RESEARCH PAPER

Development of an Anthocyanin-Loaded Nanocoating for Anti-Adhesion and ROS-Scavenging Wound Interface

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Received: 30th May, 2026; **Revised:** 11th June, 2026; **Accepted:** 15th June, 2026; **Available Online:** 18th June, 2026

ABSTRACT

Background

Wound healing is an important biological process that helps in restoring damaged skin and tissues. However, factors such as infection and excess production of reactive oxygen species (ROS) can delay the healing process. To overcome these problems, the use of natural antioxidants and biocompatible materials has gained more attention in recent research.

Objective

In this study, an anthocyanin-loaded chitosan-based nanocoating was developed for wound healing applications.

Materials and Methods

Anthocyanin, a natural pigment with strong antioxidant properties, was extracted from flower petals of Hibiscus sabdariffa plant using an acidified solvent method. The presence of anthocyanin was confirmed using UV–Visible spectroscopic analysis. Chitosan, a natural biopolymer known for its antimicrobial and biocompatible properties, was used as the coating material. The extracted anthocyanin was incorporated into the chitosan matrix to prepare the nanocoating. The antioxidant activity of the prepared sample was evaluated using the DPPH radical scavenging assay.

Results

The results showed that the anthocyanin-loaded coating exhibited good antioxidant activity. In addition, the coating also demonstrated anti-adhesion behaviour by reducing the attachment on the coated surface. The combined effect of anthocyanin and chitosan provides multiple benefits such as antioxidant protection, antimicrobial activity, and improved surface properties. These features help in reducing oxidative stress, preventing infection, and supporting tissue regeneration.

Conclusion

In conclusion, the developed anthocyanin-loaded chitosan nanocoating shows potential as an effective material for wound healing applications. This study highlights the importance of natural compounds and biopolymers in developing advanced biomedical materials.

Keywords: Anthocyanins; Anti-Adhesion; Antioxidant Activity; Biopolymer coating; Reactive Oxygen Species (ROS).

How to cite this article: Karthiga D, Pratap Kumar G, Palaniselvam K, Anantha Krishnan R, Deepika R, Deepika K, Anis Kumar M. Development of an Anthocyanin-Loaded Nanocoating for Anti-Adhesion and ROS-Scavenging Wound Interface. Int J Drug Deliv Technol. 2026;16(60s):1903-1910. DOI: 10.25258/ijddt.16.60s.170

Source of support: Nil.

Conflict of interest: None

1. Introduction

Wound healing is an important biological process that helps the body repair damaged tissues and restore normal function after an injury. When the skin is affected due to cuts, burns, or other types of damage, the body naturally starts a healing process to close the wound and protect it from external factors [1]. This process involves a series of well-

coordinated events, including inflammation, tissue formation, and tissue remodelling. Proper wound healing is essential to prevent infection and ensure complete recovery. In recent years, there has been increasing interest in developing advanced materials that can improve the wound healing process [2]. Traditional wound dressings mainly provide physical protection, but they may not actively support healing at the cellular level. Modern research focuses on designing bioactive materials that can enhance healing by controlling infection, reducing inflammation, and promoting tissue regeneration. One of the major challenges in wound healing is the presence of reactive oxygen species (ROS). While a small amount of ROS is necessary for defence against microorganisms, excessive ROS can cause oxidative stress and damage cells [3]. This can delay the healing process and lead to chronic wounds, especially in patients with conditions such as diabetes [4]. Therefore, controlling oxidative stress is an important factor in improving wound healing outcomes. Natural antioxidants have gained attention due to their ability to neutralize excess ROS and protect cells from damage [5]. Among these, anthocyanins are widely studied plant-based compounds known for their strong antioxidant activity. They are commonly found in fruits and vegetables and have additional benefits such as anti-inflammatory and antimicrobial properties [6]. These characteristics make anthocyanins suitable for biomedical applications, particularly in wound care. At the same time, chitosan is a natural polymer that has been widely used in wound healing applications due to its biocompatibility, biodegradability, and antimicrobial activity. It can form films and coatings that provide a protective barrier over the wound surface [7]. Chitosan also supports cell growth and helps in tissue regeneration. Recent research focuses on combining natural antioxidants like anthocyanins with biopolymers such as chitosan to develop advanced wound healing materials. This combination can provide multiple benefits, including antioxidant protection, antimicrobial activity, and improved tissue repair [8]. In addition, the use of nanotechnology, such as nano coatings, further enhances the effectiveness of these materials by improving stability and controlled release of active compounds [9]. Overall, the development of anthocyanin-loaded chitosan-based nano coatings represents a promising approach for improving wound healing. These materials aim to reduce oxidative stress, prevent infection, and support faster tissue regeneration. This study focuses on developing and evaluating such bioactive nano coatings for effective wound healing applications.

2. Materials

Hibiscus sabdariffa flowers (Petals), Chitosan powder, Ethanol, Acetic acid, Double distilled water, DPPH (2,2-Diphenyl-1-picrylhydrazyl) reagent, PVA (Polyvinyl Alcohol), *E. coli – 443* and *S. aureus – 902* was purchased from MTCC, Chandigarh, India. Nutrient Agar medium, Nutrient broth, Gentamicin solution was purchased from Himedia, India. Test samples, petri-plates, test tubes, beakers conical flasks were from Borosil, India.

3. Methodology:

3.1 Collection of samples

Fresh *Hibiscus sabdariffa* flower (Petals) were collected from a local source region Karur during the early morning hours to ensure maximum pigment content [10]. The samples were then thoroughly washed with distilled water to eliminate dust and other impurities. After washing, excess water was removed by gently blotting with clean filter paper. The cleaned flowers were spread evenly and subjected to shade drying at room temperature for 8 days to prevent degradation of heat-sensitive anthocyanin pigments. The drying process was continued until the flowers became completely moisture-free. The dried plant material was then finely powdered using a clean mechanical grinder to increase the surface area for efficient extraction. The powdered sample was stored in an airtight container at room temperature until further use.

3.2 Cold extraction of anthocyanin

Anthocyanin pigments were extracted using an acidified solvent extraction method. The plant material was crushed and mixed with ethanol containing a small amount of acid. The mixture was kept for some time to allow proper extraction of pigments. After extraction, the solution was filtered to obtain a clear anthocyanin extract.

The sample (HS) 10 gm and 100 ml of ethanol was taken in a beaker and incubated at 4 ^{o}C for overnight. The sample immersed in the ethanol was separated and filtered by Muslin cloth, Filter paper, and Whatman No.1 paper and dried by evaporator [11].

3.3. UV–visible spectroscopic analysis

3.3.1 Qualitative Phytochemical Analysis

The phytochemical constituents present in the anthocyanin extract from petals were analysed using standard qualitative tests. The extract was subjected to various chemical tests to identify the presence of different bioactive compounds.

1. Test for Carboxylic acid

To 1 mL of extract, 2 mL of sodium bicarbonate solution was added. The formation of effervescence indicates the presence of carboxylic acid [12].

2. Test for Tannins

To 2 mL of extract, 2–3 mL of 10% HCl was added and boiled for 5–6 minutes. The formation of red colour indicates the presence of tannins[13]

3. Test for Steroids

To 0.5 mL of extract, 5 mL of chloroform was added followed by an equal volume of concentrated H_2SO_4. The formation of red colour in the upper layer and yellow-green colour in the lower layer indicates the presence of steroids

4. Test for Flavonoids

To 0.5 mL of extract, 4 mL of 1% ammonia solution was added followed by 1 mL of concentrated H_2SO_4 [14]. The formation of yellow colour indicates the presence of flavonoids.

5. Test for Glycosides (Born- Trageru's Test)

To 2 mL of hydrolysate, 3 mL of chloroform was added and shaken well. After separation of layers, 1 mL of 10% ammonia solution was added. The formation of pink colour indicates the presence of glycosides

6. Test for Proteins (Bradford Method)

To 500 µL of extract, 5 mL of Bradford reagent was added and incubated in the dark for 10–15 minutes

7. Test for Phenol (Ferric Chloride Test)

To 500 µL of extract, 5 mL of distilled water was added followed by a few drops of 5% ferric chloride solution. The formation of dark green colour indicates the presence of phenols.

8. Test for Saponins

To 100 µL of extract, 10 mL of distilled water was added and shaken vigorously for 15 minutes. The formation of a stable foam layer indicates the presence of saponins.

9.Test for Alkaloids - Mayer's test

To a few millilitres of extract, 2–3 drops of Mayer's reagent were added along the sides of the test tube. The formation of a white creamy precipitate indicates the presence of alkaloids.

10. Test for Saponification

To 1–2 mL of 10 N sodium hydroxide solution, 2 mL of extract was added and boiled for 2 minutes. The formation of soap-like material indicates a positive result.

11. Test for Gum and mucilage

To 100 µL of extract, 2 mL of distilled water was added, followed by 2 mL of absolute alcohol with constant stirring. The formation of a white cloudy precipitate indicates the presence of gums and mucilage.

12. Test for flavanoglycoside

To 0.5 mL of extract, 5 mL of ethanol was added followed by a few drops of magnesium sulphate and concentrated HCl. The formation of pink colour indicates the presence of flavanoglycosides.

13. Test for Carbohydrates

To 0.5 mL of extract, 0.5 mL of Benedict's reagent was added and boiled for 2 minutes. The formation of coloured precipitate indicates the presence of carbohydrates.

14. Test for resins

To 0.5 mL of extract, 3 mL of copper sulphate solution was added and shaken for 1–2 minutes. The formation of green precipitate indicates the presence of resins.

15. Test for Biuret [15]

To 2 mL of extract, 1 drop of 2% $CuSO_4$ solution was added, followed by 1 mL of 95% ethanol and 2–3 sodium hydroxide pellets. The formation of pink colour indicates a positive result.

3.3.2 Anthocyanin analysis

The extracted anthocyanin solution was subjected to UV–Visible spectroscopic analysis to study its absorption characteristics. The analysis was carried out using a UV–Visible spectrophotometer. The sample was placed in a cuvette, and the absorbance was recorded over a wavelength range of 400–700 nm. Distilled water was used as a blank for baseline correction. The spectrum obtained was used for further interpretation in the results and discussion section.

3.4 Preparation of chitosan bio-nanofiller

Chitosan solution was prepared by dissolving 1 g of chitosan powder in 100 mL of 1% (v/v) acetic acid solution [16]. The mixture was stirred using a magnetic stirrer at 800 rpm for 4 hours at room temperature to ensure complete dissolution. The solution was then filtered to remove any undissolved particles.

3.5 Preparation of anthocyanin-loaded chitosan nanocoating

3.5.1 Film preparation

About 0.5 gm of Polyvinyl alcohol (PVA) was taken in a beaker and 10 ml of distilled water was added to it. The solution was heated and stirred simultaneously. About 0.5 gm of Chitosan was taken in another beaker and was dissolved in 1% Acetic acid solution, which was then filtered [17]. The PVA and Chitosan solution was taken in equal ratios, and mixed completely, to which 1 gm of the HS extract was added. The solution was stirred gently and poured in a petri plate and dried to obtain the film.

3.6 Antioxidant activity (DPPH assay)

The antioxidant activity was preliminarily assessed using a qualitative DPPH assay. 0.1 mM DPPH solution was prepared using methanol and transferred into a test tube.

To this, a small volume of the anthocyanin extract (1 mL) was added and mixed gently. The reaction mixture was allowed to stand at room temperature for 20–30 minutes in the dark.

3.7 Antibacterial activity

Petri plates containing 20 mL of nutrient agar medium were prepared and inoculated with 24-hour-old bacterial cultures (*E. coli* and *S. aureus*), adjusted to 0.5 McFarland standard.

Sterile discs were placed on the agar surface, and test samples HS (with extract) and PVA (without extract) were applied onto the discs. Gentamicin was used as a positive control [18].

The plates were incubated at 37°C for 24 hours, after which the diameter of the zone of inhibition was measured in millimetres.

3.8 Antifungal activity

Petri plates containing 20 mL of potato dextrose agar (PDA) medium were prepared and inoculated with 72-hour-old fungal cultures (*Candida albicans* and *Aspergillus niger*).

Sterile discs were placed on the agar surface, and test samples HS (with extract) and PVA (without extract)

were applied onto the discs. Amphotericin B was used as a positive control.
The plates were incubated at 28°C for 72 hours, after which the diameter of the zone of inhibition was measured in millimetres [19].

3.9 Anti-adhesion study

S. No.	Name of the Sample	Phytochemical compound	Result
1.	Hibiscus sabdariffa (petals)	Resins	-
2.		Carboxylic acid	+
3.		Tannins	-
4.		Steroids	-
5.		Flavonoid	-
6.		Carbohydrates	+
7.		Glycosides	+
8.		Saponification	+
9.		Protein	+
10.		Phenol	-
11.		Biuret	-
12.		Saponin	-
13.		Gum	-
14.		Flavanoglycosides	+
15.		Alkaloids	-

The anti-adhesion property of the developed coating was assessed indirectly based on its antimicrobial activity. The coating samples were subjected to antibacterial and antifungal assays using the agar diffusion method.
The inhibition of microbial growth around the samples was considered as an indicator for potential anti-adhesion behaviour.

4. Results and discussion

4.1 Collection process

The Hibiscus sabdariffa flowers (petals) were collected, washed, dried and powdered using mixer grinder. (Fig:1)

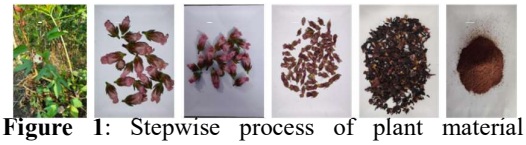


Figure 1: Stepwise process of plant material preparation including a.) collection, b.) shade drying, and powdering of *Hibiscus sabdariffa* flowers for anthocyanin extraction.

4.2 Anthocyanin extraction

Anthocyanin was successfully extracted from *Hibiscus sabdariffa* using an acidified solvent extraction method [20]. The obtained extract showed a characteristic reddish coloration, indicating the presence of anthocyanin pigments. This confirms the successful extraction of bioactive compounds required for further formulation. (Fig:2).

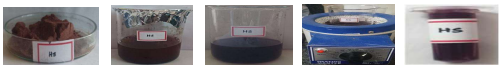


Figure 2. Stepwise cold extraction of anthocyanin from *Hibiscus sabdariffa* (HS) sample including solvent mixing, incubation, filtration, heating, and final extract formation.

4.3 Phytochemical analysis

Figure 3: Phytochemical analysis of *Hibiscus sabdariffa* extract showing different test reactions for the detection of various phytochemicals.
A- Carboxylic Acid, B- Tannins, C- Steroids, D- Flavonoids, E- Glycosides, F- Proteins,
G- Phenols, H- Saponins, I- Gums and Mucilage, J- Flavanoglycosides, K- Carbohydrates,
L- Resins, M- Biuret, N-Saponification, O- Alkaloids

Table 1. Qualitative phytochemical screening of *Hibiscus sabdariffa* (HS) extract showing the presence (+) and absence (–) of various bioactive compounds.

4.3.1 UV–Visible Analysis

The extracted sample was analysed using UV–Visible spectroscopy to confirm the presence of anthocyanin. The spectrum exhibited a primary absorption peak at around 520 nm, which is characteristic of anthocyanin pigments and corresponds to the red to purple coloration of the compound [21]. In addition, a secondary absorption peak was observed at approximately 340 nm, which indicates the presence of aromatic ring structures in the anthocyanin molecule. These spectral features confirm the successful extraction and presence of anthocyanin in the sample (Fig 4).

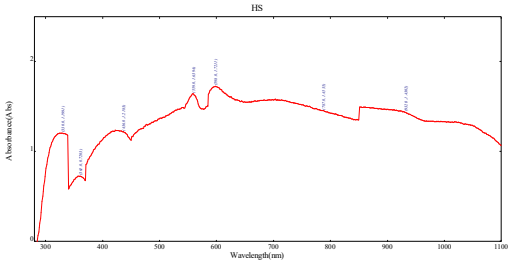


Figure 4. UV–Visible analysis confirming the presence of anthocyanin in the extract.

4.4 Antioxidant activity (DPPH ASSAY)

The antioxidant activity of the anthocyanin extract was evaluated using the DPPH assay. The DPPH solution exhibited a deep purple colour, indicating the presence of stable free radicals. Upon the addition of the anthocyanin extract, a colour change from purple to pale yellow was observed in the test tube. This colour transition indicates the reduction of DPPH radicals by the anthocyanin extract, demonstrating its free radical scavenging ability.

The change in colour confirms the hydrogen-donating capacity of anthocyanin compounds, which neutralize free radicals and convert them into a stable form. The observed results suggest that the anthocyanin extract possesses significant antioxidant potential, which may contribute to reducing oxidative stress in wound healing applications (Fig:5).

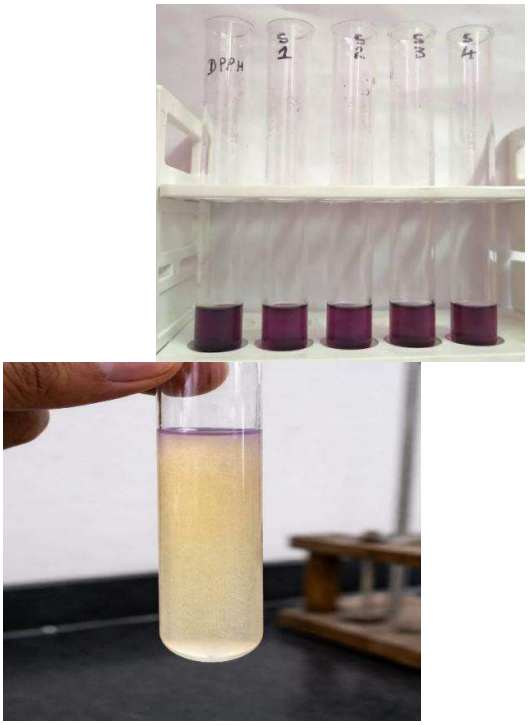


Figure 5: DPPH assay showing colour change indicating antioxidant activity.

4.5 Film formation

The chitosan–PVA film was successfully prepared by solvent casting method [22]. The film appeared uniform and flexible. The anthocyanin-loaded film showed a slight colour change compared to the control film, confirming the successful incorporation of the extract into the polymer matrix (Fig:6).

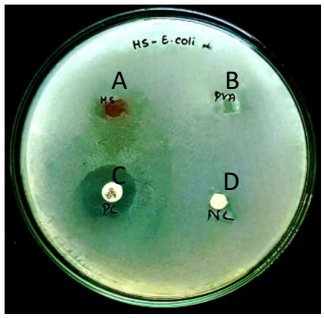
Without extract

With HS extract

Figure 6: Film formation of chitosan–PVA nanocoating: comparison between (a) film without anthocyanin extract and (b) film incorporated with *Hibiscus sabdariffa* (HS) extract.

4.6 Antibacterial activity

The antibacterial activity of the prepared nanocoating was evaluated using the agar disc diffusion method. The results showed that the HS sample exhibited a zone of inhibition of 14.8 mm against *E. coli* and 16.25 mm against *S. aureus*, indicating effective antibacterial activity. In contrast, the control sample (PVA) showed no zone of inhibition. In contrast, the control sample (PVA) showed no inhibition. The observed antibacterial effect may be attributed to the combined action of anthocyanin and chitosan, which possess natural antimicrobial properties (Fig:7,8).



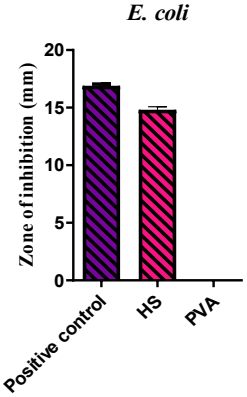


Figure 7: Effect of sample HS against *E. coli.*

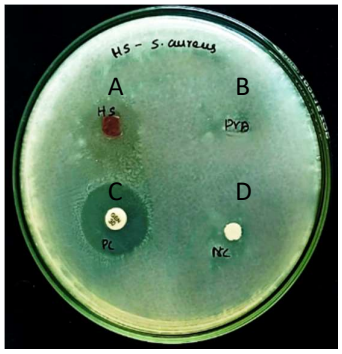


Figure 8: Effect of sample HS against *S. aureus.*
A- Anthocyanin extract, B- Polyvinyl Alcohol, C- Positive control (Standard antibacterial agent) D- Negative control (No treatment / blank disc)

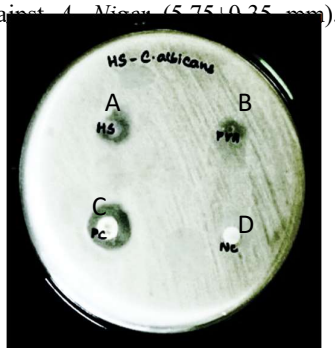
Table 2: Mean± SD of zone of inhibition obtained by sample HS against *E. coli, S. aureus*

S. No	Name of the test organism	Name of the test sample	Zone of inhibition (mm) Mean± SD		
			PC	HS	PVA
1.	*E. coli*	HS	16.9±0.14	14.8±0.28	0
2.	*S. aureus*		19.05±0.07	16.25±0.35	0

SD – Standard Deviation, *Significance - $p < 0.05$

4.7 Antifungal activity

The antifungal activity of the prepared sample HS was evaluated against Candida albicans and *Aspergillus Niger*. The results revealed that the HS sample exhibited moderate antifungal activity against *C. albicans* with a zone of inhibition of 10.85±0.21 mm, whereas a lower activity was observed against *A. Niger* (5.75±0.35 mm). The positive control showed higher inhibition, while the PVA sample (without extract) exhibited minimal to no activity. These findings indicate that the presence of the extract in HS contributes significantly to antifungal efficacy, showing statistically significant results ($p < 0.05$) (Fig:9,10).



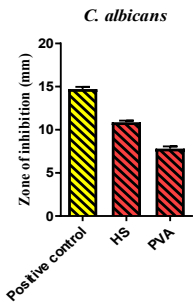
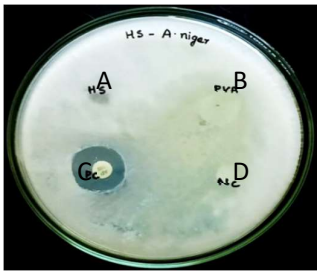


Figure 9: Effect of sample HS against *C. albicans*



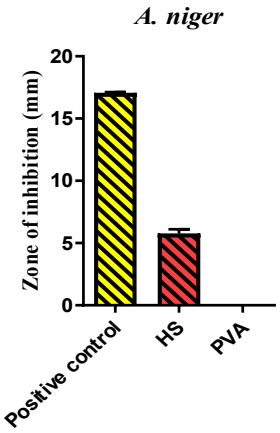


Figure 10: Effect of sample HS against *A. Niger*
A- Anthocyanin extract, B- Polyvinyl Alcohol, C- Positive control (Standard antifungal agent)
D- Negative control (No treatment / blank disc)

Table 3. Mean ± SD of zone of inhibition obtained by sample HS against *C. albicans* and *A. Niger*

S. NO	Name of the test organism	Name of the test sample	Zone of inhibition (mm) Mean ± SD		
			PC	HS	PVA
1.	*C. albicans*	HS	14.7±0.28	10.85±0.21	7.8±0.28
2	*A. Niger*		17.05±0.07	5.75±0.35	0

SD – Standard Deviation, *Significance - $p < 0.05$

4.8 Anti-adhesion study

The anti-adhesion property of the developed coating was evaluated based on its antimicrobial activity. The coating showed effective antibacterial and antifungal activity, indicating a reduction in microbial growth compared to the control sample [22]. This suggests that the coating may possess anti-adhesion characteristics by limiting microbial attachment on the surface**.**

4.9 Discussion

The results of this study confirm that anthocyanin was successfully extracted from *Hibiscus sabdariffa* and used to prepare a chitosan–PVA nanocoating. The reddish colour of the extract indicates the presence of anthocyanin [23]. Phytochemical analysis showed the presence of compounds such as glycosides, proteins, carbohydrates, flavanoglycosides, and carboxylic acids, which may contribute to its biological activity. UV–Visible analysis showed a major absorption peak around 520 nm, which confirms the presence of anthocyanin pigments [24]. In addition, another peak was observed around 340 nm, indicating the presence of aromatic ring structures and other phenolic compounds. These results confirm the successful extraction and stability of anthocyanin [25].

The DPPH assay showed a colour change from purple to pale yellow, indicating good antioxidant activity. This suggests that the extract can scavenge free radicals and help in reducing oxidative stress [26]. The prepared film was uniform and flexible, and the colour change confirmed the incorporation of the extract into the polymer matrix. The antibacterial results showed good activity against *Escherichia coli* and *Staphylococcus aureus*, while antifungal activity was moderate against *Candida albicans* and lower against *Aspergillus niger*. The anti-adhesion study suggests that the coating can reduce microbial attachment. Overall, the developed nanocoating shows antioxidant, antimicrobial, and anti-adhesion properties, indicating its potential use in wound healing applications.

5. CONCLUSION

In this study, anthocyanin was successfully extracted from *Hibiscus sabdariffa* and incorporated into a chitosan–PVA nanocoating. The presence of anthocyanin was confirmed by UV–Visible analysis, and the extract showed good antioxidant activity in the DPPH assay.

The developed coating exhibited effective antibacterial activity and moderate antifungal activity. It also showed anti-adhesion properties, which help in reducing microbial attachment.

Overall, the prepared nanocoating demonstrates good potential for wound healing applications by providing antioxidant, antimicrobial, and protective effects. Further studies can be carried out to improve its performance for advanced biomedical use.

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