



Research Article

Extraction, characterization & applications of natural dyesBushra Khan^{1*}, Ramya Sindhyani¹, Anushi Divan², Sejal Rathod³¹Dept of Biotechnology, K.C College, Mumbai, Maharashtra -400001, India.²Jai Hind College, Churchgate, Mumbai, Maharashtra 400020, India³Kishinchand Chellaram College, Churchgate, Mumbai, Maharashtra 400020, India.

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Abstract: Artificial dyes, widely used in everyday items, come with side effects not known to the common man. They are toxic, harmful & mostly carcinogenic. There has been a worldwide increase in the demand of natural dyes due to its therapeutic properties & no known side effects. The current research aimed at extracting dyes of different colors from natural plant sources, carrying out its characteristic analysis & its applications. The dyes were tested for their anti-microbial ability and were found to be inhibitory to common organisms like *S. typhi*, *C. diphtheriae* and *S. aureus*, etc. Phytochemical tests were performed and they confirmed presence of several important metabolites like Phenols, Tannins, Terpenoids, and many more. The dyes were tested for anti-oxidant activity using the FRAP assay and antioxidant levels ranging from 0.62 to 2.00 were obtained. Preparations of cosmetics like lip balms, was carried out, and dyes were also tested as natural food coloring agents. The results indicated that the dyes caused inhibition of wide range of bacteria and proved to be good sources of anti-oxidants. They departed a natural color to food items and hence can replace artificial food colorings. Thus the use of natural dyes should be promoted, as they are cheap, biodegradable and feasible.

Keywords: Natural Dyes; Antimicrobial; Phytochemicals; Anti-Oxidant; Cosmetics; Food Coloring

Introduction

Dye is a natural or synthetic substance used to impart color to, or change the color, of textiles, paper, leather, and other materials, such that the coloring is not readily altered. ⁽¹⁾ Artificial dyes have been used widely since its inception. They are used in everyday items like clothes, food products, cosmetics, etc. The harmful effects of these dyes, however, go unnoticed by the people. In recent years, due to the scientific development, the adverse effects of these dyes are being discovered, and they have been found to cause or contribute to various allergic reactions, toxicity, mental disorders like ADHD, tumor formations, etc. Recent studies have shown that many commonly used artificial dyes contribute to cancer, including brain and testicular tumors, colon cancer, and mutations. ⁽²⁾ These dyes not only pose a risk to the biotic components, but also are responsible for degradation of abiotic components like soil & water. The contamination of soils and waters by dye-containing effluents is of great environmental concern. ⁽³⁾

Dyes obtained from natural materials like plant leaves, roots, bark, insects, etc were the only dyes used for the coloring of textiles & other products, until the discovery of the first synthetic dye in 1856. Rapid research in synthetic chemistry, supported by industrialization led to the development of synthetic alternatives, which gradually pushed the natural dyes into a stupor. However, recent trends of irrational use of artificial dyes and numerous environmental and health issues have once again revived interest in

production & use of natural dyes. Natural dyes are eco-friendly since they are renewable and biodegradable. ⁽⁴⁾ Natural dyes are harmonizing & soothing to the human eye. Some of its constituents are anti-allergens, safe for skin contact and are mostly non-hazardous to human health. ⁽⁵⁾

Beetroot (*Beta vulgaris*) is high in immune-boosting vitamins & minerals; it is also good for the bones, liver & kidneys, and it reduces risk of heart disease by modulating the blood pressure. ⁽⁶⁾ The petals of Rose (*Rosa*) are beneficial to the skin & cure dry and patchy skin; they are also used in health tonics. Carrot (*Daucus carota*) is a rich source of vitamins A, C, K, B-8, as well as pantothenic acid & folate, and ions like potassium, iron, copper, and manganese. It is also a good source of antioxidants. ⁽¹⁰⁾ Marigold (*Tagetes*) is famous for its use in herbal ointments. It is a rich source of flavonoids and anti-oxidants. Pomegranate peels (*Punica granatum*) are effective detoxifying agents. It acts as a combat tool against heart diseases, and in prevention of skin cancer. It is also known to cure coughs & sore throats. ⁽⁹⁾ Henna (*Lawsonia inermis*) plays essential role in detoxification, skin protection, boosting hair health, etc. Spinach (*Spinacia oleracea*) is high in protein, zinc, fibre, vitamins, etc. Its health benefits include skin care, eyesight, regulated blood pressure etc. It is a good source of water-soluble vitamins like thiamine, riboflavin, niacin & ascorbic acid, and contains enough amount of vitamin C to prevent and cure scurvy. ⁽⁷⁾ Peppermint (*Mentha piperita*) has

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significant anti-microbial and anti-viral ability, strong anti-oxidant and anti-tumor actions, and also exhibits anti-allergenic potential to a certain extent⁽⁸⁾.

Bioactive compounds in plants are instrumental for various beneficial activities of the plant.⁽¹¹⁾ Phytochemical assays are often considered to be the first step for characterization of a plant depending on the presence of the various metabolites. A positive response of the plant sample for the presence of Terpenoids, Flavonoids & Glycosides indicates its potential use for defence against microorganisms, since these metabolites impart the ability to inhibit the growth of organisms.⁽¹²⁾⁽¹³⁾⁽¹⁵⁾ Phenolic compounds possess properties like anti-aging, anti-cancer, anti-inflammatory, anti-atherosclerosis, and provides cardiovascular protection.⁽¹⁴⁾ Coumarins have bacteriostatic and anti-tumor activity, they mediate inflammatory responses, and act as anti-coagulants.⁽¹⁶⁾ Quinones provide protection against reactive oxygen species (ROS) & have a diverse role in therapeutics, including anti-cancer agents and anti-aging and arteriosclerosis.⁽¹⁷⁾ Free radicals are highly reactive, and are capable of damaging cells. They are associated with inflammatory diseases like rheumatoid arthritis and respiratory disorders. Various studies have demonstrated the adverse effects of anti-oxidant supplements, such as high risk of cancer, increased mortality, etc. Several nutrients in food and/or plants, like vitamin C and E, beta-carotene, phenols, flavonoids, etc. are commonly studied dietary anti-oxidants.⁽¹⁸⁾

Anti-microbial agents are essentially important in reducing the burden of infectious diseases. Alternative anti-microbials are needed, and this has led to a re-evaluation of ancient remedies of plants and plant-based products. The use of plant extracts and its phytochemicals can be of great importance in treatment of microbial infections⁽¹⁹⁾. With regards to the increased scope of natural dyes, its study, production, and usage, the present research is designed for the extraction of dyes from natural plant sources, studying its essential characteristics and carrying out applications of the same.

Materials and Methods

Selection & collection of plant materials: Eight different plant sources were chosen for extraction of dyes of 4 different colors. Beetroot & Rose for Red, Pomegranate peels & Henna for Brown, Spinach & Peppermint for Green, Carrot & Marigold for Orange.

Extraction of natural dyes: Dyes were extracted using both water & organic solvents. Water-based dyes were prepared by blanching, grinding & filtering the plant material using distilled water.⁽⁴⁾⁽⁵⁾ They were further concentrated using

lyophilization. Organic solvents were used for extraction of the color imparting phytochemical. Methanol was used for beetroot, rose, marigold, and henna.⁽²⁰⁾⁽²¹⁾ Ethanol was used for pomegranate peels, while petroleum ether was used for carrot. Spinach & peppermint were extracted using acetone as solvent.⁽²²⁾⁽²³⁾⁽²⁴⁾

Qualitative phytochemical analysis: The water-based dyes were used for phytochemical screening. Phytochemicals like proteins, glycosides, terpenoids, quinones, coumarin & saponins were analysed.⁽¹¹⁾ Phenols, tannins, carbohydrates, and flavonoids were also analysed.⁽²⁵⁾⁽²⁰⁾

Antimicrobial ability: The ability of the dyes to inhibit bacterial growth was tested using the Agar dilution method. 1g of concentrated dye mixed with 2ml 20% DMSO was incorporated into molten nutrient agar butts which were poured onto petriplates. 6 common pathogenic organisms, namely, *E. coli*, *S. typhi*, *P. aeruginosa*, *S. aureus*, *S. faecalis* and *C. diphtheriae*, were spot inoculated on the plates and incubated to check for growth.⁽²⁶⁾⁽²⁷⁾

Anti-oxidant activity: The ability of the dyes to scavenge free oxygen radicals was determined using the FRAP (Ferric Reducing Ability of Plasma) assay.⁽²¹⁾

Applications of the dyes:

Use as Food coloring agents: Noodles were colored by boiling them in water containing the dye for 5-10 minutes; while, doughs of white flour were prepared using dye mixed with water.

Cosmetic production (Lip-balm): Natural ingredients like aloe gel, almond oil, beeswax, rice powder & cornstarch were mixed well. 5-6 drops of the dye was added and mixed until a good consistency was obtained. The prepared lip-balms were then stored at 4°C and RT.

Results

The extracted dyes showed a good color imparting ability and were of the desirable consistency.



Natural dyes obtained from- Spinach, Peppermint, Beetroot, Rose, Carrot, Marigold, Henna, Pomegranate peels. (L to R)

Qualitative phytochemical analysis: Phytochemical analysis revealed the presence of important metabolites like Proteins, Carbohydrates,

Phenols, Tannins, Terpenoids, and Glycosides. These are major metabolites present which are responsible for several additive properties of the plants.

Fig. 1: Tabular representation of phytochemical analysis of the dyes.

Dyes Compound	Beetroot	Rose	Pom peel	Henna	Spinach	Pepper mint	Carrot	Marigold
Proteins	+	+	+	+	+	+	+	-
Carbohydrates	+	+	+	+	+	+	+	+
Flavonoids	-	+	+	-	+	+	+	+
Glycosides	+	+	+	+	+	+	-	+
Terpenoids	+	+	+	+	-	+	+	-
Saponins	+	+	+	+	+	+	-	+
Phenols	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	-	+
Quinones	+	+	+	+	-	+	-	-
Coumarin	+	+	+	-	-	-	-	+

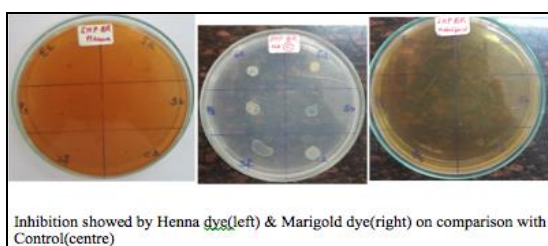
Key: '+' = Present; '-' = Absent

Antimicrobial ability: The dyes were examined for their antimicrobial ability by the Agar Dilution method. Organisms like *S. aureus* and *P. aeruginosa*, were inhibited by all the dyes. While, *S. typhi*, *E. coli*, *C. diphtheriae* and *S. faecalis* were inhibited by most of the dyes. The following is a tabular representation of the antimicrobial ability of the dyes as observed, upon comparing with the controls.

Fig. 2: Tabular representation of the antimicrobial ability of the dyes

Organism Dyes	<i>E. coli</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. faecalis</i>	<i>C. diphtheriae</i>
Beetroot	++	++	++	++	++	++
Rose	+	++	++	++	+	++
Pom peel	+	++	++	++	+	++
Henna	++	++	++	++	++	++
Spinach	+	++	++	++	+	+
Peppermint	++	++	++	++	++	++
Carrot	++	+	+	++	+	+
Marigold	++	++	++	++	++	+
Media Control	+	+	+	+	+	+
DMSO Control	+	+	+	+	+	+

Key: '++' no growth '+' growth



Anti-oxidant activity: The anti-oxidant activity of the dyes was tested using FRAP assay & ascorbic acid as standard. Highest anti-oxidant activity was shown by dye obtained from beetroot, followed by henna, rose and carrot dye. Other dyes also showed good anti-oxidant activity.

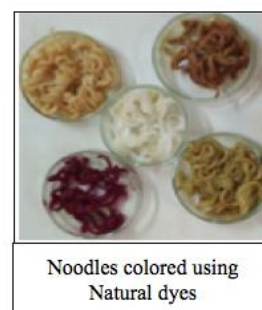
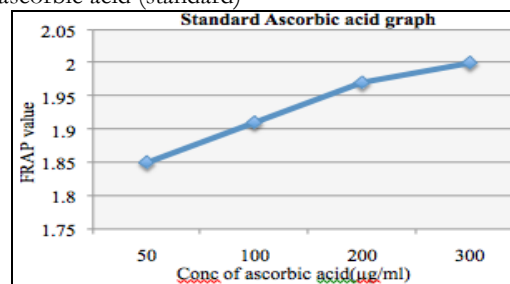
Applications of the dyes:

Use as Food coloring agents: The noodles & white flour dough that were prepared using the dyes retained a good color, and no considerable change in taste was imparted to the food products.

Fig. 3: FRAP values of the dyes in descending order of the anti-oxidant activity. (Higher the FRAP value, greater the anti-oxidant activity)

Dyes	FRAP value
Beetroot	2.00
Henna	1.99
Rose	1.98
Carrot	1.97
Pom peel	1.94
Spinach	1.86
Marigold	0.90
Peppermint	0.62

Fig. 4: Graphical representation of FRAP values of ascorbic acid (standard)



Cosmetic production (Lip-balm): The prepared lip-balms were found to be stable upon storage at RT & 4°C for upto 4 weeks. They had a good consistency and gave a vibrant color. Also, since the dyes were prepared using all natural ingredients, they gave a refreshing and soothing feel to the skin.



Discussion

The dyes extracted from natural sources are rich sources of important bioactive compounds. Plants like Henna, Beetroot, Carrot, Spinach, etc are known to have therapeutic properties. The extracted dyes showed prominent and lucid colors.

Upon testing for presence of various phytochemicals, the dyes showed presence of important metabolites like proteins, tannins, carbohydrates, phenols, glycosides and many more. These phytochemicals are known to have therapeutic properties, like protecting the cellular systems from oxidative damage, modulating immune system, preventing cancer and lowering the risk of chronic diseases like cardiovascular diseases, etc. The antimicrobial ability of the dyes was tested using the agar dilution method & the dyes showed inhibition to common pathogenic organisms like *Staphylococcus aureus*, which is a common skin infection causing bacterium, and, *Pseudomonas aeruginosa*, which is causes various nosocomial infections. Organisms like *Streptococcus faecalis*, *Salmonella typhi*, *Escherichia coli*, and *Corynebacterium diphtheriae*, that are the causatives of various afflictions were also inhibited by most of the dyes. Being extracted from natural plant sources, the dyes were found to be rich in anti-oxidants. The highest anti-oxidant level found spectrophotometrically was that of the Red beetroot dye (FRAP value = 2.00). The other dyes obtained were also found to have good anti-oxidant activity. The food products colored using the dyes showed no change in taste & retained a vibrant color. Also, the cosmetic (lip-balms) prepared using the dyes were found to be stable upon storage & gave a refreshing and soothing feel to the skin.

Natural dyes are on the rise ever since the harmful effects of artificial dyes have begun to uncover. Presence of important metabolites and high anti-oxidant activity are an indication of the beneficial purposes these dyes will serve in food industries. The ability to inhibit commonly pathogenic organisms is proof of the medicinal & therapeutic properties of these natural dyes. Natural dyes can, thus, replace artificial dyes in the near future.

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