CHAPTER 3
RESEARCH METHODOLOGY

Anthocyanin pigment

Aim

To extract and characterize the natural pigment (anthocyanin) from fresh red rose petals using 95% ethanol and to evaluate its physicochemical properties, including solubility, pH stability, thermal stability, and water resistance.

Objectives of the Study

The main objectives of the present study were:

- To extract natural pigment from fresh red rose petals using 95% ethanol.
- To prepare an ethanolic extract by maceration using a mortar and pestle.
- To observe the physical characteristics of the obtained pigment extract.
- To study the solubility of the extracted pigment in different solvents.
- To investigate the pH stability and colour behaviour of the pigment.
- To study the thermal stability of the extracted pigment.
- To investigate the water resistance of the extracted pigment.
- To assess the suitability of red rose petals as a source of natural pigment.

Materials required

- Fresh red rose flowers
- Ethanol 95%
- Distilled water
- Dilute HCl and dilute NaOH

Apparatus required

The following apparatus were used:

- Mortar and pestle
- Conical flask(250mL)
- Beakers
- Measuring cylinder
- Funnel
- Normal filter paper
- Glass rod
- Weighing machine
- Water bath

- Test tubes and test-tube stand
- pH meter
- Aluminium foil

Principle

Anthocyanins are naturally occurring water-soluble flavonoid pigments responsible for red, purple, violet, and blue colours in many plant materials. The coloured compounds present in red rose petals can be extracted into a suitable polar solvent such as ethanol.

This pigment is more stable in acidic condition and can be extracted efficiently using acidified ethanol.

Extraction procedure

1)Fresh red rose flowers were collected for the experiment.

2) The petals were separated from the flowers, and unwanted portions such as sepals and stalks were removed. The petals were washed thoroughly with distilled water to remove dust and other adhering impurities.

3) The cleaned petals were allowed to dry to remove excess surface water.

4) Exactly 10g of fresh red rose petals were weighed using a weighing balance.



Preparation of the Extract

1)The fresh rose petals were cut into small pieces and transferred into a clean mortar.

2) A small quantity of 95% ethanol was added to the petals, and the petals were thoroughly crushed using a mortar and pestle.

3)Grinding was continued until the petals were finely macerated and the ethanol developed a deep red-pink colour.

4) The remaining required quantity of 95% ethanol was gradually added during grinding to facilitate extraction of the pigment.





5) The resulting rose-petal and ethanol mixture was transferred into a clean conical flask.

6) The conical flask was covered with aluminium foil and the mixture was kept at room temperature for approximately 24hours. The flask was gently shaken from time to time to facilitate contact between the petals and solvent.

Filtration and concentration of extract

7) After approximately 24hours of extraction,the mixture was filtered using a funnel and ordinary filter paper. The filtrate was collected in a clean beaker.

8) The filtered extract was then placed in a water bath maintained at approximately 40-45°C. The extract was gently concentrated until its volume was reduced to approximately 8-10mL. Care was taken not to boil the extract.



9) After concentration, the extract was transferred to a clean conical flask and was used for further characterization.



Anthocyanin Pigment Extract

Characterization Methods

The extracted red rose pigment was characterized by studying its physical characteristics, solubility, pH stability, thermal stability, and water resistance.

1.Physical Characteristics

The obtained pigment extract was visually examined for its colour, physical state, appearance, odour. The observations were recorded to obtain the preliminary characteristics of the extract.

2.Solubility Test

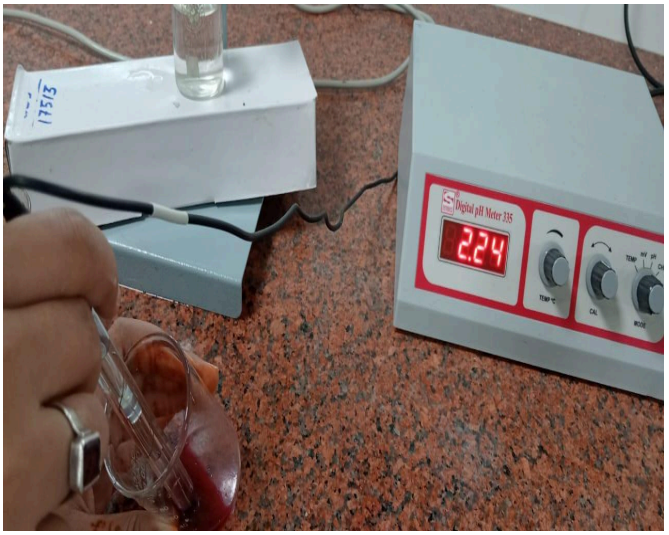
The solubility of the pigment extract was tested using selected solvents such as distilled water, 95% ethanol and acetone. About 1 mL of pigment extract was mixed with 2 mL of each solvent in separate test tubes. The degree of dissolution and colour of each mixture were observed and recorded.



3.pH Stability Test

The stability of the pigment at different pH conditions was studied. The colour of each sample was recorded initially and after 1 hour to observe changes in colour and pigment stability.





4. Thermal Stability Test

The thermal stability of the pigment was studied by exposing separate samples to 40°C, 60°C, and 80°C using a water bath. A control sample was maintained for comparison. Colour changes were observed and recorded after 30 minutes to assess the effect of temperature on pigment stability.

5.Water Resistance Test

A piece of cotton cloth was dipped in the prepared rose-petal pigment extract and allowed to absorb the pigment for a specified period. After the required time interval, the coloured cloth was washed with distilled water. The cloth was then observed for colour retention, colour fading.The observations were recorded to assess the water resistance of the extracted natural pigment.



Curcumin Pigment

Aim

To extract the natural pigment curcumin from turmeric powder using ethanol and to characterize the obtained curcumin-containing extract on the basis of its physical and chemical properties.

Objectives of the study

The main objectives of the experiment are:

- To extract the natural pigment curcumin from turmeric powder using ethanol as an extraction solvent.
- To obtain a coloured curcumin-containing extract through a simple solvent extraction method.
- To observe the physical characteristics and colour of the extracted pigment.
- To study the solubility behaviour of the extracted pigment.
- To investigate the effect of pH on the colour of the extracted pigment.
- To study the thermal stability of the extracted pigment under selected experimental conditions.
- To investigate the resistance of the pigment to water or washing.
- To record and interpret the observations obtained during the characterization of the extracted pigment.

Materials Required

- Turmeric powder-5g
- Ethanol-50mL
- Distilled water

Apparatus required

- Conical flask
- Measuring cylinder
- Weighing balance
- Glass rod
- Funnel
- Filter paper
- test tubes and test tube stand
- Dropper
- pH-meter

Principle

Curcumin is a naturally occurring yellow-orange polyphenolic pigment present mainly in the rhizomes of Curcuma longa (turmeric). It is responsible for the characteristic yellow colour of turmeric.The principle of the experiment is based on solvent extraction. Ethanol is used as the extraction solvent to dissolve curcumin and other soluble constituents from turmeric powder. When turmeric powder is brought into contact with ethanol, the soluble pigment components migrate from the solid plant material into the solvent. Gentle shaking at regular intervals increases the contact between the turmeric particles and ethanol and facilitates extraction.

After the extraction period, the mixture is filtered to separate the insoluble turmeric residue from the coloured ethanolic extract. The resulting yellow-coloured filtrate is used for further characterization.

Extraction Procedure

1)5g of turmeric powder was accurately weighed using a weighing balance.

2) The measured turmeric powder was transferred into a clean conical flask.

3) 50mL of ethanol was added to the turmeric powder.

4) The turmeric powder and ethanol were mixed thoroughly using a glass rod to ensure proper contact between the plant material and the solvent.

5) The conical flask was closed and the mixture was allowed to stand for approximately 30-60minutes for extraction.During the extraction period, the flask was gently shaken every 5-10 minutes to improve contact between the turmeric powder and ethanol and facilitate the transfer of pigment into the solvent.During extraction, the ethanol gradually developed a characteristic yellow to yellow-orange colour, indicating the extraction of coloured compounds from the turmeric powder.

6) After completion of the extraction period, the mixture was filtered using filter paper.



7) The insoluble turmeric powder residue was separated from the coloured filtrate.

8) The resulting yellow-coloured filtrate was collected in a clean container and used for further characterization studies.



Characterization Methods

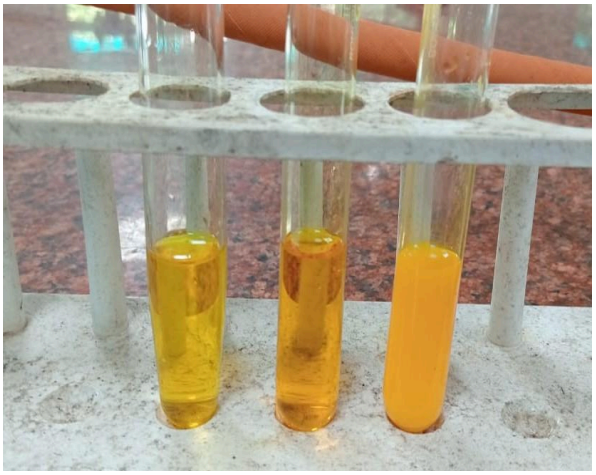
The extracted curcumin-containing pigment from turmeric powder was characterized by studying its physical characteristics, solubility, pH stability, thermal stability, and water resistance.

1. Physical Characteristics

The obtained curcumin extract was visually examined for its colour, physical state, appearance, odour. The observations were recorded to obtain the preliminary characteristics of the extracted pigment.

2. Solubility Test

The solubility of the curcumin extract was tested using selected solvents such as distilled water, ethanol, and acetone. About 2mL of the pigment extract was mixed with 4mL of each solvent in separate test tubes. The degree of dissolution, colour intensity of each mixture were observed and recorded.



3. pH Stability Test

The stability of the curcumin pigment under different pH conditions was studied.The colour of each sample was recorded initially and after 1 hour to observe changes in colour and pigment stability.





4. Thermal Stability Test

The thermal stability of the curcumin containing pigment was studied by exposing separate samples to 40°C, 60°C, and 80°C using a water bath. A control sample was maintained for comparison. Changes in colour and appearance were observed and recorded after 30minutes to assess the effect of temperature on pigment stability.



5. Water Resistance Test

A piece of cotton cloth was dipped in the prepared turmeric/curcumin pigment extract and allowed to absorb the pigment for about 5-10 minutes. After the specific time interval, the coloured cloth was washed with distilled water. The cloth was then observed for colour retention and colour fading.



