

Comparative study of sonochemical and conventional methods for extracting natural red dye from *Delonix regia* petals: Food applications and stability evaluation

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Abstract

This study investigates the extraction of natural red dye from the petals of *Delonix regia* flowers, comparing sonochemical and conventional methods to optimize dye yield, characterize its chemical properties, and assess its potential in food applications. Three extraction techniques, i.e., magnetic stirring (500 rpm at 50°C for 60 min), probe sonication (20 kHz, 650 W for 30 min), and ultrasonic water bath (40 kHz, 500 W at 80°C for 60 min) were evaluated under fixed operational parameters. The ultrasonic water bath method at 80°C for 45 min, using water as the solvent, yielded the maximum dye concentration and high color intensity, with UV-visible spectrophotometry revealing prominent absorbance peaks at 480 nm and 530 nm, indicative of the red dye's color profile. FTIR analysis confirmed the presence of functional groups, including hydroxyl, aromatic, aldehydic, and polyphenol compounds. It revealed the formation of polymerised anthocyanins that exhibited pH-dependent structural variations across a range of pH values (3–10). The red dye extract, rich in flavonoids (78.23 ± 5.67 µg GAE/mg) and total phenolic compounds (69.19 ± 1.02 µg QE/mg), demonstrated significant antioxidant properties, surpassing the synthetic antioxidant Butylated hydroxyanisole (BHA) at 250 µg/ml in DPPH radical scavenging activity. Incorporating the extracted dye into a jelly candy formulation highlighted its functional potential, with the most intense coloration observed at pH 3. This study not only establishes the ultrasonic water bath-assisted extraction method as the most efficient and eco-friendly approach for obtaining natural red dye but also emphasizes the dye's promising applications as a natural food colorant with antioxidant benefits, offering a sustainable alternative to synthetic dyes.

1. Introduction

Color is one of the most visually appealing attributes of food and plays a crucial role in influencing consumer acceptance [1]. In the food industry, colorants not only enhance visual appeal but can also contribute to food safety, nutritional value, and sensory perception, including taste [2]. Recently, there has been a growing emphasis on the use of natural additives in food processing, particularly bioactive compounds and natural colorants, due to their health benefits and consumer preference for clean-label products [3]. In addition to being present in leaves, flowers, fruits, vegetables, and various plant parts, these pigments are also found in animal tissues such as skin and eyes, as well as in structural components of fungi and bacteria [4]. The major classes of pigments derived from vegetables and flowers include betalains, chlorophylls, carotenoids, and flavonoids [5, 6]. Dyes can be classified based on their chemical structure, molecular configuration, and industrial application. Broadly, they are categorised into two main types: natural and synthetic. The majority of synthetic dyes are derived from petrochemical sources [7]. Common classes of synthetic dyes include azo, anthraquinone, and triarylmethane compounds, each offering a wide range of vibrant colors and extensive applications in various industries. The use of synthetic dyes in industrial applications poses significant risks to both human health and the environment. As noted by [8], these dyes are classified as persistent pollutants, capable of contaminating water bodies, disrupting aquatic ecosystems, and diminishing the aesthetic quality of natural environments. Biomagnification and accumulation of synthetic dyes in the food chain can also have toxicological effects on marine plants and animals, with potential consequences for human health [9]. Clinical studies and animal toxicity research have highlighted the detrimental behavioral effects associated with the consumption of synthetic food colorants, particularly among children [10]. There is an urgent need for revaluation and further research, as the current permissible

daily intake limits for food dyes may not be sufficient to safeguard the neurobehavioral health of children at risk. The detrimental effects of synthetic dyes on both the environment and human health are increasingly recognised, highlighting the urgent need for environmental remediation and the adoption of green chemistry approaches [11]. In contrast, natural dyes offer a wide spectrum of soothing colors, with the added benefits of safety, non-toxicity, and antimicrobial properties [12]. Natural dyes, including those with antibacterial, antifungal, UV protection, and flavour-enhancing properties, are valued for their distinct aesthetic appeal. They are differentiated from synthetic dyes by their renewable sources and environmentally friendly production methods [13, 14]. Although anthocyanins have the potential to replace synthetic colorants, their color stability is influenced by factors such as pH, temperature, oxygen, and light exposure. Natural dyes exhibit limited chromatic stability under varying manufacturing, formulation, and storage conditions [15]. Chemical and enzymatic processes can degrade these dyes, leading to the formation of colorless compounds or structural changes [16]. In industrial food processing, color degradation can be triggered by hydrogen peroxide, a byproduct of oxygen-sensitive molecular autoxidation, as well as bisulfite, a commonly used preservative [17]. Food scientists are currently working on developing novel methods to control anthocyanin reactions and minimise their degradation, aiming to produce more stable and aesthetically pleasing colors [18]. The structural complexity of anthocyanins, particularly through acylation, enhances the stability of blue hues and promotes color durability by facilitating intramolecular co-pigmentation and self-association [19]. Hydrophobic interactions and Van der Waals forces facilitate the transfer of aromatic acyl groups to the anthocyanidin core structure in acylated anthocyanins, leading to intramolecularly associated color [20]. The pyrylium ring and acyl groups of the flavylium cation help protect the chromophores from water's nucleophilic attack, thereby preventing the formation of chalcone or pseudo-base structures [21]. A bathochromic shift occurs when the acid-base balance equilibrium shifts to the blue quinoidal base form. *In vitro* chemical and enzymatic acylation processes can lead to the formation of acylated anthocyanins in various plants and edible flowers [22].

The Gul Moha (*Delonix regia*), commonly known as the Flame Tree or Flamboyant, is a large ornamental tree native to Madagascar. It belongs to the *Fabaceae* family, specifically the subfamily *Caesalpinioideae* [23]. It is characterised by its fern-like, bipinnately compound leaves and vivid red flowers resembling a peacock's display. Anthocyanins, betalains, chlorophylls, and carotenoids are plant-derived pigments that are widely used across various industries, including food additives, textile dyes, animal feed, pharmaceuticals, and cosmetics [24]. Natural red dye can be extracted from *D. regia* flowers. Previous studies have demonstrated the potential of these flowers as a source of colorants for food products, textiles, and leather [25]. This study aims to enhance the efficiency of dye extraction and its application in coloring food jelly. The relative color strength of the extracted dye was assessed to determine the optimal extraction conditions and maximise color yield. Variations in solvent composition during different extraction procedures resulted in differing levels of chromatic intensity [26, 27]. The non-toxic and non-allergic properties of natural dyes are crucial for applications involving sensitive uses. There is a growing demand for safe, non-toxic coloring methods, particularly for health-sensitive products such as children's toys, textile and leather garments, and food coloring.

Ultrasound has been employed as a cost-effective and environmentally friendly technique for dyeing food and textile materials, as well as for extracting natural dyes from plant sources. This method helps reduce the

consumption of chemicals, time, energy, and effluent generation during the dyeing process [28]. Plant-derived natural dyes offer a cost-effective and environmentally sustainable alternative for use in textiles, food products, and cosmetics [29]. Ultrasound-assisted extraction is an emerging technology that improves the efficiency of heat and mass transfer, thereby enhancing dye yield and process performance. This method has been applied in the field of extraction, particularly in a comparative study on the recovery of colorants from beetroot using ultrasound in combination with static and magnetic stirring techniques [30]. Notably, the use of ultrasonic technology significantly enhanced the extraction efficiency of beetroot colorants. Phenolic compounds were effectively extracted using an ethanol-water solvent system combined with ultrasound-assisted extraction [30]. The primary objective of this study is to enhance the extraction efficiency of natural dye from *D. regia* flowers. Additionally, the study examines the impact of different extraction methods and solvents on dyeing properties, pH stability, dye quality, total polyphenol and flavonoid content, anthocyanin concentration, microbial safety (yeast and mold presence), and antioxidant activity. The findings aim to support the broader industrial application of natural dyes in textiles, food products, cosmetics, and sustainable colorants.

2. Materials and methods

2.1. Chemicals and media

Analytical-grade chemicals and solvents used in this study were obtained from SRL India. Ethanol and methanol (99.9% purity) were employed as solvents. Culture media were obtained from HiMedia Laboratories (Mumbai, India). Laboratory-grade Butylated hydroxyanisole (BHA), used as a reference antimicrobial agent, was obtained from Hi-Media Laboratories (Mumbai, India). Commercial-grade China grass (Agar Agar) was received from a local market.

2.2. Collection and preparation of *D. regia petals*

Fresh flowers of *D. regia* were collected from Ponnoli Nagar village (latitude 11° 39 °N, longitude 78 8 °E) in the Salem districts of Tamil Nadu, India. The petals were carefully separated from the peduncles and cut into small pieces to facilitate efficient extraction. The red pigment was subsequently extracted from these fragmented petals.

2.3. Ultrasonic water bath (USW)-assisted extraction of natural red dye

Following the method of [31], 50 g of *D. regia petals* were placed in a glass container and extracted using 150 mL of different solvents: Milli Q water, ethanol, methanol, and a 1:1 (v/v) mixture of ethanol and methanol. The glass vial containing the petal-solvent mixture was placed at the center of an ultrasonic water bath (LABMAN, LMUC-25, India, 24-litre capacity), and sonication was carried out at 40 kHz, 500 W, and 60°C for 30 min. The container was covered with aluminium foil during all extraction procedures to minimise solvent evaporation. Following dye extraction, the suspension was centrifuged at 5000 rpm for 10 min using a Remi C-24plus centrifuge (India). The resulting supernatant was analysed using a UV-Vis spectrophotometer (UV-

1800, Genesys 180, Thermo Fisher Scientific, USA) to record absorbance spectra in the wavelength range of 200–800 nm.

2.4. Ultrasonic probe (USP)-assisted extraction of natural red dye

As described by [31], 50 g of *D. regia* flower petals were placed in a glass extraction vessel containing 150 mL of the respective solvent. The vessel was then subjected to ultrasonic extraction using a probe sonicator (LABMAN, Pro-650, India) operating at a frequency of 20 kHz and an input power of 650 W for 30 min. During the experiment, the ultrasonic probe was operated in a pulse mode with 10 sec on and 3 sec off cycles. A temperature sensor attached to the probe monitored the internal temperature of the vessel, which increased to 35°C due to the applied input power. After sonication, the mixture was centrifuged, and the absorbance of the resulting supernatant was measured according to the previously described protocol.

2.5. Magnetic stirrer-assisted extraction of natural red dye

Extracted the red dye from *D. regia* petals using the above-indicated solvent systems. For each extraction, 50 g of petals were combined with 150 mL of the respective solvent in a glass extraction vessel [32]. The mixture was then placed on a magnetic stirrer and agitated at 500 rpm at 60°C for 30 min to facilitate the release of the red dye from the flower petals into the solvent under continuous stirring and heating. After the extraction, the resulting solution was centrifuged, and the absorbance of the supernatant was measured.

2.6. Gravimetric analysis

Samples obtained from the control, direct heating, ultrasonic water bath, and ultrasonic probe extractions were filtered and spread onto pre-washed, dried, and pre-weighed glass plates. The extracts were then dried in a hot-air oven until complete water evaporation. After cooling in a desiccator, the plates were reweighed. This drying and weighing process was repeated until a constant weight was achieved. The yield of the colorant extract was calculated based on the weight of the dried extract relative to the initial weight of the plant material using the following Eqs. (1–4):

$$\text{Natural dye yield (\%)} = \frac{\text{Natural dye extract obtained (g)}}{\text{Amount of flower material used (g) (\%)}}$$

1

$$\% \text{ Improvement due to ultrasonic waterbath} = \frac{(\% \text{ yield of (ultrasonic waterbath} - \text{Control)})}{\% \text{ yield of control}}$$

2

$$\% \text{ Improvement due to ultrasonic probe} = \frac{\% \text{ yield of (ultrasonic probe} - \text{Control)}}{\% \text{ yield of control}}$$

3

$$\% \text{ Improvement due to direct heating} = \frac{\% \text{ yield of (direct heating} - \text{Control)}}{\% \text{ yield of control}}$$

4

2.7. FTIR analysis

Fourier Transform Infrared (FTIR) spectroscopy (Thermo Fisher, Summit Lite, USA) was employed to identify the functional groups present in the extracted *D. regia* red dye within the transmittance range of 500–4000 cm^{-1} .

2.8. Effect of pH on the color stability of natural red dye

The initial investigation aimed to evaluate the color stability of *D. regia* red dye extracted using different techniques across a range of pH values. Test samples were prepared by mixing 5 mL of the red dye extract with 4.5 mL of distilled water in glass vials. The pH levels were adjusted to 3, 4, 5, 6, 7, 8, 9, and 10 using 0.1 M HCl and 0.1 M NaOH solutions. Color changes were visually monitored across the pH range. pH measurements were carried out using a calibrated pH meter (Hanna Instruments, South Korea), which was standardised with buffer solutions at pH 4.01, 7.01, and 9.01 prior to use. Subsequently, the absorbance of the dye solutions at each pH level was measured to determine the corresponding wavelength shifts [6].

2.9. Preparation of red dye jelly candy at different pH levels

A standardised recipe was used to prepare jelly candies colored with *D. regia* red dye extracted using various techniques at different pH levels. In a 100 ml beaker, 1g of agar (plain china grass), 0.5 g of sugar, 0.05 g of gelatin powder, a pinch of salt, and 5 mL of coconut water were combined. The mixture was then microwaved using a Samsung microwave oven (900 W magnetron power, 2450 MHz frequency) for 1 min. Subsequently, 45 ml of *D. regia* red dye solution, pre-adjusted to the desired pH, was added to the jelly base. The resulting mixture was placed into candy molds and allowed to solidify at 4°C for 24 h [33].

2.10. Color coordination test

In the CIELAB color space, the L^* , a^* , and b^* coordinates represent the lightness black-white, red-green, and yellow-blue axes, respectively. The colour strength (K/S) of the colored agar jelly was measured using a Data Color 600 spectrophotometer (Data Colour Company USA) with a 10-degree standard observer and illuminant D65. The test results were determined based on the standard deviation of eight measurements taken from different locations on the sample. The color strength (K/S) value was calculated using Eq. (5) [34].

$$K/S = (1-R)^2 / 2R \quad (5)$$

Where R is the observed reflectance

2.11. Shelf-life assessment of colored jelly candy against microbial spoilage

The gummy jelly samples were carefully sealed in plastic zip-lock bags lined with aluminum foil and stored at 25°C for 30 days to assess storage stability. After 30 days, all the bags were retrieved for analysis. Yeast and

mold counts were performed on 10 g of each sample using standard microbiological methods, as described by [35].

2.12. Assessment of total polyphenols, flavonoids, and anthocyanins in natural extracts

The total phenolic content was estimated using the Folin-Ciocalteu method, as described by [36]. Briefly, 2.5 mL of 10% Folin-Ciocalteu reagent was mixed with 0.5 mL of *D. regia* red dye extract and allowed to react in the dark. Subsequently, 2 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added to the mixture, which was then kept in the dark for 1 h. The reaction tubes were placed in a water bath at 45°C for 5 min, followed by immediate cooling in an ice-water bath. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer. The total polyphenol content of each fraction was expressed as milligrams of gallic acid equivalents per milligram of red dye ($\mu\text{g GAE/mg}$) based on a gallic acid standard curve. The total flavonoid (TF) content in the red dye was determined using the method outlined by [36]. To 0.5 mL of red dye, 0.1 mL of 10% AlCl_3 was added, followed by the addition of 0.1 mL of 1 M CH_3COOK and 4.3 mL of Milli Q water. The mixture was incubated for 30 min, and the absorbance was measured at 415 nm using a UV-Vis spectrophotometer. A calibration curve was constructed using Quercetin as the standard. The total flavonoid content was expressed as micrograms of Quercetin equivalents per milligram of red dye ($\mu\text{g QE/mg}$) based on the calibration curve. To estimate the total anthocyanin content, the red dye extract from *D. regia* was filtered and diluted with water to achieve an optical density (OD) within the instrument's optimal range. The absorbance was measured at 520 nm after the diluted extract was incubated in the dark for 2 h. The total anthocyanin content was calculated using the following Eq. (6).

Total anthocyanin content (mg)/100 gm =

$$\frac{\text{OD} \times \text{DV} \times \text{TEV} \times 100}{\text{SV} \times \text{SW} \times 51.56}$$

(6)

where OD represents the optical density, DV is the diluted volume used for the OD measurement, TEV refers to the total extract volume, SV is the sample volume used for analysis, and SW denotes the sample weight in grams. The constant 51.56 corresponds to the extinction coefficient (E value) of the principal constituent, cyanidin.

2.13. Antioxidant activity assay

The antioxidant activity of the aqueous floral dye from *D. regia* was evaluated using the DPPH free radical scavenging assay. The methodology was adapted from [37, 38]. In this assay, the stable DPPH radical was reacted with the *D. regia* red dye in a methanol-based solution to assess its free radical scavenging capacity. Experimental solutions were prepared by mixing various concentrations of the dye (25, 50, 100, 200, 300, 400, and 500 $\mu\text{g/ml}^{-1}$) with 3 ml of absolute methanol and 0.3 ml of a 0.5 mM DPPH solution dissolved in methanol. DPPH reduction occurs when it interacts with an antioxidant molecule capable of donating a hydrogen atom. After 30 min of incubation, the absorbance (Abs) was measured at 517 nm. Butylated hydroxy anisole (BHA) was used as a positive control. The percentage of DPPH radical scavenging activity was calculated using the following Eq. (7):

$$S_a (\%) = (A_b - A_s) / A_b \times 100\% \quad (7)$$

Where A_b is the absorbance of the control solution (3.6 ml of DPPH solution and 0.4 ml of methanol), and S_a is the absorbance of the test sample (3.6 ml of DPPH solution and 0.4 ml of the sample solution).

2.14. Statistical analysis

The data obtained from the *D. regia* flower dye extract was analysed using one-way ANOVA and expressed as mean $\pm$ SD. Statistical significance between groups was determined using Duncan's multiple-range test, with differences considered significant at $p < 0.05$.

3. Results and discussion

3.1. Extraction of natural red dye from *D. regia*

(Fig. 1a) shows the *D. regia* tree in full bloom, with clusters of vibrant flowers appearing in bunches from May to July. (Fig. 1b) illustrates a close-up of the flower structure, highlighting four spoon-shaped petals along with a distinct petal (approximately 5.6 ± 0.5 cm in length) indicated by a yellow arrow, as well as five smaller sepals. The *D. regia* flower extract contains a diverse range of bioactive compounds, including flavonols, carotenoids, anthocyanins, tannins, saponins, beta-sitosterol, flavonoids, carotene hydrocarbons, steroids, alkaloids, ketocarotenoid and phenolic acids, all of which are associated with antimicrobial and antioxidant properties [39]. An ethanolic extract of *D. regia* flowers has demonstrated chemoprotective properties and has been evaluated for its effectiveness against liver cancer and hepatotoxicity induced by chlorinated compounds, both of which are major contributors to liver damage [40].

(Fig. 2a) shows that among the three extraction techniques using different solvent systems, the ultrasonic water bath with water as the solvent yielded a higher amount of red dye from *D. regia* flowers, followed by methanol (CH_3OH), ethanol ($\text{C}_2\text{H}_5\text{OH}$), and their combination. (Fig. 2b) illustrates that the highest dye yield was obtained using the ultrasonic probe with water as the solvent. (Fig. 2c) These results are further confirmed, showing that magnetic stirring also yielded a higher amount of dye when water was used as the solvent. The inset images in all three figures depict the color intensity of the extracted dye. Polyphenols and flavonoids were extracted from *Delonix Regia*. flower extract using an ultrasonic water bath, as described by [41]. According to [42], the extraction efficiency of the colorant increased significantly by 13–100% when ultrasound was employed to facilitate the extraction of natural dyes from various plant materials [41] found that a 1:1 ethanol-water mixture combined with 80 W ultrasonic power and a contact time of 3 h enhanced both yield and extraction efficiency [42]. Similarly, [32] reported that increasing the extraction time to 1 h at 100 W ultrasonic power and 80°C resulted in higher dye extraction values. Pre-treatment of marigold and nasturtium petals with 50% ethanol followed by 6 min of microwave heating resulted in improved dye yield [43]. UV-Vis spectrophotometry, which analyses a sample's light absorption or transmission across various wavelengths, provides insights into its electronic structure and molecular interactions and is commonly employed to investigate the chemical composition of floral dye compounds [44]. This method accelerates the extraction process. The highest color intensity was observed in extracts obtained using water in an ultrasonic water bath, followed by ethanol, methanol, and water without ultrasonic treatment. This technique

has proven effective for the successful extraction of natural floral dyes. Constant agitation enhances the extraction yield of colorant compounds by improving contact between the solvent and flower petals. UV-Vis spectral analysis indicates that extraction using a magnetic stirrer produces the most intense red dye across various solvents. In the case of *D. regia* dye, two characteristic absorption peaks were observed: one at 530 nm corresponding to betanin and another at 480 nm attributed to betaxanthin.

3.2. Effect of pH on the stability of red dye

To assess the pH stability of the red dye extracted from *D. regia* flowers, three extracted methods were employed: ultrasonic water bath (Fig. 3a), ultrasonic probe (Fig. 3b), and direct heating with magnetic stirring (Fig. 3c). The resulting extracts were analysed using a UV-visible spectrophotometer. The red dye extracted using the ultrasonic water bath at pH 3 exhibited two distinct absorption peaks at 530 nm and 352 nm (Fig. 4a), indicating the presence of anthocyanins. At pH levels 4–6 and 8, a single prominent peak was observed at 352 nm, while at pH 9, two peaks appeared at 324 nm and 352 nm, further confirming the presence of anthocyanins. However, at neutral pH, no prominent peak was observed, which may be attributed to a change in the chemical structure or degradation of the anthocyanin. Alternatively, the absence of the peak could indicate that the dye was not present in the extract. Similar absorption peaks were observed in the ultrasonic probe-mediated extraction (Fig. 4b) at pH 3–6, indicating the presence of anthocyanin.

At pH 8–10, a distinct peak at 412 nm was observed, which can be attributed to the presence of both chlorophyll a and b, in addition to the anthocyanins. The extract at pH 5 showed the maximum absorbance ($1.32 \pm \text{SD}$), which was significantly higher than the values observed at other pH levels ($p < 0.05$). A notable decrease in absorbance was observed at pH 8 and 9. Direct heating with magnetic stirring showed a peak at 350 nm across all pH levels (Fig. 4c), suggesting the presence of specific pigments or compounds that absorb light in this wavelength range, possibly indicating the presence of flavonoids or other similar molecules. Our results are consistent with those reported by [45, 46], where UV-Vis spectra of a natural dye extracted from *Brassica napus* flower petals revealed the presence of flavonoid and carotenoid pigments, as well as broad absorption bands.

Additionally, [47] indicated that pH level significantly influences the stability of natural dyes in flowers, particularly anthocyanins. Anthocyanins, which are water-soluble pigments responsible for the vivid colors in plants, typically appear red and are more stable at lower pH values. However, they degrade and shift to blue or other hues at higher pH values [47]. A flower dye exhibiting UV-Vis absorption peaks at 352 and 530 nm at pH 3 is most likely to contain anthocyanins [48]. The color and absorption spectrum of anthocyanins is significantly influenced by pH. At lower pH levels, such as pH 3, anthocyanins typically appear red or purple, and their absorption peak shifts towards shorter wavelengths [49, 50]. In the absence of a covalent bond with another phenolic component, monomeric anthocyanin interacts with bisulfate, resulting in the formation of a colorless sulphonate molecule [51].

3.3. Effect of pH on the color coordinates of jelly candies

(Fig. 5a) shows the color variations of jelly candies prepared using red dye extracted from *D. regia* flowers by three different methods: ultrasonic water bath (Fig. 5b), ultrasonic probe (Fig. 5b), and direct heating with magnetic stirring (Fig. 5c), adjusted with varying pH. The corresponding color coordinates values of the jelly

candies are presented in Table 1. Color intensity was higher at pH 3 and 4 (Fig. 5a) compared to the control jelly candies displayed in the center.

The color coordinate values of jelly candies colored with pH-adjusted red dye from *D. regia* flowers indicate that pH 3 and 4 yielded more intense coloration than higher pH levels (5–10), as shown in Tables 1, 2, and 3, for the ultrasonic water bath, ultrasonic probe, and direct heating with magnetic stirring, respectively. The enhanced color intensity observed at pH 3 and 4 is attributed to the stable, non-degradative nature of anthocyanin dye molecules under acidic conditions. Overall, the color strength of the pH-adjusted jelly candies followed the trend: pH 3 > pH 4 > pH 5–10. Lower pH levels likely preserve more functional groups in anthocyanins, contributing to greater color stability and intensity compared to higher pH levels. The color coordinate values L^* , a^* , and b^* of jelly candies prepared with *D. regia* flower-derived natural dyes at varying pH levels (3–10), using an ultrasonic water bath, ultrasonic probe, and direct heating with magnetic stirring extraction methods, are presented in (Fig. 5a, b, c and Tables 1–3,) respectively. Across all three extraction modes, pH 3 exhibited the most stable color, characterised by a significant decrease in L^* values, indicating a deepening of the color shade compared to pH levels 5–10. The a^* and b^* values further revealed that the jelly candies prepared using the ultrasonic probe at pH 3 and 4 exhibited notable intensity and vibrant colors. The positive a^* and b^* values reflect shifts toward red and yellow hues, respectively, contributing to visually appealing shades. These color parameters are detailed in Tables 1–3 for each extraction method. These values indicate that the jelly candies are microbiologically safe from a food technology standpoint, as microbial growth remains within acceptable limits Tables 1–3. pH levels below 3 and 4 classify the products as highly acidic, which may contribute to a noticeably sour or acidic taste on the palate.

Our studies established a clear relationship between total acidity and pH, highlighting their influence on the properties of the product. The findings indicate that *D. regia* flower extract exhibits significantly higher antioxidant activity under acidic conditions (pH 3) compared to alkaline conditions (pH 10). The acidity of jelly candies plays a critical role in determining their taste, shelf stability, and texture. Deviations from the optimal pH range can negatively affect both texture and shelf life. However, as shown in Tables 1–3, pH variations showed no insignificant impact, and storage did not alter the visual appearance of the products. Additionally, the water activity was low enough to inhibit microbial growth, resulting in minimal changes in moisture content and water activity during storage.

3.4. FTIR analysis of red natural dye from different extraction methods

(Fig. 6a-d) present the FTIR spectra of red dye extracted from *D. regia* flowers using different methods: ultrasonic water bath (Fig. 6a), ultrasonic probe (Fig. 6b), direct heating with magnetic stirring (Fig. 6c), and conventional water extraction (Fig. 6d). The ultrasonic water bath extract (Fig. 6a) exhibited distinct absorption peaks at 3246.18 cm^{-1} , corresponding to hydrogen-bonded hydroxyl (-OH) groups, and at 2954.72 cm^{-1} and 2841.05 cm^{-1} , associated with C-H stretching vibrations from alkanes or saturated hydrocarbons. A prominent peak at 1636.42 cm^{-1} indicates C = O stretching vibrations, characteristic of carbonyl functional groups. Additional peaks at 1456.37 cm^{-1} and 1412.15 cm^{-1} are attributed to C-H bending in alkanes or aromatic rings or possibly C-O bending. The peaks at 1103.96 cm^{-1} and 1013.23 cm^{-1} represent C-O stretching vibrations, suggesting the presence of ether, ester alcohol, phenol, or

polysaccharide components such as cellulose or pectin [52, 53]. The extract obtained using the ultrasonic probe (Fig. 6b) displayed three major peaks at 3329.89 cm⁻¹, 1653.32 cm⁻¹, and 1013.23 cm⁻¹.

These are consistent with the functional groups observed in the ultrasonic water bath method, indicating similar chemical characteristics across both ultrasonic-assisted techniques [54–56]. In contrast, the dye extracted through direct heating with magnetic stirring (Fig. 6c) exhibited two prominent absorption bands at 3358.23 cm⁻¹ and 1636.43 cm⁻¹, corresponding to O-H stretching (hydroxyl groups) and C = C or C = O stretching vibrations, suggesting the presence of carbon-carbon or carbonyl double bonds [57, 58]. The conventional water extraction method (Fig. 6d) revealed two main peaks at 3313.18 cm⁻¹ and 1641.26 cm⁻¹, both indicating O-H stretching vibrations, highlighting the dominance of hydroxyl functional groups in the extract.

3.5. Phytochemical analysis

Table 4

Phytochemical analysis of total polyphenols, flavonoids, and anthocyanins, and color coordinates of jelly candies prepared at different pH levels using *R. poinciana* flower dye extracted via ultrasonic water bath, ultrasonic probe, and direct heating with magnetic stirring.

Content	Total Polyphenols (TPP) (µg GAE/mg)			Total Flavonoids (TF) (µg QE/mg)			Total Anthocyanins (TA) (mg/100 g)		
	Water	MeOH	p-Value*	Water	MeOH	p-Value*	Water	MeOH	p-Value*
USW	69.19 ± 4.08	43.69 ± 1.27	0.0003	78.23 ± 5.67	27.65 ± 0.38	0.031	167.11 ± 4.16	84.28 ± 09.20	0.0002
USP	67.01 ± 3.89	40.32 ± 0.8	0.0005	76.13 ± 4.17	22.58 ± 0.29	0.012	159.11 ± 3.06	76.20 ± 10.01	0.003
DH	65.22 ± 5.09	36.28 ± 1.23	0.001	63.3 ± 3.02	19.18 ± 0.45	0.0003	153.01 ± 5.09	73.11 ± 11.14	0.02

Each value in the table is expressed as Mean ± SE (n = 3). Statistical significance was determined using Fisher's exact test: P < 0.05 indicates a significant difference.

In this study, the total polyphenolics, total flavonoids, and total anthocyanins content were found to be significantly higher in extracts obtained using an ultrasonic water bath with water as the solvent, yielding 69.19 ± 1.02 µg GAE/mg, 78.23 ± 5.67 µg QE/mg, and 167.11 ± 4.16 mg/100 g, respectively, followed by ethanol-mediated extraction Table 4. The ultrasonic probe-assisted extraction with water revealed total polyphenolics, total flavonoids, and total anthocyanins content in the flower extract of 67.01 ± 3.89 µg GAE/mg, 76.13 ± 4.17 µg QE/mg, and 159.11 ± 3.06 mg/100 g, respectively, Table 4. A lower number of total polyphenolics, total flavonoids, and total anthocyanin content was observed in the dye extract obtained through direct heating with magnetic stirring Table 4. This may be attributed to the enhanced extraction efficiency observed when using water as a solvent with the ultrasonic water bath, which likely facilitates a more effective release of these compounds into the water medium compared to direct heating. [59] and [37] reported a comparable total phenolic content of (26.70 mg GAE/g) for *D. regia* flowers extracted using water

as the solvent. Phenolic compounds are known to be thermo-labile. The primary phenolic acids identified in *D. regia* include gallic acid, chlorogenic acid, and protocatechuic acid [27, 60].

3.6 Antioxidant activity

A study was conducted to evaluate the free radical scavenging activity of the red dye extracted from *D. regia* flowers. The antioxidant potential of water-based extracts obtained using an ultrasonic water bath, ultrasonic probe, and direct heating with magnetic stirring was assessed *in vitro* using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay [25]. Results showed that extracts obtained via all three methods demonstrated substantial free radical scavenging activity, likely due to their higher phenolic content.

At a concentration of 250 µg/ml, the ascorbic acid equivalent antioxidant capacities of the extracts were 86%, 85%, 82%, and 80%, respectively as shown in Fig. 7. Multiple *in vitro* techniques were employed to assess the antioxidant activity of *D. regia* flower extracts and their fractions [25]. Among them, the crude pigment and hexane extract exhibited the lowest antioxidant activities. Identified phenolic acids in *D. regia* include protocatechuic acid, gallic acid, and chlorogenic acid [39]. According to [41], the antioxidant activity of *D. regia* flowers is attributed to carotenoids, anthocyanins, and polyphenolic compounds. These are complemented by flavonoids, which are well-known for their antioxidant properties. [35] also demonstrated that the antioxidant potential of sweet cherry extract correlates with flavonoid content. Similarly [61] identified myricetin as the most abundant flavonoid in *D. regia* flowers, followed by delphinidin, epicatechin, rutin, kaempferol, and quercetin. Plant-derived phenolic compounds are recognised for their ability to neutralise free radicals via mechanisms such as singlet oxygen quenching, hydrogen donation, and metal ion chelation. Notably, their electron-donating ability is enhanced in alkaline environments due to molecular deprotonation and stabilisation, which may explain the observed increase in antioxidant activity with rising pH.

4. Conclusion

This comparative study has revealed the considerable potential of sonochemical extraction as a more efficient and environmentally sustainable alternative to conventional methods for extracting natural red dye from *D. regia* petals. This suggests that anthocyanins, naturally occurring pigments responsible for the vibrant red color, are present in the dye. The dyes produced from both techniques exhibited desirable colour attributes suitable for food applications, offering a natural and perhaps healthier alternative to synthetic colourants. This signifies that anthocyanin, a naturally occurring pigment responsible for the intense red colour, is present in the dye. The extraction conditions influence both the quantity and stability of these anthocyanins, which are known for their biomedical applications, including as natural antibacterial agents. This study aims to optimise the extraction of red dye from *D. regia* flowers and enhance its coloring potential for food jelly. The relative color strength of the extracted dye was evaluated to determine the optimal extraction conditions and achieve the highest color yield. The study showed that using an ultrasonic probe during extraction increased the dye yield from *Delonix regia* petals significantly when compared to traditional methods ($p < 0.05$). At pH 5–6, the extract displayed peak stability and color vibrancy, with notable variations in how well it absorbed light and maintained its color. The study's results spotlight the possibility of employing sonochemical methods for producing efficient, natural food dyes, backed by strong statistical

analysis. An in-depth analysis of the specific compounds responsible for the colouration and their possible bioactive properties, coupled with extensive application studies across various food products, will be essential for fully harnessing the potential of *D. regia* petal extracts as a viable and appealing natural red food dye.

Declarations

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

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Ethics Statements

Ethics approval and consent to participate:

Not applicable. This study did not involve any human or animal participants.

Consent for publication:

Not applicable.

Plant collection and permissions:

Fresh *Delonix regia* flowers were responsibly collected from Ponnoli Nagar village, Salem district, Tamil Nadu (11°39'N, 78°08'E), from publicly accessible areas without disturbing the local ecosystem. The species is widely cultivated and not listed as endangered under the Indian Biodiversity Act. All procedures complied with relevant institutional, national, and international research guidelines. The plant material was collected from privately owned farmland with permission from the landowner.

CRediT authorship contribution statement

Moorthy Muruganandham: Resources, Project administration. Yuvaraj Tamilselvi: Conceptualization. Loganathan Lingeshwaran: Software, Kanagasabapathy Sivasubramanian: Formal analysis, Seema siddharthan: Data curation, Palanivel Velmurugan: Writing—original draft. Sivanraju Rajkumar: Conceptualization, review and editing.

Clinical trial number: Not applicable

“Ethics, Consent to Participate, and Consent to Publish declarations: Not applicable.”

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Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures

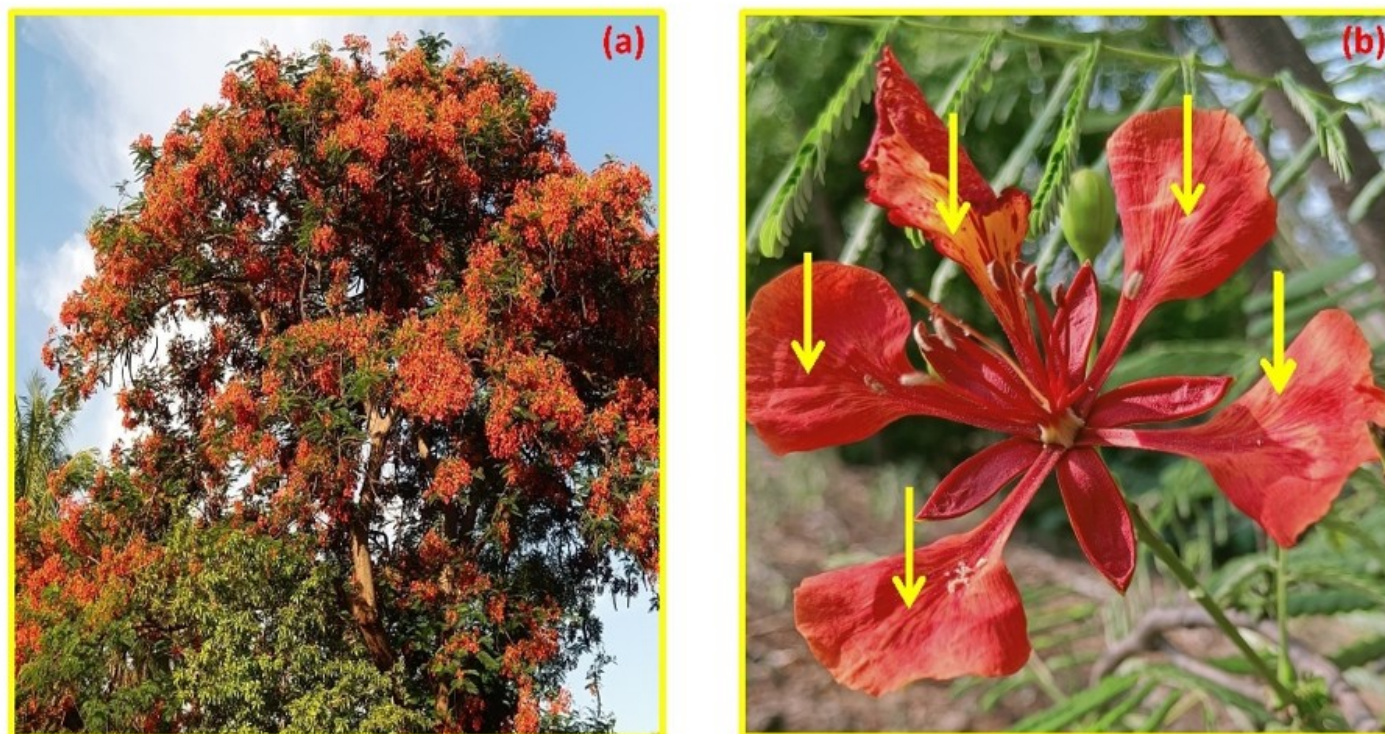


Figure 1

Delonix regia tree in full bloom (a), and a detailed view of an individual flower highlighting the vibrant red petals (b).

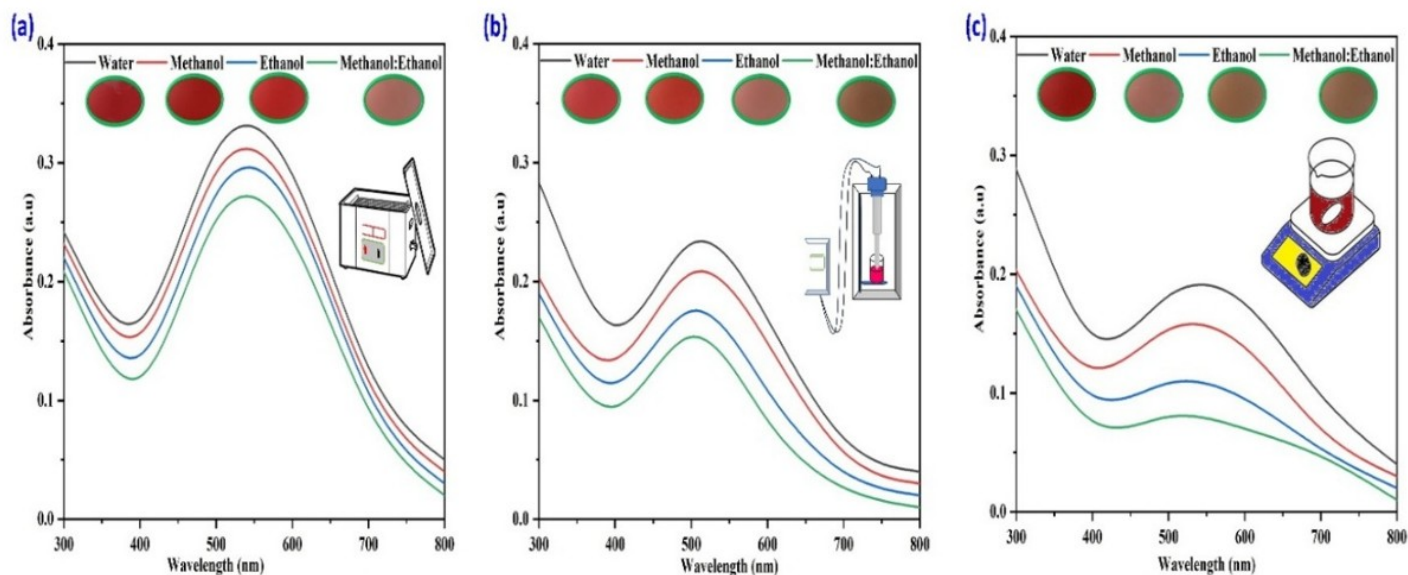


Figure 2

Extraction of red dye from *D. regia* flower petals using various solvents: Ultrasonic water bath (a), ultrasonic probe (b), and direct heating with magnetic steering (c). Data are expressed as mean $\pm$ SD ($n = 3$). Statistical differences were determined by one-way ANOVA and Tukey's test ($p < 0.05$)

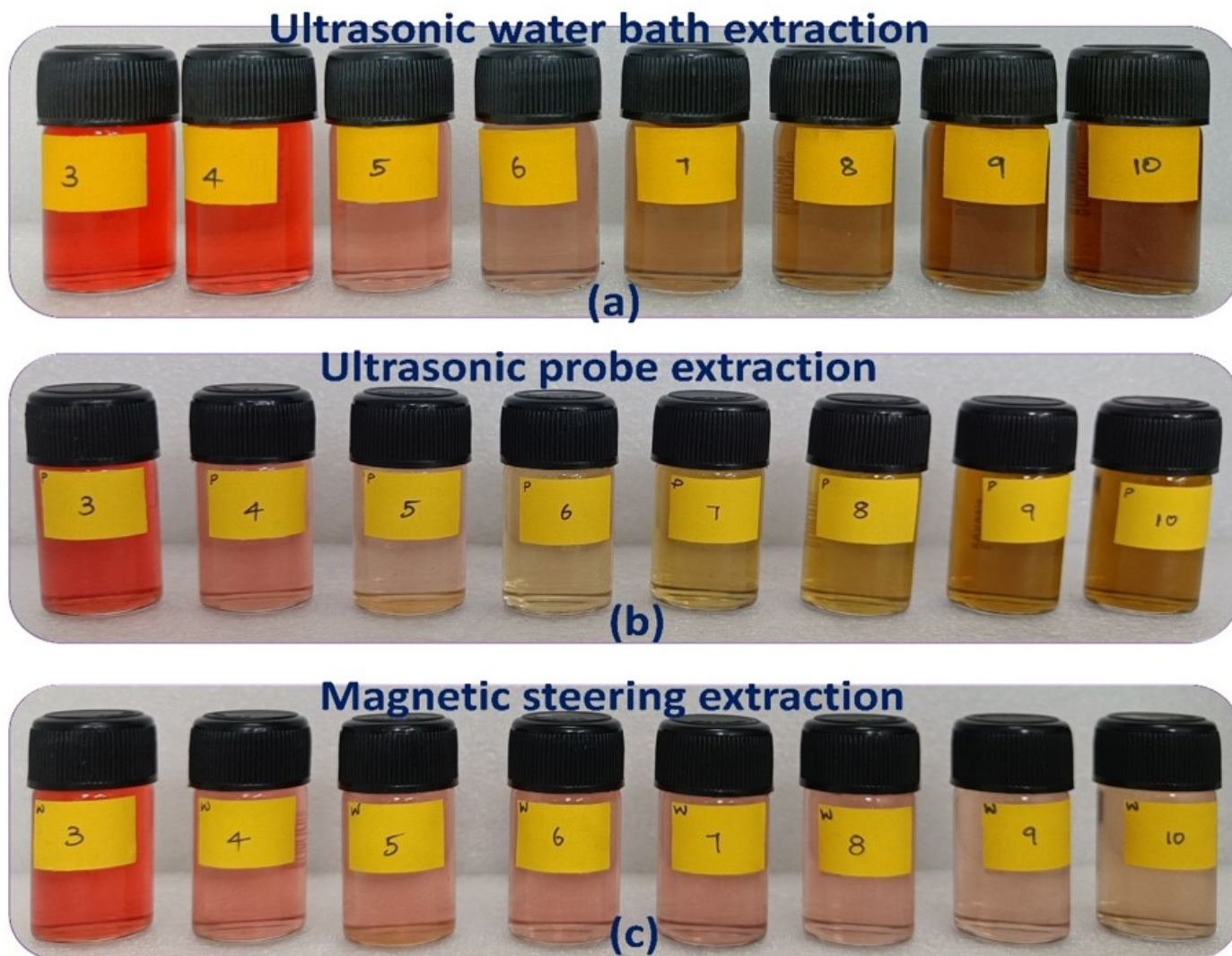


Figure 3

Images showing red dye extracted from *D. regia* flower petals at different pH levels adjusted using an ultrasonic water bath (a), ultrasonic probe (b), and direct heating with magnetic stirring (c), with water as the solvent.

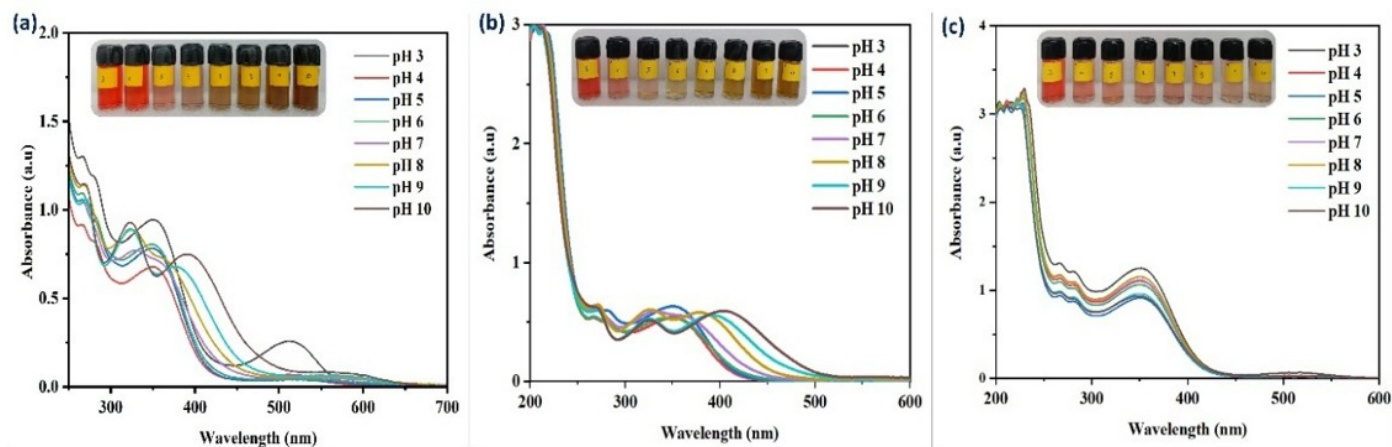


Figure 4

UV-Vis spectrum of red dye extracted from *D. regia* flower petals at different pH levels using ultrasonic water bath (a), ultrasonic probe (b), and direct heating with magnetic stirring (c), with water as the solvent.

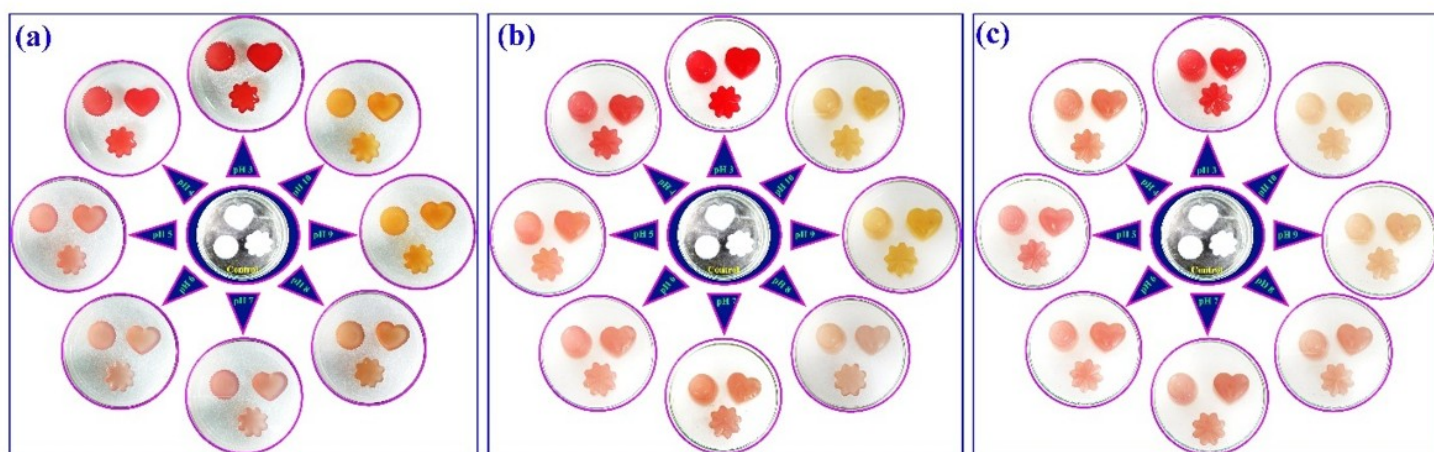


Figure 5

Preparation of jelly candies at different pH levels using red dye extracted from *D. regia* flower petals under an ultrasonic water bath (a), ultrasonic probe (b), and direct heating with magnetic stirring (c), with water as the solvent.

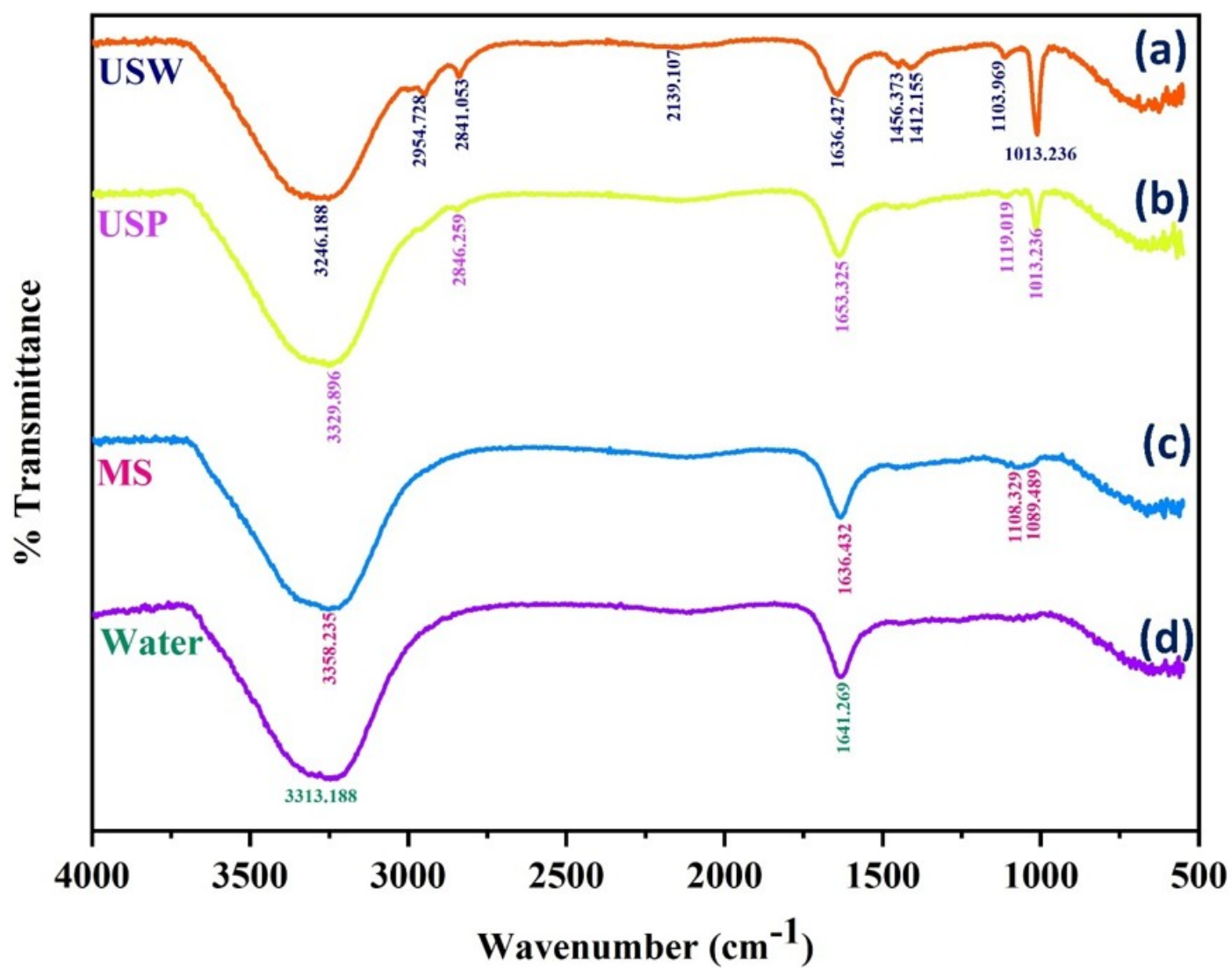


Figure 6

FTIR spectrum of red dye extracted from *D. regia* flower petals using an ultrasonic water bath (a), ultrasonic probe (b), and direct heating with magnetic stirring (c), with water as the solvent.

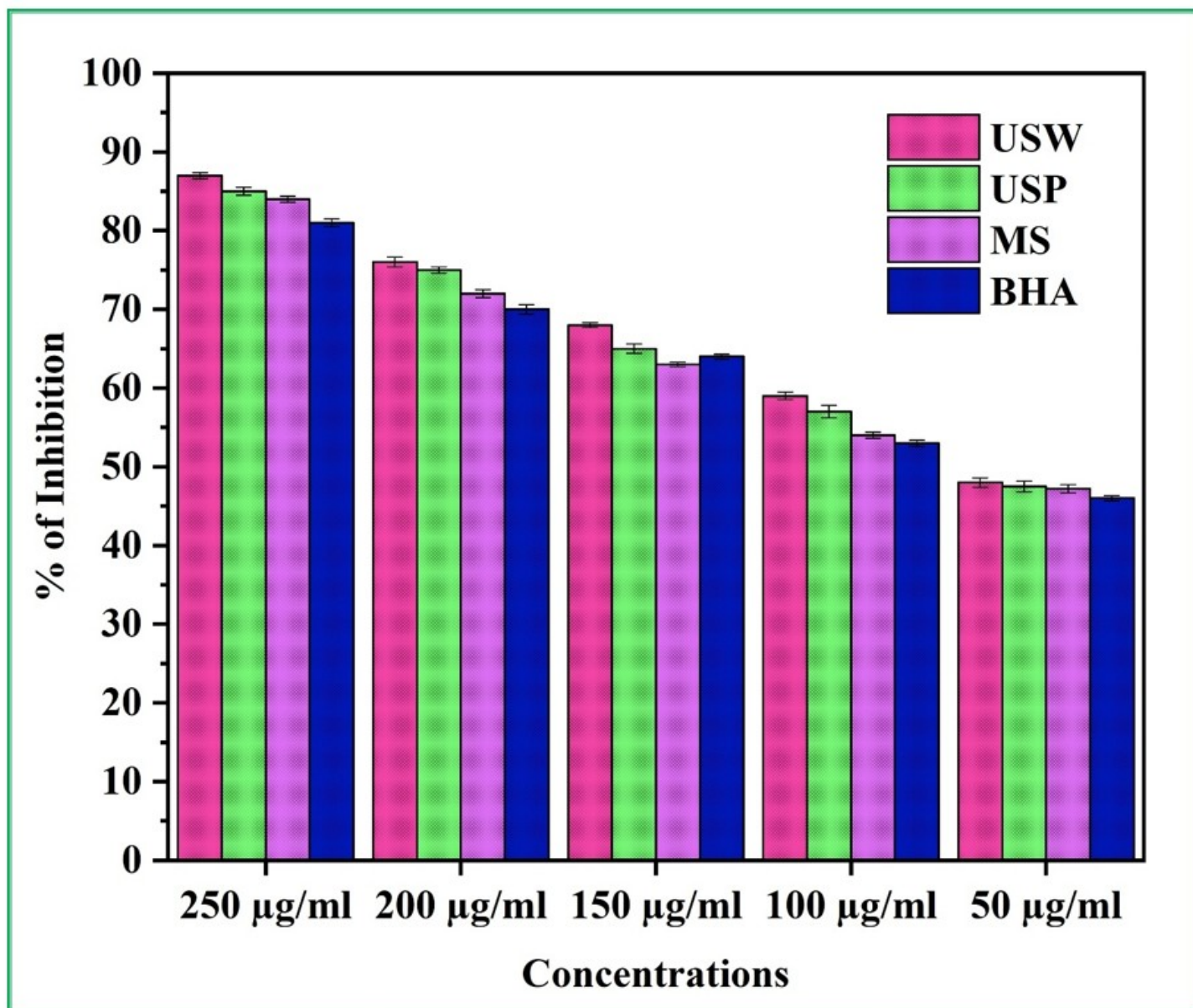


Figure 7

Antioxidant activity of red dye extracted from *D. regia* flower petals using ultrasonic water bath (a), ultrasonic probe (b), and direct heating with magnetic stirring (c), with water as the solvent. Data shown as mean $\pm$ SD ($n = 3$). Different superscript letters indicate statistically significant differences ($p < 0.05$, one-way ANOVA with Tukey's test).

Supplementary Files

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