Anthocyanin: A Natural Dye Extracted from *Hibiscus Sabdariffa* (L.) for Textile and Dye Industries

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**Abstract**

Environmental pollution is one of the major issues facing all countries throughout the world. Environmental degradation is occurring and creating crises in day-to-day life due to the increasing amount of chemicals used in industries, where even the effluents processed out after treatment also contain some trace elements. Hence the extraction of enzymes using natural methods is an alternative for the production of dye in order to reduce pollution, which in turn helps to nourish and protect the environment for future generations. *Hibiscus sabdariffa* (L.) is a rich source of anthocyanins that is further enhanced by callus formation and accumulated by increasing the sucrose concentration. Anthocyanin pigments were extracted using acidified ethanol. The dye obtained was screened by GC–MS analysis and its dyeing process used in the textile industry. The study showed certain properties affected the coloring nature depending on the cloth used. The color of anthocyanin pigment depends on the pH maintained and also shows adaptability to varied environmental conditions.

**Keywords** Anthocyanin · Environment · *Hibiscus sabdariffa* · Callus · GC–MS

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**Highlights**

- Extraction of anthocyanin by different solvent to improve the intense of coloration
- Phytochemical evaluation of plant sample to know the commercial values and medicinal values
- Plant tissue culture and callus assisted anthocyanin induction
- Amalgamation of plant oriented dye application (cloth dye, lip balm, dye sensitized solar cell).

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Introduction

Hibiscus is a broad genus that has more than 679 species that have been used for myriad purposes. *H. sabdariffa* (L.) (common name Roselle) under the family of Malvaceae contains the pigment called anthocyanin, which is water soluble, and their color varies based on the potential of hydrogen. Studies have indicated the different properties of Roselle and commercial uses based on their rich molecular pigmentation and their antioxidant and antimicrobial properties. Roselle is cultivated for their calyces and leaves, which are exported to several countries for their edible parts, including for making jelly-like substances and pickles (Fig. 1). *H. sabdariffa* pigment can produce dye after some natural processing, and it can be used as an alternative to synthetic dye, which can help limit environmental damage. Color is produced by grinding and soaking Roselle in water, and increasing and decreasing pH influences color intensity [1].

Fig. 1  a Habitat of *Hibiscus sabdariffa* L.  b calyx of *H. sabdariffa* L.
Ancient people used plants to extract natural dyes for their clothes and their beauty care, as well as for medicinal uses. There are different methods to extract the dye from *H. sabdariffa* plant parts, especially from calyces, which may be ground or fully soaked in water, because of their water soluble pigment nature, they are able to produce dye without any additives or formulation, but the intensity of the color may vary. By studying the physical and chemical characteristics of *H. sabdariffa*, we can increase the intensity of the dye by periodical and spontaneous enhancement of the potential of hydrogen [2, 3].

Plant tissue culture can also be used to reduce the time to plant maturation and simultaneously avoid the interference with food and other agricultural crops. The anthocyanin pigment obtained was further studied to determine its possible applications by treating various materials with the dye, especially on cloth, cosmetics such as lip stick, face cream, and sunscreen lotion because of its antioxidant property, and dye sensitized solar cells [4].

Many researchers have found various applications for dye obtained from Roselle using organoleptic attributes and mathematical evolution. Jian et al. reported cosmetic application of Roselle through reversing UVB induced photoaging. Characterized dye sensing of solar cell using Roselle dried extract was performed with different methods. All of these studies focused on future sustainability in specific circumstances [5, 6]. However, having a green chemical environment is important for future generations; therefore, in the present study, we sought to find a naturally sustainable way to incorporate natural dyes into the textile and dye industries [6].

**Experimental Methods**

**Sterilization of Explants**

The explants were washed with sodium hypochlorite (1.0%) and mercuric chloride (0.05%) for different time durations. The explants were transferred to a beaker containing 1% sodium hypochlorite solution with rapid shaking for 20–25 min, and the solution was changed at 5 min intervals. Then, the explants were washed with distilled water. Finally, the explants were treated with 0.05% mercuric chloride for 1–5 min. The surface sterilized explants were washed with sterile distilled water five times (5 min each). The excess water on the explants was removed using sterile tissue paper before culturing. The explants were cut into small pieces (0.5–1.0 cm) barring the cut ends and transferred to semi-solid culture medium under aseptic conditions in a laminar flow chamber.

**Anthocyanin Pigment Extraction**

The anthocyanin content from freeze-dried calyx was determined using the pH-differential method, with slight modifications. Of the overnight freeze-dried calyx,
50 mg were ground with different combinations of acidified ethanol solvent such as 1 N HCl, 1.5 N HCl, 1% citric acid, 2% citric acid, and absolute ethanol. The samples were centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatants obtained were used as crude anthocyanin pigment extracted from the calyx and were stored at 4 °C for further use. The absorbance (A) was measured at 521 and 700 nm against the blank sample [7].

**Gas Chromatography/Mass Spectrometer**

Plant extract was tested against GC/MS to confirm the presence of anthocyanin. Gas chromatography/mass spectrometry (GC/MS) is the combination of two analytical methods into a versatile technique for the identification of complex volatile materials. Gas chromatography (GC) effectively separates the different constituents of the sample for subsequent analysis and identification by mass spectrometry (MS) [8].

**Dye Preparation for Cloth**

In dye extraction, the flowers (10 g) were crushed and put in an earthen pot to which 100 mL water was added. The pot was kept undisturbed for 20–25 days, and the extract was then filtered through a piece of cloth to yield the natural dye.

**Dyeing Procedure**

The extracts obtained through the abovementioned methods were filtered and used for dyeing. Cloth used for dyeing was boiled in a NaOH solution (10%) for 15 min to remove starch from the cloth, then washed with cold distilled water. This cloth was then transferred in mordant (Myrobalan) for 30 min followed by treatment in the dye bath for 1 h. The effect of dye without mordanting the fabric was also studied. Then the cloth was treated with tepol (color fixative) and dried in sunlight.

Similarly, the effects of various mordants on the color of dye extracted from the flowers were also studied on the cloth found to retain dye the best in the above experiment. This was achieved by incorporating different mordants, including stannous chloride, ferrous sulfate, and potassium dichromate, separately, each at a concentration of 3% of the dye extract (5 mL). Cloth pieces were individually soaked with the mixture of extract–mordant solution. After 30 min of soaking, the cloth was dried in sunlight for 2 h. The sun dried cloth was further evaluated for its color, lightness, and wash fastness. Wash fastness was tested by washing with soapy water (10% w/v), and heat resistance was tested by keeping the cloth at various temperatures, viz. 50, 60, 70 °C for 30 min in the oven without water [9].
Flowchart for Staining of Anthocyanin Pigment on Cloth as a Dyeing Agent

1. Cloth boiled in NaOH solution (10%) for 15 minutes
2. Washed with cold distilled water
3. Transferred in mordant (Myrobalan) for 30 minutes
4. Treatment in the dye bath (prepared according to the methods explained in dye extraction) for one hour
5. Treated with tepol (colour fixative) and dried in sunlight
6. Cloth with best result treated with different mordants like, Stannous Chloride, Ferrous Sulphate and Potassium Dichromate, incorporated separately, each at a concentration of 3% of the dye extract (5 ml).
7. Cloth pieces individually soaked with the mixture of extract - mordant solution for 30 minutes
8. Dried in sunlight for 2 hours
9. Evaluated for colour, lightness and wash fastness
Application of Anthocyanins in the Formulation of Herbal Lip Balm

Formulation of Herbal Lip Balm

The herbal lipstick was formulated as per the method described. The ingredients used in the formulation of herbal lipstick are: beeswax, shea butter, strawberry essence, anthocyanin pigment obtained from calyx of *H. sabdariffa*, and vanilla essence [10].

Results and Discussion

Plant Tissue Culture

MS medium supplemented with varying concentrations of sucrose of 20% and 50% along with a combination of plant growth regulators such as 2,4-D (1.0 mg/L) + BAP (1.0 mg/L) showed maximum formation of callus with slight initiation of anthocyanin pigment formation in the medium containing 60% sucrose concentration (Fig. 2). The work carried out is supported by the earlier report stating that the highest formation of callus was observed in the MS medium supplemented with 2,4-D (1.0 mg/L) and BAP (1.0 mg/L) after three sub culturing [7].

Anthocyanin Pigment Extraction

After fine grinding of Roselle calyces with an acidified solvent such as 1 N HCl, 1.5 N HCl, 1% citric acid, 2% citric acid, and absolute ethanol, 1.5 N HCl showed the maximum extraction of anthocyanin (1.976%) from the calyx of *H. sabdariffa*, followed by 1.790 in 1% citric acid, 1.635 in 2% citric acid, 1.214 in 1 N HCl, and 0.784 in absolute ethanol (Fig. 3a, b). From the results of absorbance of different acidified solvent and calyx, the solvent that gives the most absorbance was 1 g of calyx extract and 5 ml of 1.5 N HCl at 1.976, and from this we can understand that 1.5 N HCl has higher color intensity and available anthocyanin (Table 1). UV analysis of the anthocyanin extracted from the calyx of *H. sabdariffa* shows peaks between the 200 and 400 nm range, as shown in Fig. 3c. The results showed that all the lipsticks were stable and had a good force of application and the breaking point reached 76.67–106.67 g. The melting points of the lipsticks containing 50%, 60%, and 70% castor oil were 56, 55, 53.5 °C, respectively, while the pH test resulted in 4.4, 4.7, and 5.2. In addition, the hedonic test showed that respondents liked the exciting color, fragrant smell, and oily texture of the lipsticks. The lipsticks themselves did not cause any irritation; therefore, they were safe to wear [11].

GC–MS of Anthocyanin Extracted from *H. sabdariffa*

GC–MS chromatogram of anthocyanin extracted from calyx of *H. sabdariffa* showed 23 peaks indicating the presence of 23 compounds. GC–MS analysis revealed the presence of compounds such as cyclootrisilixane, hexamethyl, α,β crotonolactone, pentanoic acid,4-oxo,ethyl ester, 2-oxopentanedioic acid, peonidin, butanedioic acid, hydroxy-dieth, 1,3-isobenzofurondione, pellargonidin, butanedioic acid, diethyl ester, 2-piperidinemethanol, dodecanoic acid, 1,2-benzene dicarboxylic acid, diethyl, 1,2,3-propane tricarboxylic...
acid, butanedioic acid, butanedioic acid, 3-hydroxy-2,2-DI, hexadecanoic acid, ethyl ester, 1-docosene, 9, 12, 15-octa decatrienoic acid, ethyl ester(z,z,z), E, E-1,9,17-docasatriene, eicosane, 2-methyl, cyclopentane,(4-octyldodecyl), dososanic acid ethyl ester, 4-decenoic acid ethyl ester, (z)-, 1-heptacoanol, 2-[(hexadecycloxy), methy]oxirane (Fig. 4a, b, c).

According to Sarpate et al., anthocyanin pigments extracted from fruits of Ficus racemose using acidified methanol solvent showed peaks at RT of 14.70 for peonidin and RT 25.3 for pellargonidin, which supports our study results [12]. Another report by Ovando et al. suggested that anthocyanins extracted from red grapes, plums, and capulin by MALDI-ToF MS analysis showed the presence of peonidin pigment at 301.1 in the spectral data [13].

The GC–MS analysis provided different peaks determining the presence of 15 compounds from *H. rosasinensis* flower. These compounds have biological activity namely
Fig. 3 Anthocyanin extraction. a Dried calyx of *H. sabdariffa* b Anthocyanin extraction from calyx of *H. sabdariffa* c UV analysis of anthocyanin extracted

| S. No. | Extraction and solvent | Absorbance |
|--------|------------------------|------------|
| 1      | 1 g of calyx extract & 5 ml of absolute ethanol | 0.784 |
| 2      | 1 g of calyx extract & 5 ml of 1 N acidified ethanol | 1.214 |
| 3      | 1 g of calyx extract & 5 ml of 1.5 N acidified ethanol | 1.976 |
| 4      | 1 g of calyx extract & 5 ml of 1% citric acid | 1.790 |
| 5      | 1 g of calyx extract & 5 ml of 2% citric acid | 1.635 |

ethanimidic acid, ethyl ester (31.43%), propanal, 2,3-dihydroxy (12.58%), 4H-pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6- methyl (10.69%), ethylenediamine (6.71%), o-methylisourea hydrogen sulfate (4.06%), ethene, ethoxy- (3.63%), methyl palmitate (2.99%), 7- formylbicyclo [4.1.0] heptanes (2.80%), 2-butanamine, (S)- (2.72%), 1,3,5-triazine2,4,6-triamine (2.48%), N-formyl-β-alanine (2.36%), (Z)6,(Z)9-pentadecadien-1-ol (1.70%), 1,2-ethanediamine, butanediol (1.65%), N-methyl-1-propanol, 2-methyl- (1.57%), and methanecarbothiolic acid (1.08%) [14].
Fig. 4 GC–MS of methanol extract of *H. sabdariffa*
Application of Dye to Cloth

The procedure used for application of dye to cotton cloth was given in Methods and Materials. NaOH is used to manufacture a variety of detergents and soaps used in home and commercial applications. It bleaches dirt and other unwanted material on clothes. Cloths were soaked in dye for half day, and they changed to light brown color. Cloth treated with Myrobalan + dye extract turned light reddish brown. Cloth treated with dye + K2Cr2O7 turned a shade of brown. Cloth treated with dye + FeSO4 turned a shade of blackish brown. Cloth treated with dye + SnCl2 gave light green + black (Fig. 5). According to a review, the color of anthocyanin varies with pH, which shows its adaptability to nature with varied environmental conditions. Research has shown that anthocyanin possesses antimicrobial properties. Advanced research using anthocyanin and its related gene could lead to development of textiles with antibacterial and self-fluorescence properties. Anthocyanin is also known to protect the plant in extreme weather conditions. This property could be used to develop super cloths [15]. The dye extracts applied to silk fabric with mordant-free dyeing show different colors under different pH conditions, changing between purple, blue, green, and yellow. However, the dyed colors are light and the dyeing rate is low. Metal mordant such as Sn in chelation enhances the dye depth and improves the fastness of the dyed silk fabrics, especially in silk fabrics dyed by premordanting and metamordanting [16].

Dye Sensitized Solar Cell

A dye sensitized solar cell is an imitation of a biological system, namely the photosystem of plant cells. Plant cells synthesize their own food by photosynthesis, and through this process, they make carbohydrates from sun light, water, and carbon dioxide.

\[
\text{Carbon dioxide + water + sun light} \rightarrow \text{carbohydrates}
\]

The overall process involves many chemical combinations and bonding of atoms of molecules, especially electrochemical processes. Dye sensitized solar cells work based on the electron transfer mechanism, and they are economically and performance wise stable (Fig. 6).

Length of the FTO glass = 5 cm
Breadth of the FTO glass = 9 nm
Cell in this experiment measured 5 cm by 9 nm.
Area of the glass is equal to

\[
\text{Area (A) } = 5 \times (9 \times 10^{-7}) = 0.0000045 \text{ m}^2
\]

Solar cell power

\[
P_s = \text{Si} \times A
\]

\[
\text{Si } = 1000 \text{ w/m}^2 A = 0.0000045 \text{ m}^2
\]

\[
PS = 1000 \text{ w/m}^2 \times 0.0000045 \text{ m}^2
\]

\[
Ps = 0.0045 W
\]

Dye sensitized solar cell from H.sabdariffa dye gives the result,
Fig. 5 Application of anthocyanin pigment on cloth

a. Treated with 10% NaOH + H₂O
b. Treated with myrobalan + anthocyanin
c. Treated with K₂Cr₂O₇ + anthocyanin dye
d. Treated with FeSO₄ + anthocyanin dye
e. Treated with SnCl₂ + anthocyanin dye
The maximum theoretical efficiency $E$ of the solar cell is estimated to be

\[ V_{OC} = 1.1 \]

\[ I_{SC} = 0.37 \]

\[ E = \frac{P_{Max}}{P_S} \]

The maximum theoretical efficiency $E$, of the solar cell is estimated to be
The maximum theoretical efficiency of solar cell is 46.86%.

Earlier studies have reported that the dyes have shown absorption in a broad range of the visible region (400–700 nm) of the solar spectrum and appreciable adsorption onto the semiconductor (TiO2) surface. The DSSCs made using the extracted dyes have shown that the open circuit voltages (oc) varied from 0.430 to 0.610 V and the short circuit photocurrent densities (sc) ranged from 0.11 to 0.29 mA cm\(^{-2}\). The incident photon-to-current conversion efficiencies (IPCE) varied from 12 to 37%. Among the four dyes studied, the extract obtained from teak has shown the best photosensitization effects in terms of the cell output [17].

### Anthocyanin Pigment for Lip Balm Formation

Lip balm or lip stick and any other cosmetic materials are highly sought after by humans. Cosmetics and perfume must undergo toxicity and stability tests [18, 19] (Table 2).

A double heating system was used to improve their ability to form an emulsion. It was also to prevent the loss of chemical and physiochemical properties of added samples. Sample pH was determined after adding all ingredients, and all cosmetics has an optimal pH of 5.8–7.0. With an increase in the acid–base balance, the irritating potential for skin is greatly elevated. The results showed that application of herbal dye lip care products using anthocyanin rich *H. sabdariffa* (L.) gives different colors when adding dyes with different pH, where 1.5 N HCl, 1% citric acid, and 2% citric acid showed a pH of 5.6 to 6 and showed no irritation when applied on skin (Fig. 7). Mayuri et al. have reported that using plant extracts in cosmetics is becoming more common and the natural lip balms are made with base oils, extracts, colors, and flavoring agents. They are also evaluated for the variations in the temperature, flavors, and smoothness during their application and for adherence properties [20].

![Math equation](https://example.com/math_equation.png)

\[ E = \frac{P_{\text{Max}}}{P_S} \]

\[ E = \frac{0.2109 W}{0.0045 W} = 46.86\% \]

The maximum theoretical efficiency of solar cell is 46.86%.

| S. NO | Ingredients                  | Importance               | Quantity (gm) |
|-------|-----------------------------|--------------------------|---------------|
| 1     | Beeswax                    | Glossy & hardness        | 12 g          |
| 2     | Shea butter                 | Blending properties      | 25 g          |
| 3     | Strawberry essence          | Flavoring agent          | 1.5 g         |
| 4     | *H. sabdariffa* sample     | Coloring agent, antioxidant agent | 3 ml |
| 5     | Vanilla essence             | Preservative             | 0.1 g         |

Table 2 | Ingredients for formulation of lip balm using anthocyanin pigment obtained from *Hibiscus sabdariffa*
**Conclusion**

*Hibiscus sabdariffa* (L.) is a rich source of anthocyanins and a medicinal plant used to make a variety of home and commercial products. Anthocyanin obtained from *H. sabdariffa* was studied for dye applications to reduce the use of chemical dyes in the fields of fabrics, automobiles, etc. Most of the studies reported on the acidified solvents used to extract and make intense coloration from plant materials. The use of plant tissue cultures are another boon to the synthesis of anthocyanins and production of the pigments through callus induction, and thereby treat the callus with increased sucrose content.

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**Fig. 7** Lip balm prepared using anthocyanin pigment extract from calyx of *H. sabdariffa*  
**a** Before usage of wax  
**b** After usage of wax
Different types of explants of *H. sabdariffa* were inoculated on MS medium supplemented with different hormone concentrations and hence initiation of callus was achieved. The callus obtained were further treated with increased sucrose concentration for the synthesis of anthocyanin pigment in the callus. Thus, the obtained anthocyanins from calli and calyx of *H. sabdariffa* was extracted by acidified solvent, which showed maximum extraction of the pigment. The pigment was used as a dyeing agent on various applications such as solar cells, lip balm, and coloration as dye for cloth. Thus, these applications help to prevent environmental degradation and water pollution. As chemical industries increase the use of chemicals, the effluent processed out after the treatment still contains trace chemical elements. Hence the extraction of enzymes using natural methods are an alternative for dye production to reduce pollution and to some extent to nourish and protect the environment for the future generations.

**Author Contribution** B.S. and B.L. contributed in data writing and designing of the study. B.S. and S.C. contributed in the preparation of the manuscript. M.S.A. contributed in revision of the manuscript. All the authors are responsible for the final manuscript.

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**Data Availability** Not Applicable.

**Declarations**

**Ethical Approval and Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors report no competing interest.

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