Evaluation of Anthocyanin Content, Color Stability, and Antibacterial Activity of Purple Corn Extract for Application in Colorimetric Sensors

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Abstract. In recent years, increasing attention has been paid to plant-derived compounds as natural alternatives to synthetic chemicals in food, cosmetic, and healthcare applications. Purple corn (*Zea mays L.*) has attracted considerable interest due to its high anthocyanin content, which not only imparts a vibrant color but also exhibits antioxidant and antibacterial properties. This study aimed to evaluate the multifunctional potential of purple corn extract as both a natural pH indicator and an antibacterial agent. Anthocyanins were extracted from purple corn and analyzed using UV-Visible spectrophotometry. The extracts were tested in buffer solutions across a pH range of 2.0–12.0. The extracts color exhibited distinct transitions: reddish-purple under acidic conditions (pH 2.0–6.0), grayish-purple under neutral to slightly basic conditions (pH 7.0–9.0), and bluish-green under alkaline conditions (pH 10.0–12.0). Despite these variations, the maximum absorbance was observed at 520 nm, particularly at pH 1.0 and 4.5, where measurements were conducted for anthocyanin. FTIR spectroscopy confirmed the presence of functional groups including hydroxyl, alkyl, alkene, and aromatic ring, which are characteristic of phenolic and anthocyanin compounds. Quantitative analysis showed that total anthocyanin content peaked at 177.10 ± 24.68 mg/L, corresponding to increased extract concentrations. Antibacterial activity was assessed against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) using serial dilution method. The extract demonstrated bactericidal properties at concentrations ranging from 7.0–14.0 %w/v. Overall, the study highlights the potential of purple corn extract as a natural, eco-friendly pH-sensitive dye with significant antibacterial activity, suitable for application in food packaging, cosmetics, and biomedical fields.

1 Introduction

Thailand possesses rich biodiversity, particularly in vegetables and fruits that are widely consumed and utilized. Each plant species offers unique beneficial properties. Many Thai fruits and vegetables can be consumed fresh and are also used in traditional medicine. For example, tomatoes, papayas, and guavas are rich in antioxidants that help reduce the risk of chronic diseases such as cancer and heart disease [1]. In addition, fruits like pomelo, mangosteen, and mango are highly nutritious and can be incorporated into health products due to their abundance of vitamins and flavonoids [2].

Studies on red- and purple-colored fruits and vegetables have shown that they contain anthocyanins flavonoid their purple or red coloration. Anthocyanins antioxidant properties help protect cells from oxidative stress and reduce the risk of various diseases, particularly those related to vascular and neurological degeneration [3]. Plants such as purple corn, eggplant, red cabbage, and blueberries are rich sources of anthocyanins and hold great promise for diverse applications [4].

Anthocyanins are also notable for their pH-sensitive color changes, shifting from red in acidic conditions to purple or blue under alkaline conditions [5]. Beyond their color-changing ability, anthocyanins exhibit additional bioactivities including anti-inflammatory, anti-cancer, and anti-aging effects, making them promising agents in the prevention of chronic diseases such as cardiovascular disorders [6]. Research interest in anthocyanins continues to grow due to their safety and potential for incorporation into health-related products such as pharmaceuticals and dietary supplements [7].

In addition to these health benefits, anthocyanins have been increasingly recognized for their antimicrobial activity. Several studies have demonstrated that anthocyanins can inhibit the growth of various bacterial species by disrupting cell membranes, altering intracellular functions, and interfering with nucleic acid synthesis [8]. Specifically, anthocyanins extracted from purple corn have shown strong inhibitory effects against

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both Gram-positive and Gram-negative bacteria, making them attractive candidates for natural antibacterial agents in food preservation and biomedical applications [8].

Purple corn (*Zea mays L.*) has gained attention as a rich source of anthocyanins, especially in its cob, which contains high levels of these compounds. In addition to antioxidant activity, purple corn also contains phenolic compounds that contribute to disease prevention, such as cancer and cardiovascular diseases [9]. Research has shown that anthocyanins extracted from purple corn cobs using ultrasonic-assisted extraction and acidified solvents can yield up to 7,869.26 μg CGE/g dry weight, highlighting its potential as a potent antioxidant source [10]. Furthermore, purple corn extract has demonstrated strong antibacterial activity against common foodborne and clinical pathogens [8].

Due to its pH-sensitive color-changing properties and high antioxidant content, purple corn is highly suitable for development as a natural pH indicator. The extract can serve as a colorimetric sensor capable of accurate pH detection in an environmentally friendly manner [11]. Applications include food packaging, cosmetic formulations, medical diagnostics, and chemical sensing [12].

This study aims to evaluate the multifunctional properties of purple corn extract, focusing on its potential as a colorimetric pH sensor and antibacterial agent. The sensor is expected to be safe, eco-friendly, and adaptable for future applications in industrial, medical, and nutritional fields [13].

2 Materials and Methods

2.1 Materials

Purple corn extract was obtained from T.S.T. Twin Product (Thailand). The bacterial strains used in this study were *Escherichia coli* (*E. coli*) ATCC 25922, a Gram-negative bacterium, and *Staphylococcus aureus* (*S. aureus*) ATCC 25923, a Gram-positive bacterium. All reagents and chemicals used were of analytical grade.

2.2 Preparation of purple corn extract

Different quantities (5, 10, 15, and 20 g) of dried purple corn extract powder were each dissolved in 200 mL of distilled water. The mixtures were stirred and heated at 60 °C for 90 minutes. After extraction, each sample was filtered and centrifuged at 5,000 rpm for 10 minutes to remove particulates. The resulting supernatant was used for subsequent analyses.

2.2.1 Determination of total anthocyanin content in purple corn extract

Purple corn extracts were prepared by dissolving 5, 10, 15, and 20 g of purple corn extract in distilled water. For total anthocyanin determination, the extracts were diluted with buffer solutions at pH 1.0 (potassium chloride-hydrochloric acid) and pH 4.5 (sodium acetate-acetic acid) in accordance with the pH differential method. The absorbance of each extract was measured using a UV-Visible spectrophotometer (Analytik Jena, Specord 210 Plus). These buffers provided controlled acidic environments that ensured accurate anthocyanin quantification while minimizing any interference from strong acids. The total anthocyanin content was then calculated using Equation 1. All extract concentrations (5, 10, 15, and 20 g of dried purple corn powder) were used in subsequent experiments, including pH stability and antibacterial activity tests, in order to determine the optimal concentration based on performance outcomes. The extract obtained from 20 g, which yielded the highest anthocyanin content, was included to evaluate its effectiveness compared to lower concentrations.

$$\text{References Anthocyanin (mg/L)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \qquad (1)$$

where A is (A520 nm - A700 nm) pH 1.0 - (A520 nm - A700 nm) pH 4.5, MW is molecular weight of cyanidin-3-glucoside (g/mol), DF is dilute factor, "ε" is molar absorptivity (L•mol-1•cm-1) and l is optical path length of the cell (cm)

2.2.2 Color Stability of Anthocyanins in Purple Corn Extract at Different pH

The color stability of anthocyanins in purple corn extract was assessed using buffer solutions with pH values from 2.0 to 12.0. Color changes were visually observed, and absorbance spectra were recorded using a UV-Visible spectrophotometer (Analytik Jena, Specord 210 Plus).

This pH range was chosen because anthocyanins ars unstable below pH 2.0 and above pH 12.0 Staying within this range allowed clear color changes to be observed without degrading the pigment.

2.3 Characterization

2.3.1 FTIR analysis

The purple corn extract was analyzed using Fourier Transform Infrared Spectroscopy (FTIR) with a Perkin Elmer Spectrum One spectrometer. A drop of the extract was placed onto a potassium bromide (KBr) plate, which was then covered with another KBr plate to form a thin film. The sample was mounted on the sample holder and scanned in the mid-infrared region from 400 to 4000 cm^{-1} to obtain the FTIR spectrum

The spectrum was interpreted to identify characteristic absorption bands associated with anthocyanin compounds. These included a broad O-H stretching band near 3399 cm^{-1} (hydroxyl groups), C-H stretching around 2926 cm^{-1} (alkyl groups), aromatic C=C stretching at 1499 cm^{-1} and C-O bending vibrations near 1040 cm^{-1}, consistent with phenolic and glycosidic structures such as cyanidin-3-glucoside. The band assignments were based on published literature [14]

2.3.2 Determination of the Minimum Inhibitory Concentration (MIC) of Purple Corn Extract

The purple corn extract was used in liquid form, not as a powder; thus, the use of %w/v refers to the mass of dried purple corn powder originally dissolved in a given volume of solution. This unit was retained to reflect the concentration of the initial solid material in the extract preparation.

A 96-well microtiter plate was used to determine the minimum inhibitory concentration (MIC) of purple corn extract against Gram-negative *E. coli* and Gram-positive *S. aureus*. Positive control wells contained only tryptic Soy Broth (TSB), while negative control wells contained only purple corn extract without any bacterial onoculum. 14.0% w/v purple corn extract solution was pipetted 100 µL in column 1. TSB was added to columns 2–10 and 12, 50 µL per well. A serial two-fold dilution was performed to prepare extract concentrations of 14.0, 7.0, 4.0, 2.0, 1.0, 0.5, 0.2, 0.1, 0.06, and 0.03% w/v, each well was inoculated with 10^6 CFU/mL of bacterial suspension. After incubation at 37°C for 24 hours, turbidity was visually inspected and compared with the controls. Wells showing no visible turbidity were considered as exhibiting bacterial growth inhibition.

2.3.3 Determination of the Minimum Bactericidal Concentration (MBC) of Purple Corn Extract

A 10 µL aliquot of purple corn extract concentrations ranging from 0.03 % to 14.0% w/v, prepared as described in section 2.3.2, was pipetted onto solid agar medium. After MIC determination, 10 µL from wells that showed no turbidity were plated onto TSB agar and incubated at 37°C for 24 hours. The lowest concentration with no visible bacterial colony was recorded as the MBC.

3. Results and Discussion

3.1 Total anthocyanin content in purple corn extract

The UV-Vis absorbance spectra of purple corn extract at pH 1.0 and pH 4.5 are presented in Figure1 Across all extract concentrations, the maximum absorbance was consistently observed at 520 nm, which corresponds to the characteristics absorption of anthocyanins.

At pH 1.0, the spectra of the 15 g and 20 g samples showed little difference, which may be due to anthocyanin aggregation or optical saturation under highly acidic conditions. Anthocyanins tend to self-associate in flavylium cation form at low pH, limiting further increase in absorbance even with higher concentration [15]. In contrast, at pH 4.5, absorbance increased linearly with extract amount, indicating fewer molecular interactions and greater pigment stability in mildly acidic conditions [16].

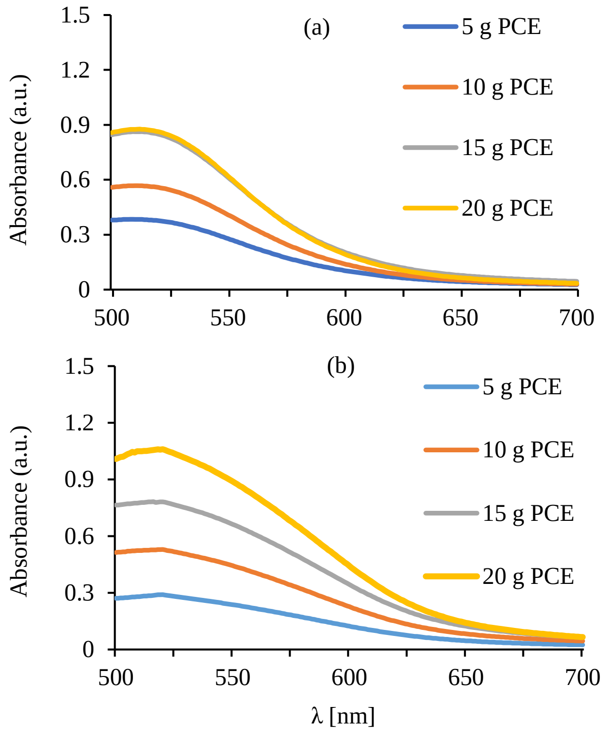

Fig. 1. UV-Vis absorbance spectra of purple corn extract solution at (a) pH 1.0 and (b) pH 4.5

As shown on Figure 2, the total anthocyanin content increased with extract weight, reaching 177.10 ± 24.68 mg/L in the 20 g sample. These results confirm that anthocyanin yield depends on extract concentration.

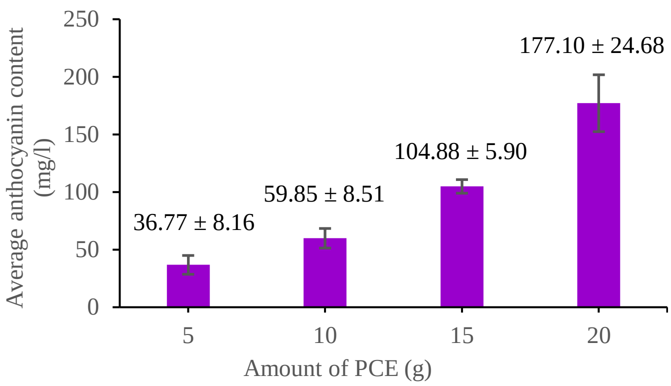

Fig. 2. The anthocyanin content in purple corn extracts weighing 5, 10, 15 and 20 g

3.2 Color Stability of Anthocyanins in Purple Corn Extract at Different pH

The results of the color change study of anthocyanins in purple corn extract in buffer solutions at pH 2.0–12.0 showed that at pH 2.0, 3.0, 4.0, 5.0, and 6.0, the extract appeared reddish-purple. At pH 7.0, 8.0, and 9.0, the color shifted to grayish-purple, while at pH 10.0, 11.0, and 12.0, the extract exhibited a darker bluish-green hue.

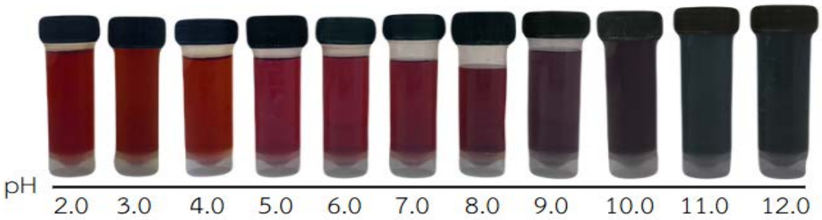

Fig. 3. Change color of Purple corn extract solution in buffer solution pH 2.0-12.0

The color transitions from red (pH 2-4) to reddish-purple (pH 5-8), deep purple (pH 9-10), and bluish-green (pH 11-12) is attributed to structural transformations of anthocyanins into flaylium cations, quinoidal bases, and chalcone forms. These pH dependent changes align with previous findings by Zhao et.al., who demonstrated how anthocyanins shift both in structure and color across varying pH conditions [17].

Similar behavior has been reported in anthocyanins from red cabbage, butterfly pea, and black rice, which also exhibit vivid and reversible pH-responsive color changes [18, 19]. These findings support their potential use in natural pH indicators.

3.3 Characterization

3.3.1 FTIR

The FTIR spectrum of purple corn extract revealed distinct absorption peaks at 3399 cm-∫ (OóH stretching), 2926 cm-∫ (CóH stretching), 1499 cm-∫ (C=C stretching), and 1040 cm-∫ (aromatic ring CóH deformation). These peaks correspond to functional groups commonly found in anthocyanin. The broad peak at 3399 cm-∫ is associated with hydroxyl groups present in phenolic compounds, while the peak at 2926 cm-∫ indicates C-H stretching from aliphatic chains. The absorption at 1499 cm-∫ represents aromatic C=C bonds, and the 1040 cm-∫ band is attributed to C-O stretching or bending vibrations in glycosidic linkages.

These spectral features confirm the presence of hydroxyl, alkyl, and aromatic ring structures, consistent with anthocyanins such as cyanidin-3-glucoside. No absorption bands related to nitrogen-containing groups were observed. The observed FTIR peaks are consistent with those reported in recent studies of anthocyanin-rich plant extracts, including the identification of hydroxyl, aromatic, and glycosidic groups [14].

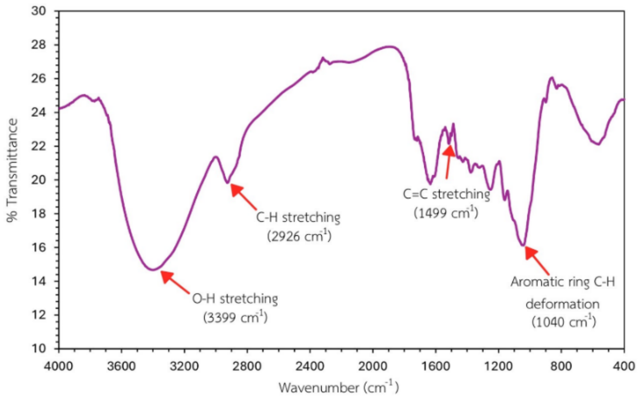

Fig. 4. FTIR spectrum of Purple corn extract solution

3.3.2 Minimum Inhibitory Concentration (MIC) of Purple Corn Extract

The experimental results showed that purple corn extract at concentrations of 7.0–14.0 % w/v effectively inhibited the growth of both Gram-negative *E. coli* and Gram-positive *S. aureus*. In the MIC assay, the bacterial inoculum was standardized to 10^6 CFU/mL, and the cultures were incubated at 37°C for 24 hours. Samples containing 7.0–14.0% w/v extract remained visibly clear, indicating no bacterial growth, similar to the negative control (TSB only). In contrast, lower concentrations and positive control (TSB with bacteria but without extract) showed visible turbidity due to bacterial proliferation.

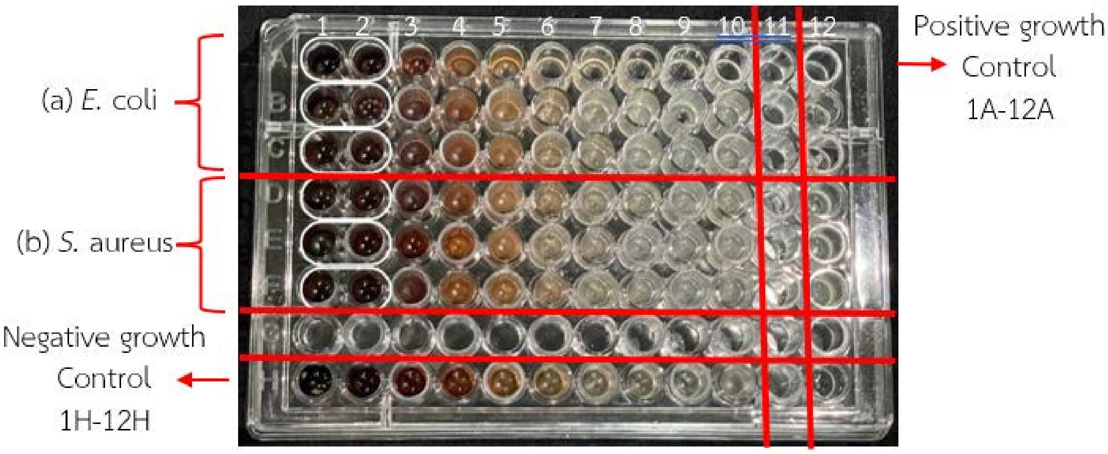

Fig. 5. MIC of Purple corn extract

3.3.3 Minimum Bactericidal Concentration (MBC) of Purple Corn Extract

The MBC of purple corn extract was determined by subculturing 10 µL from MIC wells that showed no turbidity onto TSB agar plates. After incubation at 37°C for 24 hours, the plates were examined for bacterial growth. The results demonstrated that purple corn extract at concentrations of 7.0–14.0% w/v showed no visible bacterial colonies for both Gram-negative *E. coli* and Gram-positive *S. aureus*, confirming that the extract at these concentrations exhibited bactericidal activity. In contrast, lower concentrations produced visible colonies, indicating killing of the bacteria.

The findings indicate that the MBC of purple corn extract is 7.0% w/v for both Gram-negative *E. coli* and Gram-positive *S. aureus* test organisms.

4 Conclusions

This study demonstrated the multifunctional potential of purple corn extract as both a natural pH indicator and an antibacterial agent. The extract exhibited distinct and visually perceptible color transitions across a wide pH range (pH 2.0–12.0), which is consistent with the known pH sensitivity of anthocyanins reported in prior studies on red cabbage and hibiscus [18, 19]. Spectrophotometric analysis revealed a maximum absorbance at 520 nm and confirmed a high anthocyanin content up to 177.10 ± 24.68 mg/L. These findings are in agreement with previous reports on anthocyanin-rich plant sources [6, 7] and further highlight the concentration-dependent nature of the extract's colorimetric response. FTIR spectroscopy additionally verified the presence of phenolic and aromatic functional groups, reinforcing the conclusion that the extract contains key structural features typical of anthocyanins.

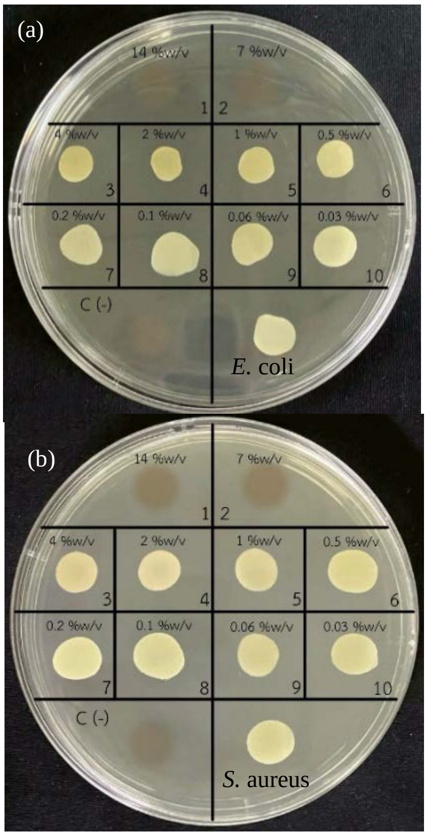

Fig. 6. MBC of Purple corn extract

Moreover, the extract exhibited significant antibacterial activity against both *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive), with minimum inhibitory and bactericidal concentrations observed at 7.0% w/v. These results support its potential as a dual-function bioactive component, particularly for applications in food safety, smart packaging, and antimicrobial materials.

Future research should focus on incorporating the extract into biopolymer films or sensor matrices, evaluating its functional stability under practical storage and usage conditions, and investigating its antimicrobial efficacy against a broader spectrum of microorganisms. Overall, purple corn extract offers a promising, sustainable, and multifunctional natural alternative for use in food, cosmetic, and healthcare industries.

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