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A REVIEW ON APPLICATION OF NATURAL PIGMENTS AS AN ALTERNATIVE TO TOXIC SYNTHETIC DYES IN STAINING BIOLOGICAL SAMPLES

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ABSTRACT

Staining is a technique used to add color to microbes, animal tissue, and plant tissues to enhance the optical contrast using stains or dyes. Most of the stains which are currently used in bacteriological, mycological and histopathological studies are chemically synthesized, known as synthetic dyes. Despite of knowing the deleterious effects of synthetic dyes on humans and the environment, we continue to use them due to their ready availability, efficiency and most importantly, they are economically priced with diverse range of colors. The indiscriminate use of synthetic dyes in laboratories and diagnostic centers has resulted in water, air, land pollution with hazardous effect on biotic life. In this review we have discussed on the various plant-based natural pigments which are a safe eco-friendly alternative to toxic synthetic dyes. It is indeed very important to replace toxic chemical-based stain with non-toxic biodegradable natural dyes which can be easily prepared and environment friendly.

Keywords: Synthetic dyes, natural pigments, plant dyes, hazardous

INTRODUCTION

The microscopic study of microbial cells and tissue in their natural state is not informative as the cells lack color of their own and without contrast which makes it impossible to detect cellular structures and distinguish the unique characteristics without artificially treating the specimens **[3]**.

Staining is a technique that creates a contrast in samples at microscopic levels and finds application in various fields of histology, cytology, microbiology and in plant studies. It plays a major role in the diagnosis of a disease. A dye is an organic compound made up of auxochrome and chromophore groups, which are linked to a benzene ring and is mainly used for coloring non-biological samples while a stain is an organic compound containing both auxochrome and chromophore group and is mainly used for coloring biological and microbiological samples **[33]**.

A auxochrome group imparts ionic properties to the stain or a dye, e.g. –OH, –COOH, SO3H, –NH2, –NH(CH3) etc. The auxochrome does not produce any color but deepens the color of a chromogen. A chromophore is an unsaturated group that absorbs and reflects light at a specific angle e.g., azo, keto, nitro, nitroso, thio, ethylene etc. It is responsible for imparting the color to the dye molecule. A molecule lacking a chromophore would be colorless **[22]**.

Dyes are broadly classified into natural and synthetic dyes based on their origin.

Natural dyes

Natural dyes are organic compounds are derived from naturally occurring sources such as plants insects, animals and minerals without any chemical treatment The preparation and production of natural dyes is environmentally friendly, and they are biodegradable making it easy and safe for disposal. Not all natural dyes are safe and nontoxic as few of them can trigger an inflammation reaction or allergic reaction when exposed to skin or when inhaled. Some natural dyes in combination with a mordant can be toxic. Mordants are used to increase the efficiency of the dye to bind to the sample for example – aluminum, copper and ores of iron **[21]**.

Examples of some natural dyes

1) **Plant dyes**

The different parts of plants like the leaves, steam, roots, bark, fruits, vegetables and flowers, are sources of coloring pigments which can serve as a dye. Some commonly used plant based dyes are Henna leaves which gives a orange red pigment, Carechu from resin for brown pigment, Fustic from the wood of the fustic tree gives a yellow pigment, Indigo from leaves and stems of the indigo plant for blue pigment, Logwood gives black pigment, Turmeric roots gives a yellow coloured pigment. Floral dye plays an important role for dying of textile material because it provide color as well as fragrance **[20]**.

2) Animal and insect dyes

Levantine sea snails of the family Muricidae are source of purple and blue dyes. Cochineal insect – *Dactylopius coccus* for crimson red pigment, *Kermes licis* for red pigment, Murex snail for purple coloured pigment are few examples of dyes from animal origin **[49]**.

3) Mineral dyes

Few examples of mineral dyes include iron bluff, iron black, manganese bistre, chrome yellow. Malachite is used as a green pigment for staining bacterial endospores, It is found in nature along with azurite, manganese oxide is used as a black pigment derived from colored clays and earth oxide, Compounds of iron and cyanide for blue pigment **[49]**.

Synthetic dyes

Synthetic dyes are derived from organic or inorganic compounds and are widely used in the field of textile, paint and printing. They are artificially produced from coal tar and hence known as coal-tar dyes. Acid dye, basic dye, reactive dye, direct dye, sulfur dye, are examples of synthetic dyes. Majority of stains used in microbiology are synthetic in nature and manufactured from aniline. Examples: Methylene blue, safranin, crystal violet, etc. The use of synthetic dye quinoline as a coloring agent in confectionery, soft drinks, and other food and beverages is accepted in a few countries. Synthetic dyes have reported to have a lot of hazardous effects on the ecosystem **[16, 41]**.

Synthetic dyes are extensively used in wide range of industries amongst which textile processing industries are the major consumers **[23]**.

Dyes influence every sphere of human life, be it food, leather, cosmetics, or the drug industry. By 1900, with an increase in demand, synthetic dyes gained immense attention and replaced natural dyes due to a variety of reasons such as low-cost production, easy availability, easy application, more color stability, resistance to light, pH changes, oxygen, etc. Considering their importance and large-scale industrial use, we cannot neglect their drawbacks.

Some of these are described below **[2]:**

They are not environmentally friendly as their synthesis involves extreme conditions such as high pH, high temperature, strong acids, and heavy metal catalysts. The most common substrate for dye production is petroleum, which is a non-renewable source of energy. Synthesis generates a large amount of effluent which contains toxic chemicals generated as side products.

The increasing dependency of various industries on synthetic organic dyes, and their solubility in water, are a cause of great concern; due to inadequate environmental legislature, industrial effluent is discharged into water bodies without proper treatment. Although dyes constitute only a small proportion (less than or equal to 1 ppm) of the industrial effluent, they are quite stable and are resistant to light, various chemicals, and biological activities owing to their complex structure, remaining as such for a long period. Hence their accumulation causes an increase in biochemical oxygen demand (BOD), affecting the pH and chemical oxygen demand (COD) **[36]**.

In addition, their increase affects the color of water bodies and affects absorption properties of planktonic life (decrease in photosynthetic activity). It is also seen that some of the dyes impound metal ions and cause toxicity in marine animals **[28]**.

These dyes also react with various other organic by-products of the effluent, forming harmful aromatic complexes that result in mutations and cancers in aquatic animals. Recent studies have shown that dyes have genotoxic, cytotoxic, and mutagenic properties when exposed in greater concentrations to living organisms. They can also cause DNA fragmentations, allergies, skin irritations, and malfunctioning of various organs when acted upon by different biotic and abiotic factors due to the formation of toxic breakdown products **[40, 29]**.

Some synthetic dyes form covalent bond to the fibres like cotton, rayon, and soy. There are not requiring harmful mordants. The toxicity of a dye considers the structure of the dye rather than the dyeing process. Heavy metals containing dyes and cancer-producing dyes cause high impact and are objectionable. Dyes that cause allergic reactions are not considered low impact. Dyes that pass eco- standards such as bluesign, GOTS, OekoTex 100 and Cradle to cradle and are compliant to the ZDHC MRSL have been assessed thoroughly for toxicity and should be chosen over dyes that have not. Acute toxicity involves oral ingestion and inhalation; the main problems of acute toxicity with textile dyes are skin irritation and skin sensitization, caused mainly by reactive dyes for cotton and viscose, few acid dyes for polyamide fibres and disperse dyes for polyester, polyamide and acetate rayon.

The reactive dyes which have proved to cause respiratory trouble and/or skin sensitization in workers on occupational **[8]**, exposure has been identified by ETAD (order to minimize the risk of exposure to these dyes, dye dust should be avoided. This may be achieved by using liquid dyes, low-dusting formulations and by using the appropriate personal protective equipment. After dyeing and fixation, reactive dyes have completely different toxicological properties because the reactive group is no longer present and the high wash fastness of the dyed fabric ensures that no dye is exposed to the skin of the wearer. Consequently, consumers wearing textiles dyed with reactive dyes have reported no cases of allergic reactions.

2. Disperse dyes, showing low perspiration fastness, are responsible for causing allergic reactions. Polyester dyed with disperse dyes does not in general pose a problem since the perspiration fastness is high. However, problems can arise with polyamide or acetate rayon dyed with disperse dyes since the low perspiration fastness allows the dyes to migrate to the skin. Basic dye are toxic and can cause allergic dermatitis, skin irritation, mutations and even cancer **[11]**.

3. Cationic dyes can cause increased heart rate, shock, vomiting, cyanosis, jaundice, quadriplegia, heinz body formation and tissue necrosis in humans **[48]**.

4. Anionic dyes have negative ions due to the excess presence of the OH- ions in aqueous solution. Anionic dyes are water soluble and they include acid dyes, azo dyes, direct dyes and reactive dyes. Reactive dyes attach to their substrates by a chemical reaction (hydrolysis of the reactive groups in the water) that forms a covalent bond between the molecule of dye and that of the fibre. Anionic dyes removal is the most challenging task as they produced very bright colours in water and show acidic properties **[10]**.

5. Reactive dyes contain reactive groups such as vinyl sulphone, chlorotriazine, trichloropyrimidine,and difluorochloro-pyrimidine that covalently bonded with the fiber during the dyeing process **[25]**. Moreover, azo dyes represent the largest class of reactive dyes used in the textile industry followed by anthraquinone and phthalocyanine classes **[51]**. Azo dyes have the largest variety of dyes and under anaerobic conditions, the dye's linkage can be reduced to form aromatic amines which are colourless but can be toxic and carcinogenic. It was estimated that 130 of 3,200 azo dyes in use can form carcinogenic aromatic amines during degradation process **[52]**.

The commonly used staining techniques in medical laboratories include Grams's staining, Lactophenol cotton blue staining, Giemsa stain and Wright's stain. In histopathology the commonly used staining involves a combination of haematoxylin and eosin. These commonly used chemicals can turn harmful based on the dose, time of exposure and other factors, imposing minor health effects from allergies to serious irreversible damages to human and animals. Studies have reported toxic effects of commonly used standard dyes having carcinogenic, genotoxic and immuonotoxic and cytotoxic effects **[7]**. Crystal violet (CV) the primary stain of grams staining finds its application also in textile industry, also used as coloring agents of plastics, gasoline, varnish, fat, oil and waxes, is a recalcitrant known persist long in the environment and cause toxic effects on aquatic and terrestrial life. Several studies have reported the carcinogenic effect of the CV dye and its use is prohibited in food industry and aquaculture. Researchers have shown that CV dye causes moderate eye irritation, sensitization to light, permanent damage to cornea and conjunctiva, as it contains a cationic dye, which is highly toxic to mammalian cells. In extreme cases it may lead to respiratory and kidney failures as well **[4, 26]**. Dyes like aniline, auramine O, basic fuchsin, ponceau 2R, Congo red, diaminobenzidine and chlorazol black E, are reported to be carcinogenic **[9]**.

The synthetic dyes disposal is a major concern as they are a threat to our environment and are one of the significant source of water and soil pollution as they are resistant to ordinary treatment process and can persist for a long time in the environment affecting the various organisms in the ecosystem. The synthetic dyes can cause severe health hazards when they entire the food chain such as skin irritation, digestive tract irritation, nausea, vomiting, liver and kidney damage, etc. they can reduce seed germination, root and shoot length in plants, and also inhibit the activity of microorganisms contributing soil fertility thus creating an imbalance in the nature **[27, 26]**.

Natural dyes can be the best alternative solution in order to overcome the above problems as they are biodegradable, safe in terms of toxicity. Plants can be a great source of natural dyes as they are available in various colours and can find application in medical, textile, and other industries. some Plants used as an alternative to toxic synthetic chemicals is listed in **Table 1.1** below.

Table 1.1: List of plant dyes used as an alternative to synthetic dyes

Sl. No.	SYNTHETIC DYE	PURPOSE	NATURAL ALTERNATIVE	REFERENCE
1	**Crystal Violet**	**For staining gram negative cells**	**Myrtle (*Myrtus*) and Stigma (*Isatis*) extracts**	**[13]**
	Safranin	**Counter stain in gram staining**	***Rosella* (hibiscus), *Juglans* (walnut) extracts**	
2	**Wright's stain**	**Blood smear**	***Curcuma longa* Rhizome exatract**	**[6]**
	Gentian vio	**Cheek cells**		
	Methylene	**Spinal cord cells**		
	Eosin	**Cycloid scales**		
	Safranin	**Plant tissues**		
3	**Wright's stain**	**Ova of Intestinal Parasites**	***Hibiscus sabdariffa* and *Azadirachita indica***	**[30]**
4	**Neutral red, Safranin, Carbol fuschin**	**Counter stain in grams staining (*Lacto bacillus* spp and *Esherichia coli*)**	**Henna (*Lawsonia inamis*) leaves extract**	**[14]**
5	**Eosin**	**Histology (Rabbit lung tissue)**	**Daucus carota (purple black carrot)**	**[37]**
6	**Lactophenol cotton blue**	**Fungal staining (*Aspergillus niger, Rhizopus stolonifer, Pencillium notatum*)**	***Hibiscus sabdariffa* extracts**	**[17]**
7	**Hematoxylin and Eosin**	**Histological section (kidney, liver and testes tissues)**	**Red onion (*Allium cepa*)**	**[18]**
8	**Cotton blue**	**Mycological stain (*Rhizopus, Microsporum gypseum and Aspergillus niger*)**	**Red Beetroot Pomace (*Beta vulagris*)**	**[45]**
9	**Heamtoxylin and Eosin**	**The cross section of the *piper betle* L.**	***Erythrina crista-galli* flower**	**[44]**
10	**Heamtoxylin and eosin**	**The cross section of *Zea mays* and *Helianthus annuus***	**Indigenous Berberis Pachyacantha Kochne**	**[19]**
11	**Safranin and picric acid-anilin blue**	**Wood anatomy**	***1. Enantia chloratha 2. Harugana madagascariensis 3. Hibiscus sabdariffa 4. Sarcocephalus latifolius 5. Sphenocentrum jollyanum 6. Sorghum bicolor***	**[1]**
12	**Heamtoxylin and Eosin**	**Testicular tissue**	***Lonchocarpus Cyanescens* leaves**	**[5]**
13	**Heamtoxylin and Eosin**	**Rat liver tissue**	***Syzygium cumini* friut**	**[39]**
14	**Heamtoxylin and Eosin**	**Tissue skin biopsy**	***Hibiscus Sabdariffa***	**[34]**
15	**Safranin and picric acid-anilin blue**	***Cola gigantean* wood**	***1.Bixa orellana 2. Pterocarpus osun 3. Curcuma domestica 4.Lonchocarpus cyanescens***	**[1]**

16	**Heamtoxylin and Eosin**	**Nerve Rat tissue staining**	**Black Mulberries (*Morus Nigra*)**	**[47]**
17	**Heamtoxylin and Eosin**	**Epithelial tissue, keratin, collagen fibers, muscle, adipocytes, blood vessels and red blood cells (RBC), cartilage and bone**	***Curcuma longa***	**[24]**
18	**Heamtoxylin and Eosin**	**Liver, lung and kidney tissue.**	***Ceratonia Siliqua* Bark**	**[31]**
19	**Heamtoxylin and Eosin**	**Rat tissue**	***Cola acuminate* seed**	**[42]**
20	**Safranim**	***Allium ascalonicum* chromosome**	***Hylocereus polyrhizus* puply**	**[12]**
21	**Safranin**	**Plant tissue**	***Hylocereus costaricensis* pulpy**	**[50]**
22	**Heamtoxylin and Eosin**	**Epithelial tissue, keratin, collagen fibers, muscle, adipocytes, Blood vessels and red blood cells (RBC), cartilage and bone**	***Curcuma longa* rhizome**	**[43]**
23	**Heamtoxylin and Eosin**	**Tilapia skin Tissue**	***Rhizopora sp.* bark**	**[35]**
24		**Skin tissue**	***Intsia bijuga* wood**	**[15]**
25	**Safranin**	**Melinjo plant tissue**	***Curcuma domestica* Turmeric rhizome**	**[38]**
26	**Safranin**	**Plant tissue (Allium cepa L)**	***Clitoria ternatea*.L flower**	**[46]**
27	**Heamtoxylin and Eosin**	**Wistar rat brain tissue**	**1. *Hibiscus sabdariffa* 2. *Sorghum bicolor***	**[32]**

CONCLUSION

Substituting the toxic chemical based synthetic dyes with natural plant based safe alternative is the need of the hour in creating ecofriendly environment and healthy lifestyle. The use of plants as a natural dye in biology, medicine, textile and food industry has to be explored and this research can be of significance in minimizing the hazardous effects of synthetic dyes.

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Conflict of interest

Authors disclose no Conflict of interest.

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