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Extraction of Natural Dye from Red Rose Flowers

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Abstract

In this research work, locally available red flowers of the red rose are selected for the extraction of anthocyanin. Ethanol solvent was used to extract anthocyanin from flowers. Different methods are used to obtain natural dyes from flowers. FTIR, pH, and UV tests were performed to characterize the extracted anthocyanin. The pH differential method was used to measure the total anthocyanin content in red rose flowers. The color of the solution is deep red under acidic conditions. The anthocyanin content determined in red rose was 138.68 mg/L. Natural dyes are extracted and applied to 100% pure cotton for fabric dyeing. Mordants play an important role in enhancing the color strength of the dye obtained from red rose flowers. In this project we use the aluminum acetate, potassium dichromate, aluminum sulphate; ferrous sulphate; and tannic acid are used as mordant to increase the color strength of natural dye. Washing and rubbing with 20 surfactants test is carried out, we conclude the strength of color is increased with the help of mordant chemical components. In this research work, locally available red flowers of the Red rose are selected for the extraction of anthocyanin. Ethanol solvent was used to extract anthocyanin from flowers. Different methods are used to obtain natural dyes from flowers. FTIR, pH, and UV tests were performed to characterize the extracted anthocyanin. The pH differential method was used to measure the total anthocyanin content in red rose flowers. The color of the solution is deep red under acidic conditions. The anthocyanin content determined in red rose was 138.68 mg/L. Natural dyes are extracted and applied to 100% pure cotton for fabric dyeing. Mordants play an important role in enhancing the color strength of the dye obtained from red rose flowers. In this project we use aluminum acetate, potassium dichromate, aluminum sulphate, ferrous sulphate, and tannic acid as mordants to increase the color strength of natural dye. Washing and rubbing with 20 surfactants test is carried out, we conclude the strength of color is increased with the help of mordant chemical components. Additionally, the dyed fabrics exhibited good color fastness properties, indicating their potential for sustainable textile applications. The use of red rose-derived anthocyanins as natural dye provides an eco-friendly alternative to synthetic dyes, reducing environmental hazards and promoting green chemistry.

Keywords: Cotton fabrics, red rose flowers, ethanol, sample preparation, extraction, mordant

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INTRODUCTION

Nowadays, we have great research work on anthocyanins. Dyeing is a complex, specialized in science. Anthocyanin pigments are soluble in water and anthocyanin pigments are present in fruits, vegetables, leaves, flowers, and nuts. The colors of anthocyanin is blue, red, or purple, which are found in plants, fruits, especially flowers in acidic condition [1, 2]. The stability of anthocyanins depends on factors, such as pH, light, temperature, and their structural composition. They are highly unstable and prone to degradation. Chemically, anthocyanins are based on the flavylium cation (2-phenyl-1-benzopyrilium), which can be linked with hydroxyl (–OH) and/or methoxyl (–OCH_3) groups,

along with one or more sugar molecules [3]. In plants, six main types of anthocyanins are commonly found: cyanidin, peonidin, pelargonidin n, delphinidin, malvidin, and petunidin. Among these, cyanidin is widely present in flowers and fruits, and it is considered a natural organic compound. Pelargonidin pigment generates orange color and it is used in food and industrial dyes. Peonidin pigments have antioxidants and metabolite, it gives red-purple color from flowers. Delphinidin pigment presents in foods in blue, red color and have antioxidant properties. Petunidin originates from Delphinidin and is an O-methyl anthocyanidin of the 3-hydroxy type. Malvidin pigments color is red and found in flowers; malvidin is an O-methyl anthocyanidin [4–6]. A natural dye is synthesis from plants and flowers that has recently gained economic advantage because of their nontoxic and biodegradable nature [5]. Most natural dyes have antioxidant properties and natural dyes do not cause any sort of irritation. The health benefits of natural dyes are many, so they are used in pharmaceutical [6]. The applications of anthocyanin have been as an excellent antioxidant enlarged on nutritional, cosmetic, and pharmaceutical industry, such as heart disease, cancer, and age-related eye diseases, might be significantly reduced by diets rich in lutein [6–9]. Some pieces of research have been done to investigate the potentials of anthocyanin in flowers and fruits as natural colors. The shades of anthocyanin extracts from berries and flowers are also promising source for textile dyes. [9, 10] The shades made from natural dyes are generally soft, shiny, and pleasing to the human eye. Using different mordant chemical components can give a different color which also depends on the source of the dye. They are also obtained from renewable sources. The use of natural dyes does not pose a disposal problem, as they are biodegradable [11, 12].

Fabrics dyed with anthocyanin extracts can be very useful in developing clothing products that protects people against allergies and skin irritation and can replace synthetic dyes [13–15] in kids' garment and foodstuffs for safety purpose.

LITERATURE REVIEW

Anthocyanins are natural plant pigments that provide health benefits to both humans and animals. They are easily recognized by their red, blue, or purple coloration, commonly found in berries, red grapes, apples, pears, radishes, and red or purple cabbage. Anthocyanins may also be consumed through their use as a food coloring and as nutritional supplements, acquired as anthocyanin-rich fruit extracts, powders, and purified compounds. Plants make anthocyanins specifically when light intensity is high, but temperatures are low adequate to slow photosynthesis. They produce anthocyanins to preserve themselves against the blemish effects of gathering more energy than they can use.

METHODOLOGY

Materials and Methods Materials

Fresh flowers of red rose are collected from farmers, the cotton fabric that was available from local markets in Jalgaon.

Chemicals

- Ethanol for extraction.
- Sodium carbonate and Tween 20 for cotton scouring.
- Calcium carbonate, tannic acid, aluminum sulphate, ferrous sulphate, potassium dichromate, aluminum acetate, etc., for used mordant.
- Hydrochloric acid, sodium acetate, potassium chloride for test sample preparation.

Extraction Methods

Extraction from red rose Most common solvent used for extraction of anthocyanins is ethanol. Ethanol is considered as a generally safe extraction medium, because of toxicity issue (Figure 1).

The Soxhlet extraction method was used to avoid the need for solvent and residue filtration. About 15 g of red rose petals were placed in the extractor thimble, and 150 ml solvent was added to a round-

bottom flask, fitted with a condenser using a high-water flow rate. The extraction was carried out for 6 hours, after which the volume of the obtained solution was measured. The samples were then analyzed to determine the total anthocyanin content.

Figure 1. Extraction of anthocyanin from red rose petals using Soxhlet method. Soxhlet extraction.

Ultrasound Assisted Extraction

At this stage, the amount of 5 g of red rose flowers petals, flower petals were mixed with 50 ml of ethanol, the extracting solvent in 100 ml conical flask. The ultrasound chamber having a frequency of 22k Hz using sonic power for 60 minutes. The temperature during extraction period stabilized at 45ºC for indirect sonication of the conical flask of sample immersed in an ultrasound chamber at half height of the solution. After completing a scheduled time of extractions, the flask was taken out and cooled at room temperature. The flower extract was filtered passed through Whatsman paper and solution was collected. Samples were used to measure the total content of anthocyanins.

Alkaline Extraction of Red Rose Flowers

At this stage, the amount of 5 g of red rose sample was taking and with 100 ml of ethanol solvent was poured into conical flask and add 0.40 g sodium hydroxyl and kept on stirring and heating at 100ºC for 30 min. After the extraction time, the flask was removed and cooled to room temperature. The contents were filtered using filter paper, and the samples obtained were analyzed for total anthocyanin content. The same extraction process was repeated for red rose flowers under identical conditions.

Alcoholic Extraction of Red Rose Flowers

"At this stage, 5 g of red rose sample was mixed with 50 ml of ethanol and 50 ml of distilled water in a conical flask, then stirred and heated at 100°C for 30 minutes. After heating, the flask was cooled to room temperature, and the contents were filtered through filter paper. The resulting samples were analyzed to determine the total anthocyanin content. The same extraction procedure was repeated for red rose flowers in the stirrer-heating extraction method, 15 g of red rose sample was mixed with 150 ml of ethanol in a conical flask and kept under stirring and heated at 50°C for 1 hour. After the extraction period, the flask was cooled to room temperature, and the contents were filtered through filter paper. The obtained samples were then analyzed for total anthocyanin content.

Acetone Extraction of Red Rose Flowers At this stage, 5 g of red rose sample was mixed with 100 ml of acetone in a conical flask and subjected to stirring and heating at 60 °C for 1 hour. After completion, the flask was cooled to room temperature, and the contents were filtered using filter paper. The filtered samples were then analyzed to determine the total anthocyanin content.

Scouring Cotton Fabrics

Cotton fabrics were scoured in a solution of 1.0 g/L sodium carbonate and 5 ml/L surfactant (Tween 20) for 30 minutes, then thoroughly rinsed with tap water and dried in the shade at room temperature.

Pre-Mordant Method Procedure

- Take a beaker 1000 ml fill a beaker with enough water to hold cotton.
- Add and mix 10 g calcium carbonate with boiling water.
- Add cotton as per required fabrics deep and leave it for 30 minutes, stirring carefully and constantly to avoid streaks.
- After completing the schedule, remove it, rinse lightly, and dry at room temperature.

Mordanting Procedure

- Take a beaker 1000 ml fill a beaker with enough water to hold cotton.
- Add and mix mordant as per required with temperature (shown in Table 1).
- Add 100 g cotton fabrics deep and leave it for 30 minutes, stirring carefully, and constantly to avoid streaks.
- After completing the schedule, remove it, rinse lightly, and apply red rose dye on it.

Dyeing

The conventional dyeing method was applied to both mordanted and non-mordanted fabrics. Cotton samples were directly dyed with the extracted dye and dried at room temperature. The dyed fabrics were then evaluated using a washing test with the surfactant (Tween 20).

Table 1. Mordanting procedure.

Descriptions S.N.	Mordant (Chemicals)	Pre-Mordanting	Quantity in g	Weight of Cotton g/	Temperature in ºC	Time in Minutes
1	Potassium dichromate	With premordanting	1	7	55	45
2	Aluminum sulphate	With premordanting	1	7	55	45
3	Aluminum sulphate	Withoutpremordanting	1	7	55	45
4	Tannic acid	With premordanting	6	9	80–90	60
5	Copper sulphate	With premordanting	1	5	80–90	30
6	Ferrous sulphate	With premordanting	0.1 (for light Color)	5	55	30
7	Ferrous sulphate	With premordanting	0.5 (for dark color)	5	82	45
8	Tannic acid + Aluminum sulphate	With premordanting	2 + 1	7	At room temperature	45
9	Stannous Chloride	With premordanting	0.5	5	70	45
10	Potassium dichromate + Aluminum sulphate	With premordanting	1+1	7	70	60
11	Aluminum acetate	With premordanting	1	7	110	30
12	Aluminum acetate	Without premordanting	1	7	110	30

Analysis of Anthocyanin Pigment

Determination of Total Monomeric Anthocyanin Pigment Content of Red Rose Flowers by the Ph Differential Method

The total anthocyanin content in red rose flowers was estimated using the pH differential method, a spectrophotometric technique that measures absorbance at pH 1.0 and 4.5 [16].

Analysis of UV FTIR, ZP, PS

- *Total Anthocyanin Content Measurement by UV spectroscopy:* The total anthocyanin content in flowers was determined using the pH differential method, a spectrophotometric technique that measures absorbance at pH 1.0 and 4.5 with the help of a pH meter standardized using pH 4.0 and 7.0 buffer solutions. Buffer solutions of pH 1.0 (0.025 M potassium chloride) and pH 4.5 (0.4 M sodium acetate) were prepared, and each sample was diluted with these buffers to obtain an absorbance reading between 0.2 and 1.4 AU. Absorbance was recorded at 520 nm and 700 nm using a UV-VIS spectrophotometer (UV-2600, USA). The total anthocyanin content (mg/L) was expressed as cyanidin-3-glucoside and converted to mg anthocyanin per 100 g of sample using the following equation.

$$\text{Anthocyanin pigment } \left(\frac{\text{mg}}{\text{l}}\right) = \frac{\text{A x MW x DF x 103}}{\varepsilon \text{ x 1}}$$

where:
$A = (A_{520nm} - A_{700nm})$ pH 1.0 – $(A_{520nm} - A_{700nm})$ pH 4.5.
M_W (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside.
D_F = dilution factor.
ε is the molar absorbance of cyanidin-3-glucoside (26900).
1 = cell path length in (1 cm).
10^3 = factor for the conversion from g to mg [17, 18].

- *Fourier transform infra-red spectroscopy:* prove the presence of anthocyanins, the purified extract was examined by FTIR apparatus (ALPHA-11), Bruker, Germany.
- *Particle size and zeta potential:* measurements were carried out using a Zeta Sizer Nano ZS 90 (Malvern Instruments, Malvern, UK) with the dynamic light scattering (DLS) technique at a fixed scattering angle of 90° and a temperature of 25 ± 1°C. For the preparation of the test solution, all

dilutions were made in 50 ml volumetric flasks. The test sample was added using a dropper, with a maximum of 1 ml extract solution diluted in 50 ml of distilled water

- *Washing test of dyed cotton:* Applied the dye extract on the pre mordant and Mordant cotton fabrics, dried it at room temperature. A washing test done on dyed fabrics 1st wash was just washed with tap water, and dry at room temperature. 2nd washed with tween 20 and rubbed it, dry at room temperature. Again, deep in hot water with temperature is 40°C–45°C for 10 minutes, after completed the schedule time removed it, and the cotton dry at room temperature. Mordanted cotton should be used immediately because some mordants are very sensitive to light.

RESULTS AND DISCUSSION

pH Detection of the Extracted Solutions

pH Detection of Samples

- pH of ultrasound assisted extraction (Rose) = 6.14.
- pH of Soxhlet extraction (Rose) = 6.34.
- pH of stirrer with heating extraction (Rose) = 6.26.
- pH of acetone extraction (Rose) = 5.95.
- pH of alkaline extraction (Rose) = 9.68.
- pH of alcoholic extraction (Rose) = 5.72.

UV-Visible

Total anthocyanin content or absorbance is calculated by using above formula (Table 2).

Table 2. Total anthocyanin content.

S.N.	Extraction Process	Samples	Absorbance at 520 nm	Absorbance at 700 nm	Anthocyanin Contents in mg/l at 520 & 700 nm
1	Ultrasound assisted extraction at 45°–50°C	5 g red rose flower's petals in 50 ml ethanol (pH 1.0) for 30 min.	0.089	–0.007	101.95
		5 g red rose flower's petals in 50 ml ethanol (pH 4.5) for 30 min.	–0.016	–0.022	
		5 g red rose flower's petals in 50 ml ethanol (pH 1.0) for 1 hour.	0.124	–0.001	82.659
		5 g red rose flower's petals in 50 ml ethanol (pH 4.5) for 1 hour.	0.012	–0.002	
2	Soxhlet Extraction for 6 hours at 45ºC–50ºC	15 g red rose flower petals in 150 ml ethanol (pH 1.0)	0.122	–0.004	138.68
		15 g red rose flower petals in 150 ml ethanol (pH 4.5)	–0.002	–0.027	
3	Stirring with heating extraction (at 45°C–50°C)	05 g red rose flower's petals in 100 ml ethanol for 30 min. (pH 1.0)	–0.109	–0.002	–105.62
		05 g red rose flower's petals in 100 ml ethanol for 30 min. (pH 4.5)	0.004	–0.004	
4	Acetone extraction at (at 55°C–60°C) for 1 hour	05 g red rose flower's petals in 100 ml acetone (pH 1.0)	0.088	–0.019	83.578
		05 g red rose flower's petals in 100 ml acetone (pH 4.5)	0.003	–0.013	
5	Alkaline extraction at (at 90°C–100°C) for 30 min	05 g red rose flower's petals in 100 ml ethanol (pH 1.0)	0.005	–0.006	–2.755
		05 g red rose flower's petals in 100 ml ethanol (pH 4.5)	0.008	–0.022	

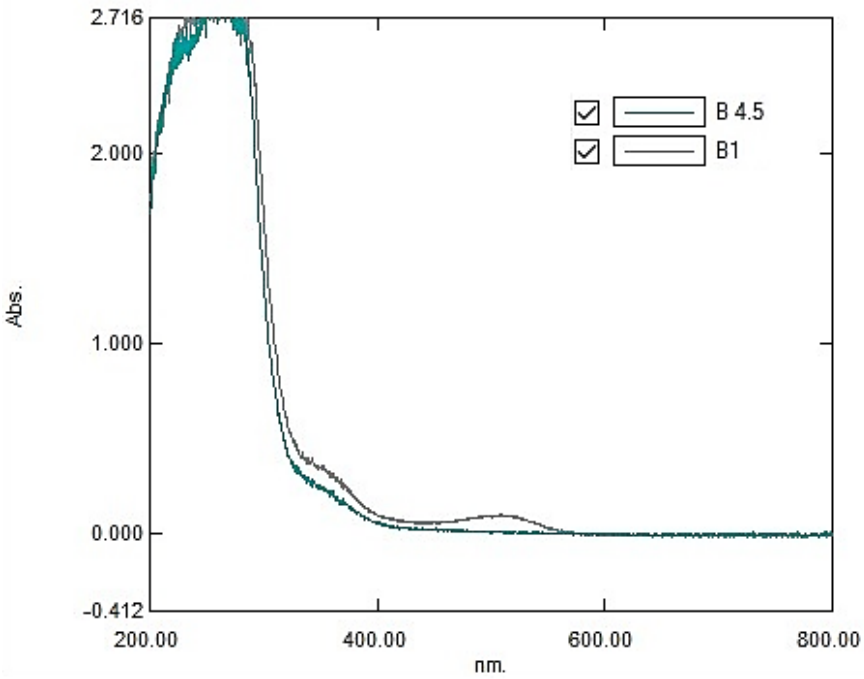

Figure 2. Graphical results of UV absorbance of red rose flowers in ethanol solvent.

The results of UV spectra of the red rose are shown in three peaks in the area 259.50 nm, 252.50 nm, and 520 nm. Appeared peak, in both pH 1.0 and pH 4.5 have the absorbance at 520 nm (highest absorbance 0.088) and 521 nm (highest absorbance 0.007), respectively (Figure 2). The maximum wavelength can be seen in Table 2. Based on the results of spectra UV, in the area λ 520 nm (highest absorbance 0.088) of pH 1.0 originated from carbonyl groups compound (C=C). The wavelength of 520 nm is the wavelength of anthocyanin cyanidin types.

FT-IR Spectroscopic Study

Graph shows (Figure 3) the analysis, to understand the appearance peaks in the FTIR below, step-by-step process can be used.

From the IR analysis can be seen that functional groups present in the red rose ethanol extract. The existence of the aromatic group of spectra UV is amplified by the IR spectrum which shows the C=C aromatic absorption at wave number 1654 cm^{-1} and 880 cm^{-1}, 666 cm^{-1} which indicate the presence of aromatic C–H bond. In addition, the aromatic group of IR also found in the O–H bonds phenolics appear in wave numbers 1384 cm^{-1} and 1327 cm^{-1}. C–O group detected at 1045 cm^{-1}. The hydroxyl group at wave number 3330 cm^{-1} and N–H absorption stretching appear at wave numbers 2973 and 2887 cm^{-1} which make up a group the sugar.

Analysis of Anthocyanin Pigments by Zeta Potential and Particle Size

The zeta potentials and particles size of anthocyanin are shown in Table 3.

- *Preparation of Test Solution:* Perform all dilutions in 50 ml volumetric flasks, use dropper for addition of the test sample. The maximum test portion added should be 1 ml extract solution and 50 ml distilled water.

Zeta potential analysis showed that the anthocyanin solution carried a negative charge. This indicates interactions between anthocyanins and water, allowing absorption, and release of anthocyanins. Such properties highlight their potential for future applications as a nanoplatform for drug storage and delivery, as well as in medical, food, and sensing fields. Shows the particle size distribution by intensity of anthocyanin pigment is about 200%. It means their intensity got better values as per the requirement used in food, pharmaceuticals, and cosmetics industries.

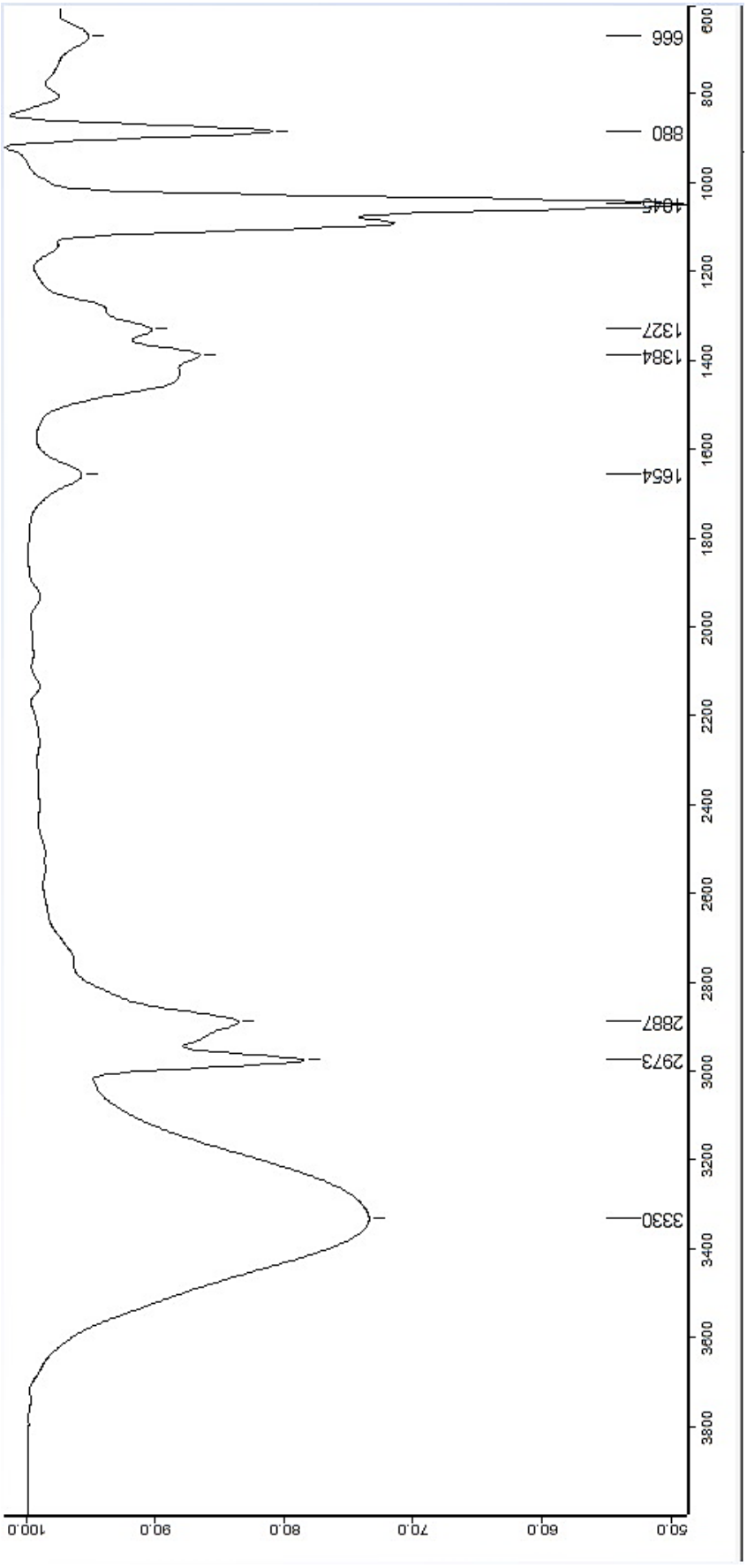

Figure 3. The graph of red rose flowers ethanol extraction.

Table 3. Results of zeta potential and particle size.

S.N.	Sample by Extraction Process	Samples	Zeta Potential Distribution in mv	Particle Size Distribution by Intensity in %
1	Ultrasound Assisted Extract at 50°C	5 g red rose flower's petals in 50 ml ethanol for 1 hour	6.66	79.13
		5 g red rose flower's petals in 50 ml ethanol for 30min.	7.04	82.24
2	Soxhlet Extraction at 50°C for 6 hours	15 g red rose flower's petals in 150 ml ethanol	–20.2	117.7
3	Alkaline extraction at (at 90°C–100ºC) for 30 min.	05 g red rose flower's petals in 100 ml ethanol	–16.0	99.11
4	Alcoholic extraction at (at 90°C–100ºC) for 30 min.	05 g red rose flower's petals in 50 ml ethanol & 50 ml D.W.	–23.6	223.4

Washing Test of Dyed Cotton

The results for the color change of mordanted and mordanted samples dye extract from red rose flower dyed cotton fabrics were the color changing effect was observed. Its color strength became a little, which is the dye was faded. When we used the different mordent chemical component or metal salts, it gives the better color strength. Different mordents were directly used on some fabrics without using pre mordant component (calcium carbonate), the results were better color strength and color changing effect was also obtained.

Different mordant chemical components were used on some fabrics with pre mordant component (calcium carbonate) can shift dye colors because it has a higher pH, the results were increased the color strength and color changing effect was also obtained. Using different mordant chemical components can give a different color which also depends on the source of the dye. The minimal color change observed in the washing test of red rose dye on cotton fabric can be attributed to the absence of reactive functional groups and the relatively small molecular size of the dye extract, which results in low affinity for textile fibers.

CONCLUSIONS

The anthocyanin was successfully extracted from red rose flowers by using different extraction methods with different solvents. Anthocyanin pigment is stable at low pH (in acidic condition) and should be kept in the dark. Other factors found to affect the pigment stability were light and elevated temperature which caused increased pigment degradation. Characterization using UV, FTIR ZP, and PS shows the characteristic of anthocyanin compared by the standard of anthocyanin and gives the better results, the highest absorbance found in red rose flower its 138.68 mg/l. 3-glucosides of cyanidin were found to be the most abundant anthocyanins in red rose flowers. ZP has obtained negative charges without adding nano particles and PS has obtained good results that are applicable for a drug storage and delivery nano platform that is adaptable to medical, food and sensing. Dyes on cloths show the better effect of color change in washing test and the color strength was greatly improved by using different mordants chemical components and calcium carbonate has shift dyes color because its higher pH. Using different mordant chemical components can give a different color which also depends on the source of the dye. They are also obtained from renewable sources. The use of natural dyes does not pose a disposal problem, as they are biodegradable.

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