PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(sp1): 01–11
https://doi.org/10.14719/pst.8023

HORIZON
e-Publishing Group

RESEARCH ARTICLE

Optimization of natural dye extraction from the flowers of *Ixora coccinea* Linn. using response surface methodology

Kamaleshwaran N K[1], Sundharaiya K[2*], Rajadurai K R[1], Sivakumar K P[3], Amirtham D[4], & Shanthanu R[5]

[1]Department of Floriculture and Landscape Architecture, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam 625 604, India

[2]Department of Fruit Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam 625 604, India

[3]Department of Food Science and Nutrition, Community Science College and Research Institute, Tamil Nadu Agricultural University, Madurai 625 104, India

[4]Department of Plant Biotechnology, CPMB & B, Tamil Nadu Agricultural University, Coimbatore 641 003, India

[5]Department of Floriculture and Landscape Architecture, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore 641 003, India

*Email: sundharaiya@tnau.ac.in

OPEN ACCESS

ARTICLE HISTORY
Received: 02 March 2025
Accepted: 17 March 2025
Available online
Version 1.0 : 07 April 2025

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Kamaleshwaran NK, Sundharaiya K, Rajadurai KR, Amirtham D, Sivakumar KP, Shanthanu R. Optimization of natural dye extraction from the flowers of *Ixora coccinea* Linn. using response surface methodology. Plant Science Today. 2025; 12(sp1):01–11. https:/doi.org/10.14719/pst.8023

Abstract

Present study optimized the extraction of natural dyes from *Ixora coccinea* Linn. (Rubiaceae) flowers to address the increasing environmental threats posed by using synthetic dyes. This method employed Box-Behnken design using response surface methodology to examine extraction factors like the extraction temperature (40-80 °C), extraction time (2-4 hr) and solvent-to-sample ratio (10-30 mL/g) to maximize the total monomeric anthocyanin content. The statistical analysis demonstrated that extraction temperature and solvent-to-sample ratio were the two main factors affecting the anthocyanin yield ($p < 0.0001$). The study showed a statistically strong validity with an adjusted R^2 of 0.9712 and a predicted R^2 of 0.9611, confirming excellent predictive ability. The optimal extraction conditions were set at 80 °C, 3 hr and 10 mL/g for maximum anthocyanin content of 66.67 mg c3gE/L. Gas Chromatography-Mass Spectroscopy studies of the ethanolic extract revealed a total of 38 phytochemical compounds including n-butyric acid 2-ethylhexyl ester (33.55 %), D-mannitol (25.01 %) and dl-α-tocopherol (18.02 %) being prominent. This study concludes successful optimization of extraction factors for *I. coccinea* flower dye and makes it a sustainable alternative over synthetic colorants having several industrial applications.

Keywords

GC-MS; *Ixora coccinea*; natural dye; optimization; response surface methodology; total monomeric anthocyanin content

Introduction

Colors play a vital role in our routine life and representing different cultures and feelings throughout the world. The colorants include both pigments and dyes (1). Natural dyes extracted from plants are biodegradable, nontoxic and do not harm the environment. In contrast, synthetic dyes that originate from petroleum-based chemical products are harmful to the environment and thus result in environmental pollution (2). The application of synthetic dyes has led to significant problems for both health and the environment (3). Synthetic dyes that are washed away are persistent in aquatic ecosystems because of their greater stability. They tend to reduce the depth of light penetration in water, thus interfering with the photosynthetic activity of small algae and phytoplankton as well as lowering gas solubility, resulting in the loss of aquatic ecosystems (4). As a consequence, there is an increasing awareness of

the harmful effects associated with synthetic dyes, which has led to a surge in demand for natural dyes that are both eco-friendly and non-toxic (1).

Natural dyes are colors that are extracted from living forms such as plants, microbes, animals and insects. People are showing a growing preference for environmentally sustainable products in their daily lives, including clothing, food, beverages and pharmaceuticals that utilize natural dyes. It is evident that only some natural dyes serve as textile dyes in the commercial industry. In the food industry, a substantial shift towards natural colorants is evident, driven by the rising popularity of healthy eating, which calls for replacing synthetic colorants with natural ones (5). These natural dyes also have other properties, such as repelling insects, neutralizing odors, acting as antifeedants and inhibiting the growth of microorganisms. Additionally, it has properties such as fluorescence and offers protection against UV radiation (6). Various naturally colored pigments are extracted from flower petals ranging from soft pastels to bright and bold hues (7). Natural dyes extracted from nontraditional flowers provide diverse income and sustainable livelihoods for small-scale farmers through the cultivation and utilization of wasteland (8).

I. coccinea Linn. belonging to the family Rubiaceae is a rounded shrub that is grown as a decorative plant which is native to India and Southeast Asia. It is referred as Idly poo in Tamil, Rangon in Bengali, flame of wood in English and Bandhaka in Sanskrit. Cycloartenol esters (9) found in its flowers have anticancer, cytotoxic, hepatoprotective, antibacterial and wound-healing (10) properties. Ixorene (11), ixorapeptide I, ixorapeptide II and quercitrin constitute some of the compounds found in the leaves that exhibit cardioprotective, antinociceptive, antioxidant, antidiarrheal, antiasthmatic, hypoglycemic and hypolipidemic properties. Furthermore, the roots have antioxidant qualities (12). The plant has been used for a variety of traditional medical purposes. Its leaves have been used as a remedy for diarrhoea. The roots have been used to treat several illnesses, such as fever, blisters, chronic ulcers, hiccoughs and other skin diseases. The flowers have also been used to treat dysentery and catarrhal bronchitis (13). Floral petal extracts from *Ixora coccinea* Linn. can effectively serve as natural dyes for cotton fabrics, with methanolic extracts yielding deeper colors than aqueous extracts (14). This study successfully optimized the dye extraction process from *I. coccinea* flowers using the Box-Behnken design within the response surface methodology.

Materials and Methods

2.1. Plant materials collection and preparation

The flowers of *I. coccinea* for this study were collected from the Botanical Garden of Tamil Nadu Agricultural University, Coimbatore. As seen in Fig. 1, the raw material was cleaned, washed, size reduced, shade dried and pulverized. To ensure the ideal conditions for the flower's effective drying, the flower's size was then reduced using scissors. The flowers of *I. coccinea* were dried for ten days in the shade. Further, the dried flower was pulverized using a mixer and sieved (180-micron thick mesh) to create a conducive environment for the efficient extraction of dye.

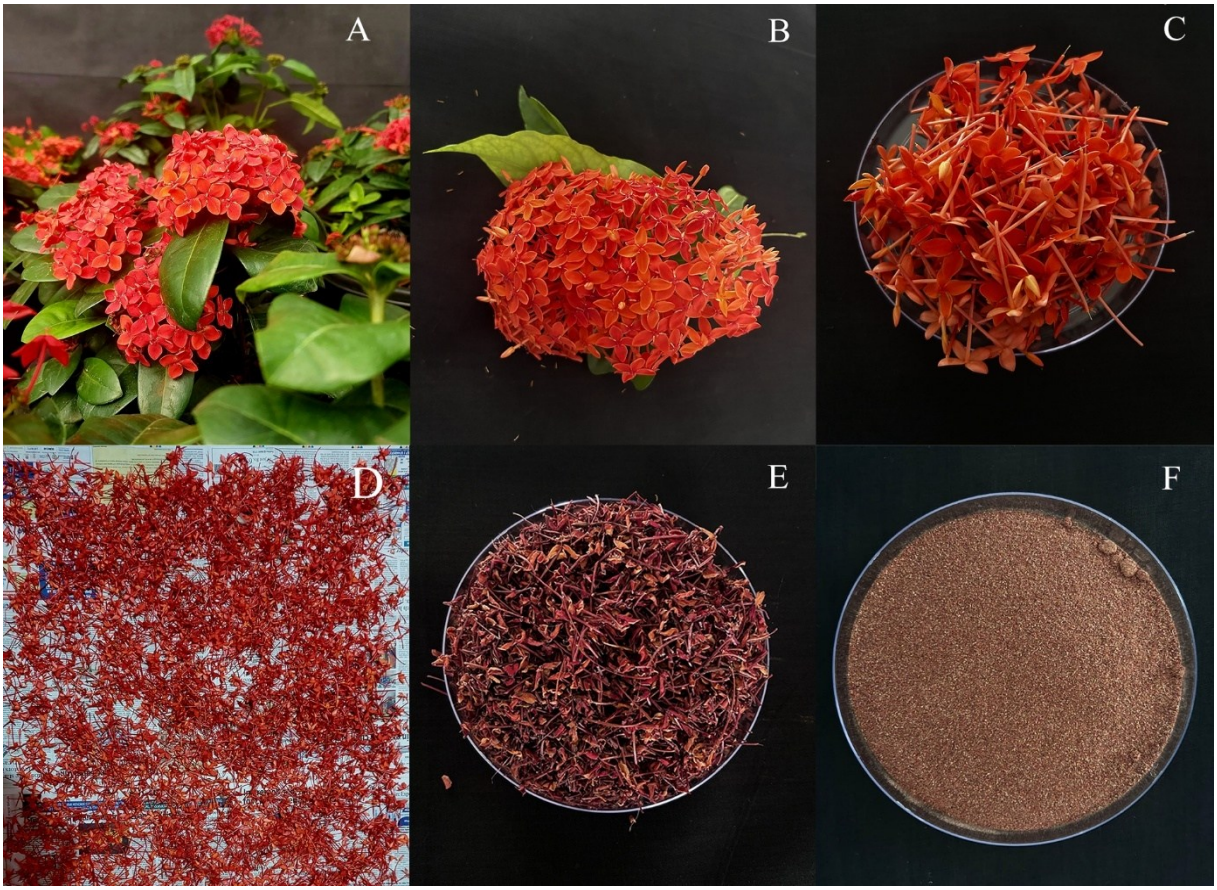

Fig. 1. Raw material preparation: *I. coccinea* plant (A), *I. coccinea* flowers (B), Cleaned and washed flowers (C), Shade drying (D), Dried flowers (E), Powdered flower sample (F).

2.2. Experimental design and optimization of dye extraction

To optimize the extraction of natural dye from *I. coccinea* flowers, the Box–Behnken approach of response surface methodology experimental design with three independent variables (extraction temperature, extraction time and solvent-to-sample ratio) at three levels (3^3) was used (15, 16). Design expert version 13 software was used for the optimization of Box -Behnken design. Basically, 27 experimental runs were generated using a full factorial design with three variables at three levels (3^3) in total. However, the number of experimental runs was decreased to 17 by making use of optimization strategies. Triplicate trials were conducted in each experimental run and the results were presented as average values. The independent variables are the extraction temperature, extraction time and solvent-to-sample ratio, whereas the total monomeric anthocyanin content (mg c3g E/ L) is the response variable. The solvent-to-sample ratio (10, 20 and 30 mL/g), extraction time (2, 3 and 4 h) and extraction temperature (40, 60 and 80 °C) are the independent variables with their respective design values, as presented in Table 1.

2.3. Dye extraction from Ixora coccinea flowers

In this study, natural dye has been extracted from *Ixora coccinea* flowers by using the Automatic Soxhlet apparatus (SOCSPLUS SCS 06 RTS model) using absolute ethanol as solvent. Additionally, the method includes weighing a sample that had already been sieved followed by placing it in a cellulose thimble. Through a funnel at the top of the condenser, absolute ethanol was transferred to the extraction chamber as a solvent. After turning on the electrothermal heating mantle, the solvent evaporated and condensed down to thimble by the means of the condenser. Subsequently, the extraction lasted 2, 3 and 4 h depending on the experimental design. A rotary evaporator was used to concentrate the extracted dye from the solvent after the extraction. Fig. 2 illustrates the key steps involved in extracting dye from *I. coccinea* flowers, where A, B and C represent the Automatic Soxhlet extraction stage, rotary evaporator and extracted dye, respectively.

2.4. Estimation of total monomeric anthocyanin content

2.4.1. Preparation of buffers : The pH 1.0 buffer (0.025 M potassium chloride) was prepared in a 1000 mL beaker by weighing 1.86 g of potassium chloride and dissolving it in 980 mL of distilled water. The pH meter was used to test the pH and 0.1 N HCl was used to adjust the pH to 1.0. Then the solution was transferred to a 1000 mL volumetric flask and distilled water was added to make up the volume. The pH 4.5 buffer (0.4 M sodium acetate trihydrate) was prepared similarly by weighing 54.43 g of sodium acetate trihydrate and dissolving in 980 mL of distilled water. The pH was adjusted to 4.5 using 0.1 N HCl. Then the solution was transferred to a 1000 mL volumetric flask and distilled water was added to make up the volume (17).

2.4.2. Estimation: The total monomeric anthocyanin content of the extracted dye was measured in the pH differential method using a double beam UV spectrophotometric analyzer (17). The appropriate dilution factor was determined by diluting the extracted dye with pH 1.0 buffer until the absorbance at 520 nm was within the linear range of the spectrophotometer. Using this dilution factor, prepare 2 dilutions of the dye, one with pH 1.0 buffer and the other with pH 4.5 buffer. Calculate the absorbance of the extracted dye at 520 and 700 nm after diluting it with pH 1.0 and pH 4.5 buffers. The diluted dye is read versus a blank cell filled with distilled water. Finally, values were recorded which are calculated using the following formula and the results are expressed as milligrams per liter (mg/L) cyanidin-3-glucoside equivalents (Eqn.1).

$$\text{Total monomeric anthocyanin content (mg c3g E/L)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1}$$

Where,

A (absorbance) = (A_{520} nm - A_{700} nm) pH 1.0 - (A_{520} nm - A_{700} nm) pH 4.5

MW (Molecular Weight) = 449.2/mol for cyanin-3-glucoside (c-3-g)

Table 1. Full factorial designs for dye extraction from *I. coccinea* flowers

Factors	Units	Lower (-)	Middle (0)	Higher (+)
Extraction temperature	Celsius	40	60	80
Extraction Time	Hours	2	3	4
Solvent-to-sample ratio	mL/g	10	20	30

Fig. 2. Extraction process of natural dye from *I. coccinea* flowers.

DF = Dilution factor

ε = Molar extinction coefficient of cyanidin-3-glucoside (26900 mol^{-1} cm^{-1})

1 = path length of cuvette in cm

10^3 = conversion factor (g to mg)

2.5. Characterization of Ixora coccinea flower dye through GC-MS

The volatile components of the *I. coccinea* ethanolic dye extract were analyzed via GC-MS using an Agilent 7890A gas chromatograph with a 5975C Mass Selective Detector. The system operated with electron ionization (70 V) and an ion source temperature of 250 °C. The analysis utilized an Agilent DB5MS capillary column (30 mm × 0.25 mm × 0.25 µm) with high-purity helium (99.9 %) as the carrier gas at a flow rate of 1 mL/min. The injector operated in split mode (1:60) with 1 µL injection volume. The temperature program started at 100 °C (held for 0.5 min), ramped to 140 °C at 20 °C/min (held for 1 min), then increased to 280 °C at 11°C/min with a 20 min final hold (18). Peak area measurements and data processing were performed using Mass Hunter software. Component identification relied on comparing obtained mass spectra with reference spectra from the NIST Wiley 2008 library. PubChem was used to confirm the molecular formula and molecular weight of the identified compounds (19).

Results and Discussions

3.1. Total monomeric anthocyanin content

Three factors were studied at three different levels in the dye extraction studies for *I. coccinea*: extraction temperature (40, 60 and 80 °C), extraction time (2, 3 and 4 hr) and solvent-to-sample ratio (10, 20 and 30 mL/ g). The experimental data for these levels and parameters is presented in Table 2. Data analysis can be used to assess the impact of each parameter and its level on the anthocyanin content of dye produced from *I. coccinea* flower. The highest anthocyanin content from *I. coccinea* flowers was achieved at an extraction temperature of 80 °C, an extraction time of 3 hr and a solvent-to-sample ratio of 10 mL/g. A total monomeric anthocyanin content of 66.67 mg c3gE/L was achieved using this combination of parameters. However, the lowest dye yield of 21.09 mg c3gE/L occurred at an extraction temperature of 40 ° C, an extraction time of 2 hr and a solid-to-liquid ratio of 20 mL/g. Comparing the results obtained by altering each parameter from the lowest to the highest values makes it obvious that the solvent-to-sample ratio had a major influence on the anthocyanin content (20). Decreasing the solvent-to-sample ratio from 30 mL/g (highest) to 10 mL/g (lowest) resulted in an increase in anthocyanin content from 22.96 to 66.67 mg c3gE/ L. This highlights how important it is to lower the solvent concentration for it to effectively interact with the flower powder. Increasing the extraction temperature from 40 °C (lowest) to 80 °C (highest) resulted in a considerable increase in anthocyanin from 21.04 to 66.67 mg c3gE/ L. This suggests that an increase in temperature allows the dye present in the flower to readily dissolve in solvent, leading to a higher yield. The extraction time had a smaller impact on the anthocyanin content than other factors. Highest anthocyanin content (66.67 mg c3gE/ L) was observed at 3 hr. The anthocyanin content dropped to 38.35 mg c3gE/L when the duration was extended to 4 hr. This suggests that extending the extraction period to 3 hr raised the anthocyanin content. On the other hand, the anthocyanin content decreased when the duration was extended over 3 hr. In general, the solvent-to-sample ratio and extraction temperature exhibited significant impacts on the anthocyanin content, while the extraction time had a comparatively smaller effect (21).

3.2. Analysis of variance (ANOVA) and fit summary

In this study, the ANOVA performed (Table 3) has two main functions. To confirm that the observed effects are not the result of chance, it first attempts to evaluate the statistical validity of the data obtained. Second, it seeks to create a model for the dye extraction procedure that can provide insight into how the parameters and anthocyanin content relate to one another. The study's adjusted R^2 value was 0.9712, meaning that the factors (extraction temperature, extraction time and solvent-to-sample ratio) employed may be responsible for around 97.12 % of the variability in the anthocyanin content. Conversely, predicted R^2 evaluates how well the model can forecast new data points or

Table 2. Total monomeric anthocyanin content of natural dye extracted from *I. coccinea* flower

		Factor 1	Factor 2	Factor 3	Response 1
Std	**Run**	**A: Temperature Celsius**	**B: Extraction Time Hours**	**C: Solvent-to-sample ratio mL/g**	**Total Monomeric Anthocyanin Content mg c3gE/L**
1	9	40	2	20	21.09
5	2	40	3	10	35.35
7	16	40	3	30	24
3	12	40	4	20	25.14
9	7	60	2	10	43.22
11	10	60	2	30	28.55
13	3	60	3	20	34.47
14	4	60	3	20	30.65
15	6	60	3	20	35.65
16	8	60	3	20	29.77
17	11	60	3	20	31.43
10	5	60	4	10	56.08
12	17	60	4	30	22.04
2	14	80	2	20	39.42
6	1	80	3	10	66.67
8	13	80	3	30	26.96
4	15	80	4	20	38.35

Table 3. ANOVA for optimization of natural dye extraction from *I. coccinea* flower

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2260.39	9	251.15	61.01	< 0.0001	significant
A-Extraction temperature	541.53	1	541.53	131.55	< 0.0001	
B-Extraction time	10.88	1	10.88	2.64	0.1480	
C-Solvent-to-sample ratio	1244.26	1	1244.26	302.26	< 0.0001	
Residual	28.82	7	4.12			
Cor Total	2289.21	16				

observations. The predicted R^2 in this study is 0.9611, indicating that the model's predictions are consistent with the observed data and reasonably accurate. The accuracy and durability of the regression model are demonstrated by the small (0.0101) difference between the adjusted R^2 and the predicted R^2. This result suggests that the model can successfully represent the underlying patterns and relationships in the dye extraction process, as illustrated in Fig. 3 and does not overfit the observed data.

The F value derived from the ANOVA indicates the model's significance (22). The model's F value in this case is 61.01, indicating strong significance ($p < 0.0001$). This suggests that the observed effects on the anthocyanin content are probably to be the result of noise or random variations (0.0001 or less). The model is therefore regarded as statistically significant, indicating that the anthocyanin content is significantly influenced by the selected factors (extraction temperature, extraction time and solvent-to-sample ratio) taken together. Among the individual factors, extraction temperature (A) and solvent-to-sample ratio (C) exhibited significant effects ($p < 0.0001$). The knowledge of the relative influence of each factor on the anthocyanin content can be derived from the final equation, which is expressed in terms of coded factors. The factors A (extraction temperature), B (extraction time) and C (solvent-to-sample ratio) are represented by their respective coefficients (+8.23, +1.17 and 12.47) in the equation. These coefficients indicate the direction and magnitude of the influence of each factor on the anthocyanin content, as indicated in (Eqn. 2).

Total monomeric anthocyanin content = + 32.39 + 8.23 A + 1.17 B - 12.47 C (Eqn.2)

Accordingly, the coefficient of A says that increasing the extraction temperature (A) by one unit (coded level) increases 8.23 units in the anthocyanin content. Similarly, increasing the extraction time (B) by one unit increases the anthocyanin content by 1.17 units and increasing the solvent-to-sample ratio (C) by one unit decreases the anthocyanin content by 12.47 units. Therefore, it can be inferred that the solvent-to-sample ratio has a stronger influence on the anthocyanin content than others. This implies that maximizing the anthocyanin content of *I. coccinea* flowers involves reducing the solvent-to-sample ratio.

3.3. Interaction effect of factors on anthocyanin content

3.3.1. Extraction temperature and extraction time : The combined interaction effect of the extraction temperature (°C) and extraction time (hr) on the total monomeric anthocyanin content is depicted in 3D and contour mapping in Fig. 4. To study the interactive effect of the variables

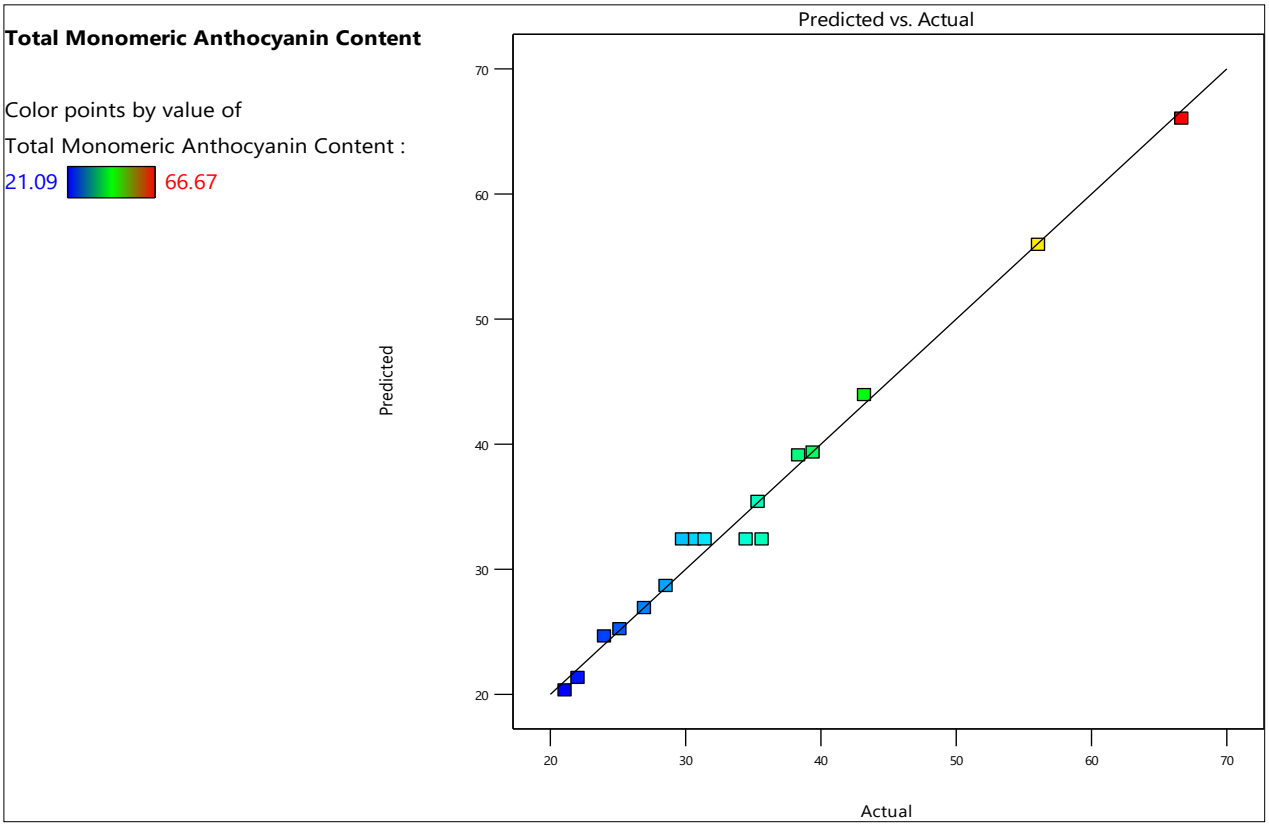

Fig. 3. Predicted and actual values for optimization of extraction of dye.

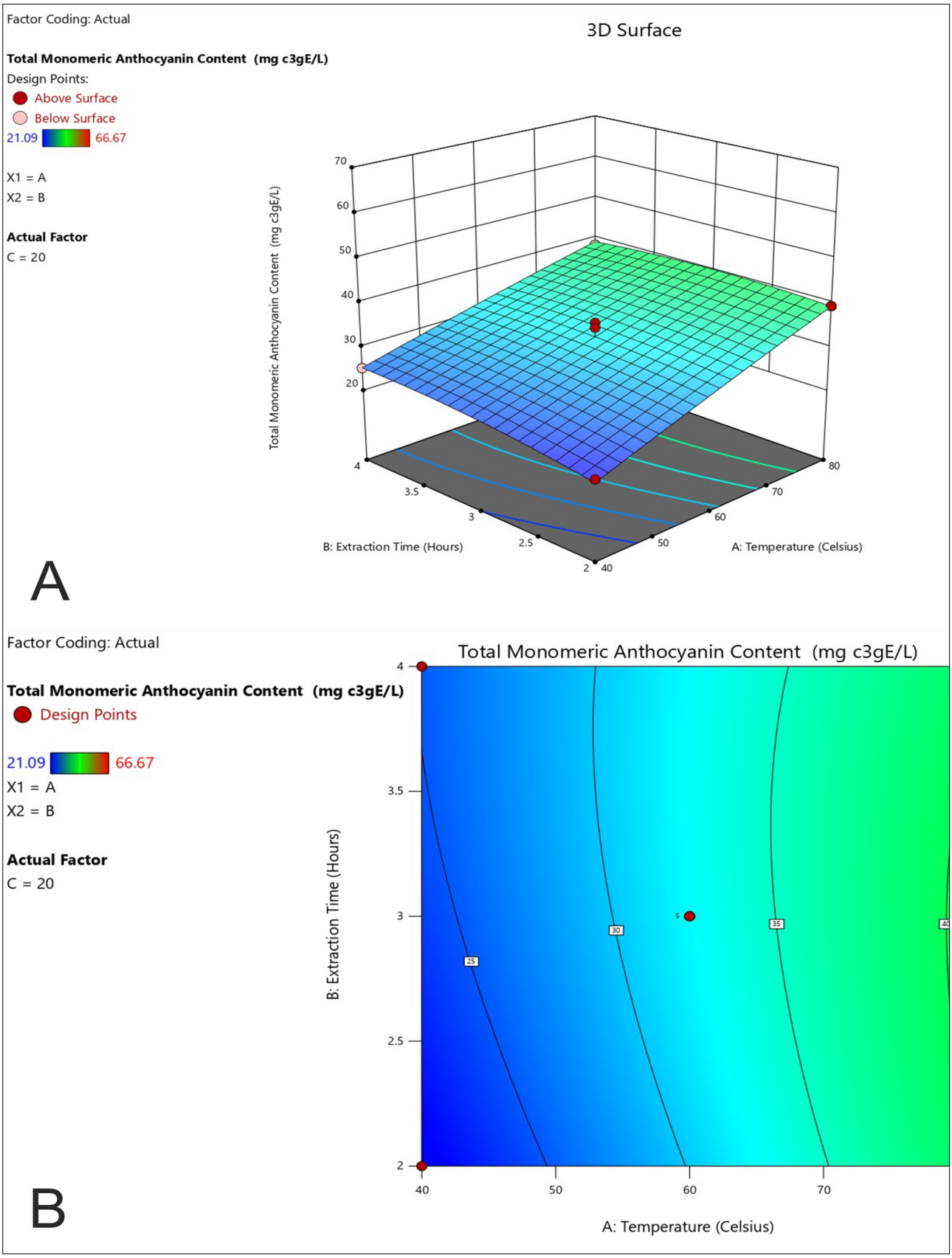

Fig. 4. Interaction effect between extraction temperature and extraction time (A) 3D response surface and (B) contour map.

influencing the extraction processes on the response variable, which is the total monomeric anthocyanin content, the Box-Behnken design approach's response surface methodology was adopted. The solvent-to-sample ratio was maintained constant at 20 mL/g to examine the interaction between the two factors and the anthocyanin content. The 3D view and contour map showed that, at a minimum extraction temperature of 40 °C, drop-in extraction time from 4 to 2 hr resulted in a decrease in the anthocyanin content of the extracted dye from 25.14 to 21.09 mg c3g E/L. The primary factor that affects anthocyanin production is the extraction temperature. The yield of anthocyanins can be increased by raising the extraction temperature. It is clear that the extraction temperature is the primary factor affecting the pigment yield (23). The anthocyanin content increased from 21.09 to 39.42 mg c3g E/L and from 25.14 to

38.35 mg c3g E/L for 2 and 4 hr, respectively, when the extraction temperature was raised from 40 °C to 80 °C. Improved mass transfer dynamics are responsible for the increased anthocyanin content observed at high temperatures. As temperature rises, two key phenomena occur simultaneously: the pigments become more soluble and the solvent's viscosity decreases. These changes facilitate greater mobility of molecules and more effective dissolution of the target compounds, ultimately leading to a more efficient extraction process (24).

3.3.2. Extraction time and solvent-to-sample ratio: The Box-Behnken design approach's response surface methodology was used to examine the interaction between the variables extraction time (hr) and solvent-to-sample ratio (mL/g) on the response variable, total monomeric anthocyanin content. The interaction effect of the two factors on the anthocyanin content of the extracted dye is depicted in 3D and contour maps in Fig. 5. At the constant extraction temperature of 60 °C, the 3D image and contour map reveal a maximum anthocyanin content of 56.08 mg c3g E/L at an extraction time of 4 hr and the solvent-to-sample ratio of 10 mL/g. This was performed to study the interaction effect. However, with a solvent-to-sample ratio of 30 mL/g and an extraction time of 4 hr, respectively, the lowest anthocyanin concentration of 22.04 mg c3g E/L was noted. This highlights how the solvent-to-sample ratio and extraction time are directly and inversely correlated with the anthocyanin content, respectively.

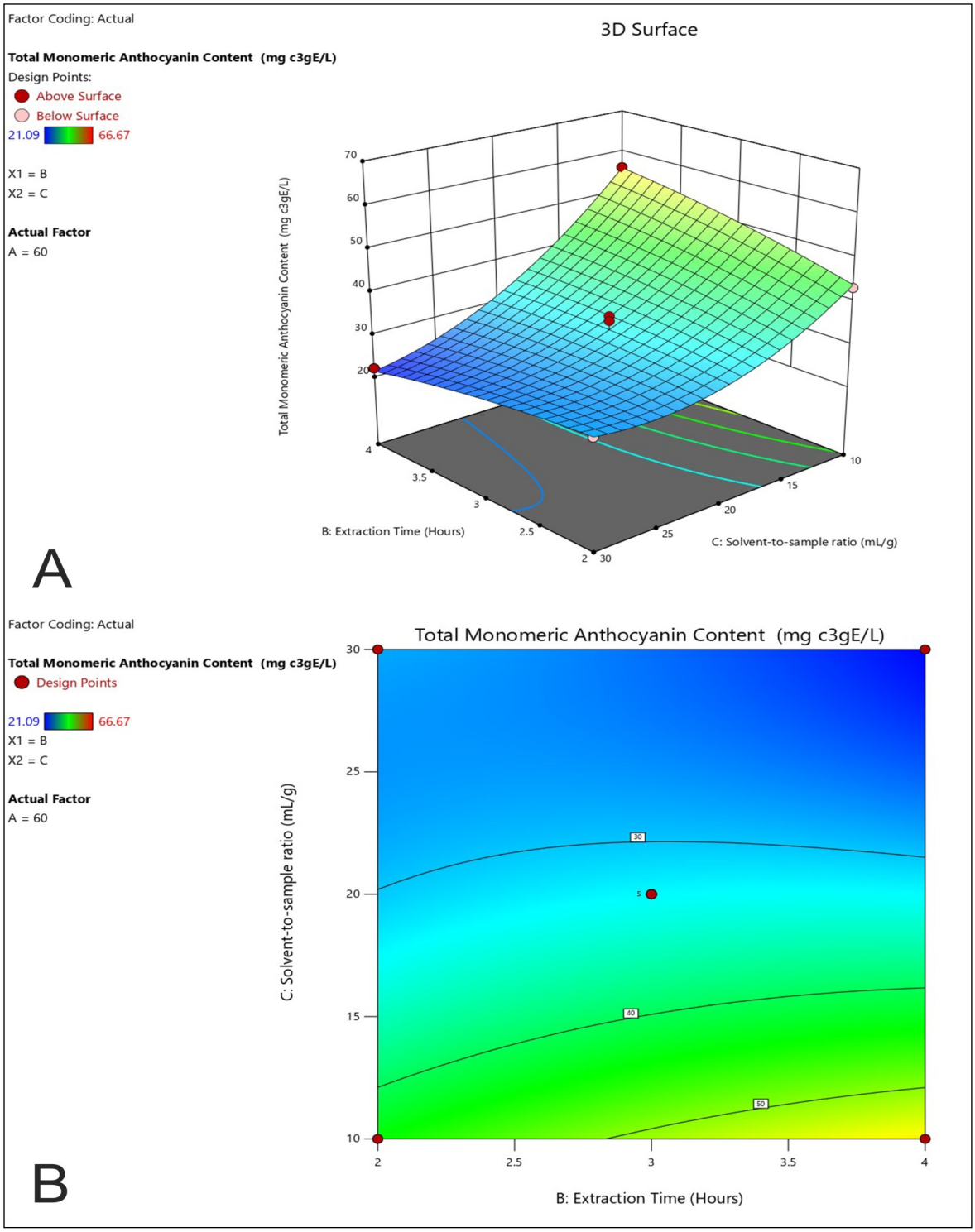

Fig. 5. Interaction effect between extraction time and Solvent-to-sample ratio (A) 3D response surface and (B) contour map.

The anthocyanin content increased from 43.22 to 56.08 mg c3g E/L when the sample's extraction time was extended from 2 to 4 hr at a solvent-to-sample ratio of 10 mL/g. The extraction time has an impact on the anthocyanin content. The longer the solvent and sample were in contact, the greater the chance of mass transfer (25). Similarly, the anthocyanin content increased from 28.85 to 43.22 mg c3g E/L when the solvent-to-sample ratio decreased from 30 mL/g to 10 mL/g at a 2-hour extraction time. The maximum amount of solvent had successfully diffused into the solid (sample) at a concentration of 10 mL/g. As a result, the red dye dissolved readily up to a concentration that was restricted by the solid's characteristics (26). As the liquid-to-solid ratio increased further, a decrease in the yield of total anthocyanin content was observed. The primary cause of this decline is the increasing solvent proportion, which lowers the solid-weight ratio and lowers the extracted anthocyanin's density thus decreasing the overall anthocyanin (27).

3.3.3. Extraction temperature and solvent-to-sample ratio : The response surface plot and contour map in Fig. 6 shows the interaction effect between extraction temperature and solvent-to-sample ratio. The extraction time of 3 h was maintained constant. At a maximum extraction temperature of 80 °C, reducing the solvent-to-sample ratio from 30 mL/g to

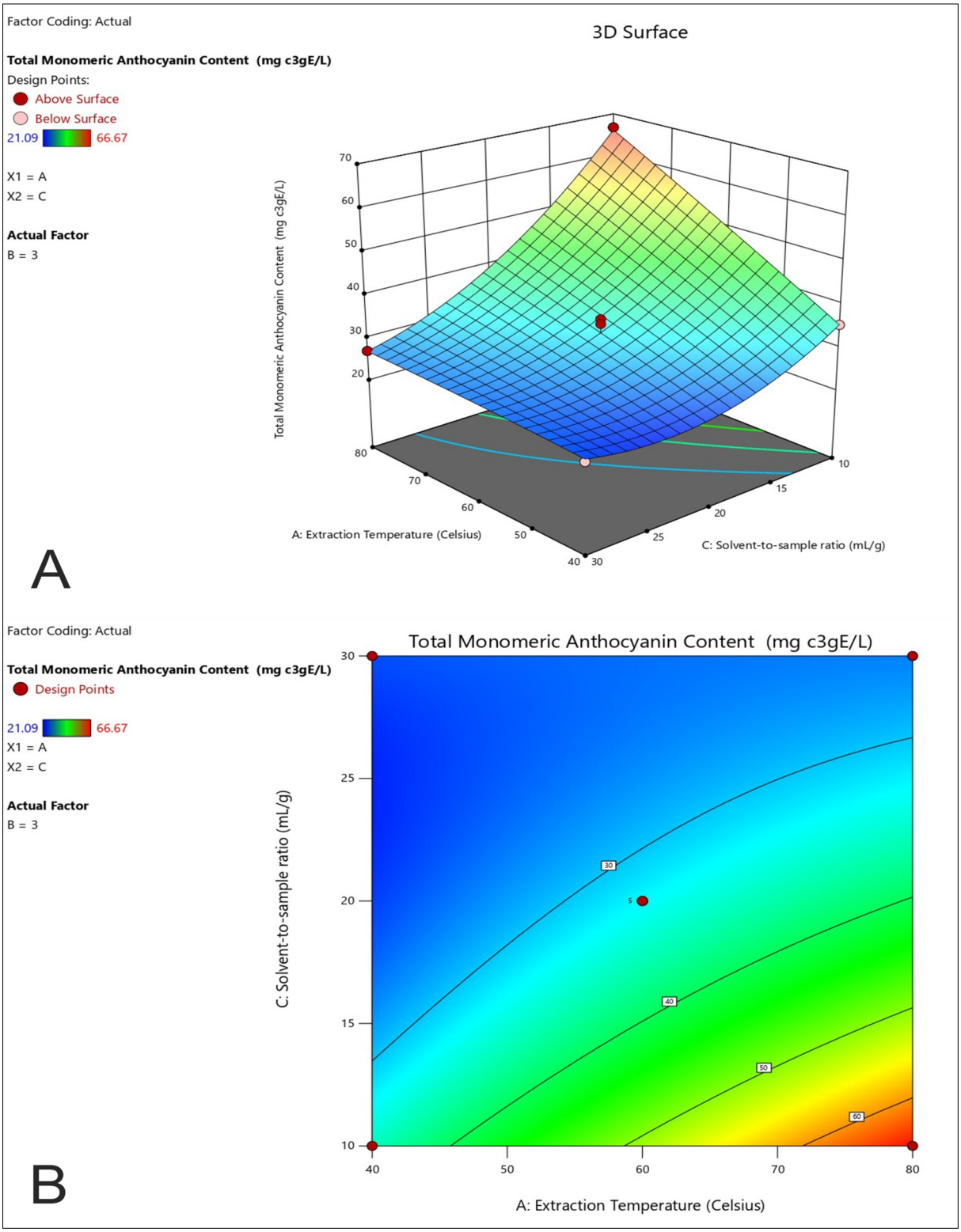

Fig. 6. Interaction effect between extraction temperature and Solvent-to-sample ratio (A) 3D response surface and (B) contour map.

10 mL/g was observed to increase the anthocyanin content of the extracted dye from 26.96 to 66.67 mg c3g E/L. Earlier research established that a 20 mL/g solvent-to-sample ratio provided optimal conditions for anthocyanin extraction, which aligns closely with current findings. The increase in temperature caused the cell walls to burst, which enhanced anthocyanin solubility and reduced the extract's viscosity by raising intracellular pressure (28).

3.4. Characterization through GC-MS analysis: Purified extract fractions of *I. coccinea* were analyzed using gas chromatography-mass spectrometry (GC-MS). This analysis revealed the presence of several phytochemical compounds in the pigment, which may support the plant's medicinal properties and other significant applications. The identities of these components were confirmed by comparing characteristics such as molecular weight, peak area, molecular formula and retention time with data from the NIST Wiley 2008 library. Among 38 identified compounds, the major compounds based on area % are n-Butyric acid 2-ethylhexyl ester (33.55), D-Mannitol (25.01), dl-α-Tocopherol (18.02), β-Sitosterol (6.38), n-Hexadecanoic acid (3.36), 4,5-Dimethylthiazole S-oxide (2.20), Germacyclopentane (1.76). Table 4 lists all the compounds included in GC-MS analysis. Among the detected compounds, n-Butyric acid 2-ethylhexyl ester is identified as the most abundant. Related compounds such as n-Hexadecanoic acid and β-Sitosterol were also found through the GC-MS analysis of the methanolic extract of *I. coccinea* that has significant antidepressant activity (29, 30).

Conclusion

Utilizing response surface methodology, the study optimized the extraction process of natural dye from *I. coccinea* flowers, determining that the optimal conditions were an extraction temperature of 80 °C, an extraction time of 3 hr and a solvent-to-sample ratio of 10 mL/g. These conditions led to a maximum anthocyanin yield of 66.67 mg c3gE/L. The study underscores the potential of *I. coccinea* as a sustainable alternative to synthetic dyes, with GC-MS analysis identifying bioactive compounds that could enhance its industrial applications. Future research should investigate the dye's stability, scalability and commercial potential in the textile, food and pharmaceutical industries. Additionally, assessing its compatibility with eco-friendly mordants and its biodegradability could further solidify its role in sustainable practices.

Table 4. GC-MS analysis of *I. coccinea* flower dye

S. No.	Retention time	Chemical Compound	Molecular Formula	Molecular Weight (g/mol)	Area %
1	4.798	Ethanethiol	C_2H_6S	62.134	0.18
2	5.009	2-Cyclopenten-1-one	C_5H_6O	82.1005	0.16
3	5.587	Methanamine	CH_3NH_2	31.06	0.84
4	6.242	2-Propen-1-amine	C_3H_7N	57.0944	0.04
5	6.287	Ethene	C_2H_4	28.05	0.31
6	6.531	2,5-Furandione	$C_4H_2O_3$	98.06	0.26
7	7.375	4H-Pyran-4-one	$C_5H_4O_2$	96.08	0.65
8	7.753	3-ethyl-Pyrimidine	C_7H_9N	107.15	0.28
9	7.986	2-Hydroxyethyl propyl sulfide	$C_5H_{12}OS$	120.213	0.11
10	8.042	2,4-Thiazolidinedione	$C_3H_3NO_2S$	117.13	0.13
11	8.486	Isothiazole	C_3H_3NS	85.12	0.04
12	8.664	2-Butynamide	C_4H_5NO	83.090	0.09
13	9.020	1,3-Cyclohexadiene-1-carboxaldehyde	C_7H_8O	108.1378	0.20
14	9.764	Benzenamine	$C_6H_5NH_2$	93.13	0.87
15	10.097	2-ethoxy-2-Propenoic acid	$C_5H_8O_3$	116.11	0.04
16	10.153	5H-1-Pyrindine-3-carboxylic acid	$C_{11}H_{13}NO_2$	191.23	0.11
17	10.186	2,4-Thiazolidinedione	$C_3H_3NO_2S$	117.13	0.05
18	10.286	4,5-Dimethylthiazole S-oxide	C_5H_7NOS	129.18	2.20
19	10.397	2-Amino-4-methyl-4-pentenoic acid	$C_6H_{11}NO_2$	129.16	0.15
20	10.475	3,4-Difluoroaniline	$C_6H_5F_2N$	129.11	1.76
21	10.753	3-Methyl-1,2,4-Thiadiazole	$C_3H_4N_2S$	100.15	0.21
22	10.808	Benzoic acid	$C_7H_6O_2$	122.123	1.18
23	10.930	9,12-Octadecadiynoic acid	$C_{18}H_{28}O_2$	276.41	0.54
24	11.053	3-Methyl-thiazole	C_4H_7NS	101.17	0.82
25	11.797	Methylguanidine	$C_2H_7N_3$	73.0971	0.04
26	12.564	n-Butyric acid 2-ethylhexyl ester	$C_{12}H_{24}O_2$	200.3178	33.55
27	13.008	N-Ethylformamide	C_3H_7NO	73.0938	0.21
28	13.208	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	3.36
29	13.530	Guanidine	CH_5N_3	59.07	0.59
30	13.863	Ethyl 4-methylbenzoate	$C_{10}H_{12}O_2$	164.2011	0.50
31	14.274	D-Mannitol	$C_6H_{14}O_6$	182.17	25.01
32	14.686	Propanamide	C_3H_7NO	73.095	0.29
33	15.863	2,4-Pentadienenitrile	C_5H_5N	79.10	0.10
34	15.863	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430.71	18.02
35	16.874	isocyanato- methane	C_2H_3NO	57.0513	0.04
36	17.007	3-(Methylthio)propanoic acid methyl ester	$C_5H_{10}O_2S$	134.197	0.45
37	20.407	β-Sitosterol	$C_{29}H_{50}O$	414.71	6.38
38	20.896	6(2h)-Benzofuranone	$C_8H_6O_2$	134.13	0.24

Acknowledgements

I would like to express my sincere gratitude to Dr. J. Rajangam, Ph.D., Dean (Horticulture), Horticultural College and Research Institute, Tamil Nadu Agricultural University - Periyakulam, for his invaluable guidance and support throughout the preparation of this research article. His insightful feedback and constant encouragement have been instrumental in shaping the direction of this work.

Authors' contributions

All authors were involved in research work, literature review, compiled the research findings, drafted the manuscript, revised and finalized the article. All authors have read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Yusuf M, Shabbir M, Mohammad F. Natural colorants: Historical, processing and sustainable prospects. Natural Products and Bioprospecting. 2017;7:123-45. https://doi.org/10.1007/s13659-017-0119-9
2. Adeel S, Rehman F-U, Rafi S, Zia KM, Zuber M. Environmentally friendly plant-based natural dyes: extraction methodology and applications. Plant and Human Health, Volume 2: Phytochemistry and Molecular Aspects. 2019:383-415. https://doi.org/10.1007/978-3-030-03344-6_17
3. Mittal J. Permissible synthetic food dyes in India. Resonance. 2020;25(4):567-77. https://doi.org/10.1007/s12045-020-0970-6
4. Bose P, Rajasekaran A. Decolourization of textile dyes using bacterial consortium. Int J Sci Res Environ Sci. 2016;4(1):0017-22. https://doi.org/10.12983/ijsres-2016-p0017-0022
5. Křížová H. Natural dyes: their past, present, future and sustainability. Recent Developments in Fibrous Material Science. 2015:59-71.
6. Kumar J, Sinha AK. Resurgence of natural colourants: a holistic view. Natural product research. 2004;18(1):59-84. https://doi.org/10.1080/1057563031000122112
7. Baig U, Khatri A, Ali S, Sanbhal N, Ishaque F, Junejo N. Ultrasound-assisted dyeing of cotton fabric with natural dye extracted from marigold flower. The Journal of the Textile Institute. 2021;112(5):801-8. https://doi.org/10.1080/00405000.2020.1779907
8. Geissler S. Economic aspects of natural dyes. Handbook of Natural Colorants. 2009;8:367. https://doi.org/10.1002/9780470744970.ch21
9. Ragasa CY, Tiu F, Rideout JA. New cycloartenol esters from Ixora coccinea. Natural Product Research. 2004;18(4):319-23. https://doi.org/10.1080/14786410310001630519
10. Philomina N, Kumar SP. Antimicrobial activity of *Ixora coccinea* flowers. Asian J Microbiol Biotechnol Environ Sci. 2011;13:605-7.
11. Ikram A, Versiani MA, Shamshad S, Salman KA, Ali ST, Faizi S. Ixorene, a new Dammarane Triterpene from the Leaves of *Ixora coccinea* Linn. Records of Natural Products. 2013;7(4):302.
12. Surana A, Aher A, Pal S. *In vitro* and *in vivo* antioxidant activity of *Ixora coccinea*. J Med plants Res. 2013;7:3071-5.
13. Torey A, Sasidharan S, Latha LY, Sudhakaran S, Ramanathan S. Antioxidant activity and total phenolic content of methanol extracts of *Ixora coccinea*. Pharmaceutical Biology. 2010;48(10):1119-23. https://doi.org/10.3109/13880200903490505
14. Ghurde MU, Padwad M, Deshmukh V, Malode S. Extraction of natural dye from *Ixora coccinea* (Linn.) flowers for cotton fabric colouration. Int J Sci Res. 2016;5(1):1272-6. https://doi.org/10.21275/v5i1.NOV153003
15. Guddi K, Sur S, Sarkar A. Analysis of floral biomass utilization feasibility of *Ixora coccinea* for its cost-effective application as a natural colorant. Biomass Conversion and Biorefinery. 2024;14(14):15661-75. https://doi.org/10.1007/s13399-022-03668-7
16. Sinha K, Saha PD, Datta S. Extraction of natural dye from petals of Flame of forest (*Butea monosperma*) flower: Process optimization using response surface methodology (RSM). Dyes and Pigments. 2012;94(2):212-6. https://doi.org/10.1016/j.dyepig.2012.01.008
17. Lee J, Durst RW, Wrolstad RE, Kupina. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants and wines by the pH differential method: collaborative study. Journal of AOAC International. 2005;88(5):1269-78.
18. Mohan C, Dinakar S, Anand T, Elayaraja R, SathiyaPriya B. Phytochemical, GC-MS analysis and Antibacterial activity of a Medicinal Plant Acalypha indica. Int J Pharm Tech Res. 2012;4(3):1050-4.
19. Igwe K, Nwankwo P, Otuokere I, Ijioma S, Amaku F. GCMS analysis of phytocomponents in the methanolic extract of *Moringa oleifera* leave. J Res Pharm Sci. 2015;2(11):1-6.
20. Larasati ID, Carrera C, Lioe HN, Estiasih T, Yuliana ND, Ray HRD, et al. Anthocyanin extraction from roselle (*Hibiscus sabdariffa* L.) calyces: A microwave-assisted approach using Box-Behnken design. Journal of Agriculture and Food Research. 2024;18:101480. https://doi.org/10.1016/j.jafr.2024.101480
21. Bekele N, Abewaa M, Mengistu A, Adino E, Takele T, Yilma M, et al. Optimization of natural dye extraction from the root of *Rumex abyssinicus* plant using response surface methodology. Results in Chemistry. 2024;8:101577. https://doi.org/10.1016/j.rechem.2024.101577
22. Ravikumar K, Pakshirajan K, Swaminathan T, Balu K. Optimization of batch process parameters using response surface methodology for dye removal by a novel adsorbent. Chemical Engineering Journal. 2005;105(3):131-8. https://doi.org/10.1016/j.cej.2004.10.008
23. Fan G, Han Y, Gu Z, Chen D. Optimizing conditions for anthocyanins extraction from purple sweet potato using response surface methodology (RSM). LWT-Food Science and Technology. 2008;41(1):155-60. https://doi.org/10.1016/j.lwt.2007.01.019
24. Yolmeh M, Najafi MBH, Farhoosh R. Optimisation of ultrasound-assisted extraction of natural pigment from annatto seeds by response surface methodology (RSM). Food Chemistry. 2014;155:319-24. https://doi.org/10.1016/j.foodchem.2014.01.059
25. Oktaviyanti ND, Kartini K, Hadiyat MA, Rachmawati E, Wijaya AC, Hayun H, et al. A green extraction design for enhancing flavonoid compounds from *Ixora javanica* flowers using a deep eutectic solvent. Royal Society Open Science. 2020;7(10):201116. https://doi.org/10.1098/rsos.201116
26. Veggi PC, Santos DT, Fabiano-Tixier A-S, Bourvellec Cl, Meireles MAA, Chemat F. Ultrasound-assisted extraction of polyphenols from jatoba (*Hymenaea courbaril* L. var stilbocarpa) bark. 2013.
27. Izirwan I, Munusamy T, Hamidi N, Sulaiman S. Optimization of microwave-assisted extraction of anthocyanin from Clitoria ternatea flowers. International Journal of Mechanical Engineering and Robotics Research. 2020;9(9):1246-52. https://doi.org/10.18178/ijmerr.9.9.1246-1252
28. Chan CH, Lim JJ, Yusoff R, Ngoh GC. A generalized energy-based

kinetic model for microwave-assisted extraction of bioactive compounds from plants. Separation and Purification Technology. 2015;143:152-60. https://doi.org/10.1016/j.seppur.2015.01.041

29. Surana AR, Wagh RD. Phytochemical analysis and antidepressant activity of *Ixora coccinea* extracts in experimental models of depression in mice. Turkish Journal of Pharmaceutical Sciences. 2018;15(2):130. https://doi.org/10.4274/tjps.14622

30. Dontha S, Kamurthy H, Mantripragada B. Phytochemical and pharmacological profile of Ixora: a review. International Journal of Pharmaceutical Sciences and Research. 2015;6(2):567.