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# STUDY ON ISOLATION AND PURIFICATION OF ANTHOCYANINS AND ITS APPLICATION AS pH INDICATOR

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## ABSTRACT

Extraction and purification of Anthocyanin pigments from Black Raspberries (Rubus occidentalis) was successfully carried out. Homogeneous and fine powder was obtained from the liquid nitrogen blending process. After extraction and purification of the plant material, a colored solution was obtained. Depending on the nature of the predominant anthocyanin and its concentration, the coloration of the solution is pink/red or purple. The solution obtained after extraction was dark brownish red coloured. Two different methods were used for extraction viz. extraction with acetone/chloroform partitioning and extraction with methanol. In the acetone/chloroform procedure, the upper phase shows colouration due to extraction of pigment in acetone while chloroform containing layer is only slightly coloured. The extraction was followed by partial purification procedures using Column chromatography and TLC. The column chromatography process was optimized for methanol extracts. The fractions collected from column analysis were subjected to thin layer chromatography. TLC analysis revealed the presence of Anthocyanins in all fractions in varying concentrations but only fraction-4 was shown to have the $R_f$ value close to that of pure Anthocyanins. Anthocyanin as natural pH indicator was studied.

**KEY WORDS** -Anthocyanin, Flavanoids, Thin layer Chromatography, pH-indicators.

## INTRODUCTION

Anthocyanins are water-soluble vacuolar pigments that may appear red, purple, or blue according to pH.[2]. In addition to traditional markets for consumption of fresh and processed black raspberry fruit, there is a long history of its use as a natural colourant and dye because of its high anthocyanin level.[8,10]. They belong to a parent class of molecules called flavonoids synthesized via the phenylpropanoid pathway; they are odorless and nearly flavorless, contributing to taste as a moderately astringent sensation [19,20] .Anthocyanins occur in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits.. Anthocyanins are derivatives of anthocyanidins which include pendant sugars.[9]. Anthocyanins represent one of the most widely distributed classes of flavonoids in plants. The difference in chemical structure that occurs in response to changes in pH is the reason that anthocyanins are often used as pH indicator, as they change from red

in acids to blue in bases. This unique property of these pigments has been exploited as an application of anthocyanins during the project work [16].

## 2. MATERIALS AND METHOD

### 2.1. Plant materials:

*Rubus occidentalis* is a deciduous shrub growing to 2–3 m tall, with prickly shoots. The leaves are pinnate, with five leaflets on leaves strong-growing stems in their first year, and three leaflets on leaves on flowering branchlets. The flowers are distinct in having long, slendersepals 6–8 mm long, more than twice as long as the petals. The round-shaped fruit is a 12–15 mm diameter aggregation of drupelets; it is edible, and has a high content of anthocyanins [15,23].

### 2.2. Extraction and purification of anthocyanins:

The plant material was frozen with **Liquid Nitrogen** and powdered using a Blender suitable for use under extremely low temperatures. The use of liquid nitrogen minimizes anthocyanin degradation by lowering the temperature and providing a nitrogen environment. The fine powder maximizes pigment recoveries due to its high surface area and favors disruption of cellular compartments. later on from the crushed material anthocyanin was extracted by following Methanol and Acetone-chloroform extraction method[4,16]. Later on the extract from respective methods was subjected to column chromatography and the absorbance was checked for fraction exhibiting pinkish colour(checked for all the fractions). TLC was also done for that fraction using particular mobile phase.[12]

### 2.3. pH indicator application

Anthocyanin exhibit various colour at different pH. So it can be use as natural source for pH indication [11,14,17]. The Anthocyanin extract was accurately measured as 1ml and was taken in different, clean test tubes. The pH of solution in each test tube was adjusted by 1N HCL and 1N NaoH sequentially from 1 to 12 using the acid and base and pH meter for accurate pH adjustments. The contents in each tube were properly mixed and the colour development was observed and recorded [1].

## 3. RESULTS AND DISCUSSION:

### EXTRACTION:-

**TABLE 1.** Extraction: Final volume made in acidified distilled water

| METHOD | PM (gm) | E-1 (ML) | E-2 (ML) | TOTAL-E (ML) | FINAL VOL (ML) |
|---|---|---|---|---|---|
| Acetone-Chloroform extraction | 250 | 300 | 300 | 600 | 600 |
| Methanol Extraction | 250 | 782.5 | 782.5 | 1565 | 1600 |

### COLUMN CHROMATOGRAPHY:-

**TABLE.2. 1.**SAMPLE A-(METHANOL METHOD EXTRACT)

| FRACTION NO. | COLOUR |
|---|---|
| 1 | Dark red |
| 2 | Pinkish red |
| 3 | Light pink |
| 4 | Fluorescent Yellow |

**TABLE 2.2.** SAMPLE B-ACETONE

### METHOD EXTRACT

| FRACTION NO. | COLOUR |
|---|---|
| 1 | Dark red |
| 2 | Pink WITH YELLOW RING |

### THIN LAYER CHROMATOGRAPHY:-

**TABLE 3.1**

| Mobile phase: (for 10 ml) | Amount (ml) | Result |
|---|---|---|
| Conc HCL:FORMIC ACID:WATER | 1.9**:** 3.96**:**4.14 | NO CLEAR BAND SEPARATION WAS OBTAINED |
| n-Butanol:Acetic acid:water | 4**:**1**:**5 | Below table3.2 |

**TABLE 3.2.** Solvent run-6 cm

| COLOUR | DISTANCE MIGRATED | Rf value |
|---|---|---|
| Faint blue | 3.9 | 0.65 |
| Pink | 1.5 | 0.25 |
| Blue | 3,5 | 0.58 |
| Faint blue | 4 | 0.66 |

**pH result :-**

**Table 4:** Results of pH application

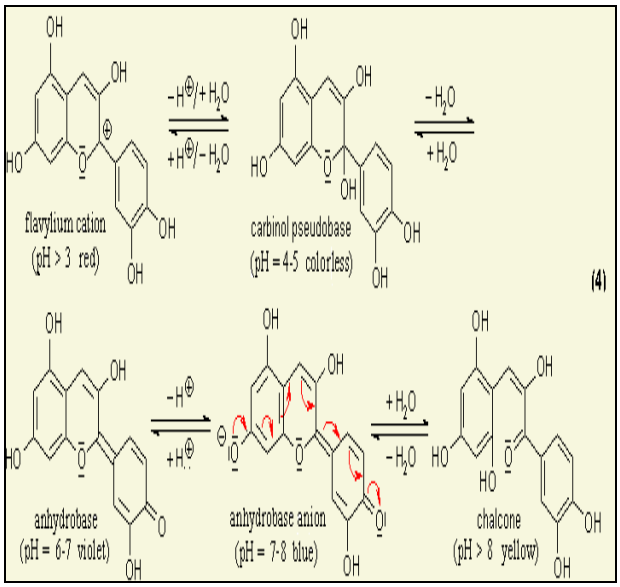
| pH | Colour |
|---|---|
| 1. | Dark red |
| 2. | Dark pink |
| 3. | Pinkish red |
| 4. | Faint pink |
| 5. | Violet |
| 6. | Faint violet/blue |
| 7. | Faint green |
| 8. | Faint green |
| 9. | Bluish green |
| 10. | Green |
| 11. | Yellowish green |
| 12. | Yellow |

### 3.1 Anthocyanins as pH Indicators

Formed, recovery of the flavylium form cannot be achieved by simple re-acidification. As is discussed before, the colour of Anthocyanin pigments changes drastically with change in pH value [7, 22]. The color of anthocyanins depends on the acidity of the medium. At acidic pH = 1-3, anthocyanidins exist predominantly in the form of the red flavylium cation (2-phenylchromenylium cation). Increasing the pH leads to a decrease in the color intensity and the concentration of the flavylium cation which undergoes hydration to produce the colorless carbinol pseudobase (hemiacetal or chromenol). The conjugated 2-benzopyrilium system is disrupted due to a nucleophilic attack of water at the 2-position of the anthocyanidin skeleton. A rapid proton loss of the flavylium cation takes place as the pH shifts higher. Now the equilibrium is shifted toward a purple quinoidal anhydrobase at pH < 7 and a deep blue ionized anhydrobase at pH < 8. When pH increases further the carbinol form yields, through opening of the central pyran ring, the light yellow chalcone. The color of the alkaline solutions can be reverted by changing the pH back to acidic. The anthocyanidin equilibrium forms shift back to the equilibrium where the red colored flavylium cation predominates [3,18,21].

The anthocyanidin system undergoes a variety of molecular transformations as the pH changes. In aqueous solutions, anthocyanidins exist as essentially five molecular species in chemical equilibrium that are already mentioned in above paragraph: red flavylium cation, colorless carbinol pseudo base, purple quinoidal base, blue quinoidal base anion, and yellowish chalcone. The changes in pH values reflect in the transformation amongst these ions ultimately resulting in the changes in the colour of the solution containing Anthocyanins[5,6,13].

**Fig.1** - Structures of cyanidins in aqueous solution under varying pH




The photograph shown below explains the application of Anthocyanins as pH indicator. The colours shown by each test tube were noted down accurately.

**Fig:2-** application of Anthocyanins as pH indicator.

## 4. CONCLUSION

The application of purified Anthocyanins as natural pH indicator was studied. The colour of Anthocyanin pigments changes drastically with change in pH value. The color of anthocyanins depends on the acidity of the medium. At acidic pH = 1-3, anthocyanidins exist

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