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Evaluation of the potential of purple eggplant peel extract (*Solanum melongena* L.) as a pH indicator for *Escherichia coli*

Evaluasi Potensi Ekstrak Kulit Terung Ungu (Solanum melongena L.) sebagai Indikator pH Pertumbuhan Escherichia coli

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ABSTRACT

Anthocyanins are natural pigments found in various plants and fruits, known for their ability to change color according to the pH of their environment. This property can be utilized as a pH indicator in observing carbohydrate fermentation reactions by bacteria. This study aims to evaluate the potential of purple eggplant peel extract (*Solanum melongena* L.) as an alternative pH indicator to monitor bacterial growth. Purple eggplant peel was extracted using 80% ethanol containing 1% HCl, stored for 24 hours at 4 °C, centrifuged, and concentrated using a rotary evaporator at 35 °C. The extract obtained was used as a pH indicator in Tryptic Soy Broth (TSB) media for *Escherichia coli* growth. This test used two treatments; namely purple eggplant peel extract added into TSB media before bacterial inoculation and purple eggplant peel extract added after bacteria grew on TSB media. The results showed that purple eggplant peel extract effectively showed a clear color change according to different pH levels. However, when applied to bacterial growth media, the addition of purple eggplant peel extract showed no significant color change on TSB media, neither before nor after bacteria growth. Thus, to effectively utilize this extract as an indicator in bacterial growth media, further research is needed to explore methods to maintain the stability of the pigments contained in purple eggplant peel extract when added to bacterial growth media.

Keywords: anthocyanin, E. coli, pH Indicator, purple eggplant peel, Solanum melongena

INTISARI

Antosianin merupakan pigmen alami yang terdapat dalam berbagai jenis tanaman dan buah-buahan, dikenal karena kemampuannya berubah warna sesuai dengan pH lingkungannya. Sifat ini dapat dimanfaatkan sebagai indikator pH dalam mengamati reaksi fermentasi karbohidrat oleh bakteri. Penelitian ini bertujuan untuk mengevaluasi potensi ekstrak kulit terung ungu (*Solanum melongena* L.) sebagai indikator pH alternatif dalam memantau pertumbuhan bakteri. Kulit terung ungu diekstraksi menggunakan etanol 80% yang mengandung 1% HCl, disimpan selama 24 jam pada suhu 4 °C, disentrifugasi, dan dikonsentrasikan menggunakan *rotary evaporator* pada suhu 35 °C. Ekstrak yang diperoleh digunakan sebagai indikator pH dalam media *Tryptic Soy Broth* (TSB) untuk pertumbuhan *Escherichia coli*. Pengujian dilakukan dengan dua perlakuan, yaitu penambahan ekstrak kulit terung ungu ke dalam media TSB sebelum inokulasi bakteri dan setelah bakteri tumbuh di media TSB. Hasil penelitian menunjukkan bahwa ekstrak kulit terung ungu mampu menunjukkan perubahan warna yang jelas sesuai dengan variasi pH. Namun, saat diaplikasikan pada media pertumbuhan bakteri, penambahan ekstrak tidak menunjukkan perubahan warna yang signifikan pada media TSB, baik sebelum maupun setelah pertumbuhan bakteri. Oleh karena itu, untuk dapat memanfaatkan

ekstrak ini secara efektif sebagai indikator dalam media pertumbuhan bakteri, diperlukan penelitian lebih lanjut guna mengeksplorasi metode mempertahankan stabilitas pigmen dalam ekstrak kulit terung ungu saat ditambahkan ke media tersebut.

Kata kunci: antosianin, E. coli, indikator pH, kulit terong ungu, Solanum melongena

INTRODUCTION

The use of natural pigments as pH indicators has been extensively studied, particularly in food packaging, biosensors, and antimicrobial applications. Anthocyanins, abundant in plant materials such as purple sweet potatoes (Sohany et al., 2021), purple yam (Aquino & Morales, 2021), and eggplant peels (Zearah, 2024), exhibit pH-sensitive color changes that make them ideal for intelligent packaging and spoilage detection. While anthocyanin-based pH indicators have been incorporated into chitosan films (Bilgiç et al., 2019), starch-based materials (Sohany et al., 2021), and biosensors for chemical detection (Arifin et al., 2022), their potential as direct bacterial growth indicators remains unexplored. This gap is particularly evident in microbiological applications, where real-time pH monitoring could provide insights into bacterial metabolism and proliferation.

Purple eggplant (*Solanum melongena* L.) peels contain high concentrations of anthocyanins, particularly delphinidin derivatives, which have demonstrated strong antioxidant and antimicrobial properties (Zearah, 2024). Prior studies have confirmed the pH-sensitive properties of anthocyanins extracted from eggplant, showing distinct color variations across different pH levels (Yong et al., 2019). However, most applications have been confined to food packaging or general pH sensing. The potential of eggplant peel extract as a pH indicator for bacterial growth, particularly for *Escherichia coli* in culture media, remains unexplored. Given that bacterial metabolism significantly alters the surrounding pH, using anthocyanin-based indicators could provide a natural and effective alternative to synthetic dyes for microbial monitoring.

Existing studies have demonstrated the antioxidant and antimicrobial potential of eggplant peel anthocyanins (Yong et al., 2019; Arifin et al., 2022), but their effectiveness in microbial culture media is not well understood. While synthetic pH indicators such as bromocresol purple (BCP) are commonly used in bacterial growth media, concerns over toxicity and environmental impact have driven interest in plant-based alternatives (Suhartati et al., 2021; Novitriani, et al., 2017). The shift toward natural indicators aligns with the increasing demand for eco-friendly, biocompatible alternatives for laboratory and industrial applications. Moreover, *Escherichia coli* is a widely studied model organism in microbiology, produces acidic or alkaline metabolic byproducts depending on its growth phase and available nutrients. The ability to visually monitor these pH shifts using an anthocyanin-based system would provide a simple yet effective tool for microbial research, potentially improving methods for assessing bacterial viability and metabolic activity in environmental and clinical settings.

This study aims to evaluate the potential of purple eggplant peel extract as an alternative pH indicator to monitor *E. coli* growth in Tryptic Soy Broth (TSB) medium. By characterizing the colorimetric changes of the extract in response to bacterial metabolic activity, this research seeks to bridge the gap between anthocyanin-based pH indicators and their applications in microbiological studies. The findings could contribute to the development of natural, sustainable alternatives for bacterial growth monitoring, with implications for food safety, environmental monitoring, and clinical diagnostics.

MATERIALS AND METHOD

Tools and materials

The study utilized the following tools and materials: measuring flask, beaker, test tube, UV-Vis spectrophotometry, pH-meter, analytical balance, cuvette, peeler, ring stand, bunsen burner, autoclave, 1000 mL Erlenmeyer flask, magnetic stirrer, centrifuge, rotary vacuum evaporator (Biobase RE-2010), distilled water, potassium chloride (KCl), sodium acetate (CH3CO2Na), purple eggplant peel (*Solanum melongena* L.), 80% ethanol, 37% HCl, and *Escherichia coli*. As a comparison medium, Tryptic Soy Broth (TSB) was also used to grow *E. coli* ATCC 25922.

Sample preparation

Sample preparation followed the protocol outlined by Yong et al. (2019), purple eggplant peel was thoroughly washed and peeled. One hundred grams of the peeled eggplant exocarp were macerated in 500 mL of 80% ethanol containing 1% HCl. The mixture was stored at 4 °C for 24 hours. The resulting extract was centrifuged at 8000 x g for 15 minutes. The supernatant was concentrated on a rotary vacuum evaporator at 35°C.

Anthocyanin concentration calculation

The concentration of anthocyanin was determined using the differential pH method described by Kusumawati (2020) and Unawahi et al. (2022). This method employs 0.025 M KCl (pH 1.0) and 0.4 M sodium acetate (pH 4.5) buffers (Giusti and Wrolstad, 2001; Anggraini et al., 2019). One milliliter of extract was mixed with 4 mL of each buffer solution. The UV-Vis spectrophotometry was set to measure absorbance at 530 nm and 700 nm, with distilled water used as a blank.

The total anthocyanin concentration was measured according to the following equation:

$$Total\ anthocyanin\ (mg/L) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times L} \quad (1)$$

Where, MW is the molecular weight of Cyanidin-3-Glukoside (449.2 g/mol), DF is the dilution factor (5), 1000 is the conversion factor from grams to milligrams, E is the molar absorptivity of Cyanidin-3-Glukoside (26,900 L/mol·cm), and L is the cuvette width (1 cm) (Horincar et al., 2019).

Preparation of 0.04% BCP indicator

A 0.04-gram portion of the BCP indicator (Bromocresol Purple) was dissolved in 100 mL of distilled water and filtered using filter paper (Suhartati et al., 2021). To assess its pH sensitivity relative to the experimental extract, preliminary pH measurements were conducted, ensuring that the standard BCP solution exhibited comparable color transitions across the tested pH range.

Preparation of pH series of purple eggplant peel extract in TSB media

To determine the color series, name, and color code at different pH levels, varying volumes of purple eggplant peel extract were added to 10 mL of TSB medium in test tubes. The concentration variations of purple eggplant peel extract (5%, 10%, 20%, and 30%) were adapted with minor modifications from the study by Aquino & Morales (2021), which investigated anthocyanins from *Dioscorea alata* L. peel. The pH of the extract was adjusted using 1 N HCl or

30% KOH. The colors and their corresponding HEX codes were determined using the Color Name Finder website (ArtyClick, 2020).

Preparation of alternative pH indicators

To examine how purple eggplant peel extract affects bacterial growth and medium colors, two experimental treatments were conducted. In the first treatment, the extract was added to TSB medium before inoculating bacteria. Four and a half grams of TSB medium were mixed with 150 mL of distilled water, then distributed into 10 mL test tubes. Purple eggplant peel extract was added in volumes of 0.5 mL (T1.4), 1 mL (T1.3), 2 mL (T1.2), and 3 mL (T1.1) to each tube. A control treatment used a 0.04% BCP indicator added to TSB medium (B1.1). All of the media were autoclaved at 121°C for 15 minutes, then inoculated with *E. coli* at a concentration of 2% from the *E. coli* liquid culture and incubated for 18 hours. Observations were made of the resulting color changes

In the second treatment, the extract was added to the medium after bacterial growth in 18 hours. After 18 hours of incubation at 37°C, 2-3 drops (T3.1), 0.5 mL (T3.2), and 1 mL (T3.3) of extract were added to the each tube. Color changes on the surface of the liquid media were observed, with the control using a 0.04% BCP indicator

RESULTS AND DISCUSSION

Extraction of purple eggplant peel

The extraction of purple eggplant peel was performed using the maceration method. Initially, 500 mL of 80% ethanol solvent, added with 1% HCl, was used to soak the purple eggplant peel for 24 hours at 4°C. Post maceration, the mixture was centrifuged at 8000 x g for 15 minutes to separate the solid residues. The resulting liquid extract was then concentrated using a vacuum rotary evaporator at 35°C to obtain a solvent-free purple eggplant peel extract solution. This extract transferred into a dark glass bottle and stored at 4 ºC for 24 hours to maintain its stability (Todaro et al., 2008).

(a) (b)

Figure 1. Results of purple eggplant peel extract, description: (a). Eggplant peel in the solvent bath, and (b). Purple eggplant peel extract that has been filtered.

The initial pH of the purple eggplant peel was 6, but it dropped to 2 after maceration. This acidic extract was then tested as a potential alternative acid-base indicator in carbohydrate fermentation assays.

Anthocyanin concentration in purple eggplant peels

For the extract diluted threefold, the absorbance values at pH 1.0 were 0.835 at 530 nm and 0.682 at 700 nm. At pH 4.5, the absorbance values were 0.030 at 530 nm and 0.014 at 700 nm. The measurement at 700 nm was particularly important to detect any residual particulates in the sample, as a clean sample should have an absorbance value below zero at this wavelength (Pratiwi & Priyani, 2019). Based on these spectrophotometric readings, the total anthocyanin concentration in the purple eggplant peel extract was calculated to be 38.4 mg/L.

Determination of the pH range of purple eggplant peel extract in TSB media

To determine the pH range of the purple eggplant peel extract, the extract was adjusted with 30% KOH, and the resulting color changes were observed using the Color Name Finder website (ArtyClick, 2020). Table 1 below summarizes these results, including the color, color name, and HEX code for each pH level. Additionally, different amounts of extract (0.5 mL, 1 mL, 2 mL, and 3 mL) were added to 10 mL of Tryptic Soy Broth (TSB) media. It was noted that with the addition of 0.5 mL extract, the initial pH was 3, which differed from the pH levels obtained with other quantities of extract. The intensity of the color increased with the amount of extract added.

According to Silitonga & Sitorus (2014), anthocyanins were more stable in acidic environments compared to alkaline or neutral ones. This was supported by our findings, which showed that the color of the extract remained more intense at pH levels below 4. At pH 5 and above, the color gradually turned brown, indicating decreased stability and intensity. Table 1 shows detailed observations and color data, illustrating how the extract's color changes with pH and extract concentration, highlighting the potential use of purple eggplant peel extract as a pH indicator, especially in acidic conditions.

Table 1. Color characterization of TSB media after adding purple eggplant peel extract at pH 2 to pH 9

pH	Media Color				Kode HEX				Color Name			
	0,5	1	2	3	0,5	1	2	3	0,5	1	2	3
2	NT				NT	#AA2940	#890417	#680010	NT	Deep Carmine	Red Devil	Rosewood
3					#AF665F	#A44550	#A44A4A	#800019	Coral Tree	Light Maroon	Light Maroon	Firebrick
4					#AF8670	#996A5C	#976453	#75453B	Light Taupe	Dark Chestnut	Dark Chestnut	Bole
5					#A38667	#876E4F	#875E3E	#492A14	Beaver	Dull Brown	Cocoa	Brown Derby
6					#947851	#7F6745	#785827	#4E3A11	Pale Brown	Dark Taupe	Milk Chocolate	Bronzetone
7					#907549	#715724	#644316	#212413	Dull Brown	Himalaya	Otter Brown	Rangoon Green
8					#956D30	#856227	#5E3A18	#12130D	Wood	Oak Brown	Irish Coffee	Oxyn
9					#956D30	#7A4810	#542E17	#26231E	Hawaiian Tan	Raw Umber	Brown Derby	Zeus

Notes: NT: Not Tested (it because the initially pH after TSB media mixed with purple eggplant peel extract o,5 mL was 3, not detected as pH 2).

Furthermore, the test was carried out with the extract added to the TSB media before the bacteria grew. Table 2 shows the result of color changes of TSB media mixed with purple eggplant peel extract with several treatments, namely T1.1 was extract added as much as 3 mL, T1.2 was extract added as much as 2 mL, T1.3 was extract added as much as 1 mL, and T1.4 was extract added as much as 0.5 mL.

From the test samples (T1.1 to T1.4), the color changed from green (*pre-autoclave*) to brown (*post-autoclave*). After inoculation, none of the test samples showed any further color changes in the TSB medium, either before or after bacterial growth. In contrast, the color of the control sample (B1.1), which used 0.04% BCP as an indicator, remained purple before and after autoclaved. However, after inoculation, its color change from purple to yellow.Based on this result indicated that purple eggplant peel extract mixed in the TSB media cannot be used as an indicator to detect acid production from carbohydrate fermentation, as no color changes were observed in the media before and after bacterial growth.

The lack of color change is likely due to the degradation of anthocyanin pigments during the autoclaving process. High temperatures induce structural changes in anthocyanins, shifting flavylium cations to chalcones through a two-step process: glycosidic bond hydrolysis yielding a labile aglycone, followed by aglycone ring opening to form colorless carbinol and chalcone groups. These chalcones can further degrade into simpler, colorless compounds, including substituted benzoic acids and 2,4,6-trihydroxybenzaldehyde (Oancea, 2021) . As a result, the structural breakdown of anthocyanins during autoclaving disrupts their ability to transition between different colored forms, limiting their effectiveness as pH-sensitive (Tindal et al., 2024). Similar degradation has been noted by Laksmiani et al., (2015) with anthocyanins from purple sweet potatoes degrading at 50°C, and by Silitonga dan Sitorus (2014), who found that purple eggplant skin anthocyanins are easily hydrolyzed at temperatures below 40°C. Additionally, prolonged storage of the extract could further degrade anthocyanin compounds, as reported by (Halisa, 2018).

Table 2. The characteristics of pH, color changes and bacterial growth in TSB medium mixed with purple eggplant peel extract as an indicator bacterial growth

Treatments	Before Bacterial Inoculation			After Bacterial Inoculation		
	Color Changes		Initial pH after Autoclave	Color Changes	*E. coli*	pH after inoculation
	Before autoclave	After autoclave				
B1.1	Purple #5C1834	Purple #5C1834	5.0	Yellow #733E1C	Grow	3.7
T1.1	Green #212413	Brown #732608	5.0	Brown #732608	Grow	3.2
T1.2	Green #644316	Brown #984000	5.0	Brown #984000	Grow	3.3
T1.3	Green #715724	Brown #A65100	5.0	Brown #A65100	Grow	3.2
T1.4	Green #907549	Brown #9A5B18	5.0	Brown #9A5B18	Grow	3.4

Notes: B1.1: Control (0,04% BCP 0.5 mL); T1.1: Extract 3 mL; T1.2: Extract 2 mL; T1.3: Extract 1 mL; and T1.4: Extract 0.5 mL

These findings contrast with the research by Novitriani et al., (2017), which successfully used torch ginger flower extract (*Etlingera elatior*), also containing anthocyanins, as a pH indicator. In their study, 0.9 mL of the extract was added to sugar media, and 3 mL of this mixture was autoclaved in each test tube. After bacterial inoculation and incubation, the media changed from colorless to pink. Similarly, Suhartati et al., (2021) found that purple cabbage extract, when added

in a volume of 5 mL to each test tube containing 10 mL of sugar media, inhibited bacterial growth due to the antioxidant properties of flavonoids.

The discrepancy between these studies and our results could be attributed to differences in the volume of extract added to the media. Novitriani et al., (2017) used a smaller volume of extract (0.9 mL) per test tube, while in our study, varying volumes of purple eggplant peel extract (0.5 mL, 1 mL, 2 mL, and 3 mL) were mixed with 10 mL of TSB media in each test tube. The higher volume of anthocyanin pigments in our media might have increased the likelihood of pigment degradation during autoclaving.

Overall, while purple eggplant peel extract shows potential as a general pH indicator, its effectiveness as an indicator of bacterial growth is compromised by the conditions required for media preparation. Future research should explore alternative methods to preserve anthocyanin stability during sterilization or test lower volumes of extract to determine optimal conditions for maintaining pigment integrity.

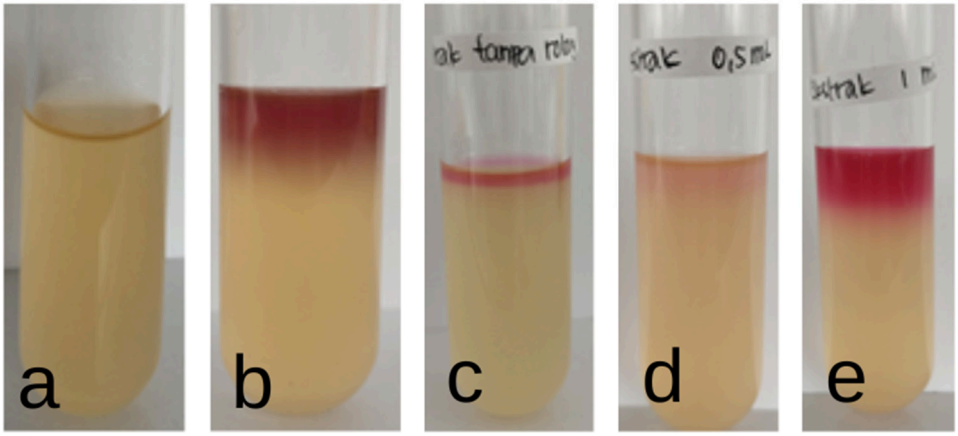

Figure 2. The characteristics of pH and surface color changes of TSB Containing *E. coli* after dropped purple eggplant peel extract. Notes: (a) TSB medium containing *E. coli* incubated for 18 hours before the extract was added; (b) B2.1: Control with 0,04% BCP (2-3 drops); (c) T3.1: Extract added (2-3 drops); (d) T3.2: Extract added (0,5 mL); (e) T3.3: Extract added (1 mL).

Figure 2 illustrates the characteristics of pH and Surface Color Changes of TSB Containing *E. coli* after Dropped Purple Eggplant Peel Extract. All samples, including the control, exhibited a uniform pH reduction from 7 to 5. The control sample (Tube B) treated with 0.04% bromocresol purple (BCP) formed a thick, uniform purple ring without noticeable stratification. In comparison, the test samples treated with 2–3 drops (Tube C), 0.5 mL (Tube D), and 1 mL (Tube E) of purple eggplant peel extract demonstrated purple ring formation with distinct visual variations. Notably, the intensity of the purple ring increased with higher extract concentrations, suggesting a dose-dependent response.

These findings highlight the presence of active compounds, such as anthocyanins, in the purple eggplant peel extract, which interact with *E. coli* metabolic products, amplifying the color change. Tube E, treated with 1 mL of the extract, produced a purple ring comparable in intensity to the control (Tube B), underscoring the extract's potential as a pH indicator. These observations align with the findings of Suhartati et al., (2021) who reported a similar phenomenon using purple cabbage extract. Their study demonstrated that clear media developed a pink ring upon the addition of purple cabbage extract,

suggesting that the extract can act as a pH indicator. However, the reaction of purple eggplant peel extract in our study was less conclusive.

Therefore, while purple eggplant peel extract indicated pH changes across various ranges, further research is warranted to elucidate the underlying mechanisms and validate its application in microbiological studies.

CONCLUSION

In conclusion, the study demonstrated that purple eggplant peel extract (*Solanum melongena* L.) effectively indicated pH changes in TSB medium without bacterial inoculation, showing clear color variations across different pH levels. However, when applied to bacterial growth media, the extract did not exhibit significant color changes, either before or after bacterial growth. To enhance the utility of purple eggplant peel extract as a pH indicator in bacterial growth media, future research should focus on improving the stability of its pigments. Further research is required to investigate alternative sterilization techniques for its extract that preserve the stability and bioactivity of anthocyanins. Modifications to the extract that increase resistance to autoclave conditions and long-term storage could help preserve its pH-sensitive properties. Additionally, optimizing the quantity and distribution of the extract in the medium may lead to more accurate and consistent detection of pH changes, thereby expanding its potential applications as a natural pH indicator in microbiological studies.

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