NATURAL DYE EXTRACTS OF ANSOSILA (*IMPATIENS TINCTORIA*) ROOTS FOR COLORATION OF COTTON FIBERS

Burka Ahmed Woya

A Thesis Submitted To Department of Applied Chemistry

School of Applied Natural Science

Presented In Partial Fulfillment of the Requirement for the Degree of Master of Science in Industrial Chemistry

Office of Graduate Studies

Adama Science and Technology University (ASTU)

June, 2023

Adama, Ethiopia

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Burka Ahmed Woya

Advisor: Gemechu Deressa Edossa (Associate Professor)
Co-advisor: Defaru Negera Duke (Assistant Professor)

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Declaration

I hereby declare that this master thesis entitled **"Natural Dye Extracts of Ansosila (*Impatiens tinctoria*) Roots for Coloration of Cotton Fibers"** is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

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We, the advisors of this thesis, hereby certify that we have read the revised version of the thesis entitled **"Natural Dye Extracts of Ansosila (*Impatiens tinctoria*) Roots for Coloration of Cotton Fibers"** prepared under our guidance by **BURKA AHMED** submitted in partial fulfillment of the requirements for the degree of Master of Science in Industrial Chemistry.

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_____	_____	_____
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We the advisors of the thesis entitled **"Natural Dye Extracts of Ansosila (*Impatiens tinctoria*) Roots for Coloration of Cotton Fibers"** and developed by **BURKA AHMED;** hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

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LIST OF ABBREVIATIONS

AASTU	Addis Ababa Science and Technology University
AATCC	American Association of Textile Chemists and Colorists
ASTU	Adama Science and Technology University
CI	Color Index
FT-IR	Fourier Transforms Infrared Spectroscopy
IR	Infrared Absorption Spectroscopy
ISO	International Organization for Standardization
K/S	Color Strength
MLR	Material-to-Liquid Ratio
POM	Post Mordanting
PRM	Pre Mordanting
I. tinctoria	*Impatiens tinctoria*
SEM	Scanning Electron Microscopy
SM	Simultaneous Mordanting
SoANS	School of Applied Natural Science
UV-Vis	Uv-Visible Spectroscopy
CIE	International Commission On Illumination
HTHP	High Temperature and High Pressure
w. o. f	Weight of fabric

ABSTRACT

Color is one of the most important factors in the attractiveness and marketability of textile products. Until recently, the majority of cotton garments were colored using synthetic dyes like reactive, acid, etc., which are typically not good to the environment. This study focuses on extracting natural dyes from Ansosila root and identifies eco-friendly methods for dyeing cotton fabrics. Aqueous extraction was used to extract natural coloring pigments. Impatiens tinctoria was used as a bio-based dye to color cotton garments. In order to improve the dyeing strength, dyeing experiments were conducted both chemical-free and employing salts of metals including iron, aluminium, and copper. The maximum absorption peak showed at 445nm. Functional groups that correspond to phenolic compounds associated with –OH (3390 cm^{-1}), C-O (1047 cm^{-1}), olefinic (alkene) associated with trans-C-H out-of-plane bend (959 cm^{-1}), aromatic ring (aryl) group related to C=C-C aromatic ring stretch (1598 cm^{-1}), primary and secondary amino group (1598 cm^{-1}), vinyl C-H in-plane bend vibration is attributed to the band at 1419 cm^{-1} and common inorganic sulfate ion (1116 cm^{-1}) were all able to be identified through FT-IR analysis. The color fastness qualities of extracted coloring compounds were used to evaluate their dyeing performance. The findings were good and the dye displayed a darker shades. The outcome also demonstrates that cotton fabrics colored with the same dye extract but with various types of mordants produced fabrics with a variety of colors. The mordanting mechanism of the extracted dye was also proposed. Fastness values ranging from good to excellent were noted (3-5). Fortunately, unmordanted fabric showed unusual very good fastness performances. Mordanting with $FeSO_4$ showed better fastness performances. All the fastness performance test results were in commercially acceptable range. Given that the dyes outstanding color fastness, it is recommended that natural dyes derived from Ansosila root can be used well as coloring agents for cotton fabrics.

***Key words**: Extraction, eco-friendly, natural dye, Impatiens tinctoria, cotton fibers, mordant*

CHAPTER ONE

1. INTRODUCTION

1.1 Background of the Study

The physical and psychological effects that a certain wavelength of light has on the brain when it reflects off an object with a specific light source present can be referred to as color. This means that the perception of color is the result of an observer's brain processing the wavelength of light that is reflected from an object when illuminated by a specific light source. A standardized scientific method of color theory, including a mathematical/empirical formula to arrange the incidence of light/standard illuminant for absorption and reflection of the color on and from the object, is used to detect and measure the color value's specific reflectance or other quantified values to record and communicate color information for reproducibility and matching (P. Samanta 2022).

The color appearances of textiles, leather, molded plastics, and other object are just as important as their physical characteristics. Prior to the creation of synthetic dyes, natural colors were obtained from sources such as plants, animals, soils, insects and minerals. A variety of pigments have been used to color human and animal flesh, hair, teeth, bones, fabrics, medicines, food, cosmetics, plastics, inks, photography, paper, and wood in a variety of hues (Berhanu and Ratnapandian 2017; Asim Kumar 2018).

Dyes for textiles are composed of complex synthetic organic compounds. Approximately 10 million tons of synthetic dyes are used each year, resulting in a large amount of hazardous waste and unfixed dyes produced during production and application, leading to serious health risks and impacting the environment (Teklemedhin 2018). Dyes made from toxic heavy metals like cadmium are a major issue for human health (Aneesiya K. Rajan and L. Cindrella 2018). They are common industrial environmental pollutants during their synthesis and later during dyeing (Asim Kumar 2018). Several classes of synthetic dyes can be applied to cotton fibers, including vat, sulfur, azo, direct and reactive dyes. The dyeing of cotton with these dyes generally uses large quantities of dyes and auxiliaries (A. Haji 2019).

Due to their specific advantages and limitations, natural dyeing of various textiles and leathers has continued mainly for specialty products, while synthetic dyes have been used on a large scale for general textiles (Khan, Hussain, and Jiang 2018). In an eco-friendly environment, it is very important to ensure that people live in a hygienic and fresh world. People's needs are not met solely by providing food, clothing and shelter, but it is important that the environment is clean and livable with clean, safe air and water. The large scale production and extensive application of synthetic dyes cause significant environmental pollution, thereby destroying an environmentally friendly atmosphere. Legislation on air and water pollution is becoming more stringent (Choudhury 2018).

The increased awareness for environmental friendliness and sustainability is bringing the momentum back towards natural dyes. Lately, most of the research work focuses on the production method and application of stable and environmentally friendly natural dyes (Aneesiya K. Rajan and L. Cindrella 2018). Natural dyes can dye wool and silk fibers in a simple procedure (Sutlović *et al*. 2020). Researchers are studying different methods and technologies for the preparation of cotton fibers with the aim of improving the dye-ability with natural dyes. Ultrasonic energy, enzymes, mineral mordants and biomordants have been used in several studies to improve the dye-ability of cotton fibers with various natural dyes (A. Haji 2019). Figure 1 shows the different types of dyes.

Figure 1: Different types of Dyes (Source: https://www.google.com/search?q=dyes)

Approximately 4×10^4 different synthetic dyes and pigments are used industrially, and about 4.5×10^4 tons of dyestuffs are produced worldwide (Pereira and Alves 2012). A recent resurgence of research and development in the production and application of natural dyes is observed due to the growing popularity of a more natural lifestyle based on naturally sustainable goods. A large number of plant and animal or insect sources have been identified for color extraction and their diversified uses in textile dyeing (A. K. Samanta, Awwad, and Algarni 2020) and functional finishing (Ibrahim, Eid, and Sharaf 2019), food coloring, cosmetics, dye-sensitive solar cells, histological staining, pH indicator and various other application disciplines(Güzel and Karadag 2021). In addition to above mentioned applications, there is a growing interest for using natural dyes to dye leather, to stain wood, pulp and some plastics, to add color to cosmetics, to dye hair (Shahid, Shahid-ul-Islam, and Mohammad 2013).

Azo dyes are the largest and more versatile class of dyes, accounting for up to 50% of the annual production. Dyes are extensively used in many fields of up-to-date technology, e.g., in various branches of the textile industry, the leather tanning industry, paper production, food, color photography, pharmaceuticals and medicine, cosmetic, hair coloring, wood staining, agricultural, biological and chemical research, light harvesting arrays, and photo-electro-chemical cells (Pereira and Alves 2012).

Dye is utilized as an activator in dye activated solar panels which utilize sensitization of the cell to transform visible light into electricity. Performances of these cells depend on dye. Sensitization is an important application of natural dye. Natural dyes are cutting down high cost of metal complex sensitizers and also replacing expensive chemical synthesis process through simple extraction process. In photo-electro-chemical water splitting, titanium dioxide-based semiconductors is used. To promote the efficiency of photo-electro-chemical reaction natural dye sensitizers are used due to their environmental friendliness and low cost (Verma and Gupta 2017).

Since ancient times, natural dyes have been used extensively for color food substrates, leather, and natural protein fibers like silk, wool, and cotton (A. K. Samanta and Agarwal 2009). Both food producers and consumers are becoming more interested in using natural pigments as food coloring, and there has been a lot of interest in the creation of novel natural colorants. Lawson is a name for henna. It is a reddish-orange pigment that has been used to dye textiles, as well as skin, hair, and other body parts. Henna has attracted a lot of scientific attention recently for use in dyeing textiles, largely because of how well it works with nature. Henna doesn't harm the environment and has slight chemical reactivity (Shahid, Shahid-ul-Islam, and Mohammad 2013). Figure 2 shows some application areas of natural dyes.

The seeds of henna are used to produce the yellow-orange dye known as annatto, also known as *Bixa orellana*. It is environmentally friendly and highly biodegradable, with little toxicity. The main compounds present in all portions of henna are carotenoids, apocarotenoids, terpens, terpenoids, sterols, and aliphatic compounds, which have been reported to have a variety of pharmacological effects. Solar cells, leather, foods, textiles and other industries all use annatto (Verma and Gupta 2017).

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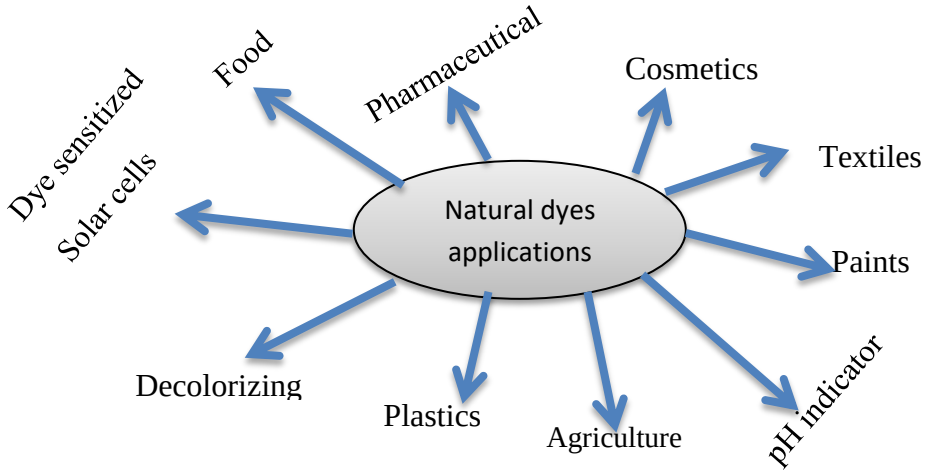


Figure 2: Some application areas of Natural Dyes

The process of applying color to fiber stock, yarn or fabric is called dyeing. There may or may not be through penetration of the colorant into the fibers or yarns. The dyeing of a textile fiber is carried out in a solution, generally aqueous, known as the dye liquor or dye bath. For true dyeing

to have taken place, coloration of fabric and absorption are important determinants. The coloration must be relatively permanent: that is not readily removed by rinsing in water or by normal washing procedures. Moreover, the dyeing must not fade rapidly on exposure to light (Humans 2010).

The process of attachment of the dye molecule to the fiber is one of the absorption: that is the dye molecules concentrate on the fiber surface. There are four kinds of forces by which dye molecules are bound to the fiber. These are: (a) Ionic forces, (b) Hydrogen bonding, (c) Van der Waals forces and (d) Covalent chemical linkages (Martin Scrivener and Phillip Carmical 2019). The chemistry involved in a dye binding to a fiber is complex. Direct bonding, H-bonding, and hydrophobic interactions are all involved. By creating a chemical link between the dye and the fiber, mordants aid in the binding of dyes to fabric while also enhancing a dye's staining and fastness capabilities. Within the fiber, mordants create soluble dye compounds that are insoluble. The dye molecule coordinates with the metal ion when specific functional groups are present in the right places. Coordination is typically accomplished by two hydroxy groups or by a hydroxy group that has a carbonyl, nitroso, or azo group in a neighboring position. Although the tints lack brilliance, the mordant dyes offer a wide spectrum of hues with outstanding resilience to wet treatments (Vankar PS 2000). In any dyeing process, whatever the chemical class of dye being used, heat must be supplied to the dye bath; energy is used in transforming dye molecules from the solution to the fiber as well as in swelling the fiber to render it more receptive. The technical term for this process is exhaustion (Sutlović *et al.* 2020).

The study plant *Impatiens tinctoria* belongs to *Impatiens* genera and that of Balsaminaceae family. It is known by English vernacular name, Balsamine or by the vernacular name in Amharic, "Insosila" or "Ansosila". The genus consists of upright perennial herbaceous plants that grow to approximately 2 meters tall with well-developed bulbs and rhizomes (figure **3**). It is native to East Africa and found in many parts of Ethiopia. Flora biodiversity assessment studies recorded as *I. tinctoria* is one of the most abundant herb species in Bonga Forest, Oromia, and Southern Tigray Region of Ethiopia (Degu et al. 2020).

Figure 3: The *I. tinctoria* A) parts above the ground B) roots

In Ethiopia women chop or mash the inside of the tubers (roots) of *I. tinctoria* A. Rich in to a paste to dye the palms and nails of the hands and feet a dark reddish color (Figure **4)**. It is considered a beauty treatment similar to that of henna. It also helps to control fungal infections like ringworm that cause athletes foot (tinea pedis) and to toughen the skin (Degu et al. 2020).

Figure 4: Dyed palms by a paste of *I.tinctoria* roots

1.2 Statement of the Problem

Environmental pollution is one of the biggest and most important problems of the modern world. Almost all the processes used by man to produce goods and services lead to the production of environmental pollutants. These pollutants are released into the air, water and soil and have harmful effects on the health of humans, plants, animals and microbes. The paint industries use toxic metal salts as a source of color when producing dyes. It is of great concern that the production and application of synthetic dyes generates a huge amount of waste and unfixed dyes, causing severe health hazards and disturbing the ecological balance of nature (Choudhury 2018). Alternatively, we should look for green processes that lead to the production of environmentally friendly products. We must also turn our attention to ways to eradicate and reduce existing environmental pollution. Therefore, for a sustainable human society, we need green chemistry and environmental restoration.

Nowadays there is a big trend to dye and finish fabrics in an ecological way with natural products. Various natural dyes have been used to dye protein fibers, but a small number can dye cotton fibers due to cellulose's low affinity for natural dyes. Natural colors are beneficial because they are gentle on the fabric, brilliant sheen, soothing effect on fibers, mix-match coordination with the ability to produce a wide range of colors, environmentally friendly dyeing techniques, wide change in gamut of colors, exceptional color ideas, customary harmonization, renewable, sustainable, and biodegradable and zero disposal problems. They are also non-toxic, non-allergenic and non-carcinogenic compared to their synthetic dye counterparts (Güzel and Karadag 2021; Jabasingh 2019). Besides enabling paints with unique colors, some natural dyes also impart functional properties such as antibacterial, deodorant and ultraviolet protection to substrates (Khan, Hussain, and Jiang 2018).

The other benefits include the potential for these plants to grow in wastelands, or as guests in gardens, with less cost and effort. They are a viable alternative to synthetic dyes, which depend on fossil fuels. In Ethiopia, there is a large amount of plants available for the production of natural dyes, but due to lack of knowledge of the process involved in harvesting and processing, the plants are used at low levels. However, the coloring plants in Ethiopia have not been scientifically identified or documented. Given the future and the high biodiversity of the country,

it is to be expected that some plants will have important color components for dyeing textiles (Kechi, Chavan, and Moeckel 2013). *Impatiens tinctoria* is economically important because it is popular as a cultivated ornamental plant. It is traditionally used to color the nails, palms, hands and feet. For all that, this species has not yet been studied in detail for its coloring properties and chemical compositions.

1.3 Objectives of the Study:

1.3.1 General objective

The main objective of this study is to extract natural dye from Ansosila (*Impatiens tinctoria)* for coloration of cotton fibers.

1.3.2 Specific objectives

The specific aims of this study are to:

- Extract natural dyes from the roots of *Impatiens tinctoria* by aqueous extraction technique.
- Dye cotton fiber using the extracted dye with different mordents and without mordent.
- Evaluate the strength and fastness of the dye using standard and universally accepted Methods such as ISO 105-C10:2006 for fastness to washing, ES ISO 105-E04 for color fastness to perspiration and ISO 105-X12:2002 for fastness to rubbing.
- To characterize the compounds responsible for the color displayed by crude extracts of *I. tinctoria* by UV-visible and FT-IR spectroscopies.

1.4 Significance

Our country Ethiopia is struggling to reduce the products imported from abroad and increase the export of its products abroad. Therefore, when we come to the products in the industry, quality is one of the most important concerns we should always be concerned about. So if Ethiopian dye industries can replace some of the heavy and toxic metal salts used for color formation with naturally available plant parts, both animal health and the environmental ecosystem will not be affected. Therefore, if this project is applied, the country will benefit from introducing foreign exchange after export, and also from creating a clean environment for all living things and ecosystem in the universe.

1.5 Scope of the Study

Due to limitations of resources and time the scope of this study was limited to gathering of samples which serves as the first step in the research's goal. Aqueous extraction method was used as the extraction solvent in this method. The sample was evaluated using Fourier transform infrared spectroscopy (FT-IR), UV-visible spectroscopy (UV-visible), and its use for dyeing cotton garments. Using performance testing tools for color intensity and color fastness, the dye's efficacy on materials were also examined.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 History of Dye Usage

The practice of coloring predates the creation of written records. In Europe, it has been practiced since the Bronze Age (Baishya, Talukdar, and Sandhya 2012). Leather and textiles have been often dyed with synthetic dyes since 1856 (Berhanu and Ratnapandian 2017). Early dyeing methods included pressing crushed pigments onto fabric or tacking plants to it. West Africa has practiced weaving since the 10th century, which uses cotton and natural dyes to create clothes. Ancient handwritten religious texts and the traditional clothes worn by monks in monasteries were both inscribed in vibrant hues. Due to their employment in some of the most significant rites and events, dyed clothing has a specific effect on African people (Jabasingh 2019). The discovery of colored textile remnants during archaeological excavations in various parts of the world demonstrates the practice of dyeing in prehistoric societies. Up until the 19th century, only natural dyes were used to color fabrics. Sir William Henry Perkin made the unintentional discovery of the first synthetic color, mauveine, in 1856 when he was synthesizing quinine-based medications to treat malarial fever. It comes in larger variety of colors with clearer shades (Yusuf 2019).

2.1.1 Dyes in Textiles

Color is one of the most important factors in the attractiveness and marketability of textile products. Color additives, primarily dye molecules from prehistoric human civilizations are applied to make things more aesthetically pleasing and colorful (Yusuf 2019). In particular for dyeing textiles, organic natural colorants have a long history of use. A dye is an organic substance made up of an auxochrome, which subtly changes color, and a chromophore, which gives it color. A binding site for the fiber is created by the auxochrome, which also renders the dye soluble. Textiles are dyed using a paste or solution (Benli 2022).

Figure 5: Colored Textiles

Dyeing processes can be used either to color fibers and yarns before they are made into fabric or to color the fabric itself. The dye also provides a way to decorate fabrics. To be useful for dyeing fabrics, a dye must be strongly colored, provide products that are colorfast or resistant to color change or loss during use and maintenance and must be soluble or capable of being solubilized in water or othe medium in which they are applied, or they must themselves be molecularly dispersible in the fibers of the fabric (Shariful Islam, Alam, and Akter 2020).

2.2 Classification of Dyes

Human is always fascinated with by colors, because we are living in a colorful world. We could see so many colors in plants and their flowers. We eat colored food stuffs and use numerous colored materials in our daily life. These dyes are all aromatic compounds. Not all aromatic compounds are colored. Aromatic compounds which absorb light of wavelength range 350-700 nm (visible light) only are colored. This nature of absorption of visible light by aromatic compound depends on their structure (Martin Scrivener and Phillip Carmical 2019).

The classification of textile dyes has become essential due to the remarkable increase in the type and number of dyes. Dyes are usually classified by their Color Index (CI), developed by the Society of Dyes and Colorist (1984), which is edited every three months. It lists dyes firstly by a

generic name based on its application and color, then by assigning a 5-digit CI number based on its chemical structure (Benkhaya, M' rabet, and El Harfi 2020).

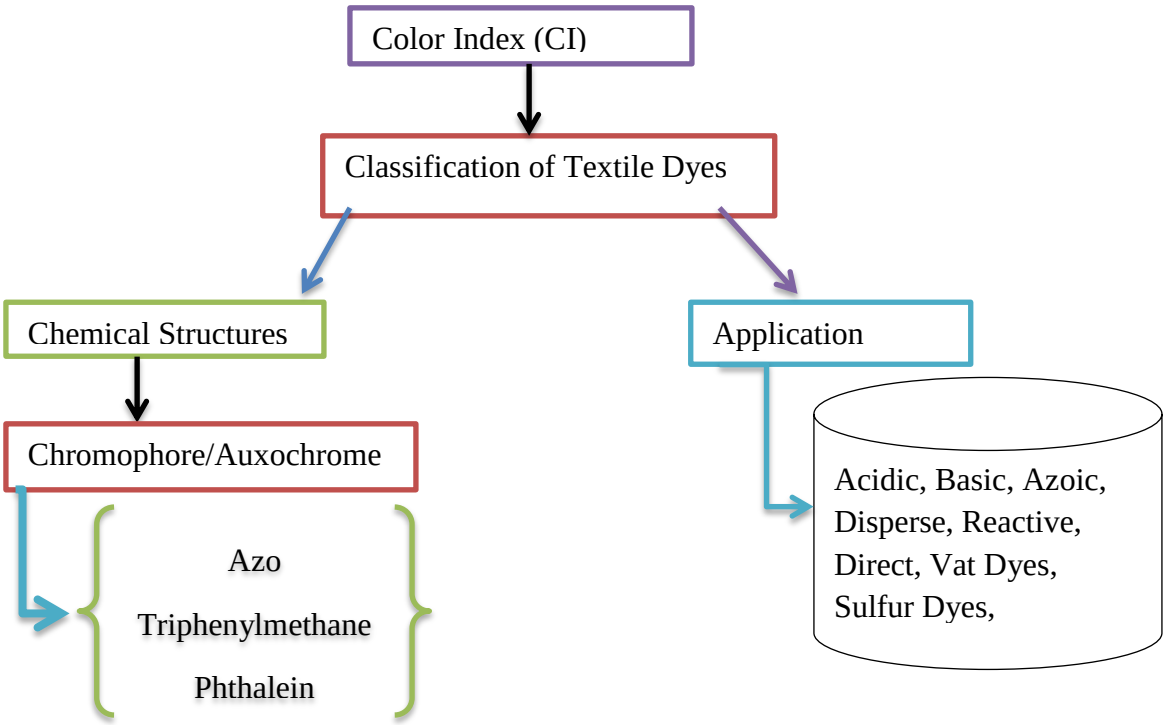


Figure 6: Classes of dyes based on CI number

The chromophore and auxochrome groups are the principal structural components responsible for the absorption of light in dye molecules. The foundation of the theory is the idea that chromophores are frequently electron-withdrawing groups. The majority of the times, auxochromes are electron releasing groups that are connected to one another by a conjugated system. In essence, the donor-acceptor chromogen idea was created (Gregory 1990).

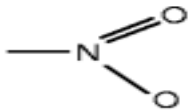
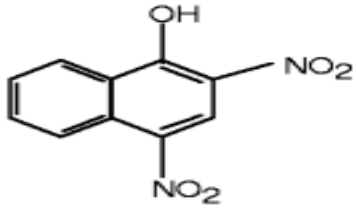
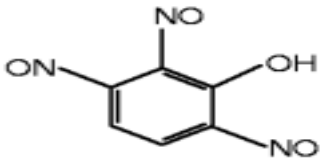
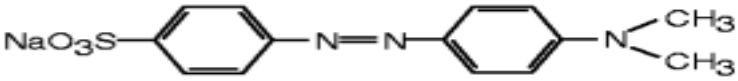
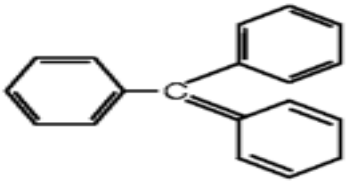
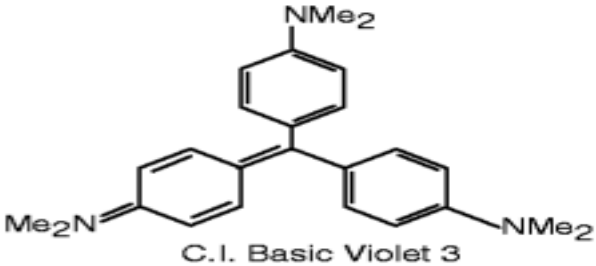
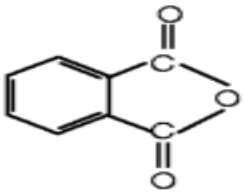
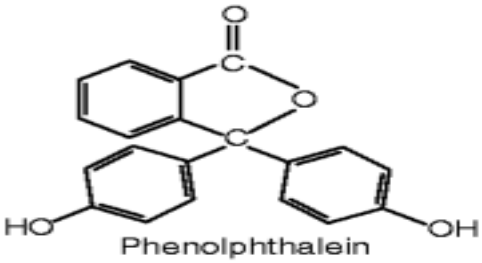
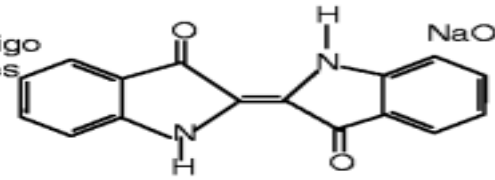
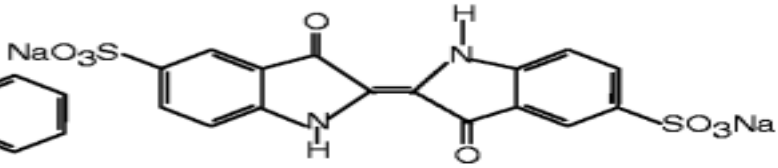
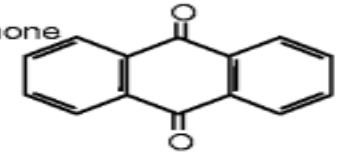
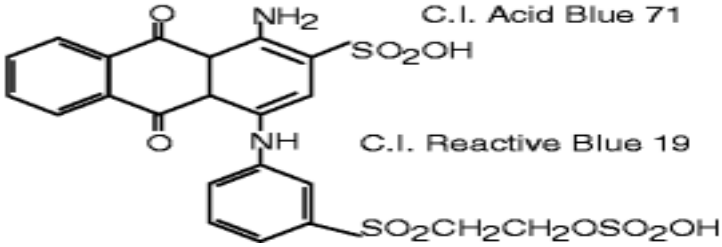
Additionally, it was discovered that by enhancing the chromophore's ability to draw electrons, augmenting the auxochromes' ability to release electrons, and lengthening the conjugation, it was possible to achieve a bathochromic shift of the color, or an absorption band that was shifted to a larger wavelength. Indeed, the basic meaning of the word auxochrome is color enhancer (Pereira and Alves 2012).

Although it lacks a formal theoretical foundation, the chromophore and auxochrome theory is suggested as a straightforward approach to explain the origin of color in dye molecules. The active site of the dye is the chrmophore, it can summarize the spatial localization of atoms absorbing light energy. Chromophores often contain heteroatoms such as N, O, and S with unbound electrons. According to this definition, the carbonyl (C=O), thiocarbonyl (─C═S), diazo (-N=N-) (Afatt et al., 2018), azomethine (-CH=N) (afatt et al., 2012), nitroso group (-NO), ethylenic bond (-C=C), amino group (NR_2), acetylenic bond and nitro (NO_2) groups are the most significant chromophores (Affat 2021; Pereira and Alves 2012).

Some auxochromes include $–NH_3$, -COOH, HSO_3 and –OH groups. These groups also have the important property of giving a higher affinity to the fiber. The chromogen, which is an aromatic structure (normally benzene, naphthalene or anthracene rings), is part of a chromogen-chromophore structure along with an auxochrome (Pereira and Alves 2012; Karabulut and Atav 2020; Kumar et al. 2021).

In general, the dyes consist of an assembly of chromophoric, auxochromic groups and conjugated aromatic structures (Benkhaya, M' rabet, and El Harfi 2020). Table 1 shows the classification of dyes based on the chromophore present adopted from (Kiron 2021).

Table 1: Classification of dyes based on the chromphore present

Class	Chromophore	Example
Nitro dyes		 C.I. Acid Yellow 24
Nitroso dyes		 Fast Green O
Azo dyes		 Methyl Orange
Triphenyl-methane dyes		 C.I. Basic Violet 3
Phthalein dyes		 Phenolphthalein
Indigo dyes		 C.I. Acid Blue 71
Anthraquinone dyes		 C.I. Reactive Blue 19

The complex, unsaturated chemical compounds known as dyes absorb light and give their color in the visible spectrum (380-750). A dye is also a material that has the chromophoric property of absorbing a portion of the visible spectrum. The absorption of electromagnetic waves by the chromophore is due to the excitation of the electrons of a molecule. Conjugated double bonds create a chemical structure that is favorable to light absorption. Dyes have color because they absorb visible light and contain a conjugated structure, which gives them the ability to stabilise organic molecules (Benkhaya, M' rabet, and El Harfi 2020; Humans 2010).

Table 2: Wavelength of light absorption versus color in organic dyes

Wavelength (nm)	**Color Absorbed**	**Color observed**
400-435	Violet	Yellow-Green
435-480	Blue	Yellow
480-490	Green-Blue	Orange
490-500	Blue-Green	Red
500-560	Green	Purple
560-580	Yellow-Green	Violet
580-595	Yellow	Blue
595-605	Orange	Green-Blue
605-700	Red	Green-Blue

Natural dyes (substantive/direct and non-substantive/additive dyes) and synthetic dyes can both be categorized according to their sources (figure 7). For non-substantive/additive dyes, like madder, to adhere to fibers, a mordant (a substance that fixes a dye) is required. These kinds of dyes are the most typical ones which have been in use for at least 2,000 years. The fabric doesn't need to be pretreated before using substantive dyes, such as indigo, orchil, and turmeric (Martin Scrivener and Phillip Carmical 2019).

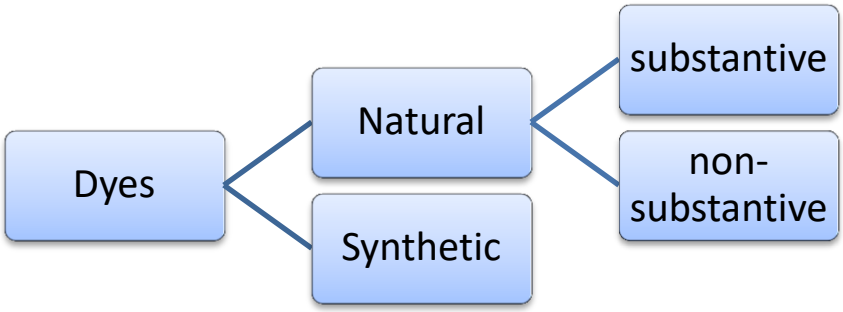


Figure 7: Scheme of Dyes class based on source

2.3 Synthetic Dyes

Dyes derived from organic or inorganic compounds are called synthetic dyes. They quickly replaced traditional natural dyes, offering a wide range of new colors and giving them better properties (Kiron 2021). In the past, dyes were made individually by harvesting fruits, vegetables and other natural items; cooking them and soaking fabrics in the dye bath. This long and arduous process was common practice until the middle for the 19th century. In 1856, William Perkin, an English chemist working in London, accidentally discovered a purple dye while trying to synthesize quinine by reacting aniline sulfate with an oxidizing agent. This purple dye, which Perkin called Mauve, was found to color silk, and although not particularly fast, it became popular (Pereira and Alves 2012; Gurr 2012).

Today, the industry almost exclusively uses synthetic dyes because they retain their color better and cost less than natural dyes. Perkin's discovery showed chemists that dyes and pigments could be made synthetically in the laboratory and that it no longer necessary to search for natural products to use as dyes. In addition industrial use, professional and private dyeing of clothes and household decorations based on the different types of dyes and their ability to resist fading is widely used. AS Storey (1985) indicates, until the second half of 19th century, dyes were mainly obtained from plant materials and some mineral dyes, but rarely from animal sources. Most synthetic dyes are toxic due to their complex chemical structures and very resistant to degradation. Synthetic dyes are widely used in textile dyeing, paper printing, color photography, pharmaceuticals, food, cosmetics and leather industries (Ali 2010).

2.3.1 Environmental Impacts of Synthetic Dye Applications

The textile industry is one of the largest producers of pollutants from liquid effluents due to the large amounts of water used in dyeing process (Kalyani *et al*. 2009). Textile dyes are complex synthetic organic compounds. They are common industrial environmental pollutants during their synthesis and later during dyeing. For the textiles dyed or printed with synthetic dyes, one should look for suppliers whose products possess eco-labels issued by well-known authorities (Choudhury 2018).

All dyes do not bind to the fabric easily; hence depending on the class of the dyestuff, they are poured to wastewater from 2% for basic dyestuffs up to 50% for reactive dyestuffs, leading to severe contamination of surface and groundwater in the vicinity of the dyeing industries (figure 8). An estimated 2.8 x 10^4 tons of textiles dyes are discharged into textile industrial wastewater each year worldwide. Dye-containing waste from the textile industry is highly colored and therefore visually recognizable (Chen *et al.* 2003).

Figure 8: Effluent from textile Industry
(Source: https://www.google.com/search?q=dye-effluents)

At today's level of scientific advances, natural dyes are only a viable option for small-scale applications and can complement synthetic dyes as an environmentally friendly option for the environmentally conscious consumer and a livelihood tool for various color stakeholders value chain of natural dyes (Pereira and Alves 2012).

2.3.1.1 Acute Toxicity of Dyes

The main issues with acute toxicity with textile dyes are skin irritation and skin sensitization, which are primarily brought on by reactive dyes for textiles and viscose, a small number of acid dyes for polyamide fibers and disperse dyes for polyester, polyamide and rayon acetate (Chavan 2013).

2.3.1.2 Chronic or Genotoxicity of Dye Intermediates

Due to their large-scale production and wide application, synthetic dyes can cause significant environmental impacts and pose serious health risk factors. In general, when the body is warm and the pores of the skin have opened to allow perspiration, fabrics and clothing come into direct contact with our skin, which is why some sensitive and toxic chemicals are often absorbed by the epithelial tissues of the skin (Yusuf 2019). The main potential long-term health risk associated with some textile dyes is genotoxicity. This was made clear when it was discovered that plant workers who worked on the production of specific dyes between 1930 and 1960 had a high prevalence of bladder cancer. The precise substances in question were benzidine, 2-naphthylamine, fuchsine, and auramine. Most industrialized nations have implemented strict rules governing the handling of all known carcinogens, which has led to almost all dye businesses terminating manufacture of these compounds (Chavan 2013).

A thorough examination of the dyes has shown that some categories of dye intermediates or metabolites (decomposition products), such as benzidines, are associated with carcinogenicity. Based on the molecular structure of the dyes, Whaley (1987) evaluated a variety of them for evidence of their potential danger. Out of the 1,460 dyes analyzed 585 dyes, or roughly 40% of

the total, had known or discovered structural information. These known dyes were predicted to be dangerous in 55% of cases and questionable in safety in 13% of cases based on an analysis of the likely development of degradation products on reduction. A focused research effort has been made since 1984 to create dyes based on less hazardous intermediates. Before the invention of the multiple assays presently available to identify environmental effects, the traditional intermediates were adopted many years ago. However, given the prevalence of dyes that appear to be harmful, responsible dye manufacturers have removed offending colors from their product lines. This calls for dyers to exercise caution when choosing dyes and handle all dyes with care (Yusuf 2019).

2.3.1.3 Aquatic Toxicity and Effect on Photosynthetic Activity

Synthetic dye-containing industrial effluents decrease light penetration in receiving water bodies, which negatively impacts the photosynthetic activities of aquatic flora and the food source for aquatic creatures. Because a very tiny concentration of synthetic dyes in water (≤ 1ppm) is extremely visible and affects the aesthetic value, transparency and gas solubility of water bodies, color is typically the first contaminant to be identified in wastewater. They absorb and reflect the sunlight that enters the water, preventing photosynthesis and aquatic creatures from growing (Pereira and Alves 2012). A receiving water body that has a thin layer of discharged dyes on its surface experiences a reduction in Dissolved Oxygen (DO) levels, which has an impact on the aquatic life there. Additionally, dye-containing effluents raise the contaminated water's Biochemical Oxygen Demand (BOD) (Chavan 2013). Figure 9 shows the ecological effects of synthetic dyes.

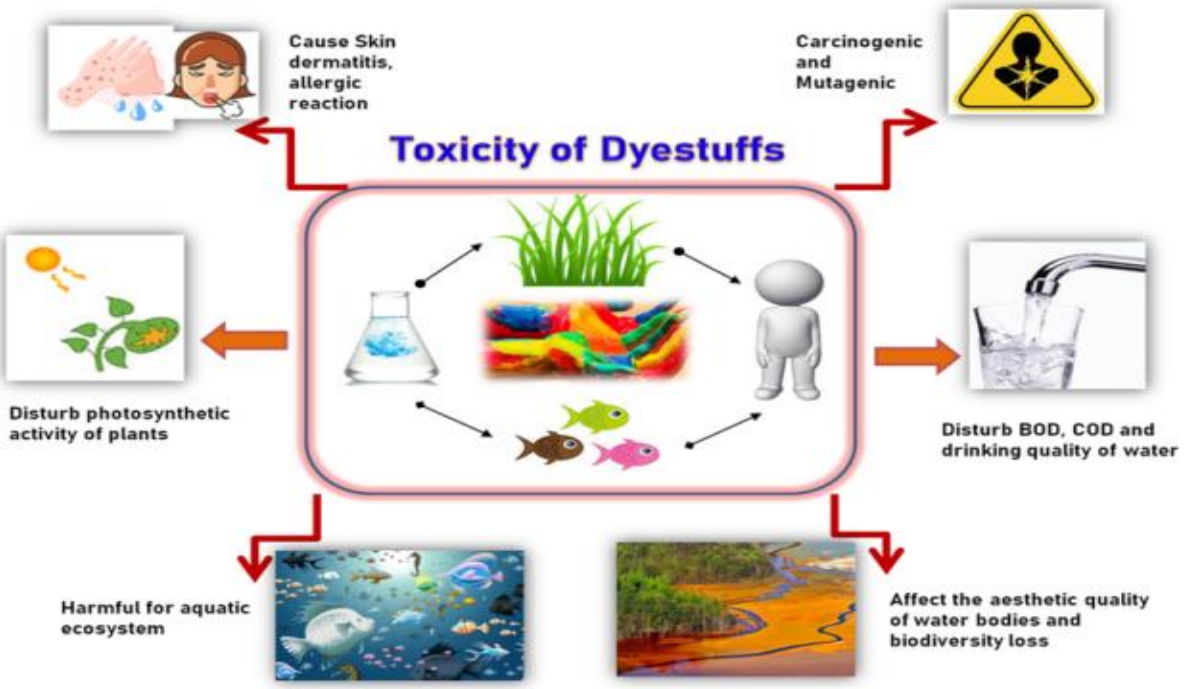


Figure 9: Toxic effects of dyes in biotic and abiotic system

Toxicity is determined by testing animals (usually rats) to determine the single oral dose of colorant that will kill 50% of the population in 14 days. The results were expressed in mg/kg bodyweight and were called lethal dose (50) or LD_{50}. On the basis of these tests, the toxicity rating of 4461 dyes belonging to various classes of dyes is performed by Barkclay and Buckley (1996). Aquatic toxicity is also expressed as LC_{50} (measured in mg/l) which gives the concentration of a chemical that causes 50% of an experimental population of fish to die during 48hrs (Chavan 2013).

These sensitive and toxic substances are discharged in the form of wastewater entering rivers, canals, pipelines and if not properly treated can cause serious pollution of water resources. Thus, the materials discharged into aquatic ecosystems are effluents that contain many organic pollutants with strong/dark color, low visibility, high biochemical oxygen demand, low dissolved oxygen concentrations and low biodegradability (A. K. Samanta, Awwad, and Algarni 2020).

2.3.1.4 Adsorbable Organic Halogens (AOX) Emission

Adsorbable Organo-halogens (AOX) is a measurement of the amount of organic halogens present in a sampled area, such as sewage waste, water, or landfill soil. The amounts of fluorine in the sample are not taken into account as the test assesses chlorine, bromine, and iodine as equivalent halogens (Choudhury 2018). Colorants containing organic halogens (with the exception of fluorine) are measured as AOX. Vat, disperse and reactive colorants are more likely to contain halogens in their molecule. The originally bound halogen content can be up to 12% by weight for some vat dyes (Gurr 2012). Numerous organic halides are produced when halogen-containing compounds are used in procedures like water treatment, bleaching, or even general synthesis to form the finished product. These organic halides are discharged in wastewater from the paper, chemical, and oil sectors and finally end up in a landfill or the ocean. Many AOX molecules do not naturally degrade in the environment and are classified as persistent organic pollutants (POPs) (Choudhury 2018).

2.3.1.5 Heavy Metal Emission

Metals can be present in dyes for two reasons. Firstly, metals are used as catalysts in the production of certain colors and may be present as impurities. Secondly, in certain dyes, the metal is chelated with the dye molecule, forming an integral structural component. The release limits for heavy metals are strict since they can be toxic to animals and aquatic life. Metal complex dyes contain chelated chromium, cobalt, copper and nickel. Certain cationic dyes contain zink and traces of mercury, cadmium and arsenic may be present as impurities from intermediates (Chavan 2013).

2.3.1.6 Impact on Growth of Crops and Plants

The textile industry's effluents, among the many types that are industrially released as waste, are of particular concern due to their serious and diversified environmental risks, such as those that are mutagenic and carcinogenic in nature (Stewart 2017). The diversity of the living environment is impacted by the contribution of each poisonous component to toxicity, which changes with its dilution and dispersion into groundwater. At the cellular and molecular levels, living things are

frequently exposed to environmental factors that could be genotoxic. Studies on genotoxic potential are crucial to forecast how certain substances may affect animals, plants, and ultimately people (Pereira and Alves 2012).

According to Wang and Keturi (1990), root-shoot ratio and seed germination tests have been employed as a quick, easy, and reproducible method to look into the harm done by hazardous substances found in various polluted wastewater reservoirs. Five plant seeds were used in a study by Umesh *et al.* (2017) to determine the effects of synthetic textile effluent on germination and root/shoot elongation (Chen *et al.* 2003). It was proposed that the phytotoxicity bioassay can serve as both a monitor of environmental contamination and an effective toxicity test for synthetic textile dye effluent. The study's findings regarding the toxicity of dyes also point to the necessity of removing textile dyes from industrial effluents before releasing them into the environment. Similarly, other study showed crops cultivated on soil contaminated with heavy metals can collect large amounts of trace elements, which could cause certain health concerns to consumers (A. K. Samanta, Awwad, and Algarni 2020).

Crop and plant intake of minerals and their accumulation are influenced by a variety of elements, including soil metal concentrations, solubility indices, and plant species. Because they have higher mineral content than other vegetables, leafy vegetables require more heavy metal absorption and accumulation. This capacity has been attributed to leafy vegetables' higher rates of transpiration and translocation compared to non-leafy vegetables, where the transfer of metals from root to stem and finally to fruit takes longer and causes less accumulation than in leafy vegetables (Shabbir and Naim 2019).

2.4 Natural Dyes

The majority of natural coloring substances are either inorganic such as clays, earth, minerals, metal salts and even semi-precious stones like malachite or organic dyestuffs, which have historically been classified into two categories: those with an animal origin and those with a plant origin (Pereira and Alves 2012). All-natural colors were typically used in past. In earlier times, alizarin and indigo were two examples of natural colors. The leaves of the indigo plant, *Indigofera tinctoria,* and the dyer's wood weed, *Isatis tinctoria,* are used to produce indigo, which is likely the oldest dye now in use (Kumar et al. 2021).

Natural dyes can be obtained directly from agriculture, wastes/by-products of agriculture or forestry (e.g. industrial wool bark), as well as wastes from the food and beverage industry pressed berries, residual distillation, pomade, skin, shells, etc (Jabasingh 2019). Synthetic dyes are by far the most frequently used colorants in industry. However, there are now more initiatives to replace them with natural dyes that are safer and more sustainable due to environmental and sustainability issues (Hirko and Getu 2022).

2.4.1 Benefits of Natural Dyes

Natural dyes are used in textiles for their color as well as their antibacterial, UV protection, anti-inflammatory and insect repellent qualities (Elsahida *et al.* 2019). Because the phytoconstituents, coumarins, polyphenols, flavonoids and sterols are strong antioxidants and act as anti-mutagenic agents with antimicrobial properties, natural dyes and fabrics dyed with them are the preferred brand in the textile industry. Functional dyes are used in fabrics to integrate the dyeing and finishing processes, creating a more effective method that uses less water and energy. Natural dyes are used for more than just textiles; they are also used to color food, medicine, crafts, toys, and the tanning process for leather. Many dye plants are also employed as medicines in various healing systems (Jabasingh 2019). They can be viewed as green and environmentally friendly alternatives to synthetic dyes because they have historically been valued for their functional properties (Hou *et al.* 2017).

2.4.2 Ethiopian Plants as a Sources of Natural Dyes

Natural dyes have been used to color textiles in Ethiopia for a very long time, as shown by the colorful artwork created on canvas before the 19th century and the colorful historic cultural clothing discovered in Orthodox churches and museums. More than 6,500 to 7,000 plant species, 15% of which are endemic, are thought to exist in Ethiopia (figure 10) (Erana 2022).

Figure 10: Traditional Ethiopian painting with natural colors (Erana 2022)

There are many different plant species on the African continent that can be used to make natural colors. Textile dyeing with natural materials has a long history in Ethiopia. The clothing is decorated with dyes made from numerous plants that may be found around the nation. Locals in Tigray and Oromia use the Qerte plant (*Osyris quadripartita*) to color a baby clothes (ankelba). Africa's subtropical and temperate climates are home to the *sumac or sumac* plant, which grows fruits in dense, scarlet clusters from which a dye is extracted. For the extraction of natural dyes, Ethiopian frequently use the leaves of *Combretum collimum,* the bark of *Ficus sycamore, Syzygiumguineense* and *Eriobotrya japonica,* the flowers of *Tagets minuta* and *Scadoxus multiflorus* (Jabasingh 2019). Some dye plants in Ethiopia are listed in table 3 below.

Table 3: The Amharic and scientific names of some dye plant sources

S.No	Amharic name	Scientific name	Plant part used	Geographical distribution	Reference
1	Abalo	*Combretumcollinum*	Leaves	Ethiopia, Southern - Africa, Madagascar…	(Kechi, Chavan, and Moeckel 2013)
2	Bamiba	*Ficus sycamores*	Bark	Ethiopia, Kenya, Sudan, Egypt, Eritrea…	
3	Dokima	*Syzygium guineense*	Bark	Ethiopia & many parts of Africa…	
4	Fesson	*Scadoxus multiflorus*	Flower	Ethiopia, Zimbabwe, South Africa …	
5	Gimea	*Tagets minuta*	Flower	Ethiopia, Eritrea, Djibouti, Kenya	
6	Gesho	*Rhamnus prinoides*	Leaves	Occurs from Ethiopia to South Africa	
7	Nech Girar	*Acacia sp.*	Flower	Ethiopia, Madagascar, Pacific Islands....	
8	Inkoy	*Flower Ximenia A.*	Flower	Native to tropics including Ethiopia…	
9	Tikur – inchet	*Pygeum Africanum*	Leaves	Ethiopia and most African countries....	
10	Warica	*Ficus sycomores*	Bark	Ethiopia, Kenya, Sudan, Egypt…	
11	Wonbela	*Eriobotrya japonica*	Bark	Ethiopia, Egypt, Kenya, Sudan ….	
12	Zembaba	*Stereospermum*	Leaves	Ethiopia, Zambia, D.R.of Congo…	

2.4.3 Limitations of Natural Dyes

Natural dyes have drawbacks that prevent their use and advancement despite their many benefits. These include challenges with tonal reproduction and standardization, the need for professional expertise, cost-prohibitive prices, low color yield, the requirement for stains and their additional costs, a lack of sufficient scientific support, an inability to precisely understand the technical details of their extraction processes, skin rashes brought on by exposure to the sun, sweat and air, a lack of sufficient color fastness, and performance evaluations that are insufficient for use in the modern textile industry. Natural dyes are difficult for reproduction since they vary from crop to crop, location to location, species to species and maturity period (Karabulut and Atav 2020). Almost all natural dyes, with a few exceptions, require the use of mordants to bind to the textile substrate. Natural colors are therefore more expensive than synthetic dyes (Ragab, Hassabo, and Othman 2022). While dyeing, a significant quantity of mordant remains undistributed in the residual dye bath, creating a significant challenge for the removal of effluent. For a given amount of fabric to be colored, more natural dyes may be needed than synthetic dyes. For instance, five grams of synthetic dye can color one gram of cotton, whereas 230g of natural dye is required to color the same amount of fabric. Due to these drawbacks, artificial synthetic dyes were developed in the late 19th century, which destroyed the massive, booming market for natural dyes (Güzel and Karadag 2021; Jabasingh 2019).

2.4.4 Sustainability of the Use of Natural Dyes in the Textile Industry

Sustainability of the use of natural dyes in the textile industry achieved through connecting economic, social and environmental aspects by increasing creativity in technology, ensuring supply chain sustainability increasing capacity and productivity and enhancing the entrepreneurial spirit (Elsahida *et al.* 2019). Figure 11 shows the elements of sustainability of natural dyes used textile industry along with the driving forces and their goals.

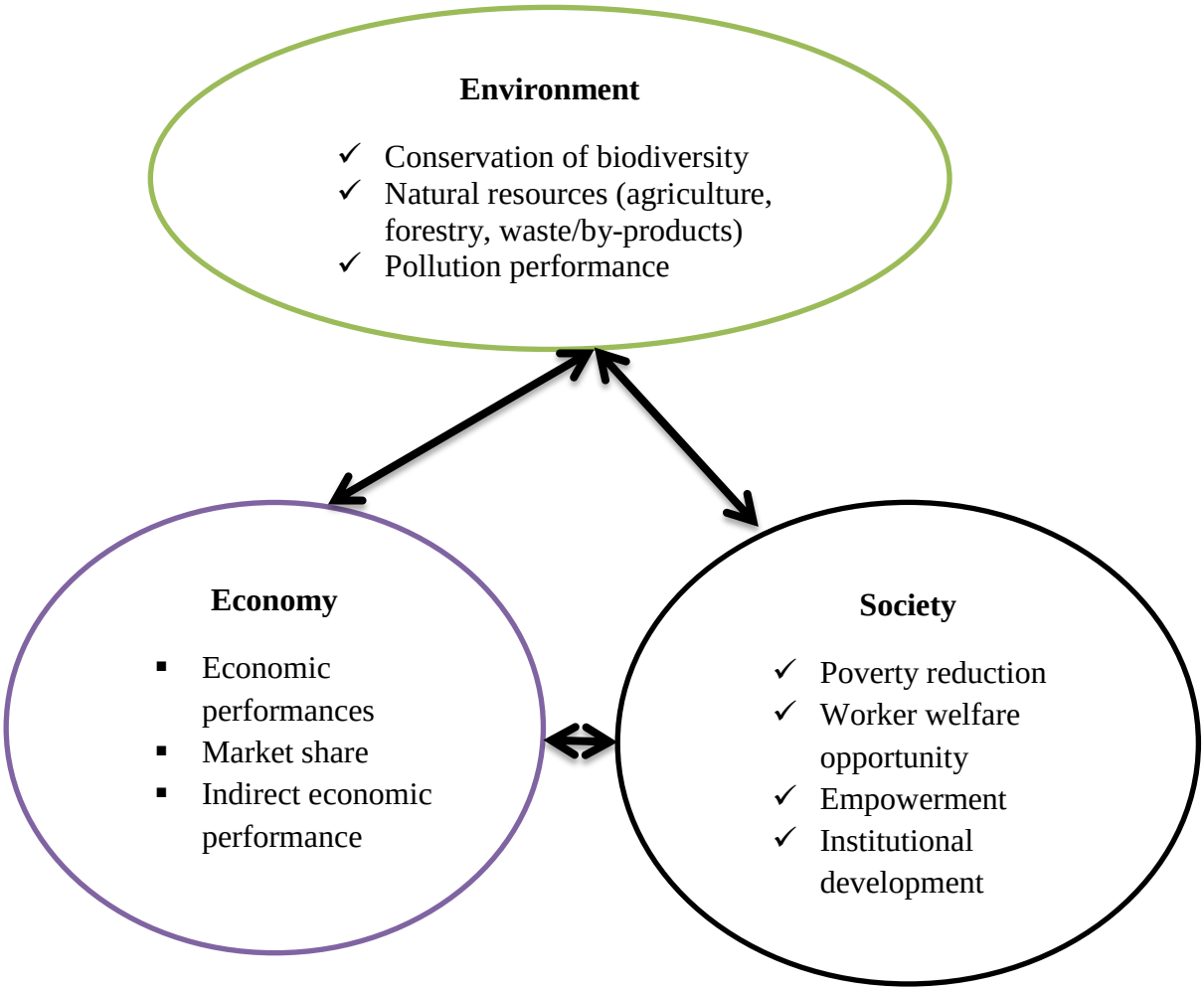


Figure 11: Elements of the sustainable natural dyes used in textile industry

2.5 Technological Progress in Dyeing Fabrics with Natural Dyes

The wet chemical processing of textiles which involves a variety of chemical agents and physicochemical procedures includes dyeing as a crucial step. Environmental issues brought on by the wet textile processing industry's rapid growth have opened the path for continuous interest in creating cleaner manufacturing techniques to produce textile products that are both cost-effective and value-added (Jia *et al*. 2017). The situation has changed significantly during the past fifteen years as a result of studies on the potential use of natural dyes in fabric dyeing

procedures undertaken by various research groups. Today, substantially better and more sophisticated information is accessible on natural dyeing techniques (Ren *et al*. 2019). Due to the growing focus on issues like water pollution, the sustainability of raw materials and processed goods, biodegradability and environmental attributes, natural dyeing in textiles is currently of great importance in both research fields and the textile industry (Shariful Islam, Alam, and Akter 2020).

It has been attempted to do research on painting processes employing both standard and alternative exhaustion approaches, including plasma treatment, ultrasound, microwave, pad painting, high temperature and high pressure (HTHP) painting, touch painting, and other optimized procedures. The recovery of traditional dyeing techniques, the variability of natural resources for dyeability, the improvement of processing for natural dye extraction, strategies for improving color fastness or dyeing properties for naturally dyed fabrics, the adaptation and optimization of natural dyeing methods and the use of contemporary technologies in the various stages of the natural dyeing process of fabrics have all been the subject of numerous research papers (A. K. Samanta, Awwad, and Algarni 2020). Figure 12 shows cleaner dyeing of textiles using plasma treatments.

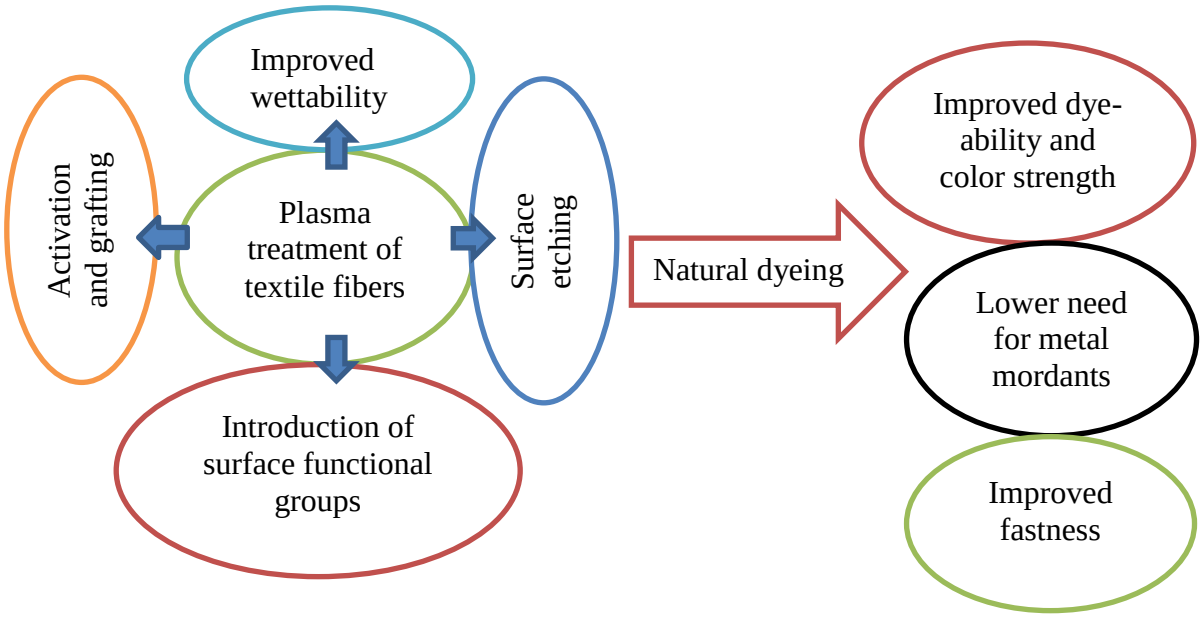


Figure 12: Cleaner dyeing of textiles using plasma treatment and natural dyes

Environmentally friendly processing methods should be considered in any process based on the use of natural dyes, and the whole process should be competitive in terms of chemical, energy, waste, and wastewater utilization (Bureekhampun and Maneepun 2021; Shahid, Shahid-ul-Islam, and Mohammad 2013).

2.6 Factors Influencing Dye Adsorption

The dye adsorption process is influenced by a variety of variables, including temperature, solution pH, mordanting, contact time, adsorbent dosage and initial dye concentration. As a result, it is important to investigate how the aforementioned factors might be optimized. The creation of industrial-scale dye treatment procedures can benefit from condition optimization (Azari *et al.* 2020).

2.6.1 Temperature

The sorption of color particles to surge towards the fabric's surface during the coloring process depends critically on temperature. In several types of fabric tests, the increase in temperature between 30℃ and 90℃ has been reported to result in higher color assimilation. The increased depletion yields are the result of the higher atomic vitality of color atoms and fiber at the elevated temperatures. Additionally, using greater temperatures causes more fiber swelling and color atom complete breakdown, which improves color dissemination into fibers. Low dyeing temperatures may prevent dye molecules from rushing to the cloth. The dye molecules' kinetic energy may be reduced as a result of sorption on the fabric (Aminoddin Haji *et al.* 2020).

2.6.2 Time

In the majority of investigations, the dye adsorption capacity and rate of removal efficiency began quickly and subsequently decreased until the sorption system reached equilibrium. The primary stages of adsorption are characterized by the existence of abundant and vacant active sites and/or reaction sites on the adsorbents. These sites eventually become occupied and saturated, leading to equilibrium (Azari *et al.* 2020). As the period rose from 20 minutes to 60

minutes, the color intensity of the samples treated with natural dyes increased. When the dyeing time was increased to 90 minutes, it started to diminish. After 60 minutes, it appears that the dyeing process has reached equilibrium. Long-term heating upsets the equilibrium and can cause dyed yarn to stripe, which lowers K/S values. The H-bonding between the dye molecules and the fabric may become weaker with prolonged dyeing. This drop in color intensity may also be explained by the natural dye molecules' partial breakdown brought on by extended exposure to high temperatures (Aminoddin Haji *et al.* 2020). Low dyeing temperatures may prevent dye molecules from rushing to the fabric. The dye molecules' kinetic energy may be reduced as a result of sorption on the fabric. So, for fabric dyeing, 75 minutes is the recommended dyeing period for optimal color strength (Kiran *et al*. 2020).

2.6.3 pH

One of the key factors influencing dye speciation, the size of electrostatic charges, the level of ionization, and surface properties during the adsorption process is solution pH (Azari *et al*. 2020). The pH of the dyeing bath is crucial to the dyeing process. On the colored fabric, high color strength is attained at the ideal pH. The ideal pH's function is to fasten dye molecules' movement towards the surface of the cloth for sorption. The more acidic the pH, the greater damage cloth sustains due to the structure of acid. Because dye molecules jump towards the cloth surface for sorption, a moderate pH is therefore good for dyeing fabric. Moderate pH does not harm the surface of the fabric (Kiran *et al.* 2020).

2.6.4 Mordanting

Since most natural dyes do not have a strong affinity for textile fibers, especially cellulosic (cotton), mordanting is a step that must be added. Mordants serve as a link between the fiber and the dyestuff since they have an attraction for both textile fibers and dyes (Saxena and Raja 2014). In order to (a) set the dye, (b) achieve a full color palette, (c) prevent natural dyes from fading, (d) improve their fastness capabilities generally, and/or (e) lighten, deepen, or dull a color, morses (mordants) are frequently used when using natural dyes. Mordants help the dye adhere to the fabric more effectively. By utilizing various mordants, the same dye can produce a variety of

shades (Rekaby, Salem, and Nassar 2009). Mordanting can be classified on the basis of application time of mordants. These include pre-mordanting (before dyeing), simultaneous (simultaneous) mordanting (during dyeing) and post-mordanting (after dyeing) (Chungkrang and Bhuyan 2020).

.

CHAPTER THREE

3. MATERIALS, MEDHODS AND EXPERIMENTAL DESIGNS

3.1 Materials

3.1.1 Plant part

Among hundreds of dye-yielding plants in Ethiopia, Ansosila (*Impatiens tinctoria)* was chosen for the study because its ready availability recommends it for use in the textile and other areas of human endeavors. The root of Ansosila (*Impatiens tinctoria)* was obtained from Gujicha kebele, Arsi, Oromia Regional State, Ethiopia.

3.1.2 Fabrics

Cotton fabric (canvas) purchased from Adama garment industry was used in the dyeing study. It's mass per unit area was 144.3 g/m^2.

3.1.3 Chemicals

All laboratory grade chemicals and reagents used for the extraction of *Impatiens tinctoria* were purchased commercially from local chemical markets. Aluminum sulfate ($Al_2(SO_4)_3.18H_2O$, Sigma Aldrich), ferrous sulfate ($FeSO_4.7H_2O$, 99%, Sigma Aldrich) and copper sulfate ($CuSO_4$, 98%, Sigma Aldrich) were used as mordants to fix dyes on fabrics and induce a variety of shades. Acetic acid (CH_3COOH, 99%, Sigma Adrich) was used to control the pH of the dye bath. Distilled water was used to extract plant roots and to prepare the solution and for washing. Soda Ash (Na_2CO_3) was used to scour the fabric. NaOH and H_2O_2 were used to bleach the fabric. A commercial detergent was used to wash the cotton fabric after dyeing.

3.1.4 Equipment and testing devices

Table 4: Equipment and testing devices with their functions

Equipment and testing devices	Function
Digital electronic balance	To measure weight of samples
Whatman filter paper	To filter extracts from the residue
Dye bath	To dye the fabric
Launder meter TF418	To test the color fastness to wash
Crock meter TF410	To test Color fastness to crocking/rubbing
Perspirometer TF416A	To test the color fastness to perspiration
Ultraviolet (UV)-visible spectrophotometer	To measure the Absorbance
pH-meter	To measure pH
Fourier Transform Infrared (FT-IR)	To identify functional groups in a molecule

3.2 Methodology and Experimental Designs

3.2.1 Study Sites Description

The extraction of the plant roots of *Impatiens tinctoria* was carried out at Applied Chemistry Laboratory of the Adama Science and Technology University (ASTU). UV-Vis characterization was performed at ASTU Applied Chemistry Laboratory and FT-IR was performed at Bahir Dar University. Color strength and fastness tests were examined at Ethiopian Textile and Garment Industry and Development Center, Kality, Addis Ababa.

3.2.2 Collection and Preparation of the Plant Sample

Indigenous and locally available root of *Impatiens tinctoria* (1kg) was harvested in the vicinity of the Gujicha kebele, Arsi, Oromia Regional State, Ethiopia. The collected plant material (1kg) was washed with water, the outer part peeled off and grated and chopped to fine paste (since the sample is in wet form) (Benli 2022).

3.2.3 Extraction of natural dyes

The extraction of Ansosila plant root was done according to the procedure in figure 13 below.

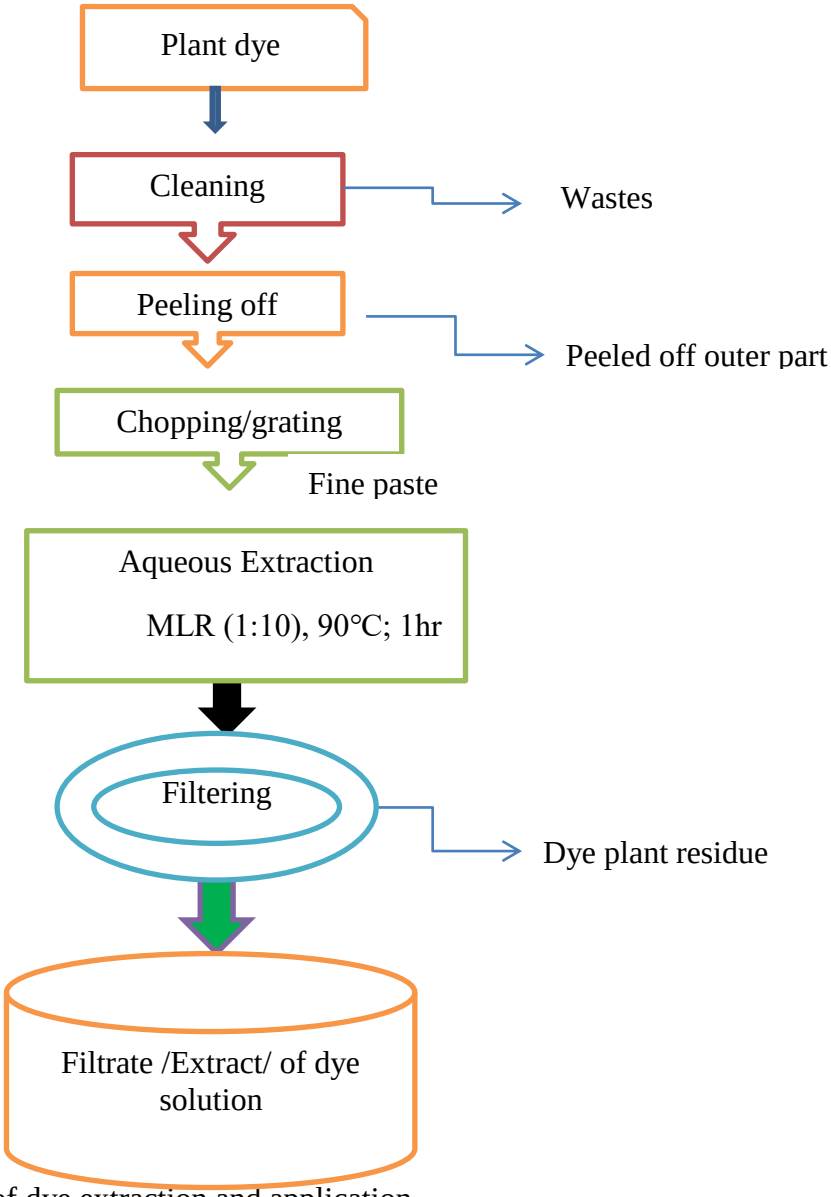


Figure 13: Flow sheet of dye extraction and application

The chopped Ansosila root was mixed with distilled water with MLR of (1:10) and boiled for 1hr with gradual increment of temperature to 90℃ (Chungkrang and Bhuyan 2020). Afterward the resulting solutions were filtered to remove the residue, cooled and stored for dyeing. The pH of the solution was also recorded (Ragab, Hassabo, and Othman 2022).

3.2.4 Fabric pretreatment

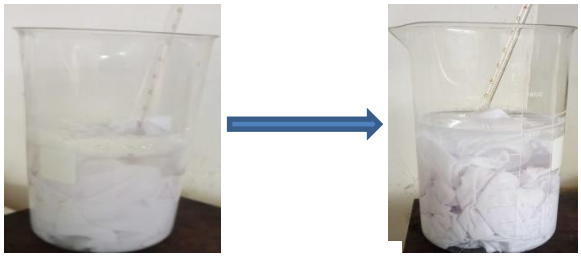
Each cotton fiber is a single cell that develops from the epidermis of the seed (Kamrmakar, 1999). About 94% of cotton fiber is cellulose. As reported in Cook (1993) cotton can be used in all types of garments and household fabrics, being used in boots and shoes, carpets and curtains, and clothing and hats (Davulcu *et al*. 2014).

3.2.4.1 Scouring

Starch, dirt, and grease that were found in the materials were removed by scouring. The fabrics were weighed before they were scrubbed. Soda ash made for 2% of the cloth weight, whereas detergent accounted for 5.5%. After vigorous stirring, warm water was added to the salts at a liquor ratio of 1:10. After adding the pre-weighed cloth, the temperature was maintained at 85 °C for an additional 45 minutes while stirring (Asaduzzaman *et al.* 2016). Figure 14A shows the scouring of cotton fabrics.

3.2.4.2 Bleaching

Bleaching is a chemical process used to get rid of the substrate's natural coloring. Organic compounds with conjugated double bonds are the source of natural color. By performing chemical bleaching, the chromophore is broken, most likely removing one or more double bonds in this conjugated system. The scoured fabrics were bleached using 10 g of NaOH and 8 ml of H_2O_2 in 800 ml of distilled water, sustained at 90℃ for 40 min. After washing the cloth in sterile water, the fabrics were neutralized with acetic acid (CH_3COOH) (Asaduzzaman *et al.* 2016). Figure 14B shows the bleaching of cotton fabrics.



A

Scouring

Soda ash + Detergent + fabrics

85°C; 45mins

B

Bleaching

NaOH + H_2O_2 + scoured fabrics

Figure 14: Fabrics pretreatments

3.2.5 Mordanting

Cotton needs to be mordanted since it is harder to dye than wool and silk because it lacks the amino- and carboxyl-groups that serve as attachment sites for dye molecules (Saxena and Raja 2014). Using the aforementioned mordanting techniques, 15% each of copper (II) sulfate, aluminum sulfate, and ferric (II) sulfate were added to the textile fabrics. This was carried out for 1hr at 75°C, with liquor ratio of 1:40 and constant stirring acts as the firm fixative agent. Cotton samples that had been treated were pressed and dried (Davulcu *et al.* 2014; Baaka 2022; Otutu *et al*. 2019; Benli 2022). Figure 15 illustrates the pre-mordanting and dyeing of cotton fabric.

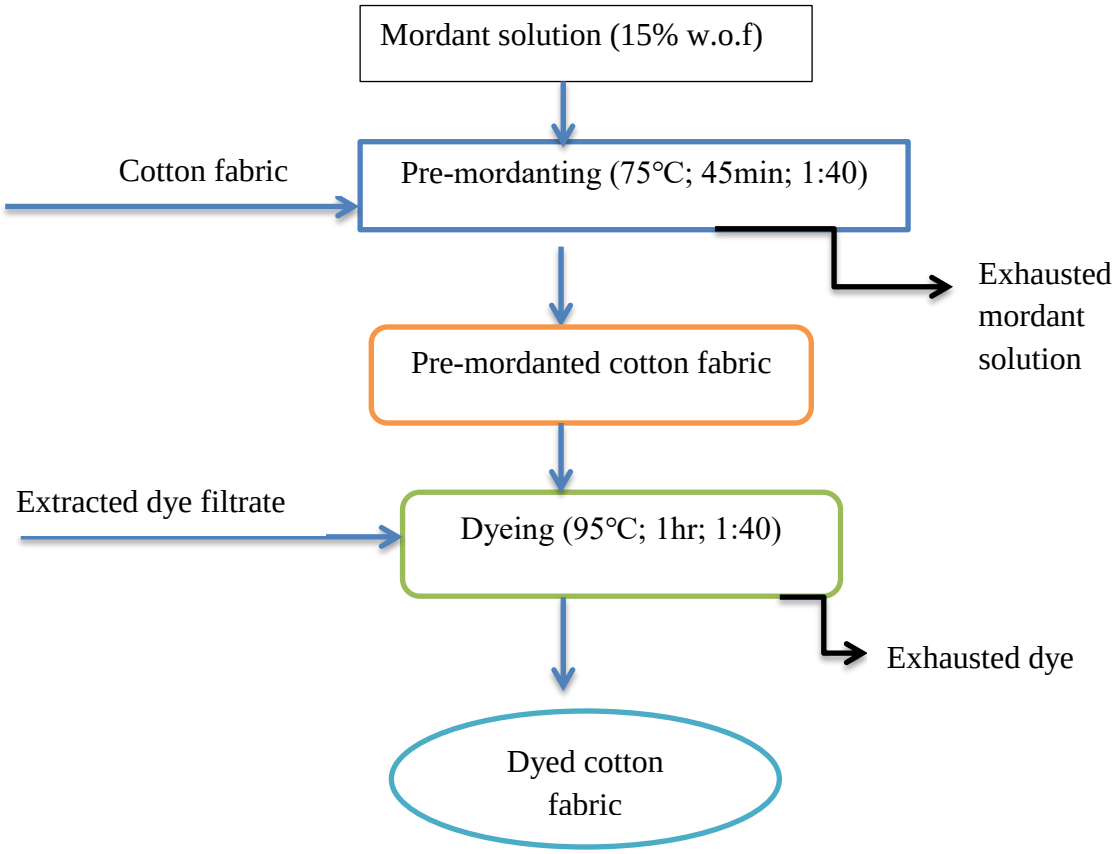


Figure 15: Simple illustration of pre-mordanting and dyeing methods.

3.2.6 Dyeing

Dyeing was done both with and without mordanting procedures to examine the dyeability of cotton by the plant dye. Cotton fabrics were applied with natural dye using pre-mordanting, simultaneous-mordanting and post-mordanting methods. The pre-treated cotton fabrics were individually soaked in ansosila-based bio-dye. In order to obtain the colors, no environmentally damaging solvent was employed. Only water was utilized in all coloring operations. Each sample of mordanted and unmordanted cotton was heated for 60 min at 95°C with a dye bath of liquor ratio of 1:40 at neutral pH. The cotton samples were then taken out of the dye baths and given a 10 min cold rinse with distilled water. The samples of naturally dyed cotton were then dried at room temperature (Davulcu *et al.* 2014; A. Haji 2019; Ren *et al.* 2019).

3.3. Characterization Techniques

3.3.1. Phytochemical Screening Tests

The filtered crude extract of *Impatiens tinctoria* was examined for the presence of secondary metabolites such as flavonoids, terpenoids, saponins, alkaloids, tannins, phenolic compounds and carbohydrates using standard techniques and reagents (Shaikh and Patil 2020).

3.3.1.1 Test for Flavonoids

It was determined whether flavonoids were present or absent using the ammonia test procedure. 5 ml of dilute ammonia solution was added to aqueous filtrate of plant extract followed by addition of concentrated H_2SO_4. The appearance of a yellow color, which vanished upon standing, demonstrates the existence of flavonoids.

3.3.1.2 Test for Saponins

In a test tube, 10 ml of extract was mixed with 10 ml of distilled water. The mixture was vigorously shaken to check for steady persistent growth. The presence of saponins is indicated by the creation of 1 cm layer of foam.

3.3.1.3 Test for Tannins

In a test tube, a 10 ml extract was heated with 10 ml of distilled water before being filtered. After adding two drops of ferric chloride, the color of the solution was checked. Tannins are present when a brownish green or blue-black coloring forms.

3.3.1.4 Test for Alkaloids

Wagner's test was used to determine whether alkaloids were present. Wagner’s reagent (iodine in potassium iodide) was applied along the test tube's sides to a few mL of the extract. The development of a creamy white/yellow precipitate implies successful outcomes.

3.3.1.5 Test for Terpenoids

10 ml of the extract was mixed with 2 ml of chloroform and 1ml of concentrated sulfuric acid. The formation of a monomer that gave reddish brown color is a beneficial consequence for the terpenoids.

3.3.1.6 Test for Phenols

To determine whether phenolic chemicals were present, the ferric chloride test was used. A few drops of 5% ferric chloride solution were added to the extract aqueous solution. Phenols are present when dark green or blueish black color forms.

3.3.1.7 Tests for Reducing Sugars

To determine whether reducing sugars were present, Benedict's test was used. Benedict's reagent and 0.5 mL of filtrate were heated for 2 minutes. Green/yellow and red color development indicates the presence of reducing sugar.

3.3.2 The UV-Vis Absorbance

Using a quartz cell with a path of 1cm, the UV-P9 (UQS2102010) spectrophotometer was used to measure the UV-Vis absorbance of the extracted solution. To create the sample's specific absorbance spectra, absorbance measurements in the range of 200-800 nm regions were taken. By measuring absorbance at the wavelength of the maximum peak or at a specified wavelength, the extracted solution was evaluated.

3.3.3 FT-IR Spectroscopy Analysis

FT-IR spectral analysis was used to determine the functional groups in the natural dye. The concentrated fraction of *Impatiens tinctoria* root extract was analyzed for the functional groups present, using Fourier Transform Infrared (FT-IR-84000S) spectroscopy. The FT-IR spectrophotometer was operated at a spectral range of 400-4000 cm^{-1} with a maximum resolution of 0.9 cm^{-1}.

3.3.4 Assessment of Color Fastness for Dyed Cotton Samples

Following dyeing, fabric samples were tested for color fastness, including fastness to rub, fastness to wash and fastness to perspiration using the AATCC's recommended gray-scale techniques (in accordance with ISO 105/A03) (Rasel *et al.* 2022).

3.3.4.1 Fastness to Wash

The AATCC test method ISO 105-C03 was employed to evaluate the samples' wash fastness. The "wash fastness" property of a dyed sample prevents the color from fading when the dyed material is washed or cleaned. This technique's washing agent was a 1:1 solution of ordinary soap and sodium carbonate. The required quantity of soap solution was added to a container, together with a prepared 4×10 mm specimen, to make the MLR 1:50. The specimen was subsequently cleaned in a launder-o-meter for 30 min at 60 ℃. A gray scale was used to test the fastness values to fading and staining.

3.3.4.2 Fastness to Rubbing

Both dry and wet rubbing fastness was assessed using a manual rubbing fastness tester machine. According to ISO 105 X12:2002 test procedures, rubbing fastness testing was performed on the dyed samples. To perform a dry rub, a colored sample was rubbed 10 times at a speed of 60 cycles per minute against a standard white cloth to see if the color has faded. The same process was used to determine the wet rubbing fastness. The standard grading system for various colored fabrics is then used to compare the faded color on the standard white cloth.

3.3.4.3 Fastness to Perspiration

Color fastness of dyed or printed fabrics, against perspiration was determined by exposing the fabric to the action of both alkaline and acidic reagents, while in contact with undyed adjacent fabrics on both sides. The AATCC Test Method (ISO 105-E04) was employed to assess a fabric's colorfastness against perspiration. In this study, a perspirometer called TF416A was utilized to assess the specimen's colorfastness to perspiration using acid and basic solutions. The specimens were prepared with the size of 4×10 mm.

CHAPTER-FOUR

4. RESULTS AND DISCUSSION

The procedure for dyeing cotton with *Impatiens tinctoria* with and without mordants and mordanting methods were covered in this thesis. The experimental results on the impact of the mordants are then discussed in accordance with the interaction mechanism. Existences of secondary metabolites, UV-Visible and FT-IR characterizations of *Impatiens tinctoria* were clearly discussed. Finally, the observations from the dyeing experiments are clarified using the modeling results.

4.1 Extraction of Natural Dyes

This work uses deionized water to extract pigments from *Impatiens tinctoria* root. Figure 16 shows the whole procedures undertook to extract the dye from the plant.

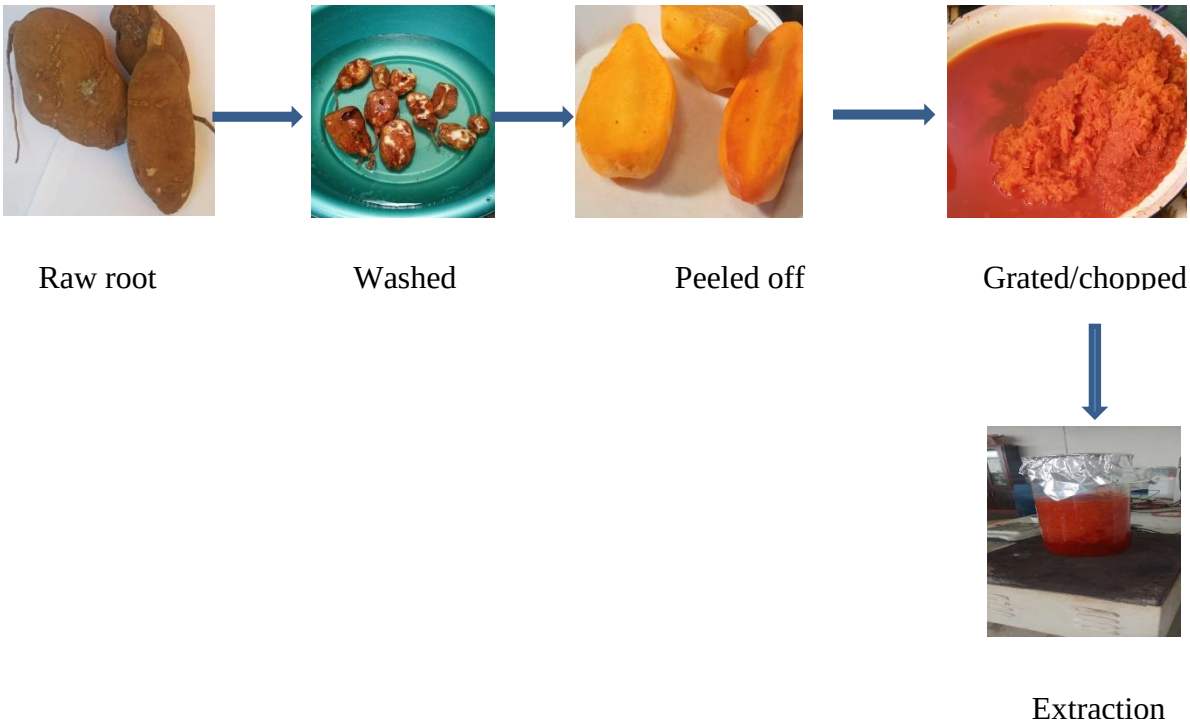


Figure 16: Extraction procedures

Figure 17B displays pictures of the pigment extracts and the by-products (figure 17A) of the extraction procedure. The aqueous extracted dye had a pH of 5.4 at 25 °C. The acidic pH of dye extracts may be caused by the presence of acidic compounds such as gallic acid or tannic acid as well as additional coloring components in each source (Ayele *et al.* 2020). When acid dyeing is used, a low pH aids the hydrogen bonding that attach acid colors to protein fibers like silk, wool and nylon. However, cellulose fibers do not attach to acid dyes like cotton fibers do. At a pH that is a little high, cellulosic fibers can be colored. When cotton fiber is activated by a high pH, a cellulosate anion is created. So, the extracted dye solution was neutralized to pH using dilute solution of sodium carbonate before dying.

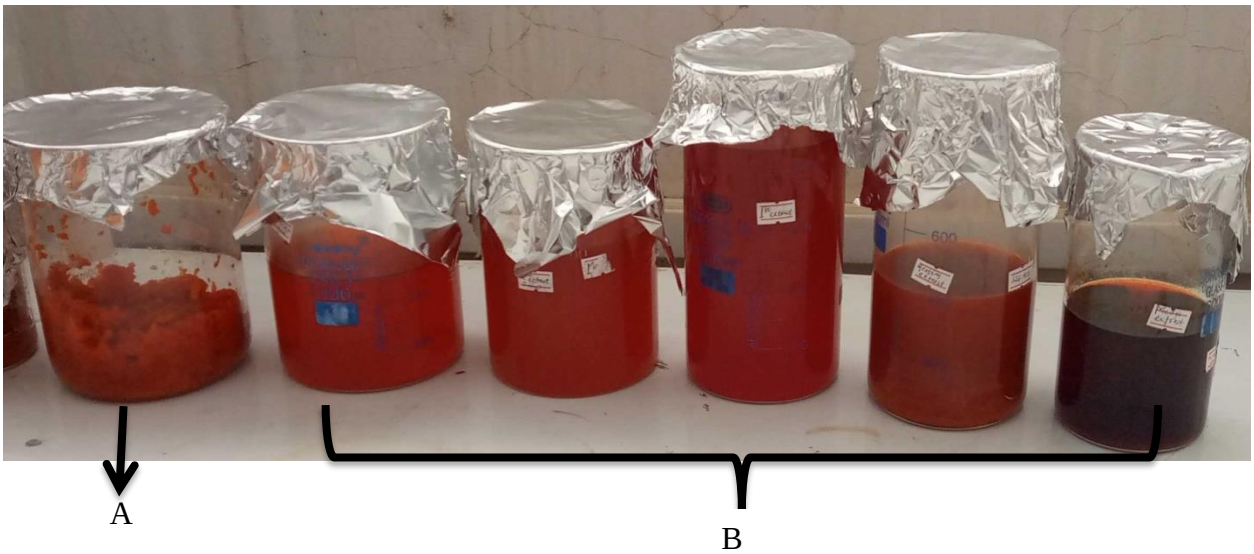


Figure 17: Residue (A) and extracts of natural dyes of *Impatiens tinctoria* root (B)

4.2 Phytochemical Screening Test

Several categories of phytochemicals were investigated as part of phytochemical analysis. The outcomes of the phytochemical testing are displayed in Table 5 and figure 18. The *Impatiens tinctoria* roots' aqueous extract underwent a phytochemical screening test, which revealed the presence of terpenoids, flavonoids, tannins, alkaloids, reducing sugar and phenol but not saponins. The fact that this plant has been used for coloring may be due to the presence of many kinds of phytochemicals. Brown colorations show the vital role of flavonoids and tannin

molecules in the coloring process. Terpenoids are a source of tastes, fragrances, and odours (Jelagat, Cherutoi, and Mecha 2022).

Table 5: Phytochemicals screening test results of aqueous extract of *Impatiens tinctoria* root

No.	Phytochemicals	Observation	Test results
1	Flavonoids	Magenta red color observed	Positive
2	Terpenoids	Greyish color observed	Positive
3	Saponins	No formation of stable foam	Negative
4	Tannins	Brown color	Positive
5	Alkaloids	Formation of precipitate	Positive
6	Phenol	Bluish black color present	Positive
7	Reducing sugar	Green color development	Positive

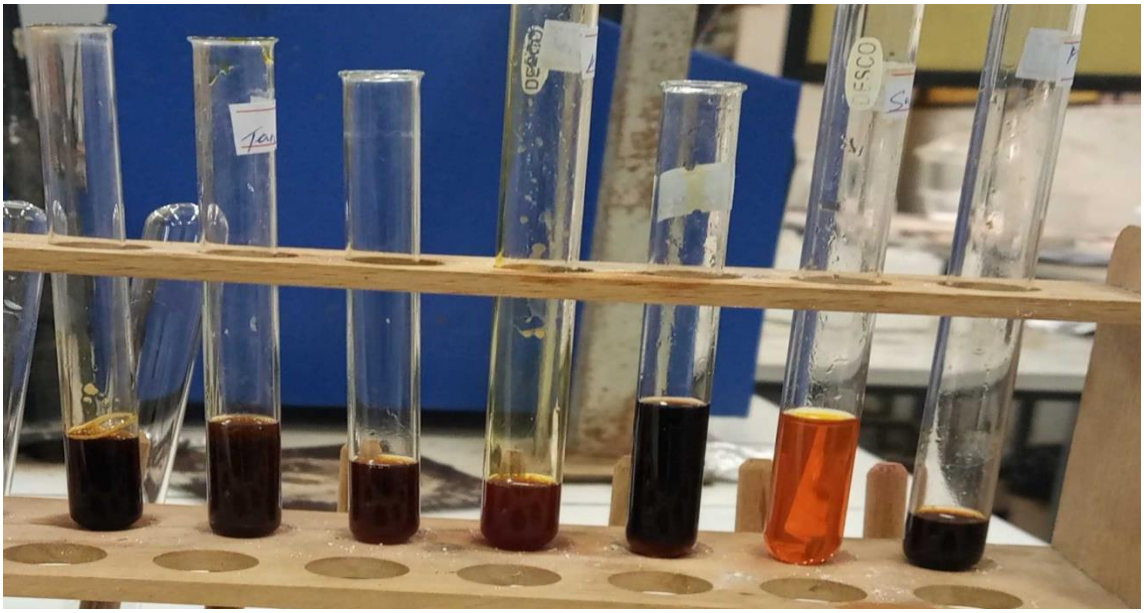


Figure 18: Phytochemical tests of aqueous roots extract of *Impatiens tinctoria*

4.3 Fabric pretreatment

Making ready the cotton cloth for scouring and bleaching is the first stage in the dyeing, printing and finishing processes. In order to create a fabric that is sufficiently hydrophilic, scouring cotton textiles is an essential step in the wet processing of textiles. During scouring, wax and other hydrophobic materials were eliminated from the fabric. The materials were prepared for other processes like bleaching and dyeing once these contaminants were removed through scouring. Cotton fibers are off-white in hue due to the presence of color bodies; bleaching is the process of removing these color bodies from fibers (Morshed *et al*. 2014).

Bleaching has as little fiber loss as possible while transforming multicolored materials into white cloth. Bleaching agents are used for this. The colorants that are removed by the bleaching agents are either get smaller or oxidized, leaving behind a permanent whiteness. After bleaching, the material looks whiter. In addition to other crucial factors, the effectiveness of different bleaching sequence combinations is evaluated in respect to weight loss, reflectance, flexibility, and light fastness. Cotton's natural coloring components can be removed with strong oxidant hydrogen peroxide without causing unintended oxidative harm to the fibers. In an alkaline medium the following equilibrium equation is set up.

$$H_2O_2 + NaOH \rightarrow Na^+ + HO_2^- + H_2O \ (1)$$

To increase the concentration of each perhydroxyl (HO_2^-) ion, the bleaching agent, and hence increase the bleaching activity, alkali has the effect of shifting the equilibrium to the right, according to this equation. Before the dyeing procedure, the pretreated materials were neutralized with acetic acid from their alkaline state.

4.4 Fabric Mordanting and Dyeing Process

According to the experimental findings, *Impatiens tinctoria* root extract was successfully used to color cotton garments. Due to various mordanting techniques, the fabric developed a variety of color shades. Figure 20 displays photographs of cotton fabrics colored with *Impatiens tinctoria*

root extract both with and without a mordant. Because one of the goals of mordanting is to bind the dye on the cotton fabric in the form of metal complex formation, which leads to an increase in dye uptake, these observations were expected. Mordants and colorants have an affinity for one another, and cellulosic fabrics create stable complexes with the colorants in the matrix of the fabric (Jelagat, Cherutoi, and Mecha 2022).

According to Kechi, Chavan, and Moeckel (2013), this is because the metal ions of mordants function as electron acceptors from electron-donating groups of dye to establish coordination bonds with the dye molecule, rendering them insoluble in water. This is attributed to more dye sites provided by mordanting process. These may be justified on the basis that natural coloring agent contains ionizable groups such as –OH and CO_2H. In aqueous solution, these –OH groups become soluble because of their conversion in anionic systems. Mordants with metal ions with vacant orbitals of the suitable energy have the ability to create complexes between fiber, mordant and dye molecule known as chelate that shifts the natural dye and, is most effective with natural fibers (Ayele *et al.* 2020). This is depicted schematically in Figure 19.

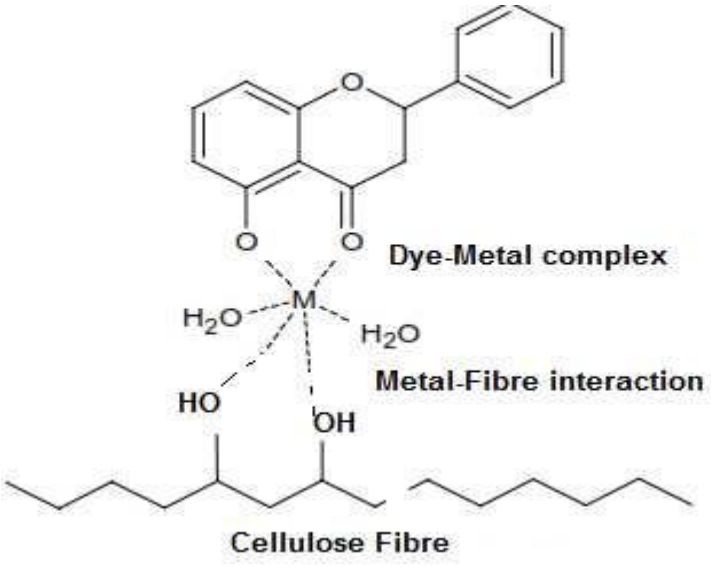


Figure 19: Schematic representation of Dye-Metal-Fiber interaction (Ayele *et al.* 2020)

The main steps involved in dyeing cellulosic substrates are reduction of insoluble dyes, (e.g., vat, sulfur, indigo) to the soluble leuco form, impregnation of the cellulosic fibers with the dye solution, dye absorption and penetration within the cellulosic structure, fixation of the dye

on/within the cellulose (cell, -OH) structure via chemical bonds with its –OH groups, i.e., dyeing sites (Figure 19), hydrogen bonds and Van der Waals forces. The vacant locations on the metal ion interaction with the fiber can be occupied by functional groups like hydroxyl, amino or carboxy on the fiber. As a result, the metal ion forms a ternary complex in which one site is attached to the fiber and the other site is coupled to the dye (Baaka 2022).

The type of metal salt employed for complex formation affects the color that is acquired after mordanting. The results and observations show that utilizing aluminum sulfate, copper sulfate and ferrous sulfate to mordant the vegetable dye yields lovely orange-brown to light red-pink hues and darker brown shades. The effects of the different types of mordants and mordanting techniques can be seen in the variations in color shades. This is due to the incorporation of the metal atom into the delocalized electron system of the dye. Metallic mordant have somewhat low energy levels, therefore, their incorporation to a delocalized system effects in reducing the overall energy. The absorbance of the hue and thus its color is related to this phenomena (Ayele *et al.* 2020).

The strong coordination propensity of the metal ion is what gives cotton fibers their rich, dark brown color when ferrous sulfate is present. High dye uptake is produced as a result of Fe^{2+}'s enhancement of the interaction between the fiber and the dye (Rasel *et al*. 2022). This is because the natural dyes have formed metal complexes with the appropriate functional groups *(Ayele et al.* 2020). Therefore, it may be concluded that the type of mordant significantly affects the intensity of the dyed material's color.

With ferrous sulfate, the color shade was darker, which may be associated with the change of ferrous sulfate into a ferric form by reacting with oxygen in the air. Literature had demonstrated that ferrous and ferric forms coexist in the fiber, and their spectra overlap, which results in a shift in λ_{max} and consequently, a color change to a darker shade (Shin and Lee 2006). Additionally, the tannins combined with ferrous salts to form complexes, which also result in a darker shade of fabric (Baaka 2022).

Note: The color of the undyed cotton fabric is pure white.

Figure 20: Photographs of dyed Cotton fabric samples.

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[1] PrMCS = Premordanting $CuSO_4$

[2] SMCS = Simultaneous Mordanting $CuSO_4$

[3] PoMCS = post-mordanting $CuSO_4$

[4] PrMAlS = Premordanting $Al_2(SO_4)_3$

[5] SMAlS = Simultaneous Mordanting $Al_2(SO_4)_3$

[6] PoMAlS = Post-mordanting $Al_2(SO_4)_3$

[7] PrMFS = Premordanting $FeSO_4$

[8] SMFS = Simultaneous Mordanting $FeSO_4$

[9] PoMFS = Post-mordanting $FeSO_4$

[10] unmordanted

4.4 UV-Visible Spectroscopy Analysis

UV-Vis spectrophotometric technique is used to determine the wavelength and intensity of ultraviolet and visible light absorbed by a sample. The UV-Vis spectrophotometry method's fundamental principle is based on the measurement of the wavelength and intensity of ultraviolet and visible light absorbed by the sample as a function of wavelength. In functional groups known as chromophore, the absorption of radiation promotes the excitation of electrons from the ground state to the excited state. According to the kind of electrons present in the analyzed molecule, electron excitation that takes place in UV-Vis spectrophotometry is recorded in the form of a spectrum expressed as wavelength and absorbance. The easier the electrons to excite, the greater the wavelength that is absorbed, the more electrons are excited, the higher the absorbance (Pratiwi and Nandiyanto 2021).

The UV-Vis absorption spectra of an Ansosila-based dye are displayed in Figure 21. At 445 nm, a significant absorption peak is located. In addition to the primary peak, the absorption spectra also showed a peak at 353 nm. It is observed that the sample chemical contains two chromophores. However, it's possible that one type of chromophore has more than one absorption band. Two bands with maximum values over 200 nm often suggest an aromatic system.

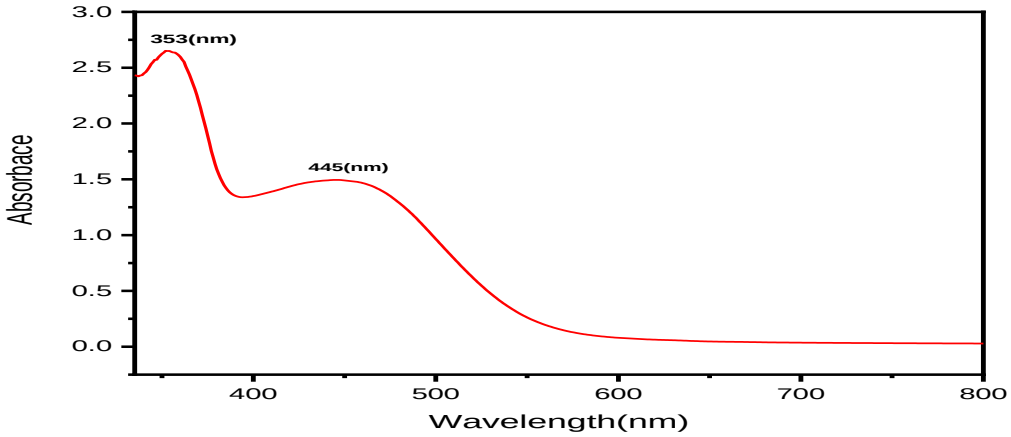

Figure 21: UV-Vis absorbance of *Impatiens tinctoria* root extract.

According to principle, UV-Vis spectroscopy is generated by electronic transition (excitation) from one electronic level to another level which is associated with simultaneous rotational and vibrational transitions as well. Absorption takes place over a range of wavelengths rather than at one fixed point and hence we get a broad peak (figure 21). The reason behind it may be the presence of molecules that have many excited states for rotation and vibration, an electronic energy level has in itself various rotational and vibrational energy states, i.e. the energy levels for these states are quite closely spaced having energy difference very smaller as compared to electronic energy levels.

The electronic transitions are generally between a bonding (σ and π) and lone-pair orbital and an un-filled non-bonding (n) or anti-bonding (σ* and π*). Since UV-Vis spectrophotometry needs the presence of a chromophore group in the molecule, the majority of applications of UV-Vis spectrophotometry methods on organic compounds are based on n → π* or π → π* (Pratiwi and Nandiyanto 2021). The chromophore that can absorb light between 300–665 nm absorption range is the result of the nitroso chromophore's presence in the dye from Ansosila root dye with usually n → π* transition which confirms the presence of chromophore with lone-pairs of electron.

4.5 FT-IR Spectroscopy Analysis

Far-IR (400cm^{-1}), mid-IR (400-4000 cm^{-1}) and near-IR (4000-13000 cm^{-1}) are the three wavenumber zones that make up the IR spectrum. Although far- and near-IR spectrums also contribute to providing information about the sample analyzed, the mid-IR spectrum is the one that is most frequently used in sample analysis (Nandiyanto, Oktiani, and Ragadhita 2019). The analysis of FT-IR data in the mid-IR spectrum was the main focus of this work.

The FT-IR spectra of *Impatiens tinctoria* root extract is displayed in Figure 22. There are more than five peaks in total, indicating that the analyzed compound is not a simple chemical. Coates (2000) reports that the broad absorption bands, which can be observed at 3390 and 3792 cm^{-1}, indicate the presence of hydrogen bonds in the sample as well as the stretching vibration of single bonds, which may contain -OH, N-H, and C-H bonds.

As opposed to the broad band that generally appears between 3250 - 3500 cm^{-1} specifically showed the hydroxyl group, H-bonded OH stretch. The –OH and C-H peaks signify the presence of many forms of carbohydrates, including lignin, cellulose, hemicelluloses and pectin as well as hydroxyl groups from alcohols. While the bond at 2358 cm^{-1} is linked to the vibration of the NH amino-related component bond, the band at 2919 cm^{-1} is attributable to the vibrational stretching of the C-H bond caused by alkanes which is methyl. These nitrogen-containing functional groups suggest that betalains, which are N-containing chemicals, are present in the roots of *S. fax-imperii* (Nandiyanto, Oktiani, and Ragadhita 2019).

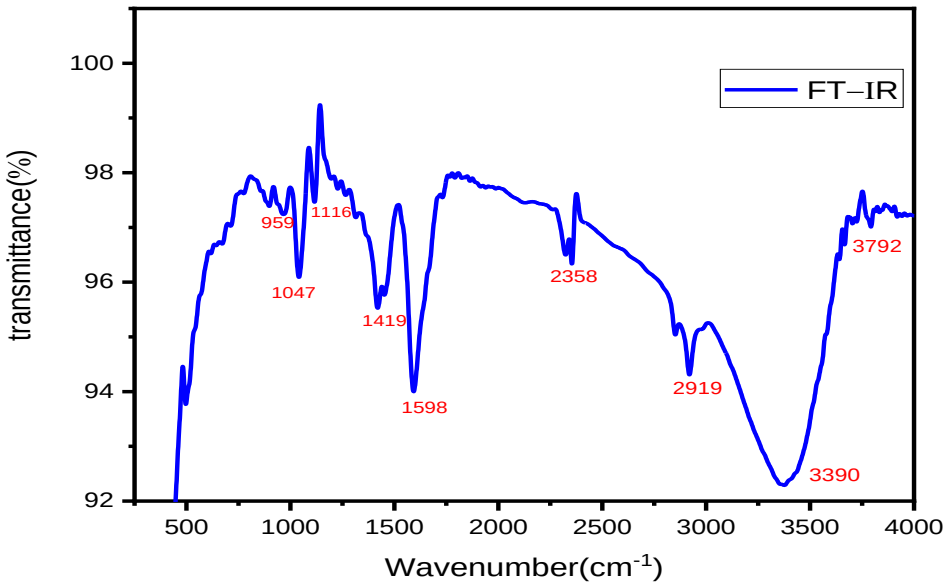

Figure 22: FT-IR spectrum of *Impatiens tinctoria* roots extract.

The secondary amine (amide) >N-H bend of the organic components in the extract may match to the band at 3792 $cm^{-}1$. C=O is relevant to band at 1598 cm^{-1}. The vinyl C-H in-plane bend vibration is attributed to the band at 1419 cm^{-1}. On the other hand, the common inorganic sulfate ion's bond may be caused by the band at 1116 cm^{-1} which absorbs between 1080 and 1130 cm^{-1}. The cyclohexane ring vibrations, primary alcohol, and stretch of the C-O may all be represented

by the band at 1047 cm^{-1}, while the trans-C-H out-of-plane bend is represented by the band at 959 cm^{-1}.

Different molecules' electrical and vibrational characteristics are influenced by one another through vibronic coupling in relation to the molecular arrangement. According to Rotich, Wangila, and Cherutoi (2022; Lee et al. 2013), vibronic coupling can predict the visible absorption spectra and color of biomolecules, including those of several biological pigments including betamins, flavins, carotenoids and anthraquinones. According to Kumar *et al.* (2018), the alterations are attributable to an increase in the number of functional groups connected to the –OH molecular bond, which causes its stretching vibration (referred to as –OH wagging in phenolic compounds). Peak changes will be expected since the –OH group assists in bonding throughout the dyeing process.

Functional groups that correspond to phenolic compounds associated with –OH (3390 cm^{-1}), C-O (1047 cm^{-1}), olefinic (alkene) associated with trans-C-H out-of-plane bend (959 cm^{-1}), aromatic ring (aryl) group related to C=C-C aromatic ring stretch and C=O at 1598 cm^{-1}, secondary amino group at (3792 cm^{-1}), vinyl C-H in-plane bend vibration is attributed to band at 1419 cm^{-1} and common inorganic sulfate ion (1116 cm^{-1}) were all able to be identified through FT-IR analysis.

4.6 Evaluation of Color Fastness

Table 6 shows the color fastness measurements of cotton samples dyed by various mordanting methods. All the color fastness tests were performed following the standard testing protocols. For consumers' expectations, colored clothing materials must have improved fastness capabilities. When fabric is correctly dyed, all of the fibers absorb the dye and the color is permanently bonded to the fiber, sustaining it from various mechanical, physical and chemical treatments. On the other hand, a cloth with inadequate colorfastness implies that the dyeing process was insufficient or that the dye chosen did not correspond to the fabric's requirements (Rasel *et al.* 2022).

Color development and dye absorption capacity of the cotton fabric were assessed in terms of CIE lab coordinates and k/s (color strength) values; as result, color fastness was conducted using the standard procedures of the AATCC's recommended gray-scale techniques in accordance with ISO 105/A03, and the colorfastness ranged from very good to excellent in the range of 3-5 against a gray-scale of 1-5 for all the tested fastness (Jelagat, Cherutoi, and Mecha 2022). The color fastness to washing, rubbing and perspiration were evaluated for the dyed cotton fabric with the dye extract from *Impatiens tinctoria*.

Table 6: Color fastness results of dyed cotton fabrics

Types of mordant	**Mordanting methods**	**Color Fastness**				
		wash	perspiration		Rubbing	
			Acid	Base	Dry	Wet
$CuSO_4$	Pre –mord	4.5	4	4	4.5	4
	Simult-mord	5	4	4	4	3.5
	Post-mord	4.5	4	4	4	3.5
$FeSO_4$	Pre-mord	4.5	4	4	4.5	4
	Simult-mord	5	4	4	4.5	4
	Post-mord	5	4	4	4.5	4
$Al_2(SO_4)_3$	Pre-mord	4.5	4	4	4.5	4
	Simult-mord	5	4	4	4	3.5
	Post-mord	5	4	4	4.5	4
No mordant	------	5	4	4	4.5	4

4.6.1 Color Fastness to Rubbing

Color fastness to rubbing/crocking is a fundamental test that assesses the degree of color transfer from dyed cloth to other surfaces by rubbing or crocking the fabric using the ISO X12 standard test protocol. A poor color fastness property is indicated by the transfer of color from the tested fabric to other fabrics, particularly to standard white cloth (Werede *et al*. 2021). In this test, a crock meter or rub fastness tester with a rubbing finger made of a 16mm diameter cylinder that moves 107mm with 9N is used.

In terms of both dry and wet rubbing, the rubbing fastness was assessed. Figure 23 shows that, for all pre-mordanting dyeing procedures, dry rubbing produced results of 4.5, with the exception of simultaneous mordanting with $CuSO_4$, $Al_2(SO_4)_3$, and post-mordanting $CuSO_4$, which produced results of 4. This is an indication of very good to excellent rating. Unmordanted cotton fabric was found to have excellent grades of 4.5 wet rubbing and grades of 4 dry rubbing fastnesses. This demonstrates that Ansosilla (*Impatiens tinctoria*) plant extract can effectively dye cotton cloth.

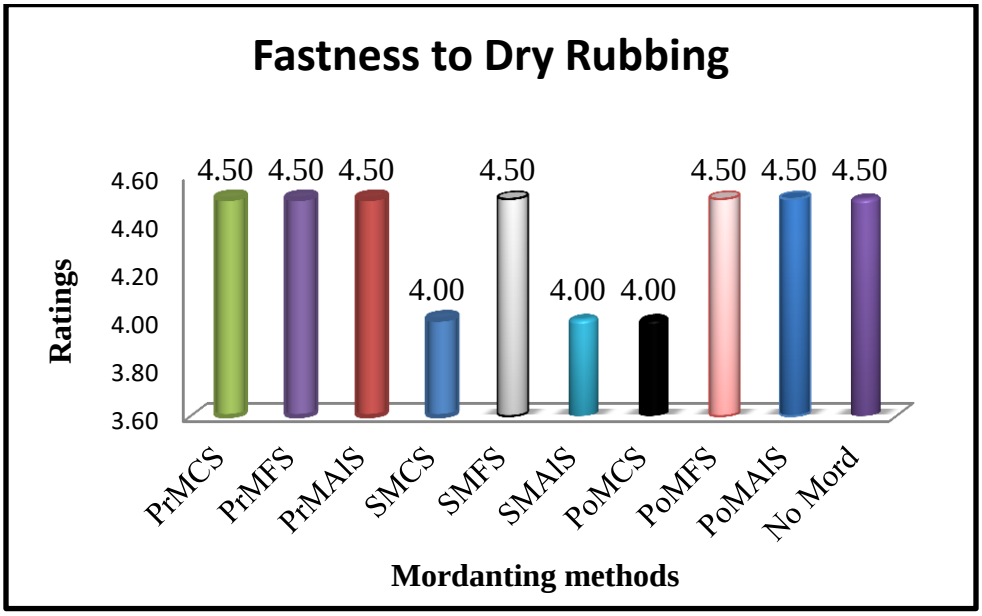

Figure 23: Fastness to Dry Rubbing

Wet rubbing in colored materials was evaluated between 3.5 and 4 (Figure 24). Cotton fabrics dyed with *Impatiens tinctoria* dye demonstrated good rub resistance for commercial use, with all of their values falling between 3.5 and 4.5. The color change grade for the unmordanted cotton was ranked to 4.5. The very good fastness to rubbing for the sample colored without a mordant can be attributed to the coloring component's affinity through H-bonding and Van der Waals forces (Md. K. Uddin *et al.* 2021).

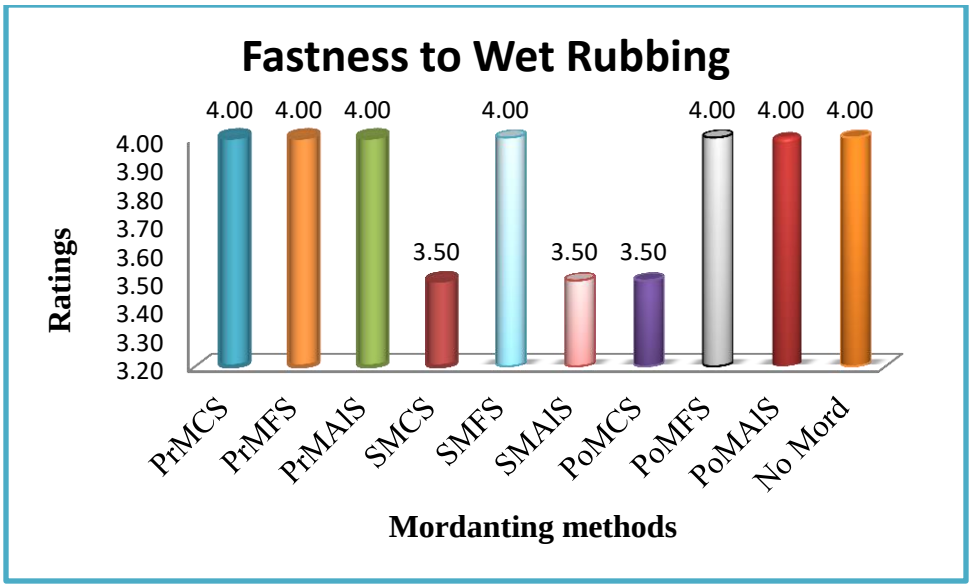

Figure 24: Fastness to Wet Rubbing

Even though both of them fell in the range of permissible gray-scale criteria, dry rubbing had produced better results than wet rubbing. The fiber was complexed with a mordant, which made the dye insoluble and made it colorfast. They demonstrated a good to very good fastness qualities in the colored fabrics.

Among the three selected mordanting techniques, premordanting and post-mordanting exhibited better rubbing performance in both dry and wet rubbing except of post-mordanting $CuSO_4$. Simultaneous -mordanting exhibited lower rubbing performances in both dry and wet rubbing rated to 4 and 3.5, respectively. However, in the case of simultaneous -mordanting with $FeSO_4$ it was as good as the others rated. So, it is clear from Table 6 that the usage of mordants and different mordanting techniques could improve the fastness properties.

4.6.2 Color Fastness to Washing

The wash fastness of the colored samples was assessed in accordance with ISO and AATCC standards for washing fastness testers. According to the wash fastness test results, both mordant and unmordant colored cotton won't stain adjacent white material when washed at 60 °C since the dye from *I. tinctoria* plants has a staining intensity that ranges from 4.5 to 5 (Figure 25).

It was discovered that washing discolored fabrics in soap led to very slight color changes. The wash fastness results are shown in table 6.

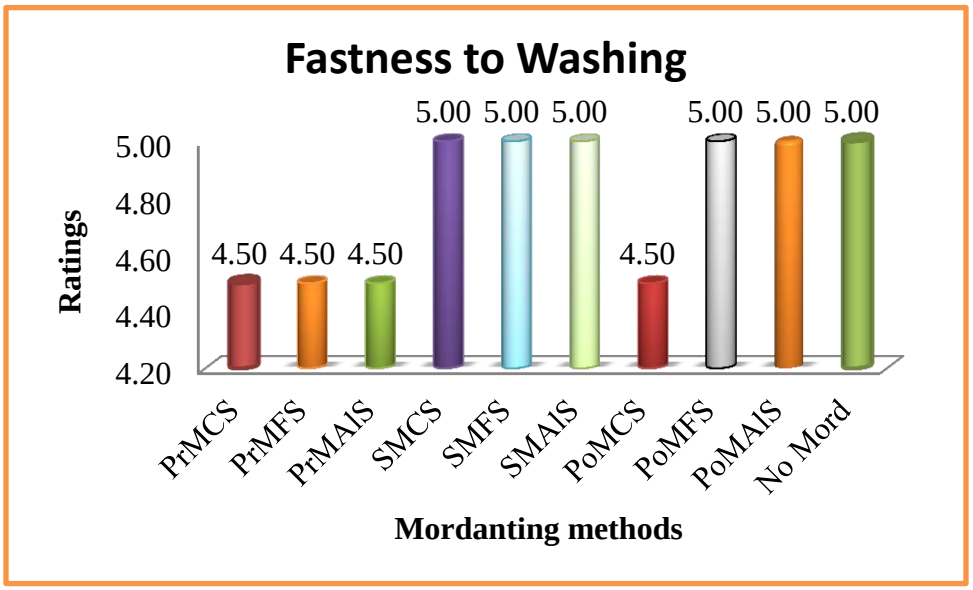

Figure 25: Fastness to Washing

Except for post-mordanting with $CuSO_4$, simultaneous and post-mordanting demonstrated improved washing fastness prior to mordanting. Premordanting techniques exhibited lower washing fastness as rated to 4.5. All dye manufacturers have demonstrated wash fastness grades of ≥ 3, which are commercially acceptable. As the rate of dye molecule diffusion and state of the dyes inside the fiber have an impact on wash fastness, dyes have a tendency for aggregating inside the fiber. This results in an increase in their molecule size and good wash fastness (M. G. Uddin 2015).

Due to the presence of tannin moieties in the dye extract, which increase the cellulosic fabric fastness by introducing extra hydroxyl and carbonyl functional groups for coordination, a stronger mordant-fabric-dye complex within the fabric matrix, large particles of water insoluble dyes and covalent bonding between the fabric and the dye colorant, the sample dyed with natural dyes had very good to excellent wash fastness.

4.6.3 Color Fastness to Perspiration

The results of the tests for color fastness to both acidic and alkaline perspiration all show excellent grades of 4 (figure 26). According to the findings, both pre- and post-mordant materials exhibit good fastness after mordanting. The outcomes support the use of *Impatiens tinctoria* cotton dyeing in perspiration-prone environments. Fortunately, our investigation demonstrated that Ansosila cotton dyeing is appropriate for wear in environments that are likely to be "sweaty".

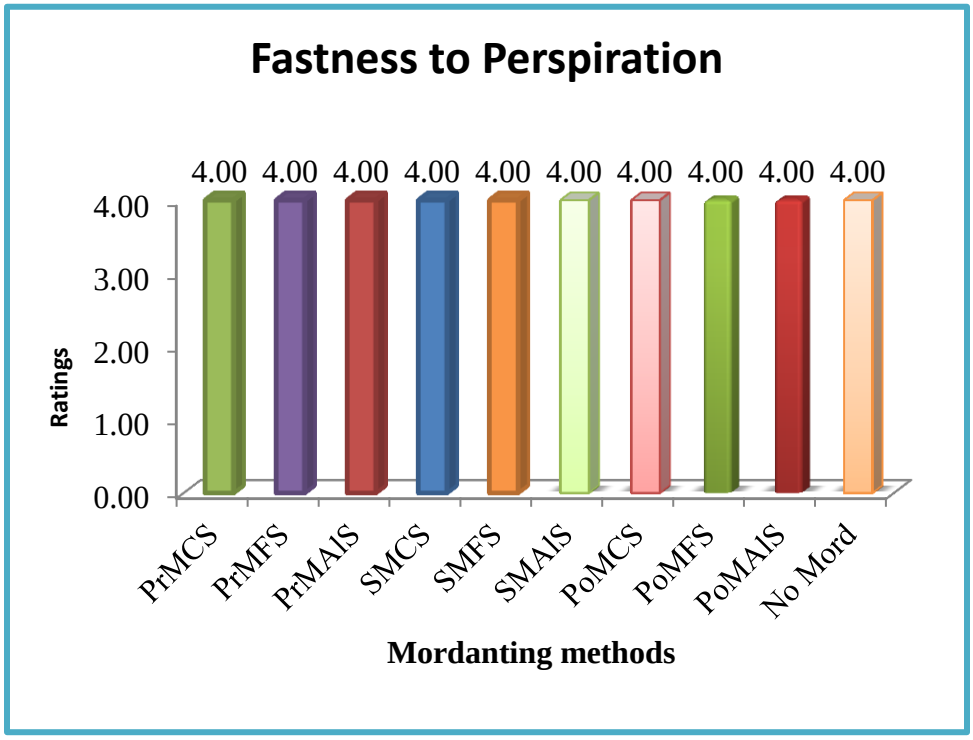

Figure 26: Fastness to perspiration

CONCLUSION

The UV-Vis absorption spectra of an Ansosila-based dye are displayed maximum absorbance at 445 nm. In addition to the primary peak, the absorption spectra also show a peak at 353 nm. So, it suggests that the sample chemical contains two chromophores. As opposed to the broad band that generally appears between 3200 - 3570 cm^{-1} specifically showed the hydroxyl group, functional groups of phenolic compounds associated with –OH (3250-3500 cm^{-1}), C-O (1047 cm^{-1}), olefinic (alkene) associated with trans-C-H out-of-plane bend (959 cm^{-1}), aromatic ring (aryl) group related to C=C-C aromatic ring stretch and C=O group at (1598 cm^{-1}), vinyl C-H in-plane bend vibration is attributed to band at 1419 cm^{-1} and common inorganic sulfate ion (1116 cm^{-1}) and the secondary amine (amide) >N-H bend of the organic components in the extract may match to the band at 3792 cm^{-1} were all able to be identified through FT-IR analysis. The *Impatiens tinctoria* roots' aqueous extract underwent a phytochemical screening test, which revealed the presence of terpenoids, flavonoids, tannins, alkaloids, reducing sugar and phenol but not saponins.

It was discovered that the dyes produced from the Ansosila root worked well as coloring agents for cotton clothing. Utilizing the dyes extracted from the roots of the *Impatiens tinctoria* plant, cotton fabric was successfully colored. Three distinct mordants and mordanting processes were used to produce varied colors of various depths. According to the findings and observations, mordanting vegetable dye with aluminum, copper, and ferrous sulfates produces gorgeous orange-brown to light red-pink colors as well as darker brown shades. All types of mordanting have a favorable effect on the fastness characteristics of dyed samples (rubbing, wash, and perspiration). Premordanting and post-mordanting among the three chosen mordanting procedures shown better rubbing performance in both dry and wet rubbing; with the exception of post-mordanting $CuSO_4$. Wet rubbing did not produce as good of benefits as dry rubbing. Prior to mordanting, washing fastness was enhanced by both simultaneous and post-mordanting, with the exception of $CuSO_4$ post-mordanting. Fortunately, our research showed that Ansosila cotton dyeing is suitable for use in settings that are likely to be "sweaty".

RECOMMENDATION

Additionally, the antibacterial and antifungal qualities of the derived dyes will be the subject of future research. Future research will involve coloring protein fibers with the colors that were extracted. The comprehensive method of dyeing using extracted dyes and an examination of the extracted dye's financial viability will also be included in the future study.

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