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Green vegetation: a promising source of color dyes

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Abstract: The present study aimed for the extraction of color dyes from different sources, such as *Brassica oleracea*, *Brassica campestris*, *Citrus limon*, *Citrus limetta*, *Tagetes erecta*, *Spinacea oleracea*, *Beta vulgaris*, *Rosa indica* and *Curcuma longa*. The leftovers of such plants were mainly used for color dye extraction and their confirmation using spectrophotometric analysis. The specific color pigments like carotenoids, anthocyanin, chlorophyll and betanin were found to be the main coloring agents that impart specific color to the samples. Among all these samples, the maximum yield was obtained from *C. limetta* aqueous peel extract, and among all the temperatures employed room temperature was found out to be the most suitable temperature for the stability of color extracts. The extracted colors were utilized in candy making and sugar syrup making and were also used for coloring various foods stuffs. Moreover, the extracted color dyes were applied for dyeing purposes on cotton cloth with alum showing better and more enhancing color fastness results than the lime.

Keywords: anthocyanin; *Beta vulgaris*; carotenoids; chlorophyll; *Curcuma longa*; *Rosa indica*; *Spinacea oleracea*.

1 Introduction

From primeval times, mankind has been fascinated by color and has left an impression on his environment in the form of painted images, which beautified his world and expressed his thoughts and feelings. In fact, color has always been a dominating element in the cultures of peoples all over the world, having been used throughout history for a great variety of purposes, not only, for

instance, to produce artworks and adorn objects or individuals, but often to designate significance and hierarchical status as well. Early civilizations such as the Romans accepted that people “eat with their eyes”. Natural dye-stuffs have been utilized for a long time. Natural dyes were found accidentally; they have a great influence on human life, and it is impossible to think of life without colors [1].

Color pigments that occur in plants are commonly referred to as natural colors. Ancient people employed color in their everyday life for coloring their body parts as they thought the colors provided an attractive look. They also dyed animal furs and skins to utilize them for clothing. Colors were also used for decorating the walls of their mud houses. For all these purposes the colors were extracted from different natural materials [2]. Four major types of color pigments are present as chlorophyll, anthocyanin, carotenoids and betalains. Natural colors have low stability when exposed to oxygen, metal, lignin, and oxidizing agents, as well as to extreme temperature and pH.

The beautiful colors that are created from natural dyes would initially appear stunning but would soon fade. Lack of color fastness, helped in promoting the discovery of mordants. Mordants are substances that aid in the absorption of dyes. Natural colors do not easily dye fabrics but require mordants to lock the dyes into the fabrics and prevent the color from vanishing. Mordants consist of chemical elements that lock the color onto the cloth stuff. It is a supplementary constituent involved in the response between the fiber and color dye which is normally utilized throughout natural dyeing procedure. Dyeing by using diverse mordants generate limited outcomes that resulted in unusual colors on the dyed fabrics [3].

Natural dyes work in combination with mordants to give a particularly good color range on different fabrics like cotton, silk, and linen [4]. Unfortunately, dyes that are extracted naturally are seldom used during dyeing. It has been generally perceived that naturally extracted colors give faint and less compatible colors compared to synthetic ones, but it has been observed that natural colors give better color shades compared to synthetic dyes [5].

The above-mentioned studies reveal that the use of natural color dyes is eco-friendly to the environment

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compared to synthetic dyes, which are composed of chemicals that might be carcinogenic and may cause serious health problems. The use of locally prepared natural dyes using cheap resources may reduce the importation of expensive chemicals which is at present a great burden to the Pakistani economy.

2 Materials and methods

The present investigation was commenced for color extraction from such sources that were mainly considered as waste, and then the experiment was performed to evaluate the utilization of the extracted colors in food and for dyeing purposes.

2.1 Plant material

For the extraction of color dyes for the various experimental analyses, plant materials (including leaves, leaf stalks, petals, and peels) were obtained from different markets and gardens around Lahore (Table 1).

For the selection of plant material, preferably cheap sources were chosen. Moreover, it was also taken into account that the samples should be free of contaminants.

2.2 Color extraction

Crude color extraction was done by following the methodology given by [6]. Briefly, the selected plant sample was washed properly with water and then dried at room temperature. When the sample was dried, the weighed amount of sample was blended with different solvents for 15–20 min, and the solvents that give highest color yield were selected. After that the blended material was filtered using cotton cloth and compressed, until all the solvent was squeezed out. The residue was again blended with a small volume of the solvent for complete color extraction.

A pinch of citric acid was added to the extracted color liquid, acting as a stabilizer. The filtrate was placed in the oven at 40–45°C for 24 h. The dried sample, in powder form or in paste, was saved in a dry sterilized jar.

The percentage (%) yield of the crude dye was determined using the equation given below:

$$Wdy = \frac{Wbe - Wae}{Wae} \times 100$$

where

Wdy = % yield of crude dye

Wbe = weight of plant material before extraction

Wae = weight of crude material after evaporation.

2.3 Thermo-stability of extracted color dye

The stability of extracted color dyes was determined at various temperatures by keeping the extracts at specific ranges of temperature. The samples were kept at the various temperatures for 15 days to ascertain the stability of the colors.

2.3.1 Procedure: The weighed amount of color sample was placed in a sterilized Petri plate, and these samples were then placed at the following different temperature ranges.

Sample 1: Color extract was kept at room temperature, 30–35°C.

Sample 2: Color extract was kept in incubator at temperature 45–55°C.

Sample 3: Color extract was kept in freezer at –15 to –20°C.

Sample 4: Color extract was kept at temperature 15–20°C.

Reweight: The samples were re-weighed after 15 days, and the loss in weight was noted.

2.4 Determination of color pigments

The amount of different color pigments in crude extracts was determined using the spectrophotometry method.

2.4.1 Determination of percent curcumin

2.4.1.1 Sample preparation

For the preparation of sample, the methodology of [7] was used. For this, 0.1 g of sample was dissolved in 25 ml of ethanol. Then the solution was filtered, and the volume was increased to 100 ml using distilled water. Afterward, about 10 ml of this solution was placed in a separate flask, and the volume was increased to 100 ml with ethanol. Absorbance was recorded at 425 nm.

Table 1: Plant materials used for color extraction.

Serial no.	Samples	Common name	Part used
1	<i>Atriplex rubra</i> (L.) Crantz	Red orchid	Leaves
2	<i>Brassica oleracea</i> L.	Red cabbage	Leaves
3	<i>Brassica campestris</i> L.	Mustard, sarson	Leaves and stem
4	<i>Citrus limon</i> L.	Lemon	Peel
5	<i>Tagetes erecta</i> L.	Marigold	Petals
6	<i>Spinacea oleracea</i> L.	Spinach	Leaves
7	<i>Citrus limetta</i> Risso	Sweet lemon, musambi	Peel
8	<i>Curcuma longa</i> L.	Turmeric, haldi	Rhizome

2.4.1.2 Calculation

The percentage (%) of curcumin and the strength of the color of a pigment was measured using the respective standards: a 425 nm, the standard value of curcumin is 0.42; the absorptive value for curcumin is $A = 16.8$; the percentage (%) of curcumin in the samples was calculated using the following formula:

$$\% \text{ Curcumin} = \frac{a \times 100}{L \times A \times W}$$

where

a = absorbance of sample at 425 nm

L = distance, that is 1 cm

A = absorption of curcumin

Color value = $a \times 1000$

2.4.2 Determination of anthocyanin

2.4.2.1 Preparation of chemicals

2.4.2.1.1 0.02 M Potassium chloride buffer

The following two solutions were required for the preparation of this buffer.

Solution A: This solution was prepared by dissolving 14.91 g KCl in 1 l of distilled water in a flask.

Solution B: This solution was prepared by dissolving 16.6 ml of HCl in 1 l of distilled water in a flask.

Then 50 ml of solution A and 50 ml of solution B were placed in a separate flask, and the final volume was increased to 200 ml with distilled water. Using HCl and NaOH, the pH of the buffer was maintained at 1.

2.4.2.1.2 0.4 M Sodium acetate buffer

For the preparation of 0.4 M sodium acetate buffer, 8.945 g of disodium hydrogen phosphate and 3.4023 g of potassium iodide were placed in a flask, and the volume was increased to 1 l using distilled water. The pH was maintained at 4.5 using concentrated solutions of HCl and NaOH.

Procedure: For the determination of anthocyanin, 1 g sample of the crude dye extract was placed in a flask, and it was dissolved in 10 ml of acidified methanol.

Solution A: 0.8 ml of the sample solution was placed in a separate flask, and 3 ml of potassium chloride buffer was added to it.

Solution B: 0.8 ml of the sample solution was placed again in a separate flask and 3 ml of sodium acetate buffer was added to it.

The absorbance of solution A was measured at 700 nm, and solution B was measured at 525 nm, whereas water was used as a blank.

The concentration of anthocyanin was determined using the formula given below.

$$A = (A_{525} - A_{700}) - (B_{525} - B_{700})$$

$$\text{Conc. of anthocyanin} = \frac{A \times MV \times Df \times 1000}{E \times l}$$

where

MV = molecular weight = 449.2 g/mol

E = absorption coefficient = 26,900/mol/cm

Df = dilution factor = 37.5

2.4.3 Determination of carotenoids: A stock solution was prepared by following the standard protocol given by [8]. For its preparation, 5 mg of crude dye extract was dissolved in 50 ml of n-hexane. The absorbance of stock solution was measured at 452 nm, while water was used as a blank.

The amount of β -carotene was calculated using the following formula.

$$\text{Carotenoids content} \left(\frac{\mu\text{g}}{\text{g}} \right) = \frac{A \times V (\text{ml}) \times 104}{\epsilon \times (p)}$$

where

A = absorbance at 452 nm

V = total volume of extract used

p = weight of sample

ϵ = extinction coefficient on hexane = 139,200 l/mol

2.4.4 Determination of betanin

2.4.4.1 Citric acid phosphate buffer

2.4.4.1.1 Solution A: 0.1 M solution of citric acid monohydrate

For preparation of this solution, 2.101 g of the citric acid was placed in a flask, and the volume was increased to 100 ml using distilled water.

2.4.4.1.2 Solution B: 0.2 M solution of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$

For preparation of 0.2 M solution of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 7.164 g of acid was placed in a flask and the volume was increased to 100 ml using distilled water.

The final solution was prepared by mixing 89.1 ml of solution A and 10.9 ml of solution B in a separate flask, and the solution was mixed to prepare a homogenized solution.

Procedure: The amount of betanin was determined following the methodology given by [9]. The 0.5123 mg crude dye extract was placed in a sterilized flask, and the volume was increased to 10 ml using citric acid phosphate buffer. The solution was allowed to stand for 10 min at room temperature. Absorption was observed at 530 nm. Total betanin content was calculated using the following formula:

$$\text{Betain content (mg/100 g)} = A \times DF \times MW \times 1000 / \epsilon L$$

where

A = absorption value at 535 nm

DF = dilution volume

L = path length of cuvette

MW = molecular weight of betanin (550 g/mol)

ϵ = extinction coefficient for betanin 60,000 l/mol.

2.5 Application of extracted dye on fabric

2.5.1 Textile materials: Cotton fabric was selected for the current study. The size of the samples was 10 cm $\times$ 10 cm.

Procedure: Pretreatment of cloth before dyeing: the cloth was heated in NaOH and detergent-containing solution for 30 min at 60°C to remove the starch. The cloth was washed thoroughly with distilled water and allowed to dry. The extracted solution of each dye was applied to cotton fabric with mordant and without mordant under the same conditions. The direct dyeing (without mordant) method was performed to find out whether the extracted dye showed affinity to cotton in the absence of mordant [10].

2.5.2 Mordants: Three different mordants as given in Table 2 were used singly or in combination to stabilize the natural dye on the cotton cloth. The mordants used were alum potassium, aluminum sulfate, and lemon.

Table 2: Mordanting recipe for textile material.

Mordants	Amount (g)	Water (ml)	Boiling time (min)	Mordanting time (min)	Mordanting temperature (°C)
Alum	1.0	200	60	30	70–90
Lemon	1.0	200	60	30	70–90

Procedure: Firstly, the weighted mordants were put into a beaker, and then water was added. The procedure was carried out over the hotplate to dissolve the mordants. The mordants were dissolved in the water at 90°C. Mordants were stirred constantly. The cotton cloth was then immersed into the mordant solution for mordanting for 30 min.

The mordant-treated cloth was not washed and was directly dyed at temperature range of 90–100°C for an hour. After dying, the fabrics were washed properly, and washing was carried out using 2 g/l detergent. The dyed samples were dried to check the washing fastness properties.

extraction of crude dye showed a maximum yield of 30.67% obtained from *Citrus limetta* peel, whereas an intermediate yield of 16.22% was obtained from *Citrus limon* peel, and the comparatively lesser yield of 14.11% was obtained from *Beta vulgaris* extract. Among peels, maximum yield of 30.67% was obtained from *C. limetta* peel. However, *Rosa indica* petals gave a yield of 18.41%, while *Spinacea oleracea* leaves gave a yield of 28.01% as indicated in Figure 3.

3 Results and discussion

Research work was conducted for the extraction of color dyes from a variety of natural sources. While selecting such sources, the main focus was to select cheap and easily available sources. The extracted color dyes were evaluated by taking into consideration the economic and commercial aspects of the plant color extract. Different solvents were employed for the extraction process, but finally the solvent that gave the maximum yield was used.

3.1 Extraction of color dye extracts

The extraction of different color dyes was done using different plant parts, such as peels, leaves, petals, and rhizomes (Figures 1 and 2). The percentage (%) of

3.2 Thermo-stability of color compound

The thermo-stability of color compounds was examined by subjecting the extracts to a variety of temperatures. The samples that were placed in the incubator showed maximum weight loss and a wide range in pH, and their physical appearances are shown in Figure 4. On the other hand, the samples that were placed in the freezer showed the highest gain in moisture and great variability in their pH. So the best and most suitable temperature for stability of samples was the room temperature, because at this temperature there was less variation in weight loss, pH, and color.

3.3 Spectrophotometric analysis

Spectrophotometric analysis was performed to verify the presence of different color pigments. Plants mainly contain a number of light-absorbing pigments, most of which are commonly associated with photosynthesis. Organic substances in plants that are reliable in giving diverse colors to plant parts are called pigments. For this analysis *Brassica oleracea*, *Brassica campestris*, *C. limon*, *Tagetes erecta*, *S. oleracea*, *C. limetta*, *B. vulgaris*, *R. indica*, and *Curcuma longa* extracts were used. Different samples showed the presence of specific color pigment at different wavelengths; for example, betanin presence was recorded at 523 nm, anthocyanin pigment was detected at 525 and 700 nm, curcumin presence was recorded at 425 nm, and carotenoid presence was recorded at 452 nm. These wavelengths showed the presence of specific color pigments in

Figure 1: Flow chart showing the dying procedure.

Figure 2: Determination of percent yield of crude dyes from different natural sources.

Figure 3: Extracted crude dyes from various sources.

Figure 4: Stability of extracts at various temperatures.

all the extracted color samples. That gives particular color to the extracts (Table 3).
This methodology helps us to determine the existence of color pigments in the crude extracts. The crude extract

is the mixture of more than two color pigments but by utilizing this methodology the presence of specific color pigments was analyzed and that is helpful in giving a particular color to the specific compound.

Table 3: Spectrophotometric analysis of different color compounds.

Extract	Absorbance (nm)	Fresh extract	Placed at room temperature	Placed in incubator	Placed in freezer
Betanin					
<i>Beta vulgaris</i> L.	523 nm	1.571±0.088	1.121±0.026	1.092±0.028	0.645±0.099
Anthocyanin (mg/g)					
<i>Brassica oleracea</i> L.	525 and 700 nm	1.519±0.072	1.14±0.035	1.17±0.04	1.13±0.00
<i>Rosa indica</i> L.		0.16±0.01	0.17±0.01	0.10±0.00	0.04±0.01
Curcumin (%)					
<i>Curcuma longa</i> L.	425 nm	60.4±6.38	52.2±10.2	37.23±6.42	9.74±2.25
Carotenoid (mg/g)					
<i>Brassica campestris</i> L.	452 nm	0.028±0.004	0.031±0.002	0.030±0.003	0.03±0.003
<i>Citrus limon</i> L.		0.028±0.001	0.027±0.000	0.027±0.000	0.024±0.000
<i>Tagetes erecta</i> L.		0.028±0.001	0.027±0.000	0.026±0.000	0.025±0.000
<i>Citrus limetta</i> L.		0.031±0.002	0.027±0.001	0.026±0.000	0.026±0.000

3.4 Application of different color compounds

Dyes provide a striking color to foods, that on the other hand looks unappealing or flavorless. It is supposed that satisfaction from food depends on “eye appeal” among many other factors and that the beautiful and attractive coloration of food are major contributing factors to the consumer’s appetite. For this analysis *B. oleracea*, *Beta campestris*, *C. limon*, *T. erecta*, *S. oleracea*, *C. limetta*, *B. vulgaris*, *R. indica*, and *C. longa* extracts were used in different food products to give striking colors to the food.

Along with the application of extracted color dyes in different food items (Figures 5 and 6), the extracted

color dyes were also used on textile fibers for dyeing purposes. For this, the extracted colors were used alone or in combination with mordants. It was found that both mordants (alum and lemon) were helpful in enhancing the color strength of extracted colors. But alum was considered to be the better mordant because it gives a sharper color result compared to the lemon. The dyed cloths were permanently dyed, meaning that on washing the cloth with water the color did not lighten (Figures 7–9). These results indicate that these extracted colors can be utilized easily in the textile and food industries for the replacement of synthetic dyes that are the major cause of many health problems.

Figure 5: Application of yellow-orange color on boiled rice. **Figure 6:** Application of yellow-orange color on boiled Spaghetti.

Figure 7: Application of color extracted from *Citrus limon*. (A) Change in colour when applied Lime and Alum as mordant (B) Colour effect without mordant.

Figure 8: Application of color extracted from *Curcuma longa*. (A) Change in colour when applied Lime and Alum as mordant (B) Colour effect without mordant.

Figure 9: Application of color extracted from *Atriplex rubra* and *Spinacea oleracea*. (A) Change in colour when applied Lime and Alum as mordant (B) Colour effect without mordant.

3.5 Discussion

The maximum amount of color extract was obtained from different samples like *Atriplex rubra*, *B. oleracea*, *Be. campestris*, *C. limon*, *T. erecta*, *S. oleracea*, *C. limetta*, *C. longa*, *R. indica*, and *B. vulgaris*. These outcomes were almost the same as those obtained by [11, 12] from leaves of *Cucurbita pepo* and by McGovern et al. [13] in color extraction from *Livistona speciosa*. An appropriate quantity of extracts can be acquired using these strategies.

Colors were extracted from fruits peels, vegetables, and petals for utilization in the food and textile industries. The appearance of food was a major factor in determining its acceptance by the consumer, who has a number of built-in expectations regarding the “proper” color for a particular type of food, also color in this regard is often the primary consideration for purchasing clothes [14].

Furthermore, as dyes were developed and experimented with, people became more adventurous and would attempt different mediums in making dyes, hence finding synthetic dyes which helped the dyeing industry to develop fast. Though synthetic dyes are colorfast, they pose a danger to the health of people owing to the harmful chemicals in them. They could cause cancer and other diseases, and so their use should be minimized or eliminated altogether. Taking this into consideration, extraction of color from various natural sources is employed nowadays. Research into the extraction and application of dyes from local plants to serve as colorants for textiles and food is therefore very critical to assess the potency of the dyes and the various medicinal and food plants for their potential to color everyday clothes, fibers, yarns, and also food items to enhance their appeal to consumers while also promoting healthy lifestyles through the use of plant-based food colors as substitutes for the chemical ones sold on the open market.

The extracted color dyes were applied for dyeing purposes on cotton cloth with or without the help of mordants. Two different mordants were used: alum and lime. It was found that alum showed better and more enhancing color fastness results than lime. The shade and fastness effect of color increased when using mordants. The extraction of brilliant yellow and red colors from *C. longa* and *Tagetes erecta* was performed by [15]. The extracts were applied as direct dyes and in the presence of iron or alum mordant [16, 17]. The shade of color was enhanced when using different mordants. Among all the mordants applied, the experimentally used alum was found to be best mordant for enhancing the shade of color [13].

4 Conclusion

The food and beverage industry releases considerable amounts of wastes which contain natural dyes. Such wastes like lemon and orange peels could serve as sources for the extraction of natural dyes for food industry usage like the extracted colors used in candy making, sugar syrup making, and coloring various foods stuffs. The results prove the potential of such wastes as a source for natural dyestuff extraction to extract color from various natural sources using barks, berries and fruits, and these colors were employed for coloring different food items like butter, candies, cakes, bakery items, and sugar syrups. The extracted colors were found to be a better substitute for the synthetic chemical dyes that cause many health hazardous problems and that also needed to be imported. The colors extracted using natural sources including wastes were considered to be the best replacement for synthetic dyes in both food and textile industries.

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