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Exploring pH-Responsive Color Changes of Anthocyanin Extracts from Four Selected Plants as Potential Natural Food Deterioration Indicators

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Abstract*— Anthocyanins are natural pigments known for their pH sensitivity, resulting in distinct chemical structures and color changes across different pH levels. This study investigated the potential of anthocyanin-rich extracts from four plant sources, namely *Antidesma bunius* (L.) Spreng fruit extract (AE), *Syzygium cumini* fruit extract (SE), *Hibiscus x archeri* Wats. flower extract (HE), and *Etlingera hemisphaerica* flower extract (EE) as natural indicators for food deterioration. The extracts were evaluated for total anthocyanin content (TAC), antioxidant activity (as DPPH radical scavenging), antibacterial activity (via disk diffusion), and pH sensitivity based on visible color changes across a pH range of 2–12. Among the tested samples, HE exhibited the highest TAC (88.89 ± 1.40 mg C3G/g extract) and antioxidant activity (25.46 ± 1.62 mg AEAC/g extract). HE (50%) demonstrated promising antibacterial inhibition against *A. hydrophila*, *S. typhimurium*, and *L. monocytogenes*, with inhibition zones of 9.07 ± 0.49 mm, 9.04 ± 1.12 mm, and 10.19 ± 0.80 mm, respectively. Moreover, HE demonstrated clear, visually perceptible color transitions across pH levels, supported by variations in ΔE and ºHue. The ΔE value increased from 3 at pH 3 to 9.55 at pH 8, while ºHue value shifted from 36.63 º to 345.96º. This significant change is linked to the extract's clear colour transition, which is red at pH 3-6, purple at pH 7, and red-purple at pH 8. These results demonstrate the HE as a promising candidate for pH indicator of food deterioration monitoring, as evidenced by its sensitivity to pH fluctuation among the tested extracts.

***Keywords*— anthocyanin extract, antibacterial, antioxidants, pH-indicator, food deterioration**

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I. INTRODUCTION

Fruits, vegetables, herbs, and cereals all naturally contain these bioactive substances. One of the most researched polyphenolic plant chemicals is anthocyanin [1]. Anthocyanin, composed of the glycosylated structure of anthocyanidins, represents an intriguing and extensively researched plant compound [2].

There are many reasons to be interested in employing anthocyanins in packaging materials, but two primary ones have fueled a great deal of anthocyanin research. The primary justification is their ability to preserve food's organoleptic qualities, which is advantageous for industrial food production. The second justification has to do with the various ways that anthocyanins contribute to health. The quantitative and qualitative composition of anthocyanins exhibits significant variation among different fruits from the same genetic background. This variation is attributable to a range of environmental factors, including agricultural practices, genetic characteristics, the type and intensity of light, and temperature [3].

Plants serve as the main origin of six prevalent anthocyanin derivatives, which are also referred to as anthocyanidins: malvidin, pelargonidin, cyanidin, delphinidin, petunidin, and peonidin. The anthocyanidins found in plants are malvidin (7%), delphinidin (12%), pelargonidin (12%), petunidin (7%), peonidin (12%), and cyanidin (50%), as shown in **Figure 1**. The three main non-methylated anthocyanidins found in nature are pelargonidin, delphinidin, and cyanidin glycosides; these glycosides make up 50% of flower pigments, 69% of fruit pigments, and 80% of leaf pigments. One of the most prevalent

anthocyanins in fruits is cyanidin-3-glucoside, but the primary anthocyanins in red grapes are malvidin [3].

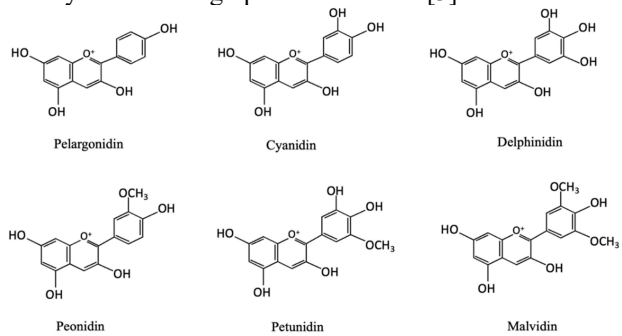

Fig. 1. The chemical structures of different types of anthocyanidins [3]

Glycosyl and aromatic or aliphatic acyl moieties alter anthocyanins, resulting in hundreds of anthocyanin compounds with varying stability and colors. Interestingly, the quantity of hydroxyl groups on the B-ring primarily determines their color, which strongly correlates with their structure. The stability and color expression of anthocyanin pigments are influenced by various factors outside the vacuole, such as pH, the co-production of colorless flavonoids, and complexation with metal ions. Several metal ions, including magnesium, copper, tin, manganese, and iron, have been reported to participate in interactions with anthocyanins by forming stable metal-anthocyanin complexes within the plant vacuoles. Once these ions accumulate to a sufficient concentration, they can significantly influence plant pigmentation. This phenomenon has been well documented in various floral species, where metal-anthocyanin complexation contributes to petal coloration. For instance, the formation of blue pigmentation in tulips has been closely associated with the involvement of Fe^{3+} ions [4]. The stability of anthocyanins is significantly influenced by the substitution pattern on the B-ring, where hydroxyl group substitutions tend to result in lower stability compared to methoxyl groups [5].

Due to their pH sensitivity, anthocyanins undergo molecular structural changes that result in distinct color variations when exposed to different pH buffer solutions [6], [7]. As shown in **Figure 2**, while anthocyanins are colorless in a slightly acidic (pH 3–6) environment, they are redder and more stable *in vitro* at low pH (often at pH < 3). A rise in alkalinity (pH > 6) causes the instability and blue color of anthocyanins [8]. The rise in pH in the vacuoles of aged tissue decreases the stability of anthocyanins and facilitates their breakdown [9].

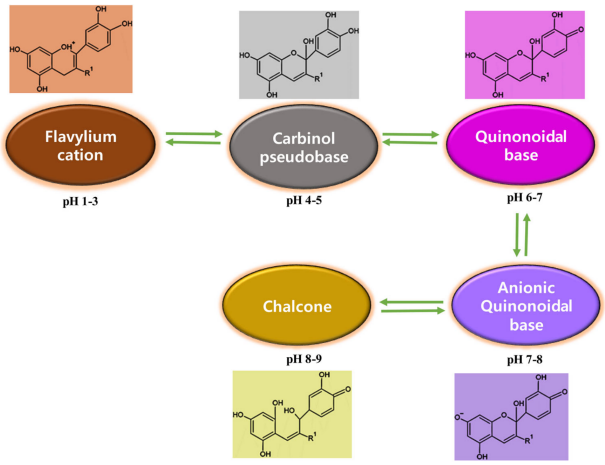

Fig. 2. pH-dependent chemical structures of anthocyanin [2]

The selection of Indonesia's tropical fruits and flowers, such as bignay (*Antidesma bunius*), jambolan fruit (*Syzygium cumini* L.), *Etlingera hemisphaerica*, and *Hibiscus x archeri* Wats. was based not only on their distinctive red-to-purple colouring, but also on the broader context of biodiversity and its rich ethnobotanical traditions. Recognized as a global biodiversity hotspot, Indonesia contains over 30,000 plant species, many of which remain unexplored for their phytochemical and functional properties [10]. The chosen plants are widely distributed across the archipelago, forming an integral part of local ecosystems and contributing to the region's floral diversity. They also play a role in indigenous cultural practices. Moreover, these species have a longstanding history of traditional use that reflects their cultural and medicinal significance in Southeast Asia. *Antidesma bunius* is frequently consumed as fresh fruit, while its juices are used to make syrups and wine, and used as a natural dye. Various parts of the plants have been traditionally utilized to treat a wide range of health concerns, including wounds, hypertension, diabetes, digestive issues, and inflammation [11]. *Syzygium cumini* is a plant of great significance in traditional medicine across Southeast Asia, as an antidiabetic, antimicrobial, antidiarrheal, antifertility, antiulcerogenic, gastroprotective, antiinflammatory, and radio-protective activities [12]. *Etlingera hemisphaerica*, a member of the ginger family, is utilized in Indonesian culinary traditions as a spice and flavouring agent. In addition, it is employed in herbal remedies due to its immunomodulatory and bioactive properties, which include antibacterial, antidiabetic, cytotoxic, and antioxidant effects [13]. Lastly, *Hibiscus x archeri* Wats. Is cultivated both for its ornamental and its use in folk medicine, including antimicrobial and anti-infective [14], [15].

Moreover, plants with such pigmentation are often rich in structurally stable anthocyanins, particularly derivatives of cyanidin and delphinidin, which are of interest for natural colorant and antioxidant applications. *Syzygium cumini* fruit is a local Indonesian fruit that contains anthocyanins [16], as

does *Antidesma bunius fruit* [17]. The content of total monomeric anthocyanin in the peel of ripe *Syzygium cumini* fruit were reported to be 64.20 µg CyE per mL extract [16]. Although detailed anthocyanin profiling of *Etlingera hemisphaerica* and *Hibiscus x archeri* Wats. is limited, their pink and red flower pigmentation strongly indicates the presence of anthocyanin compounds that may possess chemical stability.

The anthocyanin content in these fruits and flowers has sparked interest among researchers in utilizing them as indicators of food deterioration, particularly for fish deterioration. Anthocyanins derived from various natural sources, including purple sweet potato, rose, roselle, and grape, have gained increasing attention for their application as pH-sensitive indicators in intelligent packaging systems [18], [19], [20], [21]. The pH indicator film, fabricated by incorporating butterfly pea flower extract as a source of anthocyanin, demonstrated its capacity to monitor fish deterioration over seven days at a storage temperature of 4 °C. This deterioration was evidenced by a colour change from a purple-blue hue to a slight dullness, bluish grey, and olive/dark green. Concurrently, an increase in TVB-N and pH values was observed in the fish [22].

The total anthocyanin content, antioxidant and antibacterial activities, as well as pH-dependent colour changes (ΔE and hue) of extracts from locally accessible plants, such as *Antidesma bunius*, *Syzygium cumini*, *Etlingera hemisphaerica*, and *Hibiscus x archeri* Wats., are underexplored in the context of food spoilage indicators. To close this gap, the current study examined the total anthocyanin content, antioxidant and antibacterial activity, and pH sensitivity of these four anthocyanin-rich extracts using quantitative colorimetric analysis (ΔH and ºHue) over a relevant pH range. The findings offer fresh insight into the potential of underutilized tropical Indonesian plants as prospective candidates for developing anthocyanin-based markers for tracking food deterioration. Thus, the objectives of this present study were to assess the total monomeric anthocyanin, antioxidant, and antibacterial activity content, as well as the pH sensitivity of *Hibiscus x archeri* Wats. flower, *Etlingera hemisphaerica* flower, *Syzygium cumini* fruit, and *Antidesma bunius* (L) Spreng fruit extracts. The results are expected to be used as a basis for developing new food deterioration indicators from these natural sources.

II. MATERIAL AND METHODS

A. Material

Dried *Hibiscus x archeri* Wats. flower, *Etlingera hemisphaerica* flower, *Syzygium cumini* fruit, and *Antidesma bunius* (L) Spreng fruit were collected from East Java, Indonesia. The plant species were identified by Mr. Heri Susanto (Yayasan Generasi Biologi Indonesia). The dried samples were ground into a fine powder using a laboratory grinder, then immediately stored in sealed plastic containers containing silica gel. All samples were kept in a freezer until further use.

B. Methods

Extraction

The flowers and fruits powders (10 g) were extracted with 200 mL of 96 % ethanol and sonicated (1:20 w/v) using ultrasound-assisted extraction (UAE). The extraction was performed in an ultrasonic bath (Bransonic Ultrasonic Cleaner 8510E MTH, Branson Ultrasonic, USA) operating at 42 kHz for 30 minutes. The mixtures were then filtered through Whatman No.1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator (Rotavapor® R-300, Buchi, Switzerland) at 40 °C. the concentrated extract was flushed with nitrogen to remove residual solvent, transferred to amber bottles, and stored at -20 °C until further use [7]. This extraction was applied to all flower and fruit samples.

Total anthocyanin content

The pH-differential approach was utilized to assess the total anthocyanin content of *Hibiscus x archeri* Wats extract (HE), *Etlingera hemisphaerica* extract (EE), *Syzygium cumini* extract (SE), and *Antidesma bunius* (L) Spreng extract (AE) using a UV-visible spectrophotometer (Shimadzu UVmini-1240, Japan). Two buffer systems were utilized: 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5). Each reaction mixture consisted of 1.8 mL buffer solution and 0.2 mL of the appropriately diluted sample (optical density in the range of 0.1-1.2 at 510 nm). Absorbance was measured at 510 and 700 nm against a blank [7]. Absorbance was calculated as:

$$A = (A_{510nm} - A_{700nm})pH1.0 - (A_{510nm} - A_{700nm})pH4.5. \quad (1)$$

The concentration of the total monomeric anthocyanin pigment in the extract was expressed as cyanidin-3-glucoside [19].

$$TAC\ (mg/L) = A \times MW \times DF \times 1000/(\varepsilon \times 1) \quad (2)$$

where A = absorbance, MW = molecular weight (449.2); DF = dilution factor, ε = molar absorptivity (26,900). The final concentration of anthocyanins (mg/100 g) was calculated by multiplying the sample weight by the total volume of the extract.

Antioxidant activity

Ascorbic acid was used as a reference for measuring antioxidant activity, using the 1,1-diphenyl-2-picryl-hydrazyl radical scavenging activity (DPPH) method with modifications [23]. A standard calibration curve was created using ascorbic acid solutions with concentrations ranging from 2 to 10 ppm. Briefly, 2 mL of an extract with a concentration of 400 µg/ml was added to 2 mL of a solution containing 100 µg/ml of DPPH. The mixture was then kept at a temperature of 25 °C for 30 minutes. Afterward, the absorbance of the mixture was measured using a UV-vis spectrophotometer at a wavelength of 517 nm. The antioxidant activity was expressed in milligrams of ascorbic acid equivalent antioxidant capacity (AEAC) per gram of extract.

Antibacterial activity

Two Gram-negative bacteria (*Salmonella typhimurium* ATCC 14028 and *Aeromonas hydrophila* InaCC B1424 and one Gram-positive bacterium (*Listeria monocytogenes* ATCC 7644) were used. A disk containing 20 µl of an extract with concentrations of 10%, 30%, and 50% was applied to the media tryptic soy agar (TSA). Dimethyl sulfoxide (DMSO) served as the negative control, whereas chloramphenicol served as the positive control. The plates with *Aeromonas hydrophila* were incubated at 30 ˚C, while *Salmonella Typhimurium* and *Listeria monocytogenes* were incubated at 35 ˚C for 18-24 h. The clear zones were measured using a Vernier caliper. The antibacterial activity was expressed as the average size (mm) of growth inhibition zones around the disk [24].

pH response

The color changes of anthocyanin extracted from plant samples were determined at different pH values. Five mL of each anthocyanin extract was measured from pH 2 to 12. The pH was measured using a digital pH meter (pH 700 Eutech), and the samples were then photographed using a smartphone camera (Samsung Galaxy Note 10 Lite, South Korea). Buffer solutions with different pH values (2.0–12.0) were applied to measure the UV–vis spectra (Shimadzu UVmini-1240, Japan) of extracts. The color changes of the extract under different pH levels were recorded using by smartphone.

After taking pictures from the extract, the color vector values, including L* (brightness), a* (redness-greenness), and b* (yellowness-blueness), were determined using Adobe Photoshop® CS6. Total color difference (ΔE) of extracts and standard white plate (L_s* = 93.49, a_s* = 0.25, and b_s* = 0.09) according to Tabatabaei et al. (2022) [25].

Chroma ($C^* = (a^{*2} + b^{*2})^{0.5}$) and Hue angle [$H^o$ = arctg (b*/a*)] values were calculated. Chroma refers to the degree of saturation or intensity and the clarity of a color. The hue angle (H^o) represents the visible color and is measured in degrees, starting at the +a axis. A hue angle of 0^o corresponds to red, 90^o corresponds to yellow, 180^o corresponds to green, and 270^o corresponds to blue [26].

Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA) to determine significant differences among sample groups. Before conducting ANOVA, the data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. Only when both assumptions were met, ANOVA was performed, followed by Duncan's multiple range test at a significance level of $p < 0.05$. In addition, the Pearson correlation coefficient was used to evaluate the relationship between total anthocyanin content and antioxidant activity. The assumption of normality was also verified before applying the Pearson correlation. All statistical analyses were carried out using SPSS version 29 (IBM Corp., Armonk, NY, USA).

III. Result and Discussion

Total anthocyanin content

TABLE 1
TOTAL ANTHOCYANIN CONTENT OF EXTRACTS

Sample	Total anthocyanin content (mg C3G/g extract)	Total anthocyanin content (mg C3G/g dry weight)
AE	50.58±1.26^b	5.70
SE	54.92±1.45^c	8.01
HE	88.89±1.40^d	18.24
EE	33.06±1.63^a	2.22

The different letter shows that the results are significantly different ($p<0.05$). The values were the average from 3 replications. Abbreviations: *Antidesma bunius* (L) fruit extract (AE); *Syzygium cumini* fruit extract (SE); *Hibiscus x archeri* Wats. flower extract (HE); *Etlingera hemisphaerica* flower extract (EE); cyanidin-3-glucoside (C3G).

The total anthocyanin content of the extracts is presented in **Table 1**. The total anthocyanin content in each of the extracts differed significantly ($p<0.05$), ranging from 33.06±1.63 – 88.89±1.40 mg C3G /g extract. In this case, the HE showed the highest content of anthocyanin (88.89±1.40 mg C3G /g extract), significantly different in comparison to EE (33.06±1.63 mg C3G /g extract) ($p<0.05$). The total anthocyanin content of AE and SE found in this study (50.58±1.26 and 54.92±1.45 mg C3G /g extract) was higher than the results of the study conducted by Chamnansilpa et al. (2020), which were 49.5±0.2 and 44.0±0.4 mg C3G /g extract, respectively [27]. Meanwhile, the total anthocyanin content of HE (88.89±1.40 mg C3G /g extract) was higher than compared to *Hibiscus rosa-sinensis* (265.37- 316.85 mg C 3 QE /100g extract) [24].

The difference in anthocyanin content might be due to differences in the source of the plant and the extraction methods. The extraction method used in this study was ultrasound-assisted extraction (UAE), which produces higher yields compared to Chamnansilpa et al. (2020) [27], who use traditional methods to extract the fruit of *Antidesma bunius* (L.) Spreng and *Syzygium cumini* (L.) Skeels. The results of this study are supported by a study conducted by Gayan et al. (2023) [29], who reported that the utilization of the UAE approach led to greater production of total anthocyanin content compared to the traditional method. The enhanced migration of anthocyanins into the solvent can be attributed to the ultrasonic treatment, which not only disrupts plant cell walls but also generates cavitation and mechanical forces that may alter the anthocyanin structure, thus improving their release and solubility [30].

In vitro antioxidant activities

The DPPH test is a simple, quick, affordable, and widely used method for evaluating antioxidant activity. The DPPH assay entails the transfer of hydrogen atoms, with the underlying chemical process being categorized as an electron transfer reaction. The sluggish rate at which hydrogen moves from an

antioxidant to DPPH is the reason why it is considered a less relevant reaction route.

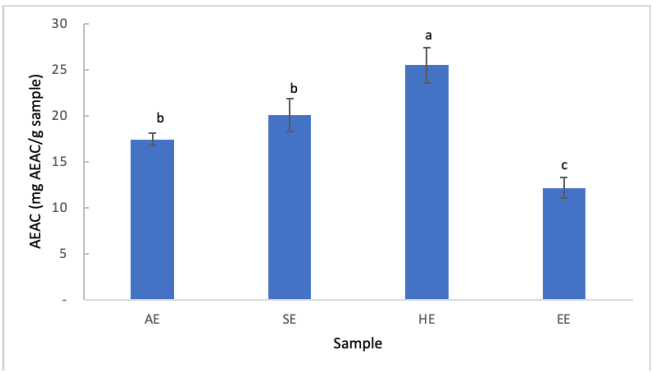

Fig. 3. DPPH radical scavenging activity. The different letter shows the results are significantly different ($p<0.05$). Legend: *Antidesma bunius* (L) extract (AE); *Syzygium cumini* extract (SE); *Hibiscus x archeri* Wats. Extract (HE); *Etlingera hemisphaerica* extract (EE). The value was averaged from 3 replications.

The antioxidant activity of all samples presented in **Figure 3**. The highest antioxidant activity was found in *HE*, followed by SE, AE, and EE, resulting in 25.46±1.62 mg AEAC/g extract, 20.08±1.77 mg AEAC/g extract, 17.39±0.67 mg AEAC/g extract, and 12.14±1.10 mg AEAC/g extract, respectively. The antioxidant activity increased significantly with increasing anthocyanin content. The phenolics play an important role in the absorption or neutralization of free radicals as well as in eliminating free radicals [31], [32], [33].

Compared to other extracts, HE showed the highest scavenging activity. There is a significant difference ($P<0.05$) revealed in AE vs. HE and EE. Due to its low anthocyanin content, EE exhibited the least action. Since the HE contains higher amounts of anthocyanins, it might have contributed to its higher free radical scavenging activity. The anthocyanin compounds contained in this fourth extract could be responsible for its antioxidant effect. Anthocyanins are a type of polyphenol that contains phenolic hydroxyl groups. These hydroxyl groups help to neutralize free radicals by generating phenoxy groups. Phenolic chemicals are inherently water-loving and contain an aromatic ring with one or more hydroxyl substituents, which enhances their antioxidant action.

TABLE 2
PEARSON CORRELATION COEFFICIENT BETWEEN TAC AND AEAC

Variable	TAC	AEAC
TAC	1	0.940**
AEAC	0.940**	1

Values are Spearman correlation coefficients (r). **P < 0.05 (2-tailed). TAC: Total Anthocyanin Content; AEAC: Ascorbic Acid Equivalent Antioxidant Capacity.

Table 2 summarizes the findings of the correlation study between TAC and AEAC. The following interpretation of correlation coefficients is based on Schober et al. (2018), namely, negligible (0.00-0.09), weak (0.10-0.39), moderate (0.40-0.69), and extremely strong (0.90-1.00) [33]. TAC and AEAC showed a statistically significant positive connection in this study ($r = 0.940$, $p < 0.001$) (Table 2) (**Figure 4**). Regression analysis further supported this correlation, showing that samples with higher anthocyanin content tend to have stronger antioxidant abilities. These results indicate that anthocyanins make a significant contribution to the antioxidant capacity of the extracts.

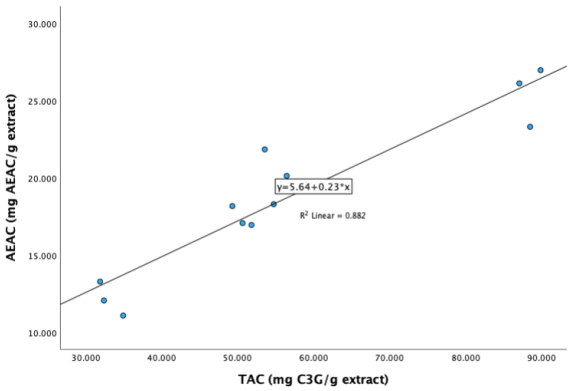

Fig. 4. Linier regression plots of TAC and AEAC

Antibacterial activity

According to the antibacterial activity tests, every extract showed inhibitory effects on a variety of microorganisms under investigation. The results, which are shown in **Table 3**, show how effective anthocyanin extracts are as antimicrobials against a variety of Gram-positive (*L. monocytogenes*) and Gram-negative bacteria (*A. hydrophila* and *S. typhimurium*). According to **Table 3**, the EE had no inhibitory zones against *S. typhimurium*, whereas the HE, AE, and SE showed broad inhibitory zones against *A. hydrophil*a (AH), *S. typhimurium* (ST), and *L. monocytogenes* (LM).

Among all the extracts, the extract concentration of 50% had the highest inhibitory activity against all bacteria. AE exhibited the largest inhibitory zones with a diameter of 20.66±0.92 mm against *L. monocytogenes* and the smallest inhibitory zones with a diameter of 13.37±1.10 mm against *S. typhimurium*. SE exhibited the greatest inhibitory diameter zones of 13.47±0.49 mm and the smallest of 9.88±0.84 mm against *A. hydrophila* and *L. monocytogenes*, respectively, when tested at the same concentration.

Antidesma bunius fruit extract often exhibited considerable antibacterial activity despite having a lower anthocyanin level than some other functional fruits. According to some studies, this is caused by a high overall polyphenolic content, primarily flavonoids, tannins, and other organic acids, which can work in

concert to prevent bacterial development in addition to anthocyanins [34], [35], [36].

TABLE 3
INHIBITION ZONE IN DISK DIFFUSION METHOD

Sample	Concentration	Inhibition zone (mm)[a]		
		AH	ST	LM
AE	10%	8.87±1.56	8.77±0.38	12.48±1.11
	30%	15.11±2.49	10.92±0.88	19.22±1.02
	50%	16.32±1.57	13.37±1.10	20.66±0.92
SE	10%	10.90±1.18	7.23±0.21	8.29±0.55
	30%	12.55±0.91	8.12±0.53	8.41±0.51
	50%	13.47±0.49	10.15±0.79	9.88±0.84
HE	10%	8.34±0.41	7.56±0.24	8.30±0.34
	30%	8.86±0.37	8.07±0.37	9.82±1.60
	50%	9.07±0.49	9.04±1.12	10.18±0.80
EE	10%	8.05±0.39	UD	8.05±0.59
	30%	8.91±0.69	UD	9.03±0.81
	50%	9.19±0.71	UD	9.25±0.59
Chloramphenicol	250ppm	27.18±1.95	23.68±1.87	17.58±2.54
DMSO	100%	UD	UD	UD

[a]Mean values of inhibition zones±SD. Legend: *Antidesma bunius* (L) fruit extract (AE); *Syzygium cumini* fruit extract (SE); *Hibiscus x archeri* Wats. flower Extract (HE); *Etlingera hemisphaerica* flower extract (EE); *Aeromonas hydrophila* (AH); *Salmonella typhimurium* (ST); *Listeria monocytogenes* (LM); Undetected (UD).

The maximum inhibitory diameter zone of HE was 10.18±0.80 mm, and the lowest of 9.04±1.12 mm was observed against *L. Monocytogenes* and *S. Typhimurium*, respectively. The HE in this study had antibacterial activity against *S. typhimurium* and *L. monocytogenes*, while the *Hibiscus rosa-sinensis* L. extract had no inhibition zone for the same bacteria. EE at a concentration of 50% exhibited the largest inhibitory zone with a diameter of 9.25±0.59 mm against *L. Monocytogenes*, while no inhibitory zone was observed against *S. Typhimurium*. Chloramphenicol exhibited antibacterial activity against all three types of bacteria, as seen by the inhibition zone diameter, serving as a positive control. Chloramphenicol is a versatile antibiotic that is effective against both Gram-positive and Gram-negative bacteria [37].

The detrimental impact of anthocyanins on bacterial cell membranes is attributed to the creation of protein-polyphenol interactions via hydrogen bonds or hydrophobic interactions. Interactions with anthocyanins can preserve the stability of membrane proteins. Furthermore, anthocyanins, which exist as flavylium ions, can either donate or take electrons at the membrane interface. This would enable them to serve as an antibacterial agent, causing detrimental effects on pathogenic bacteria [3].

pH response

We analyzed the color change and UV-Vis spectrum of anthocyanin extracts in various solutions with pH values ranging from 2 to 12. **Figure 5** shows that distinct fluctuations in the color of the anthocyanin solutions in different pH solutions were easily discernible by the naked eye. The significant change in anthocyanin color due to the pH change in the buffer solution suggests that HE was suitable as an indicator film. The color variation of anthocyanins is associated with distinct chemical compositions, which vary at different pH levels. The results of instrumental color values concerning L*, a*, b*, and ΔE are represented in **Table 4**.

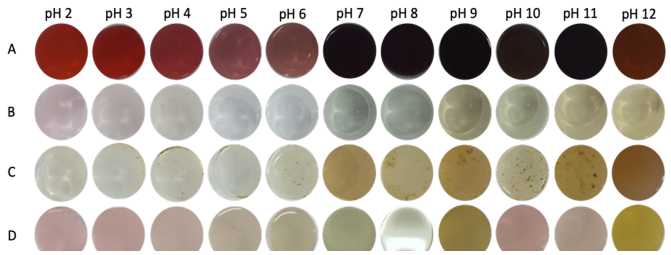

Fig. 5. Color variations of HE (A), SE (B), EE (C), and AE (D) in different buffer solutions (pH 2 to 13). Legend: *Antidesma bunius* (L) extract (AE); *Syzygium cumini* extract (SE); *Hibiscus x archeri* Wats. Extract (HE); *Etlingera hemisphaerica* extract (EE).

TABLE 4
COLOR PARAMETERS (L, a, b, AND ΔE) OF ANTHOCYANIN EXTRACTS IN DIFFERENT PH SOLUTIONS

Sample	pH	L	a	b	ΔE
AE	3	64.8	10	7	4.27
	4	66.8	5.4	9	4.34
	5	66.8	2.2	11.2	4.45
	6	64	-1.8	16	4.67
	7	62.2	-4	17	4.98
	8	59	-2.8	17	5.18
SE	3	70	3.4	1.8	4.98
	4	73	-0.4	4	4.84
	5	72.8	-1	-1.8	5.48
	6	69.6	-2.2	-1.2	5.81
	7	55.8	-4.2	3	6.74
	8	55	-4.8	4.6	6.72
HE	3	24.4	38.2	28.4	3
	4	26.4	33	15.8	4.98
	5	30.8	22	8.4	6.23
	6	30.2	18.4	9	6.54
	7	5	4.4	-2.2	9.63
	8	5.2	4.8	-1.2	9.55
EE	3	74.2	-1.6	5.4	4.69
	4	72.8	-1.8	7.6	4.63
	5	70.8	-2	5.2	5.01
	6	71	-2.4	10.6	4.56
	7	54.4	3.2	28.8	3.69
	8	62.8	-1	22.6	3.95

TABLE 5
COLOR ANALYSIS (ΔC AND ^{O}H) OF ANTHOCYANIN EXTRACTS IN DIFFERENT PH SOLUTIONS

Sample	pH	ΔC	^{0}H	Color
AE	3	12.21	34.99	Red
	4	10.50	59.04	Yellow red
	5	11.41	78.89	Yellow red
	6	16.10	96.42	Yellow
	7	17.46	103.24	Yellow
	8	17.23	99.35	Yellow
SE	3	3.85	27.90	Red
	4	4.02	95.71	Yellow
	5	2.06	240.95	Blue
	6	2.51	208.61	Blue Green
	7	5.16	144.46	Yellow-green
	8	6.65	136.22	Yellow-green
HE	3	47.60	36.63	Red
	4	36.59	25.58	Red
	5	23.55	20.90	Red
	6	20.30	26.06	Red
	7	4.92	333.43	Purple
	8	4.95	345.96	Red-Purple
EE	3	5.63	106.50	Yellow
	4	7.81	103.32	Yellow
	5	5.57	111.04	Yellow
	6	10.87	102.76	Yellow
	7	28.98	83.66	Yellow red
	8	22.62	92.53	Yellow

It is commonly believed that a color difference ΔE higher than 5 is perceptible to the naked eye. Anthocyanins' color variations can serve as a reliable deterioration indicator for food packaging. ΔE values exceeding 12 indicate a substantial absolute hue difference that can be perceived by individuals without specialized training [31]. In this study, the ΔE of AE, SE, and HE was greater than 5 in the alkali situation, indicating that it can be used as an indicator. The ΔE of HE, with the best physical characteristics, changed the most in this region (pH 5–8), indicating that good physical properties can help protect the color characteristics of the indicator and improve the detection performance of the food deterioration indicator film, as shown in **Table 5**. This is evident in the color spectrum, which is visible at the ^{o}Hue value and indicates significant color variations at pH levels of 7-8. These results strongly imply that customers in real-world food packaging applications will be able to clearly identify the colour changes caused by our indicator films. It indicates their appropriateness as real-time packaging indicators [38], [39].

Overall, the color of all examined extracts exhibited greater stability under acidic conditions. This performance adhered to the typical traits of anthocyanins, which exhibit reduced stability when the pH level rises. Anthocyanins were detected in both colorless and colorful forms under low acidic circumstances. The colorless forms included hemiketal, cis-chalcone, and trans-chalcone, while the colored forms included red flavylium cation, purple quinonoidal base, and blue quinonoidal base. As the pH level rises, the balance of the reaction moves towards the deprotonation of the flavylium cation to form the less stable quinonoidal base. Therefore, the anthocyanin exhibits reduced stability under alkaline conditions [40].

IV. CONCLUSION

This research makes a unique contribution to the field of natural pH-sensitive indicators by methodically evaluating four tropical plant extracts from Indonesia. It connects their color changes on pH and bioactivities to the practical detection needs for packaging applications. *Hibiscus x archeri* Wats. extract (HE) achieved the highest total anthocyanin content. The HE also produced the highest total yield. The value of antioxidant activity ranged from 12.14±1.10 - 25.46±1.62 mg AEAC/g extract, and HE showed the highest antioxidant value. As shown by the inhibitory diameter zones, the AE extract killed *A. hydrophila*, *L. monocytogenes*, and *S. typhimurium* the best (16.32±1.57, 13.37±1.10, and 20.67±0.92 mm), while the EE extract had the smallest. The HE has superior colorimetric performance, as demonstrated by color changes that are visible to the naked eye. As a result, *Hibiscus x archeri Wats*. flower extract is a promising source of food deterioration indicators. *Hibiscus x archeri Wats*. flower extract is a potential candidate for utilization in intelligent and active packaging as a deteriorating indicator for fresh fish. The packaging can potentially enhance fish's shelf life by incorporating ZnO nanoparticles having antibacterial properties.

Despite the promising results, this study has several drawbacks. The films' antibacterial activity was assessed using a limited range of bacterial strains, which may not represent all foodborne pathogens in real-world circumstances. Furthermore, this study did not evaluate the films' practical performance in real-world packaging systems. Future research should address these issues by integrating more microorganisms and assessing the films' efficacy under a real-world packaging system.

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CONFLICT OF INTEREST

Authors declare no conflict of interest to disclose.

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