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Review on Anthocyanins as herbal pH indicators

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ABSTRACT

In the acid-base titrations, standard synthetic indicators are used to exhibit color change at different pH intervals. Anthocyanins (natural color pigments) in plants exhibit color changes with pH variations. The aim of this study is to investigate the activity of plant extracts, Rosa indica and Rubus occidentalis, to replace the synthetic indicators. Synthetic indicators cause chemical and environmental pollution, are toxic, expensive and rarely available. Methanolic extracts of Rosa indica and Rubus occidentalis exhibit sharp and intense color change in comparison to the standard synthetic indicators phenolphthalein and methyl orange. Herbal indicators are pollution-free, non-toxic, easily available and cost-effective.

Keywords— *Anthocyanins, herbal, pH indicators, and plant extract*

1. INTRODUCTION

Fundamental chemistry laboratory technique used for the quantitative analysis of substances with unknown concentrations and using standard solutions with known concentrations is termed as titration. The substance with known concentration is termed as analyte while the standard solution is termed as the titrant[1].

The conventional varieties of titrations include acid-base titrations, redox, and complexometric titrations. Neutralization with an acid or base of known concentration is done to determine the unknown concentration of an acid or base in acid-base titration[1]. The most commonly used indicators in acid-base titrations are phenolphthalein, phenol red, bromocresol blue, methyl red, methyl orange, bromophenol blue, etc[1].

pH indicators are generally weak bases or weak acids indicate color change according to the pH of the solution to which the indicator is added. The range of pH over which the color change occurs varies with the indicator used[2]. The frequently and most commonly used acid-base indicator which has a broad range for the color change is “litmus”. It is therefore used for detecting acids and bases over a wide pH range, whereas, synthetic indicators such as methyl orange or phenolphthalein are used only when the solutions are highly acidic or basic respectively[1].

Most of the commercially available standard synthetic indicators are expensive. Additionally, they are toxic as well as flammable. Also, they show disadvantages such as availability, environmental pollution, and chemical pollution. Furthermore, the use of synthetic indicators for food applications is avoided or minimized to a greater extent due to their potentially deleterious effects on human beings[3].

To triumph over the disadvantages of synthetic indicators, researches are being carried out extensively by scientists in the field of natural products as they are less hazardous to humans, cost-effective, easy availability and eco-friendly nature[3]. Natural pigments or dyes in plants are rarely toxic, pollution-free and easy to prepare or extract. Various organic and inorganic compounds are responsible for the color property of plant parts such as anthocyanins. Anthocyanins exhibit different colors in different pH, and therefore, can be used as natural indicators[4].

Anthocyanins are vacuolar water-soluble pigments that may appear purple, red or blue according to pH. They belong to the parent class of molecules termed as flavonoids which are synthesized via the phenylpropanoid pathway. They occur in all tissues of the higher plants including stems, roots, leaves, fruits, and flowers. The difference in the chemical structure that arises due to the pH change is the reason for the often use of anthocyanins as a natural pH indicator, as they change from red in acid to blue in bases[5].

In this paper, an attempt is made to study the use of anthocyanins from two different plants as herbal indicators, substituent to synthetic indicators. The plant material studied is *Rosa indica* and *Rubus occidentalis* (black raspberries).

2. ROSA INDICA

The *Rosa indica* is a yearly flowering plant that belongs to the family Rosaceae and is well known for various pharmacological activities. It is easily available in India as well as throughout the world in enough quantity. The presence of color pigments and chemical constituents like anthocyanins, flavonoids are accountable for thought about its use as an herbal indicator[6].

2.1 MATERIALS AND METHODS

2.1.1 Plant Materials: From the Bhor region and the garden of R.D's College of Pharmacy Bhor, Pune (M.S.) fresh flowers of *Rosa* indica were collected and authenticated at the Dept. of Botany, A.T. College, Bhor.

2.1.2 Reagents: Reagents of analytical grade like hydrochloric acid (HCL), sodium hydroxide (NaOH), acetic acid (CH_3COOH), ammonia (NH_3) and phenolphthalein were procured from R.D's College of Pharmacy Bhor, Pune (M.S.). The volumetric solutions and reagents were prepared according to the Indian Pharmacopoeia.

2.1.3 Preparation of Extract: The maceration of 1 gm fresh petals of *Rosa indica* was done for 24h in 10 ml of methanol.

2.1.4 Experimental Procedure:

- Cleaning of the petals was done with distilled water and the petals were cut into small pieces followed by maceration for 24h in 10 ml of methanol.
- Preservation of the extract was done in a tightly closed glass container and stored away from direct sunlight.
- Measuring apparatus and other required instruments were calibrated and standardization of acids and bases were done according to the guidelines given in Indian Pharmacopoeia.
- Titration of 10 ml of a titrant with two drops of indicator *Rosa indica* (R. I.) against titrates was done and the color changes for the indicators were listed.
- The screening results for the strong acid-strong base (HCl - NaOH), strong acid- weak base (HCl . CH3COOH), weak acid-strong base (CH3COOH - NaOH) and weak acid-weak base (CH3COOH . NH3) were listed.
- Each titration was carried out five times by using 1N strength of acid and alkali.
- Results were recorded as mean ± SEM.

2.1.5 Results and Discussion: Equivalence point obtained for all types of titrations by methanolic extract of *Rosa indica* either exactly coincided or was very close to the equivalence point obtained by standard synthetic indicator phenolphthalein. This exemplifies that the alcoholic flower extract is advantageous as an indicator of acid-base titrations. As it gives sharp color change at the equivalence point, its use in strong acid - strong base titration was found to be more significant over the standard synthetic indicator. It was observed that indicators act reversibly and give a sharp color change in both directions. The obtained results indicated that substitution of the routinely used indicators can be done successfully by flower extract as they are simple, less hazardous to humans, cost-effective, readily available, eco-friendly, accurate, and precise and can be prepared just before the experiment.

Table 1: *Rosa indica* (R. I.)

Titrant	Indicator	Color	Titrate	Color (at end pt.)
HCL	*Rosa indica*	Colourless	NaOH	Pink
CH_3COOH	*Rosa indica*	Colorless	NaOH	Pink
HCL	*Rosa indica*	Colorless	NH_3	Pink
CH_3COOH	*Rosa indica*	Colorless	NH_3	Pink

Table 2: Volume of titrate with standard indicator

Chemicals		Titrate volume required for equivalent point with titrant (10 ml) with indicator	
Titrant (1N)	**Titrate(1N)**	**Std. Ind.**	**Rosa Indica Ind.**
HCL	NaOH	10.1 ± 0.2	10.1 ± 0.39
CH_3COOH	NaOH	9.5 ± 0.35	9.8 ± 0.41
HCL	NH_3	9.8 ± 0.62	10 ± 1.02
CH_3COOH	NH_3	10 ± 0.40	10.2 ± 0.15

3. RUBUS OCCIDENTALIS (BLACK RASPBERRIES)

Rubus occidentalis is a species of *Rubus* which is native to eastern North America. Its common name black raspberry. Other names occasionally used are wild black raspberry, black cap raspberry, thimbleberry, and scotch cap[7]. It is a deciduous shrub that grows to 2–3 m tall and has prickly shoots. The leaves are pinnate in nature. The flowers are distinct and have long slender sepals 6–8 mm long. The round-shaped fruit is an aggregation of a drupelet of 12–15 mm diameter. It is edible and is rich in anthocyanins[5].

3.1 MATERIALS AND METHODS

3.1.1 Plant Material: Black Raspberries were chosen due to their high Anthocyanin content (300mg/100gm) and availability. The fruit is perishable and requires stringent conditions for its storage.

3.1.2 Experimental Procedure:

- The plant material was frozen using Liquid Nitrogen and was powdered using a Blender which is suitable for use under extremely low temperatures.

- Anthocyanin degradation was minimized with the use of liquid nitrogen as it provides low temperature and favorable nitrogen environment.
- Due to the high surface area and disruption of cellular compartments, the fine powder maximizes pigment recoveries.
- Methanol and Acetone-chloroform extraction methods were used for the extraction of anthocyanin from the crushed material.
- Further, the extract obtained from respective methods was subjected to column chromatography and the absorbance for fraction exhibiting pinkish color was checked (checked for all the fractions).
- Additionally, TLC was done for respective fractions using a particular mobile phase.

3.1.3 Evaluation: The Anthocyanin extract was precisely measured to 1ml and was taken in different clean test tubes. The pH of the solution in each test tube was adjusted using 1N HCL and 1N NaOH sequentially from 1 to 12 using the acid and base and pH meter for accurate pH measurement. The contents in each tube were uniformly mixed and the color developed was observed and noted.

3.1.4 Results and Discussion:

a) Extraction:

Table 3: Extraction: Final volume made in acidified distilled water

Method	Plant material (gm)	Extract – 1 (ml)	Extract – 2 (ml)	Total extract (ml)	Final volume (ml)
Acetone – Chloroform extraction	250	300	300	600	600
Methanol extraction	250	782.5	782.5	1565	1600

b) Column Chromatography:

Table 4: Sample A (Methanol method extraction)

Fraction number	Color
1	Dark red
2	Pinkish red
3	Light pink
4	Fluorescent yellow

Table 5: Sample B (Acetone method extraction)

Fraction number	Color
1	Dark red
2	Pink with yellow ring

c) Thin Layer Chromatography:

Table 6: TLC

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 7: Solvent run-6 cm

Color	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

d) ph result:

Table 8: Results of pH application

pH	Color
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

Fig. 1: Application of Anthocyanins as pH indicator

3. CONCLUSION

a) The obtained results from the above study indicated that substitution of the routinely used synthetic indicators can be done successfully by flower extract as they are simple, less hazardous to humans, cost-effective, readily available, eco-friendly, accurate, and precise and can be prepared just before the experiment.

b) From the above data, the application of purified anthocyanins as a natural pH indicator was studied. The color of anthocyanin pigments showed drastic changes with a change in pH value. The color of anthocyanins depends upon the acidity of the medium. At acidic pH = 1-3, anthocyanidins exist.

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