

Original

## Characterizing and Utilizing Delphinidin from *Clitoria ternatea* as a Safer Alternative to Artificial **blue** Colourants

### Abstract

Delphinidin, a natural pigment found in *Clitoria ternatea*, was investigated as an alternative to artificial blue food colourants associated with potential health risks, including cancer. The methodology involved hydroalcoholic extraction of Delphinidin from *Clitoria ternatea* flowers, followed by lyophilization to obtain a stable powder form. Various analytical techniques were employed to characterize and assess the quality of the extracted Delphinidin, including Thin Layer Chromatography (TLC), Spectroscopy, Calorimetry, Fourier Transform Infrared Spectroscopy (FTIR), and High-Performance Liquid Chromatography (HPLC). These techniques were utilized to identify, quantify, and ensure the purity of the extracted compound. The nutritional analysis of Delphinidin was conducted to understand its potential health benefits and nutritional profile. Stability tests were performed to evaluate the shelf life and storage conditions of the formulated natural food colour. The overarching goal of the project was to introduce a safer alternative to synthetic blue food colours by utilizing Delphinidin, addressing concerns related to artificial additives and their potential health implications. Incorporating advanced analytical techniques and conducting thorough assessments, the research aimed to contribute to the development of natural and healthier food colour options, aligning with the growing demand for clean-label and sustainable food products. This endeavor not only explored the potential anti-cancer properties of Delphinidin but also offered a more sustainable and health-conscious approach to food colouring in the food industry.

**Keywords:** *Clitoria ternatea*, Delphinidin, Natural pigment, Lyophilization, Food Colour and Antioxidant Assay

### Introduction

Artificial blue colourants in food often contain synthetic additives, such as coal-tar derivatives or petroleum-based dyes <sup>[1-2]</sup>. These additives have been associated with potential health risks, including a heightened risk of cancer and various adverse effects <sup>[3]</sup>. Studies

suggest a correlation between certain artificial blue colourants and carcinogenic properties, triggering concerns about their safety<sup>[3]</sup>. In some individuals may experience allergic reactions or hypersensitivity to these artificial additives<sup>[4,5]</sup>. As a result, there is a growing demand for natural alternatives<sup>[6]</sup>, like Delphinidin from *Clitoria ternatea*, to replace synthetic blue colourants and address health and safety issues in the food industry<sup>[7]</sup>.

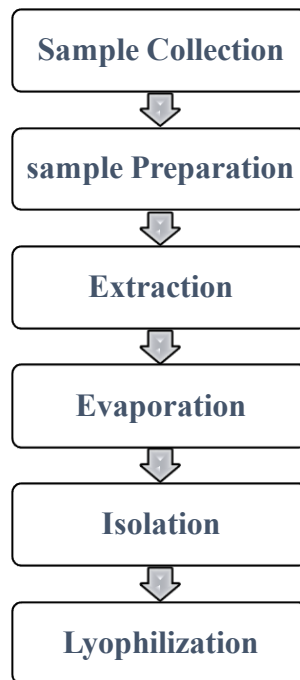
*Clitoria ternatea*, commonly known as Butterfly Pea or Blue Pea, is a flowering plant native to Southeast Asia<sup>[8]</sup>. Renowned for its vibrant blue flowers, the plant has been traditionally used in Ayurvedic and traditional medicine for its potential health benefits<sup>[9]</sup>. Rich in antioxidants, *Clitoria ternatea* is known for its anti-inflammatory properties<sup>[10]</sup>. The flowers are also valued for their natural blue pigment, Delphinidin, which is used as a food colourant<sup>[11]</sup>. Beyond its medicinal and culinary uses, Butterfly Pea is appreciated for its ornamental beauty and adaptability, making it a popular choice in gardens and landscapes<sup>[12]</sup>.

Delphinidin is a natural colourant found in certain plants, notably in the vibrant blue petals of *Clitoria ternatea* flowers<sup>[13]</sup>. Widely utilized in the food industry, Delphinidin serves as a natural blue pigment, offering an attractive hue without the use of synthetic additives<sup>[14]</sup>. Its application enhances the visual appeal of various food products, from beverages and confections to baked goods<sup>[15]</sup>. Beyond its colouring properties, Delphinidin is valued for being a safer alternative to artificial colourants, aligning with the growing demand for natural and health-conscious food ingredients<sup>[16]</sup>. The compound also brings potential nutritional benefits and is recognized for its antioxidant properties<sup>[9,17]</sup>.

This project focuses on characterizing and utilizing Delphinidin, derived from *Clitoria ternatea*, as a safe alternative to artificial blue colourants in the food industry<sup>[7]</sup>. Delphinidin, a natural blue pigment, is extracted and evaluated for its potential health benefits, specifically its anti-cancer properties and nutritional value<sup>[9,17]</sup>. By replacing synthetic additives with this natural compound, the research aims to contribute to safer food colouring options<sup>[7,11]</sup>. The exploration of Delphinidin aligns with the increasing demand for clean-label and sustainable products, offering a health-conscious approach to food colouration and addressing concerns associated with artificial additives<sup>[7,9]</sup>.

## Methodology

### List 1. Study protocol



### Sample Collection

In this study, the fresh *Clitoria ternatea* flowers were collected from the locality of Dindugal district, Tamilnadu, India.<sup>[18]</sup>

### Sample Preparation

The petals of *Clitoria ternatea* were separated and rinsed with clean water to remove any debris and impurities <sup>[19]</sup>. Then, the petals were subjected to grinding process. The grinded mixture of *Clitoria ternatea* petals were obtained <sup>[20]</sup>.

### Extraction Process

Hydroalcoholic technique was used with ethanol and water for the extraction of blue dye from grinded sample. In this technique, ethanol and water were taken in the ratio of 6:4

respectively. The grinded sample was soaked for 48 hours to obtain maximum yield. Then the liquid was strained off and the obtained yield was allowed for evaporation [21,22].

## Evaporation

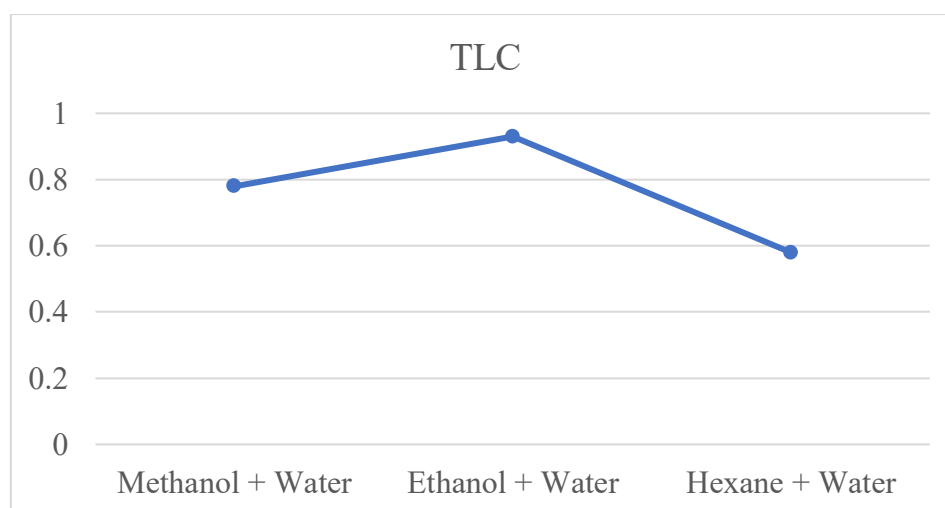
The extracted yield was heated at 80° C for 24 hours using water bath. Thus, the solvents were completely evaporated and the pure yield of blue dye was obtained [23].

## Isolation

Separates compounds based on adsorption, allowing identification and quantification; mobile phase moves through stationary phase.

## Results and Discussion

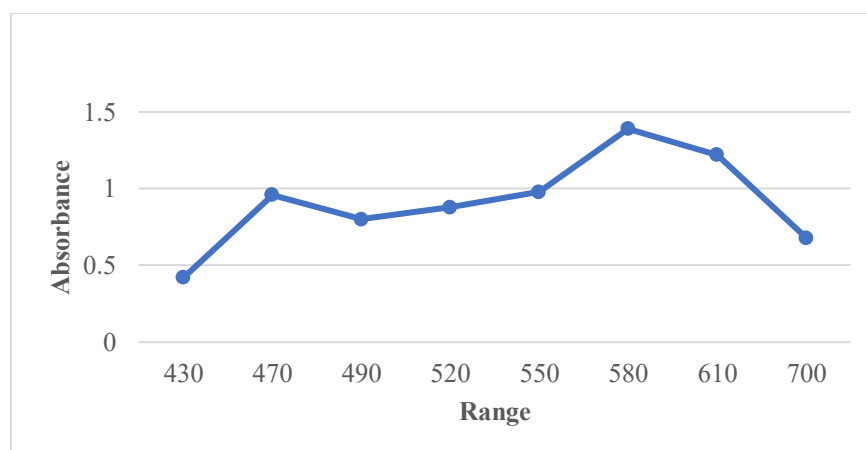
**Fig.1 Thin Layer Chromatography (TLC)**



The results of the Thin-Layer Chromatography (TLC) analysis show the retardation factor (Rf) value of a separated delphinidin compound from the flower extract separated by hexane and water in a 9:1 ratio. The spot appeared pale yellowish dark blue colour in all samples.

The results of the TLC analysis in Figure indicate that the compound separated from the flower extract has a similar polarity to delphinidin. The result identified is reference of (Kim, *et al.*, 2008).

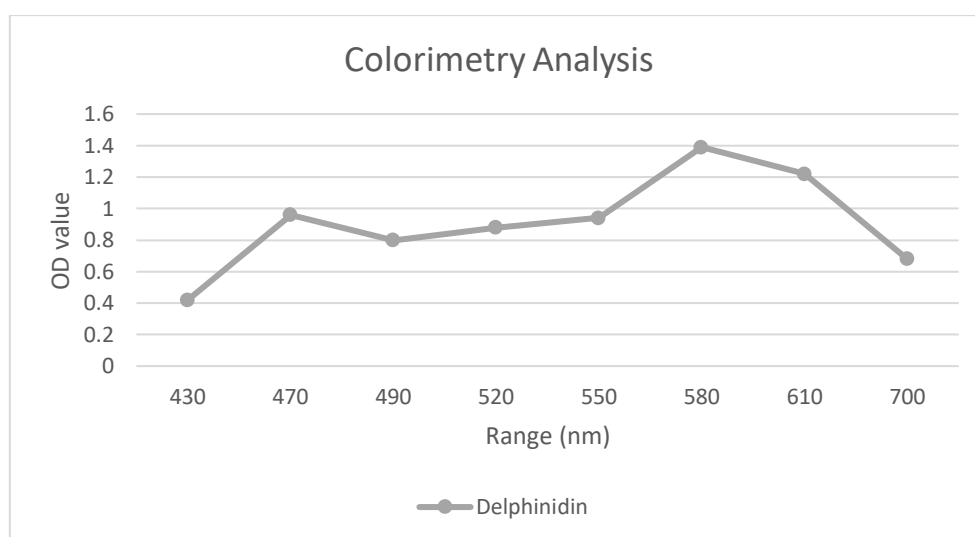
**fig.2 Spectrophotometer Analysis**



Pigments absorb light at specific wavelengths due to their unique molecular structures. The spectrophotometer measures the amount of light absorbed (absorbance) across a range of wavelengths (**Moyano, *et al.*, 2010**). This data is presented as a spectrum, with absorbance plotted on the y-axis and wavelength on the x-axis.

The highest absorbance peak at 580 nm aligns well with the expected range (530-630 nm) for anthocyanin pigments, particularly delphinidin. This suggests a strong likelihood of delphinidin being present in Sample C (**Han, *et al.*, 2015**).

**fig .3 Colorimetric Analysis**



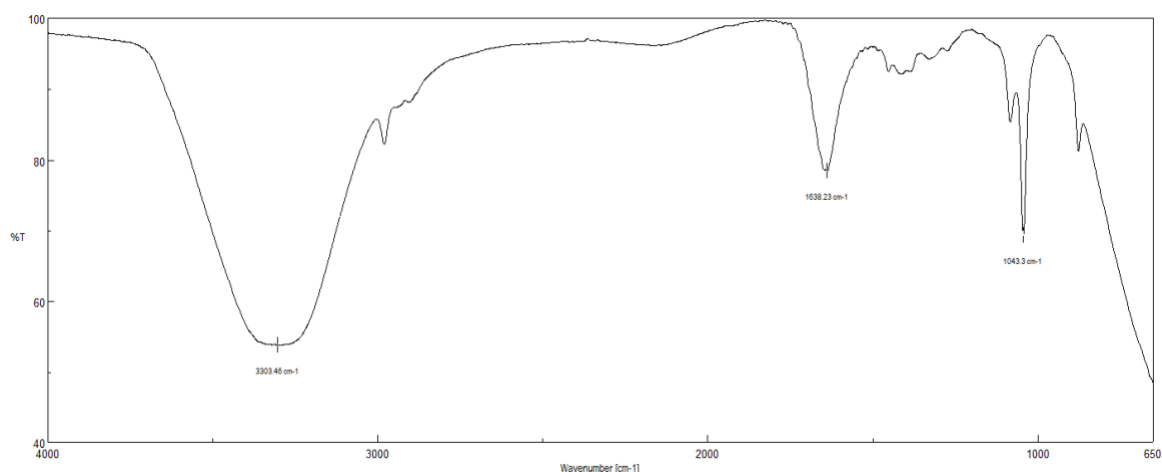
The given Colorimetric Analysis shows the concentration of delphinidin. The colourimetric data reinforces the presence of the pigment identified through UV-Vis analysis. The similar peak absorbance ranges between the two techniques (around 580 nm for delphinidin) strengthen the initial identification (**Bernardi, *et al.*, 2002**).

# FTIR Analysis

Sample	Absorption Reading (Range cm-1)	Functional Group	Functional Compound	Intensity
Delphinidin	3303.46	N-H Stretching	Amines & Amides	Medium
	1638.23	C=N Stretching	Ketones	Medium
	1043.3	CO-O-CO Stretching	Alkyl Amines	Strong-Broad

**Table 1: Interpretation of FTIR Analysis for Delphinidin**

**Fig. 4. FTIR graph**

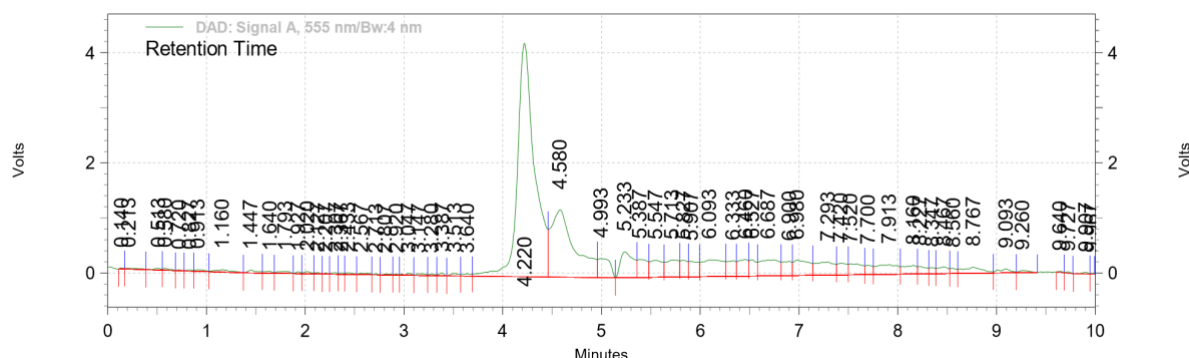


The above functional group analysis data (Table 1) offers valuable insights into the potential chemical composition of delphinidin. By analyzing the absorption readings and assigned functional groups, the functional compounds present in the sample can be identified.

The samples share some functional groups, including C=N stretching (indicative of ketones) and CO-O-CO stretching (characteristic of alkyl amines). This suggests the presence of similar core structures, potentially consistent with their classification as pigments (Blando, *et al.*, 2001). The presence of N-H stretching aligns with anthocyanin pigments. The lack of S=O stretching suggests a cleaner spectrum in this case (Bakri, *et al.*, 2016).

## HPLC Analysis

Fig. 5. HPLC graph



As the separated molecules exit the column (elute), they are detected by a specific detector depending on the type of analysis. Common detectors include ultraviolet (UV) detectors for absorbing molecules or mass spectrometers (MS) for identifying their masses.

The HPLC analysis provides promising indications for the presence of delphinidin in the sample. The highest peak at a retention time of 4.22 minutes falls within the typical range reported for delphinidin (2 - 8 minutes). The detection wavelength of 520 nm aligns with common practices for analyzing anthocyanins like delphinidin. The use of a phenyl column, while not universally employed, is suitable for separating these types of compounds. The symmetrical and narrow peak shape shows good separation from other components, further supporting the potential presence of delphinidin.

## Nutritional Analysis

Table 2 presents the results of the nutritional analysis for Samples, which are presumably extracted natural pigment. The data on carbohydrate and protein content added as the advantages of natural pigments compared to synthetic food colours.

## Stability Analysis

Pigments are primarily used for their colouring properties. Stability testing helps predict how the colour of the pigment will change over time under various storage conditions (light,

temperature). These anthocyanin pigments are more susceptible to degradation. Factors like light, heat, pH and presence of enzymes can all contribute to colour changes. Stability analysis helps identify the specific conditions that accelerate their degradation in food products.

**Table 2 : Heat Treatment (Spectrophotometer reading)**

<b>Temperature (10 mins at water bath)</b>	<b>Delphinidin (550 nm)</b>
<b>30°C</b>	2.482
<b>50°C</b>	2.484
<b>70°C</b>	2.489
<b>100°C</b>	2.492
<b>120°C</b>	2.498

Heat treatment is a common technique used in stability analysis to assess the impact of temperature on the degradation of pigment like delphinidin (Ngamwonglumlert, *et al.*, 2017).

Pigment solution (delphinidin) was prepared at a 3ml concentration in a suitable solvent (water – 1ml, Ethanol- 1ml). Divide the pigment solution into aliquots (equal portions) in separate vials or test tubes. Label each aliquot with the intended heat treatment temperature. Place the test in a water bath at different temperature range of 30°C, 50°C, 70°C, 100°C and 120°C. These ranges of temperature will elucidate how the colour of pigment withstand at various temperatures. This determines the stability of sample. Measure the absorbance of the solution using spectrophotometer at the pigment's characteristic wavelength of delphinidin at 550nm. A decrease in absorbance over time/temperature indicates degradation.

The Heat treatment results in the increases in intensity of colour in sample. This is primarily due to a thermal degradation of delphinidin. When subjected to heat, delphinidin molecules can undergo structural changes and break down into smaller compounds due to the disruption of molecular bonds. This process is accelerated at higher temperatures. As a result of these

chemical changes, the absorption and reflection of light by the pigment can be altered, leading to a shift in colour towards darker shades (Srinivas, *et al.*, 2012).

**Table 3 Light Treatment (Spectrophotometric Value – 7 Days Experiment)**

Sample	Before Exposure (nm)	After Exposure (nm)
Delphinidin (550nm)	2.362	1.564

Light exposure is a significant factor affecting the stability of many natural pigments.

Pigment solution of pigment (delphinidin) at a 3ml concentration in a suitable solvent (water – 1ml, Ethanol- 1ml). Divide the pigment solution into aliquots (equal portions) in separate vials or test tubes. The absorbance of the pigments delphinidin was observed in spectrophotometer before exposure to sunlight. Then the aliquated sample was exposed to sunlight for 7 days. The absorbance of the pigment was observed in spectrophotometer after 7 days of exposure to sunlight. Before and after exposure absorbance were compared and stability was determined.

The Light treatment results in the decreases in intensity of colour in sample. This is primarily due to a photo degradation of delphinidin. When exposed to sunlight, that results in the absorption of light energy by the pigment molecules, which triggers chemical reactions resulting in the degradation of delphinidin. This degradation may involve the breaking of chemical bonds within the pigment molecules, leading to the formation lighter-coloured compounds.

## **Application**

Fig. 6. Natural food colour Delphinidin



The extracted natural food colour Delphinidin was applied to the mojito, a popular beverage among the young communities. The colour features of mojito look exactly similar to those mojitos added with artificial food colours. This confirms that the extracted natural colour would be a great alternative for artificial blue colourant (**Katz, *et al.*, 2023**).

The natural colorant, Delphinidin used in the Mojito showed stability without altering taste. This indicates compatibility between the natural colour and the drink's flavour profile. It's suitable for enhancing the Mojito's visual appeal without compromising its taste, ensuring an enjoyable drinking experience.

## Conclusion

The surge in consumer demand for natural food ingredients has spurred investigations into alternative sources for food colouring agents. Research delves into natural pigment, Delphinidin extracted from *Clitoria ternatea*. This research illuminates a promising trajectory for harnessing natural pigment derived from *Clitoria ternatea* as eco-friendly substitutes for synthetic food colouring agents. The study enriches the burgeoning domain of sustainable food technology by presenting a tangible means to mitigate the detrimental impacts of artificial additives, concurrently elevating the nutritional value and adaptability of diverse food items. These findings underscore a significant stride towards fostering healthier food choices and environmental stewardship. Moreover, the study advocates for continued investigation to unlock further applications and refine extraction and stabilization methodologies conducive to large-scale commercial deployment. As such, this research not only advances scientific understanding but also offers tangible pathways for the food industry to embrace sustainable practices, aligning with contemporary consumer preferences and global sustainability imperatives.

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