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Development of a simple colorimetric sensor for bacteria detection

Saowapa Thumsing Niyomthai[1,*], *Nattharika* Ratchatathammanit[1], *Sunisa* Phaosung[1], *Narumol* Kreua-ongarjnukool[1], and *Pitt* Supaphol[2,3]

[1]Department of Industrial Chemistry, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, 10800 Bangkok, Thailand

[2]The Petroleum and Petrochemical College, Chulalongkorn University, 10330 Pathumwan, Bangkok, Thailand
[3]The Center of Excellence on Petrochemical and Materials Technology, Chulalongkorn University, 10330 Pathumwan, Bangkok, Thailand

Abstract. Wound care is vital to prevent infections and inflammation that can lead to chronic wounds. This study developed a novel chitosan-based wound dressing incorporated with purple corn (Zea mays L.) extract as a colorimetric sensor for detecting bacterial infections. The dressings were prepared using 1.5% w/v chitosan, 2 mL glycerol, and varying amounts of purple corn extract (5–20 g). The formulation containing 20 g extract showed the highest anthocyanin content (177.10 ± 1.77 mg/L) and desirable mechanical properties, including a tensile strength of 0.26 ± 0.04 MPa, elongation at break of 23.75 ± 1.77%, and Young's modulus of 25.33 ± 1.96 MPa. SEM analysis revealed a smooth surface with uniform extract distribution. The water vapor transmission rate (734 ± 18 g/m²/day) indicated suitable moisture permeability for wound healing. The dressing exhibited clear pH-dependent color changes orange (pH 5.0), purple-brown (pH 7.4), and dark brown (pH 9.0) enabling visual detection of infection-related pH shifts. Additionally, it showed strong antibacterial activity. These results demonstrate that purple corn extract-infused chitosan dressing is a promising smart wound care material with dual functions: pH-responsive sensing and antibacterial protection.

1 Introduction

The skin is one of the most important organs in the human body, playing a critical role in maintaining homeostasis and protecting internal organs [1]. When skin is wounded, it can cause pain, inflammation, and physiological disturbances [2]. A wound refers to damage to the skin or underlying tissues in various forms, such as abrasions, surgical incisions, or punctures [3]. While small wounds may not require intensive care and can often be managed with basic treatment, if such wounds become infected and are not properly treated, they may develop into chronic wounds. Therefore, wound dressing is an important factor in promoting faster healing and preventing infection, which is one of the main causes of delayed wound healing [4].

Wound healing involves various cell types, including keratinocytes and fibroblasts [5]. Normally, the skin's pH ranges from 4.0 to 6.0, but after injury, the pH can rise to approximately 7.4. If the wound becomes infected, the pH may further increase to around 9.0. Thus, changes in pH are closely related to wound status and bacterial infection [6].

Wound dressings help absorb exudates and prevent external contamination. Materials commonly used in commercial wound dressings include polyurethane, polyethylene oxide, and silicone. However, for patients with burn wounds or deep wounds, frequent dressing changes are necessary, which leads to higher costs [7]. To address this, biodegradable wound dressings made from biopolymers have been developed. These materials are environmentally friendly, naturally degradable, and biocompatible, such as cellulose, collagen, chitosan, casein, and starch [8].

Chitosan is a biopolymer derived from chitin through deacetylation, which con-verts N-acetyl-D-glucosamine to glucosamine [9]. Chitosan possesses key functional groups, including amino ($-NH_2$) and hydroxyl (-OH) groups, which allow it to bind with proteins and dissolve in organic acids such as acetic, lactic, and citric acid [9]. These properties make chitosan suitable for wound dressing applications due to its biocompatibility, non-irritant nature, and biodegradability [10]. Additionally, chitosan exhibits antibacterial properties and accelerates the wound healing process [11]. It can also retain moisture at the wound site, promote faster healing, reduce scar formation [12], and effectively prevent bacterial infection [13].

Recently, there has been growing interest in using natural extracts for antibacterial purposes. Purple corn extract, which is rich in anthocyanins a type of natural pigment exhibits both antioxidant and antibacterial properties [14, 15]. Notably, the structure of anthocyanins is pH-dependent, enabling their use as pH indicators to detect infection in wounds [16].

*Corresponding author : saowapa.t@sci.kmutnb.ac.th

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Purple corn (Zea mays L. var. purple corn) is native to South America, particularly Peru, and is known for its deep purple kernels rich in anthocyanins such as pelargonidin, cyanidin, and peonidin. These compounds exhibit strong antioxidant and antimicrobial properties [17]. Anthocyanins from purple corn are also more stable than those from many other sources, making them suitable for use in medical and cosmetic applications [18]. One of their key features is pH sensitivity—anthocyanins appear red in acidic conditions and turn purple or green as the pH becomes neutral or alkaline. This property allows them to serve as natural pH indicators, useful for detecting bacterial infections in wounds, which often cause an increase in wound pH [19].

This study, therefore, proposes the development of a wound dressing incorporating purple corn extract, aimed at both antibacterial activity and functioning as a pH-sensitive sensor for detecting bacterial infections in wounds.

2 Materials and Methods

2.1 Materials

Purple corn extract obtained from T. S. T. twin product. Chitosan, from crab shell was purchased from Sigma-Aldrich Germany. Acetic acid and Ethanol was purchased from Sigma Aldrich USA. All other chemical agents were of analytical grade.

2.2 Preparation of purple corn extract

Purple corn extract 5 g was dissolved in 200 mL of distilled water, then stirred and heated at 60°C for 90 minutes. The mixture was subsequently filtered, and the filtrate was centrifuged at 5,000 rpm for 10 minutes. The same procedure was repeated using 10, 15, and 20 g of purple corn extract, respectively.

2.3 Preparation of wound dressing containing purple corn extract

Chitosan (1.5 g) was dissolved in 2% v/v acetic acid solution and mixed with 2 mL of glycerol. The mixture was stirred at room temperature for 6 hours. Subsequently, 2 mL of 1% v/v glutaraldehyde was added and stirred for an additional 30 minutes. Then, 35 mL of purple corn extract solution was added to the hydrogel and stirred at room temperature for another 30 minutes. The resulting solution was centrifuged at 4000 rpm for 10 minutes. Finally, the mixture was dried at 60°C for 6 hours to obtain chitosan-based hydrogel wound dressing incorporated with purple corn extract.

2.4 Characterization

2.4.1 FTIR analysis

The wound dressing containing purple corn extract was analyzed using FTIR (PERKIN ELMER Spectrum One). The wound dressing containing purple corn extract was placed in the sample holder and scanned in the middle infrared region (400–4000 cm^{-1}), in order to investigate the infrared spectral characteristics of the wound dressing containing purple corn extract.

2.4.2 Morphological observation by SEM

Wound dressing containing purple corn extract were cut into 1 × 1 cm pieces. The cut samples were mounted onto aluminum stubs using carbon tape, ensuring that the surface of each sample was properly oriented for observation. The samples were then coated with a thin layer of gold for 1 minute prior to examination using scanning electron microscopy (SEM) to investigate their surface morphology.

2.4.3 Mechanical properties of the wound dressing containing purple corn extract

Wound dressing containing purple corn extract were cut into 1 × 4 cm pieces and tested following ASTM D882 standards using a Texture Analyzer (TA. XT Plus). The sample gauge length was set to 100.0 mm, and the load cell was set to 5 N. The crosshead speed for tensile testing was 60 mm/min. Each sample was tested three times.

2.4.4 Swelling Ratio

Wound dressing containing purple corn extract, were cut into 2 × 1 cm, were prepared and tested by weighing and recording the initial weight. The samples were then immersed in distilled water for 7 days, with the weight recorded at the following time points: 0.25, 0.50, 0.75, 1, 2, 3, 4, 5, 6, 12, 24, 48, 72, 96, 120, 144, and 168 hours. The swelling ratio was calculated using eq. 1.

$$\text{Swelling Ratio (\%)} = \frac{W_s - W_d}{W_d} \times 100 \qquad (1)$$

where Ws is weight of the hydrogel after swelling at various time intervals (g) and Wd is weight of the dry hydrogel (g)

2.4.5 Water Vapor Transmission Rate (WVTR)

Wound dressing containing purple corn extract were sealed in containers with distilled water and weighed before and after sealing. The samples were then placed in a Desiccator for 1, 2, 3, 4, 5, 6, and 7 days. Each sample was tested in triplicate. The Water Vapor Transmission Rate (WVTR) was calculated using eq. 2.

$$\text{WVTR } (g \cdot m^{-2} \cdot day^{-1}) = \frac{W_0 - W_t}{A \cdot t} \qquad (2)$$

where W_0 is Weight of the container with distilled water sealed with the hydrogel at the initial time (g), W_t is Weight of the container with distilled water sealed with the hydrogel at any given time (g), A is Cross-sectional area of the hydrogel in contact with water (m²) and t is time (day)

2.4.6 Release efficiency of purple corn extract from wound dressing

Wound dressing containing purple corn extract were immersed in phosphate-buffered saline (PBS) and shaken at 37°C for 7 days. The samples were collected and replenished at the following time intervals: 0.25, 0.50, 0.75, 1, 2, 3, 4, 5, 6, 12, 24, 48, 72, 96, 120, 144, and 168 hours. The release of the extract was measured using a UV-Vis Spectrophotometer at a wavelength of 520 nm. The release efficiency was then calculated using eq. 3.

$$\text{Release Percentage} = \frac{M_t}{M_\infty} \text{ x } 100 \qquad (3)$$

Where M_t is Actual amount of the extract in hydrogel released at time (mg) and M_∞ is Total amount of compound initially loaded or total releasable amount (mg)

2.4.7 Antibacterial Efficacy of wound dressing containing purple corn extract using the Disk Diffusion Method

The bacterial suspensions of gram-positive bacterium S. aureus and gram-negative bacterium E. coli were spread evenly on TSA. Wound dressing containing purple corn extract, which were prepared for testing, were cut into circular pieces with a diameter of 1.0 cm. The plates were incubated at 37°C until bacterial growth covered the entire plate. The inhibition zone (clear zone) was then observed, and the diameter of the inhibition zone was measured. The obtained values were used to calculate the average and standard deviation to compare the antibacterial effectiveness following ASTM E1054-08

2.5 Evaluation of pH-responsive visible color change of the wound dressing containing purple corn extract

Wound dressing containing purple corn extract were cut into 3 × 3 cm pieces and immersed in phosphate-buffered saline (PBS) solutions at pH 5.0, 7.4, and 9.0 for approximately 30 minutes. The color change of the wound dressings was visually observed before and after immersion in PBS at each pH value.

3 Result and Discussion

3.1 Characterization

3.1.1 FTIR

The difference between the purple corn extract, hydrogel, and hydrogel containing purple corn extract was observed. The basic structure of the purple corn extract contains anthocyanins, which lack nitrogen groups, and thus no N-H stretching and C-N stretching are observed. In contrast, both hydrogel with and without purple corn ex-tract show N-H stretching and C-N stretching, along with other functional groups pre-sent, which are part of the fundamental structure of chitosan. This allows for the preliminary structural identification of the compounds in the purple corn extract, hydro-gel, and hydrogel containing the purple corn extract.

3.1.2 Morphological observation by SEM

From the morphological characteristics of the wound dressing films containing purple corn extract, the naked-eye observation showed that the films had a smooth, homogeneous, and continuous surface. Additionally, the morphological characteristics of the wound dressing films with purple corn extract revealed that the extract was evenly distributed on the film. This ensures that when applied, the purple corn extract will come into direct contact with the skin and exert antibacterial effects consistently across the entire dressing.

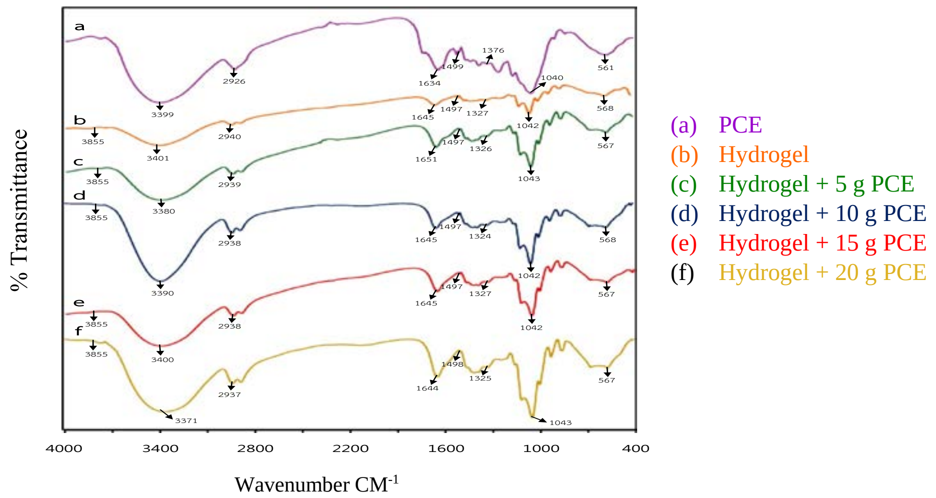

Fig. 1. FTIR spectrum of purple corn extract, hydrogel, and hydrogel containing purple corn extract

Table 1. Functional group of PCE, hydrogel, hydrogel+5 g PCE, hydrogel+10 g PCE, hydrogel+15 g PCE and hydrogel+20 g PCE

Wavenumber	PCE	Hydrogel	Hydrogel + 5 g PCE	Hydrogel + 10 g PCE	Hydrogel + 15 g PCE	Hydrogel + 20 g PCE
3865 (N-H stretching)	✗	✓	✓	✓	✓	✓
3399 (O-H stretching)	✓	✓	✓	✓	✓	✓
2926 (C-H stretching)	✓	✓	✓	✓	✓	✓
1634 (C=O stretching)	✓	✓	✓	✓	✓	✓
1499 (C=C stretching)	✓	✓	✓	✓	✓	✓
1327 (C-N stretching)	✗	✓	✓	✓	✓	✓
1040 (Aromatic ring C-H deformation)	✓	✓	✓	✓	✓	✓
561 (C-O stretching)	✓	✓	✓	✓	✓	✓

where ✓ is functional groups were observed
✗ is no functional groups were observed

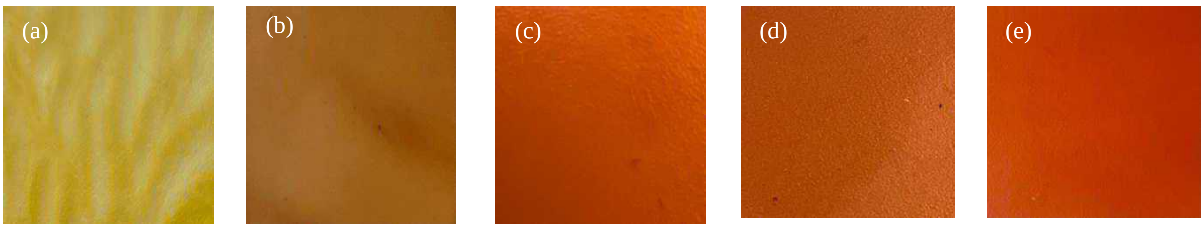

Fig. 2. Visual observation with the naked eye (a) Hydrogel, (b) Hydrogel+5 g PCE, (c) Hydrogel+10 g PCE, (d) Hydrogel+15 g PCE and (e) Hydrogel+20 g PCE

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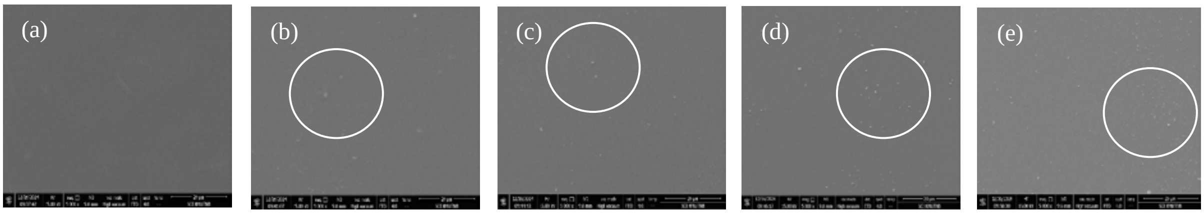


Fig. 3. SEM of surface (a) Hydrogel, (b) Hydrogel+5 g PCE, (c) Hydrogel+10 g PCE, (d) Hydrogel+15 g PCE and (e) Hydrogel+20 g PCE

3.1.3 Mechanical properties

As the concentration of purple corn extract (PCE) increases, the tensile strength, elongation at break, and Young's modulus decrease, indicating a change in the polymer structure and a reduction in mechanical strength with higher concentrations of PCE. The dressing containing 20 grams of purple corn extract exhibited a tensile strength of 0.26 ± 0.04 MPa, elongation at break of 23.75 ± 1.77%, and Young's modulus of 25.33 ± 1.96 MPa, which were lower than those of the commercially available hydro-colloid dressing, which had a tensile strength of 0.74 ± 0.02 MPa, elongation at break of 49.35 ± 3.53%, and Young's modulus of 41.59 ± 0.30 MPa. The results suggest that the dressing with a higher concentration of purple corn extract has reduced flexibility and strength. In contrast, dressings with lower concentrations of purple corn extract, such as 5 and 10 grams, exhibited better mechanical properties and retained good flexibility.

Table 2. Mechanical properties of commercial wound dressing, wound dressing and wound dressing containing purple corn extract (n=3)

Type of hydrogel	Tensile Stress (MPa)	Elongation at break (%)	Young's modulus (MPa)
Hydrogel	0.58±0.01	36.25±1.77	35.85±2.12
Hydrogel+5 g PCE	0.43±0.02	26.25±1.77	27.59±0.14
Hydrogel+10 g PCE	0.36±0.03	23.75±1.77	25.98±0.03
Hydrogel+15 g PCE	0.30±0.01	24.00±1.53	25.84±0.17
Hydrogel+20 g PCE	0.26±0.04	23.75±1.77	25.33±1.96
Commercial wound dressing[1]	0.74±0.02	49.35±3.53	41.59±0.30

[1] Commercial wound dressing (in the market)

3.1.4 Swelling Ratio

As time increases, the weight of both the wound dressing and the wound dressing containing purple corn extract swells most significantly at the 1-hour mark. The weight then slightly decreases at 4 hours, followed by an increase in weight as time progress-es. The initial decrease in swelling is due to the equilibrium of water in the dressing. Initially, the dressing absorbs water due to the driving force, and once excessive water absorption occurs, water is released to balance the chemical potential. Furthermore, it can be observed that as the concentration of purple corn extract increases, the swelling rate also increases. This is because the anthocyanins in the purple corn extract enhance the formation of hydrogen bonds with water, resulting in greater water absorption by the dressing. This affects the polymer structure, creating more pores and improving the swelling capacity of the dressing, leading to an increased swelling rate [20].

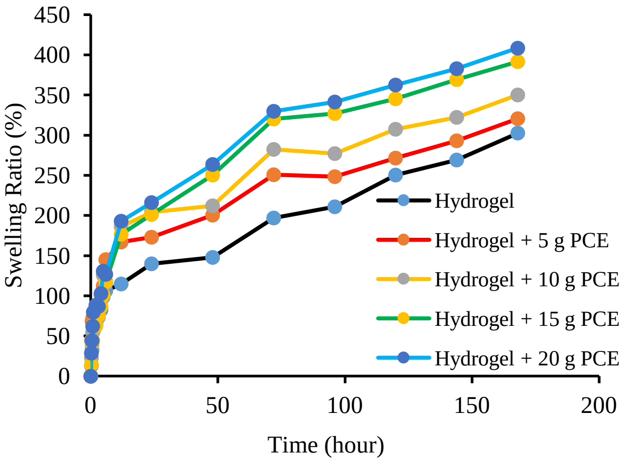


Fig. 4. Swelling Ratio at different times of the wound dressing and wound dressing containing purple corn extract (n=3)

3.1.5 Water Vapor Transmission Rate (WVTR)

The water vapor transmission rate (WVTR) of the wound dressing and the wound dressing containing purple corn extract also showed a decreasing trend over time. The WVTR was initially high but decreased within 7 days. The thickness of the film is one of the factors that affects the variation in water vapor transmission. As the thickness of the film increases, the WVTR decreases because the increased thickness lengthens the distance that water molecules must travel from one side of the film to the other. As a result, some water molecules are unable to pass through the film, thus lowering the WVTR [21]. The dressing containing 20 grams per milliliter of purple corn extract exhibited the lowest WVTR, 734 ± 18 g/m²/day, which is close to the WVTR of Te-gadermTM, a commercially available wound dressing, with a value of 862 g/m²/day, as reported by Nuutila, K. and Eriksson, E. [22]. This value is higher than the WVTR of the dressing without purple corn extract, which was 73 ± 3 g/m²/day.

These findings indicate that, within 7 days, the dressing containing purple corn extract can effectively prevent external water from penetrating the wound during the healing process.

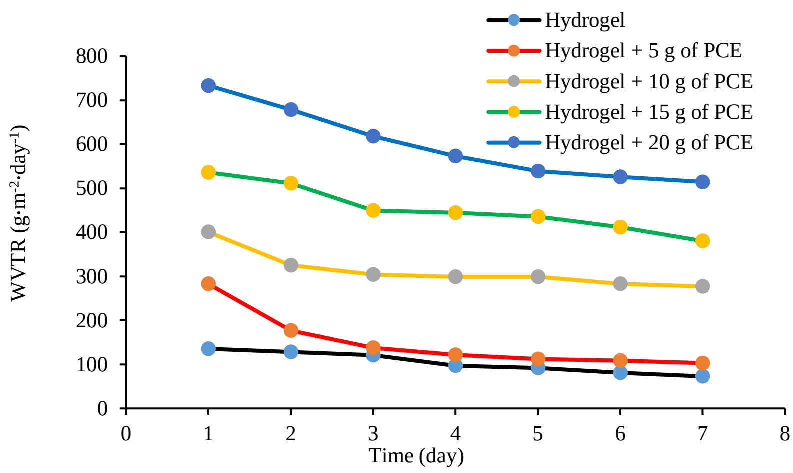


Fig. 5. WVTR of the wound dressing and wound dressing containing purple corn extract (n=3)

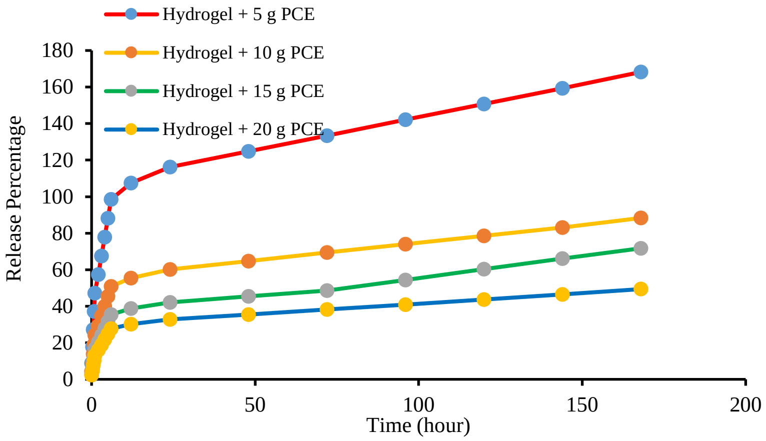


Fig. 6. Release efficiency of wound dressing containing purple corn extract (n=3)

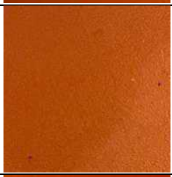
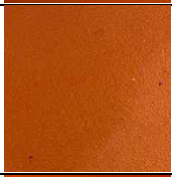
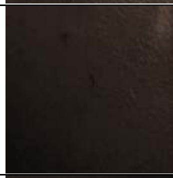
3.1.6 Release efficiency of purple corn extract from wound dressing

The wound dressings containing 5 and 10 grams of purple corn extract released the extract rapidly from 15 minutes to 12 hours. After 12 hours, the release rate slowed and became more stable. When considering the maximum cumulative release percentage of the wound dressings containing 5 and 10 grams of purple corn extract, the values were 168.25 ± 0.29 and 88.33 ± 0.22, respectively. In contrast, the cumulative re-lease percentages of the wound dressings containing 15 and 20 grams of purple corn extract were lower, at 71.73 ± 13.33 and 49.39 ± 0.58, respectively. This is attributed to the larger molecular structure of the purple corn extract in the higher concentrations, which made it more difficult for the extract to diffuse out, resulting in a lower release amount compared to the 5 and 10-gram extract dressings.

3.1.7 pH-responsive visible color change

The wound dressings containing 5 and 10 grams of purple corn extract were not able to inhibit the growth of Gram-negative *E.* coli and Gram-positive *S.* aureus bacteria. However, the wound dressings containing 15 and 20 grams of purple corn extract were able to inhibit both Gram-negative *E.* coli and Gram-positive *S.* aureus. The inhibition zone diameters for *E.* coli were 11.25 ± 0.86 and 11.97 ± 1.13 millimeters, re-spectively. It was observed that the inhibition zone for *E.* coli was smaller than that for *S.* aureus due to the more complex and asymmetric cell wall structure of Gram-negative bacteria compared to Gram-positive bacteria, which allows for greater growth of Gram-negative bacteria. This shows that the purple corn extract in wound dressings weighing 15 and 20 grams can enhance the antibacterial efficacy of the dressing against both *E.* coli and *S.* aureus.

Table 3. Visible color change of wound dressing containing purple corn extract at pH 5.0, 7.4, and 9.0

Type of hydrogel	Before the experiment	pH 5.0	pH 7.4	pH 9.0
Hydrogel + 5 g PCE				
Hydrogel + 10 g PCE				
Hydrogel + 15 g PCE				
Hydrogel + 20 g PCE				

3.1.8 pH-responsive visible color change

The wound dressing containing purple corn extract exhibits color changes. When exposed to a PBS solution at pH 5.0 or normal skin, the dressing appears orange. Upon injury, where the PBS solution has a pH of 7.4, the dressing changes to a purple-brown color. If bacterial infection occurs, with a PBS solution at pH 9.0, the dressing changes to a dark brown color.

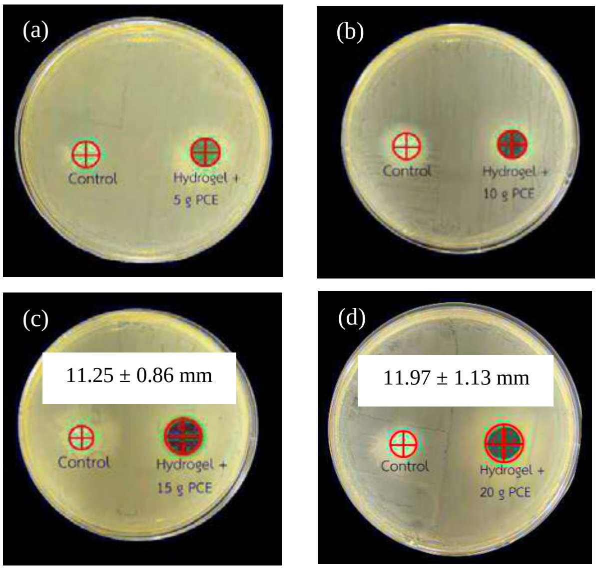


Fig. 7. Clear inhibition zone for Gram-negative *E. coli* of the wound dressing containing purple corn extract (a) 5 g PCE, (b) 10 g PCE, (c) 15 g PCE and (d) 20 g PCE

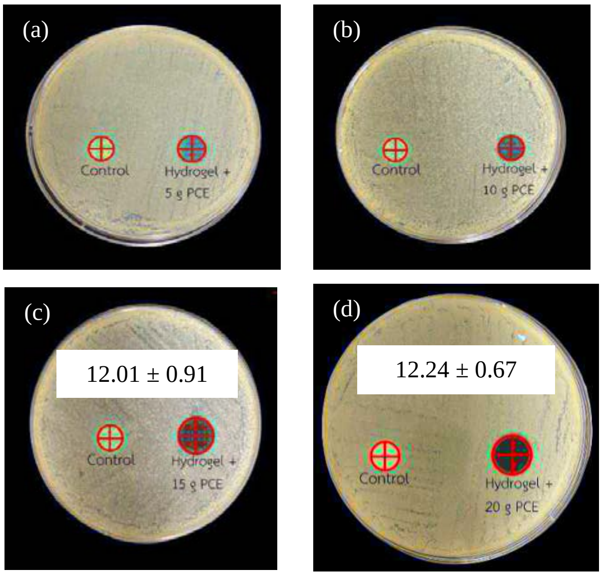


Fig. 8. Clear inhibition zone for Gram-positive *S. aureus* of the wound dressing contain-ing purple corn extract (a) 5 g PCE, (b) 10 g PCE, (c) 15 g PCE and (d) 20 g PCE

4 Conclusion

The wound dressing incorporated with purple corn extract demonstrated a uniform distribution throughout the film, ensuring direct skin contact and consistent antibacterial activity across the entire surface. The dressing exhibited a low water vapor transmission rate (WVTR) of 734 ± 18 $g \cdot m^{-2} \cdot day^{-1}$, indicating its ability to effectively prevent external moisture penetration. Over time, the weight of both the control and the extract-loaded dressings gradually increased and reached equilibrium after 72 hours. Notably, the wound dressing containing 20 g of purple corn extract was soft and flexible, showing superior handling properties compared to commercial products. In addition, the dressing exhibited visible color changes in response to different pH values appearing orange at pH 5.0 (normal skin condition), purple-brown at pH 7.4 (injured condition), and dark brown at pH 9.0 (bacterial infection) indicating its potential as a colorimetric pH sensor for wound monitoring. Furthermore, the extract-loaded dressing effectively inhibited the growth of both Gram-negative *E. coli* and Gram-positive *S. aureus*, confirming its antibacterial efficacy. Overall, these findings suggest that the purple corn extract-based wound dressing is a promising candidate for smart wound care applications combining pH-sensing and antimicrobial functions.

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