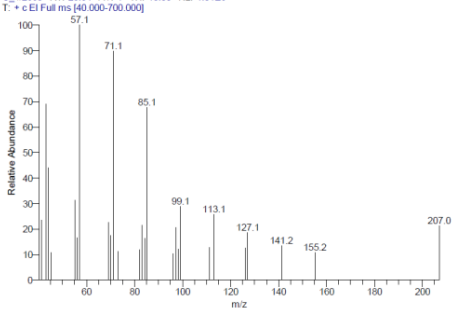


C_ #2642 RT: 25.19 AV: 1 TH: 10.00 NL: 4.37E5
T: + c EI Full ms [40.000-700.000]



C_ #2660 RT: 25.34 AV: 1 TH: 10.00 NL: 1.01E6
T: + c EI Full ms [40.000-700.000]



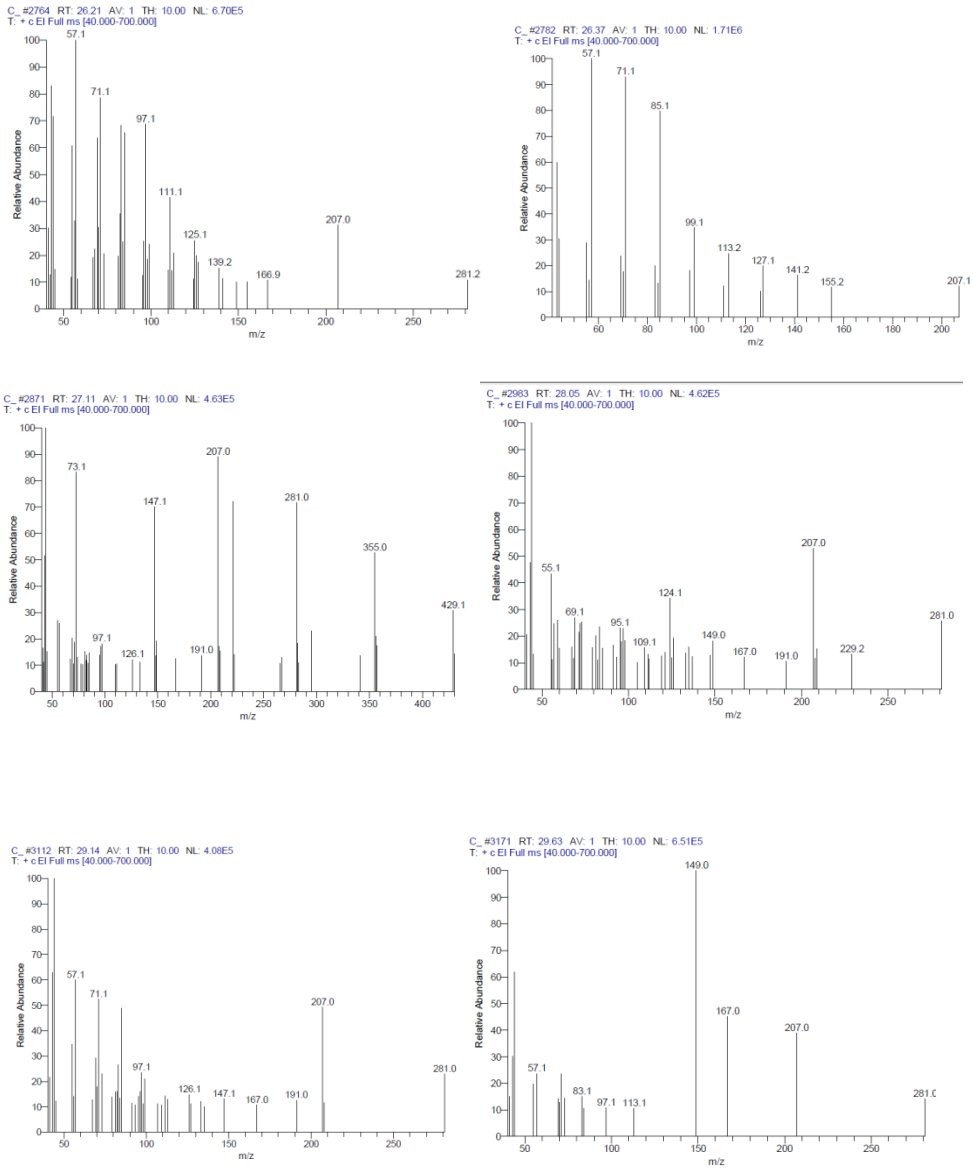


Figure 17. MS chromatogram of *Melastoma malabathricum* fruit extract.

Table 83. Compounds identified in the GC-MS analysis of *Phyllanthus reticulates* fruit extract.

Sr. No.	RT	Compound name	Class of compounds	Molecular formula	Peak area (%)
1	3.98	Acetic acid	Carboxylic acid	$C_2H_4O_2$	16.34
2	4.35	Ethanimidic acid, ethyl ester	Acid amide	C_4H_9NO	1.70
3	5.45	2- (Aminooxy), Pentanoic acid	Carboxylic acid	$C_5H_{11}NO_3$	0.72

4	6.57	Arsenous acid, tris (trimethylsilyl) ester	Ester	$C_9H_{27}AsO_3Si_3$	0.41
5	7.05	2 Pentanone, 4 hydroxy 4 methyl	Ketone	$C_6H_{12}O_2$	3.01
6	8.35	4 Ethyl benzoic acid, 2 methyl butyl ester	Benzoic acid ester	$C_{14}H_{20}O_2$	0.88
7	9.95	2,4 Dihydroxy 2,5 dimethyl 3 (2H) furanone	Ketone	$C_6H_8O_4$	1.74
8	11.15	Methyl acetoxyacetate	-	$C_5H_8O_3$	0.86
9	11.59	Arsenous acid, tris (trimethylsilyl) ester	Ester	$C_9H_{27}AsO_3Si_3$	1.30
10	12.73	Butanoic acid, 2 methyl 3 oxo, ethyl ester	Ester	$C_7H_{12}O_3$	0.81
11	13.01	2,3-Dihydro-3,5-dihydroxy-6- methyl-4H-pyran-4-one	Flavonoid	$C_6H_8O_4$	48.89
12	14.12	2,3-Dihydro-3,5-dihydroxy-6- methyl-4H-pyran-4-one	Flavonoid	$C_6H_8O_4$	1.04
13	14.64	Pentanoic acid, 3 hydroxy 4 methyl, methyl ester	Ester	$C_7H_{14}O_3$	1.42
14	15.30	1Triethylsilyloxyheptadecane	-	$C_{23}H_{50}OSi$	5.78
15	15.76	Propanoic acid, 3 hydroxy, methyl ester	Ester	$C_4H_8O_3$	1.73
16	17.57	3 Isopropoxy1,1,1,7,7,7 hexamethyl 3,5,5 tris (trimethylsiloxy) tetrasiloxane	-	$C_{18}H_{52}O_7Si_7$	1.31
17	18.01	Phenol, 3,5 bis (1,1dimethylethyl)	Phenol	$C_{14}H_{22}O$	0.43
18	18.24	Benzoic acid, 4 ethoxy, ethyl ester	Ester	$C_{11}H_{14}O_3$	0.92
19	22.03	Phthalic acid, cyclobutyl tridecyl ester	-	$C_{25}H_{38}O_4$	0.41
20	22.69	Hexadecanoic acid, 15 methyl, methyl ester	Palmitic acid ester	$C_{18}H_{36}O_2$	0.34
21	23.00	Phthalic acid, 4 cyanophenyl nonyl ester	-	$C_{24}H_{27}NO_4$	0.79

22	23.49	2 methylhexacosane	Acylic Alkane	$C_{27}H_{56}$	0.76
23	23.69	1Decanol, 2 octyl	Alcohol	$C_{18}H_{38}O$	0.71
24	24.11	Heptasiloxane, hexadecamethyl	-	$C_{16}H_{48}O_6Si_7$	4.02
25	24.38	6 Octadecenoic acid, methyl ester, (Z)	Linolenic acid methyl ester	$C_{19}H_{36}O_2$	1.15
26	24.77	14 Octadecenal	Aldehyde	$C_{18}H_{34}O$	0.62
27	28.47	1,1,1,5,7,7,7 Heptamethyl 3,3 bis (trimethylsiloxy) tetrasiloxane	-	$C_{13}H_{40}O_5Si_6$	1.90

The GC-MS analysis of the methanolic fruit extract of *Phyllanthus reticulates* revealed 27 compounds. The compounds identified along with peak area percentage are tabulated (Table 83). The major phytoconstituents were 2, 3-Dihydro-3, 5-dihydroxy-6-methyl-4 H-pyran-4-one (48.89%), Acetic acid (16.34%), 1 Triethylsilyloxyheptadecane (5.78%), Heptasiloxane, hexadecamethyl (4.02%) and 2 Pentanone, 4 hydroxy 4 methyl (3.01%) which possess biological activities. The other constituents present were 1,1,1,5,7,7,7 Heptamethyl 3,3 bis (trimethylsiloxy) tetrasiloxane (1.90%), 2,4 Dihydroxy 2,5 dimethyl 3 (2H) furanone (1.74%), Propanoic acid, 3 hydroxy, methyl ester (1.73%), Ethanimidic acid, ethyl ester (1.70%), Pentanoic acid, 3 hydroxy 4 methyl, methyl ester (1.42%), 3 Isopropoxy1,1,1,7,7,7 hexamethyl 3,5,5 tris (trimethylsiloxy) tetrasiloxane (1.31%), Arsenous acid, tris (trimethylsilyl) ester (1.30%), 6 Octadecenoic acid, methyl ester, (Z) (1.15%), 2,3-Dihydro-3,5-dihydroxy-6- methyl-4H-pyran-4-one (1.04%). The other minor constituents were Benzoic acid, 4 ethoxy, ethyl ester (0.92%), 4 Ethyl benzoic acid, 2 methyl butyl ester (0.88%), Methyl acetoxyacetate (0.86%), Butanoic acid, 2 methyl 3 oxo, ethyl ester (0.81%), Phthalic acid, 4 cyanophenyl nonyl ester (0.79%), 2 methylhexacosane (0.76%), 2-(Aminooxy), Pentanoic acid (0.72%), 1 Decanol, 2 octyl (0.71%), 14 Octadecenal (0.62%), Phenol, 3,5 bis (1,1dimethylethyl) (0.43%), Arsenous acid, tris (trimethylsilyl) ester (0.41%), Phthalic acid, cyclobutyl tridecyl ester (0.41%) and Hexadecanoic acid, 15 methyl, methyl ester (0.34%) (Figure 18-19).

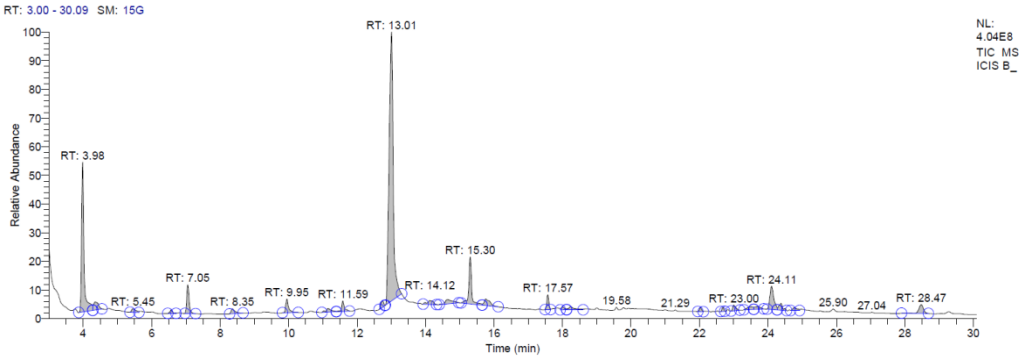
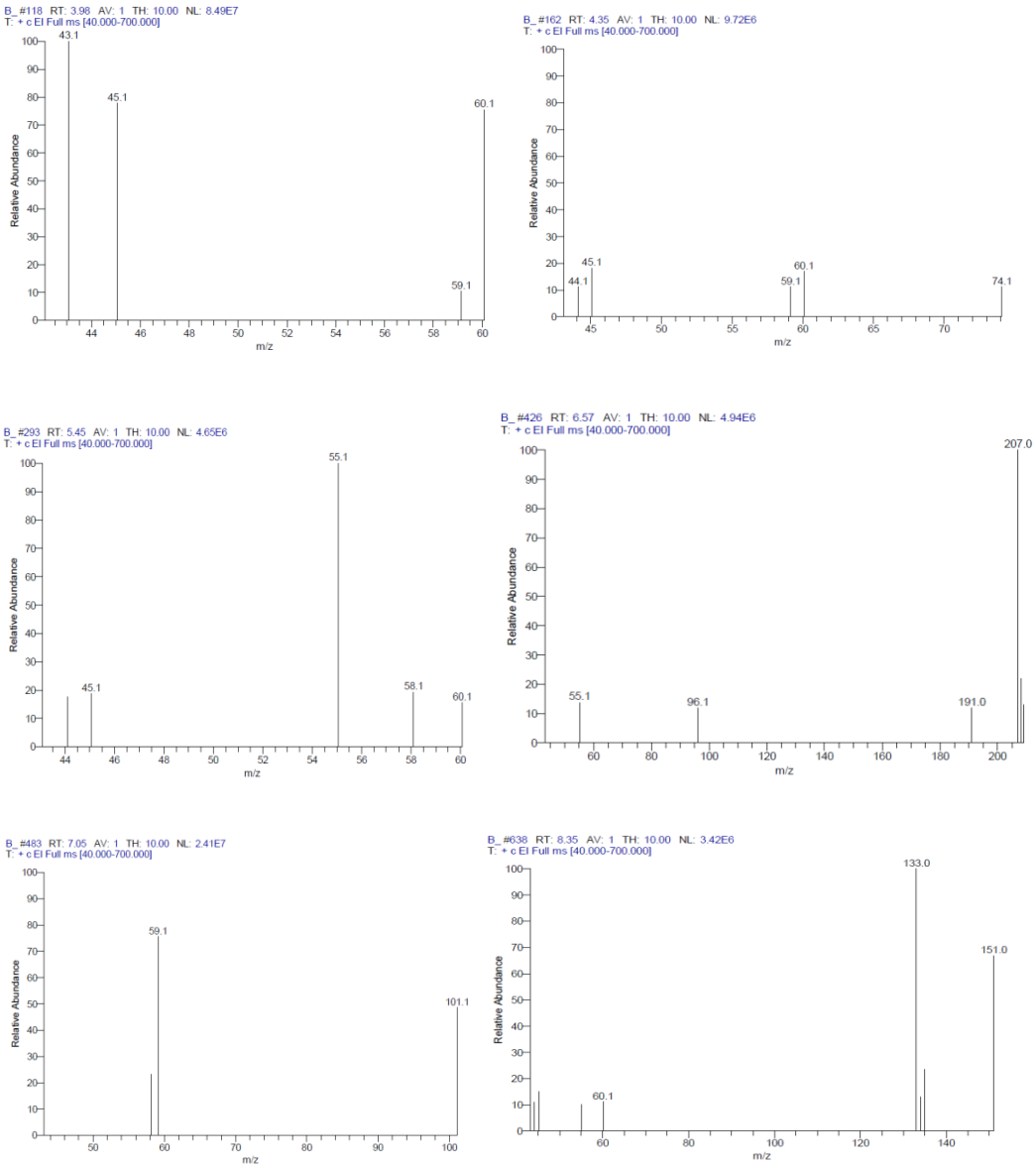
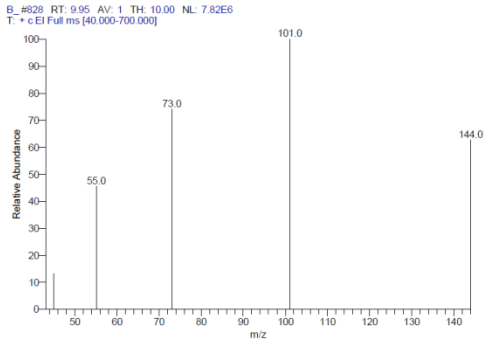
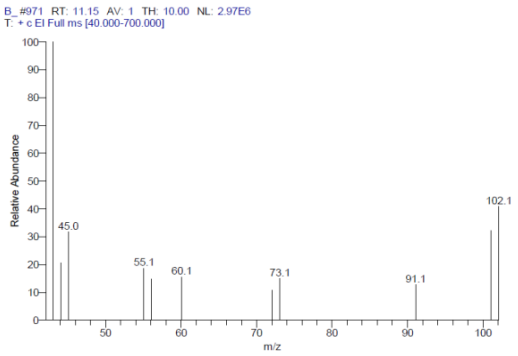
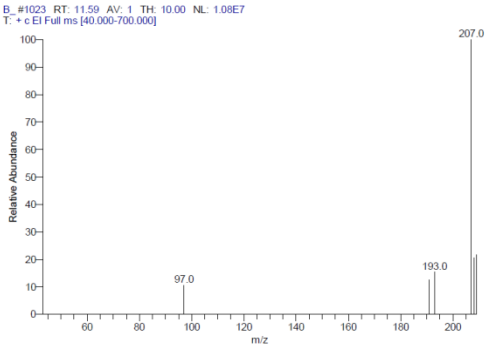


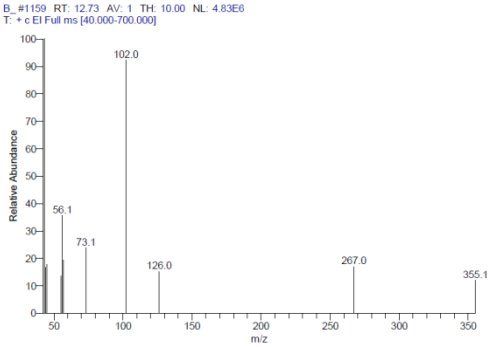
Figure 18. GC chromatogram of *Phyllanthus reticulates* fruit extract.

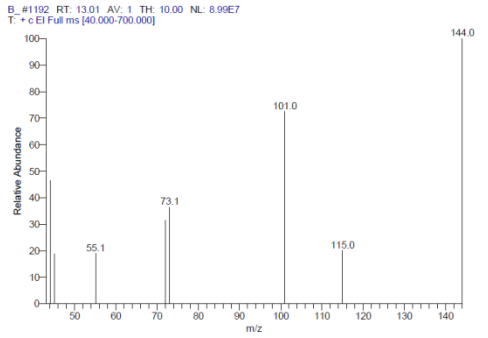


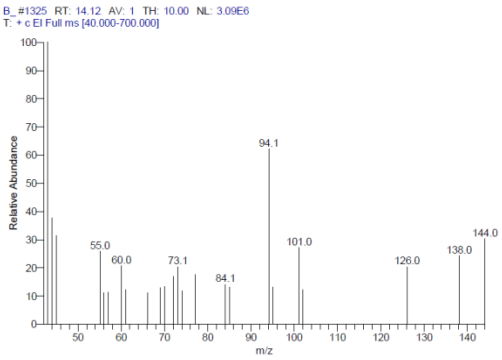

B_#828 RT: 9.95 AV: 1 TH: 10.00 NL: 7.82E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
101.0
73.0
144.0
55.0

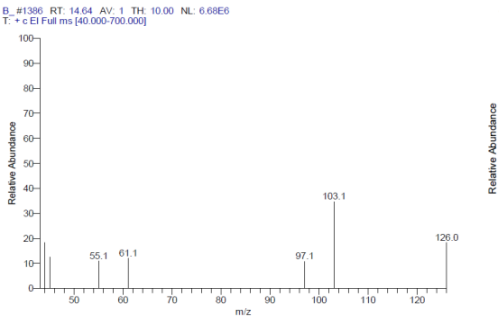

B_#971 RT: 11.15 AV: 1 TH: 10.00 NL: 2.97E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
102.1
45.0
55.1
60.1
73.1
91.1

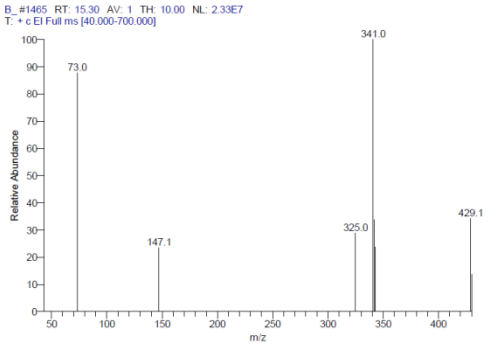

B_#1023 RT: 11.59 AV: 1 TH: 10.00 NL: 1.08E7
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
207.0
97.0
193.0


B_#1159 RT: 12.73 AV: 1 TH: 10.00 NL: 4.83E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
102.0
56.1
73.1
126.0
267.0
355.1


B_#1192 RT: 13.01 AV: 1 TH: 10.00 NL: 8.99E7
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
144.0
101.0
73.1
55.1
115.0


B_#1325 RT: 14.12 AV: 1 TH: 10.00 NL: 3.09E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
94.1
55.0
60.0
73.1
84.1
101.0
126.0
138.0
144.0


B_#1386 RT: 14.64 AV: 1 TH: 10.00 NL: 6.68E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
103.1
55.1
61.1
97.1
126.0


B_#1465 RT: 15.30 AV: 1 TH: 10.00 NL: 2.33E7
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
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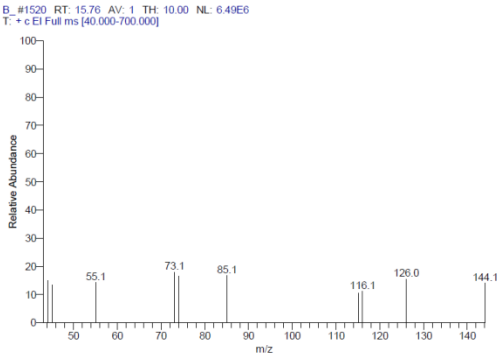
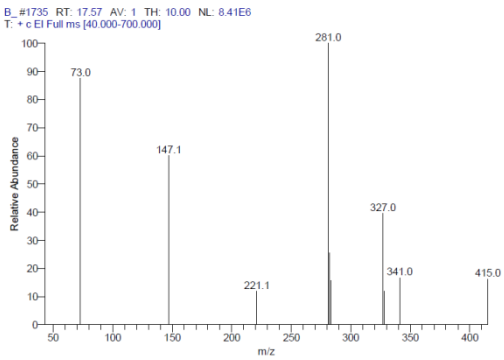
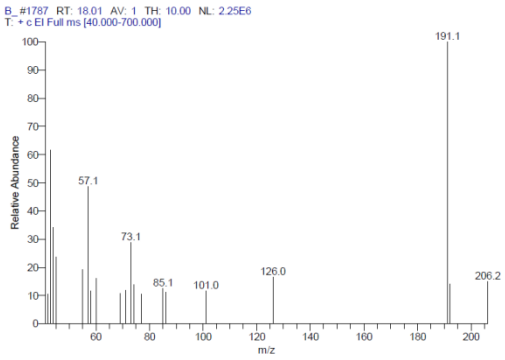
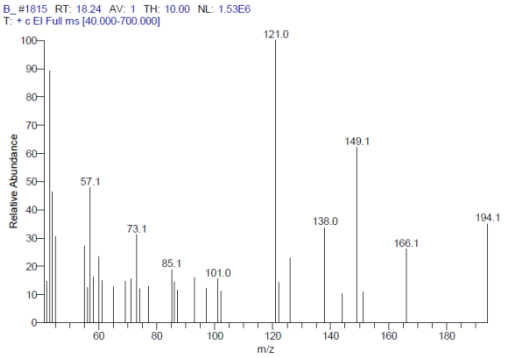
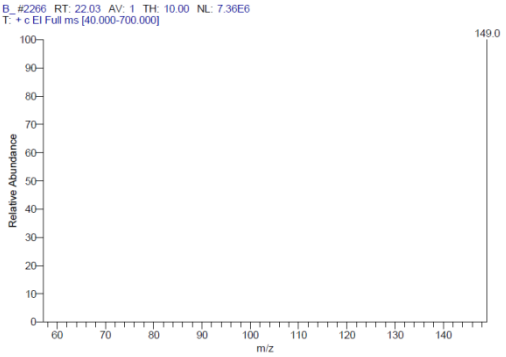
73.0
147.1
325.0
429.1

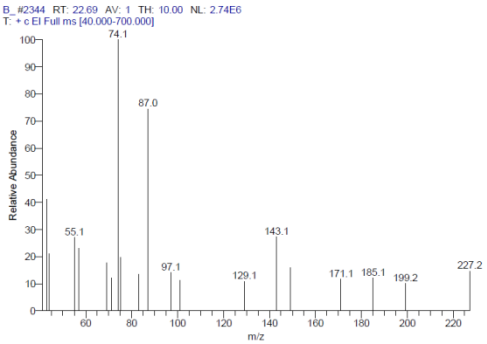

B_#1520 RT: 15.76 AV: 1 TH: 10.00 NL: 6.49E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
55.1
73.1
85.1
116.1
126.0
144.1

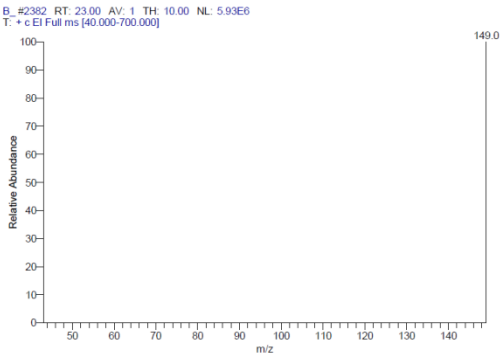

B_#1735 RT: 17.57 AV: 1 TH: 10.00 NL: 8.41E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
73.0
147.1
221.1
281.0
327.0
341.0
415.0

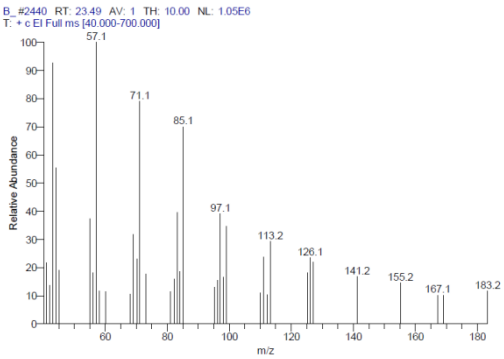

B_#1787 RT: 18.01 AV: 1 TH: 10.00 NL: 2.25E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
57.1
73.1
85.1
101.0
126.0
191.1
206.2


B_#1815 RT: 18.24 AV: 1 TH: 10.00 NL: 1.53E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
57.1
73.1
85.1
101.0
121.0
138.0
149.1
166.1
194.1


B_#2266 RT: 22.03 AV: 1 TH: 10.00 NL: 7.36E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
149.0


B_#2344 RT: 22.69 AV: 1 TH: 10.00 NL: 2.74E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
55.1
74.1
87.0
97.1
129.1
143.1
171.1
185.1
199.2
227.2


B_#2382 RT: 23.00 AV: 1 TH: 10.00 NL: 5.93E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
149.0


B_#2440 RT: 23.49 AV: 1 TH: 10.00 NL: 1.05E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
57.1
71.1
85.1
97.1
113.2
126.1
141.2
155.2
167.1
183.2

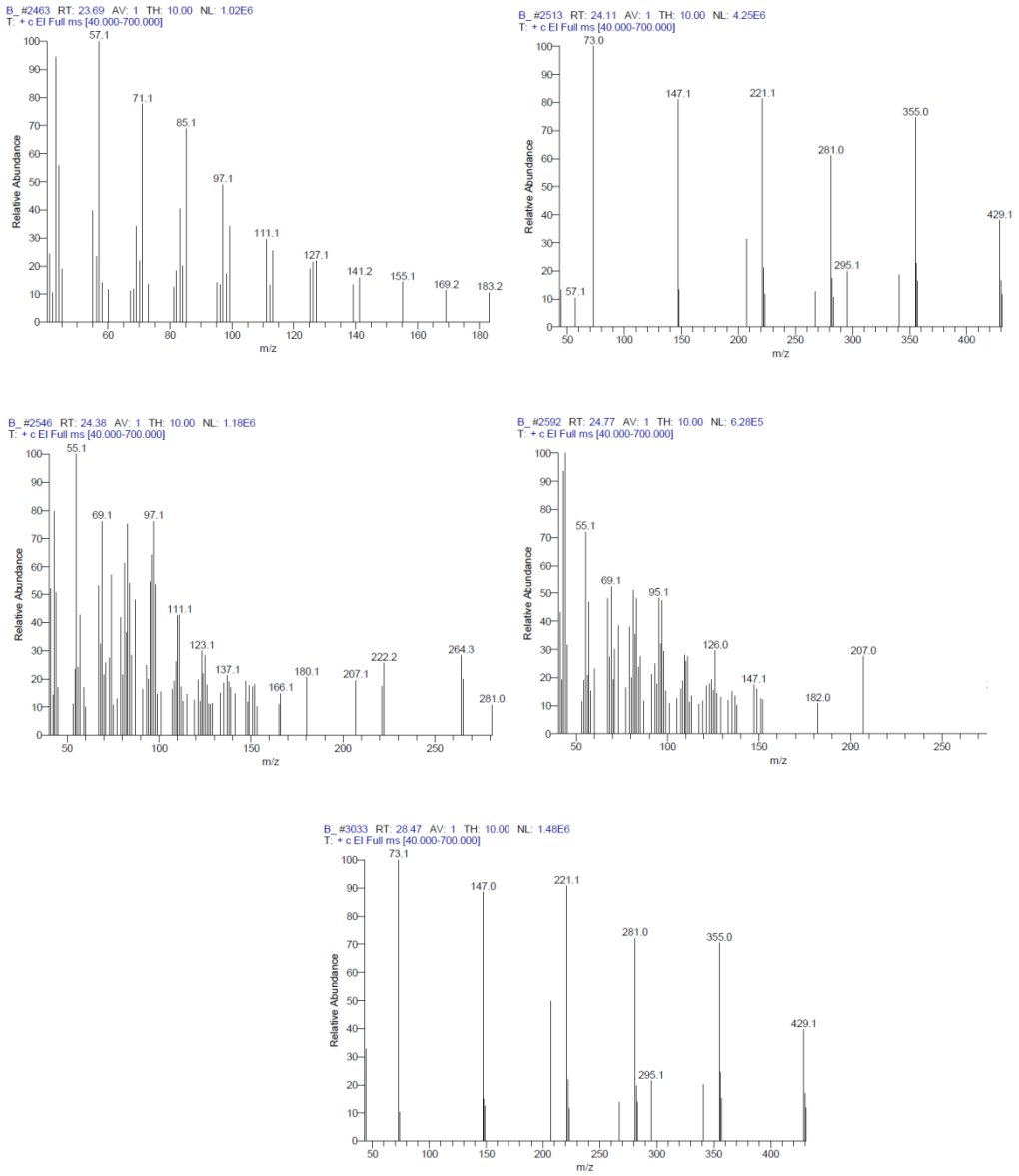


Figure 19. MS chromatogram of *Phyllanthus reticulates* fruit extract.

Table 84. Compounds identified in the GC-MS analysis of *Averrhoa bilimbi* flower extract.

Sr. No.	RT	Compound name	Class of compounds	Molecular formula	Peak area (%)
1	3.40	Formic acid, propyl ester	Carboxylic acid	$C_4H_8O_2$	0.17
2	4.35	Butanoic acid, 3methyl 2 [(phenylmethoxy)imino],	Carboxylic acid	$C_{15}H_{23}NO_3Si$	2.51

		trimethylsilyl ester			
3	5.38	2Pentanone,4 hydroxy 4methyl	Ketone	$C_6H_{12}O_2$	1.37
4	5.75	2Pentanone,4 hydroxy 4methyl	Ketone	$C_6H_{12}O_2$	4.80
5	6.07	2Pentanone,4 hydroxy 4methyl	Ketone	$C_6H_{12}O_2$	0.22
6	6.19	2Pentanone,4 hydroxy 4methyl	Ketone	$C_6H_{12}O_2$	0.29
7	6.37	2Pentanone,4 hydroxy 4methyl	Ketone	$C_6H_{12}O_2$	0.23
8	6.75	2Pentanone,4 hydroxy 4methyl	Ketone	$C_6H_{12}O_2$	2.33
9	9.78	Benzoic acid, 3 methyl 2 trimethylsilyloxy, trimethylsilyl ester	Carboxylic acid	$C_{14}H_{24}O_3Si_2$	3.85
10	12.73	Benzoic acid, 2,6 bis[(trimethylsilyl)oxy], trimethylsilyl ester	Carboxylic acid	$C_{16}H_{30}O_4Si_3$	4.28
11	15.25	Cyclohexasiloxane, dodecamethyl		$C_{12}H_{36}O_6Si_6$	6.53
12	16.14	Dodecanedioic acid, bis (tertbutyldimethylsilyl) ester	Carboxylic acid	$C_{24}H_{50}O_4Si_2$	0.87
13	17.54	Cycloheptasiloxane, tetradecamethyl		$C_{14}H_{42}O_7Si_7$	13.90
14	17.99	Phenol, 3,5 bis(1,1dimethylethyl)	Phenol	$C_{14}H_{22}O$	0.63
15	18.36	Nonadecan-1-ol trimethylsilyl ether	Fatty alcohol	$C_{22}H_{48}OSi$	0.49
16	19.56	Phenethylamine, N methyl- a,3,4tris(Trimethylsiloxy)	-	$C_{18}H_{37}NO_3Si_3$	13.67
17	20.22	1[2,4Bis(trimethylsiloxy)phenyl] 2 [(4trimethylsiloxy)phenyl]propan-1-one	Ketone	$C_{24}H_{38}O_4Si_3$	0.50
18	20.56	Hexasiloxane, tetradecamethyl	Organosiloxane	$C_{14}H_{42}O_5Si_6$	0.42
19	21.29	Cyclononasiloxane, octadecamethyl	-	$C_{18}H_{54}O_9Si_9$	9.26

20	21.90	1,1,1,3,5,7,7,7 Octamethyl 3,5 bis(trimethylsiloxy)tetrasiloxane	-	$C_{14}H_{42}O_5Si_6$	0.61
21	22.02	Phthalic acid, 4 bromophenyl octyl ester	-	$C_{22}H_{25}BrO_4$	0.25
22	22.83	1,1,3,3,5,5,7,7,9,9,11,11,13, 13,15,15 hexadecamethyl	-	$C_{16}H_{50}O_7Si_8$	6.82
23	22.99	Benzoic acid, 2 (dimethylamino) ethyl ester	Carboxylic acid	$C_{11}H_{15}NO_2$	4.00
24	23.43	Tert Butyldimethylsilyl methyl phthalate	-	$C_{15}H_{22}O_4Si$	1.28
25	24.22	Benzoic acid, 2,4 bis [(trimethylsilyl)oxy], trimethylsilyl ester	Carboxylic acid	$C_{16}H_{30}O_4Si_3$	6.77
26	24.82	Trimethylsilyl 3 methyl 4[(trimethylsilyl)oxy]benzoate	-	$C_{14}H_{24}O_3Si_2$	0.38
27	25.13	Mercaptoacetic acid, bis(trimethylsilyl)	Carboxylic acid	$C_8H_{20}O_2SSi_2$	0.14
28	25.53	Heptasiloxane, hexadecamethyl	Organosiloxane	$C_{16}H_{48}O_6Si_7$	4.97
29	26.25	Trimethylsilyl 3 methyl 4[(trimethylsilyl)oxy] benzoate	-	$C_{14}H_{24}O_3Si_2$	0.20
30	27.04	Heptasiloxane, hexadecamethyl	Organosiloxane	$C_{16}H_{48}O_6Si_7$	4.01
31	29.05	Heptasiloxane, hexadecamethyl	Organosiloxane	$C_{16}H_{48}O_6Si_7$	4.28

The GC-MS analysis of the methanolic flower extract of *Averrhoa bilimbi* revealed with 31 compounds. The compounds identified along with the peak area percentage are given (Table 84). The major components identified were Cycloheptasiloxane, tetradecamethyl (13.90%), Phenethylamine, N methyl-a, 3, 4 tris (Trimethylsiloxy) (13.67%), Cyclononasiloxane, octadecamethyl (9.26%), 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 hexadecamethyl (6.82%), Benzoic acid, 2, 4 bis [(trimethylsilyl) oxy], trimethylsilyl ester (6.77%) and Cyclohexasiloxane, dodecamethyl

(6.53%). This were followed by Heptasiloxane, hexadecamethyl (4.97%), 2 Pentanone, 4 hydroxy 4 methyl (4.80%), Heptasiloxane, hexadecamethyl (4.28%),Benzoic acid, 2,6 bis [(trimethylsilyl)oxy], trimethylsilyl ester (4.28%), Heptasiloxane, hexadecamethyl (4.01%), Benzoic acid, 2 (dimethylamino) ethyl ester (4.00%), Benzoic acid, 3 methyl 2 trimethylsilyloxy, trimethylsilyl ester (3.85%), Butanoic acid, 3 methyl 2 [(phenylmethoxy)imino], trimethylsilyl ester (2.51%), 2 Pentanone,4 hydroxy 4 methyl (2.33%). The other compounds present are 2 Pentanone,4 hydroxy 4 methyl (1.37%), Tert butyldimethylsilyl methyl phthalate (1.28%), Dodecanedioic acid, bis (tertbutyldimethylsilyl) ester (0.87%), Phenol, 3,5 bis (1,1dimethylethyl) (0.63%), 1,1,1,3,5,7,7,7 Octamethyl 3,5 bis (trimethylsiloxy) tetrasiloxane (0.61%), 1[2,4Bis(trimethylsiloxy)phenyl] 2 [(4trimethylsiloxy)phenyl]propan-1-one (0.50%), Nonadecan-1-ol trimethylsilyl ether (0.49%), Hexasiloxane, tetradecamethyl (0.42%), Trimethylsilyl 3 methyl 4[(trimethylsilyl)oxy]benzoate (0.38%), 2Pentanone,4 hydroxy 4methyl (0.29%), Phthalic acid, 4 bromophenyloctyl ester (0.25%), 2Pentanone,4 hydroxy 4methyl (0.23%), 2 Pentanone,4 hydroxy 4 methyl (0.22%), Trimethylsilyl 3 methyl 4[(trimethylsilyl)oxy] benzoate (0.20%), Formic acid, propyl ester (0.17%) and Mercaptoacetic acid, bis (trimethylsilyl) (0.14%) (Figure 20-21).

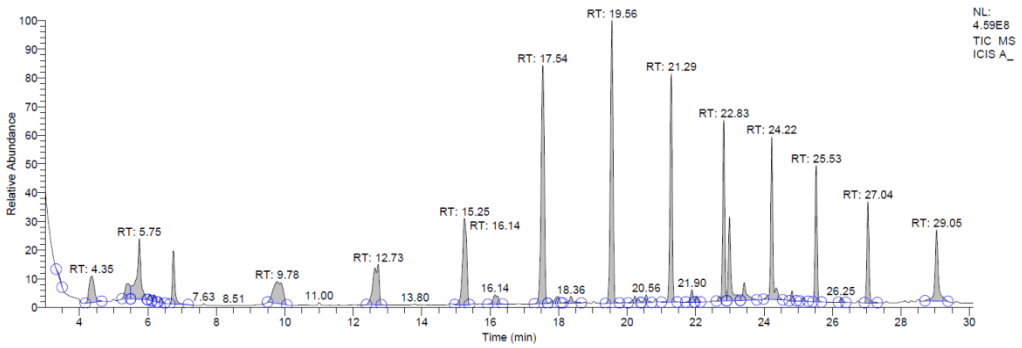
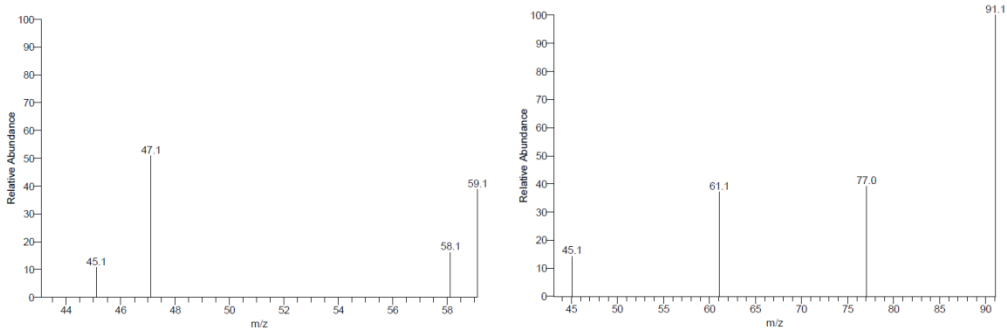
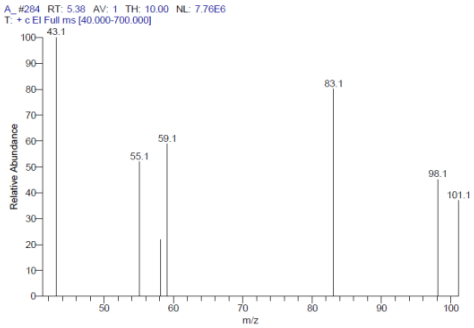
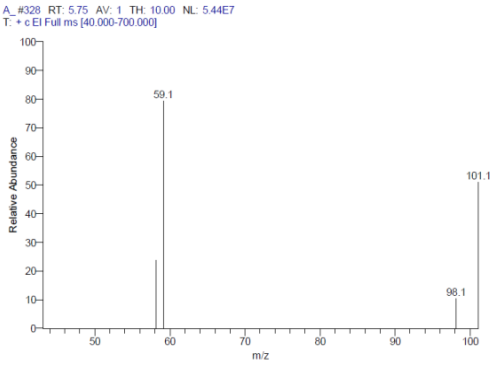
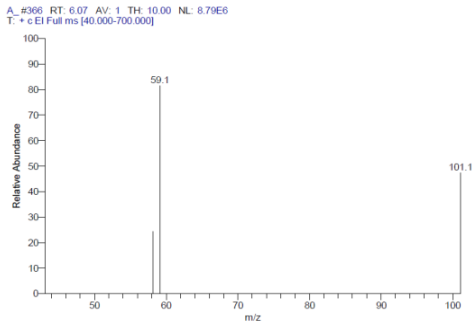


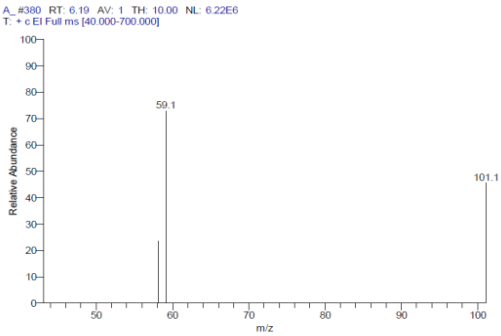
Figure 20. GC chromatogram of *Averrhoa bilimbi* flower extract.

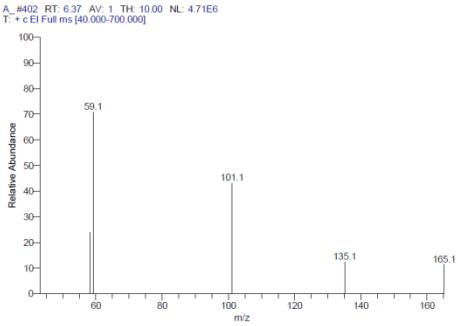



A_#284 RT: 5.38 AV: 1 TH: 10.00 NL: 7.76E6
T: + c EI Full ms [40.000-700.000]
43.1
83.1
59.1
55.1
98.1
101.1
Relative Abundance
m/z


A_#328 RT: 5.75 AV: 1 TH: 10.00 NL: 5.44E7
T: + c EI Full ms [40.000-700.000]
59.1
101.1
98.1
Relative Abundance
m/z


A_#366 RT: 6.07 AV: 1 TH: 10.00 NL: 8.79E6
T: + c EI Full ms [40.000-700.000]
59.1
101.1
Relative Abundance
m/z


A_#380 RT: 6.19 AV: 1 TH: 10.00 NL: 6.22E6
T: + c EI Full ms [40.000-700.000]
59.1
101.1
Relative Abundance
m/z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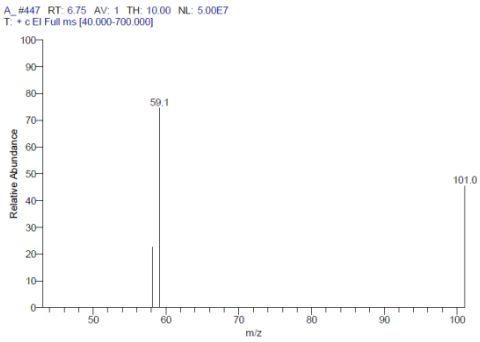
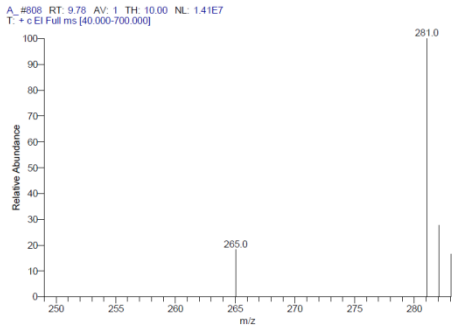
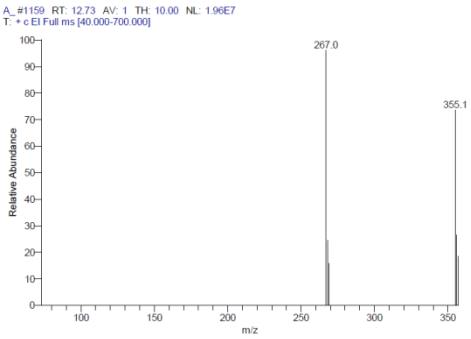
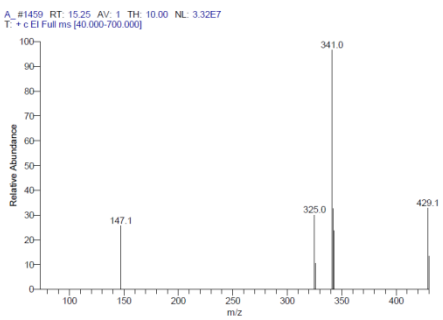
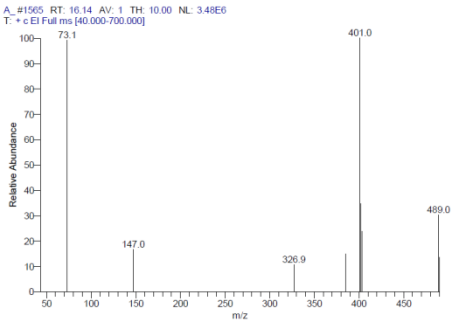
59.1
101.1
135.1
165.1
Relative Abundance
m/z

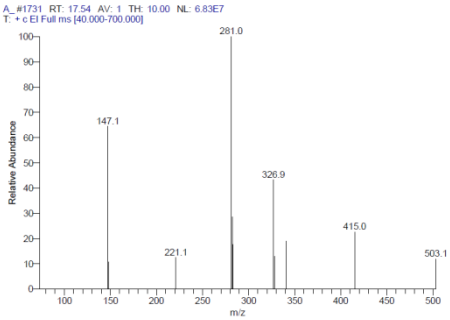

A_#447 RT: 6.75 AV: 1 TH: 10.00 NL: 5.00E7
T: + c EI Full ms [40.000-700.000]
59.1
101.0
Relative Abundance
m/z

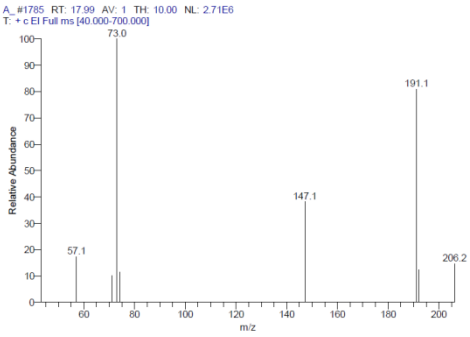

A_#808 RT: 9.78 AV: 1 TH: 10.00 NL: 1.41E7
T: + c EI Full ms [40.000-700.000]
281.0
265.0
Relative Abundance
m/z

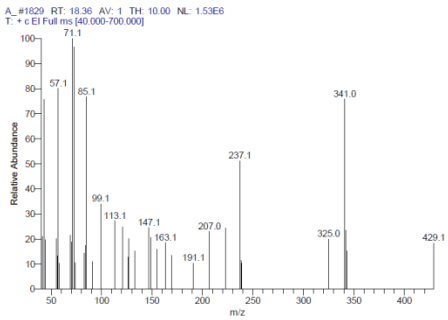

A_#1159 RT: 12.73 AV: 1 TH: 10.00 NL: 1.96E7
T: + c EI Full ms [40.000-700.000]
267.0
355.1
Relative Abundance
m/z

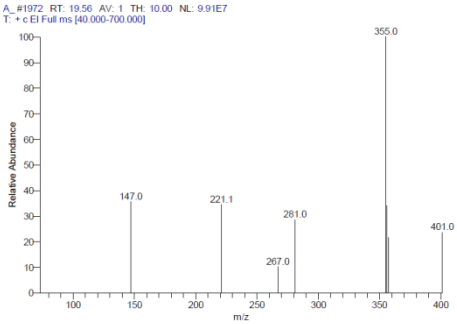

A_ #1459 RT: 15.25 AV: 1 TH: 10.00 NL: 3.32E7
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
341.0
325.0
429.1
147.1

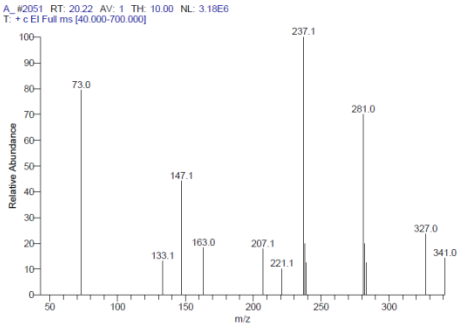

A_ #1565 RT: 16.14 AV: 1 TH: 10.00 NL: 3.48E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
73.1
401.0
489.0
147.0
326.9

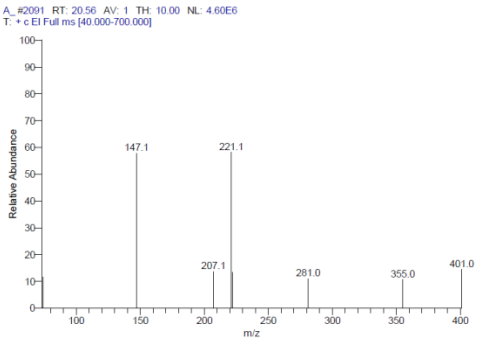

A_ #1731 RT: 17.54 AV: 1 TH: 10.00 NL: 6.83E7
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
281.0
147.1
326.9
415.0
221.1
503.1

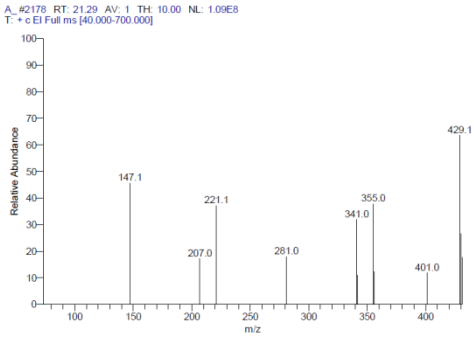

A_ #1785 RT: 17.99 AV: 1 TH: 10.00 NL: 2.71E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
73.0
191.1
147.1
57.1
206.2

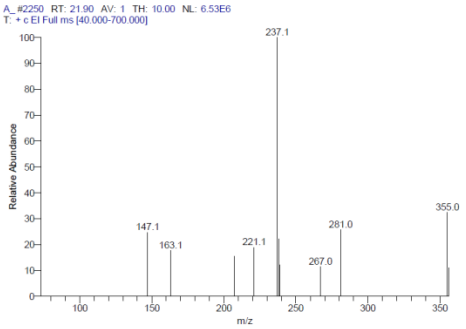

A_ #1829 RT: 18.36 AV: 1 TH: 10.00 NL: 1.53E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
71.1
57.1
85.1
341.0
237.1
99.1
113.1
147.1
207.0
163.1
191.1
325.0
429.1

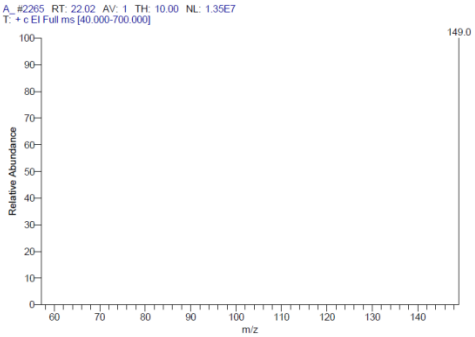

A_ #1972 RT: 19.56 AV: 1 TH: 10.00 NL: 9.91E7
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
355.0
147.0
221.1
281.0
401.0
267.0

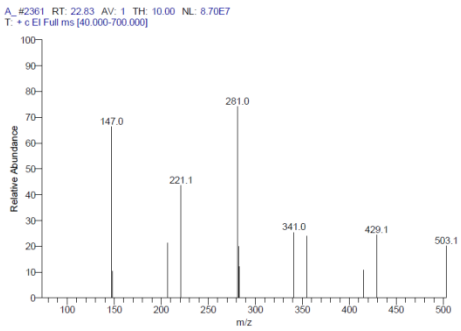

A_ #2051 RT: 20.22 AV: 1 TH: 10.00 NL: 3.18E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
237.1
73.0
281.0
147.1
327.0
163.0
207.1
341.0
133.1
221.1

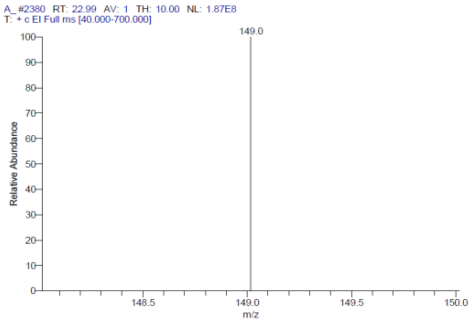

A_ #2091 RT: 20.56 AV: 1 TH: 10.00 NL: 4.60E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
147.1
221.1
207.1
281.0
355.0
401.0

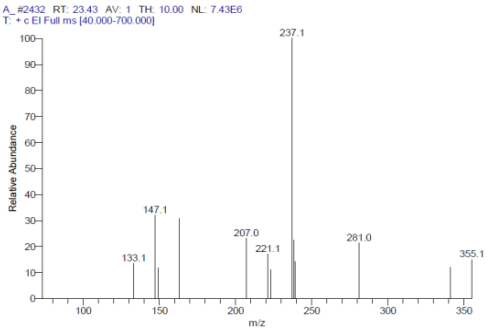

A_#2178 RT: 21.29 AV: 1 TH: 10.00 NL: 1.09E8
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
147.1
207.0
221.1
281.0
341.0
355.0
401.0
429.1

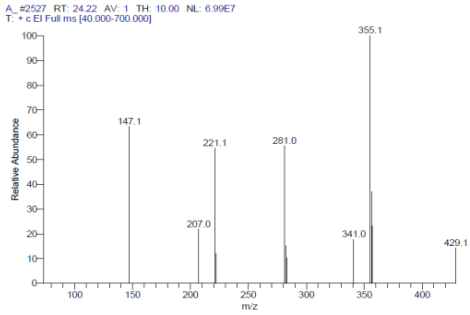

A_#2250 RT: 21.90 AV: 1 TH: 10.00 NL: 6.53E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
147.1
163.1
221.1
237.1
267.0
281.0
355.0

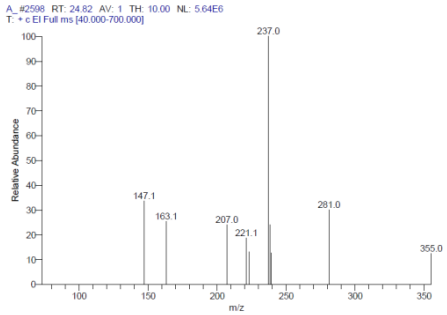

A_#2265 RT: 22.02 AV: 1 TH: 10.00 NL: 1.35E7
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
149.0


A_#2361 RT: 22.83 AV: 1 TH: 10.00 NL: 8.70E7
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
147.0
221.1
281.0
341.0
429.1
503.1


A_#2380 RT: 22.99 AV: 1 TH: 10.00 NL: 1.87E8
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
149.0


A_#2432 RT: 23.43 AV: 1 TH: 10.00 NL: 7.43E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
133.1
147.1
207.0
221.1
237.1
281.0
355.1


A_#2527 RT: 24.22 AV: 1 TH: 10.00 NL: 6.99E7
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
147.1
207.0
221.1
281.0
341.0
355.1
429.1


A_#2598 RT: 24.82 AV: 1 TH: 10.00 NL: 5.64E6
T: + c EI Full ms [40.000-700.000]
Relative Abundance
m/z
147.1
163.1
207.0
221.1
237.0
281.0
355.0

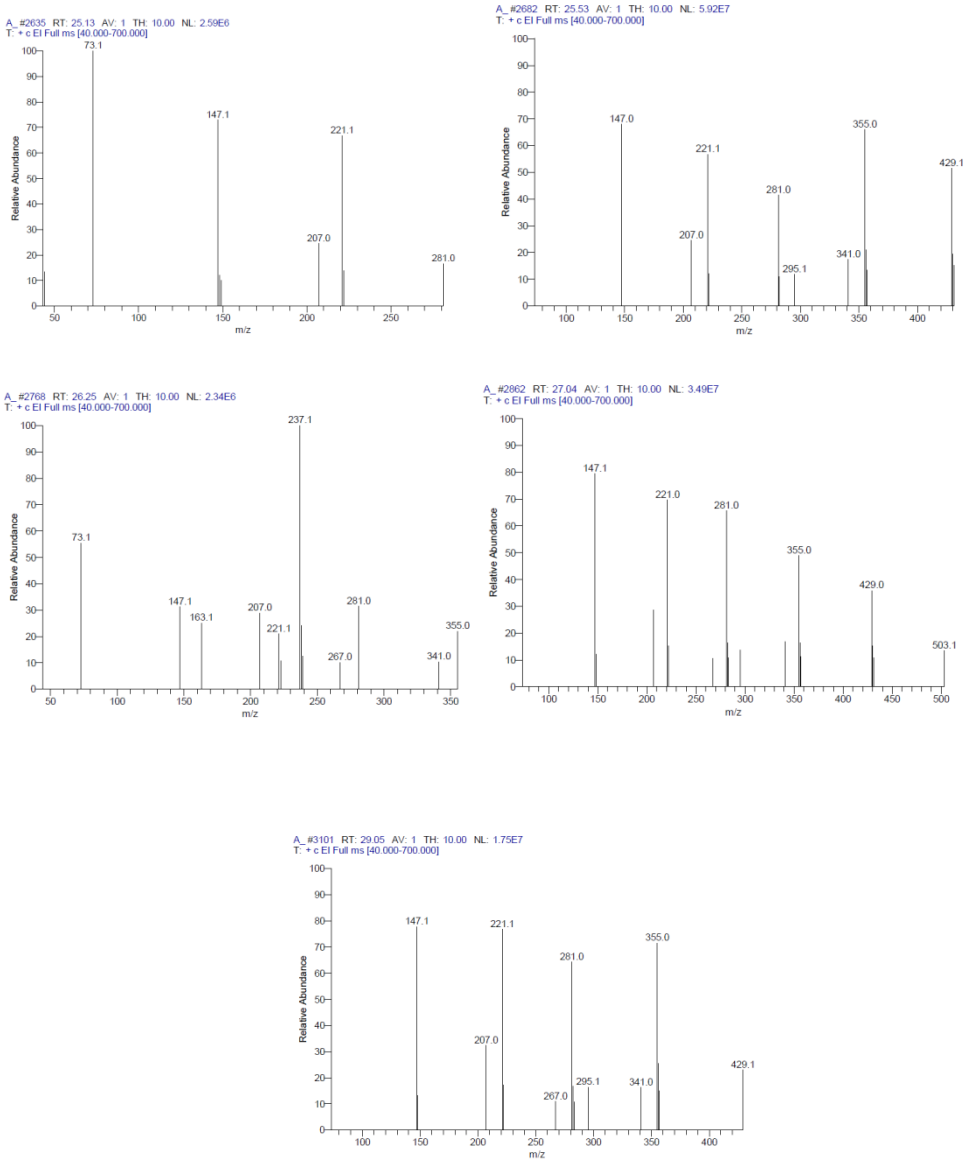


Figure 21. MS chromatogram of *Averrhoa bilimbi* flower extract.

5. DISCUSSION

5.1. Documentation of dye yielding plants

The present study reports 62 dye yielding plants belonging to 37 different families from the State of Goa. Siva (2007) reported 450 dye-yielding from India. Sharma *et al*., (2012) reported 46 dye yielding plants from Garhwal Himalayas. Sutradhar (2015) reported a checklist of 39 species of dye yielding plants belonging to 35 genera and 26 families along with their vernacular name, habit and parts used. At the same time Kikim *et al*. (2015) surveyed and documented 30 dye yielding plant species from different communities of Manipur, North Eastern India. Parisara and Kiran (2016) carried preliminary study on dye yielding plants of Bhadravathi Taluka of Karnataka and reported 29 dye yielding plants. The study was done by Kumari *et al*. (2018) wherein, they reported 51 dye yielding plants along the national highway from Parwanoo to Kaurik in Himachal Pradesh.

5.2. Optimization of extraction parameters for natural dyes

The intensity of colour increased as the material to liquor ratio (M:L ratio) was increased and reached maximum at (2g:10mL). This is because when the material to liquor ratio is less the molecules of dye collide with each other due to their congested nature thereby increasing the colour intensity. But the increase in the M:L ratio lead to no further increase in the colour intensity (Nagia and El Mohamedy, 2007). The intensity of colour increased as the dye extraction time was increased, and reached maximum at 300 min. This is because of increase in contact time between the dye and the solvent, the more dye dissolves in water due to hydrolysis but later the intensity of colour was decreased at 480 min this is because, all the dye component as almost dissolved in water and there is no further need to raise the time period of extraction (Nagia and El Mohamedy, 2007). With the increase in temperature, the colour intensity increased and reached maximum at 60°C for all the dye extracts, as the rise in temperature ruptures the cell wall and more dye component comes out into the extract. However, further increase in temperature at 80°C does not increase the intensity of colour. Similar studies were also carried out by Saravanan *et al.* (2013).

5.3. Physicochemical parameters of natural dyes

Physicochemical parameters are a vital parameter for identification and standardization of plant material for pharmaceutical purpose. Ash value is used to determine the identity and purity of plant material. The high total ash content was found in the bark of the plant species compared to the fruit pulp/rind and flowers. The high total ash value indicated the presence of inorganic constituents like silicate, oxalate, phosphate, carbonate, nitrates and chlorides, which is taken up from the soil (Kasthuri and Ramesh, 2018). The low ash content was in the fruit pulp, rind and flowers. This low ash content is attributed to greater fluid content of the fruit and flowers. Also, this may be because of the transport of minerals from roots to stems/bark and branches and finally to the flowers and fruit pulp/rind (Ranjith, 2018). The water-soluble ash gives an idea of number of inorganic compounds in these natural dye plants. Almost half of the total ash content is soluble in water for each plant species. The acid insoluble ash was a part of total ash that was insoluble in dilute HCl. The lowest acid insoluble ash value was seen in *M. malabathricum* which indicated the presence of negligible amount of siliceous matter. The fewer amounts of these three variables indicate fairly low content of inorganic matter and silica in the particular dye.

The soluble extractive value was higher in methanol, followed by water and it was least in petroleum ether. This is attributed to the polarity of different solvents. Also, this may be due to the presence of polar substances like phenols, tannins etc. in the methanol extract (Ranjith, 2018).

Fluorescence is an important phenomenon produced by various phytoconstituents present in the plant materials. Some of the substances may be converted into fluorescent derivatives by using different chemical reagents though they are not fluorescent. Hence, the crude drug powder is qualitatively assessed for fluorescence as it is an important parameter of pharmacognostical evaluation (Gupta, 2006).

5.4. Phytochemical analysis of natural dyes

The various dyes extracts revealed the presence of diverse phytoconstituents. The flavonoids were present in all the dye extracts. Tannins are present in most of the bark dye

extracts which produces brown colour shades to the fabric (Zin and Moe, 2008). The presence of saponins, alkaloids, steroids and terpenoids showed that plants are of pharmaceutical importance (Wanyama *et al*., 2011).

Most of the phytoconstituents were present in aqueous, methanol and ethanol extracts of the dyes. However, some of the phytoconstituents which were absent in aqueous extracts were present in methanol and ethanol extracts of the dyes. The phenols, flavonoids and tannins are polar in nature and thus are extracted in polar solvents (Ranjith, 2018). The chloroform, ethyl acetate and petroleum ether extract of dyes showed less phytoconstituents. Hence, methanol and ethanol extracts were considered to be the best solvents for extraction of phytoconstituents from the plants.

5.5. Staining of plant tissues with natural dyes under bright field microscopy

The dye extracts of *A. bilimbi*, *G. indica*, *M. malabathricum* and *P. reticulates* stained the vascular tissues reddish-pink in colour which was similar to the well-known synthetic dye safranin. The flowers of *A. bilimbi* contain cyanidin-3-O-beta-D-glucoside (Jusayan, 2015). Likewise, as reported in literature the fruits of *G. indica* are rich source of cyanidin-3-glucoside and cyanidin-3-sambubioside (Nayak *et al*., 2010). Also, the fruit pulp of *M. malabathricum* is known to contains cyanidin-3-glucoside and cyanidin-3, 5, diglucoside (Janna *et al*., 2006, Koay, 2008, Aishah *et al*., 2013). Cyanidin-3-glucoside is the most common anthocyanin found in the plants (Khoo *et al*., 2017). As cyanidin is magenda in colour the plant tissue took the reddish-pink stain. However, the reddish colour was more enhanced with the use of the mordants ($K_2Cr_2O_7$, $FeSO_4$, and $CuSO_4$) due to the special chelation of cyanidin with the metal salts (Wang *et al*., 2014). *Phyllanthus* species contains chemical constituents such as tannins, phenylpropanoids, terpenoids, phenolic compounds, flavonoids, alkaloids, saponins etc. (Nisar *et al*., 2018).

The anthocyanins are said to be well-known alternatives to synthetic dyes (Espin *et al*., 2000). Hence, these anthocyanins along with various other chemical compounds present in the plant extracts may be responsible for the reddish colouration. The anthocyanins are water soluble coloured pigments belonging to the phenolic group. Their water-soluble nature and their attractive orange, red and purple shades render them as a

natural colourant (Barczak, 2005). The pelargonidin (red), cyanidin and peonidin (magenda), delphinidin, petunidin and malvidin (purple) are some of the commonly distributed anthocyanins in plants (Khoo *et al*., 2017). Certain factors of dyes are taken into consideration for staining specific tissues and cell types. One of the factors is the pH of the dye. The acidic structures are stained by basic dyes whereas the basic structures are stained by acidic dyes (Avwioro, 2005). Anthocyanins are pH sensitive. They appear purple in neutral pH, blue in alkaline pH and red in acidic pH (Mazza and Miniati 1993). They are more stable in acidic medium compared to alkaline solutions (Rein, 2005). All the natural extracts *A. bilimbi*, *G. indica*, *M. malabathricum* and *P. reticulates* were acidic. Hence, all the extracts imparted reddish colouration to the plant tissues.

The stem bark of *Cassia fistula* contains anthraquinones, flavonoids, flavon-3-ol derivatives, alkaloids, glycosides, tannins, saponins, terpenoids and steroids (Ali, 2004). The main colouring component of *Cassia fistula* bark is 1, 8-di-hydroxi-6-methoxi-3-methyl anthraquinone (Ledwani and Singh, 2005). The stem bark extract of *Peltophorum pterocarpum* contains many chemicals along with tannins i.e. gallotannins (Jain *et al*., 2012) which could be an important component for staining purpose. Around 11-21% of tannin has been reported by Devi and Battu (2018) which is a pro-anthocyanidin type. The extract of *Mammea suriga* bark revealed the presence of flavonoids, phenols and terpenoids (Shastri *et al*., 2017). The yellow colour staining of plant tissues may probably be due to the presence of flavonoids in the extract. The yellow coloured (nyctanthin) is the colouring component isolated from the orange calyx of the *Nycthanthes arbor-tristis* flower (Smitha *et al*., 2014) which is responsible for bright yellow colouration of vascular tissues. The extract of mangrove barks such as *Rhizophora mucronata* and *Ceriops tagal* stained the plant tissue brown-yellow in colour due to the presence of high amounts of tannins in the extract (Duke and Allen, 2006 and Chapman, 1970). The extracts of the bark also contain flavonoids and phenolic compounds which may be responsible for yellow colouration of the tissues. Tannins are known to have large molecular mass and are water soluble phenolic compounds, which undergo some phenolic reactions i.e. chelation of metal ions and thus the tannin containing dye when used with the mordants are known to enhance the staining effect (Mongkholrattansit *et al*., 2013).

Studies have been carried out on the use of natural dyes in biological staining to understand the staining effect on both plant and animal tissues. The yellow dye extracted from the wood of *Berberis vulgaris* using water and ethanol as a solvent, was used to stain both dicot and monocot stem sections. The dye extracted in ethanol was found more effective in staining lignified tissues of plants than the dye extracted in water (Ullah *et al*., 2010). The natural dye lawsone (2-hydroxyl-1,4-naphthoquinone) was isolated from dry leaves of *Lawsonia inermis* using various solvents like clove oil, ethyl alcohol and water studied on its staining ability on stem tissue. The variations in staining property were observed using different solvents (Jan *et al*., 2011). The potential of phyto-dyes from *Enantia cholorantha*, *Harungana madagascariensis*, *Hisbiscus sabdariffa*, *Sarcocephalus latifolius*, *Sphenocentrum jollyanum* and *Sorghum bicolour* were examined to stain the wood sections and macerates. All the dyes except *H. sabdariffa* were successful in staining the wood fibres and lignified tissues (Akinloye and Illoh, 2018). The aqueous and methanol extract from *Khaya senegalensis* wood dust was used for staining rat tissue sections viz. liver, heart, intestine, lungs and kidney. All the tissues were stained red and had improved affinity for cellular cytoplasm thus giving a good contrast when compared with Hematoxylin and Eosin stained sections (Azubuike *et al*., 2018). Similar kind of study was done on rat tissues (Kusculu and Aslan, 2019). They stained the tissues with dry flower extract of Pomogranate. Differential staining was observed in nuclei and cytoplasms of all cells and epithelium cells.

5. 6. Fluorescence study of plant tissues

The unstained section of monocot and dicot stem showed auto-fluorescence, this may be due to chlorophyll or lignin content. The chlorophyll is known for its auto-fluorescence property (Papageorgiou and Govindjee, 2004).

The section stained with acridine orange dye showed green-red fluorescence in different excitation filters. The acridine orange is green-emissive dye used for cellular staining due to its good cell permeability and its capability to bind nucleic acids through electrostatic interaction. It is a selective dye that emits green light if linked to double stranded DNA (520 nm) and emits red light if linked to single stranded DNA (650 nm)

(Oliveira *et al*., 2018). It is a fluorescent dye widely used for counting the number of both living and dead bacterial cells (Natarajan, 2018).

Safranin is the universal dye that stains the lignified tissues especially xylem (Bond, 2008). Eventhough safranin is not a fluorescent dye, safranin stained sections were examined under fluorescent microscopy. In different excitation filters, safranin showed red fluorescence of vascular tissues. The tissues with high lignin content fluoresced red/orange whereas the tissues with low lignin content fluoresced green/yellow. Since, xylem is highly lignified tissue it fluoresced red in colour. Thus, the fluorescence nature of safranin provides a useful way to differentiate lignin and cellulose rich cell walls without counterstaining as required for bright-field microscopy.

The fluorescence observed in different cell components of tissues under various excitation filters is due to the colouring components of the dye extracts. The coloured pigments present are anthocyanins, flavonoids, carotenoids and phenolic compounds that fluoresce differently under various excitation filters (Parkinson and Brown, 1981). The carotenoids showed highest fluorescence peak with orange fluorescence from 350-500 nm range (Kleinegrin *et al*., 2010). This co-relates with staining of plant tissue with the carotenoids (nyctanthin) from *N. arbor-tristis* dye.

The other dyes such as *G. indica*, *M. malabathricum*, *A. bilimbi* and *P. reticulates* stained the vascular tissues red due to the anthocyanin pigment under bright-field microscopy. Anthocyanins interact with other flavonoids and related compounds to produce an increase in colour intensity and a shift in the wavelength of maximum absorbance towards higher wavelengths (Yoshida *et al*., 2009). In aqueous solution, anthocyanins exist in various structural forms depending on the pH of the medium, but at acidic pH it exists only as the flavylium ion. The flavylium ion of compound shows weak fluorescence at 520 nm (Drabent *et al*., 2007). Hence, most of the dyes being acidic in nature did not fluoresce under green excitation filter. There is scarce literature on the fluorescence study of anthocyanins. The study on red cabbage has shown that the cyanidin derivatives present in the extract fluoresce and the emission is pH dependent (Drabent *et al*., 1999).

5.7. Optimization of dyeing conditions

The temperature and time of dyeing was optimized using varying concentration. It was noted as we increase the dyeing time the fabric absorbs more dye but as we further increase the dyeing time the colour of the fabric did not increase. This could be due to the shift in equilibrium of colourant from fabric to dye (Farooq *et al*., 2013). Likewise, the increase in dyeing temperature leads to swelling of the fibres and de-aggregation of dye at higher temperature thus leading in more dye uptake. In case of material to liquor ratio (M:L ratio), the colour increases with increase in M:L ratio this is because as we increase the volume of extract, with which fabric is to be dyed, more dye molecules approaches the fabric, resulting in higher dye uptake of the fabric (Farooq *et al.*, 2013).

5.7.1. Dyeing of silk and cotton with natural dye extracts

The different shades ranging from light to dark brown, black, grey, creamish was observed on both cotton and silk fabrics dyed with *Mammea suriga* and *Ceriops tagal* dye. The chemical mordants are used for producing various shades of colour to the fabric, with single dye. Also, both dye extracts contain high amount of tannins which is responsible for brown and black colour shades (Kulkarni *et al*., 2011, Duke and Allen, 2006). The potassium dichromate produces deeper shades of colour to the fabrics and is known as brightening mordant (Mohammad *et al*., 2016). It is generally used to acquire reddish shades (Ozlenen and Leyla, 2012). The use of ferrous sulphate mordant produces black shades of colour in all the mordanting methods in both the dyes because of the strong co-ordination tendency of Fe ions that enhances the interaction between the fibre and the dye, resulting in higher dye uptake (Jothi, 2008).

5.7.2. Fastness tests of dyed fabrics

The fastness tests were better using the mordants as the wash fastness influenced with the rate of diffusion of dye from the fabric. The individual dyeing with the natural dyes showed excellent ratings for wash fastness in silk fabrics. This is due to the affinity of colouring components through H-bonding and Vander Waals forces. But dyeing the fabric with the combination of mordants has an ability to insolubilize the dye, thereby making it colour fast (Jothi, 2008). Good light and rub fastness property are due to the formation of

metal-dye complex which protects the chromophore from photolytic degradation. The ferrous sulphate mordant retained its original colour onto the fabrics even after wash, rub and light fastness tests due to its characteristic dyeing properties (Ozlenen and Leyla, 2012).

5.8. Characterization of chemical constituents

5.8.1. Spectroscopic analysis

Three methanolic extracts of *A. bilimbi*, *M. malabathricum* and *P. reticulates* was used for spectroscopy as anthocyanins are polar molecules and can be easily extracted in ethanol, methanol or acetone (Kahkonen, 2001). At pH equal or below 2, anthocyanin solutions show characteristic peak of absorption, one in the ultraviolet region (approximately 260-280 nm) and two in the visible region (approximately 415 and 490-540 nm). All the spectra of the extracted dyes were in the visible range (457-664 nm). This is because the maximum absorption of total anthocyanins occurs in the visible range (520-540 nm) (Harborne, 1958). The solvents used for spectral determination may slightly affect the position of the absorption bands. Also, the wavelengths of these absorbance peaks can differ slightly by a few nanometres among various anthocyanins, depending on the structure of each anthocyanin (Harborne, 1958).

5.8.2. Thin Layer Chromatography (TLC) analysis

Since the anthocyanins are coloured compounds it is possible to visually detect them without any chemical treatment. The different colours of anthocyanin pigments reflect the nature of their hydroxylation and methoxylation pattern. An increase in hydroxylation is accompanied by an increase in blue colour while methoxylation enhances the red colour (Jackman and Smith, 1996). The single red band (Rf = 0.32 in n-butanol: acetic acid: water) and (Rf = 0.23 in HCl: formic acid: water) is observed in the paper chromatogram which indicates the cyanidin band (Harborne, 1988, 1967, 1989; Jackman and Smith, 1996; Rice *et al*., 1986; Price *et al*., 1999; Rooyen and Bower, 2003; Galal *et al*., 2015). No such TLC study report a good separation of anthocyanidins because of the substituents of A-ring (R3, R5, R6 and R7) (Filip *et al*., 2012). Thus, from the above references mentioned, it was concluded that the red colour

spot in *A. bilimbi* (Rf = 0.35) and *M. malabathricum* (Rf = 0.32) extract was possibly due to the presence of cyanidin which corresponds to the protonatted flavilium ion of cyanidin (Harborne, 1988).

5.8.3. FT-IR analysis

The infra red spectra of unsaturated bond of unknown samples determine the chromophore in the active compound (Patel and Agarwal, 2001). It allows the analysis of a relevant amount of compositional and structural information in plants (Kogel-Knaber, 2000). The peaks between 3570-3200 cm^{-1} represent the hydroxyl group, H-bonded OH stretch. The peaks between 2936-2916 cm^{-1} belongs to the CH stretching vibrations of CH_2. The peaks from 2970-2950 cm^{-1} /2880-2860 cm^{-1} attributes to methyl C-H stretch. Whereas, the peaks from 2140-2100 cm^{-1} is due to the C=C stretching vibrations. The peaks between 1900-1700 cm^{-1} belongs to non-conjugated C=O stretching vibrations and peaks from 1750-1590 cm^{-1} represents conjugated C=O stretching vibrations. The peaks from 1405-1465 cm^{-1} indicates the CH bending vibrations of CH_2 and the peaks from 1395-1355, 1470-1430 cm^{-1} is due to CH bending vibrations of CH_3 which is related to flavonoids and aromatic rings. The peaks between 1060-990 cm^{-1} indicates aromatic C-H bonds (Coates, 2000; Oliveira *et al*., 2016). Since, the peaks in all the natural dye extracts were related to following group of compounds it indicated the presence of phenolic compounds and flavonoids in all the dye extracts.

5.8.4. GC-MS analysis

The GC chromatogram of the methanolic fruit extract of *Melastoma malabathricum* showed 24 peaks indicating the presence of 24 compounds. The extract contains compound 2 Pentanone, 4 hydroxy 4 methyl also called as diacetone alcohol which is used in paints and coatings (coil coatings, wood varnishes, architectural coatings) (Pillai and Ravirajan, 2018). The phenol derivatives like Phenol, 2, 4 bis (1, 1 dimethylethyl) was present in the extract which was also known to be present in the *Halimeda* species (Pillai and Ravirajan, 2018). The *Tectona grandis* methanolic leaf extract contains Decanoic acid methyl ester, Methyl tetra decanoate, Hexadecanoic acid methyl ester and 9-octadecanoic acid which consists of chromophore cluster, that brings colour to the substance (Qadariyah *et al*., 2018). These compounds were also present in the *Melastoma malabathricum* fruit

extract. These compounds are fatty acids and are known to possess antioxidant activity and acts as pesticide and lubricant (Hussain and Ignatius, 2010). The n- Hexadecanoic acid also called as palmitic acid is used to produce soaps and cosmetics (Benoit *et al*., 2009). The extract also contains 1 Decanol, 2 octyl which are fatty alcohols occurring naturally in plants and have antibacterial activity (Mukherjee *et al*., 2013) and antifouling activities (Kang *et al*., 2016). Eicosane is used in cosmetics and petrochemical industries. Butyl tetradecenoate is used in cosmetics (Liebert, 1990). The leaf of *Melastoma malabathricum* revealed with 16 compounds (Diris *et al*., 2017).

The GC chromatogram of the methanolic fruit extract of *Phyllanthus reticulates* plant showed the fraction of flavonoid 2, 3-Dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one which is known to possess antimicrobial and anti-inflammatory activities. It was reported in the ethanolic fruit extract of *Cucumis sativus* (Gopalakrishnan and Kalaiarasi, 2013). The extract also showed the presence of chromophore containing compounds such as 6-Octadecenoic acid, methyl ester (Z) and Hexadecanoic acid, 15 methyl, methyl ester. The octadecanoic acid has potential antibacterial and antifungal activity and pthalic acid is used in the form of aldehyde to produce chemicals like dyes, perfumes and many other useful products. The 84 chemical compounds were identified in the essential oil of *P. reticulates* (Ogundajo *et al*., 2015). The GC-MS analysis of the *P. amaras* contains dodecanoic acid, propanoic acid, 2- (tricyclo [3, 3, 1, 13, 7] dec-2-ylidene)-tetradecanoic acid, neophytadiene, hexadecanoic acid, ethyl ester and octa-decatrienoic acid, (Z, Z, Z) (Kalaiselvi *et al*., 2018). Some of these compounds were also seen in the methanolic extract of *P. reticulates*.

The compound (2 Pentanone, 4 hydroxy 4 methyl) which belongs to the ketone group was present in major amount in the methanolic flower extract of *Averrhoa bilimbi*. It was known to find its application in paints and coatings. The pthalic acid was present which was used to produce chemicals like dyes, perfumes and many other useful products. Cyclohexasiloxane, dodecamethyl is used as an ingredient for cosmetic formulation and used as a conditioning agent. It is also found in paints, lacquers, varnishes and surface treatments (Venugopal *et al*., 2012; Johnson *et al*., 2011). The chemical compound Mercaptoacetic acid, bis (trimethylsilyl) is used in cosmetic preparations. Around 49

compounds were isolated from the fruit extract of *Averrhoa bilimbi* (Suluvoy and Grace, 2017).

CONCLUSION

The present study revealed 62 dye yielding plants belonging to 37 different families from the State of Goa, India. The study reveals the optimization of various parameters for extraction of natural dyes from various plant species. The physicochemical properties of the selected dye yielding plants were studied which led to standardization of plant material for pharmaceutical purpose. The high total ash content which indicated the presence of inorganic constituents was found in the bark of the plant species compared to the fruit and flowers. The phytochemical analysis of the selected dye species revealed the presence of flavonoids in all the plant species, tannins in the bark extracts and other constituents such as saponins, alkaloids, steroids and terpenoids in the water, ethanol and methanol extracts of the plants.

The natural dye extracts from *Averrhoa bilimbi*, *Garcinia indica*, *Phyllanthus reticulates* and *Melastoma malabathricum* were effective in staining the lignified tissues of plants, which could substitute the safranin stain and dyes from *Cassia fistula*, *Peltophorum pterocarpum*, *Mammea suriga*, *Nyctanthes arbor-tristis*, *Ceriops tagal* and *Rhizophora mucronata* stained the plant tissues yellow in colour which lead to the replacement of acridine orange dye. However, the use of mordants leads to increase in staining intensity of the dye. The unstained section of monocot and dicot stem showed auto-fluorescence in all the excitation filters of fluorescence microscope. But the dye extracts from *Averrhoa bilimbi*, *Garcinia indica*, *Melastoma malabathricum*, *Cassia fistula*, *Peltophorum pterocarpum* and *Nyctanthes arbor-tristis* showed good fluorescence of cell components under most of the excitation filters and hence these dyes could be used as fluorescent dyes.

A range of appreciable shades have been developed on silk and cotton fabrics by optimizing the dyeing parameters, using chemical mordants and different mordanting methods. The post-mordanting method gave darker shades compared to pre-mordanting and simultaneous mordanting methods, hence post-mordanting method of dyeing could be adopted for *Ceriops tagal* and *Mammea suriga* dye extracts. The silk fabrics dyed better than the cotton fabrics. The colourfastness of these dyes against light, wash and rub was

better with the mordants compared to the fabrics dyed without mordants. The ferrous sulphate mordant showed excellent ratings ie. (5/5) for all the fastness tests.

The naturally dyed silk and cotton fibres revealed no damage to the physical appearance of the undyed fibres under SEM analysis.

The chromatographic and spectroscopic compositional analysis of dyes indicated the presence of phenolic compounds as major colouring constituents in dye extracts of *Averrhoa bilimbi*, *Phyllanthus reticulates* and *Melastoma malabathricum*. In the FT-IR analysis the spectral peaks revealed the presence of phenolics, especially flavonoids and anthocyanins in the extracts.

The world-wide demand of natural dyes over the synthetic dyes has been increased due to the awareness regarding high risk impact of synthetic dyes. The potential of natural dyes as a source of biological stain was scarcely identified, thus the present study recognized the fact that the natural dye extracts from the selected plant species could be successfully utilized for plant histological evaluation. Also, different appreciable shades obtained with the use of natural dye along with the mordants on the textile fibres would definitely benefit both the producers and the consumers. This idea of natural dyeing could be adopted by the traditional artisans and small enterpreneurs which would in turn generate the employment opportunities among the rural people.

SUMMARY

The present study reported 62 dye yielding plants that belongs to 37 different families from Goa, India. The highest dye yielding plant species were reported in Fabaceae. The dye from the barks of the trees was extracted by boiling the powdered bark in the water bath for 4-5 hours at 60°C. The dye from the roots was extracted by chopping the roots into fine pieces and soaking it over-night in water. The dye from the fruits is extracted by crushing the fruits using mortar and pestle. The dye from leaves is obtained by cutting the leaves into fine pieces and boiling it in water bath for 4-5 hours. The dye from the flowers and flower stalks were extracted by cutting the material into fine pieces and grinding it in mortar and pestle.

The selected dyes were subjected to optimization parameters for dye extraction. The three parameters were taken into consideration, i.e. material to liquor ratio (MLR), dye extraction time and temperature. The parameters were finalized based on the highest value of optical density measured using UV-visible spectrophotometer for each dye extracts. Material to liquor ratio (2g/10 mL), extraction time (300 min) and extraction temperature (60°C) was found to be optimum for dye extraction.

The physicochemical properties were studied for all the selected dye extracts. The physical appearance (colour) of the dye, pH of the dye, water soluble extractive, methanol soluble extractive, total ash, water soluble ash, acid insoluble ash and fluorescence analysis was carried out for all the dye extracts. The pH of all the dye extracts was found to be acidic which ranged from 2.57 to 6.10. The methanol soluble extractive was highest compared to the water and ether soluble extractive due to the polarity of the solvent. The highest ash value was seen in the bark extracts compared to the flower and fruit extracts, this is attributed to the direct transport of minerals from roots to stem/barks. The lowest amount of the total ash, water soluble ash and acid insoluble ash indicates the low contents of inorganic matter in the dye. The powder analysis plays an important role in the identification of crude drugs. Some of the reagents like 1N HCl, concentrated H_2SO_4, ethanol, methanol, 50% H_2SO_4, ammonia, acetone, chloroform, 2-propanol and picric acid showed fluorescence in the UV light.

The phytochemical tests were carried out for water, ethanol, methanol, chloroform, petroleum ether and ethyl acetate extracts of the plant species. The tests revealed the presence of various categories of chemical constituents in the plants using different solvents, which was observed after characteristic colour change. All the dye extracts showed the presence of phenols and flavonoids. However, tannins were present in the bark extracts. The dye extracts of chloroform, petroleum ether and ethyl acetate showed least number of chemical constituents whereas the solvents like water, ethanol and methanol showed maximum number of chemical constituents.

Fifteen minutes time duration was determined as the optimum staining time for the plant tissues with synthetic dyes. In mordant staining, 20 min was determined as the optimum staining time for staining with potassium dichromate and copper sulphate mordant. Whereas, for ferrous sulphate just 5 min was determined as optimum staining period, as the increase in staining time with this mordant lead to blackening of the section. The excellent staining of vascular tissues was noticed after 20 min of staining period for dyes like *Rhizophora mucronata*, *Ceriops tagal*, *Nyctanthes arbor-tristis*, *Mammea suriga*, *Cassia fistula* and *Peltophorum pterocarpum*. The clear differentiation of tissues was observed after 15 min of staining period in the dye extracts of *Averrhoa bilimbi*, *Garcinia indica*, *Phyllanthus reticulates* and *Melastoma malabathricum*.

For histochemical staining various plant sections of dicot and monocot stem were taken and stained with natural dyes, synthetic dyes and also by using the chemical mordants. The dye extracts from *Averrhoa bilimbi*, *Garcinia indica*, *Phyllanthus reticulates* and *Melastoma malabathricum* were successful in staining the plant cell components. These dyes could be used to substitute the synthetic safranin stain. Whereas, the dye extracts from *Rhizophora mucronata*, *Ceriops tagal*, *Nyctanthes arbor-tristis*, *Mammea suriga*, *Cassia fistula* and *Peltophorum pterocarpum* could be used to substitute the synthetic acridine orange dye. The mordants played an important role in enhancing the staining intensity of the natural dyes. The anthocyanins in the extracts were responsible for reddish colouration in vascular tissues and the flavonoids and tannins in the bark extract were known for yellow-brown colouration to the plant tissues.

The fluorescence study was carried out under fluorescence microscope by using five different excitation filters such as violet, blue, green, yellow and red. The autofluorescence was showed by the unstained monocot and dicot stem section but the use of natural dyes along with the mordants revealed distinct fluorescence in tissues examined under various filters. The organic dyes absorb the light in the visible region of the spectrum (λ=400-700 nm) which can be used as chromophores. The dyes which contain different auxochromes and functional groups such as amino, carbonyl, hydroxyl, carboxylic acid, sulphonic acid and nitro groups modify the ability of chromophore to absorb light. The violet excitation filter showed good fluorescence of tissues whereas the green excitation filter showed poor fluorescence with the use of most of the natural dyes.

The natural fibres like cotton and silk were dyed with the natural dyes, with and without mordants and also with the combination of mordants. Different mordanting methods such as pre-mordanting, simultaneous mordanting and post-mordanting were used for dyeing purpose. Various shades of colours were developed with the help of different chemical mordants like copper sulphate, ferrous sulphate and potassium dichromate using the bark extracts of *Ceriops tagal* and *Mammea suriga*. The dyeing conditions were standardized by using various dye concentration and varying the dyeing time. The obtained shades were described using ISCC-NBS colour system.

The different mordanting methods and mordants revealed diverse shades of colour on the silk fabrics such as moderate orange, brownish orange, greenish black, grayish olive, dark grayish olive, moderate yellowish brown, strong brown, deep orange, bluish black, light yellowish brown, strong red, brownish black, moderate brown, olive gray and olive black shades on using *Ceriops tagal* dye. The shades of colour on the cotton fabrics were moderate orange, light brown, strong brown, brownish black, pale yellow, dark brown, dark grayish yellow, reddish black, light orange yellow, strong yellowish-brown, deep brown, dark grayish brown and moderate yellowish brown using *Ceriops tagal* dye.

The shades obtained with different dyeing methods and mordants on silk fabrics using *Mammea suriga* extract were strong brown, brownish orange, brownish black, deep orange, moderate orange yellow, deep brown and dark orange yellow. Whereas, on cotton fabrics were moderate orange, dark brown, deep orange, dark grayish, light brown, light

olive gray, grayish brown, light yellowish brown, brownish orange, deep brown, grayish yellowish brown and moderate brown. The silk fabrics were known to dye better then the cotton fabrics and the post-mordanting methods gave darker shades compared to the pre and simultaneous mordanting methods.

The three fastness tests such as wash, light and rub were carried out for all the dyed fabrics. The fastness ratings were given as per the gray scale. On application of *Ceriops tagal* dye with and without mordants on silk fabrics resulted in excellent to average wash, rub and light fastness (5-2/3), while the application of same colourant on cotton fabrics resulted in (5-3) ratings for fastness tests. The application of *Mammea suriga* on silk fabrics gave excellent to very poor (5-1) wash and light fastness and excellent to moderate rub fastness. Whereas, on cotton fabrics it gave excellent to very poor (5-1/2) light, wash and rub fastness. The ferrous sulphate mordant showed excellent fastness results for silk fabrics whereas for cotton fabrics the potassium dichromate gave brilliant shades.

The methanolic extracts of the three dyes *Averrhoa bilimbi*, *Phyllanthus reticulates* and *Melastoma malabathricum* showed the presence of anthocyanins in the dye extracts which was further confirmed from the Rf values of TLC plates. The FT-IR spectra showed the characteristic peaks indicating the presence of phenolics, flavonoids and anthocyanins in the extracts.

The current study has a potential to replace the synthetic stains in laboratory for general histochemical studies. The textile studies on natural dyes and optimization of dyeing parameters can be used by the textile dyers for dyeing and marketing of natural dyes from plants, which would also lead to the employment opportunities among rural people.

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PUBLICATIONS

PAPERS PUBLISHED

1. 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.

2. Verenkar, N.G.S. and Krishnan, S. 2017. **Some potential natural dye yielding plants from the State of Goa, India.** *Indian Journal of Natural Products and Resources*, 8(4): 306-315 (***Impact factor 0.5***).

3. 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 (***Impact factor 0.4***).

4. 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 (***Impact factor 2.1***).

PAPERS PRESENTED IN CONFERENCES

1. Verenkar, N.G.S. and Krishnan, S. 2015. **Natural dyes from selected plants for anatomical staining.** Abstract of the paper presented in the National Seminar of Advances in Life Sciences, at St. Xaviers College Mapusa-Goa, December, 7-8, 2015 (***Best Poster Award: First place***).

2. Verenkar, N.G.S. and Krishnan, S. 2016. **Natural dyes from *Garcinia indica* Choisy and *Bixa orellana* L. as potential biological and textile staining.** Abstract of the paper presented in the XXVI Annual Conference of Indian Association for Angiosperm Taxonomy (IAAT) and International Seminar on Conservation and Sustainable Utilization of Bio-Diversity at Shivaji University Kolhapur, November, 7-9, 2016.

3. Verenkar, N.G.S. and Krishnan, S. 2017. **Study on natural dye yielding properties and conservation of ornamental plant *Melastoma malabathricum***. Abstract of the paper

presented in the National Conference on Science and Technology: Reaching the Unreached, at Manglore University, September, 8-9, 2017.

4. Verenkar, N.G.S. and Krishnan, S. 2017. **Natural dyes from *Peltophorum pterocarpum* and *Nyctanthes arbor-tristis* as potential biological and textile stains.** Abstract of the paper presented in the National Conference of Young Researchers, on New Frontiers in Life Sciences and Environment, at Goa-University, March, 16-17, 2017 (***Best Poster Award: Second place***).

5. Verenkar, N.G.S. and Krishnan, S. 2018. **Phytochemical analysis of crude bark extracts from selected dye-yielding plant species.** Abstract of the paper presented in the 3rd Annual International Conference on Intellectual Property: Prosecution to Litigation, at Goa College of Pharmacy, November 15-16, 2018.

6. Verenkar, N.G.S. and Krishnan, S. 2020. **Phytochemical and physicochemical analysis of selected dye yielding plant species.** Abstract of the paper presented in New Vistas in Botany, at Goa-University, February 13-14, 2020.

CONFERENCES ATTENDED

1. Verenkar, N.G.S. 2015. **National Seminar on New Frontiers in Plant Sciences and Biotechnology** organized by UGC-SAP, Department of Botany, Goa-University, Goa, January 29-30, 2015.

2. Verenkar, N.G.S. 2015. **Asian Mycological Congress**, Department of Botany, Goa-University, Goa, October 7-10, 2015.

3. Verenkar, N.G.S. 2018. **Intellectual Property Rights (IPR) Awareness Programme** organized by ASSOCHAM, Goa-University and IP office, Goa-University, Goa, December 11, 2018.

4. Verenkar, N.G.S. 2019. **Novel Vistas in Plant Science,** Department of Botany, Goa-University, Goa, January 25, 2019.

VISITS

1. Visited Bio-dye India Pvt. Ltd., at Madkhol, Sawantwadi. At Bio-dye various fabrics made from cotton, flax, jute, silk, wool, linen etc. was dyed using only bio-degradable dyes like *Indigofera tinctoria*, *Punica granatum*, *Terminalia chebula* and *Syzygium cumini*. They have employed many local villagers for weaving the yarn into fabric, rugs and tapestries.

2. Visited Bhuipal, Sattari, Valpoi-Goa. The local resident from Bhuipal, Mr. Suryakant Shankar Gaonkar prepares eco-friendly holi colours by using cornflour, rice flour and maida. He sells his products at Bicholim and Valpoi market, and recommends people to use only natural colours in order to play safe holi.

3. Visited Indigenous Native Wisdom Fest at Saraya, Goa. They found some natural dye yielding materials which on mixing together gave several different colours. These dyes were used for tie and dye technique and block printing.