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## Refined Protocol for the Isolation and Purification of BETALAINS Pigment from *Celosia argentea* y *Beta vulgaris*

Antonino Michel Lecona Jiménez<sup>1</sup>, Kalpana Nanjareddy<sup>1</sup>,  
Manoj-Kumar Arthikala<sup>1</sup>

<sup>1</sup>Ciencias Agrogenómicas, Escuela Nacional de Estudios Superiores, Unidad León.  
Universidad Nacional Autónoma de México



Antonino Michel Lecona Jiménez

Ciencias Agrogenómicas, Escuela Nacional de Estudios Superio...

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We use this protocol and it's working

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## ABSTRACT

Betalains, a group of secondary metabolites exclusive to the Caryophyllales order, are particularly abundant in plants such as beets. These compounds play a crucial role as pigments, imparting vibrant hues to different parts of the plant, ranging from roots to flowers. Beyond their aesthetic appeal, recent investigations have unveiled the multifaceted potential of betalains in diverse fields.

In biotechnology, betalains are being explored for their applications in areas such as natural dyes, food coloring agents, and even as potential alternatives to synthetic antioxidants. Their ability to exhibit vivid colors while also offering health benefits makes them particularly attractive for various industrial applications. In medicine, research suggests that betalains may possess antioxidant, anti-inflammatory, and even anti-cancer properties. These compounds show promise in mitigating oxidative stress, inflammation, and cellular damage, thereby potentially contributing to the prevention and treatment of various diseases. Furthermore, in the realm of human nutrition, betalains are increasingly recognized for their potential health-promoting effects. They are being investigated for their role in cardiovascular health, immune function, and overall well-being. Incorporating betalain-rich foods into diets may offer nutritional advantages beyond mere color enhancement.

The protocol outlined here provides a systematic approach for the extraction and purification of betalains from different types of fresh plant tissues, including tubers, flowers, and stems. By obtaining purified samples, researchers can delve deeper into the biochemical characteristics of betalains, unraveling their mechanisms of action and exploring their full potential in various applications. This meticulous methodology serves as a crucial step towards harnessing the benefits of betalains for both scientific advancement and practical use in industries ranging from biotechnology to medicine and nutrition.

## IMAGE ATTRIBUTION

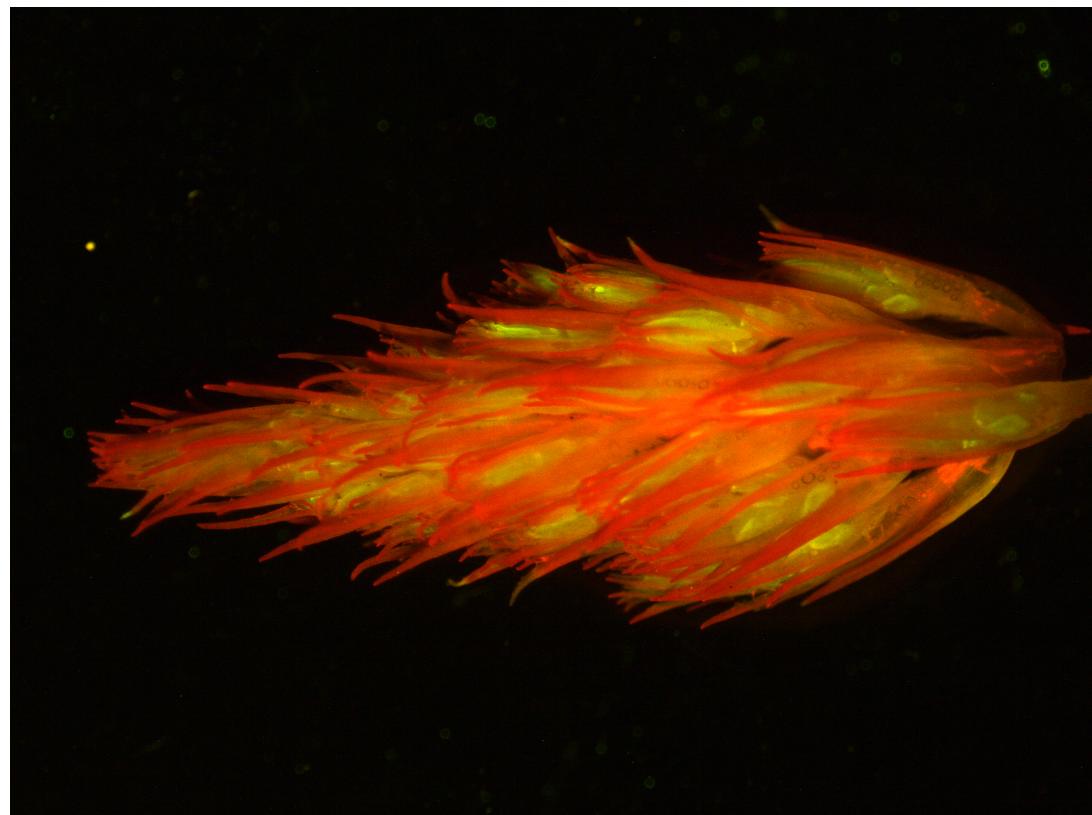


Image of betacyanin fluorescence in *Celosia argentea* inflorescence under stereoscopic microscopy.

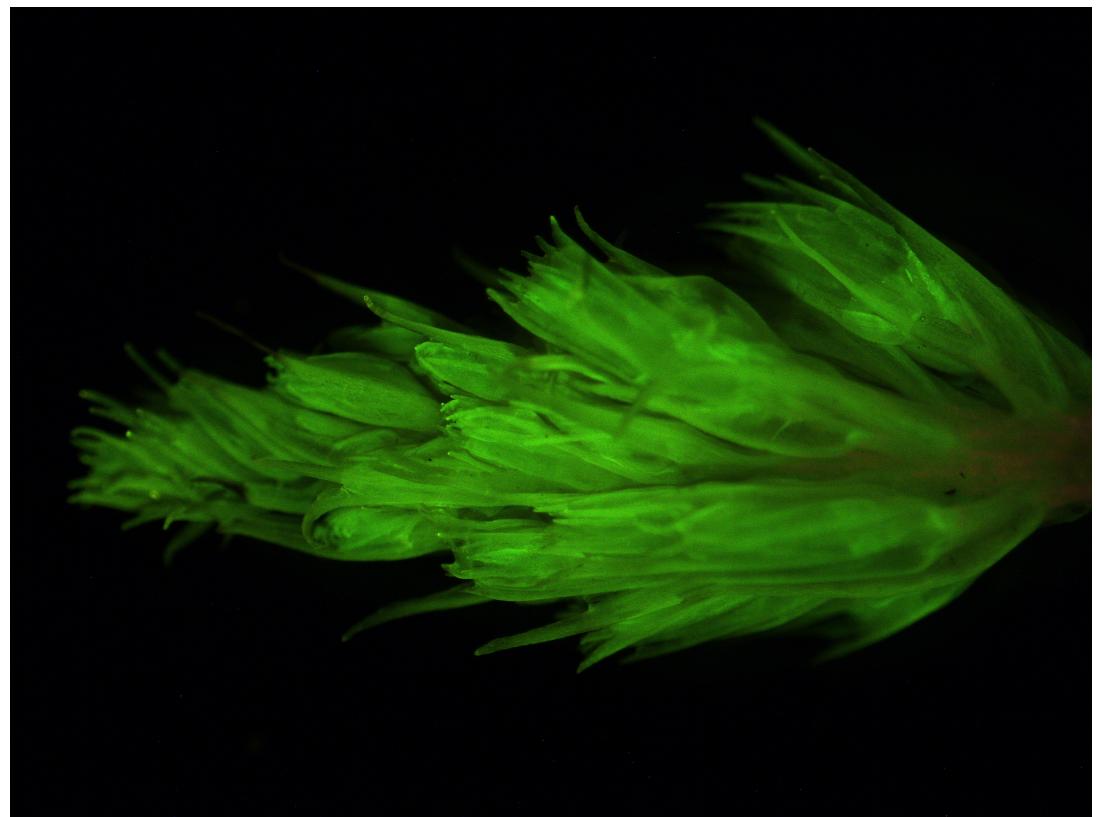


Image of betaxanthin fluorescence in *Celosia argentea* inflorescence under stereoscopic microscopy.

## MATERIALS

1. Mortar & Pistle
2. Membrane (Millipore Corp., Bedford, MA) 0.2  $\mu$ M nylon
3. 80% aqueous methane (MeOH) (20 ml H<sub>2</sub>O + 80 ml MeOH)
4. Formic acid (H-COOH (CH<sub>2</sub>O<sub>2</sub>))
5. Sodium ascorbate
6. Sephadex LH-20 column (100 x 2.5cm i.d.)
7. Sterile double-distilled water
8. Gloves
9. Centrifuge

## SAFETY WARNINGS



It is imperative to wear gloves consistently since methanol (MeOH) and ascorbic acid, both reagents utilized in the process, are deemed hazardous and carry substantial health hazards.

## BEFORE START INSTRUCTIONS

The complete procedure necessitates execution within a darkened environment owing to the light sensitivity exhibited by betalains. Furthermore, it is imperative to adhere to a maximum temperature of 4°C throughout the entirety of the process.

## Preparation of reagents

- 1 The LH-20 separation column is prepared by using methanol as the solvent, with a ratio of 3.9 ml for every 4.3 g of Sephadex column granules.
  - 1.1 The solvent/granule (v:wt) ratio may vary depending on the brand utilized. Please adhere to the instructions provided by your supplier if a ratio different from the one suggested here is specified.

## Preparation of plant material

- 2 Freshly collected plant material is washed thoroughly three times with sterile water and cut into small pieces ( $1\text{ cm}^2$ ) while ensuring the process is conducted in darkness.

**Note:** We used the flower of *Celosia argentea* or the root of *Beta vulgaris*.

- 3 The collected tissues can be stored at -20°C until use.



## Extraction process

40m

- 4 The frozen samples should be ground thoroughly in a mortar until the tissue appears colorless, which takes approximately 20 minutes.



- 5 Add 3 ml/gram (fresh weight) of freshly prepared 80% methanol + 50 mM ascorbate to the tissue and continue grinding. Collect the filtered solution in a 15 ml Falcon tube.



- 6 Centrifuge the tubes at 14,000 rpm for 10 minutes at 4°C.  
( $\text{rpm} = \sqrt{[\text{G force}/ (\text{r} \times 1.118)]} \times (1 \times 10^5}$ ... r = rotor radius in cm)

10m

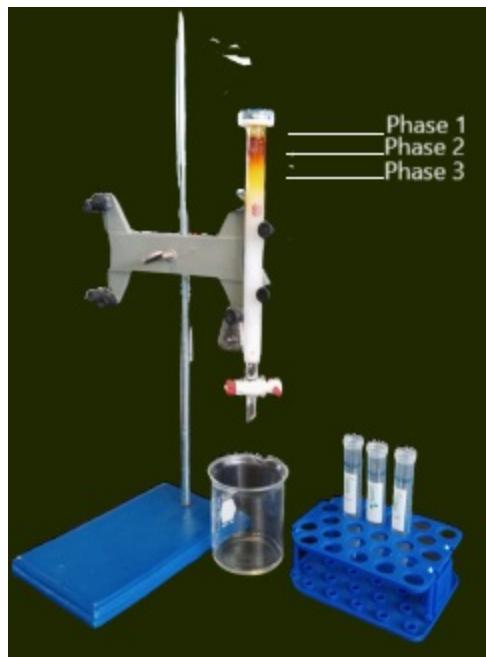
- 6.1 After collecting the supernatant, repeat step 6 if any signs of tissue contamination are observed in the sample.

- 7 The supernatant liquid is filtered through a 0.2 µM nylon membrane (Millipore Corp., Bedford, MA) under vacuum at a temperature of 22°C.

## Purification process

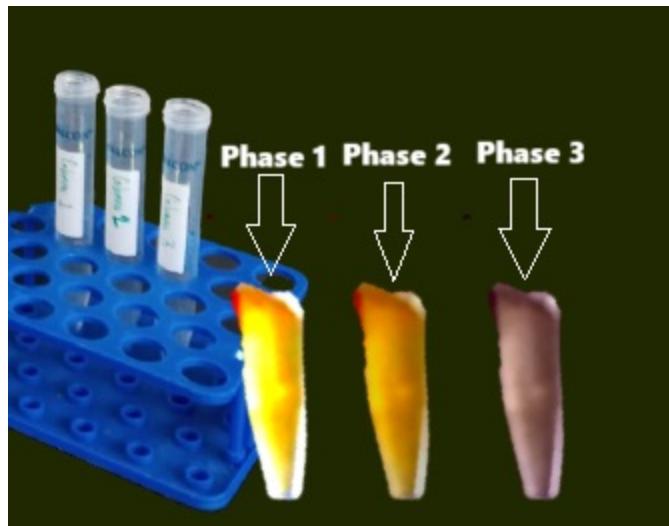
40m

- 8 The samples are transferred into a LH-20 separation column measuring 100 x 2.5 cm in diameter. The separation process is achieved through elution using water adjusted to a pH between 5 and 6 with formic acid.



**Figure 1:** LH-20 separation column showing different phases with distinct colours.

- 9 The phases collected from the separation columns are to be kept separately for individual evaluation. The filtrates can be stored at 4 degrees Celsius until they are required for subsequent steps.



**Figure 2:** Different phases collected from the LH-20 separation column.

## Spectrophotometry

- 10 The betalain content is quantified photometrically at wavelengths of 540 nm for betacyanins and 475 nm for betaxanthins, taking into account their respective molar extinction coefficients.
- 11 The purified pigment acquired through the isolation procedure offers a wide array of potential applications, with one notable example being its use as a molecular fluorophore. This capacity allows for its employment in diverse scientific endeavors, ranging from cellular imaging to biochemical assays, wherein fluorescence serves as a valuable tool for detection and analysis.
- Moreover, the purified pigment presents an invaluable resource for exploring its behavior and characteristics under different physicochemical conditions. By subjecting it to varying pH levels, temperature ranges, and other environmental factors, researchers can gain deeper insights into its stability, reactivity, and potential interactions. Such investigations not only contribute to a comprehensive understanding of the pigment's properties but also lay the groundwork for its optimized utilization in various fields, including biotechnology, pharmacology, and materials science.