Spectrophotometric investigation of natural dyes of Portulaca grandiflora and Catharanthus roseus flowers

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ABSTRACT

The study of dyes of native flowers contributes to a better investigation of these species, which can motivate new economic activities in the country. The objective of this work was to characterize by spectrophotometry the natural dyes extracted from the petals of the flowers Portulaca grandiflora and Catharanthus roseus. The extraction of the dyes was carried out by maceration of the petals of the dried flowers and under different experimental conditions to evaluate the extraction time and the type of solvent used. The dyes obtained were characterized by spectrophotometry in the UV/Visible region to evaluate the spectroscopic behavior and the maximum absorbance lengths of each flower. The extractions of the dyes from the flowers were effective in the experimental conditions evaluated, but with spectroscopic profiles showing bands in different regions and with varying intensities. The study of the spectrophotometric behavior of these flowers can help in their use as an alternative source of natural dyes.

Keywords: Natural dyes, Portulaca grandiflora, Catharanthus roseus, Spectrophotometry.

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INTRODUCTION

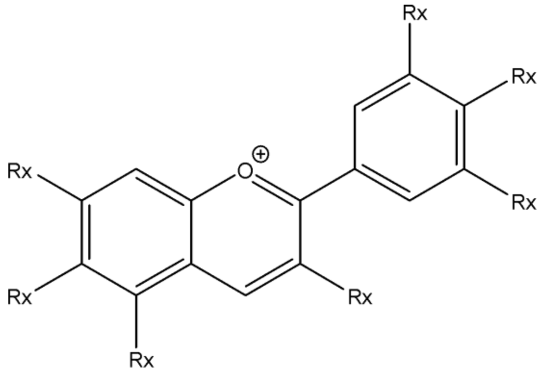
Natural dyes have been a part of human history since ancient times. Whether in cultural, artistic and/or religious manifestations, such as dyeing the body or dyeing the hair, a very common practice among indigenous peoples and which has become part of the cultural identity of these native peoples. In artistic and/or religious manifestations, cave paintings are rescued, showing that humanity searched, in its environment, for products and substances of flora to obtain color patterns for the most diverse purposes (Dias *et al.*, 2016; Granados-Balbuena *et al.*, 2024). In addition, natural dyes have been used since ancient times to dye materials such as wool, leather, silk, carpets and cotton, regardless of their origin (plants, animals or minerals) (Ebrahimi; Gheshlagh; Parham, 2024).

The use of non-allergenic, non-toxic and eco-friendly natural dyes has taken a prominent position nowadays due to the growing environmental awareness, with the aim of avoiding the dangers associated with synthetic dye sources (Ebrahimi; Gheshlagh; Parham, 2024). The vast majority of natural dye raw materials are dye plants. The roots, stems, twigs, and flowers or whole parts of certain plants are used in natural dyeing (Karadag, 2023).

These natural dyes are responsible for a wide range of colors in plants, they produce these colors through biochemical routes and utilize them to attract pollinators, protect against predators, and ultraviolet light. Natural plant pigments are divided into four main groups: carotenoids, chlorophylls, betalains, and flavonoids (Granados-Balbuena *et al.*, 2024; Kaewprachu *et al.*, 2024). Among the flavonoids, anthocyanins (Figure 1) are the most important floral pigments for the types of applications proposed in this work and are responsible for a wide spectrum of possible colors in flowers and fruits, including light yellow, scarlet, red, magenta, violet, and blue (Wang *et al.*, 2023).

The interest in developing anthocyanin-based products has grown, mainly due to their nutritional attributes, coloring power, water solubility (which facilitates their incorporation into aqueous systems) and beneficial health effects promoted by them through various mechanisms of action, including antioxidant capacity (Almeida *et al.*, 2015). Anthocyanins are primarily applied as a coloring in food, but they also have the potential to dye other products such as textile substrates, cotton, leather, silk, and human hair. In the food industry, anthocyanins can be used as pigments in products such as curds, fermented milk, low-pH beverages, wines, and solid matrices such as pancakes and omelettes (de Araújo *et al.*, 2021).

Figure 1. Basic structure of anthocyanins. Rx can be H, OH, and COOH.



Fonte: DIAS et al., 2016.

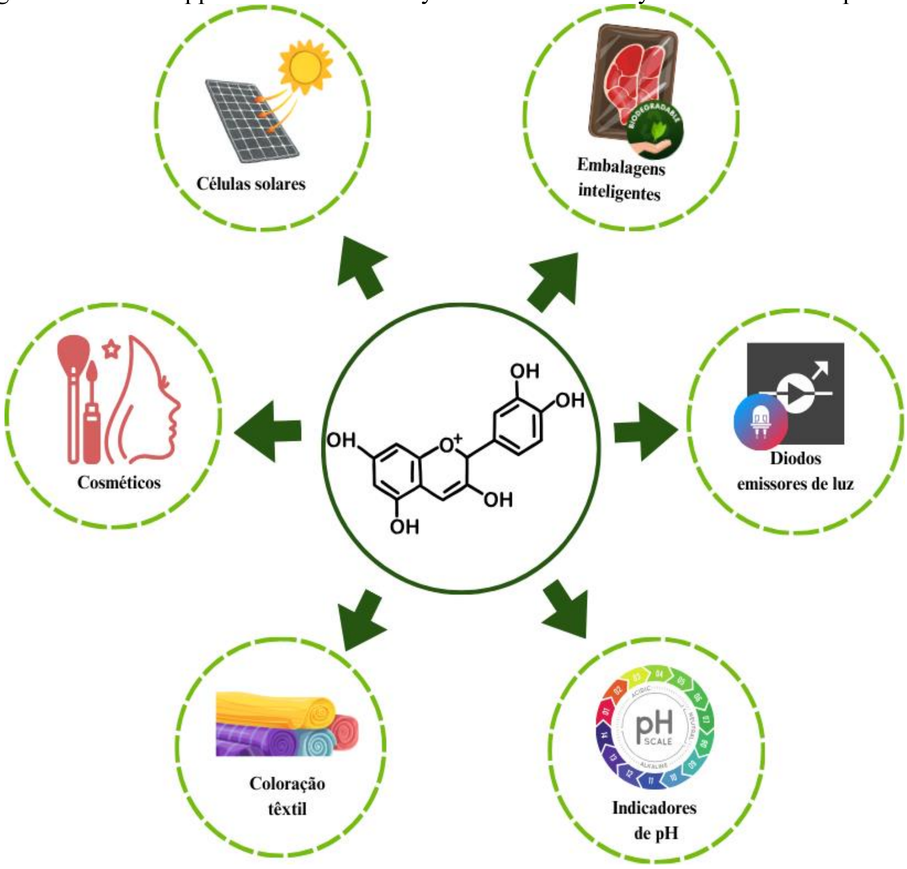
The study of native flower dyes contributes to a better investigation of these species, which may motivate new scientific discoveries. Thus, the present work aims to evaluate the best experimental conditions and characterize, by spectrophotometry in the ultraviolet to visible region, the natural dyes extracted from *Catharanthus roseus* and *Portulaca grandiflora* flowers found in the wild region of the state of Alagoas in order to evaluate the potential applicability of these dyes.

THEORETICAL FRAMEWORK

Natural dyes, obtained from plants, insects/animals and minerals, are products of renewable and sustainable biological resources, with minimal environmental impact and known since ancient times for their use (Shahid; Shahid-ul-Islam; Mohammad, 2013).

Complete biodegradation, non-toxicity and low cost are the advantages of using natural dyes for applications in various areas (Figure 2), such as in the production of organic devices such as dye-sensitized solar cells (Erdoğdu *et al.*, 2024; Mahajan *et al.*, 2024), smart packaging (Paschoa, 2016; Sheibani *et al.*, 2024), LEDs (Ohtani; Kitagawa; Matsuda, 2011) textile coloring (Sheibani *et al.*, 2024), pH indicators (Zhao *et al.*, 2024), food ingredients (Dweck, 2002), cosmetics (Portes *et al.*, 2024) among several other applications.

Figure 2. Areas of application of natural dyes that have anthocyanins in their composition.



Source: Prepared by the authors, 2024.

Anthocyanins are natural plant pigments of the flavonoid family, giving the colorful appearance (red, blue, and purple colors) of fruits, vegetables, and other foods (de Araújo *et al.*, 2021). They have properties that help maintain healthy eating habits that are demonstrated by anticarcinogenic, antiviral and antioxidant activities (Almeida *et al.*, 2015). This activity is due to its chemical structure formed by three rings, which have conjugated double bonds and hydroxyls distributed throughout the structure that enable the sequestration of free radicals, which cause cell damage and degenerative diseases (Bordignon *et al.*, 2009; da Silva *et al.*, 2007). Preclinical trials have reported the action of anthocyanins against some pathologies such as cancer, inflammation, cardiovascular diseases and obesity (de Araújo *et al.*, 2021; Saulite et *al.*, 2019; Thilavech *et al.*, 2018).

The greatest difficulty currently encountered in the use of anthocyanins as natural dyes is related to the instability of this flavonoid, since the color of the solutions depends on a series of factors such as the type of solvent used, pH, temperature, concentration, pigment structure and the presence of substances capable of reacting with anthocyanin (Phan *et al.*, 2021; Rossi *et al.*, 2022). In addition, the processes used during extraction strongly influence the stability of these compounds (Remini *et al.*, 2018).

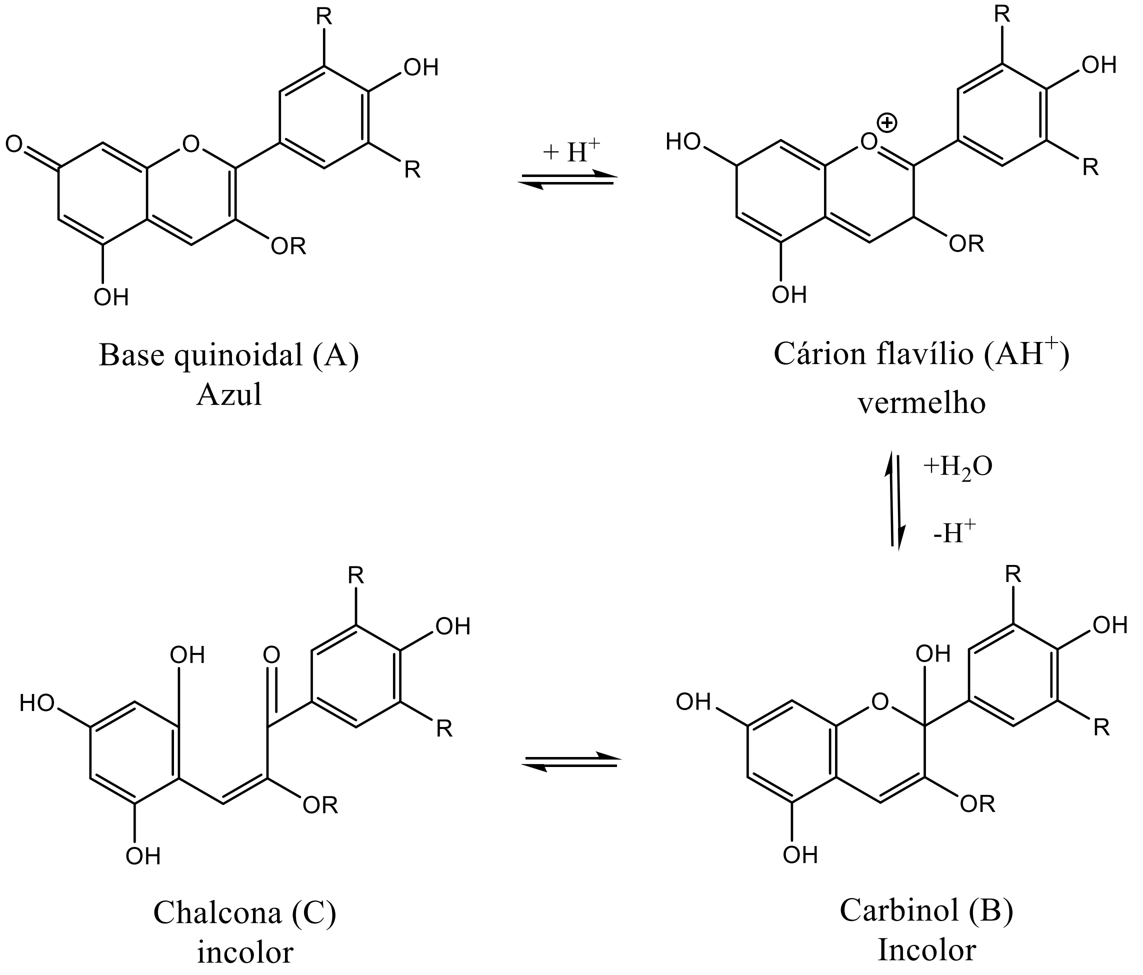
Rossi et al, (2022) reviewed the main problems found in the literature in the stability of anthocyanins present in açaí fruit, where the difficulty of ensuring the stability of anthocyanins, which modify and degrade under the influence of the pH of the medium, the temperature that is submitted in the processing stages, and exposure to light, was evidenced.

Regarding pH, Bordignon et al, (2019) evaluated the influence of the pH of the extractive solution on the anthocyanin content in strawberry fruits, verifying that the spectroscopic profile of the extracts varied according to the pH used in the extraction and found that the extraction of anthocyanins was more efficient at pH 1.0 values.

Anthocyanin extraction methods play a key role in obtaining dyes from various sources of plants, fruits, and flowers for use in a variety of applications. These methods are key to harnessing the diversity of colors found in nature and converting them into usable materials for various industries, including textiles, art, and renewable energy technologies (Mahajan *et al.*, 2024).

Absorption spectroscopy in the UV-Vis region is a technique used to characterize the properties of natural dyes, providing essential information about the presence and content of anthocyanins in the dye. This method involves measuring the absorption of light by the dye solution across a spectrum of wavelengths, offering a comprehensive view of the dye's interaction with incident light. The obtained absorption spectrum reveals the specific wavelengths of light that the dye effectively absorbs (Mahajan *et al.*, 2024). Anthocyanins can have different structural forms, which can take on different colors (Bordignon *et al.*, 2009). Data from the literature indicate that four main chemical structures may occur in equilibrium: the flavillium cation (HA+), the quinoidal base (A), the pseudobase carbinol (B) and the chalcone (C) (Figure 3). March and Scarminio (2007) reported in studies with vinegar blossoms (*Hibiscus acetosella W.*) that, according to the pH variation, there is a change in the maximum absorption in the UV-visible spectrum (Bordignon *et al.*, 2009; Levi *et al.*, 2004; March; Scarminio, 2007).

Figure 3. Structures of anthocyanins found in aqueous solution with different pH values. Flavilium cation (HA+), quinoidal base (A); carbinol or pseudobase (B) and chalcone ©.



Source: adapted from HE; GIUSTI, (2010).

Marpaung and Paramaputri (2023) studied the spectrophotometric variations of butterfly pea flower (*Clitoria ternatea L.*). At pH 1 values, the absorption spectrum exhibited two absorption bands under a UV-visible spectrophotometer, at ± 265 nm, and the second band has at ± 520 nm. The absorbance at 520 nm belongs to the AH+ flavillium cation, i.e. the only species that exists at pH 1. At pH values > 5, the band at ± 520 nm changes to 550 - 580 nm (purple quinonoid base, A) or 600 - 620 nm (blue anionic quinonoidal base, A^-).

Colorful flowers can also be a source of natural dyes (Mandal; Venkatramani, 2023). The flower of *Catharanthus roseus*, commonly known as goodnight, is grown as an ornamental flowering plant. The flowers have color variations (white, purple, peach, pink, scarlet, and red) (Kandiah; Chandrasekaran, 2021). *Catharanthus roseus* has medicinal effects, being a valuable source of antitumor agents, such as vinblastine and vincristine, used in the chemotherapy of leukemia and in the treatment of Hodgkin's disease (Piovan; Filippini, 2007). The flower of *Portulaca grandiflora* is a small herbaceous annual plant, commonly known as eleven o'clock, having several colors (white, red, purple, orange and pink). There are reports in the literature that extracts of this plant can be used as natural sources of antioxidants (Lim; Tiong; Loo, 2014).

In recent decades, many publications have reported the improvement of knowledge about plant dyes, but the benefits and potential applicability of the flower dyes highlighted in this study are still small and limited. A study of the spectrophotometric behavior may aid in the use of these flowers as an alternative source of natural dyes in the future.

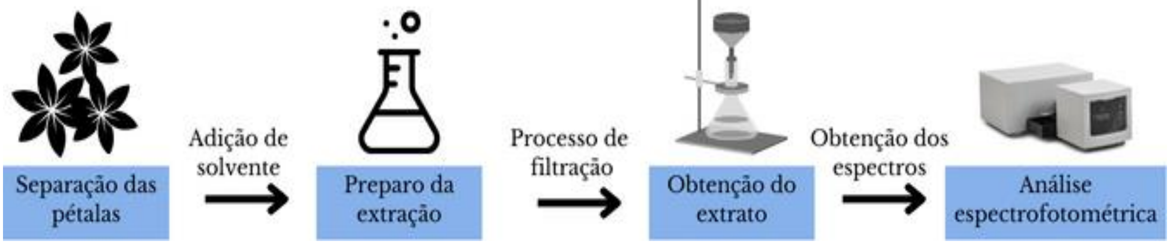
METHODOLOGY

The flowers were collected from garden houses in the city of Arapiraca, Alagoas. After collection, the flowers were washed, dried and then the petals were separated to be used.

To obtain the dyes of the flower petals, extracts with a concentration of 25% mass/volume were prepared under different experimental conditions, in order to verify in which condition the extracted dye presents the best performance. The solvents used for the extraction of the dyes were: ethanol (95%) (pH = 4.5) and acidified ethanol with 0.1 mol/L hydrochloric acid solution (pH = 2). The dyes were obtained with the flower petals at room temperature using dried petals in an oven at 60 ºC for 1 hour. In the extraction process, the flower petals were immersed in the solvent for 48 h, under agitation, where aliquots were removed 24 and 48 hours after the beginning of the process (Figure 4).

The dyes obtained were filtered in a simple system and the solutions were stored in amber vials, to avoid degradation by the action of light, and kept under refrigeration. The dyes were characterized by spectrophotometry in the UV-Visible region (300 to 800 nm) on a SHIMADZU MultiSpec-1501 spectrophotometer to evaluate the maximum absorbance lengths of each flower.

Figure 4. Flowchart of the process of obtaining and analyzing flower dyes.

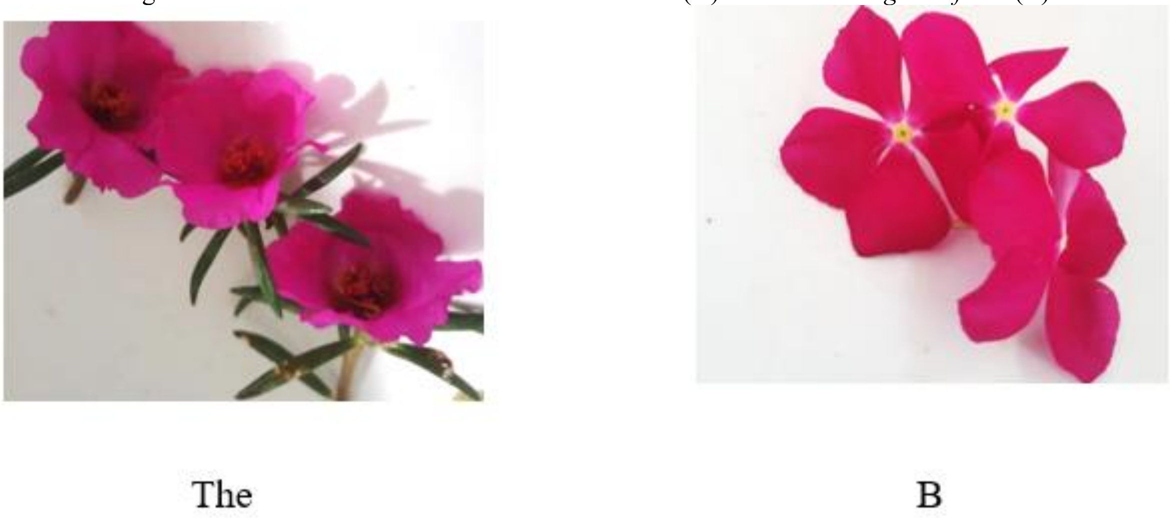


Source: Prepared by the authors, 2024.

RESULTS

The choice of flowers *Portulaca grandiflora* (eleven o'clock) and *Catharanthus roseus* (good night) (Figure 5) was made considering the fact that they are easily obtained in the northeastern region of Brazil, and because they have a color that indicates the probable presence of anthocyanins in their composition.

Figure 5. Photo of the flowers *Catharanthus roseus* (A) and *Portulaca grandiflora* (B).



Source: Prepared by the authors, 2024.

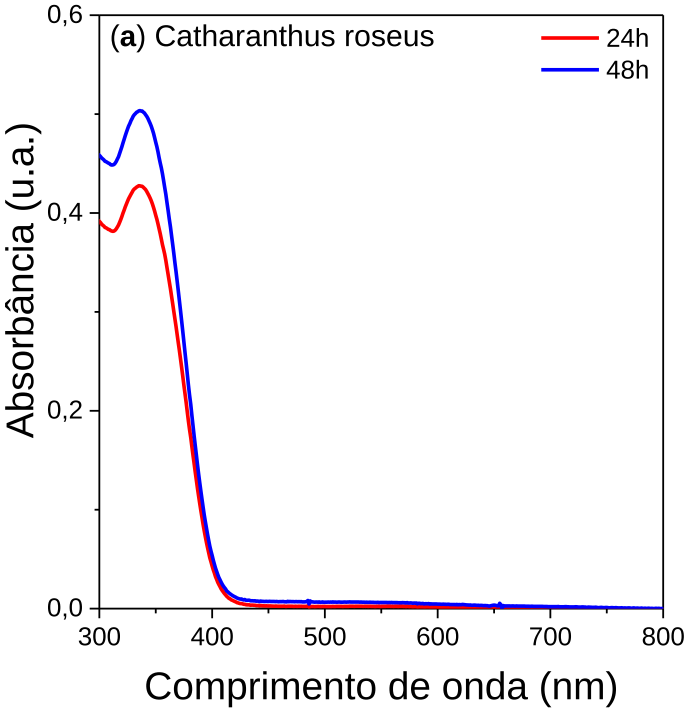
In the literature, several authors describe that dyes containing anthocyanins have a broad spectrum of absorption in the visible region and that their peak absorption is between 500 and 550 nm (Cabrera *et al.*, 2017; Sponsorship; Iha, 2010; Sampaio; Feitosa, 2016). Thus, through spectroscopy analyses in the UV-visible region, it is possible to obtain the maximum absorbances and their respective wavelengths, showing if a given dye has anthocyanins in its composition.

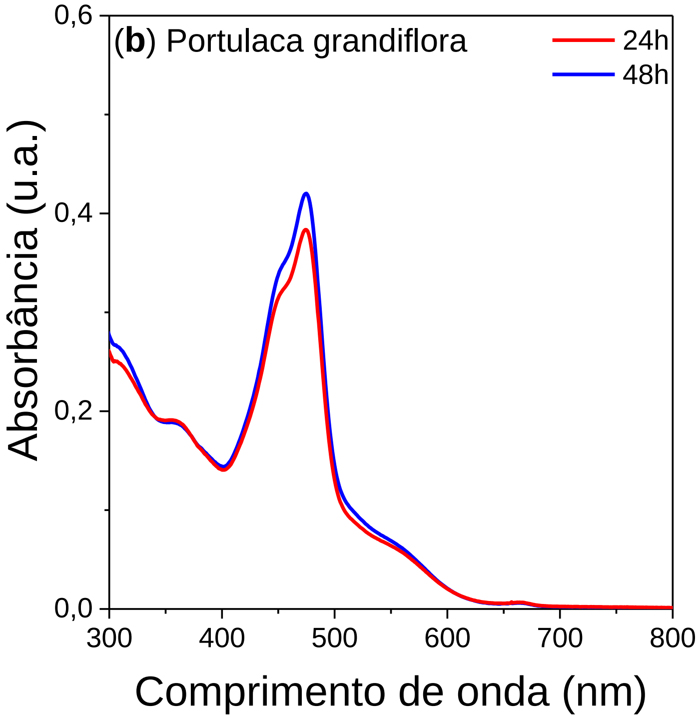
The spectroscopic profile of the dyes of the flowers of *Portulaca grandiflora* and *Catharanthus roseus* showed bands in different regions and with varying intensities, according to the extraction conditions, extraction time and plant evaluated (Table 1).

Figure 6 shows the absorption spectra of the dyes obtained from the petals of the flowers *Catharanthus roseus* (Figure 6a) and *Portulaca grandiflora* (Figure 6b) using ethanol as extraction solvent (pH 4.5) at extraction times of 24 h (red line) and 48 h (blue line). The flower dye *Catharanthus roseus* showed a single absorption band at 337 nm and a 15% increase in the absorption of this band when the extraction period was increased from 24h to 48h. On the other hand, the flower dye *Portulaca grandiflora* showed two absorption bands at 360 and 475 nm and an 8% increase in the absorption of the band at 475 nm. when the extraction time increased from 24 hours to 48 hours. The flower dyes *Catharanthus roseus* and *Portulaca grandiflora,* extracted in ethanol, did not present absorption bands characteristic of anthocyanins, since they do not have absorption bands at wavelengths between 500 and 550 nm (Feitosa; Shah; Cavalcante, 2016; Marpaung; Paramaputri, 2023).

Figure 7 shows the absorption spectra of flower dyes *Catharanthus roseus* (Figure 7a) and *Purslane grandiflora* (Figure 7b) using HCl-acidified ethanol as extraction solvent (pH 2) at 24 and 48h extraction times. Extraction of dyes with acidified ethanol revealed changes in the spectrophotometric behavior of the flower *Catharanthus roseus* with two bands at 347 and 536 nm, and the flower *Purslane grandiflora* with three bands at 381, 483 and 527 nm, compared to extraction with the solvent ethanol alone. Marpaung and Paramaputri (2023) reported that some anthocyanins, including ternatins, exhibit distinct characteristics by showing three bands in the visible region. The three bands represent all the colorful species of anthocyanins and that The bands present in dyes in the range between 500 to 550 nm are characteristic of anthocyanins and belong to the flavillium cation (Feitosa; Shah; Cavalcante, 2016; Marpaung; Paramaputri, 2023), which may indicate that the dyes obtained in acidified ethanol have these substances in their composition, however the flower dye *Purslane grandiflora* (Figure 6b) shows the best behavior, as it presented absorption bands in the anthocyanin region with higher intensities than the flower *Catharanthus roseus*.

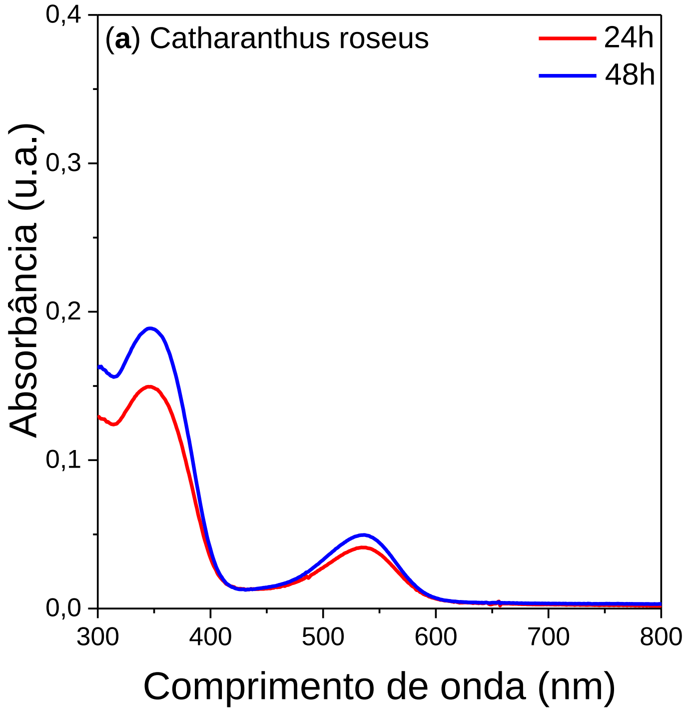
Figure 6. Absorption spectra of flower dyes *Catharanthus roseus* (a) and *Portulaca grandiflora* (b) extracted with ethanol at 24 and 48 hours.

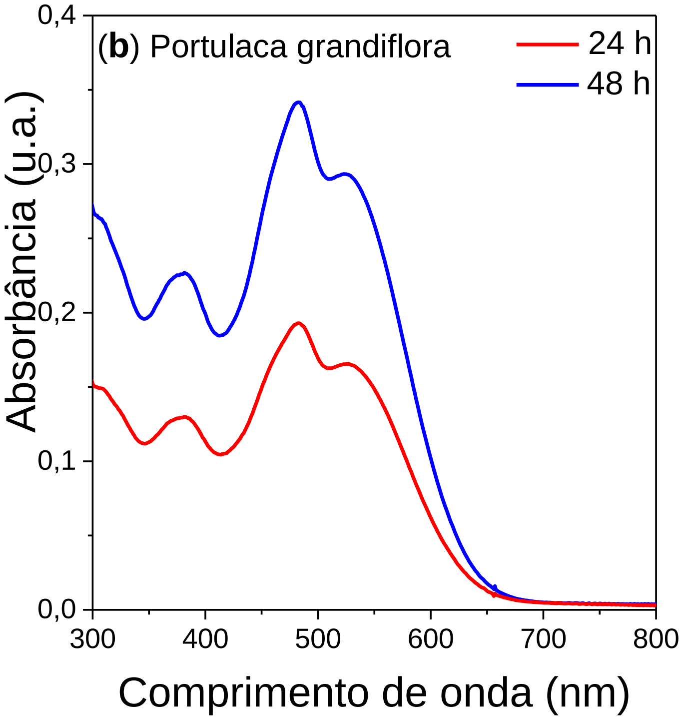




Source: Prepared by the authors, 2024.

Figure 7. Absorption spectra of flower dyes *Catharanthus roseus* (a) and *Portulaca grandiflora* (b) extracted with ethanol-HCl in 24 and 48 hours.





Source: Prepared by the authors, 2024.

In the comparison of the solvents used in the extraction of the dyes, it is possible to verify that the position and intensity of the bands varied according to the solvent used. According to the literature, for a better extraction of anthocyanins, the pH of the solution should be in the range of 2 to 3.5, since they are stable at acidic pH (Cabrera *et al.*, 2017). Wongcharee, Meeyoo and Chavadej (2007) conducted a study where anthocyanin dyes were extracted with different pH values. They studied the rosella pigment and found that at pH = 1 there was a higher efficiency of the dyes than at pH = 3. One reason for this increase in efficiency is that for pH values less than 2, anthocyanin exists in its stable form as a flavil ion. Regarding the extraction time, it is favorable to work with the dyes obtained with a time of 48 h, due to the increase in the extracted material proven by the increase in the intensity of absorbance (Wongcharee; Meeyoo; Chavadej, 2007).

Table 1. Comparison of the wavelengths of the maximum absorbance peaks for each solvent.

Solvent	**Flower Name**	**λpicos (nm)**
Ethanol	*Catharanthus roseus*	**336**
	Purslane grandiflora	**475**
Ethanol: HCl	*Catharanthus roseus*	**346 & 535**
	Purslane grandiflora	**378, 481 e 528**

Source: Prepared by the authors, 2024.

CONCLUSIONS

The extractions of the dyes from the flowers were effective in the experimental conditions evaluated, but with spectroscopic profiles showing bands in different regions and with varying intensities. A more in-depth study of the spectrophotometric behavior of these flowers may help in their use as an alternative source of natural dyes in several areas of knowledge.

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