EXTRACTION AND CHARACTERIZATION OF NATURAL DYES FROM *Euclea divinorum* AND *Erythrina abyssinica* FOR TEXTILE DYEING

BY

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DECLARATION

Declaration by the Candidate

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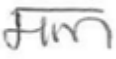
Prof. Josphat Igadwa Mwasiagi ……  ..…Date…8th June 2022……..

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This work is dedicated to Hillary Boit, Harry Kipkoech, Sherryl Chemutai and Shirley Cherotich

ABSTRACT

Most of the dyes used in the textile industry are synthetic but recently the use of natural dyes has regained interest due to health hazards associated with synthetic dyes. Synthetic dyes are non-biodegradable, carcinogenic, allergic to the skin and toxic to the environment, therefore there is need to explore natural dyes to satisfy the increasing demand for environmental friendly dyes. *Euclea divinorum* and *Erythrina abyssinica* plants have been used traditionally by Kenyan communities as a dye but their potential as a dye for textile dyeing has not been exploited. The aim of this study was to extract dyes from the root bark of *E. divinorum* and stem bark of *E. abyssinica* for textile dyeing. The specific objectives were to: extract and characterize the natural dye from *E. divinorum* and *E. abyssinica* plants; optimize extraction and dyeing conditions of the dye extracts; compare the use of bio-mordants and synthetic (metal) mordants with the natural dye extracts on cotton fabric and determine the antioxidants and antimicrobial textile finishing properties of the dyes. Natural dye extraction was done using maceration method and characterized using Fourier transform Infra- Red spectroscopy (FTIR), Gas Chromatography Mass Spectroscopy (GC-MS) and Nuclear Magnetic Resonance (NMR). Optimization of extraction and dyeing conditions was conducted using Response Surface Methodology (RSM). The independent variables were time, temperature and M:L for extraction and pH, time and temperature for dyeing and the dependent variables were absorbance and color strength (K/S) for extraction and dyeing, respectively. Dyeing with metallic (alum, ferrous and tin) and bio-mordants (rosemary and mango extracts) was executed using pre-, post- and meta-mordanting methods. Dyeing characteristics were evaluated using color fastness and color strength. Antioxidant properties of the dye on fabric was determined using 2,2 -diphenyl-1-picrylhydrazyl radical (DPPH) method. Antimicrobial assays of dyed fabric against *Escherichia coli* and *Staphylococcus aureus* strains of bacteria were done using absorbance method. FTIR analysis indicated presence of O-H (at 3297 and 3647 cm^{-1}), C-O (at 1373 and 1043 cm^{-1}) and aromatic C=C (at 1618 and 1507 cm^{-1}) functional groups for *E. divinorum* and *E. abyssinica,* respectively. GC-MS analysis of *E. divinorum* revealed that the major phytochemicals in the dye were lupeol and betulin. The NMR confirmed that the two isolated compounds were lupeol and betulin. The optimum extraction conditions for *E. divinorum* were M:L 7.5g:100mL, 84°C and 146 minutes and M:L 10.6g:100mL, 77.2°C and 131.0 minutes for *E. abyssinica.* The optimum dyeing conditions for *E. divinorum* were pH of 3.3, 82°C and 68 minutes and pH of 5.0, 69.7°C and 74.5 minutes for *E. abyssinica*. *E. divinorum* showed an excellent color fastness of 5 compared to *E. abyssinica* which was fairly good (3). The bio-mordants led to different shades of color as was achieved with metallic mordants. Rosemary improved the color strength from 0.612 to 0.911 almost similar to that of alum (0.954) for *E. divinorum*. An antioxidant activity of 72.5% and 63.1% for *E. divinorum* and *E. abyssinica,* respectively, was obtained and antimicrobial activity of 61.54% and 65.55 % against *E. coli* and *S. aureus* strains of bacteria, respectively, was observed. In conclusion the excellent color fastness of 5 of *E. divinorum* dye makes it a suitable alternative source of brown dye for dyeing cotton. This study established an important basis of bio-mordants applicable when dyeing cotton fabric with the studied natural dyes as suitable alternatives for metallic mordants. The good antioxidant and antimicrobial activity, indicates that the dyes are promising agents for future development of bioactive and protective textile. In order to maximize extraction and dyeing cotton with these natural dyes it is recommended that the optimum conditions be adopted. Mordanting process is also essential in achieving the best color fastness properties and different shades of brown and yellow for *E. divinorum* and *E. abyssinica,* respectively.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA – Analysis of variance

ATR - Attenuated total reflectance

Biomordants –Mordants from natural sources

CCD – Central composite design

CIE- Commission International de L'eclairage /International commission of illumination

DE - Direct extract

DPPH - Diphenyl-1-picrylhydrazyl radical

FTIR - Fourier Transformed Infrared

GC-MS - Gas Chromatography coupled with a Mass Spectrometer

GAE- Gallic acid equivalent

ISO - International standards organization

K/S - Colour strength

MeOH - Methanol

M:L – Material to liquor ratio

M/Z ratio - Mass to charge ratio

Natural dye – Dyes from natural sources

NMR – Nuclear magnetic resonance

QE – Quercetin equivalent

RSM – Response surface methodology

SE - Sequential extract

SEM - Scanning electron microscope

TAE – Tannic acid equivant

UV-VIS- Ultraviolet Visible

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CHAPTER 1: INTRODUCTION

1.1 Background

Dyes are colored compounds and are used to color substances. The coloring features of dyes is due to chromophores and auxochromes structures present in them (Kusumawati & Kistyanto, 2019). Chromophores are structures that have delocalized electrons and conjugated double bonds (resonance of electrons). Auxochromes are functional groups that contain non-bonding pair of electrons and they influence solubility of the dye. These characteristics of chromophores and auxochromes bring about absorption of light by the dye molecules in visible region of electromagnetic spectrum which is responsible for the color of the dye that is observed (Ezeokonkwo *et al*., 2018).

Dyes find application in various manufucturing industries such as food, textiles, cosmetics, leather, plastics, wood and drug. Dyes are categorized into natural and man-made depending on their source (Ayele *et al*., 2021). Natural dyes are acquired from natural surroundings (flora, fauna, insects and minerals) where as the man-made dyes are manufactured in industries and are basically manmade products (Benkhaya *et al*., 2017).

Natural dyes have been used for centuries by the traditional communities in arts and crafts, cultural practices and rituals as well as in textile industries. However the use of synthetic dyes in dyeing textiles replaced natural dyes after the discovery of aniline that replaced natural indigo dye (Comlekcioglu *et al*., 2015; Samanta *et al*., 2020). The use of synthetic dyes which began in the 19^{th} century spread so easily because synthetic dyes are easily producible, have diverse colors, do not easily fade due to good fastness properties, higher color strength and are cheaper compared to natural dyes (Indraningsih, 2013). As a result natural dyes became only common in handcraft

or craft worker, printers and dyers practicing dyeing in small scale since most commercial dyeing industries turned to synthetic dyes as the major source of dyes (Affat, 2021).

Global environmental awareness has raised concerns on the use of synthetic dyes in various industries such as textile, food, leather and cosmetics due to the eco-toxicological consequences associated with them (Kant, 2012; Lellis *et al*., 2019; Sharma *et al*., 2018; Valli Nachiyar *et al*., 2014) . Synthetic dyes are mainly petroleum based and therefore not renewable (Arora *et al*., 2017). Dyeing inefficiency in industries that use synthetic dyes leads to a lot of by-products with unfixed colorant. These industries release about 10 - 15% of un-adsorbed synthetic dyes as un-treated effluents to the eco-system (Rehman *et al*., 2018). Most of the synthetic dyes used in textile industries are very soluble, as a result their elimination from the environment is not an easy process (Hassan & Carr, 2018).

Synthetic textile dyes are non-biodegradable and when released to the water bodies they not only reduce rates of photosynthesis but also bio-accumulate in the aquatic plants and animals (Orts *et al*., 2018). The high rate of persistence of synthetic dyes in the environment allows them to cross whole food chains causing bio-accumulation and bio-magnification in the higher levels of the food chains such as the bodies of human beings (Khatri *et al*., 2018). In addition man-made colorants are toxic, allergic to the skin and carcinogenic since they are azo-based and they contain heavy metals that can build up in the human body therefore acting as carcinogens (Indraningsih, 2013; Rehman *et al*., 2018). As a result most countries have come up with strict environmental standards in order to curb the application of synthetic dyes (Grover & Patni, 2011; Samanta *et al*., 2020). Germany, India and Netherlands are among the first countries to ban the manufacturing and application of several specific azo-based

dyes. Germany, France, Italy and United States of America are among major importers of natural dyes (Kulkarni *et al*., 2011; Samanta, 2018).

Recent tremendous shift to natural dyes is also attributed to the eco-friendly properties exhibited by the natural dyes (Nambela *et al*., 2020). This has triggered research in the recent past on natural sources of natural dyes (Gupta, 2020; Shukla & Vankar, 2017a). Natural dyes can be obtained from food residues such as vegetables and fruits. It has been shown that carotenoids, quinones and anthocyanins natural dyes are mainly found in by products of food such as peels from carrots, mango fruits and beetroot (Phan *et al*., 2021). The fact that natural dyes are biodegradable (Gulzar *et al*., 2015) and require minimal use of chemicals during extraction makes them potential alternatives to synthetic dyes (Abou Elmaaty *et al*., 2019; Kumbhar *et al*., 2019). Consequently, there is need to discover more natural sources of natural dyes in order to satisfy the increasing demand.

The Kenyan habitat is comprised of a variety of plant species that has been utilized by different communities as herbal medicine and traditional source of natural dyes (Musyoki *et al*., 2012; Zhou *et al*., 2017). The dye yielding plants in Kenya possess a great potential for natural dyes that can be extensively exploited in the textile industry. This study focused on the textile dyeing potential of *Euclea divinorum* (Ebenaceae) and *Erythrina abyssinica (*Leguminosae) plants.

1.2 Problem Statement

Presently, the use of natural dyes is thriving worldwide as an alternative to synthetic dyes that is eco-friendly (Aggarwal, 2021; Hamdy & Hassabo, 2021; Indraningsih, 2013). During textile finishing using synthetic dyes, not every part of the colorant binds to the fabrics. About 10-15% of the unbound synthetic colorant is released into the water stream, coloring the effluent and leading to environmental pollution

(Rajmohan *et al*., 2019; Velmurugan *et al*., 2017). At the same time different kinds of solvents and chemical intermediates are used with synthetic colorants for better binding and these toxic chemicals can harm living organisms (Khattab *et al*., 2020; Yeamin *et al*., 2021). Man-made dyes have been found to be toxic, allergic to the skin and carcinogenic since they contain heavy metals that can build up in the human body acting as carcinogens (Ayele *et al*., 2021).

Several techniques for treatment of synthetic dyes have been developed, however they are all costly and time-consuming (Sadiq *et al*., 2021; Slama *et al*., 2021). To address this problem, a suitable alternative to the synthetic dyes is the eco-friendly natural dyes which are highly available at a lower cost and are environment friendly. Therefore there is need to identify more natural dyes in order to satisfy the increasing demand due to environmental awareness.

1.3 Objectives

1.3.1 General Objective

The main objective was to extract and characterize natural dyes from *Euclea divinorum* and *Erythrina abyssinica* plants for textile dyeing.

1.3.2 Specific Objectives

The specific objectives were to:

i. Extract and characterize the natural dyes from *Euclea divinorum* and *Erythrina abyssinica* plants.
ii. Optimize dye extraction and dyeing conditions of the dye extracts.
iii. Compare the use of bio-mordants and synthetic (metallic) mordants with the natural dye extracts on cotton fabric.
iv. Determine the textile finishing properties of the dye extracts on cotton fabric.

1.4 Justification and Significance of the Study

The pacifying nature and brilliant shades of natural dyes makes textile materials eye-catching and attractive to the customers (Adeel *et al*., 2017). Natural dyes are safe for human beings because they are non-toxic, non-allergic, non- carcinogenic and produce rare shades that are soft and calm. Unlike synthetic dyes whose raw materials are not renewable and are non-biodegradable, natural dyes are agro-based making them renewable and biodegradable hence addressing the problem of environmental pollution brought about by synthetic dyes (Kulkarni *et al*., 2011). Effluents from natural dyeing processes are less non to the environment (Haji *et al*., 2016) and their by-products can be used as fertilizers since they are eco-friendly.

Textiles finished with natural dyes have recently become attractive options due to their value-added features such as antimicrobial (Rather, Shahid-ul-Islam, Azam, *et al*., 2016), antioxidant (Baaka, Haddar, *et al*., 2017), antiallergenic, insect repellant properties and ultra violet protection features (Pisitsak *et al*., 2016; Shahid-ul-Islam *et al*., 2018). Cotton fabric has low and poor resistance to microbial attack. Inhibition of the microbial attack on the cotton is significant to the textile manufacturers and consumers. The ability of natural dyes to impart finishing properties to textile material makes them multipurpose agents. The advantages of natural dyes include being used to address the problems arising from the toxic synthetic dyes and hence there is need for more research on natural dyes as potential alternatives to synthetic dyes.

E. divinorum and *E. abyssinica* have been used traditionally by the local communities as a source of natural dye (Jabasingh, 2019; Maroyi, 2011). However, characterization of its textile dyeing properties has not been done.

1.5 Hypothesis

i. The extracts of *E. divinorum* and *E. abyssinica* will not dye the cotton fabric.

ii. Biomordants will not enhance the dyeing properties of *E. divinorum* and *E. abyssinica* on cotton fabric.

iii. *E. divinorum* and *E. abyssinica* dye extracts will not impart textile finishing properties on the cotton fabric.

CHAPTER 2: LITERATURE REVIEW

This literature review provides a description of natural dyes in terms of their sources, classification of natural dyes and methods of extraction of natural dyes. Types of mordanting have been discussed especially those used when dyeing with natural colorants. An insight of mechanism of fixation of natural dyes through mordants has been provided. A description of the two plants (*Euclea divinorum* and *Erythrina abyssinica*) that were studied in this research has been provided. Extensive literature on Response Surface Optimization method and textile finishing properties of natural dyes have also been provided.

2.1 Natural Dyes

In most parts of Africa natural dyes have been used in dyeing of foodstuffs, leather and natural fiber such as wool, silk and cotton (Wanyama *et al*., 2010). Traditional use of natural dyes has been a common practice in different communities in Kenya. For example, among the Somali community in Garrisa county, natural dye is an important cultural and economic activity where they mainly use it in coloring and decorating utensils, bags, mats and wood carvings (Musyoki *et al*., 2012).

Dyes and dyestuffs obtained from plants vary from one place to another depending on the geographical conditions of the region where the plant is obtained (Saha & Dutta, 2007). For a substance to be classified as colorant it must produce appropriate color and should have the ability to fix or must be capable of being fixed to textile fiber. Moreover, it should not be prone to fading away after coloring the fabric (Saha & Dutta, 2007).

Dyeing of natural fibers with natural dyes works well. Natural fibers can be of animal or plant origin. Examples of fibers that are obtained from animals are wool and silk and those from plants are cotton, ramie and jute among others (Ado *et al*., 2014).

Natural dyes have higher affinity for animal fibers compared to some plant fibers. In addition, clarity and level of fastness differs from one fiber to another (Doğan & Akan, 2018).

Figure 2.1 is showing compounds (1 to 5) isolated from natural dyes extracted from different plants. Alizarin (**1**) is a red dye that was extracted from roots of madder (*Rubia tinctoria linn*) plant (Ekrami & Goodarzian, 2010). Curcumin (**2)** dye extracted from Turmeric (*Curcuma Longla L.*) gives a yellow color on natural textile. A variety of yellow shades can be obtained depending on the dyeing parameters and mordants used. Morindone (**3**) an anthraquinone, extracted from root of *Morinda angustifolia Roxb.* is the major component of the red pigment (Aobchey *et al*., 2002). Indigo (**4**) dye which gives a blue color on textile is a substantive dye, therefore applied on textile without the use of mordants. Indigo was extracted from *Indigofera tinctoria* as the major component (Chanayath *et al*., 2002). Quercetin (**5**) has a yellow colour and is a major component of *Sophora japonica L.* (Japanese pagoda) tree (Lee *et al*., 2013).

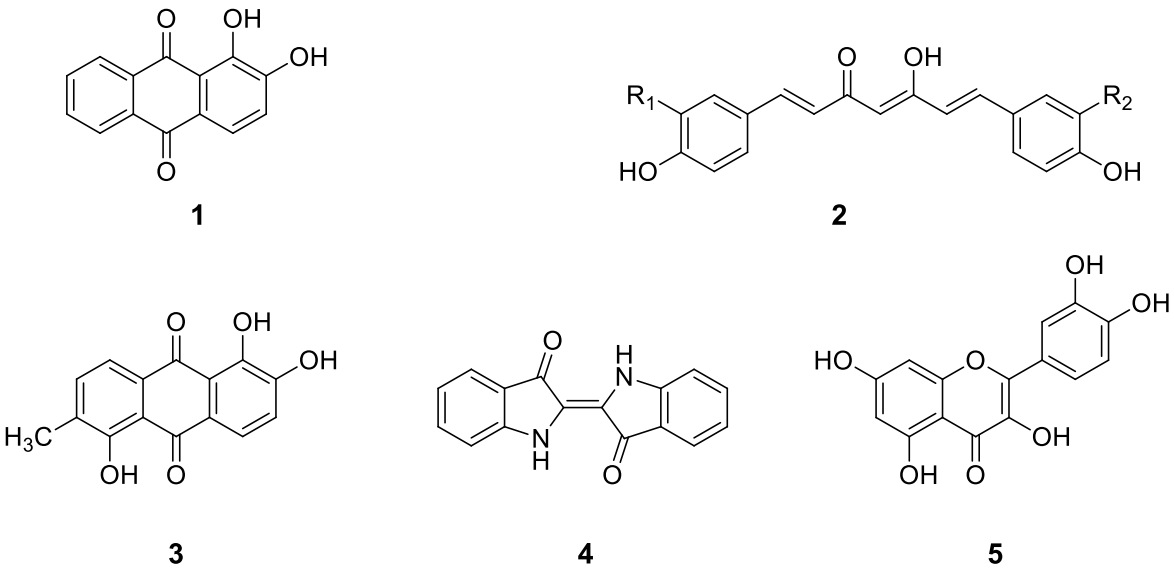


Figure 2.1: Compounds isolated from natural dyes extracted from different plants

2.2 Sources of Natural Dyes

Natural dyes have been exracted from plants, animals, Insects, microorganisms and minerals.

2.2.1 Natural Dyes Extracted from Plants

Different parts of various plants have been found to be potential sources of natural colorants. These parts include the roots (Abdel-Latif *et al*., 2015; Radwan & Mahmoud, 2015), stem bark (Jiang *et al*., 2019; Kumaresan *et al*., 2012), leaves ((Sengupta *et al*., 2015; Singh & Srivastava, 2017), seeds (El-Ghamri *et al*., 2014; Gómez-Ortíz *et al*., 2010), flowers (Al-Alwani *et al*., 2019; Singh & Srivastava, 2015; Vettumperumal *et al*., 2018; Wongcharee *et al*., 2007), heartwood (Datta *et al*., 2021; Odero *et al*., 2020; Sa *et al*., 2013) and fruits (Shanmugam *et al*., 2013; Teoli *et al*., 2016). Plant extracts can produce more than one shade of hue subject to the plant part from which the dye was extracted (Sanda & Liliana, 2021). Several studies on plants as source of natural dyes have been conducted (Agarwal & Sonia, 2021; Andriamanantena *et al*., 2021; Dutta *et al*., 2021; A. Singh & Sheikh, 2020).

Coloring of cotton cloth with natural colorants obtained from old coconut husk wastes provided brown color with good color fastness to soap washing, rubbing fastness and fastness to sunlight exposure as well as color depth. Mordanting with this natural dye resulted in light brown shade of color for alum, greenish brown color for tunjung and yellow color for napthol salt ((Kholil *et al*., 2021; Rodiah *et al*., 2022). Natural colorant acquired from *Ipomoea batatas* leaves that has been utilized in coloring cotton, nylon, silk, wool and polyester not only colored the fabrics with good color strength but also imparted anti-ultraviolet and anti-bacterial properties to the fabrics (Fang *et al*., 2022). Kandasamy *et al*., (2021) upcycled sawdust (*Pterocarpus indicus* Willd) wastes into a natural extract for dyeing pretreated cotton and silk fabrics. They

noted that the use of ultrasound extraction method and fabric pretreatment with chitosan and myrobalan enhanced the colorfastness properties along with the color strength on the cotton and silk fabrics. Dyeing wool fabric with a natural dye from agricultural wastes of *Eriobotrya japonica* L. seeds with different methods of bio-mordanting using flowers of *Folium cinnamomum camphora* and *Artemisiae argyi* as a source of the natural mordant has been done. The outcome of this study was that the use of natural mordants that are known to be rich in tannins provided better coloring properties in terms of color fastness and color strength which was attributed to the phenolic groups in the tannin molecules (Zhang *et al*., 2021). Aqueous extract of a natural dye from *Buddleja officinalis* flowers used to dye hemp fabric in presence of both metallic mordants (ferrous sulphate and potassium aluminium sulphate) and natural mordants (plant ash, gum rosin and *Chaenomeles speciosa* extract) formed different shades of yellow color ranging from light to dark yellow. This *Buddleja officinalis* natural dye extract showed promising dyeing characteristics (Yan *et al*., 2021).

2.2.2 Natural Dyes Extracted from Animals

Animals extracts is another source of natural colorants (AlAshkar & Hassabo, 2021). A common examples is lac red dye extracted from lac insect which has been used to dye wool and has shown good color fastness (Khatun *et al*., 2014). Cochineal and the kermes dyes are natural dyes obtained from insects and their major molecular components are carminic and kermesic acid, respectively, which are basically anthraquinones and produce red colors (Cooksey, 2019). Natural red dyes from cochineal insects were commonly used by Asian countries to dye clothing (Borges *et al*., 2012). (Adeel, Rehman, Pervaiz, *et al*., 2021) applied the microwave assisted method of natural dye extraction to extract the red laccaic acid natural dye from lac

insect and used the dye to color wool fabric in the presence of turmeric bio-mordants and achieved good color fastness ratings. Dyeing of woolen yarn for making carpet using a natural dye extracted from *Coccus Cacti* insect formed different shades of red color when different mordants were used. Moderate to excellent color fastness to washing and to exposure to light of the dyed woolen yarn were also noted (Ammayappan & Shakyawar, 2016)**.**

2.2.3 Natural Dyes Extracted from Minerals

These colorants are obtained from the natural minerals. Mineral dyes are mainly acquired from refined natural inorganic molecules (Mansour *et al*., 2020). Some of the significant mineral dyes are iron buff, prussian blue, chrome yellow and manganese brown. Mineral dyes have good fastness characteristics, however they have been shown to make textile stiffer hence not commonly applied in textile industry (Kumar *et al*., 2011). As a results colorants obtained from minerals mainly find application in painting. The African mineral dye with yellow, orange and grey colors was mainly used to dye hair (Adebayo *et al*., 2007).

2.2.4 Natural Dyes Extracted from Micro-organisms

Micro-organisms that are commonly used as source of natural dyes include fungi, bacteria and algae. These micro-organisms have been shown to yield colorants in form of phytochemical compounds such as quinones, carotenoids, riboflavin, flavonoids and prodigiosin (Nambela *et al*., 2020; Tuli *et al*., 2015). Natural dyes from micro-organisms are associated with biological activities such as antimicrobial and anticancer and are rich in vitamins hence they find application mainly in pharmaceutical, food and cosmetics industries (Margono *et al*., 2021). Investigations on the use of natural dyes from micro-organisms on textiles materials indicated that they have good to excellent color fastness and color strength. In addition the microbial

based natural dye imparts textile finishing properties such as antimicrobial activities, ultraviolet protection properties and antioxidant activities onto the textile material (Clement Agboyibor *et al*., 2018; Mehri *et al*., 2021; Tuli *et al*., 2015).

Fungal strains such as *Monascus, Talaromyces, Fusarium* and *Penicillium* are potential source of natural colorants that can be exploited (Clement Agboyibor *et al*., 2018; Nambela *et al*., 2020). *Monascus* fungal strain produce red and yellow colors in form of lovastatin (Margono *et al*., 2021). Yellow dyes from *Penicillium murcianum* fungus and red dyes from *Talaromyces australis* fungus that were obtained from wood samples extracted and applied on wool fabric showed good color fastness properties (Hernández *et al*., 2019). Natural pigments have been extracted from *Gonatophragmium triuniae* fungus that was isolated from *Maytenus rothiana* plant dicot (Lagashetti *et al*., 2022). Hinsch and Robinson (2018) compared the light fastness properties of wood staining fungal based natural dye with the commercial dyes on mordanted and unmordanted fabric and the results indicated that the fungal based natural dye had better colorfastness to light than the commercial dyes. Optimization of extraction of red pigment from *Cinnamomum zeylanicum* an endophytic fungus, for dyeing cotton fabric indicated that liquid medium comprising of glucose to provide carbon and yeast to provide nitrogen, produced the highest yield of the red pigment (Suwannarach *et al*., 2019).

Natural dye from green algae has been extracted and applied on wool fibers where good to excellent (4-5) color strength was obtained (El-Khatib *et al*., 2016). *Cladophora glomerata*, a species of green algae has been shown to effectively dye cotton fabric, producing light green color and upon mordanting with different metallic mordants a variety of shades such as creamy, brown and yellow were formed (Mir *et al*., 2019).

According to Nawaz *et al*., (2020) marine bacteria based natural dyes and pigments provides an alternative to the man-made colorants since they are highly compatible with the environment and are biodegradable. Purple dye has been extracted from lichens using fermentation with ammonia and used to dye silk threads. The purple natural dye showed color fastness properties against sunlight and washing with different detergents (Upreti *et al*., 2012). Silk fabric has been dyed with natural dye extract from *Streptomyces cyaneofuscatu* bacteria derived from marine and it was found to exhibitgood color fastness and color strength (Chen *et al*., 2022).

Most bacterial colorants are more advantageous compared to other natural sources of natural dyes since they exhibit easier culturing techniques, their dye and pigment extraction methods are simple making it quicker in scaling up and hence cost effective and economical (Nawaz *et al*., 2020; Numan *et al*., 2018). Natural colorants from micro-organisms have attracted the attention of researchers mainly because they are not affected by variation in temperature, pH and light and their production is not dependent of weather conditions. In addition natural dyes from micro-organisms multiply within a small duration and their extraction process require a small amount of solvent compared to other natural colorants from other sources which reduces the cost of production (Rao *et al*., 2017).

2.3 Classification of Natural Dyes

Natural dyes are categorised according to their chemical structure, method of application and color that they exhibit.

2.3.1 Based on Chemical Constituents

In this classification, the natural dyes are grouped according to correspondences in their scaffold chemical structures. These classes are anthraquinones, flavonoids,

naphthoquinones, carotenoids, indigoid, tannins, betalains, alpha-naphthoquinones, anthocyanidins and dihydropyran.

Anthraquinone natural dyes are dyes that contain the anthraquinoid structure. Examples are madder and lacs dye. Anthraquinone natural dyes are mainly red, yellow and pink. They are known to have outstanding light fastness that decrease with increase in the number of hydroxyl substituent (Adeel, Rehman, *et al*., 2019). Anthraquinone natural dyes have been extracted from *Alkanna tinctoria* (Shabbir *et al*., 2019), *Lawsonia inermis* (Sivarajasekar *et al*., 2018) and *Rubia cordifolia* (Boominathan *et al*., 2020; Mariadoss *et al*., 2020).

Flavonoids natural dyes are mainly yellow in color. They are categorized into aurones, isoflavones, chalcones and flavones. Most of the natural flavonoid colorants are derivatives of methoxy and hydroxyl substituted isoflavones and flavones. In addition flavonoids can form orange, red and light blue colors. Generally around most of the natural dyes that have been studied fall under the class of flavonoids (Brodowska, 2017). Flavonoids have poor light fastness but can be enhanced using mordants (Cristea & Vilarem, 2006). An example is flavonoid dye from weld (*Reseda luteola L*.) which has been used to dye wool fabric (Pars *et al*., 2021; Villela *et al*., 2019a) and cotton fabric (Karadag, 2021).

Di-hydropyrans are dyes whose chemical structure is almost similar to that of the flavones. They give dark shade when used to dye natural fiber. Flavanoid dyes have been extracted from *Butea monosperma* (flame of the forest) pant (Ansari & Iqbal, 2021) and *Allium cepa* onions (Nguyen & Bechtold, 2021; Volpi *et al*., 2021).

Naphthoquinones are another class of natural dyes that are mainly pink, brown and purple in color. They have shown good color fatness on textile fabric (Latos *et al*., 2019). Naphthoquinone based natural dyes can be obtained from the leaves of *Juglan*

regia and *Juglan nigra* (Dulo *et al*., 2021), *Lawsonia alba, Lawsonia innermis* (Said *et al*., 2021) and *woodfordia fructiosa* (Kafle *et al*., 2020), among other numerous plants.

Indigoid class of natural dyes contain the indigo structure and are indigo and tyrian purple in color and are known to have good fastness properties on textile fabric (Hossain *et al*., 2017). This natural dye has been extracted from *Indigofera tinctoria* L. plant and used to dye textile fabric (Pattanaik *et al*., 2020) and as a dye sensitizer for solar cells (Rajan & Cindrella, 2019).

Other classes include tannins which are polyphenolic compounds with very high molecular mass. Betalains class of natural dyes are pigments that have nitrogen atom and are derivative from the betalamic acid skeleton. Betalains are categorized into betacyanins which have red-violet color and betaxanthins which are yellow-orange in color. Anthocyanidins are basically a category of flavonoids natural dyes. Carotenoids class of natural dyes consists of molecules that are basically tetraterpene derivatives and are mainly yellow in color (Latos *et al*., 2019).

2.3.2 Classification Based on Method of Application

Categories of natural dyes that are based on method of application are direct dyes, mordant dyes, vat dyes, acid dyes and basic dyes, and disperse dyes.

Direct natural dyes are dyes that are charged, water-soluble and consist of positively and negatively charged ions that aid in attachment to the fabric (Mansour *et al*., 2020). In most cases sodium chloride and sodium sulfate is used when dyeing fabric with direct dyes in order to enhance the dye uptake by the fabric and assist in fixation of the dye to the fabric overcoming the color fastness challenge of natural dyes (Adeel, Rehman, *et al*., 2019). Examples are extracts from turmeric (Cooksey, 2017; Mirjalili & Karimi, 2013; Sahito, 2021; Umbreen *et al*., 2008), harda (Choudhury,

2018), pomegranate rind (*Punica granatum*) (Adeel *et al*., 2009; Ajmal *et al*., 2014; Basak & Wazed Ali, 2019), annatto (Chattopadhyay *et al*., 2014; Naidis *et al*., 2021; Venumbaka *et al*., 2021) and *Whitfieldia lateritia* plant (Okonkwo *et al*., 2019).

Mordant natural dyes are dyes that have poor fabric fixation ability and as result they can easily fade away. In order to enhance their fixation capability they are applied together with mordants which act as a bridge between the fabric and the dye molecules. Examples of commonly used mordants are potassium aluminium sulphate, tin chloride, iron sulphate and copper sulphate, among others (Shahid-Ul-Islam, 2017).

Vat natural dyes are not soluble in water hence reduction using sodium hydrosulphide should be done then dissolved in sodium hydroxide for them to show affinity for textile fibers. An example of vat natural dye is indigo extracted from *Indigofera tinctoria L*. plant (Shin *et al*., 2016).

Acid natural dyes are dyes that contain carboxylic group or sulphonic group in their structures. Acid natural dyes have been found to exhibit good color fastness properties and are most appropriate for dyeing of silk and wool. The fastness of this class of dyes is suitably enhanced with tannic acid. An example of acid natural dye is a dye extracted from saffron crocus (Bathaie *et al*., 2014; Mortazavi *et al*., 2012).

Basic natural dyes are dyes that form electrovalent bond with the –COOH groups that are found in textile substrates such as silk and wool. They have been found to have poor light fastness. An example of basic natural dyes is berberine (Kim *et al*., 2004; Kim & Son, 2005).

Disperse natural dyes are dyes that have small molecular mass and are slightly soluble in water. An example of disperse natural colorant is Lawsone brown dye extracted from *Lawsonia innermis* (Patil & Panchal, 2018).

2.3.3 Classification Based on Color

Natural dyes are classified on the basis of the color that they form on the textile fabric. These categories are mainly red, yellow, indigo, brown green and orange (Mansour *et al*., 2020).

2.4 Methods of Extraction of Natural Dyes

Natural dyes need to be isolated from their sources in order to be used as coloring matter. The fact that the natural dyes in the different source of dyes are not found as the only chemical unit but in a complex matrix made up of a variety of substance that are not dyes. As a result their need to extract and purify the natural dye in order to enhance its dyeing ability (Sanku *et al*., 2021). There are different methods of extracting natural dyes and a particular choice will depend on safety and cost of extraction. It is also important that an extraction technique minimizes contamination of the coloring material in various steps. Several extraction methodologies have been developed and each has its own advantages and disadvantages. The different extraction methods include aqueous extraction, alkali or acid extraction, microwave and ultrasonic assisted extraction, fermentation, enzymatic extraction, solvent extraction and super critical fluid extraction.

2.4.1 Aqueous Extraction Method

In aqueous extraction method, the source samples are first dried, and finely cut, grinded into fine powder and then the coloring matter is extracted with aqueous solution at different temperature for a specific time (Adeel, Rehman, *et al*., 2019). The content is cooled to room temperature and filtered to obtain the dye which can be used for various purposes. There are various conditions under which this technique can be applied. These are temperature, time, pH and Material to Liquor ratio, among others (Hou *et al*., 2013). To achieve a maximum yield of the natural dye the aqueous

extraction conditions should be optimized because their exact values varies for the different sources (Shafiq *et al*., 2021). Brown colorant was efficiently obtained from the bark of radiata using a solution of sodium hydrogen carbonate and used to dye cotton and silk (Mun *et al*., 2021). Aqueous extraction method was used to extract natural dyes from *Phoenix dactylifera* Linn leaves for ecofriendly dyeing of silk and cotton fabric which form different shades of red upon addition of mordants (Hossain *et al*., 2021). Yellow natural dye has been extracted from turmeric rhizomes (*Curcuma longa L.*) using aqueous extraction method (Sachan & Kapoor, 2007).

2.4.2 Solvent Systems Extraction Method

In this method the dried source of the dye is grinded to very fine particles. The crude dried powder is weighed and the dye is extracted using different organic solvents such as acetone methanol, ether, ethanol, chloroform, n-hexane, alcohol, soda ash, among others, which are sometimes mixed in the different ratios to achieve optimum yield of the natural dye (Shahid-Ul-Islam, 2017). In most cases after extraction the solvent is removed from the dye mixture using evaporation method to obtain a dry mass of the dye that can be dissolved in water during the dyeing (Adeel, Rehman, *et al*., 2019).

2.4.3 Alkali or Acid Extraction Method

Extraction of natural dyes using alkaline medium has been shown to be appropriate for those dyes that are made up of phenols (Samanta *et al*., 2020). Alkaline extraction of natural brown dye from the leaves of henna plant provided a natural dye with enhanced coloring properties on textile fabric compared to other solvents used in extraction (Ali *et al*., 2009). Alkaline extraction of natural dyes from stem of tea tree was found to produce higher yield of the dye than the aqueous method of extraction (Cheng *et al*., 2019). Effective extraction of natural dye from Kuntze flowers for dyeing cotton has been achieved (Saxena *et al*., 2012). Optimization of extraction

conditions for extraction of natural dye from Tesu (*Butea monosperma*) flowers showed the best extraction pH was acidic (4) (Singhee & Samanta, 2019). It has been shown that acidic extraction method is the most appropriate method for extract of natural dyes from bitter gourd leaves for dyeing of cotton fabric (Batool, Adeel, Iqbal, *et al*., 2022).

Acid extraction using yellow natural dye from *Cassia obovata* using chrysophanic acid was found to be the most appropriate method for extraction and dyeing of nylon fabric in presence of biomordants and metallic mordants since the extract showed superior color strength and color fastness compared to other extracts from other methods (Hasan *et al*., 2022). Acidic extraction of natural dyes from turnip (*Brassica rapa L. var. purple top*) cauliflower (*Brassica oleraceae L. var. botrytis*) and cabbage (*Brassica oleracea L.var. capitata*) and using it to dye cotton fabric, indicated that the extract from acidic extraction method had the highest color fastness and color depth compared to other methods that were used to extract the dye (Batool, Adeel, Azeem, *et al*., 2022).

2.4.4 Fermentation Method of Extraction

In this method the crushed form of the source of the natural dye is soaked in water or any other solvent for several days. Indigo dye has been extracted from *Indigofera tinctoria* L using this method where it was sprinkled with water and left to ferment for about nine months (Aino *et al*., 2018; Dutta *et al*., 2017). Bacteria fermentation of vat dyes from woad (*Isatis tinctoria* L.) lead to extraction of indigo natural dye (Milanović *et al*., 2017).

2.4.5 Enzymatic Method of Extraction

Enzymes used for extraction include pectinase, cellulose and xylase. The major advantage of using this method is that there are no solvents in the extraction process.

Mishra *et al*., (2009) extracted natural dyes from palash (*Butea monosperma*) and marigold (*Tagetes erecta*)) using enzymatic method of extraction and compared to solvent extraction. They found out that enzymatic method of extraction resulted in better dye in terms of dyeing properties than that from the solvent extraction method.

2.4.6 Microwave Assisted Extraction Method

Microwave assisted method of extraction is a technique that employs microwave form of energy to heat the extraction solvents together with the samples, hence separating the natural dye molecules from the matrix of the sample which then dissolve in the solvent (Beoletto *et al*., 2016). Microwave assisted method of extraction has helped in reducing extraction time, costs of extraction and the amount of solvent used during the extraction process (Gala *et al*., 2022). Optimization of extraction conditions using microwave assisted method of extraction for extraction of natural dye from *Miscanthus sinensis* plant for dyeing cotton fabric and paper showed that the optimum conditions for achieving the best dye in terms of color fastness and yield were 15 seconds, 540 W microwave power level and ethanol: water ratio of 1:1 ((Pinzon *et al*., 2020). Microwave assisted method of extraction has been applied in the extraction of natural dye from a mixture of Pomegranate Rind *(Punica Granatum L.)* and Turmeric Rhizome *(Curcuma Longa L.)* plant (Naveed *et al*., 2020).

2.4.7 Ultrasonic Assisted Extraction Method

Ultrasonic assisted method of extraction is a rapid and efficient technique of natural dye extraction that involves utilization of ultrasound waves to speed up the movement of solvents, leading to a rapid mass transfer of the dye from the source to the solvent hence increasing the rate of extraction. Several studies have shown that ultrasonic-assisted method of extraction of natural dyes is an effective and efficient method that results in not only high yield of the dye but also a cleaner form of the natural dye

(Righi Pessoa da Silva *et al*., 2018; Tiwari *et al*., 2010; Wang *et al*., 2020; Yuniati *et al*., 2021; Zulqarnain *et al*., 2021). Ultrasonic assisted method of extraction was utilized in the extraction procedure of natural dyes from *Hawthorn* fruits for dyeing nylon fabric and it was established that the technique boosted the effectiveness of extraction to between 20 and 70% subject to the kind of solvent that was used (Sadeghi-Kiakhani *et al*., 2021). Application of ultrasonic assisted technique in extraction of natural colorant from henna and turmeric for coloring wool fabric indicated that, ultrasound method produced colorants with higher color yield and color values compared to the conventional methods (Sheikh *et al*., 2016).

2.4.8 Supercritical Fluid Method of Extraction

Supercritical fluid extraction techniques was developed as alternative to the traditional solvent extraction of natural products. This method allows regeneration of solvents reducing toxic materials released to the environment (Adeel, Rehman, *et al*., 2019). Supercritical fluid extraction technology utilizes eco-friendly solvents such as carbon IV oxide liquid. As a result it is progressively gaining prominence over the other methods for extraction of natural dyes (Guzel & Akgerman, 2000). Supercritical fluid extraction techniques allow selective extraction of the natural dye from the sample matrix and has been found to be greener method of extraction compared to other conventional methods of extracting natural dyes (Lesellier & West, 2022). According to Vankar *et al*. (2001) extraction of natural colorants from the stem bark of eucalyptus plant using supercritical fluid extraction techniques process was a more efficient method compared to other methods.

2.5 Mordants

A mordant is a substance which enables the dye molecules to attach to the fabric. In textileindustries, mordants are applied durig dyeing so as to assist in fixing the

coloring molecules to the fabric without fading due to washing, rubbing, perspiration or exposure to light. They are mainly used when dyeing fabric that are plant based such as cotton where they enhance color fastness.

Mordants can not only help to fix the natural dye to the fiber but also generates new shades with the same dye (Har Bhajan Singh & Bharati, 2014). However, some natural dyes such indigo fix well without the support of a mordant; these dyes are classified as substantive dyes. Dyes that require the aid of a mordant to fix unto the fiber are known as additive dyes and include madder and weld (Kadolph, 2005). Additive dyes have poor fastness and fade easily on washing and on exposure to light, rubbing and perspiration hence requiring mordanting. Mordants are classified into salts or metallic mordants, Tannins and tannic acid mordants, and bio-mordants (Patel, 2011).

2.5.1 Salt or Metallic Mordants

Metallic mordants that are mainly applied by natural dyers include Alum (Potassium Aluminum Sulfate), tin (stannous chloride), Iron (ferrous sulphate) and Copper (II) sulphate (Khatun *et al*., 2014). According to Jothi, (2008) the use of mordants helps to improve light fastness of a dye to a greater extend due to the formation of transition metal complexes. The salts combine with the natural dyestuff to produce a form of dye that cannot be easily removed from the textile material, improving the color fastness properties of the natural dye ((Vankar, 2017).

Dyeing wool fabric using natural dye extract from walnut (*Juglans regia* L.) in presence of metallic alum, ferrous sulphate and stannous chloride significantly enhanced the colorimetric values and the color fastness characteristics of the natural colorant where the most superior outcome was observed in ferrous sulphate and stannous chloride mordants (Bukhari *et al*., 2017). Mordanting with metallic mordants

such as alum, stannous chloride, ferrous sulfate when dyeing wool fabric with Myrobalan (*Terminalia chebula*) natural dye extract showed substantial improvement in color strength of the dye on the fabric (Shabbir *et al*., 2016). The use of thyme and pomegranate peel natural dye extract to dye cotton fabric employing tin (II) chloride sulfate, copper (II) sulfate, potassium aluminum sulphate and iron (II) sulfate mordants indicated that the mordants affected the color values and increased the color strength on the cotton fabric (Davulcu *et al*., 2014).

Mordanting using a mixture of mordants in different proportions provides diverse hues and different color fastness (Grover & Patni, 2011). Poor light fastness of the natural dyes can be improved using ultra violet absorbers and antioxidant molecules (Cristea & Vilarem, 2006). Fading away of dyes is significantly due to ultra violet light that cause photo-degradation. Ultra violet absorbers and antioxidant molecules counteract the destructive ultra violet light.

2.5.2 Tannins and tannic Acid Mordants

Tannins and tannic Acid are polyphenols of large molecular weight. These mordants are non-toxic to the environment hence are suitable alternatives to some of the toxic metallic mordants. Post-mordanting of wool fabric dyed with coffee extract using aqueous solution of tannin mordant showed that the tannins considerably enhanced the color fastness to light of the brown dye. It was also noted that the tannin mordant increased the antibacterial and antioxidant fabric finishing properties of the dyed fabric (Hong, 2018). Microwave treated silk fabric has been effectively dyed with coconut coir natural dye extract using tannic acid mordant through post-mordanting and pre-mordanting methods (Kiran *et al*., 2020).

2.5.3 Bio-mordants

Negative environmental impacts resulting from use of metallic mordants have stirred several researches to discover new eco-friendly alternates such as bio-mordants. Examples of bio-mordants are tannin-based plant extract (Haji, 2010), tartaric acid and metal-containing extracts of plants (Bulut *et al*., 2014). Recently it has been shown that extracts from certain plants can be utilized as bio-mordants for natural dyes (Hosseinnezhad *et al*., 2020; Rani *et al*., 2020; Shahmoradi Ghaheh *et al*., 2021; Zhang, Shahid-ul-Islam, *et al*., 2022).

A study that was conducted on dyeing cotton fabric with colorant from date palm pits extract where metallic mordants (potassium aluminium sulphate, zinc (II) sulphate and copper (II) sulphate) and biological mordants (chlorophyll, gall nuts and green almond shell) were compared. The results indicated that natural biological mordants have higher enhancement of color fastness and adsorption than metallic mordants (Souissi *et al*., 2018). Adeel *et al*., (2020) dyed silk fabric with cinnamon bark (*Cinnamomum Verum*) natural colorant extract with the use of acacia, rose, henna, pomegranate and turmeric natural mordants and color fastness in terms of wash, light and rub were enhanced from rating of 3 (good) to 5 (excellent). The excellent resistance to fading by the silk fabric was attributed to formation of complexes between the molecules of the natural mordants employed during dyeing and the natural dye on the surface of the fabric. In addition a variety of novel shades of yellow were obtained using cinnamon bark natural dye.

Ecological dyeing of nylon fabric with yellow natural colorant obtained from *Cassia obovata* in presence of biomordants and metallic mordants showed that biomordants played the same role as metallic mordants in terms of different shades of color, color strength and color fastness (Hasan *et al*., 2022). The use of biomordant extracted from

gallnut (*Quercus infectoria*) which is rich in polyphenolic gallotannins, gallic acid and elagic acid was used when dyeing cotton fabric with a light yellow natural colorant acquired from babul bark (*Acacia nilotica*) was found to enhance ultra violet protection factor on the fabric (Dhanania *et al*., 2021).

Color strength and fastness characteristics in the use of bio-mordants for wool dyeing with *Adhatoda vasica* natural dye were as good as with that of the metallic mordants (Rather, Shahid-ul-Islam, Shabbir, *et al*., 2016). Wood ash bio-mordant obtained from a mixture of *Salix alba L.* and *Populus deltoides* plants has been used with a natural dye extracted from *Quercus robur* to dye wool, cotton and silk fabrics. The experiments involved dyeing with and without the mordants adopting different methods of mordanting (Geelani *et al*., 2017). Seed coat of tamarind plant that is rich in phenols has been applied as a natural mordant when dyeing silk, cotton and wool with natural dye from turmeric and was reported to work effectively (Prabhu & Teli, 2014). A comparison of mordanting using biomordants (pomegranate rind, onion peel, turmeric powder and henna leaves) and metallic mordants when dyeing cotton fabric with acidic extract of natural dyes from turnip (*Brassica rapa L. var. purple top*), cauliflower (*Brassica oleraceae L. var. botrytis*) and cabbage (*Brassica oleracea L.var. capitata*) and it was noted that the biomordants enhanced color strength and color fastness better than the metallic mordants (Batool, Adeel, Azeem, *et al*., 2022).

The potential of citrus lemon juice and colocasia esculenta bulk juice as mordants has been compared to potassium dichromate and potash alum metallic mordants during the dyeing of knitted cotton fabric with natural dye extract from Turmeric (*Curcuma longa L.*). The results showed that the color strength of the fabric dyed with biomordants was twice that of metallic mordants (Hosen *et al*., 2021). Pre and post bio-mordanting with henna, turmeric, acacia and pomegranate extracts during the

ultrasonic dyeing of cotton fabric using licorice (*Glycyrrhiza glabra* L.) extract showed superior color strength and color fastness properties compared to metallic mordants (Adeel *et al*., 2022). Barahapurkar *et al*. (2020) dyed silk fabrics using Celosia flower natural dye extract and biomordanted with extract of banana pseudostem sap and they observed that the biomordant enhanced the rub, light and wash color fastness of the dyed silk fabric. Several other studies have shown that biomordants have the potential of being used as alternatives to the toxic metallic mordants (Adeel, Rehman, Khosa, *et al*., 2021; Assefi Pour *et al*., 2020; Indrianingsih *et al*., 2021; Shahidi *et al*., 2021; Singh *et al*., 2019; Zhang *et al*., 2022; Zhang, Shahid-ul-Islam, *et al*., 2022; Zhang, Zhou, *et al*., 2022). Biomordanting with extracts from acacia, pomegranate and turmeric have have been found to demonstrate superior ability to fix natural dye extracted from Peepal (*Ficus religiosa*) on silk fabric (Habib *et al*., 2022).

Moreover, extracts from plants rich in tannin have been reported to successfully produce different colors as any other form of a mordant. *Dioscorea cirrhosa* extract a natural mordant has been used as source of condensed tannins for mordanting silk fabric and it was observed that the natural mordant increased the color values and imparted textile properties such as, antioxidant, waterproof and antibacterial properties (Yang *et al*., 2018). Investigation of dyeing characteristics of woollen yarn with natural dye extract from roots of madder (*Rubia tinctorum L*.) with various plants that are rich in tannins such as *Rhus coriaria, Pomegranat, Eucalyptus, Quercus castaneifolia* and *Terminalia chebula* as a source of biomordants using pre-mordanting and meta-mordanting methods. The tannin rich biomordants substantially improved the colorimetric values, color strength and also formed different shades of reddish brown on the woolen yarn. It was also noted that the pre-mordanting method

gave the best results compared to the meta-mordanting method (Jahangiri *et al*., 2018).

Metal hyper accumulating plants can also serve as appropriate alternatives for metal mordants (Lohtander *et al*., 2020). Plants that belong to the families of *Memecylaceae* and *Melastomacea*e have been reported to hyper accumulate aluminum and copper (Cunningham *et al*., 2011). The ability of a plant extract to fix the dye onto the fiber depends on the level of tannin and metal ions present in the extract. Tannin molecules contain phenolic hydroxyl groups that allow them to form cross-links between the dye and the fiber (Bhute, 2012). Therefore this study aimed at comparing the performance of bio-mordant with commonly used metallic mordants that are safe to the environment.

2.5.4 Methods of Mordanting

Mordanting methods are classified according to the stage at which they are applied during the dyeing process. In each method it is important to put into consideration the amount of the material to be dyed as well as the percentage of the chemical mordant. Time and temperature for each method should be adhered to (Ado *et al*., 2014). Normally when dyeing is being done for the first time with a particular dye it is important to apply the different method of mordanting in order to determine the one that gives optimum results. In most cases change in mordanting methods leads to different shades (Shabbir *et al*., 2016) as shown in Figure 2.2.

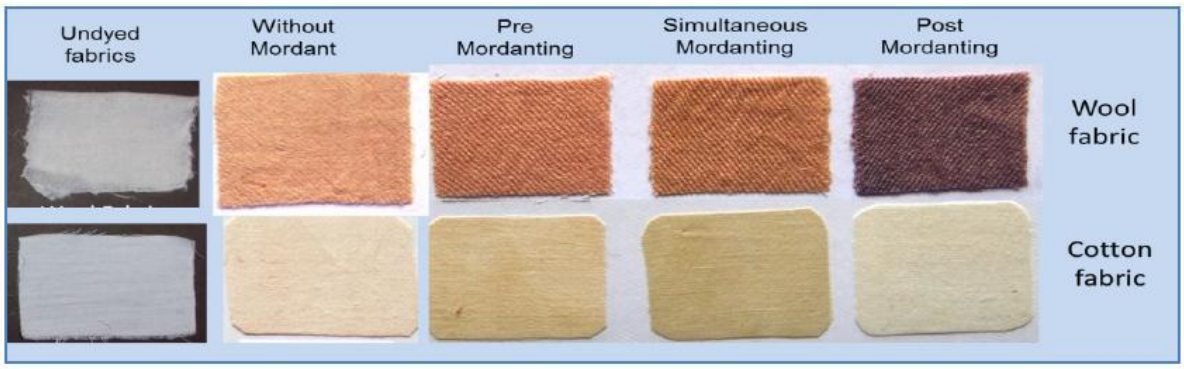


Figure 2.2: Different shades resulting from changes in mordanting methods (Geelani *et al*., 2017)

2.5.4.1 Pre-mordanting Method

In this method the mordant is applied first before the dyeing process. The mordant is dissolved in distilled water using different concentration depending on the mordant used then the solution is used to treat the textile material for a particular period of time ranging from 30 to 90 minutes and temperature of between 60 °C to 100 °C then dyed with or without washing. This conditions need to be optimized in order to obtain their optimum values. This is followed by dyeing as per the optimized dyeing conditions and the fabric is washed with soap (Janani *et al*., 2014). Different shade of brown were formed using pre-mordanting method with different percentage mixtures of natural and synthetic mordants when dyeing wool with natural dye extracted from Indian rhubarb (*Rheum emodi*) plant (Khan *et al*., 2017).

2.5.4.2 Post-mordanting Method

Post-mordanting method involve dyeing of the textile fiber first followed by mordanting. The textile fiber used in this method is preferably bleached before dyeing with the natural dye (Janani *et al*., 2014). Post-mordanting with acacia, pomegranate and turmeric extracts as bio-mordants when dyeing with natural colorant obtained from Peepal (*Ficus religiosa*) on silk fabric showed that the biomordants formed better color strength and color fastness of the reddish brown dye than the other

methods of mordanting (Habib *et al*., 2022). A study on dyeing characteristics of yellow colorants obtained from *Rheum emodi*, curcumin and *Gardenia* showed that application of ferrous mordant exerting post-mordanting method enhanced the color fastness, colorimetric properties and ultra violet protection properties of the fabric (Zhou *et al*., 2015). Post-mordanting with metallic mordants when dyeing with flower of *Hibiscus sabdariffa* dye yielded higher color chacteristics than the other methods of mordanting (Önal *et al*., 2022).

2.5.4.3 Simultaneous Mordanting Method

This method is also referred to as meta-mordanting. This is where the natural dye and the mordant are mixed together and applied to the textile fiber. The natural dye and the mordant are mixed in a dye bath and the pre-determined optimum dyeing conditions are set then the textile fiber is immersed (Ado *et al*., 2014). Simultaneous mordanting method was applied when dyeing silk material with dye from leaves of Longan plant using copper (II) sulfate, alum, stannous chloride and iron (II) sulfate (Maha-In *et al*., 2016).

2.5.5 Mechanism through which Mordants Fix Natural Dyes to the Fabric

Mordants not only enhance color fastness properties but also provide variations in color shades. It has been noted that varying the metallic mordants when dyeing with a single natural colorant results in different colors (Shukla & Vankar, 2017a). The type of the chromophore molecules of the natural dye and their electronic features influences the nature of interraction with the mordants which determines the color formed on the fabric (Manhita *et al*., 2011). The work of the mordants is to enhance the fixation of chromophores and auxochromes in the dye to the textile fabric by acting as a bridge in the formation of a complex between the fabric and the dye. Mordants which are basically metallic salts form metal complexes with the textile

fibers and the natural dyes (Kulkarni *et al*., 2011). These complexes are formed when the metal salts anchors to the fabric, attracting the molecules of the natural dyes to be anchored to the fiber (Samanta & Konar, 2011).

Improvement of the color fastness characteristics of the colored textile is due to the formation of a stable coordination complex between the metal ion and the dye molecules within fiber as shown in Figure 2.3 (Ding & Freeman, 2017). Metal ions first form coordinate complexes with the textile. The remaining un-occupied co-ordination sites of the metal after they have interacted with the fiber forms complexes with dye molecules. The metal ion acts as connection between the dye and the fabric. The stronger the coordination the higher the interaction between the fabric and the molecules of the colorant, causing an improvement in dye absorption hence better colometric features (Haji, 2012).

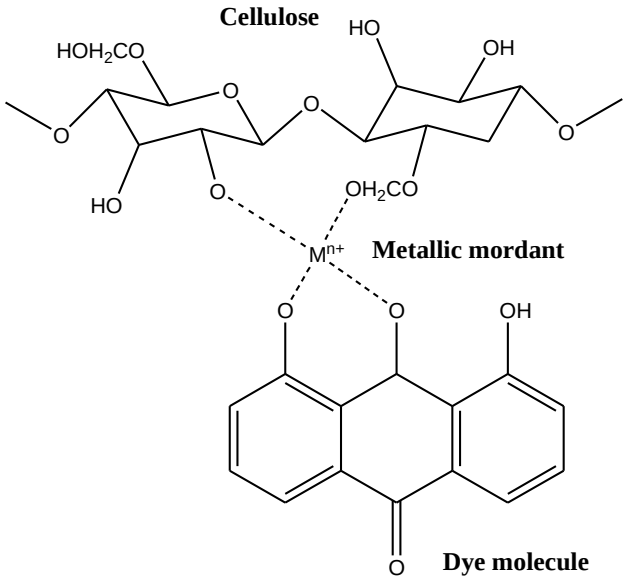


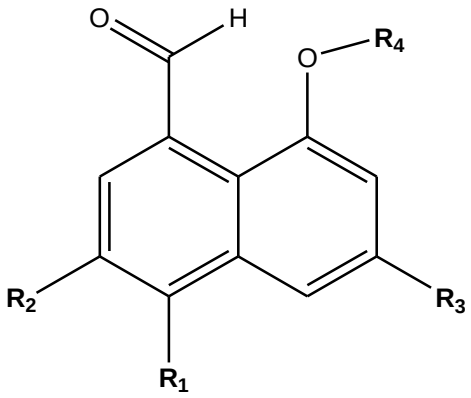
Figure 2.3: Dye-Mordant-cellulose complex

2.6 *Euclea divinorum*

Euclea divinorum Hierns (Ebenaceae) is an evergreen shrub that is mainly found along the escarpments and on rocky areas (Feyissa *et al*., 2013). *E. divinorum* plant

has medicinal values and has been used traditionally by the Kalenjin community as an anti-venom and as a purgative drug (Kigen *et al*., 2016).

Two napthalene derivatives, Eucleanal A (**6**) and Eucleanal B(**7**) (Figure 2.4) have been isolated from *E. divinorum* (Ng'ang'a *et al*., 2012). Other isolates of *E. divinorum* include: lupeol, lupene, betulin, 7-methyljuglone, isodiospyrin, shinalone and catechin (Mebe *et al*., 1998). *E. divinorum* has also been used locally as a source of natural dye. The bark of *Euclea divinorum* are identified by its gingery flavor. The roots and twigs of the plant were used as toothbrushes, mouth disinfectant and to color the lips reddish brown (Maroyi, 2011). However, the textile dyeing properties of *E. divinorum* has not been done.



6: $R_1 = R_3 = R_4 = H$, R_2 = OMe
7: $R_3 = H$, R_1 = OH, R_2 = OMe, R_4 = Me

Figure 2.4: Napthalene derivatives isolated from *E. divinorum*

2.7 *Erythrina abyssinica*

E. abyssinica is is among the commonly used herbal medicine. It has been widely used as a remedy of various microbial infections and diseases such as malaria. Phytochemistry of *E. abyssinica has* been widely done. Flavanoids, isoflavonoids and chalcones have been isolated from *E. abyssinica* and they have been found to exhibit good antiplasmodial activity (Yenesew *et al*., 2004). Musyoka *et al*. (2016) confirmed haematinic activity of *E. abyssinica* which was attributed to its phytochemical

nutrients which include flavonoids, alkaloids and minerals (calcium, chromium, potassium, iron, copper, manganese, lead, nickel, arsenic and zinc). Compounds that have been isolated from *E. abyssinica* are 5-Deoxyabyssinin II (**8**) Abyssinone III (**9**) Abyssinone V (**10**), among others (Yenesew *et al*., 2004) as shown in Figure 2.5.

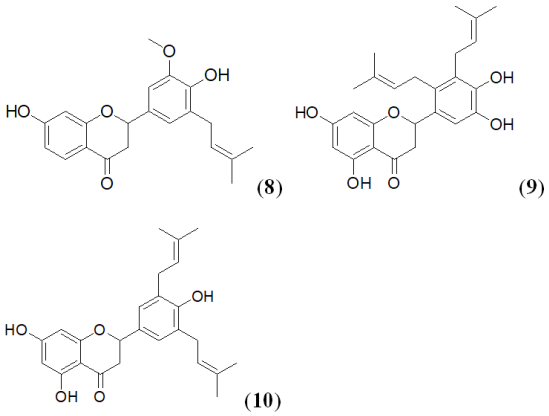


Figure 2.5: Flavonoids isolated from *E. abyssinica*

E. abyssinica belongs to Leguminosae plant family and is a multipurpose ethnomedicinal plant used to treat various ailments such as malaria, peptic ulcers, hypertension, mumps (Kigen *et al*., 2016) and tuberculosis (Obakiro *et al*., 2020). The extracts of *E. abyssinica* has shown good antimicrobial (Chitopo *et al*., 2019), antiplasmodial (Onyango & Midiwo, 2019; Yenesew *et al*., 2004) and anti-anemic activity (Musyoka *et al*., 2016).

Phytochemicals in a plant are the secondary metabolites such as alkaloids, phenols, tannins, flavonoids terpenoids, etc. In natural dyeing, the nature and quantity of this phytochemicals in a natural dye extract determines the color of the dye and its fixation ability (Mongkholrattanasit *et al*., 2013).

This plant is rich in flavonoids (Manjengwa, 2019; Yenesew *et al*., 2004) chalcones (Cui, Thuong, Lee, Njamen, *et al*., 2008) and flavanones (Cui, Thuong, Lee, Ndinteh, *et al*., 2008). (Wanyama *et al*., 2011) identified *E. abyssinica* as one of the plants in Uganda that has been used traditionally as source of natural colorants. It is among the

dye yielding plants in Kenya that are used traditionally by the local communities as a source of yellow color to dye textile material (Jabasingh, 2019). However, characterization of its textile dyeing properties has not been done. The preliminary studies showed that the color fastness for *E. abyssinica* natural dye extract on cotton fabric was not good and hence the need for pre-treatment of cotton fabric to enhance its dye-ability.

2.8 Textile Fabric Pre-treatment

Pretreatment of textile substrate before subjecting it to dyeing is a process that is basically consisting of preparation of textile for dyeing so as to improve its affinity for the dye, which leads to enhanced colorimetric characteristics such as improved color fastness.

Recently researchers have paid attention on the development and use of new technologies that would help in satisfying the future demand of natural dyes in textile industries. Pretreatment techniques are among the novel methods that would aid in achieving cost effective dyeing process with natural dyes. Examples of this techniques are ultraviolet irradiation (Vankar, 2017), gamma irradiation (Bhatti *et al*., 2010), enzyme treatment (Shanker & Vankar, 2007) and other treatment methods that are done after dyeing such as ammonia treatment (Shahid-ul-Islam *et al*., 2014).

Despite the various advantages associated with natural dyes, their use in textile industries still face challenges arising from its poor to moderate fastness properties and color strength especially on cotton fabric (Baaka, Mahfoudhi, *et al*., 2017). Cotton fabric is cellulosic in nature and is made up of several units of glucose molecules chained together. The hydroxyl functional groups on the glucose units are the points at which dye fixation and bonding take place (Baaka *et al*., 2019; Baaka, Haddar, *et al*., 2017).

Studies to enhance the low affinity of textile fiber towards natural dyes have led to textile pretreatment techniques that modify the surface of the fabric increasing its ability to absorb and retain natural dyes (Shukla & Vankar, 2017b). These modification techniques include:

2.8.1 Ultra Violet Irradiation Pretreatment of the Fabric

Surface modification of textile fabric using ultra violet radiation makes the fabric to swell allowing it to absorb more dye (Adeel *et al*., 2011; Bhatti *et al*., 2016; Zuber *et al*., 2012). Investigation of effects of pretreatment of wool yarn with ultra violet irradiation and dyeing with natural dye extracted from Cochineal insect showed the duration and strength of ultra violet radiation played a significant role in dye absorption by the wool yarn which determined the level of color fastness and color strength (Sadeghi-Kiakhani *et al*., 2020). A natural flavone dye extracted from the stem bark of Kikar (*Acacia nilotica*) plant has been used to dye ultra violet irradiated cotton fabric which showed better color properties compared to non-irradiated cotton fabric (Adeel *et al*., 2014). An advantage of ultra violet irradiation of fabric before dyeing is that there is no physical damage of the fabric, as was noted when dyeing ultraviolet irradiated cotton fabric with the greenish yellow lutein dye extracted from marigold plant (Rehman *et al*., 2017).

2.8.2 Plasma Technique of Pretreatment of the Fabric

This involves the use of plasma matter which is basically the ionized gas to slightly change the surface of the fabric where the modifying plasma particles are moved rapidly through the textile structure for a given period of time (Haji & Naebe, 2020; Poll *et al*., 2001). Plasma pretreatment of nylon and wool fabric using atmospheric air, followed by dyeing with natural dye extract from *Berberis vulgaris* showed higher

color depth and better antibacterial finishing properties which was attributed to enhanced dye absorption ability of the fabric (Haji *et al*., 2014, 2015).

Effective modification of chemical and structural morphology of wool fabric using plasma pretreatment method has been achieved, which improved the affinity of the fabrics towards the natural dye extracts from yarrow flowers and henna leaves. As a result less amount of the mordants was required during dyeing of the plasma pretreated wool fabric (Haji, 2020). Peran *et al*. (2020) observed that the color strength and color fastness to laundry of wool fabric pretreated with oxygen plasma and dyed with pomegranate peel (*Punica granatum* L.*)* natural dye extract was higher than that of untreated wool fabric. Plasma pretreatment of cotton fabric was found to enhance its hydrophilicity hence the color strength and color fastness increased due to improved absorption of natural dye extracted from the leaves of neem plant (Nithya *et al*., 2011). It has been shown that plasma pretreatment not only modifies the physical structure of the fabric but also introduces functional groups that assist in fixing the dye molecules to the fabric (Dayioglu *et al*., 2015; Haji *et al*., 2016; Krifa *et al*., 2021; Ullah *et al*., 2021; Vulpetti *et al*., 2006).

2.8.3 Microwave Technique of Pretreatment of the Fabric

Microwave pretreatment of the fabric is applied with the main aim being to reduce the time and solvents used during pretreatment (Adeel, Hussaan, *et al*., 2019). Microwave pretreated wool fiber dyed with walnut green peel natural dye showed good color properties compared to untreated wool fiber (Wang *et al*., 2021). In an investigation to determine the effects of microwave pretreatment of nylon fabric on dyeing properties of yellow natural dye extracted from *Cassia obovata* in presence of biomordants and metallic mordants, it was noted that the pretreated nylon fabric exhibited better color strength and color fastness and formed deep shades of color which was attributed to

enhanced affinity of the fabric towards the dye hence increased absorption (Hasan *et al*., 2022).

2.8.4 Enzymatic Technique of Pretreatment of the Fabric

This involves the use of enzymes such as pectinase, protease, cellulase, and lipase to enhance the surface wetting characteristics and retention properties of the fabric. Enzyme pretreatment mainly improves dyeability of the cotton fabric by increasing its ability to absorb and its adherence to dye molecules (Duran & Bahtiyari, 2007). Ecofriendly ultrasonic dyeing of enzyme pretreated cotton fabric using natural dye extracts from *Tectona grandis* and *Acacia catechu* showed increased light and wash fatness properties compared to untreated cotton fabric (Vankar & Shanker, 2008). Dyeing of cotton fabric pretreated with xylanase, neutral cellulase and acid cellulose enzymes with natural dye extract from *Acacia catechu* indicated that the absorption of the color was superior in the enzyme pretreated fabric compared to the cotton fabric pretreated with conventional chemical method (Samant *et al*., 2020). Treatment of woolen yarn using protease enzyme increased its affinity for the natural dye extract from rose pulp resulting in high colorimetric values (Bulut *et al*., 2014).

2.8.5 Gamma Irradiation Technique of Pretreatment of the Fabric

Dyeing of gamma irradiated cellulose substrate using banana floral stem extraction indicated that the gamma pretreatment improved the color fastness and ultra violet protection properties of the cellulose substrate (Islam *et al*., 2021). Dyeability of flax and cotton fabrics was found to improve when the cotton fabric was pretreated with gamma irradiation and dyed with Itodye nat pomegranate. It was also noted that the gamma radiation did not alter the surface morphology of the fabric (Chirila *et al*., 2018). In an investigation on the effects of gamma treatment on the dyeing properties of cotton material when colored with a natural colorant obtained from henna leaves

(*Lawsonia inermis*). It was established that the pretreatment substantially increased the color fastness and the antioxidant, hemolytic and antimicrobial activities (Rehman *et al*., 2012). Gamma radiation of cotton substrate has been found to increase the rub, wash and light fastness of the fabric after dyeing a natural colorant from an extract of turmeric (*Curcuma longa L.*) plant (Bhatti *et al*., 2010). Gamma irradiated cotton showed superior absorption ability of flavonoid colorant that was obtained from powdered onion shells (*Allium cepa*) which resulted in good color strength and color fastness properties (Rehman *et al*., 2013). In a study on the effects of gamma pretreatment of cotton fabric on dyeing properties of natural dye extract from henna, it was observed that gamma irradiated cotton fabric exhibited better colorimetric properties than the untreated fabric (M'Garrech & Ncib, 2009).

2.8.6 Cationization Method of Pretreatment of the Fabric

Cationization is the introduction of cations which makes the surface of the cotton fabric positively charged hence attractive to the anionic natural dye solution. Commonly used commercial cationic agents include Croscolor DRT and stabifix which are basically fixing agents (Ben Ticha *et al*., 2013). (Gargoubi *et al*., 2016) successfully enhanced the color fastness of turmeric dye extract through the modification of carbohydrate polymer of cotton fabric using dopamine cationic agent. Modified cotton fabric using Croscolor DRT provided darker shades compared to unmodified fabric (Haddar *et al*., 2014). Cationization of cotton fabric using decamethylcyclopentasiloxane agent and dyeing with a dye from cacao husk improved dye fixation and absorption resulting in higher color characteristics (Hossain *et al*., 2022).

Natural cationic agents are tannic acid and plant extracts rich in tannins such as Mimosa extract that has shown modification effects similar to other cationic agents

(Baaka *et al*., 2019). Tannic acid introduces carboxylic groups (-COOH) whose proton is more acidic than that of hydroxyl group (-OH) and can be easily deprotonated during the formation of a bond between the dye molecules and the cellulosic fabric (Baaka *et al*., 2019, p. 2; Sinha *et al*., 2016). Koh and Hong (2017) modified cotton fabric using an aqueous solution of tannic acid and improved the dyeability of the fabric significantly. Tannic acid pre-treatment of cotton fabric and dyeing with *Cladophora glomerata* natural dye extract provided darker shades than the untreated cotton fabric (Mir *et al*., 2019). Generally, cationization of cotton fabric using tannic acid has been found to increase fabrics' dye uptake, color strength and color fastness of the natural dye on the fabric (Bhuiyan *et al*., 2017). In this study tannic acid was applied in pre-treatment of cotton in order to enhance its affinity towards the natural dye extract from *E. abyssinica*.

2.9 Textile Finishing Properties of Natural Dyes

Several research on natural dyes in textile dyeing have shown that natural dyes have the potential of replacing synthetic dyes (Souissi *et al*., 2018). In addition, there has been an increasing demand for fabrics that are comfortable to the wearer and exhibit functional finishing properties. As a result, textile experts have embarked on various investigations to determine the possibility of utilising bioactive agents inherent in plants that are sources of natural dyes in production of biofunctionalized textile fabrics (Gulati *et al*., 2021). The textile functional finishing properties that have been achieved are antioxidant, antimicrobial, antifungal, ultra violet protection, deodorant characteristics and insect repellant properties.

2.9.1 Antioxidant Finishing Property

Antioxidant activity also known as radical scavenging activity is one of the most significant features of biofunctionalised textile materials since it protects the textile

material from getting damaged and safeguards the human skin from inflammation and aging due to oxidative stress caused by free radicals (Sheikh & Bramhecha, 2018). Human skin is continuously exposed to ionizing radicals which are the primary cause of skin damaging and its associated diseases (Godic *et al*., 2014). Ultra violet radiation is the main source of free radicals in the environment which accumulate to a level that the antioxidants in the skin cannot neutralize them leading to oxidative stress which may cause skin cancer among other diseases (Narendhirakannan & Hannah, 2013).

Antioxidants also referred to as free radical scavengers are substances that react with free radicals and neutralize them hence counteracting their harmful effects. Studies have shown that antioxidant molecules such as phenols and flavonoids have good anticancer activity against skin cancer (Chowdhury *et al*., 2017). The antioxidant activity of the textile fabric is achieved through deactivation of the very reactive and destructive radicals in the environment for example the oxygen and nitrogen radicals (Baaka, El Ksibi, *et al*., 2017).

Natural dyes are preferably suitable agents for bestowing the antioxidants properties to textile material because they are not toxic and do not irritate the skins ((Li *et al*., 2019). The antioxidant activity of silk fabric dyed with *Lonicera japonica Thunb* extracts increased from 24% to 96% (Shahid *et al*., 2017). Analysis of functionality of chitosan fiber dyed with lac dye indicated that the fiber had good radical scavenging activity (Liu *et al*., 2013). According to Sheikh and Bramhecha (2018) linen fabric retained an antioxidant activity of 50% after several cycles of washing, indicating that this textile property is durable. The antioxidant activity of cotton fabric dyed with a natural dye extracted from *Citrus sinensis* peel increased by over 60% compared to undyed cotton fabric (Shahid-ul-Islam & Butola, 2020).

Antioxidant biofunctionalization of wool fabric was effectively achieved by dyeing with natural dye extract from *Cinnamomum camphora* plant (Rather, Zhou, & Li, 2021). Promising radical scavenging activity has been observed in wool fabric dyed with a natural dye extracted from the bark of *Acacia nilotica*. It was also noted that the radical scavenging activity of the wool fabric dyed with *Acacia nilotica* was semi resistant to several washing cycles (Rather *et al*., 2017). Natural colorants acquired from *Terminalia chebula* and used to dye cotton fabric imparted percentage antioxidant activity of above 87% to the fabric. The antioxidant activity of the dyed cotton fabric was effective even after several washing cycles (Singh & Sheikh, 2021). Polyester fabric dyed with natural dye extracted from indigo and pomegranate showed percentage antioxidant activity of of 90% (Tambi *et al*., 2021). Yellow natural dyes extracted from *Rheum emodi*, curcumin and *Gardenia* and used to dye silk fabric imparted good antioxidant activity with fabric dyed with curcumin extraction showing the highest activity compared to the other extracts (Zhou *et al*., 2015).

Zayed *et al*. (2022) studied the antioxidant properties of cotton fabric dyed with natural extract from Psidium guajava leaves (*Psidium guajava L*.) and found out that the dye imparted antioxidant activity to the fabric. Wool fabric dyed with a natural colorant acquired from mulberry wood waste exhibited outstanding antioxidant activity which was attributed to the ellagic acid, oxyresveratrol and taxifolin phenolic compounds that make up the mulberry wood extract (Ivanovska *et al*., 2021). Ecological dyeing of nylon fabric with yellow natural colorant extracted from *Cassia obovata* imparted good antioxidant activity. Natural colorant extracted from Buddlejae Flos shrub has been used to dye cotton fabric and notable antioxidant activity was observed on the dyed fabric (Ke *et al*., 2021). Clothes touch the skin directly hence textile antioxidant activity is significant in development of bio-active

healthy textile fabrics that help in reducing the negative effects of free radicals on the human skin.

2.9.2 Antimicrobial Finishing Property

Natural dyes are made up of numerous phytochemicals that exhibit antimicrobial activity that can be retained even after absorption of the phytochemicals by the fabric (Kamboj *et al*., 2021; Selvam *et al*., 2012). Antimicrobial functionalization of textile materials is a significant practice because it provides protection to the fabric and the user against microbial attack (Rather, Shahid-ul-Islam, Azam, *et al*., 2016). Majority of the natural fibers including cotton fabric are vulnerable to attacks by various micro-organisms since it offers suitable environment in terms of nutrients, moisture and temperature that favors growth and multiplication of microbes. Textile microbial attack leads to development of skin allergic reactions, stinking clothes, color fading and rapid degradation of the textile fabric (Shahid-ul-Islam & Butola, 2020).

Research has shown that natural dye extracted from skin of peanuts imparts notable antibacterial properties to the dyed wool fabric (Rather, Zhou, Ali, *et al*., 2021). Both synthetic and natural textile fabric dyed with prodigiosin from *Serratia marcescens* bacteria exhibited remarkable antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Metwally *et al*., 2021). Antibacterial activity of cotton fabric pretreated with chitosan and dyed with natural dye extract from the skin of onions was found to be 98.03 % and 97.20 % against *S. aureus* and *E. coli* strains of bacteria, respectively, (Verma *et al*., 2021). Natural colorants acquired from *Terminalia chebula* and used to dye cotton fabric imparted percentage antimicrobial reduction potential of 94% to the fabric. The antimicrobial activity of the dyed cotton fabric was effective even after several washing cycles (Singh & Sheikh, 2021). Cotton fabric dyed with natural colorant obtained from gallnut (*Quercus infectoria* Olivier) showed

91% bacteria reduction ability against gram-positive bacteria (Güzel & Karadag, 2021). Yellow natural dyes extracted from *Rheum emodi*, curcumin and *Gardenia* and used to dye silk fabric imparted good antibacterial activity with fabric dyed with curcumin and *R. emodi* extracts exhibiting the highest activity compared to the other extract (Zhou *et al*., 2015).

Antimicrobial biofunctionalization of tussah silk fabric using lotus seedpod natural dye extract has been achieved with bacteria reduction percentage of 83.27 and 60.2% against *Escherichia coli* and *Staphylococcus aureus* strains of bacteria, respecitvely (He *et al*., 2021). Silk and cotton fabric colored with a brown colorant obtained from the bark of pinus radiata exhibited good antimicrobial properties against *klebsiella pneumoniae* and *Staphylococcus aureus* strains of bacteria (Mun *et al*., 2021). Wool fabric dyed with natural dye extracted from eucalyptus leaves exhibited significant antimicrobial activity against *E. coli* (Yılmaz, 2021). Comparison of the effectiveness of natural colorant obtained from turmeric, saffron and cinnamon in biofunctionalization of cotton fabric showed that tumeric dye had better antimicrobial activity than the other dyes (Shahidi *et al*., 2021).

Natural dye obtained from tea leaves and used to dye wool fabric imparted outstanding antimicrobial activities against *Escherichia coli* and *Staphylococcus aureus* strains of bacteria, which was attributed to the polyphenols inherent in th tea leaves (Ren *et al*., 2016). Natural flavonoid based dyes extracted from dyer's broom (*Genista tinctoria*), marigold (*Tagetes patula* L.) and weld (*Reseda luteola* L.) showed good antimicrobial properties on linen fabric (Schmidt-Przewozna & Zajaczek, 2022). Woolen yarn dyed with natural colorant acquired from *Terminalia arjuna* plant showed above 85% bacteria inhibition against various bacteria strains (Rather, Shahid-ul-Islam, Azam, *et al*., 2016). Durable percentage antimicrobial

reduction ability of cotton fabric has been attained through dyeing with natural dye extract from *Eucalyptus globulus*. The antimicrobial activity of the cotton fabric was found to increase when citric acid mordant was used and was effective up to five washing cycles (Endris & Govindan, 2021). Silk fabric dyed with natural colorant obtained from *Streptomyces cyaneofuscatu* strain of bacteria derived from marine exhibited noteworthy antimicrobial activity with 90% retention ability after several washing cycles (Chen *et al*., 2022). Polyester fabric dyed with natural dye extracted from indigo and pomegranate showed bacteria reduction ability of over 90% (Tambi *et al*., 2021).

2.9.3 Antifungal Finishing Property

Textile fabric provides favorable conditions for fungal growth. The presence of fungi on the textile fabric is the basis for discoloration and degradation of the fabric. The ability of natural dyes to impart antifungal properties to the dyed textile materials is an important feature of these dyes since the antifungal not only offers protection of the fabric against fungal attack but also to the user of the fabric (Muthu & Gardetti, 2020).

Investigations on the antifungal activity of silk, cotton and wool textile fabrics dyed with *Barleria prionitis* natural dye extracted from its aerial parts has been conducted. In the study it was evident that the dyed silk, cotton and wool textile fabrics had remarkable antifungal activity against the *Aspergillus flavus, A. parasiticus, A. niger, Penicillium canescens* and *Fusarium moniliforme* strains of fungi (Pal *et al*., 2018). It has been shown that woolen fabric that has been dyed with natural dye extract from the skin of onion (*Allium cepa*) exhibit notable antifungal activity against *Candida albicans* strain of fungi (Şapci Selamoğlu *et al*., 2017). 99.98% retention of antifungal activity against *Candida albicans* strain of fungi has been observed after five times

washing of the silk fabric colored with natural colorant obtained from gallnut (*Quercus infectoria*) and madder (*Rubia tinctorium* L.) plant (Alkan *et al*., 2017). Cotton fabric dyed with a natural extract from aloe vera showed antifungal activity against *Fusarium oxysporum* strain of fungi. The antifungal activity of the cotton fabric was effective even after fifteen cycles of washing (Korra, 2022).

2.9.4 Ultra Violet Protection Finishing Properties

Ultra violet radiation from the sun causes sunburns, browning of the skin, early aging and wrinkling, dark spots on the skin and if prolonged can cause skin cancer. Prolonged disclosure of human skin to the sun is a work-related threat to open-air labors such as those in building industry, sportspersons, agricultural workers and fishermen (El-Sayed *et al*., 2021; Grifoni *et al*., 2014; Hou *et al*., 2013). This demands the development of protective outfits that reduce or shield the dangerous effects of ultra violet radiation.

The ability of textile material to transmit ultra violet radiation depend on the morphology and physiochemical properties of the material, the dyes used to color the fabric, moisture content, thickness of the material and porosity (Vuthiganond *et al*., 2019). In order to improve the ability of the textile material to block ultra violet radiations, dyes and ultraviolet absobers are used (Shabbir *et al*., 2018). It has been shown that colored fabrics has higher protection properties against harmful effects of ultraviolet radiation than the fabric that is not dyed. Moreover the more the fabric absorbs dyes the better the protection properties against harmful effects of ultra violet radiation (Bonet-Aracil *et al*., 2016; Sinnur *et al*., 2018).

Investigation on the ultra violet protection characteristics of chitosan pretreated cotton material colored with natural colorant obtained from the skin of onion showed that the dyed fabric had good ultra violet protection factor of 84.80 (Verma *et al*., 2021).

Polyester fabric colored with natural colorant obtained from indigo and pomegranate showed ultra violet protection factor of above 270 (Tambi *et al*., 2021). Natural colorants acquired from *Terminalia chebula* and used to color cotton fabric imparted ultra violet protection propertis to the fabric which showed ultra violet protection factor that was above fifty. The ultra violet protection properties of the dyed cotton fabric were effective even after several washing cycles (Singh & Sheikh, 2021). Silk fabric colored with natural colorant obtained from *Streptomyces cyaneofuscatu* bacteria derived from marine exhibited outstanding ultra violet protection properties compared to cotton fabric (Chen *et al*., 2022). Natural flavonoid based dyes extracted from dyer's broom (*Genista tinctoria*), marigold (*Tagetes patula* L.) and weld (*Reseda luteola* L.) showed ultra violet protection properties on linen fabric (Schmidt-Przewozna & Zajaczek, 2022). Comparison of the effectiveness of natural colarants obtained from turmeric, saffron and cinnamon in bio-functionalization of cotton fabric showed that saffron colorant had better ultraviolet safety characteristics than the other colorants (Shahidi *et al*., 2021).

In the study to determine the ultra violet protection properties of cotton fabric dyed with a light yellow natural dye extracted from babul bark (*Acacia nilotica*), it was observed that the dyed fabric exhibited good ultra violet protection ability with ultra violet protection factor of 35. The use of biomordant extracted from gallnut (*Quercus infectoria*) which is rich in polyphenolic gallotannins further enhanced the ultra violet protection factor on the fabric (Dhanania *et al*., 2021). Silk fabric colored with a natural colorant extract from wood of wall nut tree showed ultra violet protection factor of over 50 (Wang *et al*., 2022). Ultra violet protection properties have been noted on silk fabric colored with a natural colorant extracted from the leaves of *Acorus gramineus* solander (Yang & Yi, 2020). Yellow natural colorants obtained

from *Rheum emodi*, curcumin and *Gardenia* and used to dye silk fabric imparted good ultra violet protection properties with fabric dyed with curcumin extract showing the most remarkable ultra violet protection properties compared to the other extracts (Zhou *et al*., 2015). Ultra violet biofunctionalization of tussah silk fabric using lotus seedpod natural dye extract has been achieved with ultra violet protection factor of 2000 (He *et al*., 2021). Natural dye extracted from watermelon rind and banana floral stem bestowed ultra violet protection characteristic to cotton fabric with ultra violet protection factor of 50 and 42 for banana dyed cotton and watermelon dyed cotton, respectively. *Sargentodoxa cuneata* natural dye imparted durable ultra violet protection properties to silk fabric (Wang *et al*., 2021). The good ultra violet protection characteristic of watermelon rind and banana floral stem was due to the polyphenols inherent in them (Rahman Liman *et al*., 2021).

2.9.5 Insect Repellent Finishing Properties

Insect destruction on textile goods such as mats, clothes, padded furniture and blankets is a severe challenge encountered in storing them and therefore leads to major loss of value which affects their marketing (Shahid-ul-Islam *et al*., 2013). As a result there has been a growing demand for insect repellents that prevents these insect destruction faced in various textile production industries (Agnihotri *et al*., 2019; Rather *et al*., 2019). In addition textile fabric possesing insect repellent finishing properties provide effective means of safeguard human body from insect bites that can transmit pathogens to them (Kamari *et al*., 2022). Consequently, inhibiting tramission of vector-borne diseases such as malaria from insects to human beings (Chavan & Pandit, 2020).

Sustainable biofunctionalization of cotton fabric to achieve insect repellant properties against mosquito has been attained using natural dye extracted from *Terminalia*

chebula with insect repellency value of 100%. The mosquito repellent property of cotton fabric was found to be effective even after twenty cycles of washing (Singh and Sheikh, 2021). Durable insect repellent properties against mosquito on cotton fabric has been attained through dyeing with natural dye extract from *Eucalyptus globulus*. The insect repellent properties on the cotton fabric was found to increase to 90% repellency when citric acid mordant was used and was effective up to five washing cycles (Endris & Govindan, 2021).

2.9.6 Solar Cells Sensitized with Natural Dyes

Solar energy has come up as one of the promising sources of energy. Solar energy is renewable and hence is eco-friendly and inexpensive compared to the other sources of energy (Ahmed & Anwar, 2022). Solar cell sensitized using natural dyes have been shown to be even more cost effective because of the application of fabrication process that is simple and cheaper (Adeel, Rehman, *et al*., 2019). Solar cells that are sensitized with natural dyes are solar cells that imitate what happens during photosynthesis in plants. Solar cells that are sensitized with natural dyes can efficiently work in limited light settings and are rarely vulnerable to losing its energy by conversion to heat energy, a feature that cannot be achieved in the traditional solar cells (Błaszczyk *et al*., 2021; Shalini *et al*., 2015). The productivity of solar cells that are sensitized with natural dyes depend on the nature of the sensitizer utilised (Sanda *et al*., 2020). The role of the natural dye solar cells is to absorb the light from the sun and convert the solar energy into electrical energy (Castillo-Robles *et al*., 2021).

The potential of application of natural dye extracts from *Arrabidaea chica*, *Euterpe oleracea*, *Bixa orellana*, *Myrcia sylvatica,* and *Genipa Americana* plants has been explored and was found to be promising (Amâncio *et al*., 2021). An investigation on solar cells sensitized with natural dye extract from pomegranate showed that the cells

exhibited electrical properties (Faraz *et al*., 2021). Solar cells sensitized with natural dye extracts from from *Alcea rosea,* Cytisus and Roselle flowers showed outstanding performance which was enhanced using zinc oxide quantum dots (Peymannia *et al*., 2021). Analysis of the ability of a natural dye extracted for inthanin bok (*Lagerstroemia macrocarpa)* to sensitize solar cell showed that it is highly effective (Khammee *et al*., 2021). Solar cell sensitized with a natural dye extracted from turmeric showed good photovoltaic characteristics (Hossain *et al*., 2017). Ethanol solution of saffron (*Crocus sativus L.*) dye extract used to sensitize solar cells improved the efficiency of the solar cells by 29% (Arof *et al*., 2017). A mixture of extracts of *Bougainvillea* sp. and *Ixora coccinea* dyes used to sensitize solar cells exhibited an outstanding performance (Lim *et al*., 2016). Natural dye extracted from harda fruit and used to fabricate solar cell showed superior photovoltaic characteristics which was attributed to the crystalline features and better surface structure of the natural dye (Yadav *et al*., 2021).

2.9.7 Deodorant Characteristics of Natural Dyes

High temperatures cause body sweats which bring about unpleasant odor. Deodorant works by impeding the development and activity of bacteria that results in bad odor on the armpit. Synthetic deodorants have been shown to cause cancer and contact dermatitis (Bhatt & Patel, 2021).Textile fabric dyed with natural dyes are potential alternatives to synthetic deodorants since they have the ability to absorb the bad odor making the wearer to remain fresh throughout the day (Adeel *et al*., 2019; Zhou *et al*., 2015). Cotton fabric dyed with natural dye extracted from chestnut shell exhibited noteworthy deodorant characteristics which intensified with rise in concentration of the colorant (Hong, 2021). Silk and cotton fabric colored with a brown dye obtained from the bark of pinus radiata showed good deodorant properties (Mun *et al*., 2021).

Deodorant properties were observed on silk fabric colored with a natural dye obtained from the leaves of *Acorus gramineus* solander (Yang & Yi, 2020). Outstanding deodorant properties have been noted on silk, wool and cotton fabrics dyed with a natural colorant obtained from coffee (*Coffea arabica L.*) sludge (Lee, 2007). Analysis of functionality of chitosan fiber dyed with lac dye indicated that the fiber had good deodorizing properties (Liu *et al*., 2013). Among the natural colorant obtained form gardenia, pomegranate, *Cassia tora. L.* and coffee sludge for dyeing and deodorizing silk, cotton and wool, pomegranate dye extract imparted the highest deodorant properties af about 99% (Hwang *et al*., 2008).

2.10 Response Surface Optimization

The structures of natural dye molecules are made up of different chromophores and auxochromes and hence have different methods and conditions of extracting the dye (Saxena & Raja, 2014). It is important to optimize extraction conditions in order to maximize the yield of the extracted dye and enhance the color strength of the dye on the fabric (Verma and Gupta, 2017).

Single factor design (one factor at a time) of optimization is simply the process of varying one factor while maintaining the others at a constant level (Abou-Taleb & Galal, 2018).

The main shortcoming of this design is that it does not put into account the effects of interactions that occur among the parameters which contribute to the entire outcome or response (Das & Dewanjee, 2018). So as to overcome this limitation, optimization in this study was carried out using Response surface methodology (RSM).

RSM is a statistical technique that is efficient and effective for optimizing processes that are known to be complex (Sinha *et al*., 2012). This technique allows the determination of individual effects as well as the interactive effects of the parameters

under study (Aydar, 2018). Central Composite Design is one of the designs of RSM that provides room for study of variables at five different levels solving the problem encountered by other designs with only three or two levels of study (Nasirizadeh *et al*., 2012). Consequently, CCD designs is a mathematical model that can precisely depict the overall process (Riswanto *et al*., 2019). Several studies have successfully utilized RSM design to optimize natural dye process extraction parameters (Sinha *et al*., 2012; Vedaraman *et al*., 2017; Yin *et al*., 2017). As a result, optimization of extraction and dyeing conditions conducted in this research was carried out using CCD design of experiment.

CHAPTER 3: MATERIALS AND METHODS

3.1 Dye Extraction and Characterization

3.1.1 Chemicals and Reagents

The solvents (methanol, ethyl acetate, hexane and dichloromethane) used for extraction were of analytical grade. Folin-ciocalteu, tannic acid, quercetin and gallic acid were used as the analytical standards. All chemicals used were purchased from Py-rex East Africa Ltd. The cotton fabric (GSM 97.1) was obtained from Rivatex East Efrica Ltd (REAL).

3.1.2 Dye Extraction

The root bark of *E. divinorum* and the stem bark of *E. abyssinica* were obtained from (0°01’59.5”S and 35°03’17.3”E) Chesumei escarpment and (0°07’36.8”N and 35°10’29.7”E) Nandi hills, respectively, in Nandi County. The samples were identified in the botany laboratory of department of Biological Sciences, Moi University, Kenya. The plant samples were cleaned with water, allowed to dry out and ground to powder. Dye extraction was done using sequential maceration of the ground samples with organic solvents (hexane, dichloromethane, ethyl acetate and methanol) and direct aqueous and methanol extraction as shown in Figure 3.1. The organic solvents were used in order of their increasing polarity. Maceration for each solvent was done for twenty four hours followed by filtration using Whatman filter paper (No. 1) and the solvents evaporated using rotary evaporator to obtain a dry solid mass whose weight was determined and percentage yield calculated. The aqueous extract was filtered and directly used in the dyeing process.

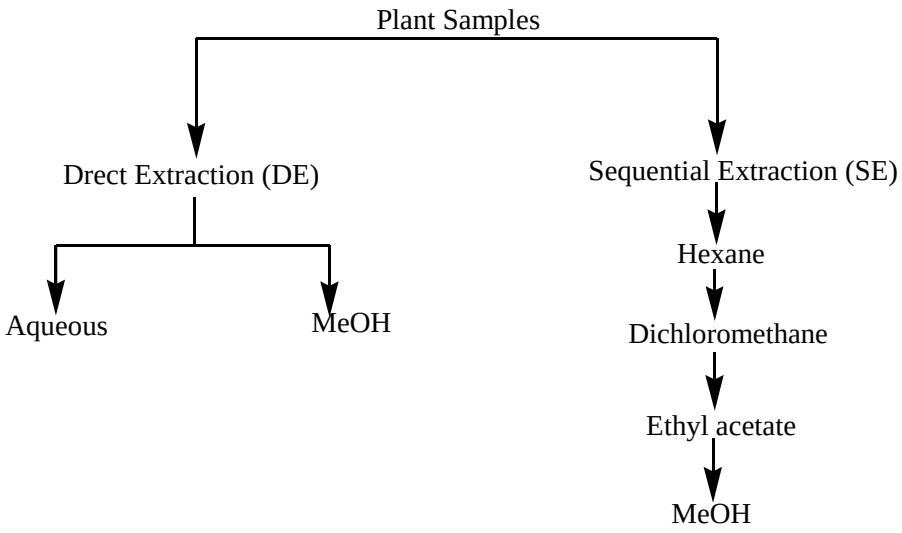


Figure 3.1: Dye extraction scheme

3.1.3 Characterization of the Dye Extracts

3.1.3.1 Qualitative Phytochemical Analysis

Qualitative phytochemical analysis was carried out according to Rajesh *et al*. (2014) and was as specifically described below.

3.1.3.1.1 Test for Phenols

A volume of 2 ml of distilled water was added to each plant extract (1 ml) then sodium carbonate (0.5 ml) was added and shaken followed by 0.5 ml of Folin Ciocalteau's reagent. A green / blue colour showed that phenols were present.

3.1.3.1.2 Test for Flavonoids

A volume of 4 ml of 1M NaOH was added to 3 ml of each plant sample extract and shaken well in a test tube. The presence of flavonoids was indicated by the formation of dark yellow colour.

3.1.3.1.3 Test for Tannins

Three drops of $FeCl_3$ (5%) was mixed with a volume of 2 ml of each plant sample extract. The appearance of a greenish solution indicated that tannins were present.

3.1.3.1.4 Test for Quinones

A volume of 1ml of concentrated sulphuric acid was slowly mixed with a volume of 1 ml of each plant sample extract. The development of red color showed that quinones were present.

3.1.3.1.5 Test for Saponins

A mass of 0.6 g of each plant sample extract was added to a volume of 2 ml of hot distilled water, after cooling the mixture was shaken thoroughly. The appearance of foam indicated that the Saponins were present.

3.1.3.1.6 Test for Terpenoids (Salkowski Test)

A volume of 2 ml of chloroform were mixed with a volume of 5 ml of each plant extract then a few drops of concentrated sulphuric acid were added slowly by the wall of the test tube. The formation of a reddish brown color at the interface indicated that the terpenoids were present.

3.1.3.2 Quantitative Phytochemical Analysis

Hexane, dichloromethane, ethyl acetate and methanolic extracts were subjected to quantitative evaluation of total tannins, phenols and flavonoids. All the spectrophotometric assays were measured by UV/Vis spectrophotometer (Model No: DU'720PC, Beckman Coulter). Measurements were done in triplicate and averaged (Baliarsingh *et al*., 2012, 2015). MS Excel software was used to draw the standard curves and calculate the correlation coefficient (R^2).

3.1.3.2.1 Determination of Total Tannins

Tannin content was estimated using the standard procedure described by Petchidurai *et al*. (2019). One mg of the sample was dissolved in 1ml of distilled water. An aliquot of 1ml of the sample was mixed with 0.5ml of 10% folin-Ciocalteau's reagent and incubated for 3 minutes. A volume of 1ml of 15% Na_2CO_3 and 8ml of distilled

water were added and incubated in the dark for 30 minutes at room temperature. UV-Visible Spectrophotometric analysis of absorbance was done at 725nm in triplicate. A stock solution was prepared using tannic acid standard (1g) in 1000ml volumetric flask. The stock solution was used to prepare tannic acid solutions in increasing concentrations (20, 40, 75, 100 and 125 mg/L). 0.5ml of each concentration was measured using a micropipette and treated like the sample then absorbance was measured. Tannic acid calibration curve was plotted and the concentration of total tannins in the sample was expressed as mg tannic acid equivalence /g (mg TAE/g) of the dry sample extract.

3.1.3.2.2 Determination of Total Phenols

Total phenols was determined by the Folin-ciocalteu method using standard procedure (Acemi *et al*., 2020; Shi *et al*., 2019). An aliquot of 0.5ml of the sample was mixed with 10% folin -Ciocalteau's reagent (2.5ml) and 15% Na_2CO_3 (2.5ml) then incubated in the dark for 20 minutes at room temperature. UV-Vis analysis of absorbance was done at 725nm in triplicate. A stock solution was prepared using gallic acid standard (1g) in 1000ml volumetric flask. The stock solution was used to prepare gallic acid solutions in increasing concentrations (20, 40, 75, 100 and 125 mg/L). 0.5ml of each concentration was measured using a micropipette and treated like the sample then absorbance was measured. Gallic acid calibration curve was plotted and the concentration of total phenols in the sample was expressed as mg gallic acid equivalence /g (mg GAE/g) of the dry sample extract.

3.1.3.2.3 Determination of Total Flavonoids

Total flavonoids was evaluated by aluminium chloride calorimetric method using standard procedure (Bahukhandi *et al*., 2019). Sample extract (1ml) was mixed with methanol (3ml), 10% aluminium chloride (0.2ml), 1M potassium acetate (0.2ml) and

5.6ml of distilled. Quercetin (1g) was dissolved in distilled water in 1L volumetric to make a stock solution of 1000mg/1L. From the stock solution quercetin increasing concentrations of 10, 20, 40, 80 and 100mg/L were prepared and subjected to similar treatment as the sample extracts. All the samples incubated at room temperature for 30 minutes then the absorbance was measured at 420nm in triplicate. Quercetin calibration curve was plotted and the concentration of the total flavonoids in the sample extract was expressed as mg of quercetin equivalent/g (mg QE/g) of dry sample extract.

3.1.3.3 Spectroscopic Analysis

3.1.3.3.1 UV-visible Spectroscopy

UV-visible spectroscopic analysis of the plant extracts were measured by UV-visible spectrophotometer (Model No: DU'720PC, Beckman Coulter) in the spectral range of 300-800 nm in order to determine the characteristic absorption spectra. The plant extracts for root bark of *E. divinorum* and the stem bark of *E. abyssinica* were filtered and 1mL was taken and diluted to 100mL using distilled water in a volumetric flask and its aliquot was analyzed as per the method of Saxena *et al*., (2012).

3.1.3.3.2 Fourier-transform Infrared spectroscopic (FTIR) Analysis

The plant extracts, the untreated, treated and dyed cotton fabrics were analysed using FTIR to confirm changes on the functional groups of the cellulosic fiber. The spectra were generated using PerkinElmer Frontier FTIR Spectrometer. The frequency regions used were 4000–600 cm^{-1} with 32 scans and 4 cm^{-1} resolution. Spectrum of air from the background was subtracted from all the generated spectra. 70% isopropyl was used to clean the attenuated total reflectance (ATR) plate before positioning the sample between the plate and the pre-mounting clamp. The absorbance was recorded in triplicate and the spectra generated using the average (Yılmaz & Bahtiyari, 2020).

3.1.3.3.3 Scanning electron Microscopy (SEM)

The morphology of pure white cotton and dyed cotton fabric was determined using Scanning electron Microscope (TESCAN VEGA 3 SEM). The samples were analyzed under low vacuum without coating. The acceleration voltage of between 5 and 20kV was used at a magnification 100μm. SEM images were generated using imaging software.

3.1.3.3.4 Gas Chromatogram Mass Spectrometry (GCMS)

A volume of 1mL of dichloromethane containing 1mg of the sample was analysed using gas chromatogram (Clarus A®500) coupled to a Clarus A ® 500 MS quadrupole mass spectrometer (Perkin Elmer Inc., USA). Gas chromatography was carried out on a 5 % diphenyl / 95 % dimethyl polysiloxane fused silica capillary column (Elite-5ms, 60 m x 0.25 mm, 0.25 mm film thickness, Perkin Elmer Inc, USA). The gas chromatograph was furnished with a split/split-less injection port that is regulated electronically. A volume of 1 μl was used as the injection volume at 250°C with 20 ml/min as the split movement rate. The flow rate for Helium (carrier gas) was kept constant at 1.2 ml/min. Ionization energy was 70 eV. The mass spectrum of the unknown compounds was compared with the spectrum of known molecules in the database of National institute Standard and Technology (NIST) library.

3.1.3.3.5 Purification

Purification of dye extracts and isolation of the major molecular components was achieved using column chromatography. The column was prepared by wet slurry method using hexane and silica gel (60-120 mesh). Methanol and the plant extract were mixed in the ratio of 1:1 then silica gel was added and the solvent evaporated to dryness. The sample was then crashed into powder and loaded to the packed column.

Hexane/dichloromethane and hexane/ethylacetate were used as solvent systems for column elution with increasing polarity. Elutes of 20ml were collected and their content was monitored using thin layer chromatography (TLC) which was done on aluminium plates coated with silica gel 60 F254. TLC spots were visualized under ultraviolet light (254 and 366 nm). Elutes with similar TLC profile were pooled together as fraction and evaporated at reduced pressure.

3.1.3.3.6 Nuclear Magnetic Resonance (NMR) Analysis

Chemical structures of the pure fractions were elucidated using ^{1}H-NMR and ^{13}C-NMR spectra that were generated on a 600 MHz Bruker Ultrashield-plus NMR spectrometer. Chemical shift values are given in δ-value (ppm) with tetramethylsilane (TMS) as the internal standard.

3.2 Optimization of Extraction and Dyeing Conditions of the Dye Extracts

3.2.1 Cotton Pre-treatment

The cotton fabric was cut into equal sizes of 1g. Wetting of the cotton fabric was done using 5g/l of non-ionic detergent for 30 minutes. Tannic acid was used as a pre-treatment agent to modify fabric as described by Vankar *et al.*, (2009). Cotton fabric was immersed in a 4% on weight of the fabric tannic acid solution prepared using distilled water. The fabric pre-treatment was done for two hours using covered conical flask, after which the fabric was cleaned with water and air dried.

3.2.2 Dyeing

Wetting of the fabric was done using 5g/L of non-ionic laundry detergent for half an hours prior to dyeing (Mohan *et al*., 2012).The natural dye extracts of the root bark of *E. divinorum* and the stem bark of *E. abyssinica* were used to prepare the dye-bath in Erlenmeyer flasks with a material to liquor (M: L) ratio 1:40 (Geelani *et al*., 2017) and dyeing was carried out according to Figure 3.2. The pH was modified with 0.1M

hydrochloric acid. After dyeing it was allowed to cool then the dyed samples were washed with cold water to remove the unfixed dyestuff then subjected to soaping with 2 g/L soap solution then cleaned with tap water and then allowed to dry out in the air (Patel & Kanade, 2019).

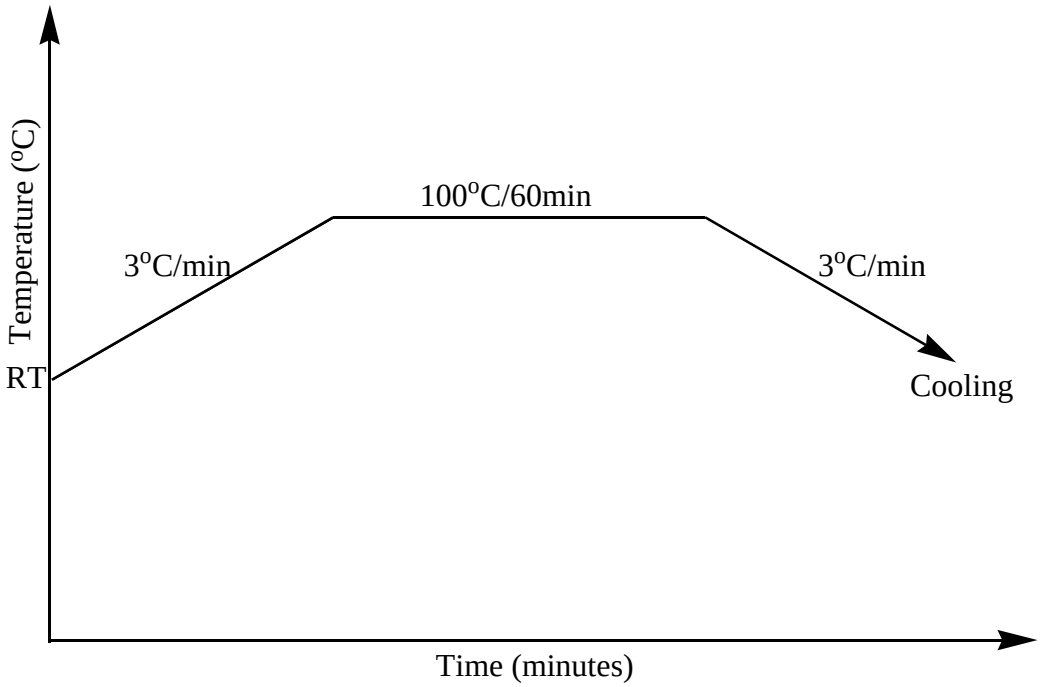


Figure 3.2: Dyeing scheme for natural dye extracts

3.2.3 Optimization

Optimization of dye extraction conditions and dyeing parameters was done with the aim of maximizing the response or the output. RSM design were employed in the optimization process.

3.2.3.1 Optimization of Dye Extraction Conditions

3.2.3.1.1 Single Factor Design

Single factor design was used as a preliminary study to evaluate the effect of every parameter on the extraction processes (Yin *et al*., 2017). Moreover this design was used to determine the range of parameters at which the experimental design optimization would be executed (Frey *et al*., 2003). Temperature (°C), Time (minutes)

and M: L (g: 100mL) extraction conditions were studied for both solvents. Direct aqueous extraction was carried out at different temperatures of 40, 60, 70, 80 and100 °C while direct methanol extraction was done at 30, 45, 50, 60 and 65 °C. M:L ratio and time for both solvents were 2,4,6, 8 and 10g:100mL and 60, 90, 120, 150 and 180 minutes, respectively (Elksibi *et al*., 2014).

3.2.3.1.2 Response Surface Methodology Design (RSM)

Optimization using RSM was carried out with an explicit target of evaluating interaction effects of the multiple factors and to determine their optimum operating conditions that yield an optimum response. The response or dependent variable used was absorbance at maximum wavelength. The design of the experiment, graphical designs, regression and other statistical analysis were carried out using Central Composite Design (CCD) of RSM using Minitab 17 software. Three factors at different five levels were studied as shown in Table 3.1 below. A total of twenty experiments were carried out in triplicate according to CCD design where randomized run order was used. The independent variables: M:L, temperature and time were represented by letters A, B and C, respectively.

Table 3.1: Experimental levels of independent process variables.

Variables	Factors	Levels				
		$-\alpha$	-1	0	+1	$+\alpha$
M:L (g:100mL)	A	0.8	2	5	8	9.2
Temperature(°C)	B Aqueous	28	40	70	100	112
	Methanol	35	40	52	64	69
Time	C	36	60	120	180	204

3.2.3.2 Optimization of Dyeing Conditions

3.2.3.2.1 Single Factor Design

A lead up study was conducted using single factor design to establish the outcome of varying every condition on dyeing processes (Elksibi *et al*., 2014) as well as the

range of the conditions at which the RSM design would be carried out (Chen *et al*., 2018). Dyeing conditions that were studied were time (minutes), temperature (°C) and pH. Dyeing of cotton fabric using *E. divinorum* and *E. abyssinica* aqueous dye extract was carried out at pH of 2,3, 4, 5 and 7, temperature of 45, 60, 75, 90 and 100 °C at a duration of 30, 45, 60, 75 and 90 minutes (Adeel *et al*., 2017).

3.2.3.2.2 Experimental Design

Response Surface Methodology (RSM) was used to test individual and interactive effects of the studied dyeing parameters on the color strength which is response (Noor *et al*., 2015). Particularly the Central Composite Design (CCD) of experiment was used to develop the optimization design with three independent variables at five levels (Table 3.2). The minimum and the maximum values of the variables were guided by single factor testing results. The experimental design and data analysis were performed using statistical techniques. The optimization experimental design developed comprised of twenty dyeing experiments each conducted five times and the response averaged (Silva *et al*., 2020).

Table 3.2: Experimental levels of independent variables

Variables	Factors	levels				
		$-\alpha$	-1	0	+1	$+\alpha$
Time (min)	A	22.5	30	60	90	97.5
Temperature (°C)	B	44	50	72.5	95	100
pH	C	1.5	2	4	6	6.5

3.2.3.3 Statistical Analysis

In order to determine the relationship between the dependent and independent factors, the data from the optimization experiment was fitted to second order polynomial equation (equation 3.1).

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC$$

Equation 3.1

Where Y is the dependent variable, M:L (A), temperature (B) and time (C) are the independent variables for extraction and time (A), temperature (B) and pH (C) are the independent variables for dyeing. β_0 is the constant term, β_1, β_2, and β_3 are coefficients of the linear terms, β_{12}, β_{13}, and β_{23} are coefficient of the interactive terms and β_{11}, β_{22}, and β_{33} are coefficients of the quadratic terms of the equation.

Adequacy of the statistical model to this optimization study was checked using Analysis of variance (ANOVA), coefficient of determination ($R^{2)}$ and adjusted correlation coefficient (R^2adj) where $R^2 = 0$ implies inadequacy and $R^2 = 1$ implies very adequate (Yolmeh *et al*., 2014). Significance at a 95% confidence level for all the terms of the second order polynomial equation and lack of fit error were statistically considered (Savic *et al*., 2014). Interaction plots were used to assess variables interactions during the extraction and dyeing process. The significance of the linear, quadratic and interaction terms of the polynomial model was evaluated using the probability p-value. The replicates of the center points (0, 0, 0) were designed in the experimental design so that significance of lack of fit error could be statistically assessed (Das & Dewanjee, 2018).

3.2.3.4 Model Validation

Optimization with the objective of maximizing the dependent variable/response was executed to obtain optimal dyeing conditions as per the statistical model. The validity of the values drawn from the desirability plot was checked for by conducting dyeing experiments under the conditions then the response (color strength) was measured. The statistically predicted response was then compared to the experimentally determined response (Haddar *et al*., 2014).

3.3 Mordanting

3.3.1 Metallic Mordants

The process of mordanting and dyeing was carried out at the determined optimal conditions. Pre, meta and post-mordanting methods were adapted from Mir *et al*. (2019). For pre-mordanting the wet cotton fabric was immersed in a 4 % on weight of the fabric solution of mordant using material to liquor ratio of 1:50 at 70°C. The mixture was stirred for 1hour followed by dyeing. In meta-mordanting the wet cotton fabric was immersed in flask containing 4 % on weight of the fabric mordant and the dye extract solution using material to-liquor ratio of 1:50 at 70°C for 1 hour. For post-mordanting method the already dyed cotton fabric was placed in flask containing 4 % on weight of the fabric solution of mordant using material to-liquor ratio of 1:50 at 70°C for 1 hour.

3.3.2 Colorimetric Measurements

3.3.2.1 The CIELAB Co-ordinates

Color characteristics of the dyed samples was measured using Spectro -Flash X-rite SP62 spectrophotometer using D65 source of light and 10° standard observer. The CIELAB coordinates measured were reddish – greenish color (+a* = red, -a* = green), yellowish - bluish color (+b* = yellow, -b* = blue), brightness (L*), color saturation (C) and hue (H*). The un-dyed cotton fabric was used as the blank. Kubelka–Munk equation (equation 3.2) was used to determine the relative color strength (K/S) values (Baaka *et al*., 2015).

$$K/S = \frac{(1 - 0.01R)^2}{2(0.00R)}$$

Equation 3.2

Where K is the absorption coefficient, S is the scattering coefficient and R is the minimum reflectance of dyed substrate samples.

3.3.2.2 Color Fastness

The capability of cotton fabric to retain the dye during washing, rubbing, on exposure to light and perspiration was determined by the respective standard color fastness tests. These were conducted according to ISO 105-C02:1989, ISO 105 A02:1993, ISO 105-X12:2000 and ISO AATCC-2009 for washing, exposure to light, rubbing and perspiration fastness, respectively. Grey scale was used to rate the color fastness between one and five where five is the best fastness (Canche-Escamilla *et al*., 2019).

3.3.3 Bio-mordanting

The mango (*Mangifera indica*) stem bark bio-mordant was extracted using the procedure described by Wangatia, Tadesse, and Moyo (2015). Extraction was done at 90 °C for one hour using 15g of the sample in 200mL of distilled water. Rosemary (*Rosmarinus officinalis*) branches were purchased from a local market, cut into small pieces and dried under the sun then ground into powder. The 2g of the rosemary powder in 100ml of distilled was used to extract the mordant at 100 °C for one hour (İşmal, 2017). Bio-mordanting was carried out by adopting the pre, meta and post-mordanting methods. For pre-mordanting method, the wet cotton fabric was immersed in a 3g/L solution of mordant using material to liquor ratio of 1:50 at 60 °C. The mixture was stirred continuously for 1 hour followed by dyeing. In meta-mordanting the wet cotton fabric was immersed in flask containing 3g/L solution of mordant and the dye extract using material to liquor ratio of 1:50 at 60 °C for 1 hour. For post-mordanting method, the already dyed cotton fabric was placed in flask containing 3g/L solution of mordant using material to-liquor ratio of 1:50 at 60 °C for 1 hour ((Erdem İşmal *et al*., 2015; Wangatia *et al*., 2015).

3.4 Textile Finishing Properties of the Dye Extract on the Fabric

3.4.1 Antioxidant Activity

The antioxidant activity of the pure and dyed cotton fabric was evaluated using DPPH radical scavenging assay according to Rather *et al*. (2017). 2.54 cm^2 of the pure and dyed cotton fabric were separately immersed in a test tube containing a volume of 50mL solution of 2,2 -diphenyl-1-picrylhydrazyl radical (DPPH) in methanol (0.15 mM) and mixed thoroughly. The samples were incubated in the dark at room temperature for 30 minutes. The absorbance of the solution was measured at 517 nm using UV-Vis spectrophotometer. The percentage antioxidant activity was calculated according to equation 3.3.

$$A\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \qquad \text{Equation 3.3}$$

Where A% is the percentage antioxidant activity, $A_{control}$ is the absorbance of the DPPH solution after incubation with the undyed cotton fabric and A_{sample} is the absorbance of the DPPH solution after incubation with the dyed cotton sample. The durability of antioxidant activity of the dyed samples was assessed by subjecting the dyed fabric to washing cycles and after every washing the antioxidant activity was determined. The washing tests were done by putting the samples into the washing solution made up of commercial detergent (2 g/L) with material to liquor ratio of 1:50. The antioxidant activity was determined after the 1^{st}, 5^{th} and 10^{th} washing cycles (Li *et al*., 2019).

3.4.2 Antimicrobial Activity

Antibacterial potential of the cotton fabrics (dyed and bio-mordanted) was assessed using absorbance method as described by (Rather, Shahid-ul-Islam, Azam, *et al*., (2016). The purchased *Escherichia. coli* (ATCC® 25922 ^{TM}) and *Staphylococcus*

aureus (ATCC® 25923™) were cultured at 37 °C for 24 hours. 1.27cm^2 of the fabric was introduced into a 25mL nutrient broth inoculated with two different strains of bacteria (*E. coli* and *S. aureus*). The broth was then incubated at 37 °C in an automated incubator shaker (Unitronic – J.P selector) for 24 hours. The absorbance of the bacteria culture media was determined at 595 nm after incubation for 24 hours. The percentage reduction in the bacteria growth was determined using equation 3.4.

$$R = \frac{(B - A)}{B} X\ 100 \qquad \text{Equation 3.4}$$

Where R is the percentage reduction in population of the microbes (antimicrobial activity), B is the absorbance of media inoculated with microbe and introduced un-dyed cotton fabric; A is the absorbance of media inoculated with microbe and introduced dyed cotton fabric. The durability of the antimicrobial activity was evaluated by subjecting the dyed fabric to washing cycles and after every washing the antimicrobial activity was determined. The washing tests were done by putting the samples into the washing solution made up of commercial detergent (2 g/L) with material to liquor ratio of 1:50. The antioxidant activity was determined after the 1st, 5th and 10th washing cycles (Shahid-ul-Islam & Butola, 2020).

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Dye Extracts and Characterization

Evaporation of the solvent led to formation of solid extracts for; hexane, dichloromethane and ethyl acetate while methanolic extract formed a paste *for Euclea divinorum*. Hexane extract was yellow in color, dichloromethane extract was light green, ethyl acetate extract was dark green and methanolic and aqueous extracts were brown in color. The results and the steps of obtaining the root bark extract of *Euclea divinorum* plant are as shown in the Figure 4.1. Percentage yield for each solvent extracts were 1.37% (hexane), 2.52% (dichloromethane), 4.13% (ethyl acetate) and methanolic 18.81%.

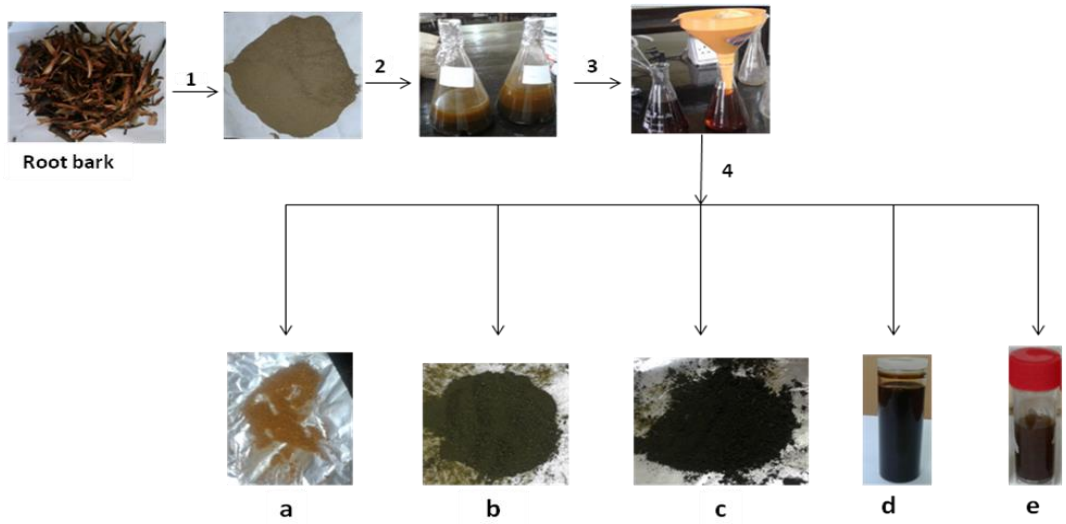


Figure 4.1: Dye extraction from *E. divinorum*; (1) grinding, (2) maceration (3) filtration, (4) solvent evaporation. (a) Hexane, (b) dichloromethane, (c) ethyl acetate, (d) methanolic and (e) aqueous extract

The Hexane, Dichloromethane and ethyl acetate stem bark extracts of *E. abyssinica were* insoluble in water and could not be used in dyeing. Direct methanol and direct aqueous extracts obtained were as shown in Figure 4.2. The percentage yield for methanolic extract was 7.12%.

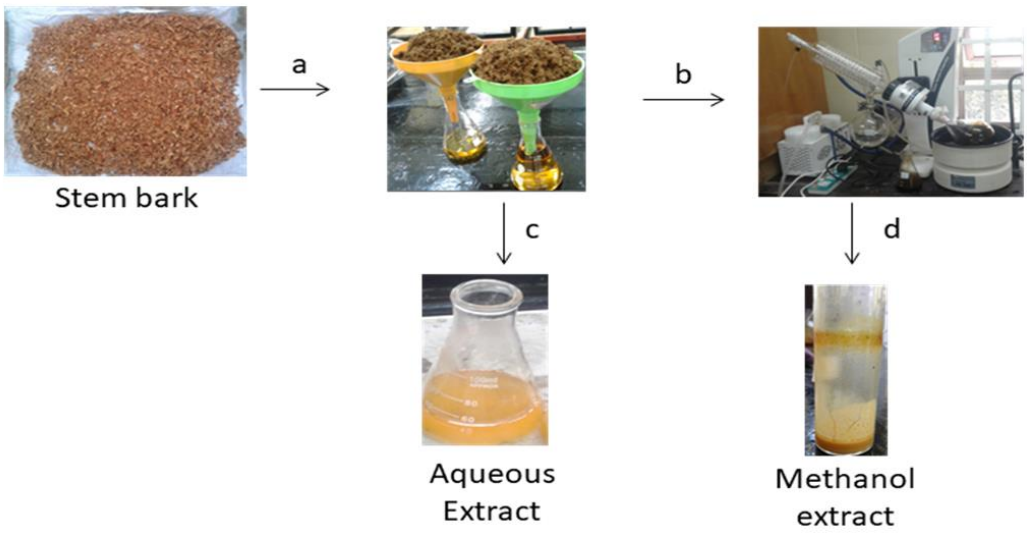


Figure 4.2: Dye extraction from the stem bark of *E. abyssinica;* (a) grinding and maceration for 24 hours, (b and c) filtration, (d) solvent evaporation

4.1.1 Phytochemical Analysis

Phytochemicals in a plant extract are responsible for the particular color produced by the natural dye. Phenols, flavonoids and tannins are the most significant classes of compounds in the dyeing process because they account for the capability of the colorant to get attached to the fabric (color fastness) and the shade formed on the fabric (Mongkholrattanasit *et al*., 2013). In addition, plants rich in tannins have been used as bio-mordants (Amin *et al*., 2020; Erdem İşmal *et al*., 2014; Prabhu & Teli, 2014) and exhibited the potential to be used as alternatives to the toxic metallic mordants that are currently in use (İşmal & Yıldırım, 2019).

Qualitative phytochemical screening studies of *E. divinorum* (Table 4.1) showed that it contains flavonoids, phenols, tannins and terpenoids as was observed by Mwonjoria *et al*. (2018). Qualitative phytochemical screening studies of *E. Abyssinica* showed it contains flavonoids, tannins and phenols as was observed by Ali (2011).

Table 4.1: Qualitative phytochemical analysis of the different solvent extracts

Plant	Solvent extracts	Tannins	Quinones	Phenols	Terpenoids	Saponnins	Flavonoids
E. divinorum	Methanol	+	+	+	+	+	+
	Ethyl acetate	+	+	+	+	-	+
	Dichloromethane	+	-	+	-	-	-
E. abyssinica	Methanol	+	+	+	+	-	+
	Ethyl acetate	+	+	+	-	-	+
	Dichloromethane	+	+	+	-	-	+

+ (present), - (absent)

The linear regression equation for the calibration curve of tannic acid was y = 0.007x + 0.0632 with R^2 = 0.9914 (Figure 6A) indicating good linear relationship within the range of detection. As a result the equation was used to estimate total tannins in the different solvent extracts. The highest tannin content (115.114 mg TAE/g) for *E. divinorum* was observed in the methanolic extract (Table 4.2). This can be attributed to the fact that tannins are polar and hence would preferably dissolve in more polar solvents than less polar solvents (Ishak & Elgailani, 2016). The presence of tannins in the dye extract is significant because it enhances the textile finishing characteristics such as antioxidant, antimicrobial and antifungal properties (Fraga-Corral *et al*., 2020).

The linear regression equation for the calibration curve of gallic acid was y = 0.0085x + 0.0242 with R^2 = 0.9938 (Figure 4.3B), indicating good linear relationship within the range of detection. As a result the equation was used to estimate total phenols in the different solvent extracts. Methanolic extract of *E. divinorum* showed the highest phenol content (123.741 mg GAE/g) followed by ethyl acetate (82.094 mg GAE/g) and dichloromethane (69.741 mg GAE/g) in that order. Comparable ranges to what was observed have been reported in other plants in the same family (Ebeneacea) as *E. divinorum* (Mekonnen *et al*., 2018). Moreover, the results were in line with the

observations made on qualitative phytochemical screening of root extracts of *E. divinorum* (Nyambe, 2014).

The linear regression equation for the calibration curve of Quercetin standard was found to be y=0.0048x + 0.0321 with R^2 = 0.9961 (Figure 4.3C) indicating good linear relationship within the range of detection. As result the equation was used to determine total flavonoids in the different solvent extracts (Table 4.2). The flavonoids content across the three solvents of *E. divinorum* was found to be lower compared to the other phytochemicals analyzed which was in agreement with qualitative phytochemical screening observations (Mwonjoria *et al*., 2018). The flavonoids content in dichloromethane (0.189 mg QE/g) was negligible which was similar to the findings of Al-Fatimi (2019) where flavonoids in dichloromethane extract were not detectable but moderate amounts were observed in methanolic extract. For *E. abyssinica,* in all the three quantified phytochemicals, methanolic extract showed the highest content as has been observed in qualitative screening of the stem bark of *E. abyssinica* (Ali, 2011). This is because flavonoids, tannins and phenols are class of phytochemicals that are polar in nature and hence would tend to dissolve in more polar solvents (Mongkholrattanasit *et al*., 2013).

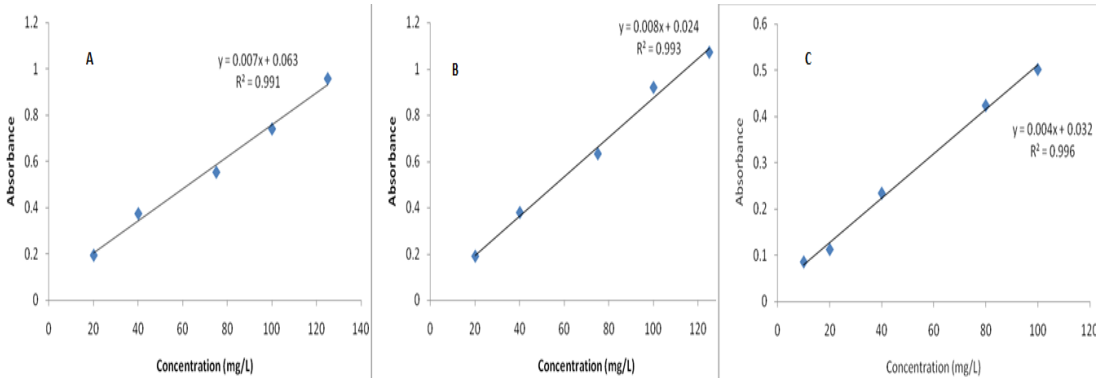


Figure 4.3: Calibration curve for (A) Tannic acid (B) Gallic acid (C) Quercetin

Table 4.2: Quantitative phytochemical content of the different solvent extracts of the dyes

Plant	Solvent extracts	Total tannins (mg TAE g^{-1})	Total phenols (mg GAE g^{-1})	Total flavonoids (mg QE g^{-1})
E. divinorum	Methanol	115.114	123.741	4.979
	Ethyl acetate	99.400	82.094	1.438
	Dichloromethane	66.740	69.741	0.189
E. abyssinica	Methanol	135.571	150.375	92.011
	Ethyl acetate	65.342	82.094	23.132
	Dichloromethane	44.393	73.682	9.114

TAE-Tannic Acid Equivalence, GAE-Gallic Acid Equivalence, QE-Quercetin Equivalence

4.1.2 Evaluation of the Dyeing Properties of the Different Solvent Extracts

The different solvent extracts of *E. divinorum* and *E. abyssinica* showed different shades of color on cotton fabric as shown in Table 4.3. The variation in the color shades of cotton fabric dyed with different solvent extract can be attributed to the difference in the phytochemical composition of each extract according to their polarity (Guinot *et al*., 2008). Hexane, dichloromethane and ethyl acetate extract for *E. abyssinica* were not soluble in water hence were not used in dyeing.

Table 4.3: The different shades of the dyed samples using different solvent extracts

	Solvent extract						
Plant	Un-dyed (control)	Hexane	DCM	Ethyl A.	MeOH SE	MeOH DE	Aqueous
A							
B		**X**	**X**	**X**			

x- Insoluble in water, A- *E. divinorum*, B- *E. abyssinica*

4.1.3 Color Fastness of the Fabric Dyed with Different Solvent Extract

From Table 4.4 it was noted that the methanolic and aqueous dye extracts of *E. divinorum* plant showed good wash fastness properties among the five solvent extracts with a value between 4 and 5 which is approaching excellent. On the other

hand, hexane and dichloromethane dye extracts showed the lowest color fastness values which do not meet the standard requirements for color fastness test to washing (Souissi *et al*., 2018). All the white cotton fabric attached to dyed samples during wash fastness testing did not show any staining and hence had an excellent value of 5. This indicates that the dyed fabric resisted the color staining and color change during the washing process (Yang *et al*., 2018). Despite the little loss of dye by the dyed samples, the white cotton fabrics were not stained which is due to the fact that the conditions for wash fastness testing were not favorable for the white fabric to absorb the dye from the solution. With regards to color fastness to light, it was observed that the fabric dyed with methanolic extract had the highest light fastness value followed by aqueous, ethyl acetate, dichloromethane and hexane (Table 4.4). Color fading due to exposure of the dyed samples to UV light was generally between fairly good and excellent which is quite acceptable in textile dyeing processes (Yusuf *et al*., 2015).

Considering color fastness to rubbing, both dry and wet rubbing fastnesses for all the samples were excellent. The white samples sewed to the dyed samples during rub fastness testing did not show any staining at all, hence a rating of 5 (Table 4.4). Consequently, these fastness results meet the requirement for colour fastness which should be a rating of 3 and above (Pisitsak *et al*., 2018). The color fastness for *E. abyssinica* natural dye extract on cotton fabric was generally not good (between 2 and 3) which is below the textile acceptable level of above 3.0. This observation necessitated for pre-treatment of cotton fabric to enhance its dye-ability and absorbance of the dye molecules.

Table 4.4: Color fastness properties of the different solvent extracts of *E. divinorum* and *E.abyssinica*

Plant	Solvent extract	Wash fastness		Rubbing fastness		Light fastness	Perspiration Fastness
		C.C	C.S	Dry	Wet		
E. divinorum	Aqueous	4-5	5	5	5	5	4
	MeOH DE	4-5	5	5	5	5	4
	MeOH SE	4-5	5	5	5	5	4
	Ethyl A.	4	5	5	5	4-5	3
	DCM	3-4	5	5	5	4	2
	Hexane	2	5	5	5	3	2
E. abyssinica	Aqueous	2	2	3-4	3-4	3	2-3
	MeOH DE	2	2	3-4	3-4	3	2-3
	MeOH SE	2	2	3-4	3-4	3	2-3

DE- direct extraction, SE- sequential extraction, c.c- color change, c.s- color staining

4.1.4 Spectroscopic Characterization of the Dye Extracts

4.1.4.1 UV-visible Analysis

The UV-Vis spectra for *E. divinorum* and *E. abyssinica* extracts indicated that the dye extracts were made of a mixture of compounds (Figure 4.4 and 4.5). The methanolic extract for *E. divinorum* showed peaks at 341 and 427nm and the aqueous extract showed a peak at 340nm (Figure 4.4). The peaks observed in these extracts are characteristic peaks of naphthoquinones (Spruit, 2010). The extract for *E. Abyssinica* showed peaks at 337, 345 and 407nm (Figure 4.5), which are characteristic peaks of flavonoids (Tsimogiannis *et al*., 2007).

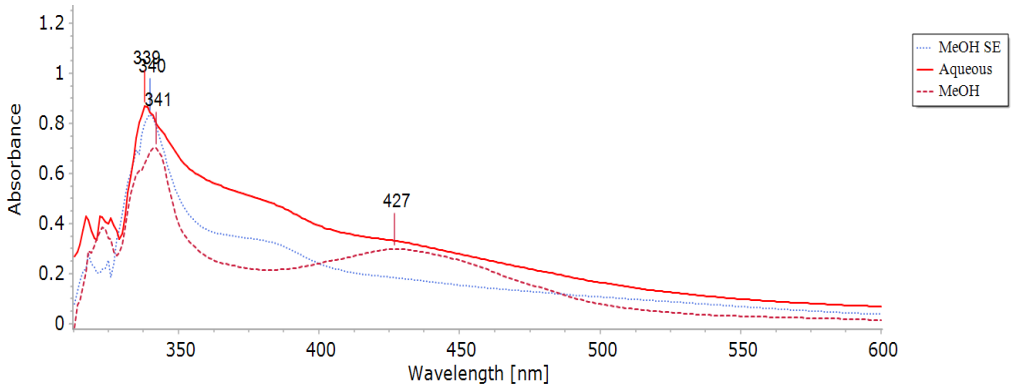


Figure 4.4: UV-Vis absorbance spectra of the extracts of *E. divinorum* plant

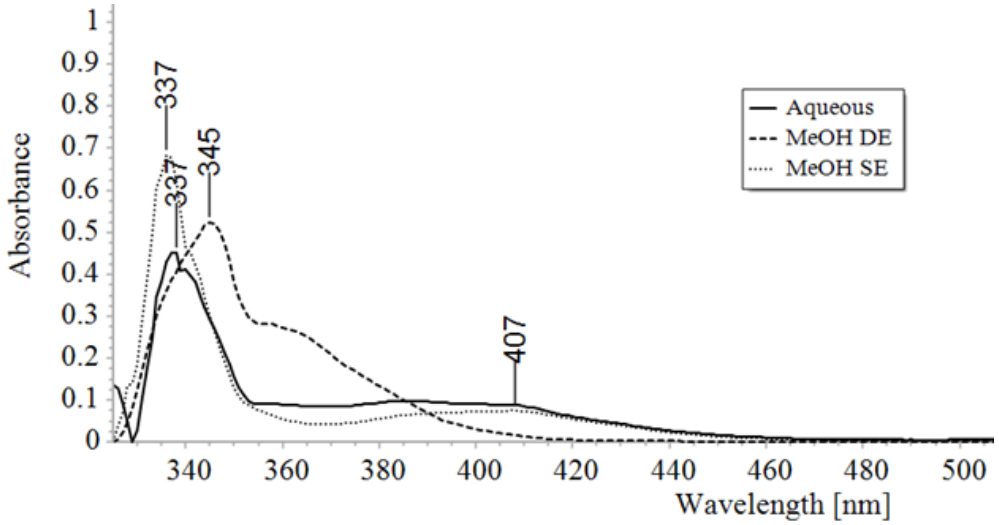


Figure 4.5: UV-Vis absorbance spectra of the extracts of *E. abyssinica plant*

4.1.4.2 Fourier Transform Infra-Red (FT-IR) Analysis

The FT-IR spectra for major peaks for the plant extract were as shown in Figure 4.6. *E. divinorum* dye extract showed a broad band at 3297 cm^{-1} corresponding to O–H bond stretching mode for carboxylic acid. The bands at 1608 cm^{-1}, 1373 cm^{-1} and 1033 cm^{-1} corresponds to C=C stretching mode, C–O–H bending mode, C–O stretching mode, respectively. *E. abyssinica* dye extract showed a peak at 3647 cm^{-1} corresponding to O–H bond stretching mode for carboxylic acid. The bands at 2364 cm^{-1}, 1507 cm^{-1} and 1043 cm^{-1} corresponds to O–H stretching mode, C=C stretching mode for aromatic rings and C–O stretching mode, respectively.

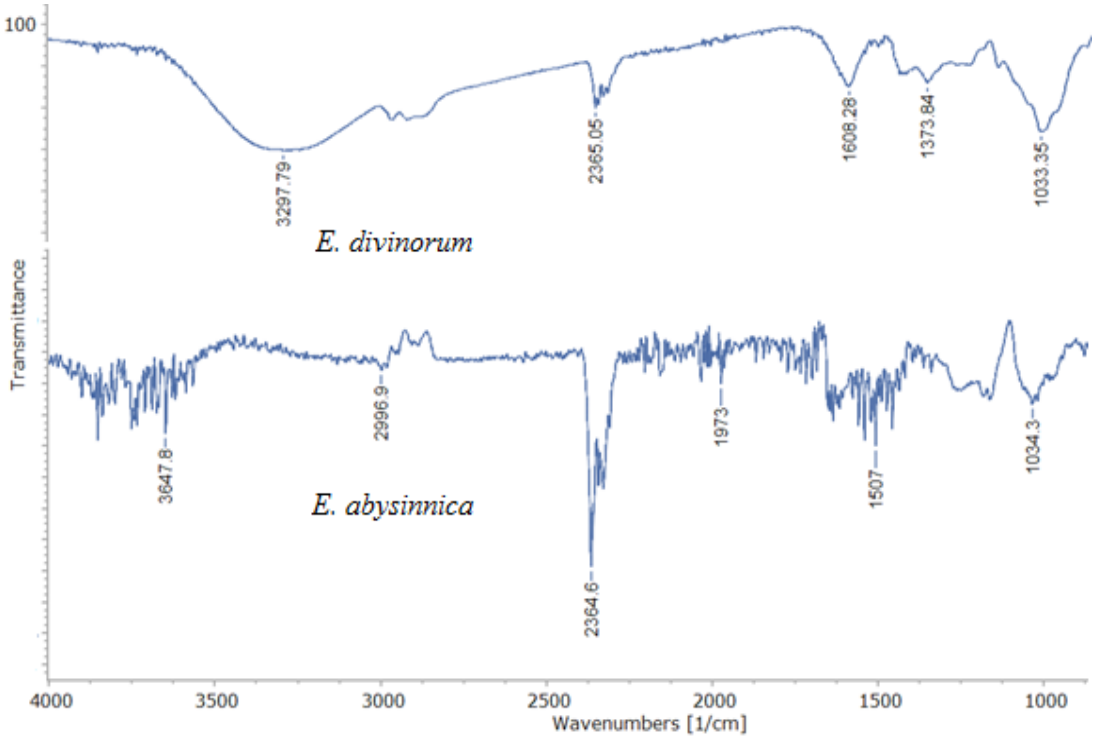


Figure 4.6: FTIR spectra of the dye extracts

4.1.4.3 Gas Chromatography and Mass Spectrometry Analysis

In this study *E. divinorum* natural dye extract was found to exhibit promising dyeing characteristics compared to *E. abyssinica* natural dye extract. *E. abyssinica* required pre-treatment procedures to be done on the cotton fabric to enhance its dyeing ability, dye absorption and color fastness. As a result the *E. divinorum* methanolic dye extract was subjected to GCMS analysis to ascertain its major molecular components that are responsible for the good coloring properties in terms of color fastness and color strength that was observed on cotton fabric. Figure 4.7 shows the gas chromatogram of the analyzed sample indicating compound retention time. Five compounds (Carbomethoxy-5,8-dimethoxy-1-tetralone, 1-Docosene, Lupeol (Lup-20(29)-en-3-ol), Betulin (Lup-20(29)-en-3, 28- diol) and Lup-20(29)en-3-ol, acetate (3β) were identified as shown in Table 4.5 were identified in the analysis. Lupeol and Betulin have been isolated from the root bark of *E. divinorum* by Mebe *et al*. (1998).

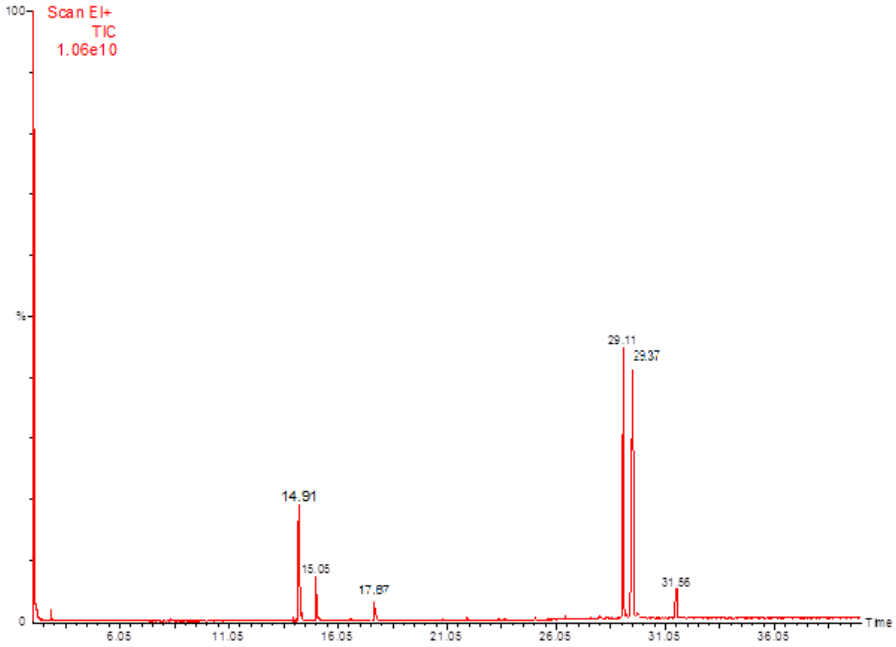
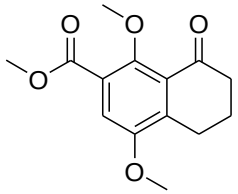
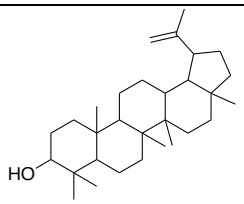
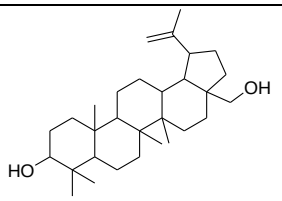
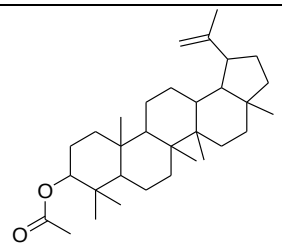


Figure 4.7: Gas chromatogram of *E. divinorum* dye extract

The mass spectrum for Lupeol showed a molecular ion peak at a mass to charge (*m/z*) of 426 and the structure was confirmed by the presence of fragment peaks at m/z 189 and m/z 207 which have been observed in other studies reporting lupeol (Carvalho *et al*., 2010). Other major fragments were *m/z* of 411, 383,218, 189, 161, 55 and 43 (Appendix D). Betulin was detected with its molecular ion peak at mass to charge (*m/z*) of 442 and upon fragmentation and loosing water molecule (-H_2O) it resulted in a peak at *m/z* 424 (Joshi *et al*., 2013). Other major fragments were *m/z* of 411,203, 189, 175, 161, 135, 121, 107, 95, 81, 69, 55 and 41 (Appendix E).

Table 4.5: Compounds in *E. divinorum* identified by GCMS analysis

Retention time	Molecular weight	Molecular formula	Compound Name	Structure
14.91	264	$C_{14}H_{16}O_5$	Carbomethoxy-5,8-dimethoxy-1-tetralone	
17.67	308	$C_{22}H_{44}$	1-Docosene	
29.11	426	$C_{30}H_{50}O$	Lupeol Lup-20(29)-en-3-ol	
29.37	442	$C_{30}H_{50}O_2$	Betulin Lup-20(29)-en-3, 28- diol	
31.56	468	$C_{32}H_{52}O_2$	Lup-20(29)en-3-ol, acetate (3β)	

4.1.4.4 Nuclear Magnetic Resonance (NMR) Analysis

E. divinorum dye extract was purified using column chromatography in order to isolate and identify the phytochemicals that make up the dye and are responsible for the color imparted by the dye molecule on the cotton fabric. Column chromatography of the ethyl acetate extract led to 29 elutes of 20ml which were subjected to thin layer chromatography and on pooling together the elutes with similar TLC profile A to G fractions were formed. The elution with solvent mixture of ethyl acetate/hexane (1:4) resulted in pure fraction D (1.02g) that was comprised of white needle like crystals (Figure 4.8A). Further purification of E with solvent mixture of ethyl acetate/hexane (1:9 followed by 1:4) resulted in pure fraction E2 (23.4mg,) that was made up of small cream-white crystals (Figure 4.8B).

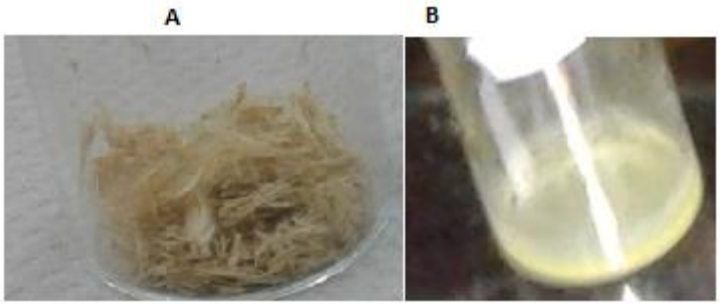


Figure 4.8: Crystals of isolate D (A) and E2 (B)

Proton and carbon NMR analysis of sample D and E were used to determine their structures and confirm their identities. NMR spectral data for compound D (Figure 4.9) were as follows: ^{1}H NMR ($CDCl_3$, 600 MHz) showed signals at δ (chemical shifts in ppm) 4.73 (1H, d, H29b, J = 1.8Hz), 4.58 (1H, d, H-29a, J = 1.8Hz), 3.16 (1H, d, H-3), 2.42-2.48(1H, m, H-19), 1.68, 1.53 (2H, m, H-2), 1.67 (3H, s, H-30), 1.66 (1H, m, H-13), 1.46, 1.35(2H, m, H-16), 1.50, 1.38 (2H, m, H-6), 1.39 (2H, m, H-7), 1.36 (1H, m, H-18), 1.31, 1.91 (2H, m, H-21), 1.25 (1H, m, H-9), 1.21, 1.42 (2H, m, H-11), 1.18, 1.37 (2H, m, H-22), 106,1.63 (2H, m, H-12), 1.04, 1.60 (2H, m, H-15), 1.03 (3H, s, H-26), 0.96 (3H, s, H-23), 0.93 (3H, s, H-27), 0.88, 1.66 (2H, m, H-1), 0.84 (3H, s, H-25), 0.79 (3H, s, H-28), 0.76 (3H, s, H-24),0.66 (1H, m, H-5). ^{13}C NMR ($CDCl_3$, 500 MHz) (Figure 4.10) showed signals at (chemical shifts in ppm) δ 150.65 (C-20), 109.06 (C-29), 77.71 (C-3), 55.43 (C-5), 50.47 (C-9), 48.23 (C-18), 47.93 (C19),42.83 (C-17), 42.70 (C-14), 40.79 (C-8), 39.74 (C-22), 39.26 (C-1), 38.71 (C-4), 38.14 (C-13), 37.07 (C 10), 35.40 (C-16), 34.27 (C-7), 29.65 (C-21), 27.68 (C-23), 27.41 (C-2), 27.35 (C15),25.18 (C-12), 20.78 (C-11), 18.62 (C-30), 18.21 (C-6), 17.42 (C-28), 15.72 (C-25), 15.56 (C-26), 15.18 (C24),14.06 (C-27).

The ^{1}H NMR spectrum of compound D showed seven singlet signals assigned to seven methyl groups at chemical shifts 1.67, 1.03, 0.96, 0.93, 0.84, 0.79, 0.76 ppm, a sextet at 2.42-2.48 ppm was assigned to H-19β which is a characteristic signal for lupeol. In addition, the doublet at δ 3.16 ppm for H-3 proton suggested a 3β-hydroxy

triterpene and the doublets at δ 4.73 and 4.58 ppm for H-29a and H-29b protons, respectively, were observed. According to ^{13}C NMR spectrum of compound D, the compound is made up of six methine, seven methyl, eleven methylene and six quaternary carbons hence a total of 30 carbon signals were observed. One oxygenated carbon (C-3, a methylene carbon) was observed at chemical shift 77.71 ppm and two olefinic carbons showed signals at 150.65 ppm and 109.06 ppm which were identified as (C-20, a quaternary carbon) and (C-29, a methylene carbon), respectively. The ^{13}C spectral data confirmed that the compound was a tri-terpenoid. The described ^{1}H and ^{13}C spectral data was compared to what has been reported in literature (Abdullahi *et al*., 2013; Jain & Bari, 2010; Shwe *et al*., 2019) and it consistently led to a pentacylic tri-terpenoid named lupeol (Figure 4.11).

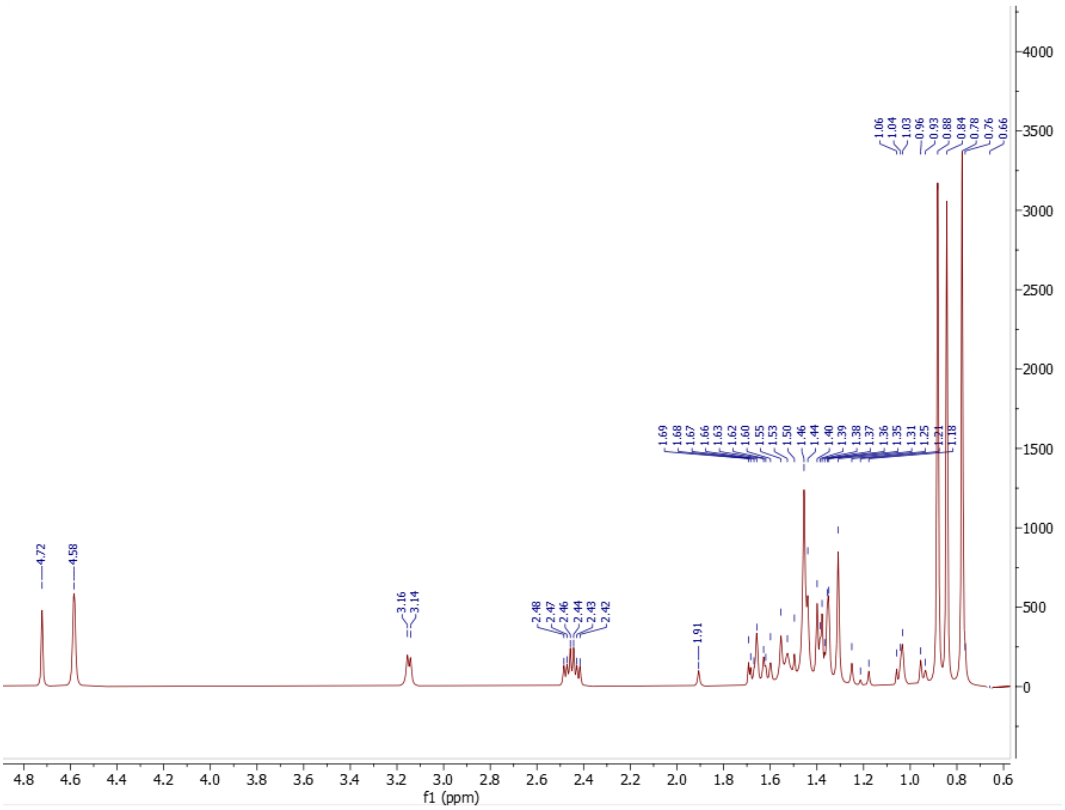


Figure 4.9: ^{1}H NMR spectrum of compound D

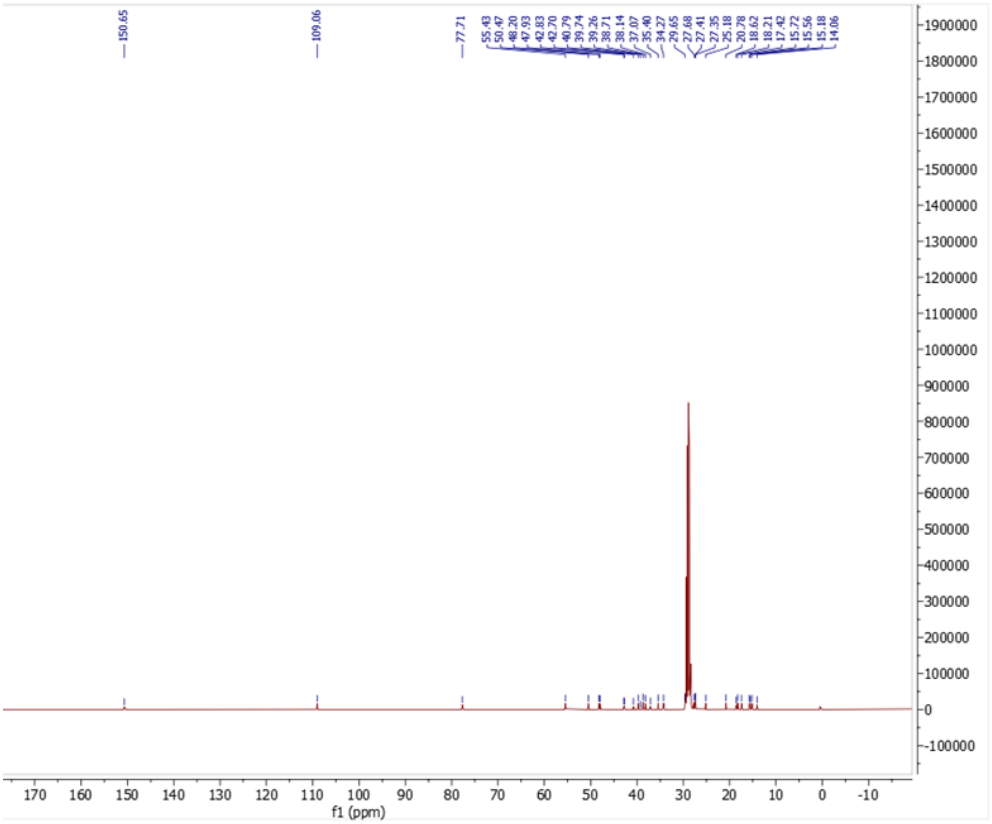


Figure 4.10: ^{13}C NMR spectrum of compound D

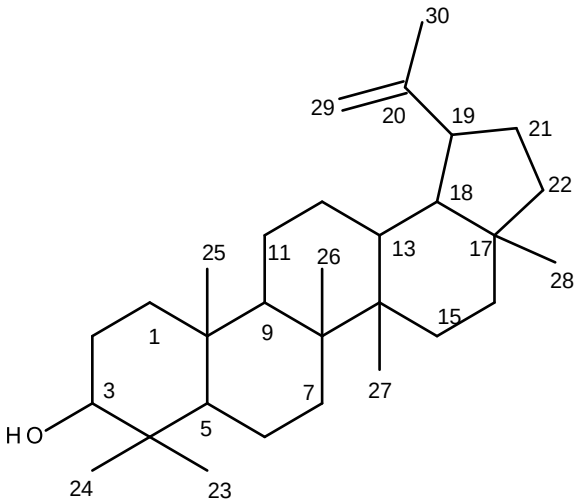


Figure 4.11: Structure of Lupeol

Spectral data for compound E2 were as follows: ^{1}H NMR (CDCl3, 500 MHz) (Figure 4.12). It showed signals at (chemical shifts in ppm) δ 4.73 (1H, d, H29b), 4.58 (1H,d,

H-29a), 3.79 (1H, d, J = 10.6, H-28b), 3.34 (1H, d, J = 10.6, H-28a), 3.16 (1H, d, J =5.4, H-3α), 2.42-248 (1H, m, H-19), 1.93-199, 1.19 (2H, m, H-16), 1.91, 1.03 (2H, m, H-22), 1.69, 1.04 (2H, m, H-15), 1.67 (3H, s, H-30), 1.66, 0.88 (2H, m, H-1), 1.63, 1.03 (2H, m, H-12), 1.62 (1H, m, H-13), 1.60, 1.53 (2H, m, H-2), 1.55 (1H, m, H-18), 1.50, 1.37 (2H, m, H-6), 1.44, 1.91, (2H, m, H-21), 1.42, 1.21 (2H, m, H-11), 1.38 (2H, m, H-7), 1.25 (1H, m, H-9), 1.04 (1H, m, H-15), 1.03 (3H, s, H-26), 0.99 (3H, s, H-27), 0.93 (3H, s, H-23), 0.84 (3H, s, H-25), 0.76 (3H, s, H-24),0.66 (1H, m, H-5). ^{13}C NMR (CDCl3, 125 MHz) (Figure 4.13) showed signals at (chemical shifts in ppm) δ 150.65 (C20),109.06 (C-29), 77.71 (C-3), 60.68 (C-28), 55.43 (C-5), 50.47 (C-9), 48.20 (C-18), 47.93 (C19),47.43 (C-17), 42.70 (C-14), 40.79 (C-8), 39.74 (C-1), 38.71 (C-4), 38.14 (C-13), 37.07 (C10),34.27 (C-7), 33.88 (C-22), 29.65 (C-21), 29.10 (C-16), 27.68 (C-23), 27.41 (C-2), 27.35 (C15),25.18 (C-12), 20.78 (C-11), 18.62 (C-30), 18.21 (C-6), 15.72 (C-25), 15.56 (C-26), 15.18 (C24),14.06 (C-27).

The ^{1}H NMR spectrum of compound E2 showed six singlet signals assigned to six methyl groups at chemical shifts 1.67, 1.03, 0.99, 0.93, 0.84, 0.76 ppm and a doublet for terminal alkene (Vinylic protons) at δ 4.73 and 4.58 ppm for H-29a and H-29b protons. The signal at δ 3.14 ppm and 3.16 ppm in form of a doublet was for α-oriented proton at H-3 of a 3β-hydroxy triterpene as well as the signal at δ 3.34 ppm and 3.79 ppm for H-28 confirmed the presence of two hydroxyl groups attached to C-3 and C-28. The ^{13}C NMR spectrum of compound E2 showed six methine carbons, six methyl groups, twelve methylene groups, six quaternary carbons and two olefinic carbons hence a total of 30 carbon signals were observed. Two oxygenated carbon (C-3) and (C-28) were observed at chemical shift 60.68, 77.71 ppm and two olefinic carbons showed signals at 150.65 ppm and 109.06 ppm which were identified as (C-20, a quaternary carbon) and (C-29, a methylene carbon), respectively. The described

^{1}H and ^{13}C spectral data were compared to what have been reported in literature (Ayatollahi *et al*., 2009; Lall *et al*., 2006; Tijjani *et al*., 2012)(Ayatollahi *et al*., 2009; Lall *et al*., 2006; Tijjani, Ndukwe, and Ayo, 2012) and it consistently led to a pentacylic tri-terpenoid named Lup-20(29)-en-3, 28- diol commonly known as betulin (Figure 4.14).

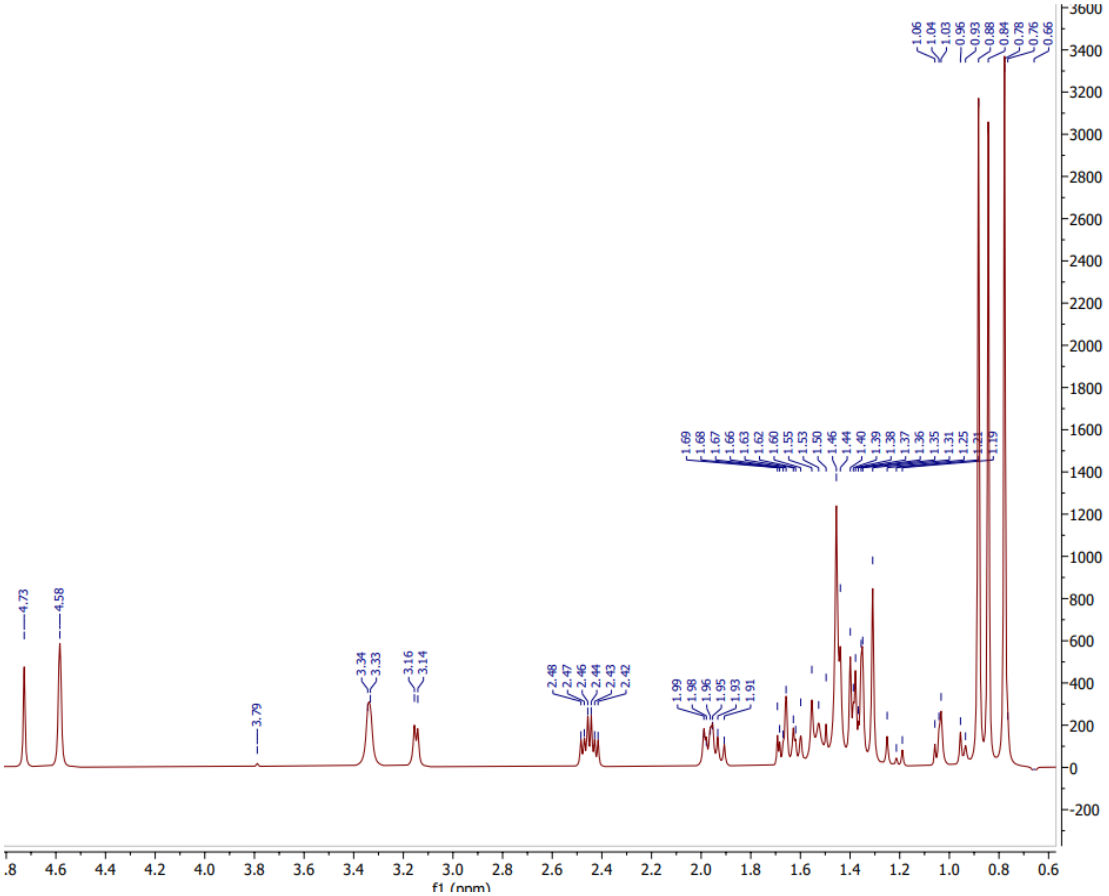


Figure 4.12: ^{1}HNMR spectrum of compound E2

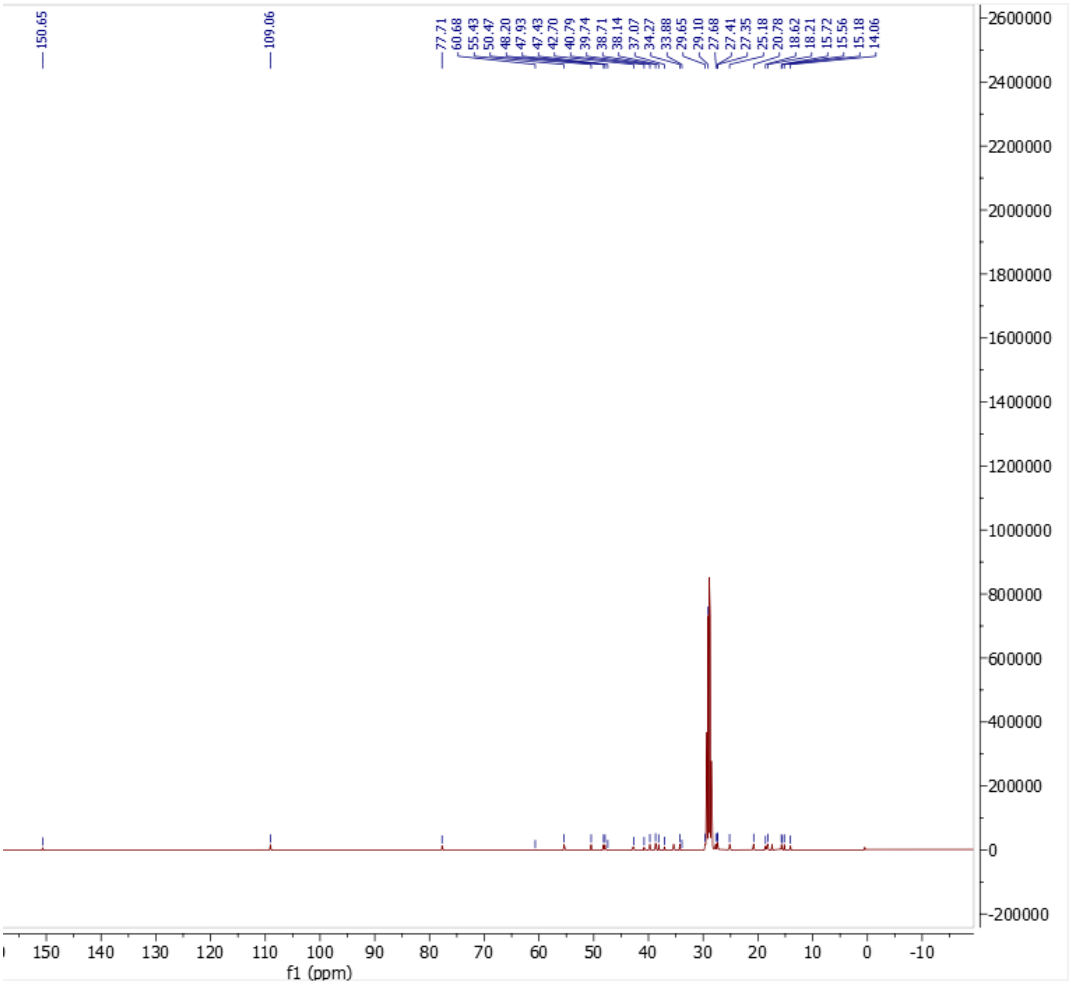


Figure 4.13: ^{13}C NMR spectrum of compound E2

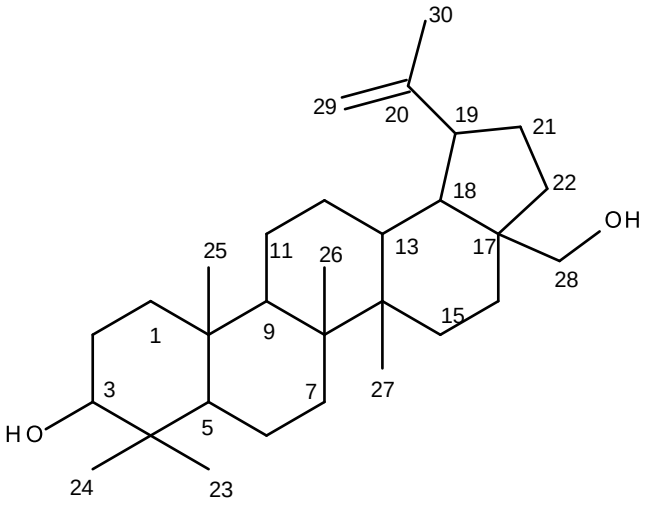


Figure 4.14: Structure of betulin

4.2 Optimization of Extraction and Dyeing Conditions

4.2.1 Optimization of Dye Extraction Conditions

4.2.1.1 Analysis of Single-Factor Design

Effect of time, temperature and M: L (ratio of the solid sample to the solvent) on the absorbance of the dye extract was as shown in Figure 4.15. The three parameters played a role on the absorbance hence concentration of the dye since there was variation when each parameter was varied.

4.2.1.1.1 Effects of Time

In both aqueous and methanolic extracts there was fast increase in color concentration in terms of absorbance as the time increased from 60 to 120 minutes (Figure 4.15A) which is due to increased contact duration between the sample and the solvent allowing more dye to dissolve (Farooq *et al*., 2013). A minimal increase in absorbance was then observed up to 150 minutes beyond which there was no change in absorbance meaning that at this point the solvent was saturated and more pigments could not dissolve in it or the dye had been exhausted in the sample (Yusuf *et al*., 2017).

4.2.1.1.2 Effects of M:L (Material to Liquor Ratio)

The influence of M: L ratio on the concentration of the dye was as shown in Figure 4.15B. It was noted that the concentration of the dye increased when the M:L was increased from 2:100 to 8:100 then the concentration started to drop. The reduction in the concentration of the dye at high ratio can be explained in terms of congestion of the plant sample in the mixture which prevents infiltration of the solvent into the solid averting dye extraction (Wang *et al*., 2020).

4.2.1.1.3 Effects of Temperature

As the temperature increases, the concentration of the dye increases up to the optimum temperature of 60 °C for methanolic extraction (Figure 4.15C) and 80°C for aqueous extraction (Figure 4.15D). The reason is as the temperature increases, solvent's kinetic energy increases hence more solvent penetrates into the sample matrix, boosting dye extraction (Rather *et al*., 2020). There was a insignificant reduction in the concentration of the dye beyond the optimum temperature because of the decomposition of some of the dye components at the high temperatures (Yin *et al*., 2017).

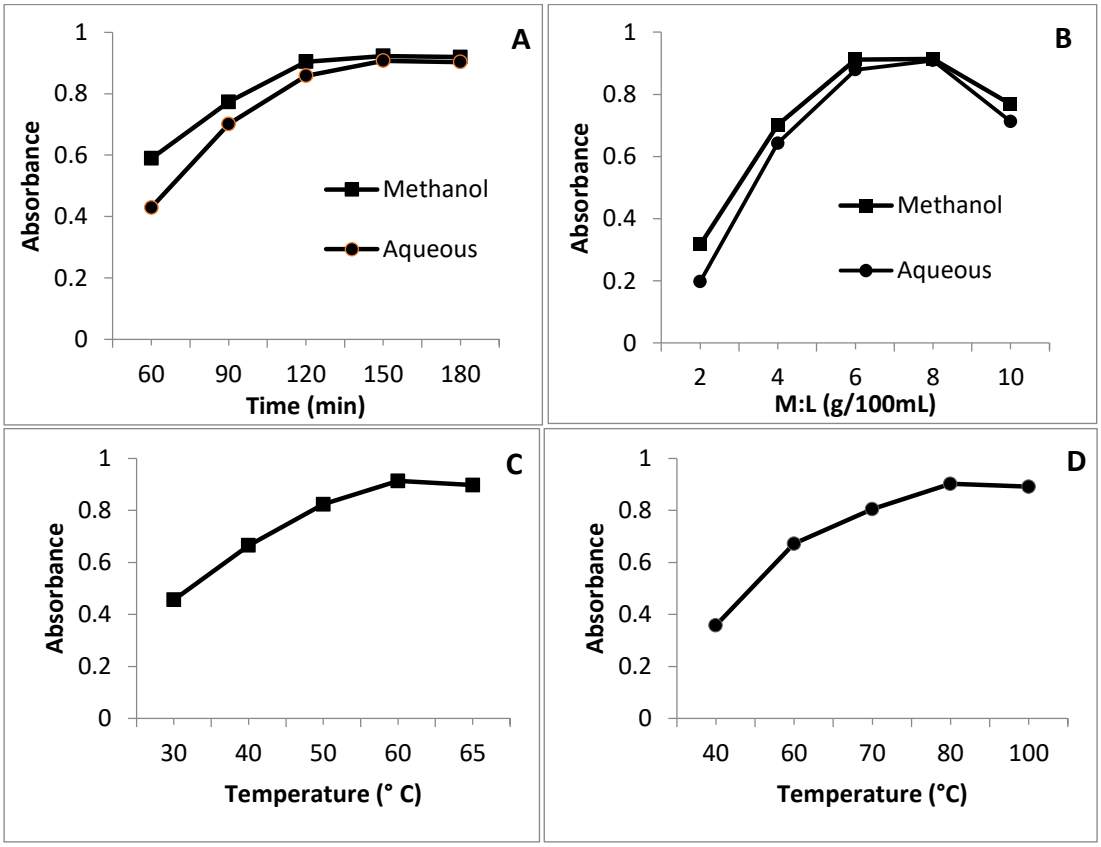


Figure 4.15: Effects of (A) Time, (B) material to liquor ratio, (C) Temperature for methanolic dye extract and (D) Temperature for aqueous dye extract on the absorbance

4.2.1.2 Response Surface Methodology

The Response Surface Methodology (RSM) design was set up and the range for the parameters was based on the single factor design results. The experimental levels of the studied factors: M:L (g of the sample per 100 mL of the solvent), time (minutes) and temperature (°C) with their responses (absorbance at λ max) were as shown in Table 4.6 and 4.7 for *E. divinorum* and *E. abyssinica*, respectively. M:L, time and temperature were coded as A, B and C, repectively. Absorbance was chosen as the response to monitor the concentration of the extracted dye because of their direct correlation (Uddin, 2015).

Table 4.6: Coded CCD design for aqueous and methanolic dye extraction from *E. divinorum*.

Run order	Aqueous Extraction Design				Methanolic Extraction Design			
	Factors			Absorbance	Factors			Absorbance
	A	B	C		A	B	C	
1	0	0	0	0.872	1	1	-1	0.877
2	α	0	0	0.934	-1	-1	-1	0.209
3	0	0	0	0.912	0	0	0	0.961
4	0	-α	0	0.123	1	0	-1	0.556
5	0	α	0	0.643	1	-1	1	0.605
6	0	0	-α	0.701	-1	1	-1	0.303
7	-α	0	0	0.180	-1	-1	1	0.313
8	0	0	α	0.933	1	1	1	0.954
9	1	-1	1	0.466	-1	1	1	0.379
10	1	1	1	0.932	0	0	0	0.912
11	0	0	0	0.912	0	0	0	0.994
12	1	1	-1	0.924	0	0	0	0.968
13	-1	-1	-1	0.075	α	0	0	0.998
14	0	0	0	0.932	0	α	0	0.889
15	-1	-1	1	0.194	0	0	0	0.972
16	-1	1	1	0.288	0	0	α	0.785
17	0	0	0	0.867	0	-α	0	0.527
18	-1	1	-1	0.276	-α	0	0	0.248
19	0	0	0	0.921	0	0	-α	0.593
20	1	-1	-1	0.372	0	0	0	0.906

Table 4.7: Coded CCD for aqueous and methanolic extraction conditions from *E. abyssinica*

Run order	Aqueous Extraction Design				Methanolic Extraction Design			
	Factors				Factors			
	A	B	C	Absorbance	A	B	C	Absorbance
1	0	0	-α	0.559	1	-1	1	0.527
2	α	0	0	0.854	-1	1	1	0.720
3	0	0	α	0.735	0	0	-α	0.543
4	-1	-1	-1	0.344	0	-α	0	0.572
5	0	0	0	0.854	0	0	0	0.901
6	0	-α	0	0.489	0	0	0	0.901
7	1	-1	1	0.482	0	0	0	0.901
8	0	α	0	0.741	-1	-1	1	0.304
9	-1	1	-1	0.462	-1	1	-1	0.415
10	1	1	1	0.521	1	-1	1	0.633
11	0	0	0	0.855	-1	-1	-1	0.294
12	-1	1	1	0.487	0	0	α	0.688
13	0	0	0	0.856	-α	0	0	0.298
14	0	0	0	0.854	0	0	0	0.902
15	0	0	0	0.854	1	1	1	0.893
16	-α	0	0	0.415	α	0	0	0.856
17	-1	-1	-1	0.298	0	0	0	0.901
18	1	1	-1	0.436	0	α	0	0.905
19	α	0	0	0.618	1	1	-1	0.813
20	1	-1	-1	0.386	0	0	0	0.902

4.2.1.3 Adequacy of the Models

Validation of the fitted model is important to make sure that it makes sufficient approximation that is closer or equal to the actual value (Al-Alwani *et al*., 2016). Normal probability plots (Figure 4.16) show the distribution of the residuals (the variation between the theoretical and the experimental response) for aqueous (A) and methanolic (B) dye extraction. The residual values in both methods were small (-2 > residual < 2) which indicated that the model prediction is highly precise (Radaei *et al*., 2014). Furthermore, the distribution of almost all the data points on the chart was nearer to the straight line with little scattering which was anticipated with normal data (Swamy *et al*., 2014), therefore the data was found to be normally distributed.

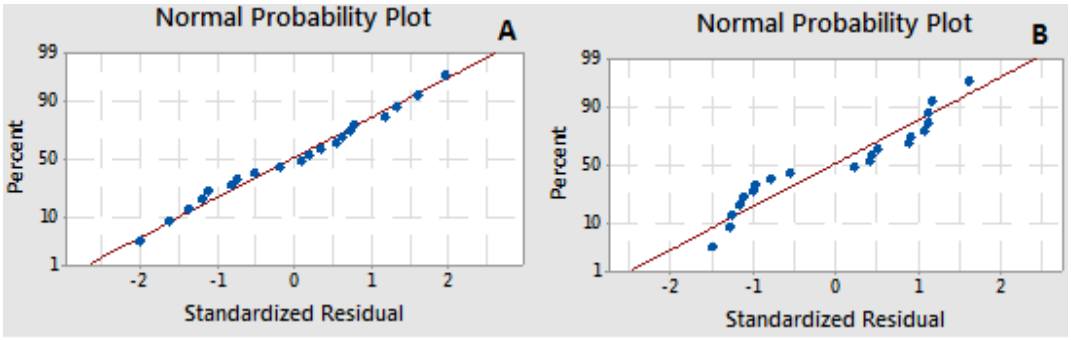


Figure 4.16: Normal probability plots: A is for aqueous and B is for methanolic extraction

The analyses of the variation of the statistical models lead to regression equations 4.1 and 4.2 for aqueous and methanolic extraction, respectively, for *E. divinorum* dye while equations 4.3 and 4.4 for aqueous and methanolic extraction, respectively, for *E. abyssinica* dye. The second order equations are in form of coded factors A, B, C and absorbance which refers to M:L, temperature, time and response, respectively. The coefficient of determination R^2 showed that the fitted models can explain over 98% of the variation in the response for all dye extractions. This implies that the statistical model used fitted the actual data well since when the value of R^2 approache 100% the model fits better (Bouatay *et al*., 2019). Adjusted R^2 which corrects for the size of the model and the number of the terms was highly closer to the R^2 for both extractions denoting that the model strongly fit to the study (Vedaraman *et al*., 2017). The predicted R^2 points out that the model has 85% ability to predict a novel observation which is a high estimation ability (Hemanthraj *et al*., 2014).

$$Absorbance = -1.81 + 0.23A + 0.043B + 0.004C - 0.019A^2 - 0.0003B^2 - 0.00001C^2 + 0.001AB - 0.00002AC - 0.000013BC \quad \text{Equation 4.1}$$

With R^2 = 99.26%, R^2 (adj) = 98.43%, R^2 (pred) = 93.90%

$$Absorbance = -2.91 + 0.1797A + 0.0892B + 0.01002C - 0.0188A^2 - 0.00085B^2 - 0.000038C^2 + 0.001771AB - 0.000038AC - 0.00001BC \quad \text{Equation 4.2}$$

With R^2 = 98.98%, R^2 (adj) = 97.85%, R^2 (pred) = 93.04%

$$Absorbance = -1.193 + 0.113A + 0.024B + 0.0081C - 0.00502A^2 - 0.00015B^2 - 0.000032C^2 + 0.001AB - 0.000028AC - 0.00002BC \quad \text{Equation 4.3}$$

With R^2 =98.25%, R^2 (adj) =96.68% and R^2 (pred) =86.88%

$$Absorbance = -1.698 + 0.1203A + 0.0454B + 0.0058C - 0.004A^2 - 0.0004B^2 - 0.000035C^2 + 0.000016AB - 0.000118AC - 0.00008BC \quad \text{Equation 4.4}$$

With R^2 = 98.02%, R^2 (adj) = 96.24 and R^2 (pred) = 85.07%

4.2.1.4 Statistical Analysis

The significance of the model and terms to the response were evaluated using the p-value of ANOVA (Tables 4.8, 4.9, 4.10 and 4.11). From ANOVA results the whole model, linear and squared model were very significant because of the p = 0.000 for both aqueous and methanolic extraction. The two way factor interaction model are also significant with p = 0.001 and 0.014 for aqueous and methanolic extraction, respectively, for both *E. divinorum* and *E. abyssinica* dye extracts since p < 0.05 is significant (Parra-Campos & Ordóñez-Santos, 2019).

Analysis of significance of individual factors in the model for aqueous extraction indicated that all the linear and squared factors had significant effects on the absorbance but for two way factor interaction terms only the interaction between M: L*temperature was significant for both *E. divinorum* and *E. abyssinica* dye extracts. The other two interaction terms had p = 0.811> 0.05 and p = 0.136 > 0.05 for *E. divinorum* meaning they did not have any influence on the variation of absorbance of the dye extract. Correspondingly, for methanolic extraction the two way interaction terms for methanolic extraction didn't show significant effects except for M: L*temperature where p = 0.02 < 0.05. Insignificance of most interaction factors to the response during extraction of dyes have been observed in other studies (Sharmila *et al*., 2019).

The lack of fit value probability value was p > 0.05 for all extractions for both *E. divinorum* and *E. abyssinica* dye extracts (Tables 4.8 4.9, 4.10 and 4.11), signifying

triviality of lack of fit in relation to the pure error hence quadratic models were reliable (Vedaraman *et al*., 2017).

Table 4.8: Analysis of variance for aqueous extraction of *E. divinorum* dye

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	10	2.09347	0.209347	120.34	0.000
Linear	3	1.08921	0.363070	208.71	*
M:L	1	0.71364	0.713637	410.23	0.000*
Temperature	1	0.34947	0.349470	200.89	0.000*
Time	1	0.02610	0.026102	15.00	0.000*
Square	3	0.91346	0.304487	175.03	0.004*
M:L*M:L	1	0.24136	0.241360	138.74	0.000*
Temperature*Temperature	1	0.55825	0.558255	320.91	0.000*
Time*Time	1	0.01187	0.011867	6.82	0.000*
2-Way Interaction	3	0.07010	0.023367	13.43	0.028*
M:L*Temperature	1	0.06534	0.065341	37.56	0.001*
M:L*Time	1	0.00011	0.000105	0.06	0.000*
Temperature*Time	1	0.00466	0.004656	2.68	0.811
Error	9	0.01566	0.001740		0.136
Lack-of-Fit	5	0.01241	0.002483	3.06	
Pure Error	4	0.00324	0.000811		
Total	19	2.10913			0.150

Table 4.9: Analysis of variance for methanolic extraction for *E. divinorum* dye

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	10	1.52645	0.152645	87.46	0.000*
Linear	3	0.85350	0.284501	163.01	0.000*
M:L	1	0.67569	0.675692	387.15	0.000*
Temperature	1	0.15009	0.150093	86.00	0.000*
Time	1	0.02772	0.027718	15.88	0.003*
Square	3	0.61621	0.205403	117.69	0.000*
M:L*M:L	1	0.23876	0.238763	136.80	0.000*
Temperature*Temperature	1	0.13030	0.130304	74.66	0.000*
Time*Time	1	0.15329	0.153294	87.83	0.000*
2-Way Interaction	3	0.03288	0.010959	6.28	0.014*
M:L*Temperature	1	0.03251	0.032512	18.63	0.002*
M:L*Time	1	0.00036	0.000365	0.2	0.659
Temperature*Time	1	0.00000	0.000000	0.00	1.000
Error	9	0.01571	0.001745		
Lack-of-Fit	5	0.01001	0.002002	1.41	0.382
Pure Error	4	0.00570	0.001424		
Total	19	1.54215			

Table 4.10: Analysis of variance for aqueous extraction for *E. abyssinica* dye

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	0.743229	0.082581	62.39	0.000
Linear	3	0.090582	0.030194	22.81	0.001
M:L	1	0.022528	0.022528	17.02	0.002
Temperature	1	0.047215	0.047215	35.67	0.000
Time	1	0.020839	0.020839	15.74	0.003
Square	3	0.645067	0.215022	162.46	0.000
M:L*M:L	1	0.275323	0.275323	208.02	0.004
Temperature*Temperature	1	0.143063	0.143063	108.09	0.002
Time*Time	1	0.109979	0.109979	83.09	0.000
2-Way Interaction	3	0.007581	0.002527	1.91	0.192
M:L*Temperature	1	0.005941	0.005941	4.49	0.060
M:L*Time	1	0.001512	0.001512	1.14	0.310
Temperature*Time	1	0.000128	0.000128	0.10	0.762
Error	10	0.013236	0.001324		
Lack-of-Fit	5	0.013232	0.002646	3780.62	0.801
Pure Error	5	0.000003	0.000001		
Total	19	0.756465			

Table 4.11: Analysis of variance for methanolic extraction for *E. abyssinica* dye

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	0.979414	0.108824	54.97	0.005
Linear	3	0.529048	0.176349	89.09	0.003
M:L	1	0.307396	0.307396	155.29	0.011
Temperature	1	0.201344	0.201344	101.71	0.002
Time	1	0.020307	0.020307	10.26	0.009
Square	3	0.406901	0.135634	68.52	0.010
M:L*M:L	1	0.175120	0.175120	88.47	0.000
Temperature*Temperature	1	0.032230	0.032230	16.28	0.002
Time*Time	1	0.130690	0.130690	66.02	0.000
2-Way Interaction	3	0.043465	0.014488	7.32	0.007
M:L*Temperature	1	0.000010	0.000010	0.01	0.944
M:L*Time	1	0.014535	0.014535	7.34	0.022
Temperature*Time	1	0.028920	0.028920	14.61	0.003
Error	10	0.019795	0.001980		
Lack-of-Fit	5	0.019794	0.003959	14845.52	0.937
Pure Error	5	0.000001	0.000000		
Total	19	0.999209			

DF-degrees of freedom, SS-sum of squares MS- mean square F- Fischer test value, p- probability value and *-significant

4.2.1.5 3D-Surface Plots Analysis

The 3D – response surface plot is a representation of the surface area of the dye absorbance against the process variables. It illustrates the effects of two factors on the dye absorbance as the third factor is kept constant (Swamy *et al*., 2014). The hold values for aqueous extraction were at constant values of M: L 5 g: 100mL, temperature 70 °C, time 120 minutes and for methanolic extraction were M: L 5 g: mL, temperature 52 °C, time 120 minutes. Figure 4.17 red and green shows that at a constant time an increase in temperature and M:L ratio causes an increase in absorbance, however higher M:L ratio diminishes the absorbance of the dye which has been observed in other studies (Al-Alwani *et al*., 2016).

At a constant temperature (Figure 4.17 blue and black) an increase in M:L and time leads to increase in absorbance of the dye in almost a linear manner. A drastic decrease in absorbance beyond the optimum values was observed because a continuous increase of the sample leads to overloading in the solvent which reduces its dissolving capacity hence preventing extraction (Anuar *et al*., 2013). At a constant M:L ratio (Figure 4.17), an increase in temperature and time favours dye extraction hence increase in dye absorbance but begins to reduce when the variables are above the optimum. Heating at extremely high temperature for a longer duration makes the dye components unstable hence they undergo degradation (Maran *et al*., 2015) or the conjugated double bonds could have been broken and so the absorbance reduces (Ben Ticha *et al*., 2016).

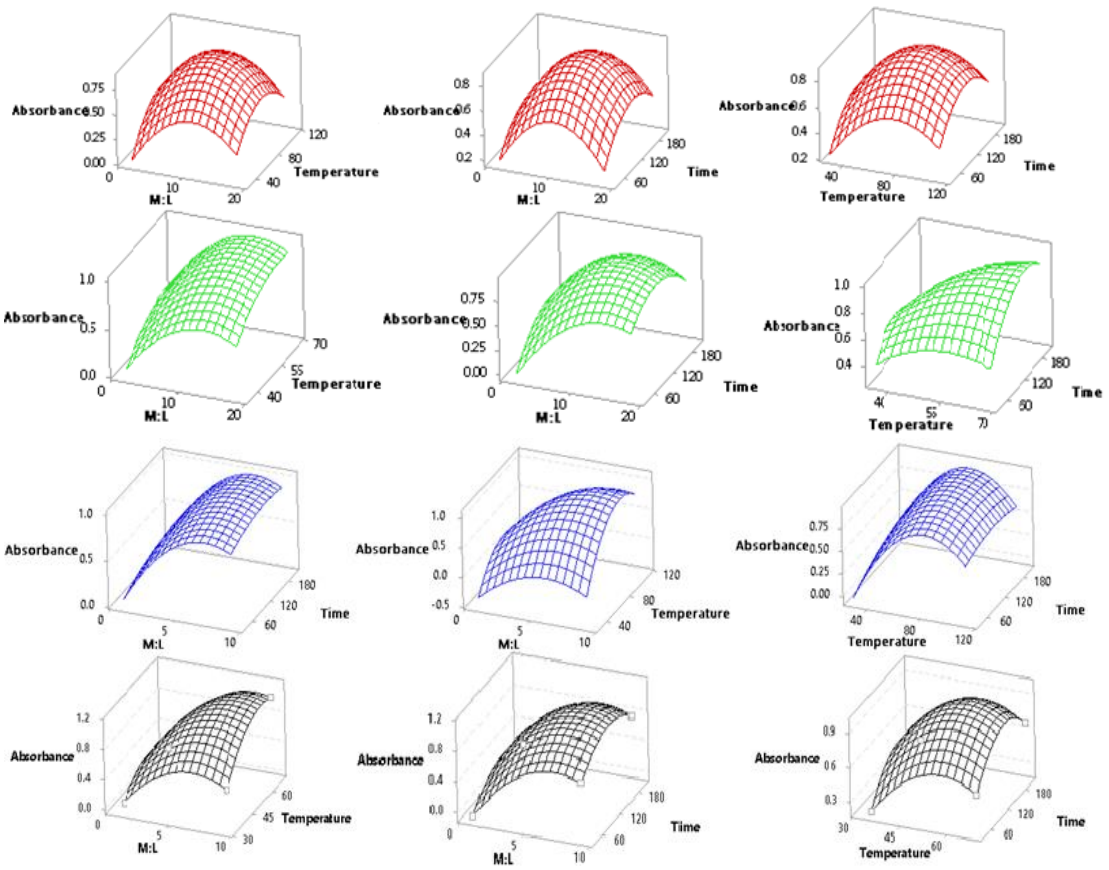


Figure 4.17: 3D - surface plots for dye absorbance for aqueous (red) and methanolic (green) for *E. divinorum* and for aqueous (blue) and methanolic (black) extraction for *E. Abyssinica*

4.2.1.6 Validation of the Optimized Conditions

optimizer of CCD design involved selection of the target, for this case was to maximize the response which resulted in Figure 4.18 with desirability values of d = 1.000 implying a response almost similar to the optimum. The desirability value is between zero and one where 0 equals to undesirable and 1 equals to desirable (Swamy *et al.*, 2014). The optimum conditions for aqueous extraction were: M: L 7.5g: 100mL, temperature 84°C and time of 146.3 minutes (Figure 4.18A) and for methanolic extraction were: M: L 7.5g: 100mL, temperature 60°C and time of 129.3 minutes (Figure 4.18B) for *E. divinorum*. The optimum conditions for aqueous extraction were: M: L 10.6g: 100mL, temperature 77.2°C and time of 131.0 minutes

(Figure 4.18C) and for methanolic extraction were: M: L 12.9g: 100mL, temperature 66.8°C and time of 142.9.3 minutes (Figure 4.18D) for *E. Abyssinica*. The statistical model was validated experimentally by carrying out the dye extraction process under the theoretical optimum conditions from the response optimizer (Aydar, 2018). The experiments were conducted in triplicate and the response averaged (Ben Ticha *et al*., 2016). The experimental absorbance was found to be 1.039 for aqueous extraction and 1.088 for methanolic extraction for *E. divinorum*. On the other hand the theoretical absorbance under optimum conditions for aqueous extraction was 1.051 and 1.092 for methanolic extraction for *E. divinorum*. The experimental absorbance was found to be 0.8722 for aqueous extraction and 1.0194 for methanolic extraction for *E. Abyssinica*. On the other hand the theoretical absorbance under optimum conditions for aqueous extraction was 0.8813 and 1.0204 for methanolic extraction for *E. Abyssinica.* The experimental and the theoretical values were in agreement. Consequently, the statistical models used in this research were confirmed to be adequate. In addition, it verified that the optimal extraction values within the study range are valid (Bahloul *et al*., 2016).

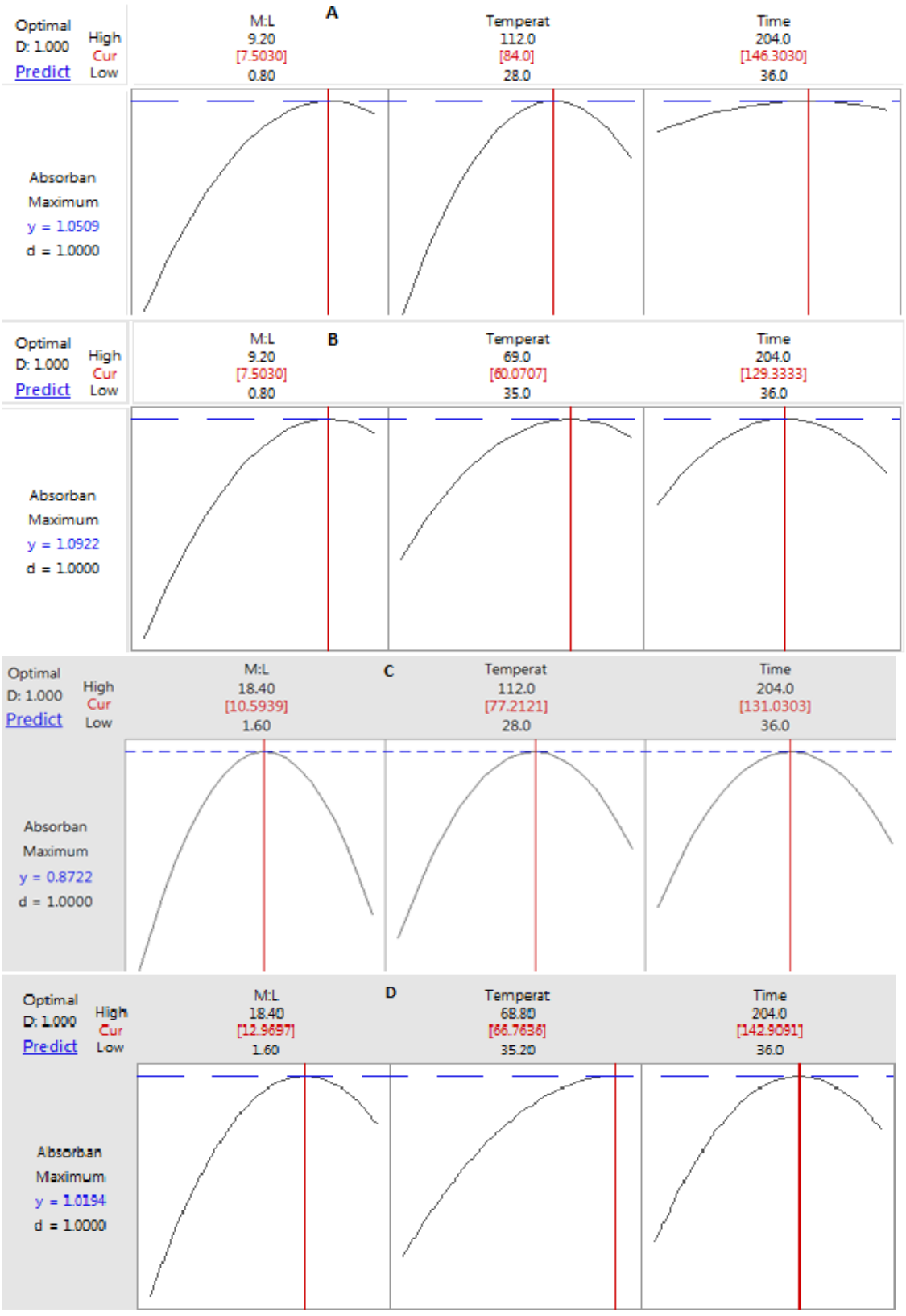


Figure 4.18: Desirability plot for optimization for aqueous (A) and methanolic (B) extraction for *E. divinorum* and for aqueous (C) and methanolic (D) extraction for *E. Abyssinica*

4.2.2 Optimization of Dyeing Conditions

4.2.2.1 Single Factor Analysis of Dyeing Conditions

Analysis of one factor at a time optimization of dyeing condition indicated that all the three factors (time, temperature and pH) affects the color strength (K/S value) of the colorant on the cotton fabric (Figure 4.19).

4.2.2.1.1 Effects of Time

Maximum color strength was observed after dyeing for 60 minutes which remained steady up to 75 minutes (Figure 4.19A). The color strength started to reduce when the time was increased beyond 75 minutes which is attributable to dye desorption that occurs when dyeing is done for long period of time as was observed by Haddar *et al*. (2018). Dye desorption occurs because of shift of equilibrium of dye uptake to the opposite direction (Syrine *et al*., 2020). In addition maintaining high temperature for a dye bath for prolonged duration destabilizes the dye molecules which are converted to colorless compounds (Kumbhar *et al*., 2019).

4.2.2.1.2 Effects of Temperature

Figure 4.19B showed that the color strength increased as the temperature was raised from 45 °C to 75 °C. As the temperature increases the fiber becomes more swollen which increases its dye-ability and dye diffusion into the fabric (Baaka, Mahfoudhi, *et al*., 2017). A gentle decrease in color strength was then observed beyond 75 °C which can be attributed to degradation of the dye molecules as a result of extreme dyeing temperatures (Baaka, Haddar, *et al*., 2017).

4.2.2.1.3 Effects of pH

The Effects of pH on the color strength were evaluated between 2 and 7 (Figure 4.19C). An increase in color strength was observed as the pH increased from 2 to 3 followed by a slight decrease between 3 and 4. The color strength then began to drop

steadily as the pH increased from 4 to 7. The optimum pH for dyeing was found to be 3 which is the appropriate acidic medium at which there is maximum adsorption of the dye molecules (Rather *et al*., 2020).

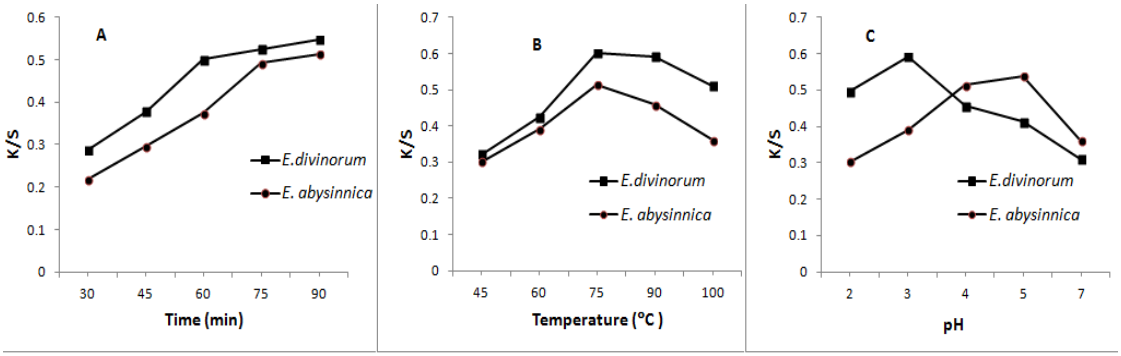


Figure 4.19: Effects of time, temperature and pH on K/S at constant 60 min, 90 °C and pH, 4

4.2.2.2 Analysis of Response Surface Optimization

The outcome of the one factor at a time design was used as a guide in selection of the range of variables for response surface optimization (Chen *et al*., 2018). Table 4.12 shows the coded and the actual levels of the parameters studied as well as the response (K/S) of surface optimization. The parameters were represented by A, B and C for time (minutes), Temperature (°C) and pH, respectively. The response is an average of five dyeing experiments conducted at each level.

Table 4.12: Coded levels of variables and the dyeing response for the CCD design

Run	*E. divinorum*				*E. Abyssinica*			
	Coded Levels			Response	Coded Levels			Response
	A	B	C	(K/S)	A	B	C	(K/S)
1	1	1	-1	0.456	0	0	0	0.515
2	-1	1	1	0.247	0	0	0	0.518
3	0	0	0	0.598	1	-1	-1	0.189
4	1	-1	-1	0.285	α	0	0	0.483
5	1	1	1	0.267	1	-1	1	0.387
6	-1	-1	-1	0.222	-1	1	-1	0.146
7	1	-1	1	0.204	-1	-1	-1	0.098
8	0	0	0	0.602	1	1	1	0.371
9	0	0	0	0.586	-1	1	1	0.207
10	0	0	0	0.614	0	-α	0	0.267
11	-1	-1	1	0.194	1	1	-1	0.222
12	-1	1	-1	0.412	0	0	α	0.501
13	0	0	α	0.367	0	0	0	0.516
14	0	-α	0	0.309	-α	0	0	0.205
15	0	0	0	0.564	0	0	0	0.515
16	0	0	-α	0.405	0	α	0	0.287
17	0	0	0	0.586	0	0	0	0.515
18	0	α	0	0.489	0	0	-α	0.203
19	α	0	0	0.479	0	0	0	0.514
20	-α	0	0	0.399	-1	-1	1	0.245

4.2.2.2.1 Regression Model for Optimization

The prediction of the effects of variation of dyeing conditions on color was represented numerically using a regression model. The model illustrates this relationship in coded factor where Equation 4.1 is for *E divinorum* and Equation 4.2 is for *E. Abyssinica*. A, B, C, K/S represent time, temperature, pH and response, respectively. According to equation 4.1 and 4.2, the Regression coefficient, R^2 of 96.27% and 98.37% indicates that this regression model fits the experimental data adequately. This is confirmed by the adjusted R^2 of 92.91% and 96.90%, which are closer to the R^2. According to the value of predicted R^2, the statistical model has 75.65% and 87.53% capability of predicting a new observation which is a good prediction ability (Hemanthraj *et al*., 2014).

$$K/S = -1.692 + 0.013A + 0.033B + 0.272C - 0.0001A^2 - 0.0002B^2 - 0.026C^2 + 0.000014AB - 0.0003AC - 0.0009BC \quad \text{Equation 4.5}$$

With R^2 = 96.27%, R^2 (adj) = 92.91%, R^2 (pred) = 75.65%

$$K/S = -1.928 + 0.012A + 0.042B + 0.213C - 0.001A^2 - 0.0002B^2 - 0.020C^2 + 0.001AB - 0.002AC - 0.004BC \quad \text{Equation 4.6}$$

With R^2 = 98.37%, R^2 (adj) = 96.90%, and R^2 (pred) = 87.53%

4.2.2.2.2 Analysis of Variance

Analysis of Variance is essential for checking the significant effects of process variables to response as well as the presence of variable interactions (Souissi *et al*., 2018). ANOVA for the color strength of the cotton fabric samples that were dyed using the *E. divinorum* dye was as shown in Table 4.13, where significance of each term was evaluated using p-value. The linear and the squared terms of the model for *E. divinorum* dye showed significant effects on the variance of the color strength with p-value < 0.05. The most significant variables for *E. abyssinica* were time and temperature (Table 4.14). Insignificance of lack of fit error (p > 0.05) for both dyes confirmed the fitness of the polynomial model to the data (Sinha *et al*., 2016). Dyeing with *E. divinorum* showed interactions between temperature and pH were also significant as was observed in the interaction plots (Figure 4.20) where the temperature-pH plots are not curving correspondingly (Baaka *et al*., 2015).

Table 4.13: ANOVA table for dyeing factors for *E. divinorum*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	0.414420	0.046047	28.68	0.000*
Linear	3	0.101418	0.033806	21.05	0.000*
Time	1	0.013619	0.013619	8.48	0.015*
Temperature	1	0.055815	0.055815	34.76	0.000*
pH	1	0.031983	0.031983	19.92	0.001*
Square	3	0.295244	0.098415	61.29	0.000*
Time*Time	1	0.046929	0.046929	29.23	0.000*
Temperature*Temperatur	1	0.056482	0.056482	35.18	0.000*
e	1	0.066516	0.066516	41.43	0.000*
pH*pH	3	0.017757	0.005919	3.69	0.051
2-Way Interaction	1	0.000741	0.000741	0.46	0.512
Time*Temperature	1	0.003321	0.003321	2.07	0.181
Time*pH	1	0.013695	0.013695	8.53	0.015*
Temperature*pH	10	0.016056	0.001606		
Error	5	0.014581	0.002916	9.88	0.103
Lack-of-Fit	5	0.001475	0.000295		
Pure Error	19	0.430476			
Total					

Table 4.14: ANOVA table for dyeing factors *of E. abyssinica*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	0.431422	0.047936	66.99	0.000
Linear	3	0.142951	0.047650	66.59	0.000
Time	1	0.062989	0.062989	88.02	0.000
Temperature	1	0.000253	0.000253	0.35	0.566
pH	1	0.079710	0.079710	111.39	0.000
Square	3	0.283736	0.094579	132.17	0.000
Time*Time	1	0.067198	0.067198	93.91	0.000
Temperature*Temperature	1	0.127069	0.127069	177.57	0.000
pH*pH	1	0.061210	0.061210	85.54	0.000
2-Way Interaction	3	0.004735	0.001578	2.21	0.150
Time*Temperature	1	0.000007	0.000007	0.01	0.923
Time*pH	1	0.002433	0.002433	3.40	0.095
Temperature*pH	1	0.002295	0.002295	3.21	0.104
Error	10	0.007156	0.000716		
Lack-of-Fit	5	0.007146	0.001429	752.25	1.322
Pure Error	5	0.000009	0.000002		
Total	19	0.438578			

DF-degrees of freedom, SS-sum of squares, MS- mean square F- Fischer test value, p- probability value and *- significant

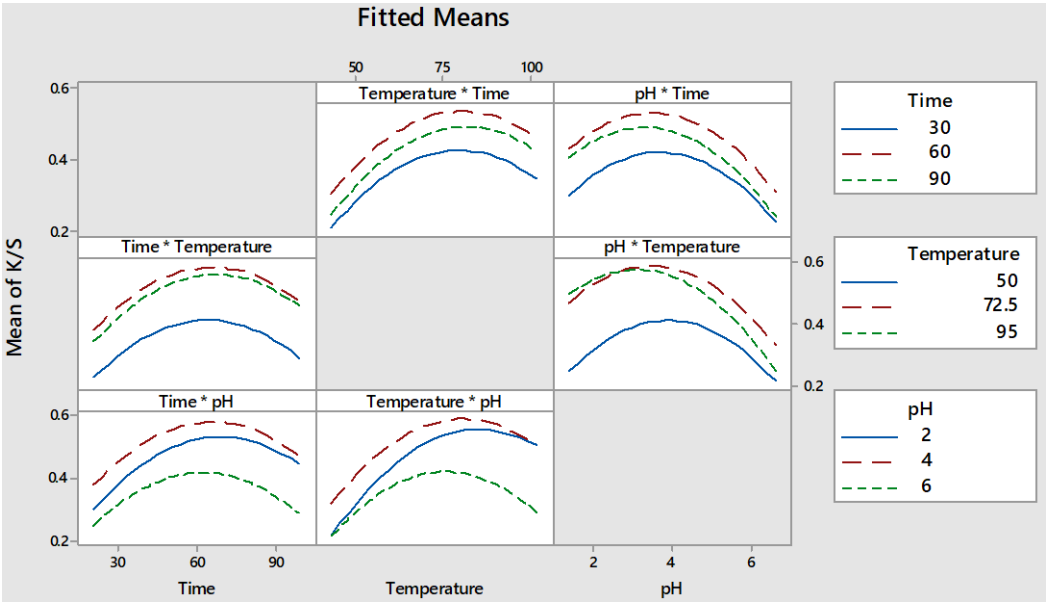


Figure 4.20: Interaction plot for K/S

4.2.2.2.3 3D-Surface Plots and Response Optimization

The 3D – response surface plot is a representation of the surface area of the color strength of the dye against the process variables. It illustrates the effects of two factors on the color strength of the dye as the third factor is kept constant (Swamy *et al*., 2014). The hold values for dyeing with *E. divinorum* dye were pH of 4, 72.5 °C and 60 minutes and for *E. abyssinica* dye were pH of 4, 70 °C and 60 minutes. At a constant pH (Figure 4.21a and d), an increase in temperature and time favors dye absorption hence increase in color strength but begins to reduce when the variables are above the optimum. Heating at extremely high temperature for a longer duration makes the dye components unstable hence they decompose (Maran *et al*., 2015). At a constant temperature (Figure 4.21b and e) an increase in pH and time leads to increase in K/S values of the colorant on the fabric but a strong decrease in color strength beyond the optimum values was observed. Figure 4.21c and f show that at a constant

time an increase in temperature and pH causes an increase in colors strength, however decreasing acidity reduces the dye uptake by the fabric.

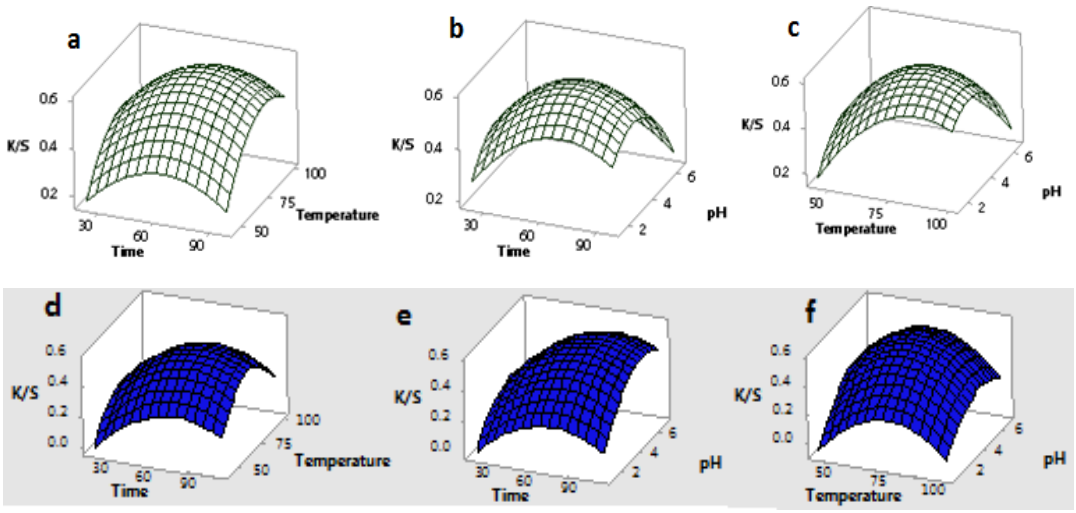


Figure 4.21: 3D-surface plots for E. *divinorum* dye (a-c) *and* E. *Abyssinica* dye (d-f)

4.2.2.2.4 Validation of the Optimized Dyeing Conditions

Optimization using response optimizer of CCD design involved selection of the target, for this case was to maximize the response with desirability values of d = 1.000 implying a response almost similar to the optimum (Swamy *et al*., 2014). The optimum conditions for dyeing with *E. divinorum* were found to be pH of 3.3, 82°C and 68 minutes (Figure 4.22A). The optimum conditions for dyeing with *E. abyssinica* were found to be pH of 5.0, 69.7°C and 74.5 minutes (Figure 4.22B). The statistical model was validated experimentally by dyeing under the theoretical optimum conditions from the response optimizer (Aydar, 2018). The experimental color strength was 0.597 and 0.501 while the theoretical color strength was 0.609 and 0.556 for *E. divinorum* and *E. abyssinica*, respectively. The two values were in agreement hence the statistical model used in this research was confirmed to be adequate (Bahloul *et al*., 2016).

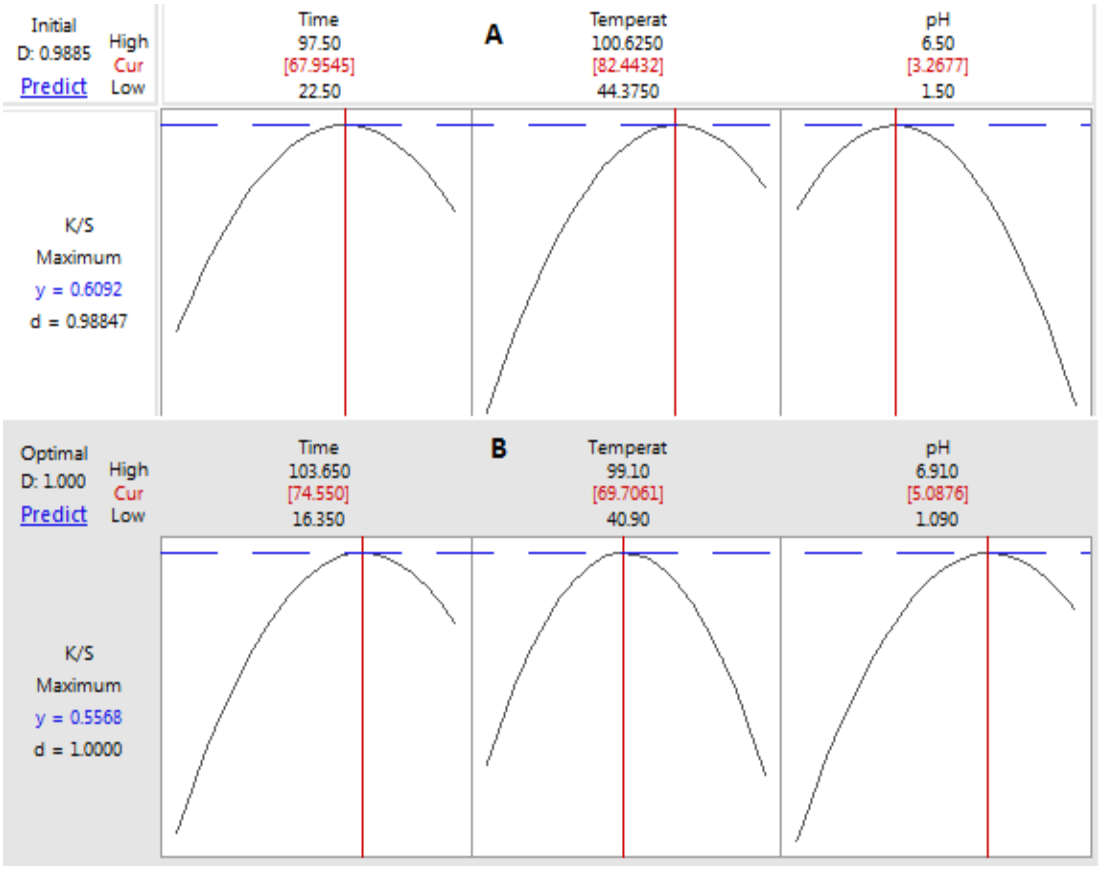


Figure 4.22: Desirability plot for optimum dyeing conditions for *E. divinorum* dye (A) and *E. abyssinica* dye (B)

4.3 Mordanting

4.3.1 Colorimetric Analysis of Pre-treated and Dyed Cotton Fabric

Fabric pre-treatment was done for *E. abyssinica* dye extract only because its color fastness was below the textile requirement of above 3. Hexane, dichloromethane and ethyl acetate extracts were poorly soluble in water therefore they were not used in dyeing of cotton fabric. Aqueous extract, MeOH direct extract (DE) and MeOH sequential extract (SE) were used to dye the untreated and treated cotton fabric and their color measurements were as shown in Table 4.15. Treatment of the cotton fabric with tannic acid before dyeing with the *E. abyssinica* natural dye enhanced its dye uptake as shown by the significant increase in the color strength compared to the untreated cotton fabric. This is because tannic acid pre-treatment cationizes the

cellulose molecules improving the fabric's ability to absorb the dye hence the increased color strength (Mir *et al*., 2019). The MeOH SE dye extract formed light yellow colors on the cotton fabric while the aqueous and MeOH DE formed greenish yellow colors. Yellow color is associated with flavonoids class of phytochemicals (Villela *et al*., 2019b) meaning the flavonoids in sequentially extracted dye (MeOH SE) were in purer form compared to the other dye extracts. Mordanting with alum and ferrous provided a variety of shades where ferrous formed darker colors as has been observed in other studies (Bukhari *et al*., 2016). The ferrous mordant showed the most improved color strength across all the dye extract which is attributed to its more empty orbitals that can accommodate more dye molecules hence increasing the concentration of the dye absorbed by the fabric as well as the formation of more stable fabric mordant complexes (Eser *et al*., 2016).

Table 4.15: Color measurements for the fabric dyed with different extracts of *E. abyssinica and* mordanted with metallic mordants

Extract	Method	Mordant	L*	a*	b*	C*	H°	K/S	Shade
MeOH SE	untreated	-	86.40	0.32	30.83	31.89	89.6	0.498	
	treated	-	87.98	-0.77	20.08	18.74	92.75	0.601	
	Pre	Alum	74.24	-2.60	50.70	49.43	93.03	0.891	
		Ferrous	42.48	0.14	31.57	31.64	91.13	2.924	
	Meta	Alum	85.87	-1.66	39.16	37.38	92.61	0.681	
		Ferrous	40.74	0.05	32.69	31.55	90.24	2.768	
	Post	Alum	87.60	0.52	16.26	28.41	92.52	0.583	
		Ferrous	48.08	-1.00	24.44	24.48	92.41	1.840	
Aqueous	untreated	-	63.80	3.13	37.09	30.53	74.69	0.487	
	treated	-	63.46	2.98	34.78	17.37	76.63	0.515	
	Pre	Alum	62.91	3.14	35.80	15.91	78.64	1.123	
		Ferrous	56.23	1.78	9.67	19.82	79.61	3.004	
	Meta	Alum	61.26	3.23	14.11	14.48	77.10	0.934	
		Ferrous	55.86	1.65	25.87	11.05	81.37	2.891	
	Post	Alum	61.51	3.45	33.11	13.60	75.32	0.673	
		Ferrous	52.86	0.87	25.79	5.68	81.54	2.137	
MeOH DE	untreated	-	62.71	2.12	47.09	31.53	71.61	0.465	
	treated	-	62.54	1.88	35.78	16.26	75.64	0.496	
	Pre	Alum	61.26	4.14	33.81	16.44	79.63	1.226	
		Ferrous	54.35	1.78	22.61	18.20	78.65	2.975	
	Meta	Alum	60.57	3.23	42.13	13.37	78.11	0.897	
		Ferrous	53.63	1.65	26.87	12.23	80.22	2.673	
	Post	Alum	60.12	3.45	34.11	14.60	76.12	0.484	
		Ferrous	50.58	0.87	26.79	06.88	82.44	2.973	

4.3.1.1 FT-IR Analysis of Pretreated Cotton Fabric

FT-IR spectra for untreated, treated and dyed cotton fabrics were as indicated in Figure 4.23. The characteristic cellulose broad peak at 3337 cm^{-1} is for O–H bond stretching mode and that at 2917 cm^{-1} corresponds to C–H bond stretching. C–O stretching mode and C–O–H bending mode were shown by the peaks at 1428 cm^{-1} and 1315 cm^{-1}, respectively. All these peaks are in relation to chemical structure of cellulosic pure cotton fabric (Ben Ticha *et al*., 2013). FTIR analysis of tannic acid treated cotton fabric showed new peaks at 1645 cm^{-1}, which correspond to carbonyl (C=O) stretching mode which confirms that the cellulose structure was modified by the acid which lead to introduction of carbonyl group that is not found in cellulose

structure (Syrine *et al*., 2020). Comparing the FTIR spectra of treated and dyed cotton fabric with that of pure cotton there was new peak at 1578 cm^{-1} corresponding to C=C stretching mode for aromatic ring confirming the attachment of the aromatic dye molecules to the cellulose structure (Kumbhar *et al*., 2019). In addition, the characteristic cellulose band at 3337 cm^{-1} is for O–H shifted to lower region of 3335 cm^{-1} which could be attributed to stronger hydrogen bonds between dye and cellulose than those between cellulose molecules (Ren *et al*., 2019).

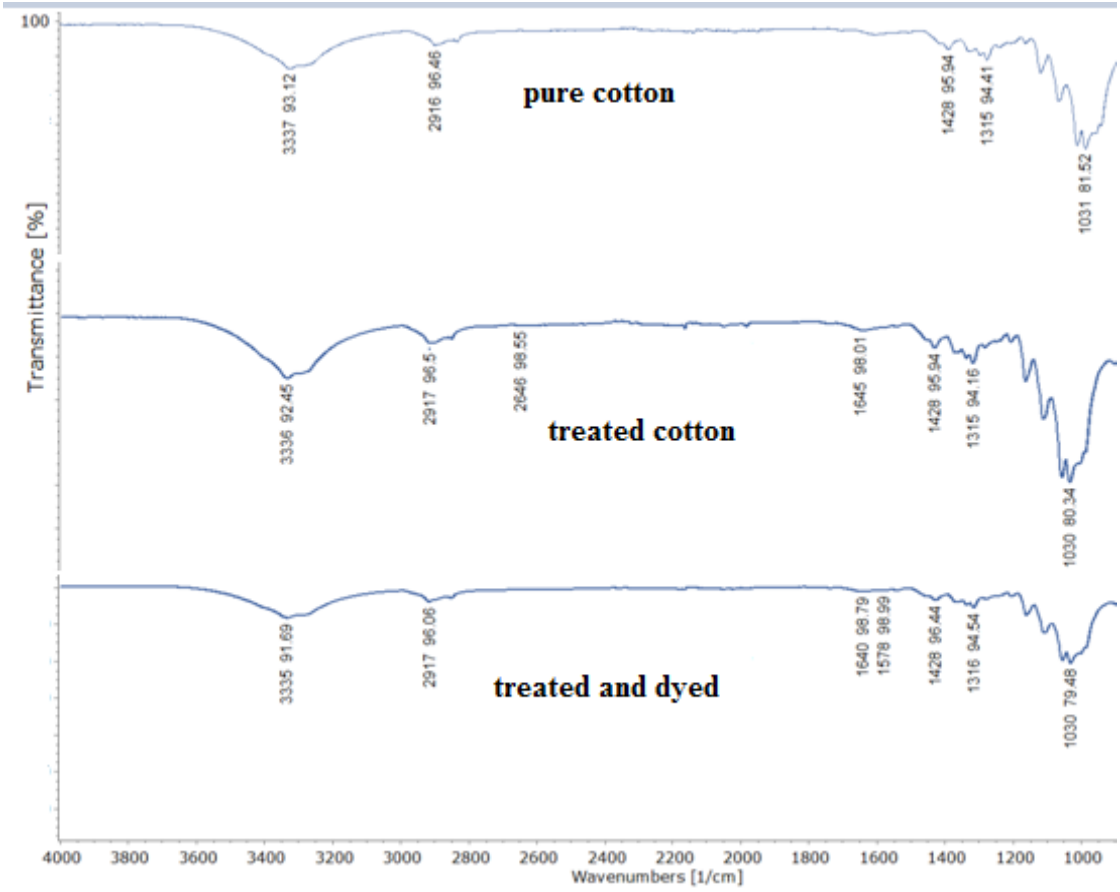


Figure 4.23: FTIR spectrum of pure, tannic acid treated and treated and dyed cotton

4.3.1.2 SEM Analysis of pre-treated cotton fabric

The morphology of the un-dyed, dyed and bio-mordanted cotton fabric were as shown on Figure 4.24. The un-dyed cotton fabric showed the typical morphology of pure cotton fabric with a continuous smooth surface across the fabric (Velmurugan *et al*., 2016) as shown in Figure 4.24A. The surface of the dyed cotton fabric (Figure 4.24B)

was found to have a rough and slightly swollen surface compared to the un-dyed cotton, which can be due to the interactions between the cellulosic molecules and the dye molecules that make the morphology uneven and swollen. The morphology of the bio-mordanted and dyed cotton fabric (Figure 4.24C) was rougher than the just dyed and un-dyed cotton fabric. This can be attributed to the fact that the molecules of the bio-mordant attaches to the fabric first then it acts as a link between the molecules of the dye and the textile material resulting in a more irregular surface of the cotton fabric. It was also noted that the surface of the dyed and bio-mordanted cotton fabric were darker than the un-dyed cotton fabric because of deposition of the dye and bio-mordant molecules on the surface of the fabric (Rather *et al*., 2016).

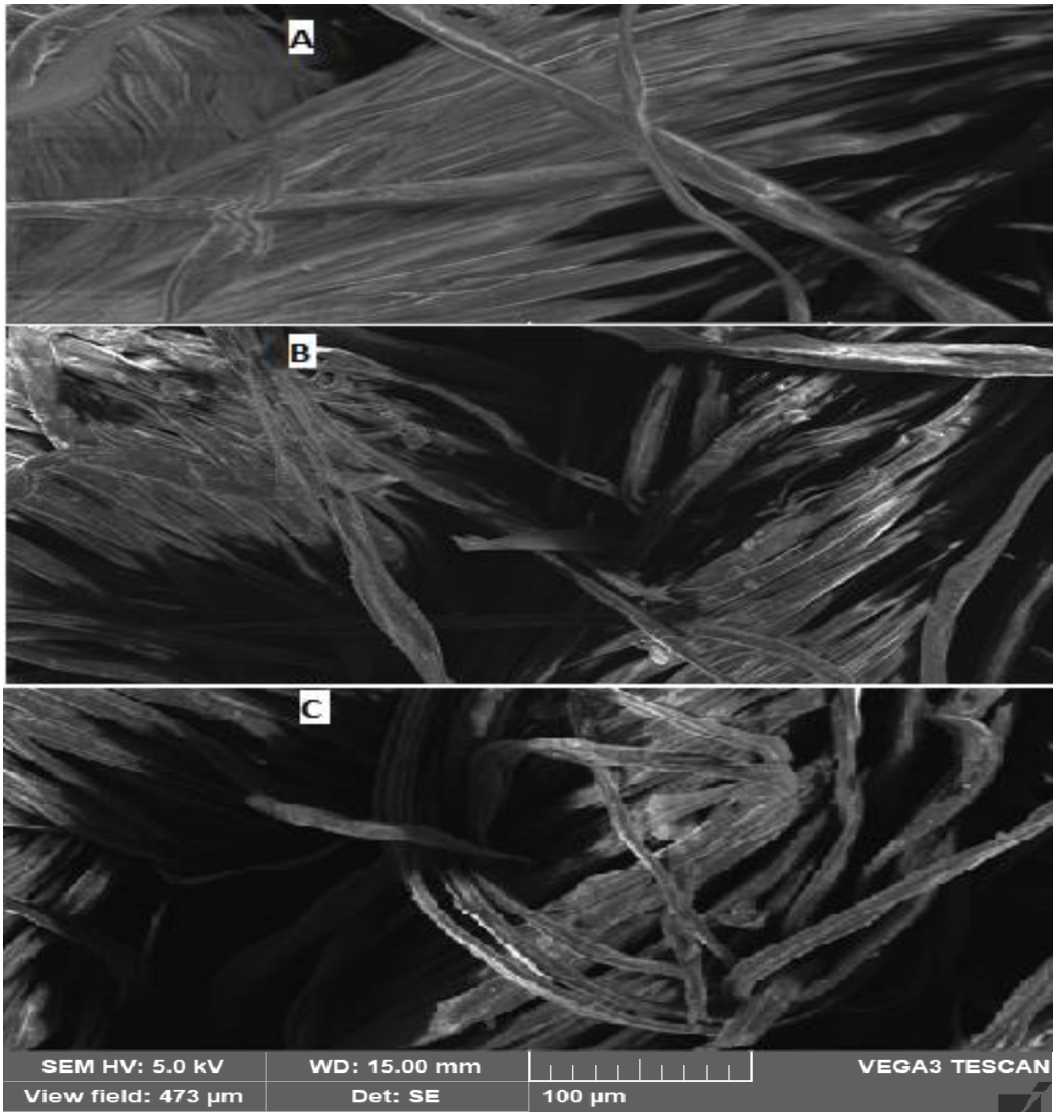


Figure 4.24: SEM images of the (A) un-dyed, (B) dyed (C) dyed and bio-mordanted cotton fabric

For *E. divinorum* mordanting was done directly without any pre-treatment of the cotton fabric since its color fastness had already been found to be good. The application of metallic mordants during dyeing with *E. divinorum* not only improved the color strength but also led to formation of diverse shades of color with the same natural dye as shown in Table 4.16. Ferrous mordant is a darkening mordant whereas tin and alum are brightening mordants (İşmal and Yıldırım, 2019) which was observed in the shades of the fabrics across all the mordanting methods.

All the mordanting methods increased the K/S values of the colorant on the fabric compared to the un-mordanted fabric, indicating that the mordants increased dye absorption since mordants act as bridges between the molecules of the dye and the fabric increasing the affinity of the cotton fabric to the dye (Baliarsingh *et al*., 2012). The higher color strengths of the fabric mordanted with ferrous is attributed to the stable metal complex that Fe^{2+} form with the fabric and dye compared to the other metal ions (Adeel *et al*., 2018). Pre-mordanting method had a higher color strength improvement compared to the other methods and hence is the appropriate mordanting method for this natural dye.

Table 4.16: Color measurements of the dyed fabric using different methods of mordanting

Method	Mordant	L^*	a^*	b^*	C^*	H°	K/S	Shade
	without	63.47	+4.63	+16.86	17.53	74.53	0.612	
Pre	Alum	62.91	+3.14	+15.71	15.90	78.60	0.954	
	Ferrous	56.23	+1.78	+9.67	9.80	79.64	3.120	
	Tin	79.43	+3.56	+14.58	15.01	76.32	0.711	
Meta	Alum	61.26	+3.23	+14.11	14.48	77.10	0.889	
	Ferrous	55.86	+1.65	+10.87	11.00	81.5	1.813	
	Tin	71.07	+2.17	+16.58	16.72	82.55	0.672	
Post	Alum	61.54	+3.45	+0.85	13.6	75.30	0.785	
	Ferrous	52.86	+0.83	+5.79	5.9	81.5	0.776	
	Tin	85.23	+3.24	+13.37	13.8	76.4	0.708	

4.3.2 Color Fastness of the Fabric Dyed and Mordanted with Metal Mordants

Color fastness test in terms of wash, rub and light was determined for the cotton fabric samples dyed with the dyes extracted with different solvent and mordanted as shown in Table 4.17. It was noted that all the color fastness properties for all the dye extracts of *E. abyssinica* was in the order of untreated<treated<mordanted. The improved fastness of the treated and dyed cotton fabric is due to modification of the cellulosic structure by the tannic acid pre-treatment agent that enhances the ability of the dye to fix to the cotton fabric (Koh & Hong, 2017). Treated, dyed and mordanted cotton

fabric showed greater improvement in fastness compared to untreated dyed cotton fabric. For example the wash fastness for aqueous extract improved from 2.5 for untreated to 3.0 for treated fabric. This is due to the formation of complexes of tannic acid and the metal mordants on the surface of the cotton which leads to the formation of insoluble precipitates with dye molecules hence enhanced color fastness characterisitcs (Bukhari *et al*., 2016).

Considering the color fastness of the cotton fabrics dyed with different solvent extracts of the natural colorant, it was noted that the MeOH SE extract showed the best fastness properties compared to the other dye extracts (Table 4.17). This can be attributed to the fact that the MeOH SE dye extract was purified during the sequential extraction process which reduces the concentration of non-dye substances present in the dye extract matrix allowing the dye molecules to bond with the cellulose on the fabric (Mansour, 2018). Pre-mordanting and meta-mordanting methods showed greater improvement of the color fastness properties as has been observed in other studies (Bukhari *et al*., 2017).

Table 4.17: Color fastness for the fabric dyed with different extracts of *E. abyssinica*

Extract	Method	Mordant	Wash fastness		Rubbing fastness		Light fastness
			CC	CS	Dry	Wet	
Aqueous	untreated	-	2.5	2.5	3.0	3.0	3.0
	treated	-	3.0	3.0	3.5	3.5	3.5
	Pre	Alum	4.0	4.0	4.0	4.0	4.0
		Ferrous	4.0	4.0	4.0	4.0	4.0
	Meta	Alum	4.0	4.0	4.0	4.0	4.0
		Ferrous	4.0	4.0	4.0	4.0	4.0
	Post	Alum	3.5	3.5	3.5	3.5	3.5
		Ferrous	3.5	3.5	3.5	3.5	3.5
MeOH DE	untreated	-	2.0	2.0	2.5	2.5	2.5
	treated	-	2.5	2.5	3.0	3.0	2.5
	Meta	Alum	3.5	3.5	3.5	3.5	3.5
		Ferrous	3.5	3.5	3.5	3.5	3.5
	Pre	Alum	3.5	3.5	3.5	3.5	3.5
		Ferrous	3.5	3.5	3.5	3.5	3.5
	Post	Alum	3.0	3.0	3.0	3.0	3.0
		Ferrous	3.0	3.0	3.0	3.0	3.0
MeOH SE	untreated	-	3.0	3.0	3.0	3.0	3.0
	treated	-	3.5	3.5	3.5	3.5	3.5
	Meta	Alum	4.0	4.0	4.0	4.0	4.0
		Ferrous	4.0	4.0	4.0	4.0	4.0
	Pre	Alum	4.0	4.0	4.0	4.0	4.0
		Ferrous	4.0	4.0	4.0	4.0	4.0
	Post	Alum	3.5	3.5	3.5	3.5	3.5
		Ferrous	3.5	3.5	3.5	3.5	3.5

The cotton fabric dyed without mordanting showed excellent rub and light fastness but fairly good (4-5) fastness to washing for *E. divinorum* (Table 4.18). The excellent color strength of the natural colorant on cotton fabric can be ascribed to the fact that *E. divinorum* is rich in tannins which are known to have good fixing ability (Mongkholrattanasit *et al*., 2013). All the mordanting methods improved the wash fastness to excellent meaning the color of the fabric did not change, even after wash fastness test. This is because the metallic ions complexes with the dye molecules forming insoluble precipitate molecules on the fabric (Bukhari *et al*., 2016).

Table 4.18: Color fastness of the fabric dyed with *E. divinorum* using different methods of mordanting

Method	Mordant	Wash fastness		Rubbing fastness		Light fastness
		C.C	C.S	Dry	Wet	
	without	4-5	5	5	5	5
Pre	Alum	5	5	5	5	5
	Ferrous	5	5	5	5	5
	Tin	5	5	5	5	5
Meta	Alum	5	5	5	5	5
	Ferrous	5	5	5	5	5
	Tin	5	5	5	5	5
Post	Alum	5	5	5	5	5
	Ferrous	5	5	5	5	5
	Tin	5	5	5	5	5

4.3.3 Colorimetric Analysis of Bio-mordanted Cotton Fabric

Considering the lightness (L^*) the mango and rosemary bio-mordants increased the lightness for the fabric dyed with *E. divinorum* from 63 to 66.33 and 67.27, respectively, providing lighter shades of brown (Table 4.19). The ability of mango and rosemary bio-mordants to form lighter shades indicates that they can be used as substitutes for brightening metallic mordants such as alum and tin, an observation that has been reported in literature (İşmal, 2017). In addition it was noted that the color strength achieved with these bio-mordants were closer to those that were mordanted with alum for example pre-mordanting with rosemary showed color strength of 0.911 while that with alum was 0.954. The color strength for the bio-mordants (mango, 0.863and rosemary, 0.911) was higher than that of tin (0.711). Bio-mordanting with *E. abyssinica* formed different shades of color, ranging between yellow and brown (Table 4.19). The bio-mordants enhanced the color strength from 0.601 to 0.762 for rosemary bio-mordant. The poly-phenols in the rosemary bio-mordants are responsible for the increased dye absorption by the cotton fabric which leads to increased color strength (Erdem İşmal *et al*., 2014). Pre-mordanting method for both

mordants showed the best color strength compared to post-mordanting and meta-mordanting methods as has been observed in other studies (Berhanu & Ratnapandian, 2017; Jahangiri *et al*., 2018).

Table 4.19: Colorimetric values of bio-mordanted and dyed cotton fabric

Plant	Method	Mordant	L^*	a^*	b^*	C^*	H°	K/S	Shade
E. divinorum		without	63.47	+4.63	+16.86	17.53	74.53	0.612	
	Pre	Mango bark	67.27	+9.66	+21.01	23.13	65.32	0.863	
		Rosemary	66.33	+8.65	+18.80	20.72	65.19	0.911	
	Meta	Mango bark	64.59	+11.82	+19.55	22.85	58.84	0.708	
		Rosemary	64.74	+10.65	+17.27	20.31	58.30	0.720	
	Post	Mango bark	66.14	+9.97	+19.18	21.64	62.51	0.691	
		Rosemary	65.76	+7.26	+16.72	18.22	66.58	0.724	
E. Abyssinica		without	87.98	-0.77	+20.08	18.74	92.75	0.601	
	Pre	Mango bark	86.06	+1.43	+15.08	15.15	84.50	0.692	
		Rosemary	86.01	+0.59	+26.23	23.13	88.26	0.762	
	Meta	Mango bark	87.54	+2.01	+14.71	23.86	88.35	0.618	
		Rosemary	86.78	+0.41	+25.19	23.01	88.38	0.663	
	Post	Mango bark	87.92	+2.24	+14.54	23.47	88.31	0.611	
		Rosemary	85.98	+0.92	+27.23	22.91	88.14	0.630	

Compound D was isolated in a reasonable amount (1.02g) and was used to dye cotton fabric and its color characteristics were compared to those of crude extract (Table 4.20). The cotton dyed with compound D showed a lighter shade (L^* = 68.78) compared to the fabric dyed with crude extract (L^* = 63.47). The shades were close to each other and hence it can be concluded that compound D is the major component of *E. divinorum* dye extract.

Table 4.20: Colorimetric values of fabric dyed with compound D

Dye	L^*	a^*	b^*	C^*	H°	K/S	Shade
Crude extract	63.47	+4.63	+16.86	17.53	74.53	0.612	
Compound D	68.78	+9.59	+17.83	15.59	76.03	0.589	

4.4 Textile Finishing Properties of the Dye extracts on Cotton Fabric

4.4.1 Antioxidant Activity of the Dyed Cotton Fabric

The percentage antioxidant activity was measured by the reduction of absorbance of DPPH. When the phenolic hydroxyl donates a proton to the DPPH radical the solution is decolorized and its absorbance reduces (Yang *et al*., 2018). The antioxidant activity of the cotton fabric samples that were assayed were as shown in Table 4.21. Dyeing with *E. divinorum* dye extract increased the antioxidant activity from 26.9% (un-dyed cotton) to 72.5% (dyed cotton) which was attributed to the molecules adsorbed by the cotton fabric from dye extract which consequently imparts the radical scavenging activity into the fabric. According to Al-Fatimi (2019), the radical scavenging activity of aqueous and methanolic root extracts of *E. divinorum* was found to be between 74.5 – 82.5% DPPH. It was also noted that the radical scavenging activity of the cotton textile samples bio-mordanted with mango (82.4%) and rosemary (85.3%) was higher than that of the un-mordanted (72.5%) fabric. This could be due to the additional good antioxidant which has been associated with the mango (Coelho *et al*., 2019; Richard, 2019; Sultana *et al*., 2012) and rosemary (Chraibi *et al*., 2020; Nieto *et al*., 2018; Rašković *et al*., 2014) extracts. The mango bark extract is made up of poly-phenols that are associated with good radical scavenging ability (Masibo & He, 2008).

Table 4.21: Antioxidant activity of the dyed sample fabric

Plant	Sample	Antioxidant activity (%)
	Un-dyed cotton	26.9
E. divinorum	Dyed	72.5
	Mango mordanted	82.4
	Rosemary mordanted	85.3
E. abyssinica	Dyed	63.1
	Mango mordanted	75.0
	Rosemary mordanted	78.6

4.4.2 The Durability of the Antioxidant Activity of the Dyed Fabric

The durability of the antioxidant activity of the dyed fabric after washing cycles was as indicated in Figure 4.25. Subsequent reduction in antioxidant activity of the dyed samples was observed as has been reported in other studies (Sheikh & Bramhecha, 2018). However, the reduction in antioxidant activity of *E. divinorum* dyed fabric became minimal after the 5th washing cycle, which is a significant attribute for this extract and can be ascribed to its good fastness properties (Sheikh and Bramhecha, 2018). The higher rate of reduction of the *E abyssinica* dyed fabric was mainly due to its poor washing fastness as was observed in fastness tests. The rate of reduction was lower in the mordanted fabric for both mango and rosemary mordants compared to un-mordanted fabric which could be due to the enhanced fastness of the dye to the fabric (Li *et al*., 2019). The rosemary mordanted fabric showed good antioxidant durability (above 80%) after the 10th washing cycle indicating that the species responsible for the bioactivity were not affected by the washing process hence remained very active (Sheikh and Bramhecha, 2018).

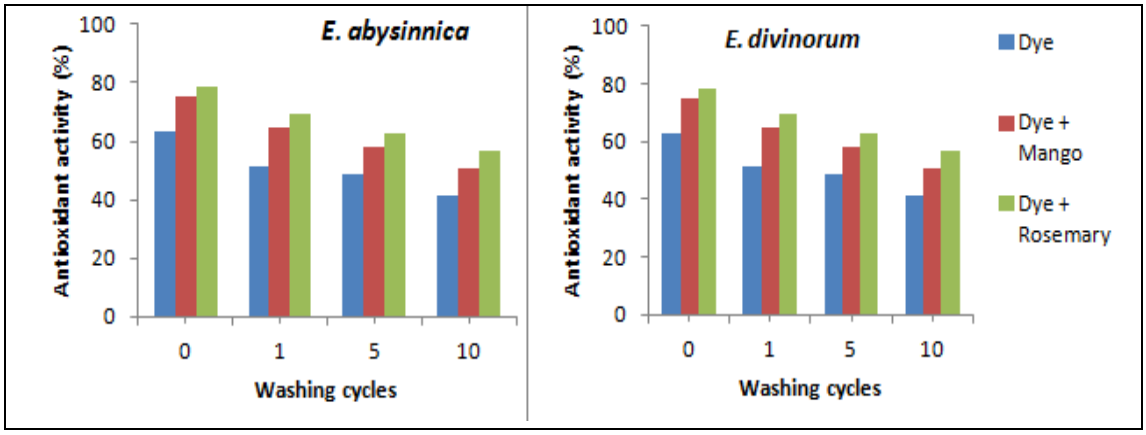


Figure 4.25: Durability of antioxidant activity of the dyed fabric after washing cycles

4.4.3 Antimicrobial Activity of the Dyed Cotton Fabric

Absorbance method is based on the fact that the absorbance of bacteria culture media increases with rise in turbidity of the media caused by the increase in the microbial growth or the number of bacteria cells in the media (Shahid *et al*., 2012). According to Table 4.22, the *E. divinorum* dyed cotton fabric showed bacteria activity of 61.54% against the Gram-negative *E. coli* and 65.55% against the Gram-positive *S. aureus.* This indicates that the dye molecules contains phenolic hydroxyl groups that form hydrogen links and covalent bonds with the microbial cells preventing growth of microbes and rupturing the plasma membrane leading to the death of the bacteria (Ren *et al*., 2018). *E. abyssinica* dye extract that has been shown to have antimicrobial activity (Chitopo *et al*., 2019) imparted microbial reduction ability to the fabric of 61.26% and 64.3% against *E. coli* and *S. aureus,* respectively. The bio-mordanted and dyed cotton fabric showed a higher bacteria reduction efficacy which could be attributed to the additional phenolic hydroxyl groups from the bio-mordant extracts that enhance the bacteria inhibition ability of the dye.

Table 4.22: Bacteria reduction (%) of the samples of the fabric

Plant	Sample	Bacteria reduction (%)	
		E. coli	*S. aureus*
	Undyed cotton (control)	-	-
E. divinorum	Dyed	61.54	65.55
	Mango mordanted	69.49	71.48
	Rosemary mordanted	72.31	72.59
E. Abyssinica	Dyed	61.26	64.31
	Mango mordanted	68.57	68.93
	Rosemary mordanted	69.11	70.06

4.4.4 The Durability of the Antibacterial Efficacy of the Dyed Fabric

The durability of the antibacterial efficacy of the dyed sample materials after washing cycles was found to decrease continuously (Figure 4.26), which has been noted in other similar research (Shahid-ul-Islam and Butola, 2020). It was observed that the

successive decrease in antimicrobial efficacy of the bio-mordanted and dyed cotton fabric after washing cycles was negligible compared to the un-mordanted cotton fabric which could be due to enhanced fastness properties. The ability of the fabric to retain its antimicrobial activity (above 50%) after ten cycles of washing indicates that the *E. divinorum* dye extract is suitable for developing antimicrobial functionalized textile fabric.

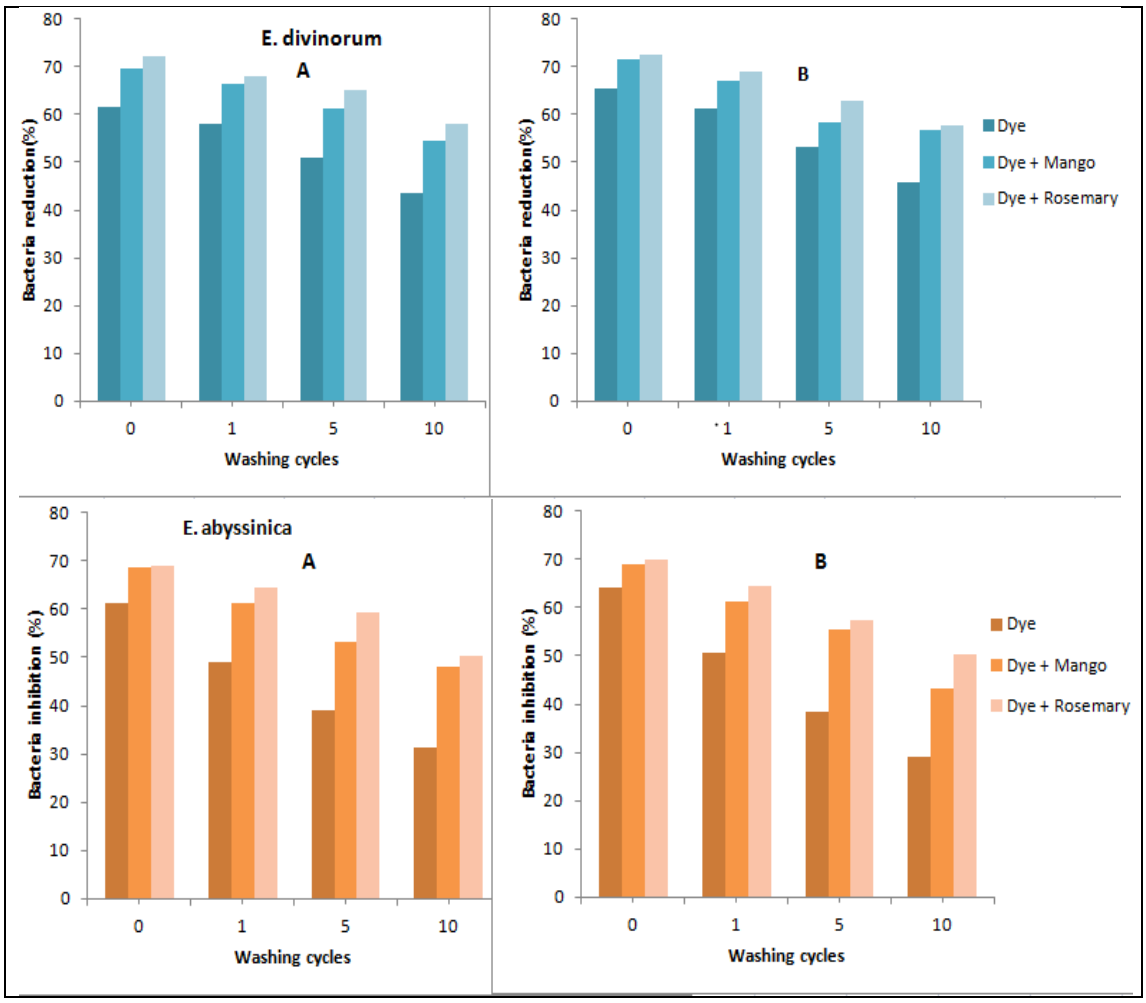


Figure 4.26: Bacteria reduction (%) of cotton fabric after washing cycles. (A) *E. coli* and (B) *S. aureus*

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The following conclusions were drawn from the results obtained in this study:

1. The phytochemicals found in *E. divinorum* and *E. abyssinica* were mainly tannins and phenols
2. *E. divinorum* natural dye extract is majorly made up of lupeol and betulin compounds as suggested by GCMS analysis and confirmed by NMR analysis.
3. *E. divinorum* root bark is suitable source of brown dye for dyeing cotton fabric as confirmed by excellent color fastness of above 4/5.
4. Among the solvents used in the dye extraction, aqueous and methanolic solvents were found to be the most appropriate for extraction of the brown and yellow dyes from *E. divinorum* and *E. abyssinica* plants.
5. The optimum conditions for dyeing with *E. divinorum* were found to be pH of 3.3, 82°C and 68 minutes and for *E. abyssinica* were found to be pH of 5.0, 69.7°C and 74.5 minutes.
6. Tannic acid pre-treatment of the fabric enhanced the dyeing characteristics of *E. abyssinica* dye extract as observed in color fastness that improved from 2 to 3.5.
7. Bio-mordants improved the color strength of the dye extract from 0.612 for *E. divinorum* to 0.863 (mango) and 0.911 (rosemary) and from 0.601 for *E. abyssinica* to 0.692 (mango) and 0.762 (rosemary). Moreover, the use of bio-mordants with different mordanting methods lead to a variety of brown shades for *E. divinorum* and yellow shades for *E. abyssinica* which was also observed with metallic mordants hence bio-mordants are suitable alternatives for some of the toxic metallic mordants.

8. Pre-mordanting method for both metallic mordants and bio-mordants is the best method for mordanting for these natural dyes compared to post-mordanting and meta-mordanting methods.
9. The dyed cotton fabric showed good antioxidant activity of 72.5% for *E. divinorum* and 63.1% for *E. abyssinica*. The dyes also imparted antimicrobial activity to the fabric where *E. divinorum* dyed fabric showed 61.54% and 65.55% bacteria reduction against *E. coli* and *S. aureus*, respectively, whereas *E. abyssinica* dyed fabric showed 61.26% and 64.31% bacteria reduction against *E. coli* and *S. aureus*, respectively. As a result the dye extracts are promising agents for future development of bioactive, protective and health textile fabric.

5.2 Recommendations

This study showed that *E. divinorum* root bark and *E. abyssinica* stem can be used as a source of brown and yellow dyes. Dyeing properties of *E. abyssinica* dye extract were enhanced through tannic acid pretreatment of cotton fabric and two bio-mordants were compared to metallic mordants that are commonly used. The antioxidant and antimicrobial textile finishing properties of the natural dyes on cotton fabric were also investigated. In order to further exploit the potential of *E. divinorum* and *E. abyssinica* as source of natural dyes for textile dyeing the following recommendations were made from this study;

1. There is need to exploit the dyeing characteristics of *E. divinorum* and *E. abyssinica* natural dye extracts on other types of commonly used textile fabric e.g silk and wool.
2. Other natural dye extraction techniques such as micro-wave assisted and ultra sound assisted methods should be investigated.

3. Other bio-mordants should be exploited to identify those that can provide other particular shades that can be obtained with the natural dyes.
4. Extraction of the natural dyes from other different parts of the plant will provide more sources of the dye and maximizing for the same plant.
5. There is need to assess other textile finishing properties such as ultra-violent protection properties, insect repellent properties and deodorizing abilities that the natural dye can impart on the dyed fabric.
6. More research on harvesting season, age of the plant, place of sampling is necessary to identify when the best conditions that dye can be obtained from the plants under study so that its potential, economic and environmental sustainability can be determined.

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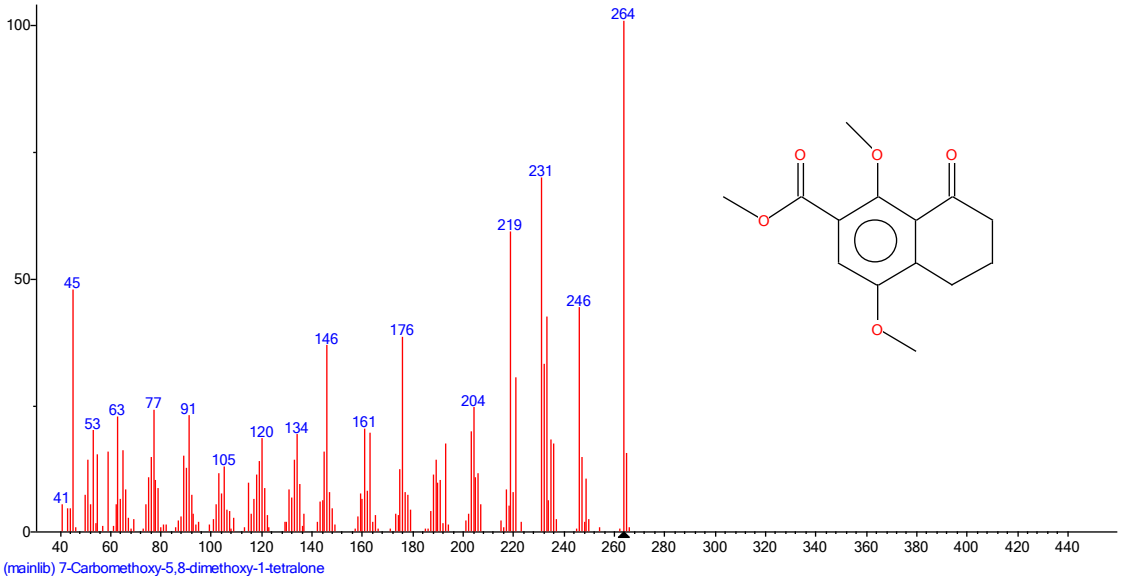
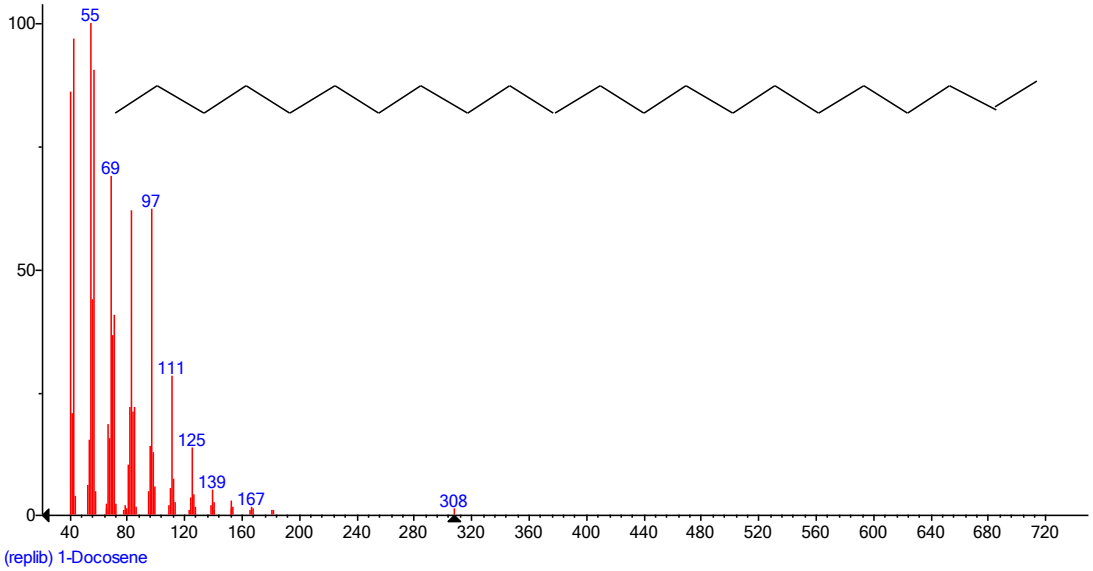
APPENDICES

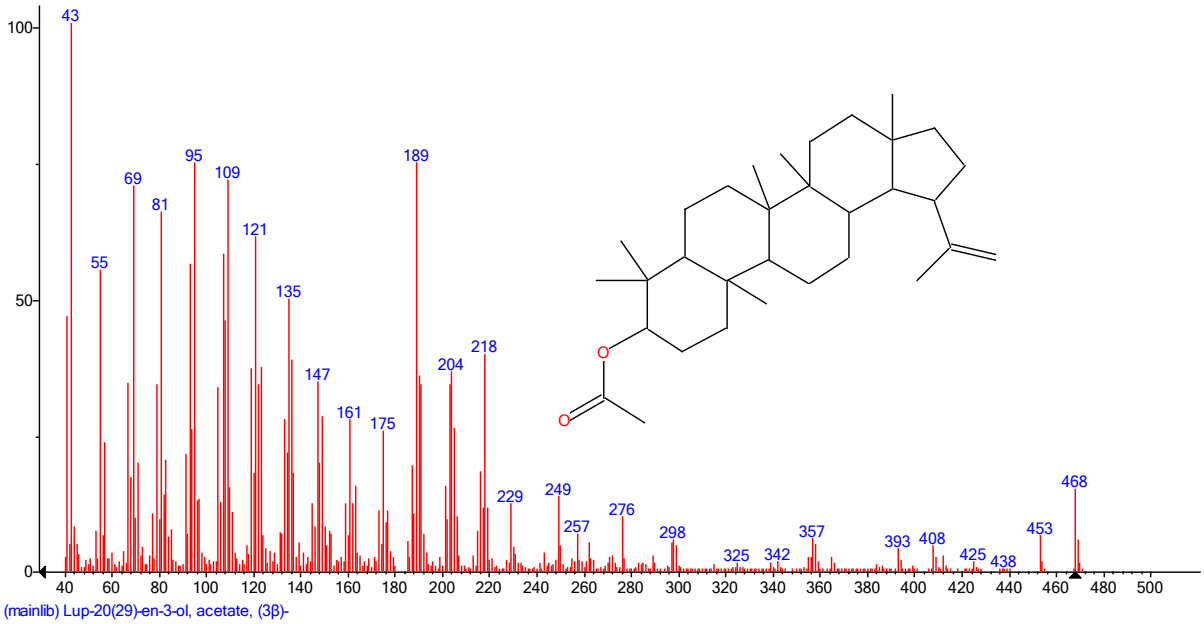
Appendix 1: GCMS Spectra for 7-Carbomethoxy-5,8-dimethoxy-1-tetralone



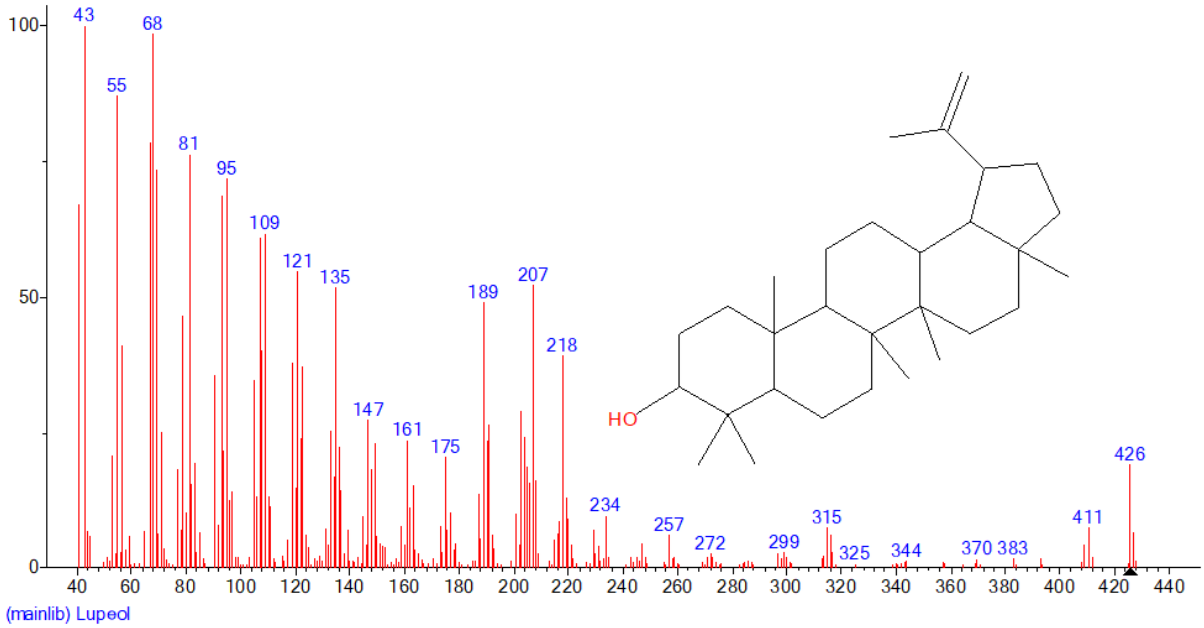
Appendix 2: GCMS Spectra for 1-Docosene



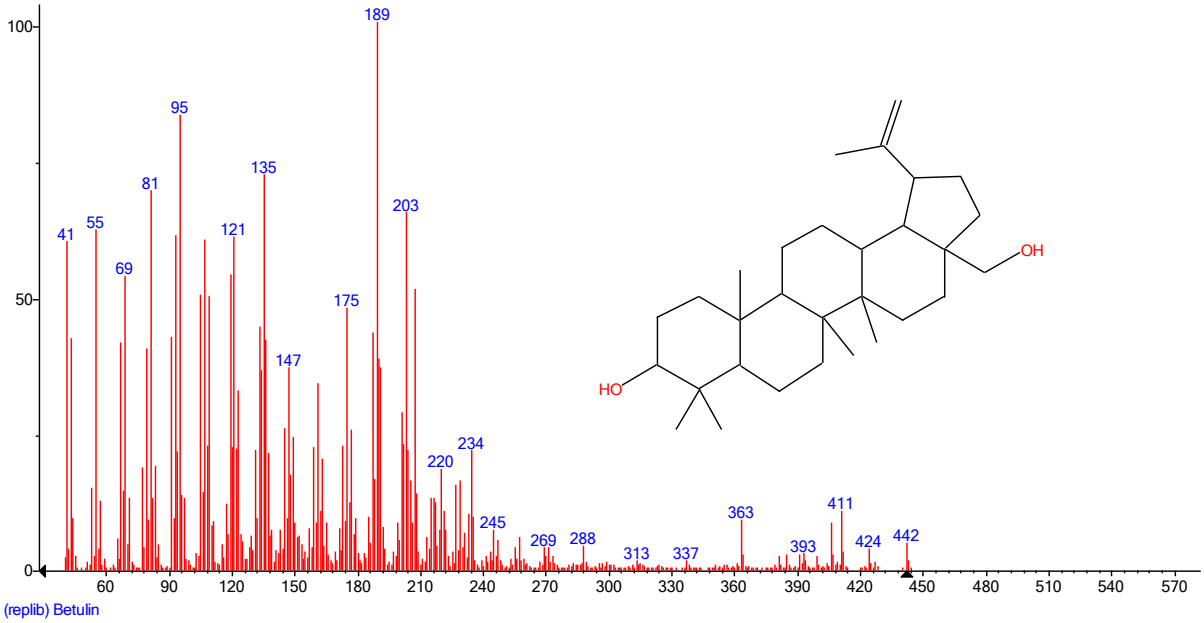
Appendix 3: GCMS Spectra for Lup-20(29)-en-3-ol acetate



Appendix 4: GCMS Spectra for Lupeol



Appendix 5: GCMS Spectra for Betulin



Appendix 6: **Research Outputs of this Study**

Journal Articles

1. Manyim, S., Kiprop, A. K., Mwasiagi, J. I., Achisa, C. M., & Odero, M. P. (2021). Dyeing of cotton fabric with *Euclea divinorusm* extract using response surface optimization method. Research Journal of Textile and Apparel, ahead-of-print (ahead-of-print). https://doi.org/10.1108/RJTA-10-2020-0115
2. Manyim, S., Kiprop, A. K., Mwasiagi, J. I., & Mecha, A. C. (2021). Eco-Friendly Dyeing of Pretreated Cotton Fabric Using a Natural Dye Extract from *Erythrina abyssinica*. Journal of Natural Fibers, 0(0), 1–13. https://doi.org/10.1080/15440478.2021.1964125
3. Manyim, S., Kiprop, A. K., Igadwa, J. I. J., & Achisa, C. M. (2020). Optimization of extraction conditions of natural dye from *Euclea divinorum* using response surface methodology. Annals of the University of Oradea. Fascicle of Textiles, Leatherwork, 21(2), 47–52.
4. Manyim, S., Kiprop, A. K., Mwasiagi, J. I., & Mecha, A. C. (2022). Dyeing characteristics of different solvent extracts of Euclea divinorum on cotton fabric.

In Advances in Phytochemistry, Textile and Renewable Energy Research for Industrial Growth (1st ed., pp. 136–142). CRC Press. https://doi.org/10.1201/9781003221968-18

5. Antioxidant and antibacterial bio-functionalization properties of cotton fabric dyed with *Erythrina abyssinica* using bio-mordants (Journal Article accepted but is still under review).

Conference Presentations

1. Virtual International Conference on Phytochemistry, Textile & Renewable Energy for Sustainable Development from 12th to 14th August 2020. Conference theme: Advancing Science, Technology and Innovation for Industrial Growth. Title of the presentation "Dyeing characteristics of different solvent extracts of *Euclea divinorum* on cotton fabric"
2. Virtual Seventh Sino-Africa International Symposium on Textile and Apparel 2021 (SAISTA) conference from 5th to 7th November, 2021. Conference theme: Green Textile Technology and Sustainable Development. Title of the presentation "Antioxidant and antibacterial bio-functionalization properties of cotton fabric dyed with *Erythrina abyssinica* using bio-mordants"