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Characterization of Red Poppy (*Papaver rhoeas* L.) Extract: An Alternative Food Colorant

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ABSTRACT

The growing interest in natural products has prompted researchers and the food industry to seek out clean additives. The red petals of the poppy plant offer a strong alternative to synthetic additives, thanks to their bioactive compounds and vibrant color. This study extracted anthocyanins from poppy petals using an acid-ethanol-water mixture and examined the nutritional properties and color stability of the resulting pigment. It was found that the poppy extract (PE) is rich in sodium and potassium minerals and contains major phenolic compounds, such as quercetin and kaempferol. The total anthocyanin content was determined to be 17.11 ± 0.60 mg cyanidin-3-glucoside g^{-1}. The antioxidant activity of PE was evaluated using DPPH, CUPRAC, and FRAP assays, with results of 0.92 ± 0.08 µg mL^{-1}, 155.98 ± 8.73 mM TE g^{-1}, and 581.94 ± 12.09 µmol TE g^{-1}, respectively. Antimicrobial activity of PE was determined on 4 pathogenic microorganisms, *Salmonella enteridis*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Staphylococcus aureus,* with the highest inhibition order. The anthocyanins were found to decrease by approximately 15% after 60 minutes of heat treatment at 100°C, and oxidative degradation (H_2O_2) increased over time but did not exceed 13%. Additionally, under different pH levels, the anthocyanins exhibited characteristic behavior, shifting in color from red to purple and then to a yellowish green. In conclusion, poppy extract has the potential to be used as a functional colorant in food products subjected to heat treatment and oxygen exposure. Furthermore, its ability to change color with pH paves the way for its application in the development of smart packaging.

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Gelincik Çiçeği (*Papaver rhoeas* L.) Ekstraktının Karakterizasyonu: Alternatif Bir Gıda Boyası

ÖZET

Doğal içerikli ürünlere olan ilginin günümüzde artması, araştırmacıları ve gıda endüstrisini temiz katkı maddeleri bulmaya yönlendirmektedir. Gelincik bitkisinin kırmızı taç yaprakları, biyoaktif bileşenleri ve rengi sayesinde sentetik katkı maddelerine güçlü bir alternatif sunar. Bu çalışmada, gelincik yapraklarından asit:etanol:su karışımı ile antosiyaninler ekstrakte edilmiş ve elde edilen renk maddesinin besleyici özellikleri ile renk stabilitesi incelenmiştir. Gelincik ekstraktının (GE) sodyum ve potasyum mineralleri açısından zengin olduğu, ayrıca majör fenolik bileşenler olarak kuarsetin ve kamferol içerdiği tespit edilmiştir. Toplam antosiyanin miktarı 17.11 ± 0.60 mg siyanidin-3-glukozid g^{-1} olarak belirlenmiştir. GE'nin antioksidan aktivitesi DPPH, CUPRAC ve FRAP testleri ile değerlendirilmiş olup, sonuçlar sırasıyla 0.92 ± 0.08 µg mL^{-1}, 155.98 ± 8.73 mM TE g^{-1} ve 581.94 ± 12.09 µmol TE g^{-1} olarak bulunmuştur. GE'nin antimikrobiyel aktivitesi 4 patojen mikroorganizma üzerinde incelenmiş ve en yüksek inhibisyon sırasıyla *Salmonella*, *Listeria monocytogenes*, *Escherichia coli*

Gıda Bilimi

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O157:H7 ve *Staphylococcus aureus* olarak tespit edilmiştir. Antosiyaninlerin 100°C'de 60 dakikalık bir sıcaklık uygulaması sonucunda yaklaşık %15 oranında azaldığı, oksidatif bozulmanın (H_2O_2) zamanla arttığı fakat maksimum %13 olduğu belirlenmiştir. Farklı pH değerlerinde ise antosiyaninler karakteristik davranışını göstererek kırmızıdan mora, ardından sarımsı bir yeşile doğru renk değişimi sergilemiştir. Sonuç olarak, gelincik ekstraktı, ısıl işlem gören ve oksijen maruziyetine uğrayan gıda ürünlerinde fonksiyonel bir renk maddesi olarak kullanılma potansiyeline sahiptir. Ayrıca pH ile renk değiştirme özelliği, bu renk maddesinin akıllı ambalajların geliştirilmesinde kullanımının önünü açmaktadır.

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INTRODUCTION

The trend toward nature is replacing synthetic additives with natural constituents as consumer awareness increases. The most commonly used edible flowers in Turkey are saffron (*Crocus sativus*), rose (*Rosa damascena*), lavender (*Lavandula angustifolia*), violet (*Viola odorata*), hibiscus (*Hibiscus sabdariffa*), verbena (*Verbena officinalis*), poppy (*Papaver rhoeas*), pumpkin (*Cucurbita pepo*), and borage (*Borago officinalis*) (Bayram et al., 2014). Among these plants, the poppy flower stands out as a beneficial alternative to synthetic additives due to its bioactive components and vibrant color. The color of *Papaver rhoeas* L. is primarily derived from its main component, cyanol, which belongs to the red anthocyanins group (Bujak et al., 2021).

The genus *Papaver* (family *Papaveraceae*) is a plant that grows in the temperate climates of Eurasia, Africa, and North America, with a general distribution in the Mediterranean region. (Mohammed et al., 2023). The *P. rhoeas (poppy)* plant is known to contain high concentrations of primary and secondary metabolites, including amino acids, carbohydrates, fatty acids, vitamins, phenolic compounds, essential oils, flavonoids, alkaloids, coumarins, organic acids, and other compounds (Hmamou et al., 2022). The stems, seeds, and petals of the poppy plant are consumed as food (Yüksel et al., 2022). The seeds are used to flavor cakes, biscuits, salads, and breads, while the green stems and leaves are used in the traditional cuisine of the Italian region, mostly in cooked products (Montefusco et al., 2015; Grauso et al., 2020). The red petals are used to make sherbet in Turkey (Yüksel et al., 2022). It is also used as a colorant in beverages (Marsoul et al., 2020). The commercial use of red petals has not been found in the literature.

The poppy (*P. rhoeas* L.) has been utilized in alternative medicine since antiquity for its bioactive compounds, which assist in promoting sleep, alleviating thoracic pain, and mitigating inflammation of the throat and tongue (Grauso et al., 2020). *P. rhoeas* extracts possess numerous pharmacological properties, including anti-inflammatory, antimicrobial, antioxidant, anti-ulcerogenic, cytotoxic, cough suppressant, antispasmodic, anti-genotoxic, anti-mutagenic, and anti-carcinogenic effects (Grauso et al., 2020; Marsoul et al., 2020). Poppy is extensively utilized in traditional Turkish medicine as a cough syrup, a depressant, analgesic, remedy for diarrhea, treatment for skin irritation, and as a sleep tea for children (İpek et al., 2023). Rhoeadine and benzylisoquinoline compounds, alkaloids derived from poppy flowers, exhibit moderate analgesic and sedative effects (Grauso et al., 2020). Poppy leaves possess alkaloids with notable anticancer properties, including those present in buprenorphine and codeine, alongside alkaloids utilized as narcotics and analgesics, such as morphine (Mohammed, et al., 2023). The dark region surrounding the capsule of the poppy flower harbors thebaine, a potent toxic alkaloid, necessitating careful removal of this section, particularly in beverage manufacturing (Ekici, 2014; Mohammed et al., 2023). Nonetheless, it has been documented that the efficacy of these highly potent alkaloids is diminished by the bioactive compounds present in the poppy flower (Bujak et al., 2021).

Coloring additives are frequently used to prevent color loss during food processing, to make food more attractive, and to improve quality. Increasing awareness of the toxicity of synthetic dyes leads manufacturers to use natural colorants. These natural colorants also attract attention with their therapeutic and medicinal properties. Natural dyes are derived from naturally occurring sources such as plants, insects, animals, and minerals (Chaitanya-Lakshmi, 2014). Plant-based colorants are preferred due to their medicinal value. The poppy flower also draws attention as a natural food coloring with its color and composition. The color of the poppy flower comes from the anthocyanins it contains, and the most abundant anthocyanin was determined as cyanidin (Ekici, 2014).

Natural colors of plant origin used in the food industry are yellow-orange (Marigold, β-carotene, lycopene, gentism, turmeric, saffron, *Sanguinaria canadensis*), brown (*Camellia sinensis*, *Lawsonia inermis*), red (Annatto, *Beta vulgaris*, paprica, grapes, *Vitaceae polistiformis*, *Alkanna tinctoria*), and purple-blue (*Centaurea cyanus*, *Indigofera tinctoria*, *Vaccinium myrtillus*) (Chaitanya-Lakshmi, 2014).

Synthetic antioxidants and preservatives are frequently used in industrial processing to extend the storage stability of food. However, it has been found that synthetic additives can lead to nutrient loss and even create toxic effects (Zhang et al., 2013). At the beginning of the 20th century, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were restricted due to their carcinogenic properties (Shi et al., 2005). In recent years, natural antioxidants and preservatives obtained from edible materials, edible by-products, and waste sources have become intriguing because they can protect the human body from free radicals and delay the progression of many chronic diseases (Zhang et al., 2013).

Antioxidants are bioactive components that protect molecules and biological systems from free radicals, such as reactive oxygen (Montefusco et al., 2015). When endogenous defense system enzymes such as superoxide dismutase, catalase, and glutathione peroxidase in the human body are insufficient against free radicals, antioxidant molecules such as phenol, flavonoid, carotenoid, vitamin C and E, and reduced glutathione should be taken externally (Montefusco et al., 2015). Wild poppy, an annual herbaceous plant belonging to the *Papaveraceae* family, is a beneficial source of antioxidants (Montefusco et al., 2015). The aqueous extract of *P. rhoeas* contains phenolic compounds such as caffeic acid, quinic acid, gallic acid, rutin, and quercetin, which are known for their antioxidant properties (Bujak et al., 2022). In addition, vitamin C, known as an antioxidant, is also present in poppy flowers (Bujak et al., 2022).

Since anthocyanins used as natural colorants have high polarity, their extraction from plant matrices is usually carried out using organic solvents (Bleve et al., 2008; Bridgers et al., 2010). It is also known that hydrochloric acid added to the extraction solvent is effective in terms of the extraction efficiency of anthocyanins (Oancea et al., 2012; Patil et al., 2009). In addition, modern extraction techniques such as pressurized liquid extraction (PLE) and supercritical fluid extraction (SPE) have limitations such as the heat sensitivity of anthocyanins and the fact that the SFE technique is particularly suitable for non-polar solvents. For this reason, solvents such as ethanol, methanol, and acetone are still used in the extraction of natural colorants today (Patil et al., 2009). However, since methanol and acetone are toxic, ethanol, which is more environmentally friendly, was used in this study.

In this study, the usability of poppy flower extract, which contains bioactive components and natural coloring substances such as anthocyanin, as a natural food coloring was evaluated. The main objective of the study was to demonstrate the potential of poppy flower extract not only for coloring but also for providing nutritional and functional properties to food products with its bioactive component composition and activity. In this direction, physicochemical properties, total phenolic and anthocyanin content, phenolic compound composition, antioxidant and antimicrobial activities, and mineral composition of the extract were investigated in detail. Color stability and degradation of anthocyanins were also evaluated. The findings indicate that poppy flower extract can be used as a functional additive as well as a coloring agent, and thus has the potential as a versatile additive.

MATERIAL and METHOD

Material

Poppy leaves of the same color and maturity were collected from Kırklareli University Kayalı Campus (41°47'23.7 'N 27°09'32.4 'E) in the first week of May 2024. The collected red leaves were separated from the black parts on the same day and subjected to extraction to minimize deterioration in quality.

Preparation of Poppy Color Extract (PE)

In the extraction of anthocyanin pigments, a mixture of acidified ethanol (0.01% HCl v/v) (Merck, Darmstadt, Germany) and distilled water (1:1) was used as the solvent. The sample-solvent ratio in the extraction process (Figure 1) has been set to 1:20 (weight/volume). It has been left to macerate in the dark and at room temperature for 72 hours. Extracts purified from the solvent in a rotary evaporator (Scilogex, RE100, USA) have been stored in amber bottles at -18 °C (Bayram et al., 2015).

Physicochemical Properties of Poppy Color Extract

The pH value of the poppy color extract was determined using a pH meter (Hanna, HI 2211-02, USA). The determination of dry matter and ash content has been established according to the AOAC (2006) method. The color parameters L* (lightness), a* (red-green), b* (yellow-blue), C*, h°, and Y values were measured using the Konica Minolta Chromo Meter device (Konica Minolta Sensing Inc., CR-5, Japan).

(a) (b) (c)

Figure 1. (a): freshly collected poppy leaves, (b): leaves separated from seeds and black part, (c): poppy leaf extract
Şekil 1. (a): taze toplanmış gelincik çiçeği yaprakları, (b): tohumlarından ve siyah kısmından ayrılmış yapraklar, (c): gelincik çiçeği özütü

As a result of color measurements, the Hunter L*, a*, and b* values do not represent the color phenomenon directly perceived by consumers in the market. Instead, the "Hue Angle" (h^o) has been determined, where red-purple colors fall within the 0°-270° angle range, yellow colors are within the 60° angle range, and blue and green colors are within the 240°-120° angle range, along with the "Chroma" value. Chroma (C*), a measure of intensity or saturation, is used to determine the degree of difference of a hue compared to a gray color with the same hue angle (h^o) and is considered a quantitative quality of colorfulness. The chroma parameter according to the CIELab system has been calculated as follows:

$$C *= \sqrt{a *^2 + b *^2} \quad \text{Eq. 1}$$

The h^o value, which expresses the tone angle according to the CIELab system, was calculated as follows:

$$h^0 = arctan\ \frac{b*}{a*} \quad \text{Eq.2}$$

Mineral Composition of Poppy Color Extract

The sample was incinerated in a microwave incineration system (Mars 6, CEM, USA) at 200 °C for 45 minutes by adding 65% (v/v) HNO_3. The sample was subjected to elemental analysis by Agilent Technologies 7700 ICP-MS (Inductively coupled plasma-mass spectrometer) against a blind sample after removal of organic compounds and inorganic compounds in acid solution. The results are given in ppb.

Bioactive Compounds

450 µL of phenolic extract (5 times diluted) was subjected to a reaction by adding 2.25 mL of distilled water, 1:9 (v/v) diluted Folin-Ciocalteau reagent, and 1.8 mL of Na_2CO_3 solution (75 g L^{-1}). After being kept in a dark room at room temperature for 2 hours, the absorbance of the color formed during the reaction was read against a blank at a wavelength of 760 nm using a spectrophotometer. The total phenolic content is expressed as mg GAE g^{-1} (Atacan & Yanık, 2017).

The total anthocyanin content (TAC) of PE is based on the pH differential method. (Wrolstad, 1993). Samples were diluted in the same ratio after being passed through a 0.45 µm filter, using potassium chloride (pH 1.0) and sodium acetate buffer (pH 4.5) to ensure that the absorbance values remained below 1.2. Absorbance was measured at 510 and 700 nm. The total anthocyanin content of PE has been calculated based on cyanidin-3-glucoside, which has a molecular weight of 449.2 and a molar absorptivity (Ɛ) of 26900 L cm^{-1} mg^{-1} (Küskü and Karaman, 2023).

The phenolic profile of PE's clear supernatant was determined using an Agilent 6460 system (Agilent Technologies, Santa Clara, California, USA) equipped with an electrospray ionization (ESI) interface in triple quadrupole liquid chromatography-tandem mass spectrometry (LC-MS/MS). The Agilent Zorbax SB-C8 column (150 mm × 3.0 mm, 3.5 µm particle size) was used for separation (Bayram et al., 2022).

Antioxidant Capacity

The antioxidant activity of poppy extract has been determined in terms of 1,1-diphenyl-2-picrylhydrazyl (DPPH), cupric-reducing antioxidant capacity (CUPRAC), and ferric-reducing ability of plasma (FRAP).

The extracts of PE were determined by adding 600 µL of 1 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) solution and incubating the mixture with a final volume of 6000 µL in the dark for 30 minutes, followed by measuring the absorbance at 517 nm. The IC_{50} value has been calculated for the radical sweeping activity (μg mL^{-1}) (Yıldız et al., 2019).

For the CUPRAC experiment, a 500μL sample solution was prepared by mixing 1000μL of 0.0075 M neocuproine (2,9-dimethyl-1,10-phenanthroline), 1000μL of 0.01 M copper (II) chloride, and 600μL of distilled water. The mixture has been incubated in the dark for one hour. Subsequently, absorbance measurements were performed at 450 nm, and the results were calculated as mM Trolox equivalents (mM TE g^{-1}) (Durmuş et al., 2020).

The iron reducing power of the samples was determined using FRAP reagent (obtained by mixing sodium acetate buffer solution, 10 mM TPTZ solution, 20 mM $FeCl_3.6H_2O$ solution at a ratio of 10:1:1 (v/v/v), respectively). 100 µL of the extract was reacted with 3.0 mL of FRAP reagent, and after being incubated at 37 °C for 30 minutes, the absorbance was measured at a wavelength of 593 nm using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The antioxidant capacity is expressed as Trolox equivalent (µmol TE g^{-1}) (Atacan & Koçak-Yanık, 2017).

Antimicrobial Activity

The disk diffusion method has been used to determine the antimicrobial activity of PE. For the analysis, strains of *Escherichia coli* O157 (ATCC 33150), *Listeria monocytogenes* (ATCC 7644), *Salmonella enteritidis* (ATCC 13076), and *Staphylococcus aureus* (ATCC 25923) were used. Bacterial cultures were prepared by inoculating each strain into Nutrient Broth medium and incubating at 37 °C for 24 hours. After incubation, the cultures blur was adjusted to the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU mL^{-1}. Standardized bacterial suspensions have been spread onto Müller-Hinton agar plates for inoculation. After the plates were dried for 15 minutes, 30 µL of PE was added to the discs with a diameter of 6 mm. Ethanol-imbued discs have been used as a control. The plates were incubated at 37 °C for 24 hours. After the incubation, the diameters of the inhibition zones formed to demonstrate antimicrobial activity were measured using calipers and recorded in millimeters (Kittisakulnam et al., 2017; Reinheimer et al., 1990).

Color Stability and Degradation

Thermal stability

The thermal stability of the flower extracts was determined using the temperature degradation kinetics method. A volume of 1 mL of the aqueous extract was made up to 9 mL with 0.1 M citrate buffer (pH 1), placed in a test tube with a screw cap, and heated in a thermostatically controlled water bath at 60, 80, and 100 °C for 60 min. The test tubes were immediately cooled to ambient temperature under running tap water, and the change in absorbance at 520 nm was measured at 30-, 45-, and 60-min. Initial absorbance values were determined before each experiment (Venter et al., 2022).

Oxidative stability

The oxidative stability of PE was determined using three different H_2O_2 concentrations (31.91, 21.27, and 10.64 mM). The predetermined amounts of H_2O_2 were rapidly added to 1 mL of flower extracts (in citrate-phosphate buffer pH 1) to form 8 mL each of 9.31, 18.61, and 27.92 mM. The change in absorbance at 520 nm was measured at 30, 45, 60, 90, 120, 150, 180, and 240 min for 240 min for each concentration of hydrogen peroxide used. The absorbance value of the sample solution prepared with the same amount of distilled water instead of hydrogen peroxide was determined and recorded as the initial value (Venter et al., 2022).

Anthocyanin degradation

Oxidative and thermal degradation, pseudo-first-order kinetic rate constants (k), were obtained by plotting ln (Ct/Co) versus time to estimate the kinetic degradation parameters of anthocyanins. The modelling equation is as follows:

$$C_t = C_0 \, exp \, (-kt) \qquad \text{Eq.3}$$

C_t, represents the number of anthocyanins at time t, and C_o indicates the initial amount (Gümüş et al., 2024).

pH sensitivity

The color change of the extract at different pH values was determined spectrophotometrically. 5 mL of the extract was taken in test tubes, and the pH of the samples was adjusted from 2 to 14 with 0.1 N HCl and 0.1 N NaOH. Samples with different colors were photographed using a smartphone camera. Spectra scans of all samples were then performed using a UV/visible spectrophotometer in the wavelength range from 400 to 700 nm (Kamer et al., 2022).

Statistically Analysis

Statistical evaluations were conducted using one-way analysis of variance (ANOVA). The all experiments were conducted in triplicate, and the differences between the samples were analyzed using Duncan's multiple range tests with SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The level of statistical significance is 95% (Erol, 2013).

RESULTS and DISCUSSION

Physicochemical Properties

Color is an important sensory characteristic in determining product quality and is closely related to anthocyanin pigments found in various plants. Minimizing pigment losses in food processing processes is very important (Karasu et al., 2015). The red petals of the poppy plant and the color parameters of the obtained aqueous extract are presented in Table 1. The L^*, a^*, b^*, C^*, h^o, and Y values were found to be higher in the leaves. The brightness value (L^*) was low in both samples. This situation may have arisen due to the quite high value of the redness (a^*) parameter. The a^* value of the poppy leaf was only 1.3 times higher than that of the color extract, while the b^* value is 1.8 times greater. The close values of redness indicate that the anthocyanins were well extracted. A high chroma value (C^*) represented a more saturated, vibrant, and pure color, while the opposite indicates a pale, grayish, or pastel color. It was observed that this value is higher in the poppy leaf in parallel with brightness. Hue angle values (h^o) represent the degree of redness, yellowness, greenness, and blueness of colors; the maximum values for these hues are 0°, 90°, 180°, and 270°, respectively. C^* and Ho values were similar to Monascus red (Mapari et al., 2006). In both samples, the Y value was closer to 0 than 100. This was an indication that they reflect less light and support the L^* value results.

Table 1. Comparison of the color parameters of poppy leaves and GE.
Çizelge 1. Gelincik çiçeği yapraklarının ve GE'nin renk parametrelerinin karşılaştırılması.

	GE	Poppy Leaves
L*	28.89 ± 0.84	38.24 ± 0.14
a*	49.56 ± 0.14	65.58 ± 0.05
b*	18.02 ± 0.03	30.92 ± 0.16
C*	52.72 ± 0.14	72.48 ± 0.16
h°	19.98 ± 0.22	25.25 ± 0.09
Y	38.27 ± 0.22	46.76 ± 0.08

Abbreviations: L*, lightness; a*, green-red color; b*, blue-yellow color; C*, Chroma; h°, hue angle.

In a study, L^*, a^*, b^*, C^*, and h^o values of ethanol: water (20:80) extract of poppy flower were determined as 14.19 ± 0.09; 3.04 ± 0.03; 2.02 ± 0.01; 3.7 ± 0.03; and 33.4 ± 0.03, respectively (Bujak et al., 2021). In another study, these values were determined as 23.23 ± 0.06; 2.51 ± 0.10; -1.18 ± 0.01; 2.8 ± 0.02; and -25.2 ± 0.05 for aqueous extract, respectively (Bujak et al., 2022). In another study where the extract was added to skin cream, it was determined that although the C^* value was low, the color was clearly noticeable and maintained its color stability over time (Bujak et al., 2021; Bujak et al., 2022). The color parameters of the aqueous extract of poppy flower were determined as 6.00 ± 0.04; 0.45 ± 0.12; 0.37 ± 0.08; 0.47 ± 0.15; and 18.57 ± 5.08 (Çelik & Göncü, 2021). In another study where ferret flower sherbet was concentrated by different methods, the best color values were obtained for the vacuum method, and L^*, C^*, and h° values were determined as 16.3, 1.59, and 307.88, respectively (Ekici, 2014). The elevated color parameters found may be attributed to methodological variances, including the solvent type and ratio, extraction duration, as well as collection timing, cultivation circumstances, and species variations. The utilization of extracts in various matrices (cream, etc.) in certain investigations may elucidate the discrepancies noted in the color parameters. The fact that the C* value of PE is quite high compared to the literature data shows that it has a more saturated, vivid, and pure color (Fig. 1-c).

The % ash content of ferret extract in dry matter was determined as 1.32 (Table 2). In a study conducted with dried poppy, ash content was determined as 15.33% (Kaya et al., 2004). It can be said that approximately 7% of the mineral substances can be recovered with the extraction method. The brix value of the extract was determined as 8.36. Considering that the total dry matter content of poppy flowers is approximately 95% (Kaya et al., 2004), it can be said that approximately 9% of the dry matter can be recovered. The pH value of the PE had been determined to be 0.58, as it was extracted with acid. According to the study conducted by Çelik and Göncü, (2021), the pH of the aqueous extract of the poppy flower was found to be neutral (7.04 ± 0.69). In another study, the pH of dried weasel was found to be 5.87 (Kaya et al., 2004). The thermal stability of anthocyanins decreases with increasing pH (Ekici, Simsek, Ozturk, Sagdic, & Yetim, 2014). In the study by Liu et al. (2018), which examined the effects of temperature and pH on the stability of anthocyanins obtained from blueberries, it was observed that anthocyanins rapidly degrade above pH 3. The low pH is important in both the extraction and stability of anthocyanins.

Mineral Compounds

Owing to the crucial roles of mineral substances in metabolism, a mineral composition analysis was performed on PE. Macroelements such as potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), and phosphorus (P) are essential nutrients necessary for maintaining normal body functions (Katarzyna et al., 2021). When the mineral composition of the poppy flower was examined, it was seen that the minerals were found in the following order from most to least: Ca, K, Na, Mg, and P (Grauso et al., 2020).

In PE, analysis was performed for P, Mn, Fe, Ni, Cu, Zn, Na, Mg, Al, K, and Ca minerals, and only Na, K, P, and Fe minerals were detected (Table 2). Mn, Ni, Cu, Zn, Mg, Al, and Ca are below the quantification limit (< 0.00 ppb). Katarzyna et al. (2021) detected K, P, Mg, and Na minerals in aqueous extracts of poppy flower petals from more to less, respectively. The mineral composition in plant extracts can vary significantly depending on environmental conditions. Various environmental conditions, including the mineral content of the soil, water availability, temperature, light level, and stress factors exposed to the plant, can cause this variability (Anjum et al., 2022). Potassium, the most important intracellular cation, is responsible for regulating the normal functioning of neurons and muscles. Along with sodium ions, it is an important part of the enzyme Na^+/K^+-ATPase, which helps with things like muscle contraction and nerve cell excitation (Quintaes & Diez-Garcia, 2015). Sodium is an electrolyte that helps the body regulate fluid balance and blood pressure. Na is vital for fluid balance and nerve conduction in the body, but excess intake can increase the risk of high blood pressure and heart disease. P supports bone and tooth health and is essential for energy production and cellular function, but excess can negatively affect kidney health. Fe, which is found in the structure of proteins such as hemoglobin and myoglobin, helps transport oxygen to cells, and this process is vital for energy production. Iron deficiency can lead to anemia (Godswill et al., 2020).

Considering the mineral substances in the poppy extract and its positive effects on health, it will increase the nutritional value of the food used as a natural coloring agent and provide a functional quality.

Table 2. Mineral and physicochemical properties of poppy extract.
Çizelge 2. Gelincik çiçeği özütünün mineral ve fizikokimyasal özellikleri.

pH	0.58 ± 0.01
% Ash*	1.32 ± 0.00
Brix	8.36 ± 0.50
P (mg/kg)*	186.60
Fe (mg/kg)*	186.00
Na (mg/kg)*	9382.77
K (mg/kg)*	5901.07

*Dry weight.

Bioactive Components

The total phenolic content (TPC) of plants used for obtaining medicinal plant extracts and natural food colorants varies between 8.9-299.5 mg GAE g^{-1} extract and 2.96-125.18 mg GAE g^{-1} dry matter, respectively (Table 4). The phenolic content of the poppy flower (45.10 ± 0.84 mg GAE g^{-1} dry matter) is comparable to that of other medicinal plants (Table 3). According to a study, the basal green leaves of the poppy plant had a total phenolic content of 1.348±0.006 mg GAE g^{-1} and an antioxidant activity of 13.26 ± 0.23 µmol TE g^{-1} (ABTS) (Montefusco et al., 2015). A study on the concentration of poppy flower syrup using various methods revealed that the vacuum approach yielded the highest results, with total phenolic content and total anthocyanin values measured at 1022.84 mg GAE kg^{-1} and 774.49 mg cyanidin-3-glucoside kg^{-1}, respectively. In contrast to our study, Marsoul et al. (2020) and Kazazic et al. (2016) found higher levels of phenolic compounds, whereas and Bayram et al. (2015); Katarzyna et al. (2021); Kostic et al. (2010) found lower levels. This situation is thought to be due to differences in extraction method, solvent and harvesting region. The examined extract's substantial polyphenol content suggests it is a potent source of bioactive compounds. Polyphenols are widely recognized for their numerous health benefits, including antioxidant, antimicrobial, anti-inflammatory, prebiotic, and anticancer properties (Katarzyna et al., 2021).

Table 3. Bioactive compound content and antioxidant capacity of poppy extract.
Çizelge 3. Gelincik çiçeği özütünün biyoaktif bileşik içeriği ve antioksidan kapasitesi

TPC*	45.10 ± 0.84 mg GAE g^{-1}
TAC*	17.11 ± 0.60 mg cyanidin-3-glucoside g^{-1}
DPPH (IC_{50})*	0.92 ± 0.08 µg mL^{-1}
CUPRAC*	155.98 ± 8.73 mM TE g^{-1}
FRAP*	581.94 ± 12.09 µmol TE g^{-1}

*Dry weight

Anthocyanins are flavonoids classified as water-soluble natural pigments. They exhibit strong absorption in the UV-visible region of the electromagnetic spectrum and are responsible for the red-blue color and its derivatives in the plant kingdom (Enaru et al., 2021). Cyanidin, pelargonidin, delphinidin, petunidin, peonidin, and malvidin are the most commonly found types of anthocyanins. Anthocyanin is a biologically active compound known for its ability to neutralize free radicals, inhibit angiogenesis, and its anticancer, antidiabetic, antimicrobial, and neuroprotective effects. Due to these properties, it is widely used in functional foods, traditional medicine, and as a food additive (Roy & Rhim, 2021). The red color of the poppy flower is due to anthocyanins, primarily cyanidin (Grauso et al., 2020). The anthocyanin content in the poppy extract was determined to be 17.11 ± 0.60 mg cyanidin-3-glucoside g^{-1} on a dry matter basis. Kostic et al. (2010) found the anthocyanin content in poppy extract lower than in our study. Bayram et al. (2015) and Ekici (2014) reported higher total anthocyanin content in poppy extract compared to our findings. The reason why our findings differ from the literature can be explained by the difference in the extraction methods applied and the different harvesting regions of the poppy flower. The anthocyanin content of poppy flowers is higher than plum, red onion, red radish, strawberry, red raspberry, red cabbage and cranberry, but lower than blueberry, blackberry and grape (Bridgers et al., 2010). Since anthocyanins have high polarity, their extraction from plant matrices is usually carried out using organic solvents such as methanol, ethanol, acetone etc. (Bleve et al., 2008; Bridgers et al., 2010). Traditional extraction of anthocyanins is usually carried out in acetone or acidified methanol solutions to obtain the stable flavylium cation with red color. It is known that hydrochloric acid added to the extraction solvent is effective in terms of the extraction efficiency of anthocyanins, and this effect is higher in ethanol: water (1:1) solvent compared to water (Oancea et al., 2012; Patil et al., 2009). In fact, a study determined that acidified solvents yielded 10-45% and 16-46% more anthocyanins than non-acidified ethanol and methanol solvents, respectively (Bridgers et al., 2010).

Phytochemical studies have revealed that the leaves of *P. rhoeas* L. contain various flavanols such as quercetin, kaempferol, myricetin, and isorhamnetin (Katarzyna et al., 2021). In our study, quercetin, kaempferol, isorhamnetin, and protocatechuic acid were detected at levels of 283.01, 125.72, 47.49, and 20.69 mg kg^{-1}, respectively. As shown in Figure 2, the poppy flower also contains trace amounts of phenolic compounds, from most to least, luteolin, rutin, hesperidin, p-coumaric acid, naringenin, caffeic acid, chlorogenic acid, myricetin and phlorizin. The poppy extract, which predominantly contains quercetin and kaempferol, is highly valuable due to the antioxidant, anti-osteoporotic, anti-inflammatory, anti-neuroprotective, thermogenic, cellular signaling modulation, gene expression, and immune response, as well as antihistaminic, anticancer, antidiabetic, hepatoprotective, neuroprotective, cardioprotective, antimicrobial, analgesic, and antihypertensive properties of these compounds (Guven et al., 2019).

The maximum suggested dosage of quercetin, the main phenolic component in poppy extract (PE), is around 1000 mg/day in dietary supplements. However, when used in combination with other ingredients like bromelain, doses of 1200-2400 mg/day have been reported (Andres et al., 2018). Human trials involving doses of 500-1000 mg/day over 3-12 weeks have not identified any pro-oxidative effects of quercetin. The long-term effects of extended exposure remain unclear (Andres et al., 2018). A study of healthy individuals demonstrated that quercetin supplementation, given at gradually increasing doses (up to 150 mg per day) over two weeks, resulted in a dose-dependent elevation of plasma quercetin levels; yet, it did not significantly affect antioxidant status, oxidized LDL, inflammation, or metabolism (Egert et al., 2008). In conclusion, the high level of bioactive components in a natural colorant is significant for both nutritional value and its capacity to prolong the shelf life of the food it improves. Still, the long-term health implications of these chemicals are still insufficiently investigated.

Studies on the phenolic composition of poppy flowers are quite limited. In a study, *Papaver rhoeas* L. contained the phenolic compounds quercetin (263 mg/kg), kaempferol (23 mg/kg), myrictein (11 mg/kg), isorhamnetin (11 mg/kg), luteolin (1 mg/kg) and apigenin (1 mg/kg), from most to least (Trichopoulou et al., 2000). When the results of Trichopoulou et al. (2000) are compared with our study, it is seen that the quercetin results are similar, our kaempferol and luteolin contents are 5.5 and 6.5 times higher, respectively, and our myricetin content is 20 times lower.

Table 4. Total phenolic content (TPC) and antioxidant activity (DPPH and FRAP) of plants (dry weight) used for obtaining medicinal plant extracts and natural food colorants.

Çizelge 4. Tıbbi bitki ekstraktları ve doğal gıda renklendiricileri elde etmek için kullanılan bitkilerin toplam fenolik madde içeriği (TPC) ve antioksidan aktivitesi (DPPH ve FRAP) (kuru ağırlık).

Herbs	TPC (mg GAE g^{-1})	DPPH (%)	FRAP (mmol Fe^{2+} g^{-1})	References
Medicinal herbs extracts				
Rosemary leaves	50.0–178.8	19.8–64.7	2.2–10.3	Ulewicz-Magulska & Wesolowski, 2019
Sage leaves	16.2–173.6	6.4–64.5	2.2–7.9	Ulewicz-Magulska & Wesolowski, 2019
Thyme herbs	42.4–240.8 (a)	12.4–79.3 (a)	0.6–15.4 (a)	Ulewicz-Magulska & Wesolowski, 2019
	46.7–59.0 (b)		16.0 mg GAE/g (b)	Vábková & Neugebauerová, 2012
Oregano herbs	104.3–325.1	28.6–87.2	6.8–22.3	Ulewicz-Magulska & Wesolowski, 2019
Basil herbs	28.2–47.5 (a)	13.9–28.3 (a)	0.9–2.4 (a)	Ulewicz-Magulska & Wesolowski, 2019
	147.0 (b)	0.49 (IC_{50}) (b)	0.54 mmol AAE/g (b)	Hinneburg et al., 2006
Melissa leaves	54.9–299.5	25.7–87.5	9.7–26.9	Ulewicz-Magulska & Wesolowski, 2019
Peppermint leaves	18.3–284.3	14.5–185.6	4.1–18.7	Ulewicz-Magulska & Wesolowski, 2019
Caraway seed	8.9–26.9 (a)	1.3–21.1 (a)	0.1–0.2 (a)	Ulewicz-Magulska & Wesolowski, 2019
	37.4 (b)			Hinneburg et al., 2006
Lovage roots	13.8–26.4	1.8–22.7	0.2–0.4	Ulewicz-Magulska & Wesolowski, 2019
Angelica roots	10.2–21.6	1.7–22.8	0.1–0.2	Ulewicz-Magulska & Wesolowski, 2019
Marjoram	51.8–236.0 (a)	6.9–53.04 (a)	1.8–7.2 (a)	Ulewicz-Magulska & Wesolowski, 2019
	67.2 (b)		16.6 mg GAE/g (b)	Vábková & Neugebauerová, 2012
Tarragon	59.5–253.5	25.6–64.7	1.9–6.3	Ulewicz-Magulska & Wesolowski, 2019
Summer savory	41.6–64.4	-	14.2 mg GAE/g	Vábková & Neugebauerová, 2012
Parsley	29.2	12.0 (IC_{50})	0.05 mmol AAE/g	Hinneburg et al., 2006
Laurel	92.0	0.49 (IC_{50})	0.46 mmol AAE/g	Hinneburg et al., 2006
Juniper	18.5	2.2 (IC_{50})	0.18 mmol AAE/g	Hinneburg et al., 2006
Cardamom	24.2	7.8 (IC_{50})	0.051 mmol AAE/g	Hinneburg et al., 2006
Aniseed	20.8	2.9 (IC_{50})	0.11 mmol AAE/g	Hinneburg et al., 2006
Fennel	30.3	2.1 (IC_{50})	0.12 mmol AAE/g	Hinneburg et al., 2006
Plants from which natural food dyes are obtained				
Centaurea cyanus (Cornflower)*	7.19	83.42	78.01(mg TE/mL)	Marian et al., 2017
*Indigofera tinctoria**	69.7	-	-	Prakash et al., 2009
*Lawsonia inermis**	75.8	-	-	Prakash et al., 2009
Curcuma longa (Turmeric)*	36.6	-	-	Prakash et al., 2009
Annatto (*Bixa orellana* L.)	0.73–1.81	29.88–94.20	-	Viuda-Martos et al., 2012
Alkanna tinctoria (ext.)	18.27	76.86 (IC_{50})	-	Das et al., 2024
Camellia sinensis (tea)*	56.63–80.27	0.03–0.04 (IC_{50})	12.40–14.83(μmol $Fe_2SO_4 \cdot 7H_2O$/mL)	Nor Qhairul Izzreen & Mohd Fadzelly, 2013
Tagetes erecta (Mexican Marigold)*	57.52–125.18	32.5–113.32 (IC_{50})	-	Youssef et al., 2020
Crocus sativus (Saffron)*	2.96–6.17	248–280 (IC_{50})	-	Ghanbari et al., 2019

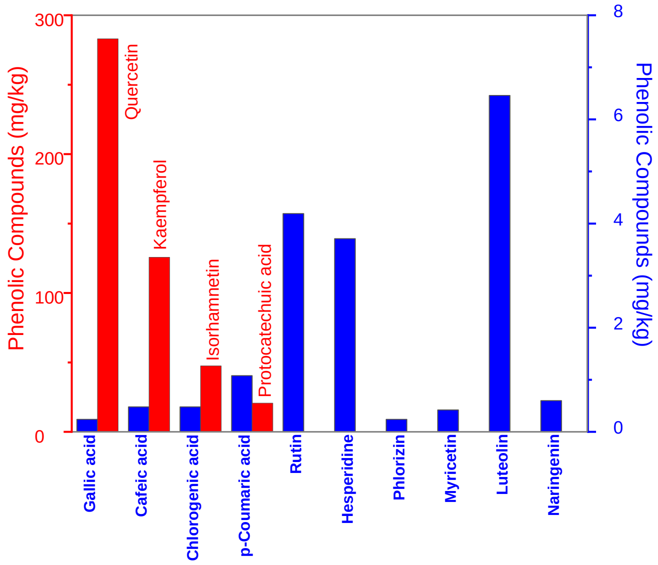

Figure 2. *Quantitative distribution of the phenolic compound profile of poppy flower extract (dry matter basis, mg/kg). Highly detected compounds are indicated by red columns (left axis), lowly detected compounds by blue columns (right axis).*

Şekil 2. Gelincik çiçeği özütünün fenolik bileşik profilinin kantitatif dağılımı (kuru madde bazında, mg/kg). Yüksek miktarda tespit edilen bileşikler kırmızı sütunlarla (sol eksen), düşük miktarda tespit edilen bileşikler mavi sütunlarla (sağ eksen) gösterilmiştir.

In their study, Bujak et al. (2021) determined the amount of quercetin in the poppy flower extract as 18 times lower (16 mg/kg), the amount of gallic acid as similar (0.2 mg/kg), and the amount of caffeic acid as 3 times (1.21 mg/kg) higher than our study. It is thought that the method they applied in extraction (10 g dry flowers/100 g ethanol: water (20:80) solution, 25 C, 20 min) is not sufficient in the recovery of phenolic compounds. In their study on poppy flower sherbet, Aydoğdu et al. (2023) found the amount of gallic acid 92 times (24 mg/kg) higher, while the amounts of protocatechuic acid (1.15 mg/kg) and rutin (1.35 mg/kg) were found 18 and 3 times lower, respectively. According to our study, quercetin, the dominant phenolic component, could not be detected in poppy flower sherbet. These results show that gallic acid can be extracted at a higher rate with water, but ethanol is important in the extraction of other phenolic compounds.

Antioxidant Capacity

The poppy plant exhibits significant antioxidant capacity, attributed to its many bioactive components and the presence of vitamins (Grauso et al., 2020). A study by Maurizi et al. (2015) evaluated the antioxidant activity of an acetone: water: acid (70:29.5:0.5 v/v/v) extract from the green leaves of the poppy plant, yielding a value of 43.89 mmol TE g^{-1} by the ORAC method. A study conducted by Hasplova et al. (2011) indicated that the DPPH free radical scavenging activity of the pure methanol extract (25 mg/mL) from poppy flowers was 85%. Table 2 presents the DPPH (IC_{50}), CUPRAC, and FRAP results, demonstrating the antioxidant activity of poppy extract. Dhiman et al. (2017) reported significantly lower CUPRAC and FRAP values for poppy, at 8.75 µmol TE g^{-1} and 2.07 µmol g^{-1}, respectively, compared to our study. Bayram et al. (2015) determined the IC_{50} value to be 55.9 µg mL^{-1}. It is thought that the difference between the literature results and the result of our study is due to the differences in the harvest regions of the poppy flower, extraction methods, and solvents. When examining the phenolic content, it can be suggested that the strong antioxidant effects of the major identified components contributed to these analysis results. The red pigment derived from poppy was found to have a considerably higher antioxidant content compared to alternative colorant sources such as red carrot (FRAP; 2.30 µmolTE/g and CUPRAC; 3.31 µmolTE/g) (Singh et al., 2018), red beet (FRAP; 63-302 µmolTE/g and CUPRAC; 62-143 µmolTE/g) (Carrillo et al., 2019), red pepper (FRAP; ~0.112 µmol TE/g) (Anaya-Esparza et al., 2021), and hibiscus (DPPH higher than 25 mg/g, FRAP lower than 90 µmolTE/g and CUPRAC lower than 110 µmolTE/g) (Nguyen et al., 2022). Additionally, when examining the medicinal plants in Table 4, the highest antioxidant activity in terms of IC_{50} was observed in *Camellia sinensis* (0.03-0.04 µg mL^{-1}), while the lowest was found in saffron (*Crocus sativus*, 248-280 µg mL^{-1}). The IC_{50} value for the poppy extract (PE) was determined to be 0.92 µg mL^{-1} (Table 3), indicating significantly higher antioxidant activity compared to many medicinal plants.

Antimicrobial Activity

The antimicrobial activity of poppy extract (PE) was evaluated against two Gram-positive and two Gram-negative pathogenic bacteria using the disk diffusion method to investigate its broad-spectrum antibacterial potential. The results, presented in Table 5, show that for Gram-positive bacteria, inhibition zones were 11.32 ± 0.25 mm for *Listeria monocytogenes* and 9.14 ± 0.14 mm for *Staphylococcus aureus*. In the case of Gram-negative bacteria, inhibition zones were 13.21 ± 2.10 mm for *Salmonella* Enteritidis and 10.29 ± 0.41 mm for *Escherichia coli* O157:H7. PE demonstrated the highest antimicrobial activity against *S. enteritidis* followed by *L. monocytogenes, E. coli* O157:H7, and *S. aureus*. These findings indicate that Gram-negative bacteria may be more sensitive to PE, possibly due to their more permeable outer membrane. Conversely, the thicker peptidoglycan layer in Gram-positive bacteria may reduce the efficacy of PE against these strains.

Table 5. Antimicrobial activity of poppy extract.
Çizelge 5. Gelincik çiçeği ekstraktının antimikrobiyal aktivitesi.

Microorganism	Inhibition zone (mm)
***Escherichia coli* O157:H7 ATCC 33150**	10.29 ± 0.41^{bc}
***Listeria monocytogenes* ATCC 7644**	11.32 ± 0.25^{ab}
***Salmonella* Enteritidis ATCC 13076**	13.21 ± 2.10^{a}
***Staphylococcus aureus* ATCC 25923**	9.14 ± 0.14^{c}

[a-c] Statistically significant differences between the means in the same column are indicated ($p < .05$).

The antimicrobial activity of the poppy (*Papaver rhoeas L.*) flower can be attributed to the presence of phenolic compounds and flavonoids in its extracts. These phytochemicals demonstrate antimicrobial properties by mechanisms such as damaging bacterial cell walls and disrupting intracellular metabolic processes (Hmamou et al., 2022). A study by Hmamou et al. (2022) reported that the total phenolic content in *Papaver rhoeas* extracts was strongly correlated with significant antimicrobial activity against various bacterial strains, suggesting that these compounds play a key role in inhibiting bacterial growth.

Bayram et al. (2015) reported that poppy flower extract did not exhibit antimicrobial activity against several strains (Staphylococcus aureus ATCC 29213, Staphylococcus aureus ATCC 25923, Enterobacter cloasea ATCC 13047, Salmonella typhimurium ATCC 14028, Escherichia coli ATCC 23897, Escherichia coli 0157:H7 ATCC 33150, Yersinia enterocolitica ATCC 27729, Pseudomonas aeruginosa ATCC 27853, Listeria monocytogenes ATCC 7644, Bacillus cereus ATCC 33019, Bacillus subtilis ATCC 6630, and two yeasts: Saccharomyces cerevisiae BC 5461 and Candida albicans ATCC 1223), including *E. coli* O157:H7, *L. monocytogenes, S. typhimurium, S. aureus, E. cloacae, Y. enterocolitica, B. cereus, B. subtilis, P. aeruginosa*, and *E. coli*. The minimum inhibitory concentration (MIC) of *Papaver rhoeas* green leaves against *S. aureus* was determined to be 39.06 µg mL^{-1} (Ünsal et al., 2009). In another study, chloroform extracts from the aerial parts of the poppy plant showed significant activity against *S. aureus* (MIC = 1.22 µg mL^{-1}), *S. epidermidis*, and *K. pneumoniae*, but no activity against *P. mirabilis* (Çoban et al., 2017). The MIC value of poppy flower extract against *Enterobacter faecalis* was reported to be 0.11 mg/mL (Marsoul et al., 2020). Furthermore, extracts from the green leaves of *P. rhoeas* were used in silver nanoparticle synthesis, and the MIC values for *S. aureus, E. coli, Bacillus subtilis, Pseudomonas aeruginosa*, and *Candida albicans* were 1.50, 0.75, 3.00, 6.00, and 0.37 mg mL^{-1}, respectively (İpek et al., 2023). This difference may be largely due to the extraction methods used. Bayram et al. carried out the extraction at 35 °C for 2 hours, whereas in our study, it was left to macerate at room temperature for 72 hours. This longer time and low-temperature extraction process may have made it possible to extract not only anthocyanins, but also alkaloids and other phenolic compounds with potentially antimicrobial effects. In addition, the observed differences may also be explained by the variation in phytochemical composition depending on the region and conditions in which the plant grows.

A study by Katarzyna et al. (2021) demonstrated that alkaloids extracted from poppy flowers significantly inhibited the growth of *S. enteritidis*, likely through disruption of the bacterial cell membrane or interference with metabolic processes. Similarly, Hmamou et al. (2022) found that phenolic compounds in poppy flower extract exhibited notable antimicrobial effects against *L. monocytogenes* by disrupting bacterial cell walls and altering membrane permeability. Çoban et al. (2017) also reported that poppy phytochemicals showed significant antimicrobial activity against *E. coli* O157:H7, suggesting that this effect may result from the synergistic actions of flavonoids and alkaloids. These phytochemicals were found to reduce bacterial viability by inhibiting protein synthesis and disrupting cellular functions. The antimicrobial activity observed in this study aligns with these previous findings, highlighting the potential of bioactive compounds from poppy flowers as natural antimicrobial agents. These results provide a strong foundation for the application of poppy flower extracts in the food and health industries.

Color Stability and Degradation

Anthocyanins are among the primary water-soluble pigments responsible for the wide range of colors in fruits and vegetables. Changes during food processing and storage can significantly affect the stability or degradation of these pigments (Karasu et al., 2015). The changes in anthocyanin content and the percentage of anthocyanins retained in poppy extract after exposure to different temperatures and durations are presented in Figure 3-A and 4-A, respectively. The extract was subjected to temperatures of 60 °C, 80 °C, and 100 °C, and the changes in anthocyanin content were measured at 30-, 45-, and 60-minutes relative to the initial levels. The results indicated that exposure to 60 °C caused less loss of anthocyanins compared to higher temperatures, while 60 minutes of exposure to 100 °C reduced the anthocyanin content by only 15.49%. A significant time-dependent decrease in anthocyanin content was observed across all temperature treatments ($p < .05$). The most substantial decline occurred during the first 30 minutes at 100 °C, while no statistical difference was found between 60 °C and 80 °C treatments during this period ($p < .05$). Overall, no significant difference was observed between the 60 °C and 80 °C thermal processes over the entire duration. At the end of 60 minutes, the percentage of retained anthocyanins in the extract was 87.60%, 85.22%, and 84.51% for 60 °C, 80 °C, and 100 °C, respectively. These findings suggest that the poppy extract demonstrates stability under low temperature long time (LTLT), high temperature long time (HTLT), and even extreme pasteurization or sterilization conditions. This opens the possibility for the use of poppy extract as a colorant in thermally processed products. Accordingly, PE has the potential to be used as a natural colorant and/or antioxidant additive in various heat-treated food products such as fruit juices, jams and bakery products. This potential, considering both the high thermal stability of PE and the functional properties of the phenolic compounds it contains, provides a basis for further research on its application.

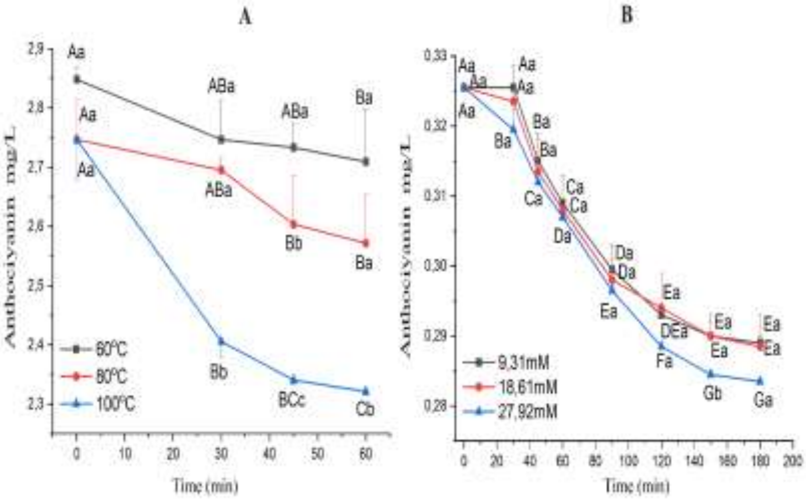

Figure 3. Changes in anthocyanin content of poppy color extract under different temperature treatments (A) and varying concentrations of hydrogen peroxide (B). (a-c Indicates statistically significant differences between different temperatures and hydrogen peroxide concentrations at the same time point ($p < .05$). A-C Indicates statistically significant differences between time points at the same temperature or hydrogen peroxide concentration ($p < .05$)).

Şekil 3. Farklı sıcaklık uygulamaları (A) ve değişen hidrojen peroksit konsantrasyonları (B) altında gelincik çiçeği özütünün antosiyanin içeriğindeki değişimler. (a-c Aynı zaman noktasında farklı sıcaklıklar ve hidrojen peroksit konsantrasyonları arasındaki istatistiksel olarak anlamlı farklılıkları gösterir (p < .05). A-C Aynı sıcaklık veya hidrojen peroksit konsantrasyonundaki zaman noktaları arasındaki istatistiksel olarak anlamlı farklılıkları gösterir (p < .05)).

Bahreini et al. (2015) studied the thermal stability of anthocyanins obtained from eggplant peel and carried out analyses at temperatures of 20, 30, and 40 °C for 1, 3, 6, 9, and 15 days. The percentage of retained anthocyanins for temperatures of 20, 30, and 40 ℃ was determined as 92, 93, and 92, respectively, at the end of the first day, and 64, 43, and 2 at the end of the fifteenth day. Fernández-López et al. (2013) studied the thermal stability of anthocyanins obtained from elderberry, red cabbage, hibiscus, red beet, Opuntia fruits, and red cochineal. The anthocyanin percentages of the aforementioned natural colorant extracts remaining after 1 hour at 50, 70 and 90 ºC were determined as 95, 90, 90, 90, 82, 100 (50 ºC); 85, 75, 70, 43, 30, 98 (70 ºC); 72, 65, 50, 43, 3, 99 (90 ºC), respectively. In this study, the retention of anthocyanins above 84% even at higher temperatures reveals that poppy flower extract exhibits remarkable stability against heat treatment, and in this respect, our study is superior compared to many natural sources in the literature.

The first-order kinetic model for anthocyanin degradation in poppy extract subjected to thermal treatment at various temperatures over specific time periods was confirmed by high R^2 values ranging from 0.91 to 0.97 (Figure 4-A). Anthocyanin degradation increased across all temperature conditions over time. When examining the degradation rate constant (k), the highest rate was observed at 80 °C (15.60 × 10^{-4} min^{-1}), followed by 100 °C (11.90 × 10^{-4} min^{-1}), with the lowest at 60 °C (4.60 × 10^{-4} min^{-1}). It can be inferred that the degradation at 80 °C was approximately 3.4 times greater than at 60 °C over time. At the end of the temperature treatments, degradation percentages of 4.88%, 6.37%, and 15.49% were recorded at 60 °C, 80 °C, and 100 °C, respectively.

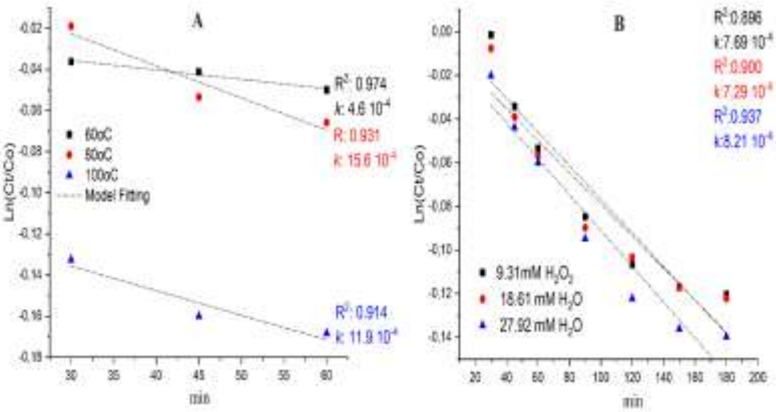

Figure 4. Degradation curves of poppy color extract under different temperature treatments (A) and varying concentrations of hydrogen peroxide (B).

Şekil 4. Gelincik çiçeği özütünün farklı sıcaklık uygulamaları (A) ve değişen hidrojen peroksit konsantrasyonları (B) altında bozunma eğrileri.

Aztatzi-Rugerio et al. (2019) observed similar color loss when they exposed betanin, a colorant derived from beets, to heat at 75 °C for 60 minutes. In comparison, only 70% of the total anthocyanin content in microencapsulated color components was retained under light at 70 °C (Sarabandi et al., 2023). Our study demonstrates that poppy extract is heat-resistant and that food processing involving thermal treatments does not significantly affect its stability. These findings suggest that the colorant derived from poppy extract is suitable for use in the production of thermally processed food products.

Hydrogen peroxide (H_2O_2) is a biological compound found in low concentrations in air, water, plants, microorganisms, and foods (Ma et al., 2011). Hydrogen peroxide and its radicals can bind to the carbon ring of anthocyanins, causing structural breakdown and degradation (Satake & Yanase, 2018). The resistance of the plant extract to oxidation and its degradation kinetics are shown in Figure 3-B and 4-B, respectively. When examining the oxidative degradation kinetics of the pigmented poppy extract in the presence of H_2O_2, the R^2 values were found to range between 0.90 and 0.93, indicating a high correlation between the experimental data and the linear regression analysis. The rate constants (k) for anthocyanin degradation over time were 7.69 × 10^{-4} min^{-1}, 7.29 × 10^{-4} min^{-1}, and 8.21 × 10^{-4} min^{-1} for the extract reacted with hydrogen peroxide concentrations of 9.31, 18.61, and 27.92 mM, respectively. The highest degradation occurred at the 27.92 mM concentration. As shown in Figure 5-B, increasing doses of H_2O_2 over time decreased the percentage of anthocyanins retained in all samples. After 30 minutes, the lowest H_2O_2 concentration (9.31 mM) retained the highest percentage of anthocyanins (99.84%). However, as the concentration increased, the retention decreased. After 3 hours, the anthocyanin retention rates were 88.65%, 88.49%, and 86.96% for the 9.31, 18.61, and 27.92 mM concentrations, respectively. While there was no significant difference between the 9.31 and 18.61 mM concentrations, the retention at 27.92 mM showed a more substantial decrease (13.04%).

The application of different doses of H_2O_2 did not cause significant changes in the anthocyanin content of poppy extract ($p < .05$). However, a statistically significant decrease in anthocyanin content occurred over time across all doses. In the first 30 minutes, no significant decrease was observed with the 9.31 and 18.61 mM H_2O_2 treatments, but after 45 minutes, all concentrations showed a statistically significant reduction ($p < .05$). Quercetin, kaempferol, and isorhamnetin are the primary phenolic compounds in poppy extract, known to be effective antioxidants against oxidative stress (Kim et al., 2014). The relatively low degradation observed is likely due to the hydrogen peroxide scavenging capacity of these phenolic compounds, which is attributed to their antioxidant properties.

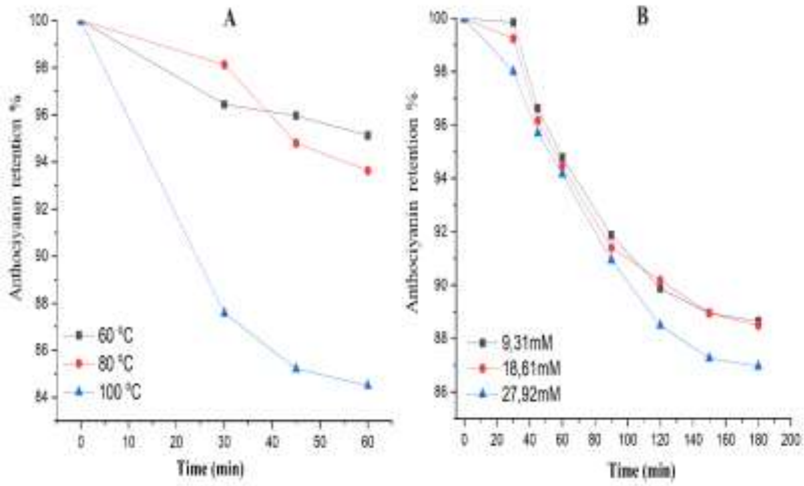

Figure 5. Percentage of anthocyanins retained in poppy color extract after exposure to different temperatures (A) and varying concentrations of hydrogen peroxide (B).
Şekil 5. Farklı sıcaklıklara (A) ve değişen konsantrasyonlarda hidrojen peroksit (B) maruziyetinden sonra gelincik çiçeği özütünde tutulan antosiyaninlerin yüzdesi.

Anthocyanins, which have a central core structure in the form of 2-phenylbenzopyrylium or flavylium cation, are classified as polyphenols and secondary metabolites (Enaru et al., 2021). Various colors such as crimson, indigo, violet, purple, and pink in flowers, fruits, and vegetables are attributed to anthocyanins. These pigments are known to change color in response to pH variations. This pH-dependent color-changing property is crucial for potential applications in monitoring food quality, determining shelf life, enhancing consumer interest, and using anthocyanins as color indicators in food packaging (Roy & Rhim, 2021).

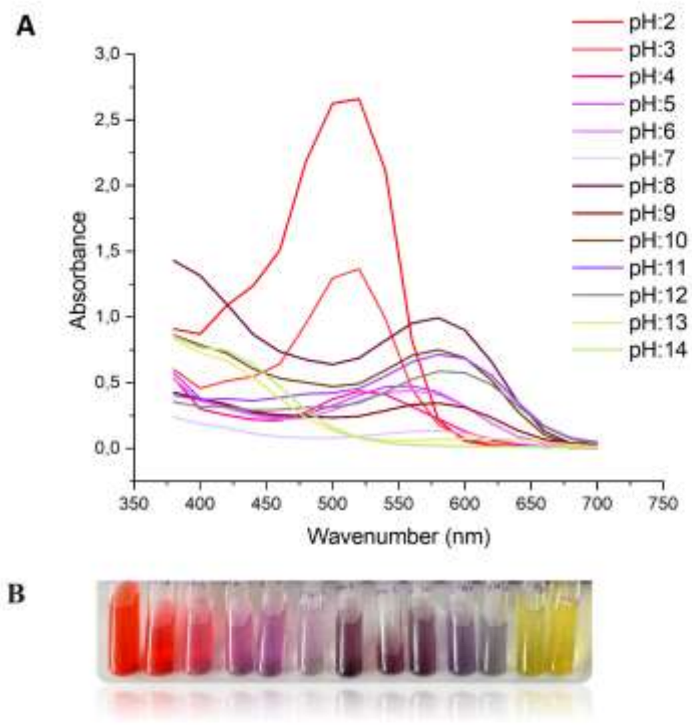

Figure 6. Spectrum scans (A) and color changes (B) of poppy color extract at different pH values.
Şekil 6. Gelincik çiçeği özütünün farklı pH değerlerinde spektrum taramaları (A) ve renk değişimleri (B).

Poppy extracts exhibited a red-pink color at pH 2-4, a more purplish (magenta) hue at pH 5-6, colorlessness at neutral pH, a brownish color at pH 8-10, a bluish tint at pH 11-12, and a greenish-yellow color at pH 13-14 (Figure 6). The red color observed at pH 2-4 is attributed to the presence of the flavylium cation, while the blue quinoidal forms dominate at pH 5-6. At neutral pH, anthocyanins shift to the quinonoidal base form, which typically shows purple and blue hues. The quinonoidal base form becomes more prominent at pH 8-10, with increasing alkalinity darkening the colors. In highly alkaline conditions, anthocyanins shift towards yellow tones, a result of further deprotonation and structural changes in the molecule. These yellow shades are due to the formation of the chalcone form (Roy & Rhim, 2021). The ability of poppy extract to maintain its stable color in acidic environments makes it suitable for use as a colorant in acidic foods. Additionally, it has potential applications as a spoilage indicator in various food packaging systems. Studies by Bakhshizadeh et al. (2023), Tavassoli et al. (2024) and Tavassoli et al. (2023) have explored the use of poppy extract as a spoilage indicator in food packaging, with promising results.

CONCLUSION

This study assessed the potential application of poppy flowers as a natural food colorant, along with their bioactive chemical composition, activity, and mineral content. The TPC and TAC content of the poppy flower were determined as 45.10 ± 0.84 mg GAE g^{-1} and 17.11 ± 0.60 mg cyanidin-3-glucoside g^{-1}, respectively. The antioxidant activity was determined as 0.92 ± 0.08 μg mL^{-1} for DPPH (IC_{50}), 155.98 ± 8.73 mM TE g^{-1} for CUPRAC, and 581.94 ± 12.09 μmol TE g^{-1} for FRAP. In terms of antimicrobial activity, the highest inhibition was observed in *S. enteritidis*, followed by *L. monocytogenes*, *E. coli* O157:H7, and *S. aureus*, respectively. The dominant phenolic compounds in the poppy flower were identified as quercetin, kaempferol, isorhamnetin, and protocatechuic acid. To emphasize the nutritional properties of the poppy flower, an analysis was conducted on 11 minerals. The most abundant minerals were sodium (9382.77 mg kg^{-1}), potassium (5901.07 mg kg^{-1}), phosphorus (186.6 mg kg^{-1}), and iron (186.0 mg kg^{-1}). The color stability results of the poppy flower indicate that, even at the maximum temperature and duration examined (100 °C for 60 minutes), 84.15% of the anthocyanins were maintained. Although no statistically significant differences were observed among the results for the H_2O_2 concentrations tested, 86.96% of the anthocyanins were still preserved after a treatment duration of 180 min. The results demonstrate that poppy flower extract has considerable antioxidant capacity and a significant concentration of phenolic components. The extract exhibited high color stability, demonstrating resistance to thermal treatment and oxidative conditions. The extract's color-changing characteristics in relation to changes in pH highlight its potential application in food packaging as a spoilage indicator. Since the red poppy is not yet cultivated in our country, the limited feasibility studies constrain the ability to perform cost analysis and assess its commercial scalability. However, future studies focusing on this area could significantly contribute to unlocking the economic potential of the red poppy, promoting its agricultural and industrial applications. Consequently, poppy flower extract can be regarded as both a cost-effective and practical substitute for the natural colorant companies. Moreover, given the limited studies on the phenolic composition of poppy flower extract, this research will provide a valuable contribution to the literature.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Ethics Statement

This study does not involve any human or animal testing.

Conflict of Interest

The authors declare no conflicts of interest.

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