Extraction, Characterisation and Pharmacological Potential of Natural Dyes: A Review

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Abstract: Plants are great source of natural dyes. These natural dyes are yielded by many plant part such as leaves, flowers, roots, seeds and sometimes microorganisms also like algae, fungi etc. These dyes are rich organic sources of printing paper and fibres also. These extracted materials are responsible for their therapeutic and curative properties also due to presence of many bio compounds in it. Natural dyes are curative and commercial also. They have many pharmacological efficiency like antimicrobial, antioxidant, Ameliorative activity, Hepatoprotective activity, no tropic activity, Analgesic activity, antiviral, anticancer, anti-inflammatory etc. These natural dyes are extracted by many extraction methods such as simple aqueous methods, alcoholic or solvent extraction method, complicated solvent systems, supercritical fluid extraction, Microwave or Ultrasonic extraction, Enzymatic extraction, fermentation extraction those are use to isolate pigments from plant parts. Characterisation of dye is main step to show the affinity of dye on its fibre. Extracted dyes are characterised by many parameters like uv-vis spectroscopy, FTIR, HPCL, GCMS, NMR techniques. So, this review revails the pharmacological and economical perspective of natural extracted dye which is useful in medical and commercial industry in the globe.
Keywords: supercritical, no tropic, extraction, Hepatoprotective, fermentation

Introduction:

Although plants provide a comprehensive band of colours, not all of the colours may be applied as dyes. Some colours are un dissolve in water while others cannot be adsorbed on fabrics whereas others colours diminish when washed or open up to air or sunlight.(Siva, et al. (2007). Naturally extracted colours are considered as dyes. Dyes are those that are gained without the practice of severe chemicals compound. Natural colours dye "become considered as an important constituent of many applications (Khan et al., 2017; Adeel et al., 2019). India having a assorted biodiversity of vegetation and rated on 11th positions in the world in class of biodiversity. There are approximately 490,000 floras in the vegetative kingdom and the vegetative kingdom is a gem of varied natural properties (Neha Grover et al, (2011)). The dye is one such natural extracted material by Plants, insects, fungi, algae and minerals. They are the big foundations of natural colorants. Plant parts such as bark, leaves, flowers, stems, roots, shoots, fruit, rind, hulls and husks rolled as natural dye resources. These bio extracted dyes from natural original source are printing worldwide renown as "dyes". They are not only excellent source of anti-oxidant, anti-bacterial, anti-deodorant and antifungal features but also plays a vital role to fill spectrum with colours.(Zia et al., 2019). Around the world, these natural colorants material also plays a essential space in textiles as these naturally extracted dyes have no discarding problems and there is freely from distinct care of their isolation and utilization (Haddar et al., 2018). Due to many benefits currently, these natural dyes have prove their worthy place in textiles, food, flavour, cosmetics and the restoration of cultural heritage (Wang et al., 2018). Natural dyes are more eco-friendly, hygienic, user friendly, and long-lasting than artificial colorants. (Sankari, M., et al,(2024)).

Object: The aim of this review study is to explore the detail explore about the extraction, classification and applications of naturally extracted dyes.

Methods and Methodology:

The resourceful data used for this review article includes of research and review articles published by reputable publishers such as Springer, Elsevier, Wiley, etc. Literature of this review was conducted by using Google Scholar and PubMed with the following keywords: dye, *natural dye*, extraction of natural dye from plant, pharmaceutical properties of *natural dye*, biochemical components of dyes, etc. Several research and review papers published in the reliable have contributed to the fundamental attention of this review paper. For instance, prior researches have detailed the description, traditional usage, extraction, phytochemical composition and pharmacological activities including antioxidant, antimicrobial etc were applied of crude dye extract of various parts of this plant and their parts were explore the information about naturally extracted dye.

Sources of Natural Dyes:

Natural dyes comprise those colorants i. e, dye and pigments that are resourced from animal and vegetable substance without chemical processing. According to (Verma, .NET al., (2017)) natural extracted dye can be extracted from different resources from plants , other microorganism and minerals. Since prehistoric times natural dyes may have a wide range of shades of colours and can be obtained from various part of plants tissues including roots ,bark, leaves to flowers and fruit, other microorganism and minerals.

These natural dyes can be classified as below according to the extraction resources:

- Natural dyes extracted from plants parts -Berry, flower, bark, leaf, seed etc.
- Natural dyes extracted from insects
- Natural dyes extracted from animal
- Natural dyes extracted from mineral sources

- **Plant Dyes**

The different plant parts i.e, roots, stem ,leaf etc are used to extract colourant from it and dyes extract from different parts of plant like Henna gives orange-red colour-from leave of henna plants, Catechu, shaded brown colour from resin (sticky substance secrete from stem of *Acacia* tree, Fustic shade of yellow from the stem of the fustic tree, Indigo shade of blue colour intake from leaves and stems of the indigo plant, Logwood a type of black colour-from the core (heart) of the log wood tree, Turmeric ,a violet shade- from the roots of turmeric plant and Saffron gives orange-yellow shade from stigmas of flower part of plant . Dyes extracted from flowers plays an important role for dying of textile substantial's because it provide ranges of colours and fragrance also. Local grown species of plants are one best resource of natural dyes. They are easily accessible in the kingdom and can be considered as no expense dyes and naturally extracted dyes are having antimicrobial, antifungal, insect repellent, deodorant, disinfectant and other therapeutic standards. Almost 500 plant species identified as good sources for natural colourant. These natural dyes are derived and extracted from several plant parts are not only replaceable but also biodegradable.

- **Animal Dyes**

Red mouthed rock shell is sourced of tyrain purple colour. Phoenician purples and biblical blues are noble and holy of all prehistoric dyeing extracted from Levantine sea snails. These mollusc are responsible for excreate purple pigmentation.

- **Mineral Dyes**

Malachite and azurite was used for a green pigment and manganese oxide are responsible for black pigment derive from colourful clays on earth surface. Many other dyes like Chrom green a composite of chromium and oxygen, Chrom red- compound of chromium and lead, Chrom yellow-a compound of chromic acid and lead and prussion blue- a compound of iron and cyanide are used as vegetable dye are product of soil surface.

- **Insect dye**

Discharge of insects and dried insect bodies are the chief source of natural dyes. For example shell of -fish provides the colouring material. Lac and cochineal are good example of natural dye obtained from carmine insect bodies and they deliversparallel colours.

Classification of natural dyes:

- **Based on Chemical Class**:

Naturally extracted plant based dyes and pigments are found infollowing types as : Polymethines, Ketones, Imines, Quinines, Anthraquinonoids, Naphthoquinones, Flavones, Flavanols, Flavanones, Indigoids and Chlorophyll.

- **Based on Application Class**:

Application of dyes on their platforming agents are another criteria of classification of natural dyes. Categorisation of natural and organic dyes into two classification in his book named as "Treatise on Permanent Colours," which was published around 160 years ago. According to (Sankari, M.,et al (2024)) some application based classification are

Mordant Dyes: The word mordant comes from the Latin word "mordere" which means "to bite". A mordant is a biochemicalcompound which can fixed on the fibrils and also perform bonding betweenfibre and natural colourants. It rolls in absorption and fixation of natural dyes and also avoids bleeding and fading of colours and improves the stronghold properties of the dyed fabrics. Mordant colours are dyes that have an affinity for mordanted fibres. The natural dyes having limited substantively for the fibrescompulsory use of the mordant which enhances the fixation of the natural dye on the fibre. Some of the important mordants used are alum, potassium dichromate, ferrous sulphate, copper sulphate, zinc sulphate, tannin, and tannic acid . Fustic, Kermes, Cochineal, are great examples.Created on the final colour fashioned with the natural dyes, these metallic mordants are further divided in to two types:

Brightening Mordents: Alum, Potassium dichromate and Tin (Stannous chloride) falls under the category of brightening mordants

Dulling Mordents: Copper sulphate and Ferrous sulphates mordants are considered as dulling mordants.

Vat Dyes: The term 'Vat Dyes' derived from the wooden fermentation tank known as a 'Vat,' which was once used to convert dye to its soluble form. Vatting is the procedurein which solubilizing of dye takes place and a stage leuco is the soluble form of dye. The soluble leuco form has a strong sympathy for natural fibresand when uncovered to airoxidation take place on the fibre. Vatting is also proficient by the use of a reducing agent like sodium hydrosulphite and an alkali compound such as sodium hydroxide. Eg: Indigo.

Direct Dyes: Turmeric a fleeting dye, has a strong effinity to cotton fibres to bind. Other natural fibres directly absorbed by this dye. Example as Turmeric, Annatto, Harda, pomegranate (Punicagranatum L.), Carthamin derived from Sunflower.

Acid Dyes: Acid dyes is a direct dye used on polyamide fibres such as wool and silk. These dyes are used in acidic environments and have either sulphuric or carboxylic groups in the molecule ex saffron.

Basic Dyes: These are cationic type of dyes 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: Barbering.

Dispersal Dyes: A dispersion dye has a low relative molecular masssolubleand significant solubilizing groups. These dyes contain hydroxyl and amino groups which give solubility to the dye. Lawsone is example of this type of dye. Many flavones and anthraquinone dyes have the potential to classify as Disperse dyes.

Based on Colour:
The dyes in the colour index are categorised based on their chemical composition. The dyes are organised by colour within each application. Natural dyes have their own area of applications. However, only some sources provide dyes that can be isolated and commercially viableand colourants that are derived from animals. The method has been developed for synthetic dyes. However, in the case of natural dyes, the dying methods vary for each dye. 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 colourswith poor fastness properties. When choosing a colour palette, it is best to start with at least one blue, one redand 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:

- **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 Indigo era species, removing the liquid and oxidising it to precipitate the colour. Indigo is also found in wood (IsatisTinctoria L.). Indigotin and Indirubin are the two major components of natural indigo. Natural indigohas a greater affinity, and coloured materials are more resistant to fading.

- **Red Dyes**: The colour index includes 32 natural red dyes. Madder (Rubiatinctorum L.), Manjeet (Rubiacordifolia L.), Brazilwood/Sappan wood (Caesalpinasappan

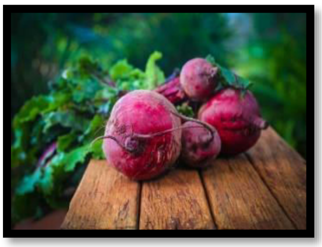
L.),Morinda (Morindacitrifolia L.),Cochineal (Coccus cactil L.)and Lac dye are the most renowned (Coccus laccase). All of these dyes are anthraquinone-based. because the colours are proneto oxidation. Turkey Red, a strong and nearly fadeless cotton dyewas discovered in Indiaand spread to Turkey. It requires around twenty different actions 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 alizarinin 1869, the usage of madder fell precipitously [Gulrajani et al., 2001]

- **Yellow Dyes**: Yellow is the most commonly used colour in natural dyes. The majority of the yellowcolourants, on the other hand, are fugitive. yellow dyes derived from Barberry (Berberisaristata), Tesuflowers(Butea frondosa, Monosperma), and Kamala are among the most significant (Mallotusphilippensis). Yellowdyes can also be found in black oak (Quercus velutina), turmeric (Curcuma longa), weld (Reseda luteola), andHimalayan rhubarb (Rheum emodi) [Gulrajani et al., 1992].

Annatto plant

Marigold

Blue peacock pea



Beetroot

Onion peels

Madder

Turmeric

Pomegranate

Rose Lawsonia L.

Inermis

Crocus sativus

Hibiscus

Extraction methods of plant natural dye:

Natural Dyes can't be applied directly from their renewable sources. Via raw resources for colouring has many boundaries. Harmless and inexpensive extraction of main dyeing plant component is most significant without upsetting the extraction conditions and stooping any contamination in various extraction techniques. Several extraction processesfor natural dye that completely comfortable with both consumer likelihood and supervisory control and are cheap in priceare becoming more widespread. Some extraction technique of natural dyes is as follows:

- **Simple Aqueous Methods**
- **Alcoholic or solvent extraction method**
- **Complicated solvent systems**
- **Supercritical fluid extraction**
- **Microwave or Ultrasonic extraction**
- **Enzymatic extraction**
- **Fermentation extraction**

- **Simple aqueous methods**

This is the one of the simplest and traditional process applied for the extraction natural colouring bio components universally. Here in this technique, the raw materials are first dried and finely cut, m grinded in the powder form and then the colouring components were extracted in water at boil. After the boiling for certain time, the content is cooled to room temperature and filtered. The filtrate is used as a dye for dyeing. The aqueous extractions of colorants liquid are carried out under various conditions such as temperature, time of extraction, pH, raw material concentration. Thus, the optimum extraction condition is determined for a particular dye by studying the maximum absorbance or optical density value at a particular (maximum) absorbance wavelength for the aqueous extracted solution using UV-Visible absorbance spectrophotometer.(MA Khan et al,(2006))(Maulik S R e al,2005)(A Konar e al,(2011))

- **Alcoholic or solvent extraction method**

None quos organic methods are used for extraction of organic dyes/antimicrobials from natural resources. In this method, a mixture of organic solvents, usually alcohols with hexane, is used for extraction of natural dyes. As an example, the roots of the authentic sample of Ratanjot were air-dried and ground to coarse powder. The powdered roots were extracted with n-hexane in soxhlet apparatus (Figure 2) till the color of the decoction became very light. Solvent was removed and the yield percentage of the hexane extraction from the roots was calculated .(A. Arora, et al (2009))

- **Complicated solvent systems**

The dried material (leaves, roots, barks, wood, resinous secretion of insects etc) are ground to very fine particles. The crude dried powder is weighed and solvent extracted using Soxhlet Apparatus, Steam Heated Extractor. Different solvents (such as Acetone, chloroform, ether, n-hexane, alcohol, soda ash, etc.) are used for Extraction. The process is carried out for 4 hours. The dye extract is evaporated in an evaporating dish over a water bath. After evaporating to dryness, the solute is weighed and the percentage yield is calculated.

- **Supercritical fluid extraction**

Now a day, safety of both producers and consumers is major necessity of any product or procedure. Accordingly, compelling regulations on the usage of hazardous, carcinogenicor toxic solvents as well as high energy costs for solvent regeneration have curtailed the growth of the natural extract industries. One of such major technologies that have emerged over the last two decades as the alternative to the traditional solvent extraction of natural products is the Supercritical Fluid Extraction Techniques. It uses a clean, safe, inexpensive, non-flammable, non-toxic, environment-friendly, non-polluting solvent, such as CO_2. Supercritical Fluid Extraction Technology is increasingly gaining importance over the conventional techniques for extraction of natural products. Supercritical fluid extraction is an advanced separation technique based on the enhanced solvating power of gases above their critical point. CO_2 is an ideal solvent in the food, dye, pharmaceutical and cosmetic industries, where it is essential to obtain final products of a high degree of purity. Main pigments of ripe tomatoes are the carotenes, compounds of colours ranging between yellow and red, i.e. α, β and γ-carotene, lycopene and Xanthophylls at very low concentrations. Experiments show the extraction and purification of natural colourant from eucalyptus bark using SCF process. Attempts has been made to standardize colourant derived from arjun bark, babool bark and pomegranate rind. Natural dye is obtained from the grape skin waste by using soxhlet extractor and later on distilled it under vacuum to obtain the concentrated dye solution.(PS Vankar et al,(2001)), (SK Bhattacharya et al,(2002))(R Patel et al,(2001))

- **Microwave or Ultrasonic extraction**

The combined effect associated with Ultra-Sound Energy is of cavitations, compressions, rarefactions, and microstreaming results in intermolecular tearing and surface scrubbing. In particular, it has been noted that, some reactions when exposed to ultrasonic energy become faster with lower temperature that is the most beneficial effect as it reduces processing time and energy consumption and improves product quality in the colouration of textiles.(Anon., (1988))(B.Ghorpade et al,(2000))(T Vandana et al,(2001))

- **Enzymatic extraction**

Enzyme assisted extraction nowadays; this state of art extraction method is being widely used due to its high efficiency comparing the conventional methods. As a recipe for enzymatic extraction technique, a 2% solution of pectinase:cellulase (2:1) was sprayed on pomegranate rind (25 g) for achieving better soaking and contact and then, was left overnight. The enzyme treated material was washed with little amount of distilled water and pH of the solution was adjusted to 10. The sample was shaken at 150 rpm for 20-80 min at desired temperature. The contents of beakers were filtered through standard test sieve to remove solid materials and the concentrated dye extract was vacuum evaporated in a rotary vacuum evaporator to about half of the original volume and finally, the concentrated liquid was spray-dried (H. C. Tiwari, et al,(2010).)

- **Fermentation extraction:**

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].

Medicinal Importance of Natural Dyes:

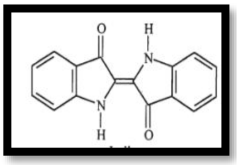
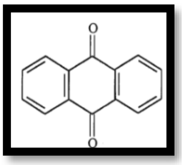
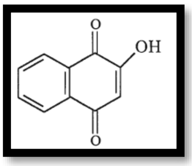
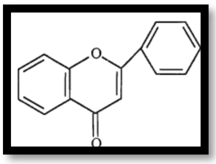
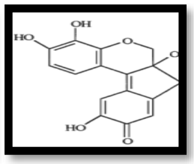
Table: 1Important natural dye yielding plants and their medicinal properties.

Plant	Part	Colour obtained	Pigment	Medicinal properties
Acacia catechu (L.f) Willd	bark	Brown/ black	Catechin, catechutanic acid	Used medicinally for sore throat and cough.
Acanthophonaxtrifoliatum L.	fruit	Black	Acantrifoside, nevadensin	Used in paralysis; roots cooked and eaten
AdhatodavasicaNees.	leaves	yellow	Adhatodic acid, carotein, quercetin	Used in bronchial infection

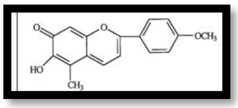
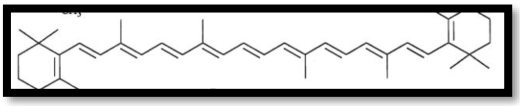
Aloe barbadensis L.	Whole plant	red	Barbaloin, aloe emodine	Fresh juice of leaves is cathartic and refrigerant used in liver and spleen ailments and for eye infections, useful in X-ray burns and other skin disorders.
Azadirachtaindica A.	Bark	brown	Nimbin, nimbinin, and nimbidin	Skin disorders, leaves considered as Antiseptic.
Bixaorellena L	seeds	Orange/red	Bixin, norbixin	Antimicrobial, diuretic, digestive stimulant, hepatoprotective, Antipyretic and antiperiodic
Butea monosperma Lam.	Flowers	Yellow/orange	Butrin	Astringent, antidiarrheal,Antidysenteric, febrifuge,aphrodisiac,purgative and anthelmintic
Capsicum annuum L	fruit	red	Capsanthin, capsorubin	Digestive, carminative, stimulant, cardiotonic, anti pyretic and expectorant.
Carthamustinctorious L	flower	Yellow/red	Carthamin	Oil applied to sores and rheumatic swelling; also used in case of jaundice
Cassia auriculata L.	flower	yellow	Di(2-ethyl) hexyl	Leaves and fruit anthelmintic. Seeds used ineye infection. Roots employed in skin disorders.
Crocus sativus L.	flower	Yellow/orange	Crocin, picrocrocin	Used as sedative and emmenagogue.
Curcuma longa L.	Rhizomes	yellow	Curcumin	Anti-oxidant, anti-inflammatory, antiplatelet, anti-cancer, anti -viral, anti -fungal, anti -bacterial effects.
Elaeodendronglaucum Pers.	bark	red	Elaeodendrol, elaeodendradiol.	To cure stomach pain.
Eugenia jambolana Lam.	Bark, leaf	red	Elgicacid, jamboline.	Decoction of bark and seeds used in diabetes
Galiumaparine L.	root	purple	Asperuloside, acumin	Infusion of herb used as an aperient diuretic,

				refrigerant and antiscorbatic
Garcinia mangostana L.	fruit	black	Mangostin, gartanin.	Used in diarrhoea and dysentery.
Indigoferatinctoria L	Leaf	blue	Indirubin, Indican	Extract used in epilepsy and other nervous disorders; in the form of ointment used for sores, old ulcers and piles. Root used in urinary complaints and hepatitis
Lawsoniainermis L.	leaf	orange	Lawsone	Antidiarrheal, antidysenteric, astringent, emmenagogue liver tonic and antifungal.
Nyctanthesarbortristis L.	flower	yellow	Rengyolone	Used in rheumatism, fever and anti bacterial.
Punicagranatum L.	fruit	yellow	Punicalgin, isopelleterine	Antibacterial, Antiviral, Astringent, Cardiac, Demulcent, Emmenagogue, Refrigerant; Stomachic and Vermifuge
Pterocarpussantalinus L	wood	red	Santalin	Hepatoprotective.
Rubiacordifolia L	root	red	Purpurin, Rubiacordone	Antitussive, Astringent, Diuretic, Emmenagogue, Expectorant and Styptic
Solanum lycopersicum L.	fruit	red	Lycopene	Anti bacterial, anti fungal, anti-mutagenic. Used in prostate cancer, arteriosclerosis and diabetes
Tageteserecta L	flower	yellow	Lutein, carotene	Emmenagogue,dispersesco ntusions. Anthelminthic,aromatic,digestive, diuretic, sedative, stomachic.

Some extracted natural dye with chemical structures:

Indigo Anthraquinonea –hydroxynap hthoquinone Flavons Dihydropyran

Anthrocynanidin Beta-carotene

Advantages:

The results of reviewed works revealed that the growing global interest toward the application of dyes/antimicrobials from natural resources is mainly due to the following advantages:

• Natural dyes/antimicrobials are obtained from low-cost renewable resources with considerable potential.

• Natural dyes produce a variety of uncommon, eye-catching, and soothing shades on textiles.

• Wide range of shades can be produced with an individual natural dye either in mixture with mordants or by variation in dyeing condition.

• Different natural constituents such asauxiliary, mordant, fixing component, etc. along with the natural dye may enhance dyeing/finishing process efficiency.

• Some natural dyes have intrinsic additional properties such as antibacterial, moth proof, anti-allergy, anti-ultra violet (UV) irradiation, etc. Natural dyes/antimicrobials are environmentally friendlier than synthetic ones because: I) in natural materials, all synthesis processes are accomplished by nature with no pollution of environment and II) these materials are readily biodegradable and do not produce hazardous effluent upon degradation in environment, and so, there is no need for further treatment of effluent before discharging into the environment.

• Colour characteristics of some natural dyes mellow with time. This characteristic imparts unique properties and appearance to the stuff dyed. An old carpet piles dyed with natural dyes could be an example.

• Majority of natural dyes/antimicrobials are extracted from wild and self-growing plants that require no additional cost for the cultivation.

Limitations:

Natural dyes/antimicrobials have some drawbacks that limit their application on textiles. Some important ones can be summarized as follows:

• Generally, natural dyes/antimicrobials have low wash, rub, light, sweat, and gas fastness on textiles probably due to their weak bonding and interaction to the textiles.

• Color matching and reproduction of color is another disadvantage of natural dyes since the quantity and quality of natural dyes steadily change with climate, plant genus, region, etc.
• The efficiency of the natural dyes/antimicrobials extraction process is generally low, i.e., just few grams of natural component per kilogram of raw materials.

Characterisation techniques:
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.

TLC (thin layer chromatography)
This method was initially proposed by Izmailov and Schreiber 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]. $Rf = 1/(1 + K')$ 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 G.H.,et al,(1964))

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. 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.(GRIFFIN, R. M. E. & SPEERS, S. J. (1999))

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; Nadiger et al., 2004].

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 agaseous 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]

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]

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].

Uv-vis spectroscopy

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]

Pharmacological activity of natural dyes:

Antimicrobial

Information about organic dyes is comes from the literature of traditional dyeing methods. The reported plants were framed for dyeing traditionally in Anatolia and some of plants were also identified for their therapeutic properties. Many of them results antimicrobial effects against tested microorganisms. The experiments were carried out for dye extraction from many plant species using water as solvent.Extracted natural dyes were screened for their antimicrobial Activity. Antimicrobial activity of a dye will vary while it is in solution or it is detained by wool yarn.Results given above showed antimicrobial effect of dyes in a growth media against selected microorganisms and for the evaluation of their efficacy, it is necessary to study the antimicrobial activity of dyed yarn wool. (Comlekcioglu, N.,et al (2017)).

Antiviral

Viral infections are responsible for several chronic and acute diseases in both humans and animals. Despite the incredible progress in human medicine, several viral diseases, such as acquired immunodeficiency syndrome, respiratory syndromes, and hepatitis, are still associated with high morbidity and mortality rates in humans. Natural products from plants or other organisms are a rich source of structurally novel chemical compounds including antivirals. Indeed, in traditional medicine, many pathological conditions have been treated using plant-derived medicines. Thus, the identification of novel alternative antiviral agents is of critical importance. In this review, we summarize novel phytochemicals with antiviral activity against human viruses and their potential application in treating or preventing viral disease. (Musarra-Pizzo, M., et al,(2021)).

Anticancer

Recent advancements in cancer research have significantly advanced our understanding of the intricate molecular mechanisms driving cancer progression, leading to the identification of new drug targets and therapeutic strategies. The active colouring and biologically active principle of Henna is found to be Lawsone (2-hydroxy-1, 4-naphthoquinone) which can serve as a starting building block for synthesizing large number of therapeutically useful compounds including Atovaquone, Lapachol and Dichloroallyllaw sone which have been shown to possess potent anticancer activities.(Pradhan, R.,et al, (2012)).

Antioxidants

Antioxidant potential of hydroalcholic bark extract of Acacia nilotica was evaluated in terms of reducing power and inhibition of peroxidation by 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity and subsequently dyed woolen yarn in terms of inhibition of peroxidation by DPPH assay and compared with that of the ascorbic acid used as positive control. Mordanting reduced the antioxidant activity of dyed woollen yarn as compared to unmordanted samples. Wash durability results proved successful antioxidant finishing of woollen yarn dyed with Acacia nilotica bark extract. Reduction in antioxidant activity on successive washing cycles follows 1st order rate equation.(Rather, L. J., et al,(2017)).

Anti-inflammatory

Chuku et al.29 conducted a study on the anti-inflammatory activity of L. inermis. In this study, twenty white cockerels were used. They used carrageenan in order to stimulate inflammation and oedema in the 7-day-old chicks. Three different concentrations of N-butanol leaf extract suspension and ethylacetate extract suspension were administered to the chicks (50, 100 and 200 mg/kg). The results of this study reported that both extracts showed a significant decrease in the level of oedema. Both extracts showed the highest anti-inflammatory activity at 50 mg/kg.(Chuku, L. C., et al, (2020))

Analgesic activity

It seems to possess high therapeutic potentialfor the treatment of various diseases making it a target for pharmacological studies aiming to validate and provide scientific evidence for the traditional claims of its efficacy.3-6 As part of our ongoing research of traditional medicinal plant, this study evaluated the analgesic and neuropharmacological activity of methanol extract of orellana (MEBO) leaves. experimental findings characterise that numerous phytoconstituents existing in MEBO demonstrate analgesic and anxiolytic activity. These results support that the MEBO has analgesic and anxiolytic properties like diazepam which act through binding to benzodiazepines site on GABA-BDZ receptor complex. Supplementary advance studies are required to identify with observed bioactivities in animal behavioural models and isolate the active phytoconstituents associated with observed bioactivities in animal behavioural models.(Aktary, N., et al,(2020))

Hepato protective activity

Plant extracts withhepatoprotective properties against toxic chemicals that cause liver injury, seeming to validate their use in folk medicine. These plants may offer new alternatives to the limited therapeutic options that exist at present in the treatment of liver diseases or their symptoms, and they should be considered for future studies. The study also identified glycosides, flavonoids, triterpenes and phenolic compounds as classes of compounds with hepatoprotective activity. The potent hepatoprotective activities of the chemically defined molecules isolated from natural origins represent an exciting advance in the search for effective liver protective agents, especially now, when there is an urgent need for new innovative drug leads. Further studies including clinical trials need to be carried out to ascertain the safety of these compounds as a good alternative to conventional drugs in the treatment of liver disease. skeletal flavonoids, anastatins A and B, were isolated from the methanol extract of Anastaticahierochuntica L. Anastatins A and B were found to show hepato protective effects on Dgalactosamine-induced cytotoxicity in primary cultured mouse hepatocytes and their activities were stronger than those of related flavonoids and commercial silybin - a known hepatoprotective compound.(Adewusi, E. A., &Afolayan, A. J. (2010))

Nootropic activity

Cognitive disorders are responsible for memory impairments, deterioration of language, motor, sensory abnormalities, gait disturbance, and seizures. Nootropic agents are being primarily used to improve memory, mood and behaviour. Swarnabhasma (gold ash), the principal constituentwhich has very low particle size of average 2.75 μm shows better memory enhancement results, asthe particle size of gold decreases absorption will increases. The Swarnabhasma has been scientifically proved for free radical scavenging activity, rheumatoid arthritis, inflammatory diseases, asthma, and immunological disorders. The ghee and honey which were used as vehicles. When Swarnaprashana administered continuously for15 days improved learning and memory of youngmice and aged mice when subject to studies usingEPM and MWM. Swarnaprashana decreases the TLin EPM. It also decreases ELT which shows betteracquisition or learning and increase in TSTQ in MWMwhich supports the improvement of memory. The formulation significantly reduces whole brain Ache activity of both young and aged mice as compared to respective control group. All these observations support the findings that the formulation Swarnaprashana was able to offer significant protection all both the models EPM, MWM, and whole brain AChE activity studied.(Warad, V. B., et al,(2014))

Ameliorative activity

The ameliorative effect can be defined as improving a better condition from a certain problem. A crude methanol extract that contains alkaloids, triterpenes and steroids, carbohydrates, tannins, cardiac glycosides, saponin glycosides and flavonoids showed a positive ameliorative effect against trypanosomiasis on different concentration ,result of this study showed that in trypanosome-infected rats, The leaves of L. interims were able to improve erythrocyte membrane integrity and maintain high erythrocyte count. Consequently, it will help in reducing the chances to get anaemia and thus intensify the productivity of trypanosome-ridden animals. (Tauheed AM, (2016))

Environmental Applications:

The characterization of eco-friendly dyes has numerous applications across various industries and fields. Some applications are as follows (Verma, S., & Gupta, G. (2017))

Textile Industry: One of the most important applications of eco-friendly dye is in the textile industry. Eco-friendly dyes can be used to colour natural and synthetic fibres, fabrics, and wardrobes. By characterizing these dyes, manufacturers can ensure the parameters of quality and performance standards, permitting the production of environmentally friendly textiles.

Paper and Printing: The paper industry can profit from eco-friendly dye classification for producing globally friendly and maintainable papers. These dyes can be used for shading in paper manufacturing and printing applications, reducing the environmental impact on printed materials.

Cosmetics and Personal Care: Eco-friendly dyes are also result applications in cosmetics and personal care products like natural hair dyes, organic lipsticksand eco-friendly nail paints. Characterization ensures their safety and compliance with regulated products.

Food and Beverages: Natural and eco-friendly dyes can be used to pigments of food and beverages. By characterizing these dyes, their safety and stability can best and raised, enhancing the manufacture of natural and organic food products.

Medical and Healthcare: Eco-friendly dyes can be castoff in medical applications,e.g, medical ,textiles and bandages where the absence of harmful substances is crucial. Characterization helps standardised their biocompatibility and safety for further use.

Education and Research: Naturally yielded dye characterization plays a noteworthy role in academic and scientific research. Researchers can experiment about the properties, behavior, and interactions of these dyes to develop new and improved nature-friendly dyeing processes.

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

Sensitization: Dye is used as a sensitizer in dye sensitized solar cells which convert visible light into electricity using sensitization of the cell. Performances of these cells depend on dye. Sensitization is an important application of natural dye. Natural dyes are cutting down high cost of metal complex sensitizers and also replacing expensive chemical synthesis process through simple extraction process. In photo electrochemical water splitting titanium dioxide-based semiconductors are used. For promote the efficiency of photo electrochemical reaction natural dye sensitizers are used due to their environmental friendliness and low cost. Fast electron injection and slow backward reactions are exhibited by dye sensitizers. A protective layer like conductive polymer layer is required for natural dye sensitizer because it is unstable in solution .Natural dye is used as photosensitizer in dye sensitized solar cells along with TiO_2 nano particles extracted from Hemigraphiscolorata (Red flame) with minimal chemical procedure and is used without further purification. anthocyanin and beta-carotene is present in this dye and it is proved by UV-Visible absorption spectroscopy and micro-Raman spectroscopic studies.

Conclusion and future perspective:

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 colour fastness eco-friendly dyes has shown that they can achieve comparable or even improved colour fastness properties compared to conventional dyes. This ensures longevity and colour 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.

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