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Ecofriendly Dyes: Extraction, Characterization and Potential Applications

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Abstract

Indians were thought to be forerunners in the technique of natural dyeing. Although indigenous knowledge systems have been practiced for many years, the usage of natural dyes has declined over generations owing to a lack of documentation and accurate understanding of the extraction and dyeing processes. As a result, natural dyes aren't commercially viable. Currently, all ecologically hazardous synthetic chemical dyes are utilized to colour textile fabrics. They are nonbiodegradable, carcinogenic, and cause water contamination and waste disposal issues. Natural colours provide a viable answer to these issues. Natural dyes are used to colour textiles, meals, medicines, and cosmetics. Dyes are also used in small amounts to colour paper, leather, shoe polish, wood, cane, candles, and other materials. Historically, dyes were obtained only from natural sources. Natural dyes, on the other hand, suffer from the inherent limitations of uniform application and dye standardization, since dyes obtained from comparable plants or natural sources are impacted and exposed to the vagaries of climate, soil, cultivation practices, and so on. As a result, standardization procedures play a critical and essential role for natural dyes to be properly commercialized and compete with synthetic dyes. This study is all about natural dyes, their extraction, characterization, applications, and their uses.

Keywords: Natural Dyes, Extraction, Characterization, Dyeing, Synthetic chemical dyes.

1.Introduction:

Although plants provide a broad spectrum of colours, not all of the pigments may be utilized as dyes. Some do not dissolve in water, others cannot be adsorbed on fibres, while yet others fade when washed or exposed to air or sunshine [Siva and Ramamoorthy, 2000]. Natural colours are those that are obtained without the use of harsh chemicals. Natural colours have become an essential component of many applications [Khan et al., 2017; Adeel et al., 2019]. India has a diverse plant biodiversity and it is rated at 11th in the world in terms of biodiversity. There are roughly 490,000 plant species in the plant kingdom, and the plant world is a treasure trove of varied natural goods [Neha Grover and Vidya Patni, 2011]. The dye is one such natural substance. Plants, insects, fungi, algae, and minerals are the big sources of natural colorants. Plant parts such as bark, leaves, flowers, stems, roots, shoots, fruit, rind, hulls, and husks serve as natural dye sources. These bio dyes of natural origin are gaining worldwide fame as dyes have not only excellent anti-oxidant, anti-bacterial, anti-deodorant, and antifungal characteristics but also cover with a spectrum of colours with bright hues [Zia et al., 2019].
Around the globe, these colorants have also a special place in textiles as these dyes have no disposal problems and there is no need for special care of their isolation and utilization [Haddar et al., 2018]. Due to such benefits nowadays, these dyes have found their excellent place in information about textiles, food, flavour, cosmetics, and the revival of cultural heritage [Wang et al., 2018]. Natural dyes are more eco-friendly, hygienic, user friendly, and long-lasting than artificial colorants. Due to the practical colouring characteristic of natural dyes, natural dyes could be replaced until the development of synthetic dyes [Kumaresan et al., 2011]. Color yield, dyeing process complexity, reproducible outcomes, restricted colours, blending issues, and inadequate fastness characteristics are some of the issues with the use of natural dyes in textile dyeing as shown in [Sachan et al.,2007]. However, these issues can be mitigated by the use of substances known as mordants. Mordants are metal salts that cause a chemical bond between the cloth and the dye [Samanta et al., 2009; Vankar et al., 2009]. Synthetic dyes are hazardous to both the environment and humans. These colours induce a variety of illnesses in humans, including liver tumours, renal damage, and heart problems [Kumar et al., 2004]. As natural colours are gradually replacing synthetic dyes. Natural dyes are not only produced from plants, but also from insects such as Cochineal and Kerriclacca, and can be used to colour food [Bhuyan et al., 2005; Sundari et al., 2015]. Shellfish and lichen are also used for the extraction of dyes. Natural dyes have developed a competitive edge over manufactured colours.

Recently, there has been a heavy interest in the use of natural dyes as a result of rigorous environmental regulations established by many nations' environmental boards and pollution control boards in response to harmful and allergic responses linked with synthetic dyes [Kamel et al., 2005]. According to research, synthetic dyes are suspected of releasing hazardous compounds that are high pollutants in both water and land, perhaps causing allergic reactions, carcinogenesis, and harm to human health. Natural dyes not only release medicinal properties but also improve the aesthetic value of the product, and they are one-of-a-kind and environmentally friendly [Neha Grover and Vidya Patni, 2011].

Many places in the world have evidence of well-developed dye industries. The dyeing skills of the ancient Egyptians, Phoenicians, and Peruvians were well renowned. From the time of the Romans until the sixteenth century, Italian dyers were among the best. Indian dyers were the best at dying cotton. Dyers in China specialized in silk dyeing. Until the development of synthetic dyes, natural dyes were a key trade item. Natural dyes had been supplanted in most uses by the early twentieth century. However, the majority of these dyes continue to be significant for artists, crafters, and niche producers [Casselman and Karen Leigh, 1980; Gordon et al., 1987; Liles et al., 1990]. Some examples of the plants producing dyes, colours, and their uses are listed in the Table.1

Table. 1. Dye producing plants with medicinal uses

S.No.	Botanical name	Family	Parts used	Colour	Medicinal use	References
1.	*Abies spectabilis (D.Don.)Spach*	Pinaceae	cone	Purple or violet	Used for curing cough	Tiwari et al., 2020

2.	*Baleria prionitis L.*	Acanthaceae	Flower	Yellow	Juice of leaves given with honey in catarrhal infections of children. paste of roots applied to boils and glandular swellings.	Banerjee et al.,2012
3.	*Eugenia jambolana Lam.*	Myrtaceae	Bark, leaf	Red	Decoction of bark and seeds used in diabetes	Smita et al., 2012
4.	*Dioscoreabulbifera L.*	Dioscoreaceae	Tuber	Pale colour	Used for ulcers, piles and dysentery	Marandi et al., 2016
5.	*Acacia catechu (L.f.) Wild.*	mimosaceae	bark	Brown/black	Kheersal is used medicinally for sore throat and cough	Siva, 2007
6.	*Bassia latifolia Roxb. /Madhuca indica J.F.Gmel*	Sapotaceae	Bark	Yellow, brown	Used in rheumatism and skin infections and as a laxative in cases of habitual constipation and piles	Vardhana, 2008.
7.	*Geranium wallichianumD.Don*	Geraniaceae	Fruit, root	Yellow, red, brown	Astringent used in toothache and eye infection	Siva, 2007.
8.	*Citrus medica L.*	Rutaceae	Bark	Black	Used for curing dysentery	Dzeufiet, et al., 2014.
9.	*Acacia dealbata Link*	mimosaceae	bark	Brown/black	Used in bronchial infection	Batiha, et al., 2022.
10.	*Bauhinia tomentosa L.*	Caesalpinaceae	Leaf	Yellow	Decoction of root bark used for inflammation of liver and as vermifuge. dry leaves, buds and flowers used in dysentery	Siva, 2007.
11.	*Heliotropiumtrigosu m L.*	Boraginaceae	Leaf	Black	Laxative and diuretic. juice applied to sore eyes; also used for boils, wounds and ulcers.	Siva, 2007.
12.	*Diospyros embryopterisPers*	Ebenaceae	Fruit	Brown	Seeds used for dysentery and diarrhoea	Rauf et al., 2017.
13.	*Acanthophonaxtrifoliatum (L.) Merr*	araliaceae	fruit	Black	Used in paralysis; roots cooked and eaten.	Chen et al., 2021.
14.	*Bauhinia variegate L.*	Caesalpinaceae	Bark	Yellow	Roots carminative, decoction prevents obesity, bark tonic and anthelmintic used in scrofula and cutaneous diseases; also used for ulcer and leprosy. Dried flowers eaten in case of diarrhoea, dysentery and piles.	Bodakhe, et al., 2007.
15.	*Euphorbia triucalli L.*	Euphorbiaceae	Wood	Red	Toothache	Mali, et al., 2017
16.	*Clitoriaternatea L.*	Fabaceae	Flower	Blue	Roots are powerful cathartic and diuretic	Gupta, et al., 2010.
17.	*Actaea spicata L.*	ranunculaceae	seed	Black, red, green	Rhizomes are used for nervous disorders and uterine tenderness	Siva, 2007
18.	*Betula utilis D.Don*	Betulaceae	Tree gum	Brown	Infusion of dark is aromatic and antiseptic; also used as a carminative.	Suberi, et al., 2021

19.	*Pistachiaintergerrim a L.*	Anacardiaceae	Flower, leaf	Yellow	Useful for asthma and other respiratory disorders and also for dysentery.	Younis, et al., 2018.
20.	*Cordia myxa L.*	Boraginaceae	Roots, leaf	Yellow, red	Astringent, anthelminthic, diuretic demulcent and expectorant, used in diseases of chest and urinary tract.	Jamkhand et al., 2013.
21.	*AdathodavasicaNees.*	acanthaceae	leaf	Yellow	Used in bronchial infection	Shamsuddin et al., 2021.
22.	*Briedeliastipularis L.*	Euphorbiacae	Fruit	Black	Decoction of the bark used for cough, fever and asthma. Leaves used in the case of jaundice	Siva, 2007
23.	*Toddalia asiatica (L.) Lam*	Rutaceae	Root	Yellow	Diaphoretic, stomachache relieving and antipyretic properties. root is also used for treatment of cough.	Alagaraj, et al.,2020.
24.	*Cosciniumfenestratu m (gaertn.) Clolebr*	Menispermaceae	Seed, bark, wood	Red	Root considered bitter tonic and used dressing wounds and ulcers.	Rai, et al., 2013.
25.	*Aegle marmelos (L.) Corr.*	rutaceae	Fruit rind	Yellow	Unripe or half ripe fruit is astringent, used as digestive and for curing stomachache diarrhoea	Rahman, et al., 2014.
26.	*Butea monosperma (Lam) Taubert*	Papilonaceae	Flower	Yellow, orange	Bark astringent, used for piles, tumour and menstrual disorders. gum is astringent and used in diarrhoea.	Jhade, et al., 2009.
27.	*Aloe barbadensis (L.) Burm.f.*	lilliaceae	Whole plant	Red	Fresh juice of leaves is cathartic and refrigerant used in liver and spleen ailments and for eye infections, useful in x-rays burns and other skin disorders.	Siva, 2017
28.	*Althea rosea Cav.*	Malvaceae	Flower	Red	Considered emollient, demulcent and diuretic, used in chest complaints	Fahamiya, et al., 2016.
29.	*Ardisia solanaceaRoxb.*	Myrstinaceae	Berry	Yellow	Roots used in diarrhoea and rheumatism	Fahamiya, et al.,2016.
30.	*Arnebia guttata Bunge*	Boraginaceae	Root	Red	Roots are also used for cough	Hosseini, et al., 2018.
31.	*Azadirachta indica A.Juzz*	Meliaceae	Bark	Brown	Skin disorders, leaves considered as antiseptic.	Wylie, et al., 2022.
32.	*Arnebiabenthami (Wall.exG.Don)*	Boraginaceae	Underground parts	Purple	Stimulant, tonic, diuretic, and expectorant used in infection of tongue and throat, and also cardiac disorders and fever.	Siva, 2017

2.Classification of Natural Dyes:

Natural dyes categorization is done in three ways: chemical class, application class, and colour as shown in (Fig.1.).

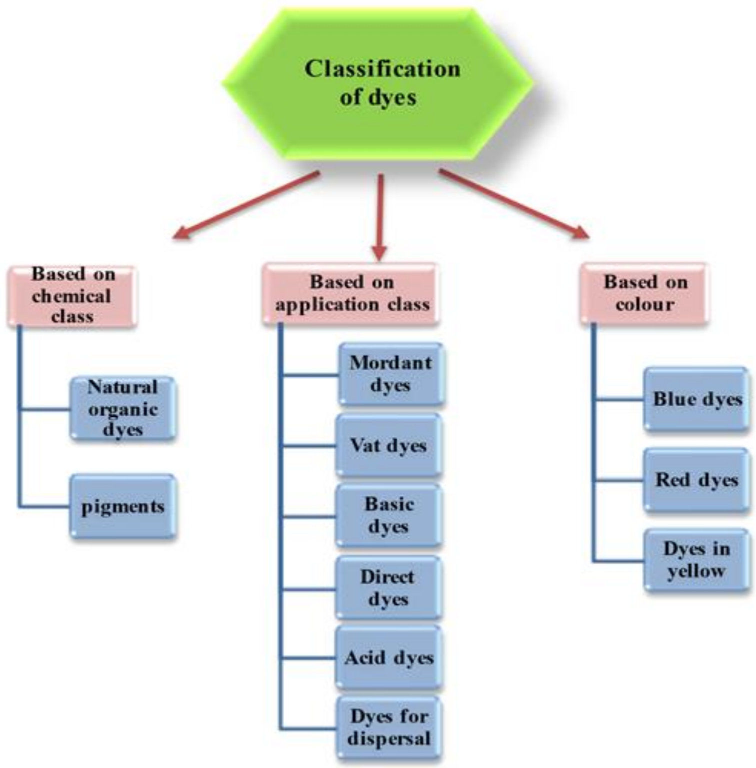

Figure 1: Classification of Dyes

2.1 Based on Chemical Class:

Natural organic dyes and pigments come in a variety of chemical types. Polymethines, Ketones, Imines, Quinines, Anthraquinonoids, Naphthoquinones, Flavones, Flavanols, Flavanones, Indigoids, and Chlorophyll are just a few examples.

2.2 Based on Application Class:

Bancroft categorised natural dyes into two classes in his “Treatise on Permanent Colours,” which was published around 160 years ago.
·**Substantive dyes:** Substantive dyes are dyes that directly colour the fibre. Indigo, Turmeric, and Orchil are examples.
·**Adjective dyes:** These dyes only colour the material when it is mordanted with metallic salt or when metallic salt is added to the dye solution. Logwood, Madder, Cochineal, and Fustic are a few examples.
The preceding categorization was superseded by the subsequent classification as direct dyes and mordant dyes. Hummel's other categorization is as follows:
·**Monogenetic dyes:** These dyes produce only one colour irrespective of the mordant.
·**Polygenetic dyes:** The colour generated by these dyes depends on the mordant used another application-based classification:

2.2.1 Mordant Dyes: Mordant colours are dyes that have an affinity for mordanted fibres. Mordant dyes include any dyes that form complexes with mordants. Fustic, Kermes, Cochineal, and other examples.

2.2.2 Vat Dyes: The term 'Vat Dyes' comes from the wooden fermentation tank known as a 'Vat,' which was once used to reduce the dye to convert it to a soluble form. Vatting is the process of solubilizing dye, and leuco dye is the soluble form of dye. The soluble leuco form has a strong affinity for natural fibres, and when exposed to air, it oxidises back on the fibre. Vatting is also accomplished by the use of a reducing agent, such as sodium hydrosulphite, and an alkali, such as sodium hydroxide. Indigo is an example.

2.2.3 Direct Dyes: For example, turmeric, while being a fugitive dye, has a strong affinity for cotton. Other natural fibres are likewise directly absorbed by this dye.
Turmeric, Annatto, Harda, pomegranate (*Punica granatum L.*), Carthamin derived from Safflower, and so forth are examples.

2.2.4 Acid Dyes: Acid dyes are a type of direct dye used on polyamide fibres such as wool and silk. These dyes are used in acidic environments and have either sulphonic or carboxylic groups in the molecule. Saffron is one example.

2.2.5 Basic Dyes: These are cationic dyes that, when ionised, produce coloured cations. They are used on polyamide fibres such as wool and silk that have been exposed to neutral or moderately acidic environments. These colours can be used on cotton that has been mordanted with tannic acid, tartar emetic, or other metal salts. Light fastness is low for these colours. Example: Barberine.

2.2.6 Dispersal Dyes: A dispersion dye has a low relative molecular mass (R.M.M.), is poorly soluble, and lacks significant solubilizing groups. These dyes contain hydroxyl and amino groups, which give the dye molecule some solubility. Lawsone is one example of such a dye. Many additional flavones and anthraquinone dyes have the potential to be classed as Disperse dyes.

2.3 Based on Colour:
The dyes in the colour index are categorised based on their chemical composition as well as their primary application classifications. The dyes are organised by colour within each application class. Natural dyes have their area.
Almost any vegetable matter might be used to produce colouring matter. However, only a handful of the sources provide colourants that can be isolated and are commercially viable. The same is true for colourants that are derived from animals. To obtain any given hue, three primary colours are required. The method has been developed for synthetic dyes. However, in the case of natural dyes, the dying methods vary for each dye, and they cannot be readily mixed to get the desired colour. However, while looking for various colours, it is preferable to have a small number of dyes with good fastness qualities rather than a large number of colours with poor fastness properties. When choosing a colour palette, it is best to start with at least one blue, one red, and one yellow. Because there are only a limited number of natural dyes available, dye selections are critical. The following section contains information on several significant natural dyes.

2.3.1 Blue Dyes: The colour index only mentions three natural blue dyes: natural indigo, sulfonated indigo, and the blossoms of the Japanese ‘Tsuyukusa,' which are mostly used to make awobana paper. Indigo is the sole suitable option among the blue natural dyes. Natural indigo is made by fermenting the leaves of different Indigofera species, removing the liquid, and oxidising it to precipitate the colour. Indigo is also found in woad (*Isatis Tinctoria L.*). Indigotin and Indirubin are the two major components of natural indigo. Natural indigo has a greater affinity, and coloured materials are more resistant to fading.

2.3.2 Red Dyes: The colour index includes 32 natural red dyes. Madder (*Rubia tinctorum L.*), Manjeet (*Rubia cordifolia L.*), Brazilwood/Sappan wood (*Caesalpina sappan L.),* Al or Morinda (*Morinda citrifolia L.*), Cochineal (*Coccus cactil L.*), and Lac dye are the most well-known (*Coccus laccase*). Except for the colours derived from Brazil and Sappan wood, all of these dyes are anthraquinone-based. Because the colours are prone to oxidation, they are unsuitable. Turkey Red, a strong and nearly fadeless cotton dye, was invented in India and spread to Turkey. It requires around twenty different procedures using wood, oil and rancid fat, charcoal, cow/sheep/dog faeces, and liquid content of the animal stomach. Unsurprisingly, only the dyers and their families lived in the villages where the procedure was carried out. Following the invention of synthetic alizarin in 1869, the usage of madder fell precipitously [Gulrajani et al., 2001].

2.3.3 Yellow Dyes: Yellow is the most commonly used colour in natural dyes. The majority of the yellow colourants, on the other hand, are fugitive. yellow dyes derived from Barberry (*Berberis aristata*), Tesu flowers (*Butea frondosa, Monosperma*), and Kamala are among the most significant (*Mallotus philippensis*). Yellow dyes can also be found in black oak (*Quercus velutina*), turmeric (*Curcuma longa*), weld (*Reseda luteola*), and Himalayan rhubarb (*Rheum emodi*) [Gulrajani et al., 1992].

3. Dyeing Process:

In comparison to cellulosic fibres, natural dyes are ideal for colouring protein fibres. Natural dyes can also be used on synthetic fibres with polar groups, such as nylon, acrylic, and viscose. Natural dyes are thermally unstable and have low chemical stability, making them unsuitable for high-temperature and pressure dyeing. The existence of hydrogen bonds and the Van der Waals force of attraction are significant factors in the fixing of natural dyes on fibre. Because natural dyes have a low exhaustion value due to their low affinity for fibre materials, common salt/salt Glauber's are added to the dye bath to boost the exhaustion of colours. The Nernst isotherm governs the sorption of natural dyes [Patel et al., 2002; Bhattacharya et al., 2000]. Natural dyes have low affinity and substantivity for cellulosic fibres like cotton and viscose [Maulik et al., 2006]. Because the absence of reactive groups in fibres and dyes prevents bond formation, mordanting is required to attach the dye to the fibre surface. Protein fibres include bond-forming groups in their structure, and the presence of carboxylic groups in natural dyes allows for bonding and bonding with fibre, resulting in high fastness qualities. Natural dyes have a lower molecular size and lack a conjugated linear structure [Samanta et al., 2008]. Natural dyes exhibit poor fatigue behaviour. Sodium chloride salt is sometimes used to boost the dye exhaustion percentage.

4. Extraction method:

The most essential processes in the production of natural colours are extraction and purification. Because the extraction procedure is so complicated, it is critical to first evaluate the solubility of the natural colours. For the extraction and separation of natural materials, the conventional chemical separation approach is frequently employed. It is primarily based on the concepts of varying solubility of active components in different solvents; however, crystallisation and other techniques can also be explored. This approach requires no additional equipment and is simple to use. The dye extraction techniques are as follows [Merdan et al., 2017; Oztav 2009].
Aqueous extraction

- Solvent extraction
- Alkaline and Acid Extraction
- Supercritical CO_2 Extraction
- Enzymatic Extraction & Fermentation
- Ultrasound assisted extraction

4.1 Aqueous extraction:

The water extraction method is traditional, straightforward, and appropriate for extracting plant components that may be distilled with steam without altering the molecular structure. To improve extraction efficiency, coloured materials are chopped into small pieces, crushed into powder, and kept overnight in a steel container to relax the cell structure [Merdan et al., 2017] [Selvam et al., 2015]. These compounds are incompatible with water and just marginally soluble, they also have a specific vapour pressure at 100°C. When the water boils, the material may be transported away with the steam. Following condensation, it is separated using an oil-water separator to remove the water and extract the plant components necessary. Depending on the results of the extraction, the boiling and separating procedure may be repeated. The dye and thin plant leftovers may be readily separated using a trickling filter. However, boiling may impair the colour output of temperature sensitive dyes, therefore a moderate temperature is advised. This technique produces dyes that may be utilised in textile applications.
This technique has a lengthy processing time, high water use, and temperature [Samanta et al., 2009]. Similarly, the fractionation process fractionates liquid components based on their varied boiling points before refining and

purifying them. This approach is simple for extracting flowers from plant sources. One researcher successfully recovered pigment from the African marigold flower by boiling it in distilled water for two hours and then filtering it [Jothi, 2008].

4.2 Solvent extraction:

This method is simple, the equipment investment is minimal, the technology is simple to learn and understand, and it has the most practical applications. It is simpler to purify and remove solvents from extracted dyes, as well as reuse them. When compared to the aqueous technique, alcohol extraction is more efficient [Merdan et al., 2017; Borges et al., 2012].

Depending on the nature of the native colourant, water-soluble pigments are generally extracted using water or hydrophilic organic solvents such as ethanol, methanol, and acetone. Fat-soluble pigments, on the other hand, are extracted using hexane, dichloromethane, and petroleum ether. The solvent's solubility in the solute to be dissolved should be high, as should the solubility of other solutes. Dry leaves were finely crushed in a grinder before being exposed to Soxhlet extraction with a solvent in this technique. At 60°C, the cycle is repeated three times. The cooled extract was then filtered via filter paper, and the solvent was evaporated using a rotary evaporator.

Impurity solubility is low, solvent supplies are plentiful and non-toxic, and the boiling point is suitable for recycling. Specific extraction methods include the widely used decocting method, leaching method, reflux extraction method, percolation method, and continuous reflux extraction method. The degree of grinding, extraction duration, temperature, equipment, and solvent selection are all elements that influence the extraction. Low-temperature processing causes less deterioration. The primary drawbacks of this technique include poisonous residues, greenhouse gas emissions, and processing problems caused by chlorophyll and waxy compounds [Merdan et al., 2017; Alwani et al., 2015].

4.3 Alkaline and Acid Extraction:

Some natural colours with glycosides in their structure can be treated with weak acids and alkalis. The extraction process is accelerated by the addition of these acids and alkalis owing to glycoside hydrolysis. Furthermore, this procedure boosts the colour yield. The colours containing phenolic groups, and alkaline extraction yields superior results [Liu et al., 2009]. Once the extraction procedure is complete, acids can be used to precipitate the product. In addition, alkaline extraction may be used to extract lac dye from lac pest and safflower leaf. [Yusuf et al., 2017].

4.4 Supercritical CO_2 Extraction:

Supercritical fluid is the most complicated process, yet it has the benefits of both liquids and gases, such as high density and viscosity, reduced surface tension, and higher solubility, which increases fast with pressure. It can enter the matrix of extraction materials and is a highly efficient extraction process [Prabhu and Bhute, 2012]. Extraction and solvent removal are integrated into a single device that identifies the process flow and increases production efficiency. It also offers a few benefits, such as quick extraction speed, high selectivity, the ability to extract and segregate at room temperature or low temperatures, and it is environmentally friendly [Borges et al., 2012]. It is a two-step process that extracts a dense gas, often carbon dioxide, above its critical temperature (31°C) and critical pressure (74 bar). The natural product is pulverised before being loaded into the extractor. A high-pressure pump (10034 bar) delivers carbon dioxide to the extractor. A pressure reduction value is used to send the extracted charged carbon dioxide to a separator (60120 bar). The extract precipitates out in the separator at lower temperatures and pressure. The extract-free carbon dioxide stream is injected multiple times to ensure that all of the colour material from the natural product is extracted effectively.

Earlier, in industrialized nations, research on supercritical fluid extraction technology was discovered, and it was primarily found in the extraction process of hops, caffeine, and other chemicals. Since 1984, Japan has used supercritical technology in the extraction and purification of raw materials for food flavours, cosmetics, medicinal goods, and cigarettes, but China has just recently begun to use supercritical technology. However, greater results have been obtained in China, where many textile and agricultural institutions have begun to employ supercritical technology to extract chlorophyll [Luinstra, 2008].

4.5 Enzymatic Extraction & Fermentation:

Recent advancements in biotechnology are gaining popularity for extracting useful components from natural plants. There is a variety of suitable enzymes that can degrade plant tissues gently, speed up the release of active components, and increase the extraction rate. For example, cellulase can degrade cellulose, hemicellulose, and other substances, causing localised loose and swelling changes in the cell wall and cytoplasmic structure, increasing the diffusion of effective components in the cell to the extraction medium and promoting pigment extraction efficiency. The primary parameters influencing the enzyme's action are temperature and pH. The enzyme extraction technique offers the benefit of gentler extraction conditions as well as stable physical and chemical characteristics of active components. An enzymatic process can alter the structure of Geniposide in natural gardenia yellow pigment to create gardenia red and blue pigment. The enzymatic approach extracts anthocyanins at a rate that is approximately 72% greater than the solvent method. This technique is appropriate for colours derived from hard plant sources such as bark and roots. [Xiangyuan et al., 2011].

4.6 Ultrasound assisted extraction:

Ultrasound is thought to be one of the most efficient and energy-efficient sources of electricity, allowing for considerably quicker extraction and colouring [Sivakumar et al., 2009]. The two forms of ultrasound are characterised by frequency range: power ultrasound (20-100 kHz) and diagnostic ultrasound (1-10 MHz). In the case of ultrasonic technology, when the acoustic pressure rises, micro-bubbles become apparent, expand in size, oscillate at a higher frequency, and eventually burst. Microjets and shock waves will collide near a solid surface as a result of this phenomenon [Sheikh, 2016].

UAE was performed by dissolving dried and powdered samples in methanol or other solvent in a flask and placing it in an ultrasonic bath for 30 minutes. The temperature of the extraction began at 2040°C and decreased to 60°C after one hour of extraction. The extraction was done two to three times, and the extracts were collected each time. There are substantial variations in the capacity of various sections of biological cells to absorb microwave energy, resulting in local heating of cells in the microwave field. Parts that are rich in free water, such as liquid cells, are rapidly heated by microwave radiation, and the water vaporises [Rahman et al., 2013]. The cell wall or membrane cannot withstand high internal pressure, microwave irradiation causes cell deformation. Microwave-assisted extraction (MAE) offers the benefits of high yield, high selectivity, short time, and low energy consumption [Gomes et al., 2020]

5. Characterization Methods:

Dye characterization involves a multifaceted approach that takes into variables such as molecular composition, colourfastness, and application appropriateness. Methods, such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), Fourier transform infrared spectroscopy (FTIR), gas chromatography–mass spectrometry (GC MS), nuclear magnetic resonance (NMR), x-ray diffraction (XRD), UV-visible spectrophotometer. (Fig.2.)

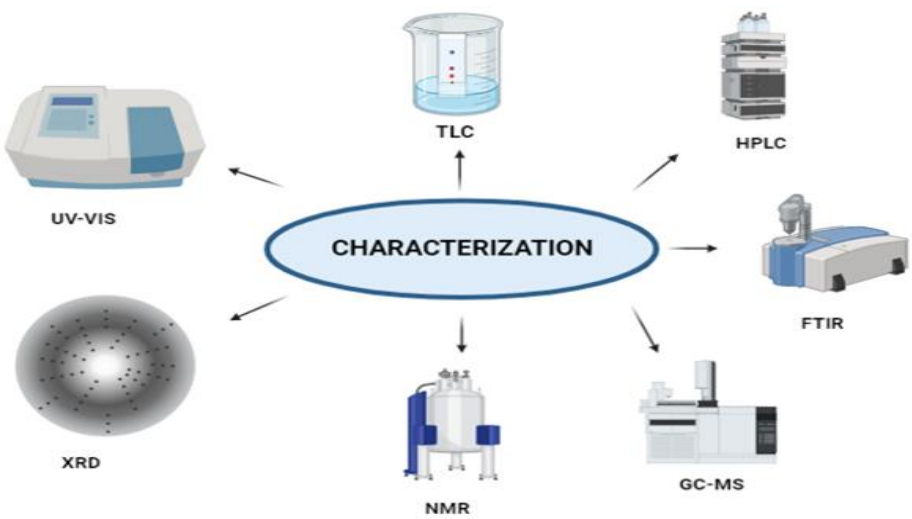

Figure 2: Characterization of natural dyes (Created via Biorender).

5.1 Thin Layer Chromatography (TLC):

This method was initially proposed by Izmailov and Schraiber in the 1930s, but it has only recently acquired prominence as a consequence of the work of Stahl, a German pharmacy lecturer who invented a spreading mechanism to generate homogeneous layers of adsorbent on glass plates. To get consistent results, he also standardized the production of appropriate adsorbents. Thin-layer chromatography is a micro-analytical technology that separates compounds by adsorption or partitioning on a tiny layer of adsorbent put on a glass plate. The stationary phase is present in TLC as a thin layer on top of an inert rigid support. TLC phases include selecting an appropriate chromatographic stationary phase, applying the sample, selecting a mobile phase, developing, visualizing, and detecting. TLC separation entails placing the material to be analysed as a distinct spot on the chromatographic plate. After the solvent in which the sample was applied evaporates, the plate is formed by letting a mobile phase pass through the stationary phase by capillary action, taking the components contained within the sample with it. The stationary phase retains these components to varying degrees, resulting in separation. Plates are dried with a drier, and bands are seen under UV lights before being scanned in a TLC scanner. Chemical elution characteristics can be expressed as Rf values, which are a measure of the relative distance travelled by each compound from the origin to the solvent front [Robards et al., 1994].

$$\mathbf{Rf = 1/\ (1 + K')}$$

Rf = Ratio of distance travelled by solute to distance travelled by solvent front. K's value measures the ratio of time spent by solute in the stationary phase to time spent in the mobile phase. [Rettie et al., 1964].

5.2 High Performance Liquid Chromatography (HPLC):

HPLC is a very effective separation method that is widely utilized in all branches of analytical research. It can separate a wide range of molecules with molecular weights ranging from a few hundred to a few million. It entails the chemical bonding of a hydrophobic, low-polarity stationary phase to an inert solid such as silica. The analyst has access to a diverse set of stationary phases, as well as a nearly unlimited set of mobile phase compositions. This adaptability raises the difficulty of the development procedure for a new analytical separation. The procedure is carried out at a high velocity and pressure decrease. The column is shorter and has a smaller diameter, but it has a greater number of equilibrium phases. It is especially useful for fractionating large molecular weight molecules, as well as thermally unstable chemicals and products that cannot be volatilized without breakdown.

When compared to TLC, HPLC likewise has high initial set-up costs and considerable operating expenditures. Another difficulty that arises when samples are put into an HPLC system in solution is the possible incompatibility between the extraction solvent and the separation conditions (Griffin et al., 1999). TLC is difficult to analyze when two dyes have the same Rf value. Dyes of various colours with the same retention time, on the other hand, can be quantified using HPLC if their spectra do not interfere. To identify the eluting analytes, a variety of methods can be employed; the most frequent is UV-visible absorbance spectrophotometry, although mass spectrometry has also been utilized in the study of fibre dyes. The resultant separation is recorded as a graph, traditionally on a chart recorder but today electronically on a computer, known as a chromatogram.

5.3 Fourier Transform Infra-Red spectroscopy (FTIR):

FTIR spectroscopy is commonly used to identify materials in their gaseous, liquid, and solid forms. It is yet another analytical tool for determining the chemical composition of a dye. It can also be used to determine the structure of the dye's organic and inorganic components. It generally indicates the presence of functional groupings in a sample [William Kemp, 1986]. Materials that are transparent to IR radiation, such as sodium chloride and potassium bromide, are used to make sample cells. In comparison to conventional IR devices, Fourier Transform Infra-Red Spectrometers offer faster data gathering and improved signal-to-noise ratios through signal averaging. The monochromator of IR instruments is replaced with an interferometer in FTIR [Bhattacharyya et al., 1997; Harvey David, 2000; Nadiger et al., 2004].

5.4 Gas chromatography-mass spectrometry (GC MS):

The components to be separated are divided into two phases in gas chromatography, one of which is a stationary bed with a large surface area and the other of which percolates through the stationary bed. Gas-solid chromatography is the more formal name for the separation procedure when the immobile phase is a solid. In a gaseous solution, this method is typically employed to separate volatile chemicals [McLafferty, 1980]. Gas liquid chromatography (GLC), which is more widely used and will be employed in this experiment, uses a

porous material as the stationary phase that is covered in an absorbing liquid. Several different chemical molecules can be separated using GLC. The sample must be volatile and must not break down during the vaporization process to meet the fundamental requirements for GLC. Breakdown of the sample is typically not an issue because the vaporization takes place in an immobile atmosphere [Silverstein et al., 1981].
The variances in the sample vapor's solubility in the liquid determine whether a mixture can be divided into its constituent stationary phase. A slight layer of the paper chromatography is applied over large-surface-area solid particles before being uniformly stacked into a column. The carrier gas flows continuously through the column while transporting solute particles in the gaseous phase. The column is wrapped such that it may be precisely temperature-controlled within the oven. The strength of the GCMS technique is that each component of a mixture is structurally identified by the sensor, in addition to being quantitatively separated and detected. Unlike in traditional GC, substances can also be recognized by the mass spectra to compare the lag phase to a standard. Most of the time, an unidentified can also be located based only on its spectra, opposing the need to use retention time requirements [Skoog et al., 1998].

5.5 Nuclear Magnetic Resonance (NMR):

The energy absorbed by changes in the nuclear electron spin is detected using the spectroscopic method known as nuclear magnetic resonance (NMR). The analysis of both nucleic acids and proteins using NMR spectroscopy has yielded novel insights into the dynamics and chemical kinetics of these systems. NMR's ability to offer order at the atomic level on the dynamics of both nucleic acids and proteins over an extraordinarily large variety of time scales, from seconds to picoseconds, is one of its key features. It is not necessary to crystallize the material for NMR research because NMR may also reveal atomic-level information about the structure of both nucleic acids and proteins in the solution. Hence, if a molecule cannot form a crystal or a crystal structure determined by X-ray crystallography, NMR offers a technique for getting structural information. The detected NMR absorption peaks must be attributed to a specific protein atom in practically all investigations. Although wavelength transfer methods are widely understood, they do take a lot of effort to collect and analyze the necessary data. NMR has a restricted range of protein and nucleic acid sizes that can be investigated [Zhang et al., 2010; Harris et al., 2001; Harris et al., 2008].

5.6 X-ray Diffraction (XRD):

An analytical method known as X-ray diffraction (XRD) is quick and is generally used to determine the phase of crystalline materials. It also gives information on the dimensions of a unit cell. The material under analysis is finely powdered, and homogenized, and the bulk composition is calculated on average. Constructive interference between homogeneous X-rays and a solid sample is the foundation of X-ray diffraction. A cathode beam tube produces the X-rays, which are then purified to provide monochromatic radiation, focused by collimation, and pointed at the sample. When the circumstances follow Bragg's Law (n=2d sin), the action of the incident light with the sample results in a scattering ray. This law proves a relationship between the strength of electromagnetic radiation and the crystallite size and scattering direction in a crystalline sample. Afterward, the diffracted X-rays are found, examined, and tallied. Due to the powdered material's random orientation, all potential lattice diffraction directions should be obtained by screening the specimen at a total of 2 angles. Each material has a specific set of d-spacings, converting the diffraction pattern to d-spacings enables mineral identification. This is often accomplished by comparing the d-spacings with accepted reference patterns. The production of X-rays in an X-ray tube is the foundation of all diffraction techniques. The sample is hit with these X-rays, and the light-refracting rays are captured. The ratio between both the incident and light-refracting rays is a major element in all types of diffraction. Beyond this, the apparatus for particle and monocrystalline diffraction differs [Brady et al., 1995].

5.7 UV-Visible Spectrophotometer:

The measurement and analysis of electromagnetic radiation that is absorbed or released when molecules, atoms, or ions in a sample transition from one energy state to another is known as spectroscopy. UV spectroscopy is a form of absorption spectroscopy, a molecule absorbs light in the ultra-violet range (200–400 nm), which causes the electrons to be excited from their initial state to a greater energy state. This instrument's operation is rather simple. A mirror or diffraction grating separates a red beam of visible and/or ultraviolet light into its wavelengths. A half-mirrored device divides every homogenous (single wavelength) stream into two similar intensity beams. One light, the sample beam, which is magenta, travels through a tiny, clear cuvette that contains a mixture of the substance under investigation in an opaque solvent. The reference beam, which is coloured blue, travels through a similar cuvette that only contains solvent. Then, electronic detectors measure and compare the light beam intensities. [Godinho et al., 2011; Hadjadj, 2015].

6. Applications:

The characterization of eco-friendly dyes has numerous applications across various industries and fields (Fig.3.). Some of the key applications include:

6.1 Textile Industry: One of the most significant applications of eco-friendly dye characterization is in the textile industry. Eco-friendly dyes can be used to color natural and synthetic fibres, fabrics, and garments. By characterizing these dyes, manufacturers can ensure they meet quality and performance standards, enabling the production of sustainable and environmentally friendly textiles.

6.2 Paper and Printing: The paper industry can benefit from eco-friendly dye characterization for producing environmentally friendly and sustainable papers. These dyes can be used for coloration in paper manufacturing and printing applications, reducing the environmental impact of printed materials.

6.3 Cosmetics and Personal Care: Eco-friendly dyes are also finding applications in cosmetics and personal care products, such as natural hair dyes, organic lipsticks, and eco- friendly nail polishes. Characterization ensures their safety and compliance with regulations.

Figure 3: Applications of natural dyes (Created via Biorender).

6.4 Food and Beverages: Natural and eco-friendly dyes can be used to color food and beverages. By characterizing these dyes, their safety and stability can be ensured, enabling the production of natural and organic food products.

6.5 Medical and Healthcare: Eco-friendly dyes can be used in medical applications, such as medical textiles and bandages, where the absence of harmful chemicals is crucial. Characterization helps ensure their biocompatibility and safety for medical use.

6.6 Education and Research: Eco-friendly dye characterization plays a significant role in academic and scientific research. Researchers can study the properties, behavior, and interactions of these dyes to develop new and improved eco-friendly dyeing processes.

6.7 Environmental Monitoring: Eco-friendly dyes can also be used as tracers in environmental monitoring studies, helping to track water flow, identify sources of pollution, and assess the impact of industrial activities on the environment.
Eco-friendly dye characterization has broad applications in diverse industries, ranging from textiles and fashion to healthcare and environmental monitoring. As sustainability becomes increasingly vital, the demand for ecofriendly dyes and their characterization will continue to grow, promoting a greener and more sustainable future. [Adeel et al., 2019].

7. Future prospects:

The current work offers a summary of recent developments and viewpoints on a few natural dyes and pigments. The discussion includes some of the essential characteristics of colorants, their chemical makeup, and their manufacture. Future potential and the specifics of the dyeing technique are also described. Also mentioned are some of the fundamental problems with using natural colorants. Here, the classification of organic colorants and their pharmacological potential are explored together with a summary of their use. There is a discussion of a few contemporary techniques for extracting natural colorants and dyeing processes. An appropriate colorant must function well on the substrates, be commercially viable, and be biodegradable. Natural colorants are nontoxic, biodegradable, and simple to extract, among other advantageous qualities. Some of the colouring agents also have medical qualities, which makes them ideal colouring agent options. Despite these advantageous qualities, their poor performance and low yield make them uneconomical.

Natural colorants have become possible alternatives to dangerous synthetic dyes due to their less laborious manufacture and extraction processes and environmental friendliness. More studies should be done to optimize the manufacturing of colorants from natural resources. Some of the more recent uses for natural colorants are emphasized, including those in dye-sensitized solar cells, cell imaging, anticancer, biosensors, corrosion inhibition, etc. Plants, minerals, and animals that produce colours are widely available in India. Therefore, a huge production can be reached by improving their cultivation or processing. It is possible to investigate using bacteria and insects to produce colorants. It is also possible to investigate the usage of natural mordants to enhance the performance of colorants.

8.Conclusion:

After a comprehensive analysis and characterization of eco-friendly dyes, it is evident that these sustainable alternatives offer promising prospects for the textile and dyeing industries. Throughout this study, several key conclusions have emerged Environmentally Responsible Eco-friendly dyes are a significant step forward in promoting sustainable practices in the dyeing industry. They are derived from renewable resources, use nontoxic and biodegradable ingredients, and reduce the overall environmental impact compared to conventional synthetic dyes. The use of reduced water consumption eco-friendly dyes requires less water during the dyeing process due to improved dye uptake and fixation properties. This can contribute to substantial water savings, addressing one of the critical challenges faced by the textile industry. Energy-Efficient Eco-friendly dyes often require lower temperatures and shorter dyeing times, leading to reduced energy consumption during the dyeing process. This can contribute to lower greenhouse gas emissions and help mitigate climate change. The characterization of improved color fastness eco-friendly dyes has shown that they can achieve comparable or even improved color fastness properties compared to conventional dyes. This ensures longevity and color retention in the dyed fabrics, reducing the need for frequent re-dyeing and extending the lifespan of textiles. Biocompatibility Eco-friendly dyes are generally safer for both human health and the environment. They do not contain harmful chemicals or heavy metals, reducing the risk of allergic reactions and pollution in wastewater. The successful characterization of Potential for Scalability eco-friendly dyes indicates their potential for widespread adoption in the textile industry. With further research and development, these dyes can be refined, scaled up, and made more cost-effective. Consumer Demand the growing awareness and concern for environmental issues have led to an increasing demand for sustainable and eco-friendly products. The textile industry's adoption of eco-friendly dyes can cater to this demand and align businesses with responsible consumer preferences. In conclusion, eco-friendly dyes represent a positive step towards achieving a more sustainable and environmentally conscious dyeing process in the textile industry. By reducing water consumption, energy usage, and harmful chemical release, these dyes offer a viable and responsible alternative for the future of textile coloration. Embracing eco-friendly dyes can foster positive change, promoting a greener, cleaner, and more ethical approach to textile production and ultimately contributing to a healthier planet for generations to come.

Declaration of Interest Statement

Consent to Participate

Informed consent was obtained from all individual participants included in the study.

Conflict of interest

The authors declare no conflict of interest.

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