*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25 No. 02 (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

# Phytochemical Isolation, Characterization, And Biological Evaluation Of Dye-Yielding Plants

**Vipul Kumar 1, , Dr Jigar Y. Soni 2, , Dr Gitam singh 3**

1 Faculty of Basic and Applied Sciences, Department of Chemistry, Madhav University, Abu Road, Sirohi, Rajasthan, India.
2 Faculty of Basic and Applied Sciences, Department of Chemistry, Madhav University, Abu Road, Sirohi, Rajasthan, India.
3 Faculty of Agriculture and Science, Department of Chemistry, Madhav University, Abu Road, Sirohi, Rajasthan, India.
Corresponding author: E-mail address: valiyadav1988@gmail.com

**ABSTRACT**
Natural dyes derived from plants have long been used in traditional textile dyeing, food coloring, and medicinal applications. Due to the growing environmental and health concerns associated with synthetic dyes, there is an increasing global interest in the revival and scientific exploration of plant-based dyes. This review provides a detailed overview of the phytochemical isolation, structural characterization, and biological assessment of compounds obtained from various dye-producing plants. It summarizes recent advancements in extraction techniques, chromatographic isolation methods, and spectroscopic tools used to identify bioactive compounds. Additionally, the review highlights the biological potentials of these natural dyes, including their antioxidant, antimicrobial, anticancer, and anti-inflammatory activities. The findings support the significance of dye-yielding plants as valuable sources of eco-friendly colorants with potential therapeutic benefits.

**Keywords:** Natural dyes, dye-yielding plants, phytochemicals, bioactive compounds, spectroscopic characterization, biological activities, sustainable dyes, antimicrobial potential, antioxidant properties.

## INTRODUCTION

The resurgence of interest in natural dyes is driven by increasing awareness of the toxicity and environmental hazards of synthetic dyes. Plant-derived dyes are considered safer, biodegradable, and often possess medicinal properties. Historically, natural dyes were widely used until the discovery of synthetic alternatives, which eventually dominated the global market. However, concerns about the carcinogenicity, allergenicity, and non-biodegradability of synthetic dyes have led researchers back to nature for safer and more sustainable options.

Dye-producing plants not only provide vibrant colors but are also rich in phytochemicals such as flavonoids, tannins, anthraquinones, and carotenoids, which can exhibit significant biological activities. This review explores the processes involved in isolating and characterizing these compounds and emphasizes their biological relevance.

## NATURAL DYE

In their most basic form, dyes are organic chemical compounds that may change their colour when exposed to light. These compounds' vibrant colours are retained in things by physical absorption, mechanical retention, and the formation of covalent chemical bonds or complexes with metals or salts. Farnfield (1988) states that dyes are colourants with substantivity for a substrate, which may be achieved either naturally or by reactants. Cloth, paper, wood, varnishes, leather, ink, fur, food, medicine, cosmetics, and the list goes on and on and on. Dyeable materials include almost everything imaginable. Natural dyes and synthetic dyes, both created via chemical processes, are the two most common types of dyes. Gupta (1990) states that a natural dye is one that is produced from materials that may be found in nature, such as plants, insects, or minerals. According to the Society of Dyers and Colourists, UK, natural dyes are colourants that are obtained from plants, animals, or minerals and go through little chemical processing. The beauty and intrigue of human existence have been enhanced by natural phenomena such as colour. Feelings, human traits, the change of the seasons, festivities, and the zeal with which humans live their lives are all intended associations. The ancient humans must have been able to perceive a myriad of natural sounds. The use of natural hues for colouring has increased throughout human history due to the improvement of dyeing procedures. In India, natural dyes have been used for cosmetics and dyeing for a long time (Shrivastava, 1989). Natural dyes were widely used by many ancient civilisations, such as the Aztecs, Egyptians, Greeks, and Indians. Natural colours like annatto, saffron, and turmeric (Curcuma longa) were still widely used to colour dishes.

## HISTORICAL TRADITION

Some dyes and the practice of dying as an art form have their roots in primordial times. For many years, the artistic practice of textile dying has had widespread acclaim and financial backing. Natural resources were used in the creation of cave paintings, ornaments made of shell and feathers, and painted skins. Prehistoric people used ochre-based paints in

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Vol 25, No. 02 (2024)
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Article Received: Revised: Accepted:

cave paintings; these pigments came in a variety of hues, including black, white, yellow, and reddish. Their dates are known. Many ancient cultures relied on natural colours, such as the Aztecs, Indians, Egyptians, and Greeks. Dyeing methods evolved with the development of civilisations. Although they could have been discovered by accident, it is difficult to imagine modern civilisation without these hues. So deeply ingrained in human traditions is their usage. According to Krishnamurthy et al. (2002), dyeing was a frequent technique in cultures that developed further. In the past, people would apply adhesives made of plants or rub crushed pigments over cloth to provide colour. The fabric was dyed using natural dyes made from crushed fruits, berries, and other plants; this gave it lightfastness and waterfastness (resistance) as the procedures continued. India was an early adopter of natural dyeing techniques during the era of the great epics. The nation is proud of its long history of traditional arts and crafts and its status as a pioneer in the production of natural dyes. It is still a leading producer of these colours in the modern day. Famous ancient dyes used in India from around 2500 years ago include madder, a red dye made from the roots of the Rubia tinctorum L. plant, turmeric, saffron stigmas, Indigofera tinctoria L. leaves, saffron, and blue indigo. Henna, tyrian purple, cutch, and logwood were among the many natural ingredients used to colour fabrics (Shenai and Saraf, 1991). Confirmation of this was provided by the unearthing of red-dyed cloth during the Harrappan civilisation excavations at Mohenjodaro. The vibrant ceramics found during this period clearly demonstrates the use of mineral dyes. The colourful clothing of the time is described in epics like the Mahabharata and the Ramayana. A striking item is the yellow "Pitambar," a robe that is worn by both sexes and used to adorn representations of gods. There is a description of natural dyes in Atharveda. The Bhrugu Samhita was written using natural hues. Dated to the first century A.D., the frescoes of Ajantha were made using natural colours. Ancient humans had colourful garments, as seen in later murals. The exquisite dying techniques used by Ancient Indian weavers have been acknowledged since the seventeenth century, when their vibrant patterns and colours first appeared on clothing. Methods for resist-dyeing were probably first developed in this country. Mordants, first utilised by the Indians, transformed dyes into an integral part of fabric rather than only a decorative accent (Belfer, 1972).

**EMERGENCE OF SYNTHETIC DYES**

Indigo and coal tar were among the many sources of a basic compound that Hofmann proved to be its identity in the early 1840s. Anil is the Arabic term for indigo, and it was subsequently renamed aniline. He analysed significant natural substances, such as dyestuffs, and worked on reactions that produced various amino compounds. Originally called Tyrian Purple after the legendary Levantine dye, the synthetic colour quickly gained popularity. Following the explosion in research, production, and use of synthetic dyes caused by the accidental 1856 discovery of a synthetic dye dubbed "Mauve" by German scientist W.H. Perkin, the use of natural colourants came to a sharp decline (Holme, 2006). The French artist Verguin used aniline and tin chloride to make a rose-colored dye called "Fuchsine" in 1858. This dye was the progenitor of the Triphenylmethane family. Prior to 1900, over hundred colours were documented, and subsequent studies uncovered many more variations. The rapid development and widespread use of several so-called aniline dyes greatly expanded the palette of vibrant textile hues. A blue dye was produced by a mixture that Girard and de Laire found while investigating the aniline red reaction. Alizarin, quinine, and a plethora of other possible new hues were thought to have originated from coal tar compounds about 1850. Researchers from Germany, France, and the United Kingdom considered converting naphthalene into alizarin, a dihydroxyanthraquinone (White, 1956). Caro developed azo dyes such as chrysoidine and others in the middle of the 1870s. The first phthalein product, eosin, was released in 1874. It was also known as brominated fluorescein. The production of phthalic anhydride caused a dramatic increase in the demand for naphthalene, a plentiful compound extracted from coal tar. Natural dye usage dropped dramatically around the turn of the twentieth century due to the proliferation of coal tar dyes and manufactured indigo blue substitutes (Singh, 2001).

**DECLINE OF NATURAL DYES**

Because synthetic dyes could be mass-produced at reduced costs and were more widely available in the early 1900s, natural dyes were mostly forgotten (Saravan and Chandramoham, 2011). There is much less need for natural colours now that synthetic dyes are so common and can be mass-produced. Natural dyes were less often utilised due to their relatively high cost, a lack of scientific input, and conservative attitudes. The traditional artisans were known for their unwavering commitment to their trade and their strict adherence to traditional methods. Fabric dying and dye manufacture were undocumented activities known exclusively to certain civilisations. Practical education was the most common means of passing this knowledge along from one generation to the next, rather than broadcasting it to the outer world. As interest waned in future generations due to poor returns on investment, folk knowledge of textile dying also diminished. Even while plants have long been studied for their therapeutic properties, very little effort has gone into researching them as a potential source of natural dyes or creating procedures for making and dying using natural dyes. As a result, there is now very little known about the hundreds of plant species that were once believed to have inherent colouration.

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**ADVERSE IMPACTS OF SYNTHETIC DYES**

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**REVIVAL OF NATURAL DYES**

Many sectors are seeing a renaissance in the use of non-toxic bioresource products due to the increased awareness of health and environmental problems. Therefore, production processes for everyday goods must be more eco-friendly. More and more, shoppers throughout the world are considering environmental impacts when they buy products, particularly clothing. Natural dyes have recently taken centre stage, as people are more concerned about the environmental impacts of synthetic dye production and consumption (Kumar et al., 1990; Glover, 1995). More and more people across the world are worried about the effects these chemicals might have on people's and the planet's health in the last few years. Developed nations have also seen a rise in interest in sustainable lifestyles and natural (Green) products. Natural dyes are regaining popularity in the textile business due to rising consumer awareness of synthetic colours' potential carcinogenic, mutagenic, and sensitising effects (Paul et al., 2003). The present worldwide trend towards eco-friendly and biodegradable items has put natural dyes in great demand (Bhuyan et al., 2004). In terms of the export performance of natural dyes, the rise of India's textile industry has been really spectacular (Cautisicos, 2006). The dye and textile industries have recently been at the forefront of developing more modern products that meet the needs of the fashion industry while also being environmentally conscious. Due to the synthetic dyes and pigments industry's pollution and toxicity issues, numerous countries have returned to making their own natural dyes and pigments. Agricultural byproducts and other sustainable alternatives are highly sought after. Natural dyes have a lot of untapped potential for printing and dying a variety of materials, and most commercial dyers and textile exporters are reassessing this potential in light of the rise of niche markets. Because of this, there has been a recent uptick in the usage of natural dyes on natural fibres and an increase in studies investigating this method for creating more environmentally friendly textile hues (Samanta and Agarwal, 2009). Many people are interested in using natural colours since they are biodegradable and are safe for the environment (Prusty et al., 2010).

**RECENT TRENDS**

Concerns over the impact of certain synthetic dyes on both the workers who apply them and the people who use the goods coloured with them have led to fresh study into the use of natural dyes on textiles. You may find natural dyes in a wide variety of products, including clothing, food, medicine, art, paint, and cosmetics. Many industrialised countries are pouring resources into the research and development of natural dyes since they are the most eco-friendly and healthiest alternative. Natural dyeing of different fabrics has mostly continued in the decentralised sector for specialist items because to the advantages of both synthetic and natural dyes (Shukla et al., 2000). In an effort to combat the environmental risks posed by synthetic dyes, a number of commercial dyers and small textile export enterprises have begun to consider switching to natural dyes for their routine textile printing and dyeing (Glover et al., 1993). There is a significant domestic and international demand for methods of producing natural dyes and pigments. Dye manufacturers have lately shifted their focus to produce more eco-friendly items in response to rising demand for goods that meet both aesthetic and ecological standards.

As the demand for textile dyeing continues to rise, there is a fine line to walk between the compatibility of different finishing materials and treatments and the application processes used to impart desirable attributes to fabrics. People all around the world are always looking for better fabrics that will keep them dry, clean, and odor-free while also making them feel fantastic. Now is the moment to consider fresh approaches to creating multipurpose sanitary textile products. Recent articles (Sarkar, 2004; Hwang et al., 2008; Lee et al., 2008) discuss the potential of natural colourants to provide textiles many uses, such as antibacterial, insect repellent, deodorants, and UV protection. Singh et al. (2005) and Katho et al. (2004) are two examples of studies that cover this subject. The cosmetics industry has seen a recent uptick in the use of natural dyes as a substitute for synthetic dyestuffs. In addition to having fewer negative effects, natural dyes may have extra beneficial properties, such as moisturising the skin, shielding it from UV radiation, and slowing down the ageing process (Samanta and Konar, 2011; Bhandari, 2011).

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**BRIEF CHEMISTRY AND PROPERTIES OF DYES**

**Principles of Color Chemistry**

Dye composition differs from that of most organic compounds in four key respects: absorption of visible light (380-750 nm), presence of a chromophore (a group that imparts colour), conjugation of carbon and hydrogen bonds, and the stabilising effect of electron resonance (E.N. Abrahat 1977). In the absence of any one of these characteristics in the molecular structure, colour is rendered ineffective. Most dyes include auxochromes, also known as colour aids, which are groups that are different from chromophores. Carboxylic acids, sulfonic acids, amino groups, and hydroxyl groups are all examples of auxochromes. While they don't really produce colour, they may cause a colorant's colour to change, and their primary use is to influence dye solubility. Table 1.1 shows the absorbed light wavelengths in relation to the primary and secondary colours. Measuring the amount of light that a substance absorbs allows one to determine its apparent colour. If a substance absorbs violet light (400-435 nm), for example, its complimentary hue, yellow, will look as by it.

Resonance, chromophoric groups, conjugated systems, and light absorption are some of the other components that contribute to colour (Figure 1.3). Figure 1.1b's structures show how crucial an extended conjugated system is for producing colours. A chromophore cannot impart colour to an organic compound when used in isolation; rather, it must be a component of the conjugated system. Figure 1.4 clearly shows that an azo group between methyl groups does not produce any colour, but the same azo group between aromatic rings produces a yellow-orange hue. Incorporating chromophores into conjugated systems allows for colour production, as shown in the figures.

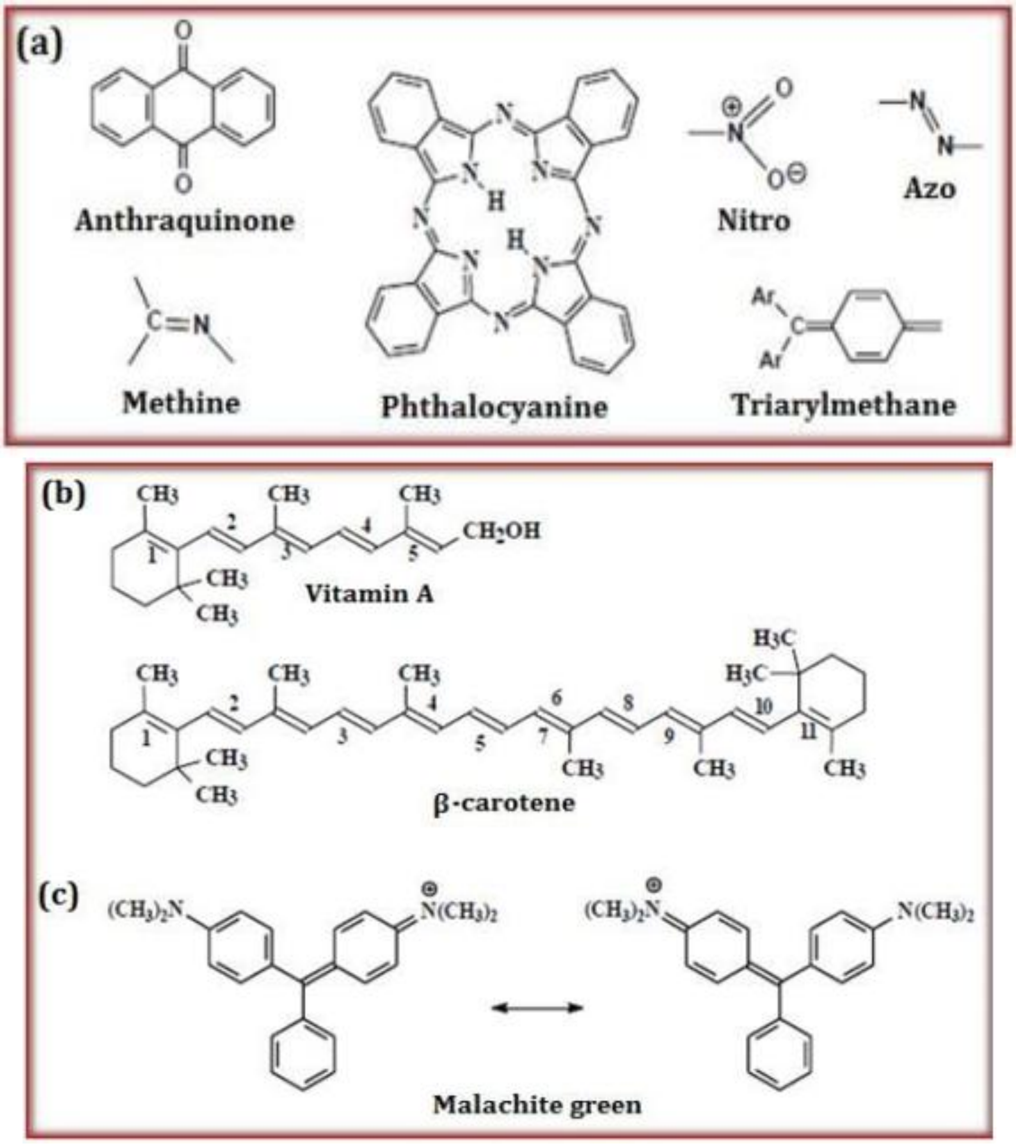


**Figure 1: Organic dyes contain chromophores, vitamin A and β-carotene have conjugated systems, and malachite green has resonance structures.**

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**Pigments versus Dyes**

The classification of organic colourants as dyes or pigments is based on their solubility (R.L.M. Allen 1971). Dyeing materials are soluble in water and organic solvents, while pigments are not. The main distinction between the two is this. Compatible substrates may be coloured using dyes. If the polymer and pigment are not mixed before the creation of the fibre or moulded product, pigments can only colour the surface of a polymeric substrate, unlike dyes.

**Designing Dyes: Some Considerations**

The vast majority of organic dyes, which are often referred to as azo dyes, include one or more azo groups. Direct and reactive colours are often used to dye cellulose substrates like paper, rayon, linen, and cotton, whereas dispersion colours are used to dye hydrophobic substrates like acetate and polyester. Protein and polyamide substrates, including wool, silk, and nylon, are the most typical targets for acid dyes.

In most cases, two steps are required to create azo dye. A diazo compound is formed when an aromatic amine undergoes diazotisation, changing its moiety from $Ar-NH_2$ to $Ar-N_2^+$. In diazo coupling, an aromatic amine, phenol, naphthol, or methylene active molecule is combined with the diazo compound to produce the azo dye ($Ar-N_2^+ + Ar'-OH \rightarrow Ar-N=N-Ar'-OH$). This procedure may be used to make azo dyes and pigments. See Figure 1.6 for a typical structure of a pigment or dye.

**CRITERION FOR CLASSIFICATION OF DYES**

The chemical makeup or application technique of colours is one way to categorise them. Experts in the field of dye chemistry use the former method and refer to their products by names such as azo dyes, anthraquinone dyes, and phthalocyanine dyes. People who work as dye technologists are responsible for both the creation and usage of dyes, such as reactive dyes for cotton and dispersion dyes for polyester. For instance, azo dispersion dye is often used for polyester and phthalocyanine reactive dye for cotton, as stated by Hunger (2003).

**EQUIPMENT AND MANUFACTURE**

Having several steps in a single process or reaction is typical. In Figure 1.5, we can see the main steps that go into making dyes and intermediates.

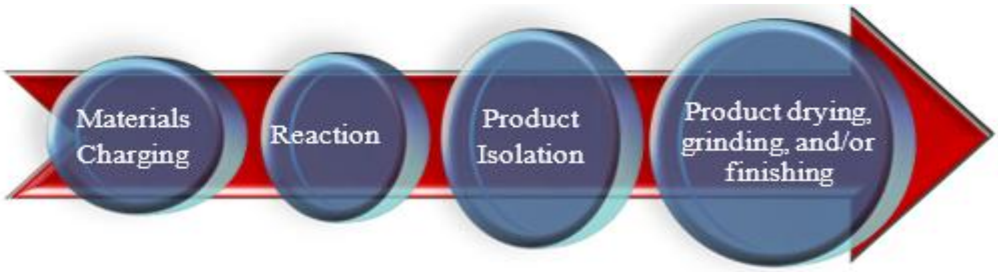


**Figure 2: Process flow for the production of dyes and intermediates**

The reactor, the facility's nerve hub, is where the unit operations that create the dyes and intermediates typically occur (Hunger, 2003). The workup phases are the ones that follow a response. While details may differ from product to product, in general, intermediates (which may be utilised without drying if possible) often have simpler finishing procedures than colourants. The reaction containers, which are shaped like bombs, are composed of steel. The linings might be lined with cast iron, stainless steel, brick, carbon blocks, rubber, or glass (enamel). The reactions that result in the pigments and intermediates happen in these receptacles. Mechanical agitators, condensers, pH sensors, thermometers, and recorders of temperatures are common accessories for these containers. Their capacity ranges from 2 to 40 cubic meters, or around 500 to 10,000 gallons. Oil and Dowtherm are high-temperature fluid examples; to cool a coil or jacket, you may use air, cold water, or chilled brine. Another option for heating is steam or hot water. When reacting with water-based solutions in containers without jackets, it is usual practice to heat the solution with steam and cool it with ice or heat exchangers. For the sake of operational simplicity, reaction vessels in a plant may span many levels.

Using mechanisms such as pumps, gravity, or the mechanical propulsion of inert gases like air, machines may transfer things from one location to another. Centrifuges, filter boxes, continuous belt filters, recessed-plate or plate-and-frame versions, and other filter presses are used to extract particles from liquids. Various fabrics, such as dyneel, cotton, and polypropylene, adorn these presses. Frames and plates are often made of materials such as wood, cast iron, polyethylene, hard rubber, or polyester.

**TOXICOLOGICAL AND ENVIRONMENTAL CONSIDERATIONS**

The dye-substrate affinity is crucial since synthetic dyes can't be commercialised unless they pose little danger to end-user health. That is why molecular design places an emphasis on environmental safety. Therefore, the ingredients used to create synthetic colours should not include substances that are known to be detrimental to health. The Salmonella test, the de facto standard for carcinogenicity and mutagenicity, has identified a large class of aromatic amines as being among these substances (Anon, 1996). Dye designers must, therefore, take into account the potential genotoxicity of metabolites generated in mammalian systems.

**CONCLUSION**

Dye-yielding plants represent a valuable resource not only for sustainable coloring agents but also for novel bioactive compounds with potential pharmacological applications. Advancements in phytochemical extraction, compound isolation, and biological evaluation continue to highlight the multifaceted benefits of natural dyes. Integrating traditional knowledge with modern scientific approaches can further broaden the commercial and therapeutic use of these plant resources.

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