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# NATURAL PIGMENT AS AN ALTERNATIVE TRACKING DYE FOR GEL

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## ABSTRACT

Bromophenol blue can cause irritation with redness and pain when it comes in contact with the skin; can causes irritation to the respiratory tract and leads to allergic reaction. Symptoms may include coughing and shortness of breath. Natural pigment anthocyanin was extracted from the samples clitorea ternatea and tecoma stans by normal centrifugation process. So, this study is aimed to find alternative tracking dye for natural sources to replace the synthetic Bromophenol blue dye. Natural pigment derived from the plant sources including chlorophyll, carotenoids, flavonoids, anthocyanin, quinones, betacyanin etc. Natural dye have some similar characters to Bromophenol blue In this project I reported the stability of anthocyanin pigment at different pH temperature and demonstrated the effectiveness of pigment in tracking dye in agarose gel electrophoresis. That has content maintain their color over a wide range of pH 3 to 7. This flower sample is used as the tracking dye in place of Bromophenol blue. Every flower and leaves contain pigments but among them only some will have capacity to act as effective dye. Since, anthocyanin pigments are similar to Bromophenol blue, anthocyanin rich flowers like clitorea ternatea are used for the experiment.

**Keywords**: Clitorea Ternatea, Tecoma Stans, Tracking Dye, Bromophenol Blue, Gel Electrophoresis, Natural Pigments.

## I. INTRODUCTION

Nature is the only source which doesn't cause any disaster or damage to all lives. DNA isolation and band observing is an important process involved in the major research and laboratory. Dye is mainly synthesized by chemicals extensively used in many fields, such as textile industries, leather industry, paper production, food technology, agricultural experiments. Bromophenol blue and Xylene cyanol is a dye that has been used as a tracking dye in agarose or polyacrylamide gel electrophoresis Bromophenol blue is a chemical dye which may cause cancer and affection soil, there is further research going on this topic. It is a well-known fact that colorants from synthetic sources can be harmful and cause allergies for human. Therefore, interest in natural dyes has increased considerably during the last few years. Loading dyes are important in molecular biological experiments as they give colour and density to the sample and allow monitoring the progress of the sample on the gel. So, this study is aimed to find alternative tracking dye for natural sources to replace the synthetic Bromophenol blue dye. Natural pigment derived from plant sources including chlorophyll, carotenoids, flavonoids, anthocyanin, quinones, betacyanin etc. Gel electrophoresis is the technique used to separate and sometimes purify molecules, especially proteins and nucleic acids that differ in size, charge, and conformation. It is one of the most widely used in molecular biology technologies. Electrophoresis is the movement of the particles in the solution on electric field. DNA is negatively charged pole, it rate of migration of molecules depends on two factors, its shape and charge to ratio. As SDS adds to the negative charge of the protein the sample moves towards the anode in an electric field. In this project I reported the stability of anthocyanin pigment at different temperature, pH and demonstrated the effectiveness of the pigment in tracking dye in agarose gel electrophoresis. Every flower and leaves contain pigments but among them only selective will be have capacity to act as a effective dye.

## II. MATERIALS AND METHOD

The study was carried with fresh flowers samples such as Clitorea ternatea (blue pea) and Tecoma stans (yellow elder) from Hindustan college of arts and science Coimbatore.

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**Sample 1** **Sample 2**

**EXTRACTION OF PIGMENT:**

The flower samples were collected from the plant. 5-10 grams of flower sample were added into 5ml of water, ethanol and methanol were centrifuged at 10,000 rpm for 5-10 minutes to get clear extract of the anthocyanin and finally stored at 4°C. The resulting supernatant solution was considered to be tracking dye stock (Archana Deka et al.,2000).


**Sample 1** **Sample 2**

**EXTRACTION OF SAMPLE:**

**pH STABILITY:**

An effect of pH on stability of the anthocyanin was measured in a pH range from 2 to 12 by adding HCl and NAOH. Anthocyanin (10µl) was added to 2990 distilled water of different pH. After setting the pH ranges, the absorbance of the solution was measured from 400 and 600 using colorimeter .


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**THE EFFECT OF PH**:

The stability of the flower petal extracts of flowers of purplish blue was studied at different pH 2 to12. The extracts was produced pinkish red solution at 3-7 range while at pH from 8 and 9, the extracts produced blue solutions. Beyond pH 10, the solution was green to yellow color.

Anthocyanin is relatively stable at low pH (acidic condition) which gives the red pigment.

**TEMPERATURE STABILITY:**

A exact volume of 1ml of samples were taken in the Eppendorf tubes and kept the samples with different temperatures ranging from 20 C,40°C,60°C, 80°C, and 100°C for 1 hour and then absorbance values were measured using colorimeter at 540 nm.


**DETERMINATION OF MOISTURE CONTENT:**

To check the moisture content, Petri plates along with aluminium foil has to be taken. A volume of 1 ml of extract was added to that aluminium foil and measures the wet weight of sample kept the samples in hot air oven for 1 hour. After, 1 hour, the samples were completely dried and measure the dry weight of the sample.

**Wet weight**


**Dry weight**

**CONFIRMATION TEST FOR ANTHOCYANIN:**

2N HCl

A volume of 1ml sample extraction was added to 2ml of 2N HCl for 5 minutes, and the result was observed, it was appeared as pink-red in color.

**AMMONIA:**

After the colour change 2ml of ammonia was added to the sample extraction, then the color changed to purplish blue colour, after 5 minutes the result was observed.

**QUANTIFICATION OF ANTHOCYANIN:**

At last calculate the total anthocyanins by the absorbance at the appropriate wavelength according to Lambert-Beer law. The absorbance values of the samples to be tested is gradually diluted, so that the final sample absorbance value reading is <1.2. The details are noted as follows. Take samples of different volumes, dilute to

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the same volume with potassium chloride buffer solution (0.025M, pH 1.0), and equilibrate them in a dark environment at room temperature for 30 min. Finally, measure absorbance of the samples with different dilutions.

**Calculation of anthocyanin concentration**:

The concentration of anthocyanin pigment can be obtained using the following formula:

Anthocyanin pigment (mg/L) =- (A MWDF * 1000) (ε* 1)

**PLASMID DNA ISOLATION FROM E. coli:**

Grow the bacterial culture in LB medium at 37°C overnight with shaking. Transfer O/N culture to eppendorff tubes and spin at high speed for 1 minute. To the pellet resuspension solution was added and vortex it. Then Lysis solution was added and mixed gently by inverting tubes. Neutralizing solution was added and centrifuge at high speed. To the supernatant, ice cold ethanol was added and spin down plasmid DNA precipitate at high speed. Resuspend the pellet to 50µl TE buffer.

**AGAROSE GEL ELECTROPHORESIS:**

**PREPARING THE GEL BED:**

1. Close off the open ends to a clean and dry gel bed (casting tray) by using rubber dams|tape.
2. Place a well-former template(comb) in the middle of the notches. Make sure that the comb sits firmly and evenly across the bed
3. Use 250 ml flask to prepare the gel solution and add the following components to flask such as buffer concentrate, Distilled water and Agarose powder.
4. Swirl the mixture to disperse clumps of agarose powder.
5. With a marking pen, indicate the level of the solution volume on the outside of the flask.
6. Heat the mixture to dissolve the agarose powder.At The final solution should appear clear without any undissolved particles.
7. Cool the agarose solution to 55°C with careful swirling .If detectable evaporation has occurred, add some distilled water to make the solution up to the original volume as marked on the flask in step 5.
8. Seal the interface of the gel bed and tape that to prevent the agarose solution from leaking.
9. Pour the cooled agarose solution into the bed and make sure the bed is on a level surface.
10. Allow the gel to completely solidify and it will become firm and cool to touch after approximately 20 minutes.

## III. RESULTS AND DISCUSSION

The sample were crushed and centrifuged at 10,000 rpm for 5 minutes. The solutions were filtered using normal filter paper and the extracts were stored at 4°C.

**Temperature stability:**

A volume of 1 ml of samples was taken in Eppendorf tubes and kept the samples with different temperatures ranging from 10°C,20°C, 50°C and 100°C for 10 min and then absorbance values was measured using colorimeter at 540 nm. The readings were plotted in the graph for every sample and identified that the pigment was unstable at high temperature, when compared to normal temperature. They reported that decreased in copigmentation of anthocyanin at high temperature.

Sample 1- clitorea ternatea

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**Water**

| Temperature | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---|---|---|---|---|---|
| 10 | 0.480 | 0.456 | 0.438 | 0.415 | 0.388 |
| 20 | 0.412 | 0.397 | 0.353 | 0.335 | 0.310 |
| 30 | 0.383 | 0.345 | 0.380 | 0.335 | 0.254 |
| 40 | 0.313 | 0.290 | 0.300 | 0.280 | 0.190 |

**Ethanol**

| Temperature | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---|---|---|---|---|---|
| 10 | 0.565 | 0.480 | 0.445 | 0.385 | 0.310 |
| 25 | 0.446 | 0.410 | 0.380 | 0.304 | 0.258 |
| 50 | 0.386 | 0.360 | 0.297 | 0.255 | 0.217 |
| 100 | 0.287 | 0.257 | 0.210 | 0.180 | 0.164 |

**Methanol**

| Temperature | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---|---|---|---|---|---|
| 10 | 0.536 | 0.470 | 0.475 | 0.415 | 0.389 |
| 25 | 0.471 | 0.396 | 0.350 | 0.348 | 0.310 |
| 50 | 0.380 | 0.261 | 0.246 | 0.205 | 0.201 |
| 100 | 0.182 | 0.205 | 0.176 | 0.180 | 0.161 |

Sample 2 Tecoma stans

**Water**

| Temperature | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---|---|---|---|---|---|
| 10 | 0.530 | 0.476 | 0.434 | 0.410 | 0.392 |
| 20 | 0.461 | 0.382 | 0.356 | 0.346 | 0.310 |
| 30 | 0.315 | 0.270 | 0.238 | 0.245 | 0.231 |
| 40 | 0.202 | 0.190 | 0.178 | 0.155 | 0.129 |

**Ethanol**

| Temperature | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---|---|---|---|---|---|
| 10 | 0.535 | 0.470 | 0.456 | 0.410 | 0.384 |
| 25 | 0.457 | 0.389 | 0.370 | 0.353 | 0.296 |
| 50 | 0.370 | 0.295 | 0.270 | 0.254 | 0.177 |
| 100 | 0.286 | 0.181 | 0.160 | 0.130 | 0.090 |

**Methanol**

| Temperature | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---|---|---|---|---|---|
| 10 | 0.474 | 0.430 | 0.403 | 0.382 | 0.345 |

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| | | | | | |
|---|---|---|---|---|---|
| 25 | 0.380 | 0.354 | 0.330 | 0.235 | 0.214 |
| 50 | 0.266 | 0.235 | 0.265 | 0.179 | 0.179 |
| 100 | 0.175 | 0.160 | 0.135 | 0.085 | 0.065 |

**Confirmation test for Anthocyanin**

The presence of the anthocyanins has been demonstrated by adding 2 mL of the sample extract with 2 mL of 2 N HCI. The appearance of pink-red colour that turns into purplish blue after addition of ammonia indicates that the presence anthocyanins. By observing the results, I found that the anthocyanin was absent in tecoma stans (yellow elder) sample and the anthocyanin pigment presnt in the clitorea ternatea (blue pea) sample. So, I continued the balance test with clitorea ternatea sample. Determination of moisture content.

Moisture content study for the anthocyanin pigment samples

| S.NO | Fresh samples (clitore ternatea)with | Wet weight in grams | Dry weight in grams |
|---|---|---|---|
| 1 | Water | 0.99 | 0.03 |
| 2 | Ethanol | 0.92 | 0.04 |
| 3 | Methanol | 0.91 | 0.03 |

**pH stability**

Samples (10µl) was added to 2990 distilled water of different pH, after setting pH 2- 12, measured the reading in colorimeter at 400 nm. The anthocyanin pigment from clitorea ternatea was stable showing pinkish red colour between the pH ranges of pH 2-8, tecoma stans in brown color show stable between pH 3-5.

Stability of the anthocyanin in clitorea ternatea (fresh) samples was stable at pH 3-8, after pH 8 the absorbance was decreased. The readings were measured at 500 nm using calorimeter. Anthocyanin pigment pH values are calculated, that pH value of 4-8 have good results. Anthocyanins are more stable at low pH (acidic conditions) which gives a red pigment.

**Water**

| **pH** | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---|---|---|---|---|---|
| 2 | .083 | .088 | .090 | .091 | .098 |
| 3 | .098 | .097 | .092 | .093 | .095 |
| 4 | .092 | .098 | .098 | .121 | .163 |
| 5 | .141 | .142 | .134 | .118 | .112 |
| 6 | .098 | .197 | .085 | .080 | .071 |
| 7 | .107 | .104 | .102 | .091 | .063 |
| 8 | .100 | .098 | .095 | .086 | .067 |
| 9 | .081 | .112 | .105 | .093 | .090 |
| 10 | .074 | .108 | .103 | .096 | .082 |
| 11 | .033 | .127 | .122 | .108 | .094 |
| 12 | .041 | .130 | .129 | .117 | .068 |

Stability of anthocyanin pH in water

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**Ethanol**

| pH | Day 1 | Day 2 | Day 3 | DAY 5 |
|---|---|---|---|---|
| 2 | .113 | .104 | .098 | .067 |
| 3 | .107 | .105 | .093 | .072 |
| 4 | .116 | .102 | .098 | .074 |
| 5 | .164 | .170 | .173 | .179 |
| 6 | .182 | .176 | .170 | .153 |
| 7 | .214 | .203 | .191 | .167 |
| 8 | .150 | .144 | .142 | .103 |
| 9 | .132 | .134 | .145 | .178 |
| 10 | .149 | .154 | .160 | .172 |
| 11 | .133 | .127 | .118 | .074 |
| 12 | .139 | .130 | .102 | .083 |

Stability of anthocyanin pH in ethanol

**Methanol**

| Ph | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---|---|---|---|---|---|
| 2 | .099 | .084 | .081 | .063 | .051 |
| 3 | .087 | .082 | .072 | .065 | .042 |
| 4 | .102 | .086 | .062 | .059 | .048 |
| 5 | .151 | .144 | .141 | .140 | .112 |
| 6 | .140 | .135 | .128 | .126 | .122 |
| 7 | .123 | .114 | .112 | .105 | .100 |
| 8 | .130 | .120 | .115 | .104 | .097 |
| 9 | .180 | .162 | .133 | .130 | .105 |
| 10 | .213 | .191 | .178 | .163 | .112 |
| 11 | .192 | .163 | .132 | .125 | .098 |
| 12 | .174 | .163 | .093 | .097 | .077 |

**Quantification test for anthocyanin**

AC (Anthocyanin Content)(mg/L) = (A* MWDF * 1000) (ε* 1)

| | |
|---|---|
| Water | .090 |
| Ethanol | .098 |
| Methanol | .084 |

**FTIR analysis**

The Fourier Transform Infrared (FTIR) spectral analysis was done to identify the major functional groups presented in the extracted pigment (DeSouza et al., 2003).

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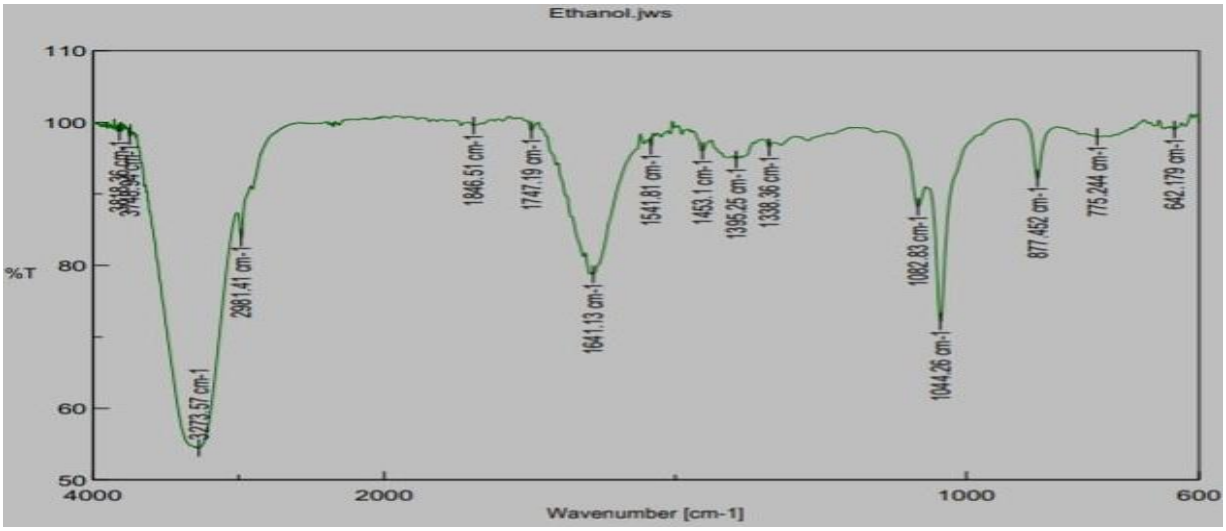
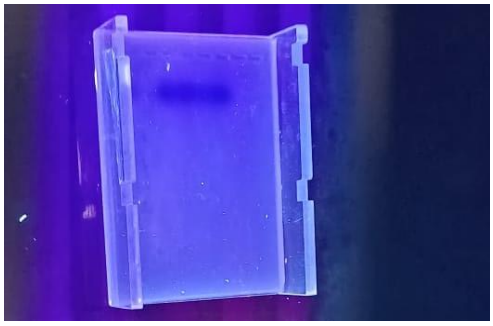


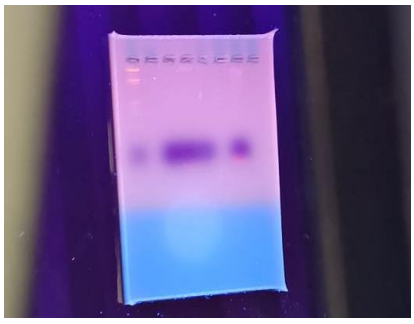

The peaks in the range of 3335-2938 cm −1 indicate the O-H functional group of sugars and phenols corresponding to the occurrence of anthocyanin compounds.

## IV. CONCLUSION

**Agarose Gel Electrophoresis:**

The anthocyanin was used as an alternative tracking dye to bromophenol blue in agarose gel electrophoresis. This was evaluated by using concentration of anthocyanin. The gel was examined under UV transilluminator after running for 45 minutes at 100 Volt. The effectiveness of anthocyanin pigment was determined after the DNA bands were for formed.





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