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Development of smart bandages from gelidium extract for efficient wound healing

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The prolonged and acute wound healing processes continue to remain a clinical issue due to the risk associated with the presence of infection, lack of efficient wound evaluation tools, and requirement of efficient and sustainable patient therapeutic interventions. Traditional wound coverings offer passive protection and can lack real-time monitoring of infection, pH changes, and healing status, which can compromise the treatment outcomes. The necessity to make medical device designs more environmentally friendly, mitigate antimicrobial resistance, and monitor healing have resulted in a rise in the demand of intelligent wound dressings for several environmentally friendly ones. This study therefore seeks to address these gaps by coming up with a smart biochromic therapeutic dressing that is formulated using natural beetroot stalk and *Gelidium acerosa* extract that have pH-sensitive colorimetric sensing and bioactive release to enhance wound management at large scale.

Keywords: Anthocyanins, Carboxymethyl cellulose (CMC), pH-responsive materials, Biodegradable dressing, Wound monitoring

Introduction

Wound healing is a complex biological process that can be divided into four overlapping stages: hemostasis, inflammation, proliferation, and tissue remodeling[1]. Contemporary wound care is not only meant to preserve the wound but also to promote healing and monitor the progression of the wound[2]. Plant extracts, honey, and oils constitute the most common types of traditional treatments that do not provide specific wound-status monitoring[3]. Modernized medicated dressings and regulated drug delivery systems enhance clinical outcomes; however, traditional dressings tend not to accommodate wound changes and can lead to discomfort[4]. Smart wound dressings can react to physiological signals, such as pH, which is acidic (4-6), whereas chronic wounds have an alkaline (7-9)[5]. pH-responsive dressings can be visually monitored in real-time using color indicator shifts. The natural pigments of fruits and vegetables (anthocyanins) are color-dependent on pH because of molecular changes, and thus, they make the best biocompatible and environmentally friendly wound monitoring[6].

Marine macroalgae, such as *Gelidium acerosa*, are abundant in bioactive compounds, such as flavonoids and tannins, and have antimicrobial and wound healing properties[7]. Carboxymethyl cellulose (CMC) is a biodegradable polymer, which has an elastic hydrogel that can maintain moisture, serve as a hemostatic, and heal tissues[8]. It is designed in a manner that allows the integration of the therapeutic and sensing agents.

This study uniquely integrates marine macroalgal bioactives with plant-derived anthocyanin-based pH sensing within a single CMC hydrogel matrix to achieve simultaneous wound monitoring and therapeutic functionality. In this study, anthocyanins and metabolites from seaweed were extracted and identified and a hydrogel was developed and tested for antioxidant, antidiabetic, and pH-sensitive effects. The structural characteristics and the wound healing ability were evaluated under simulated condition in SEM. The end result is a biodegradable dressing that is sustainable and effective for advanced wound care.

Materials and methods

Extraction of anthocyanin from beetroot stalk

Fresh beetroot stalks were purchased from a local market in Avadi (Lat.: 13.1067: Long.: 80.0970) Chennai (Fig. 1). The moisture content was removed by drying the chopped beetroot stem at approximately 50°C. After drying, the sample was ground into a powder using a blender[9]. The water and acidified methanol (0.1 % acetic acid in methanol) were distilled and subjected to extraction at a 1:10 ratio of sample to solvent and swirled for 40 min in a magnetic stirrer in a beaker. This mixture was then filtered and stored at 4°C for further analysis[10].

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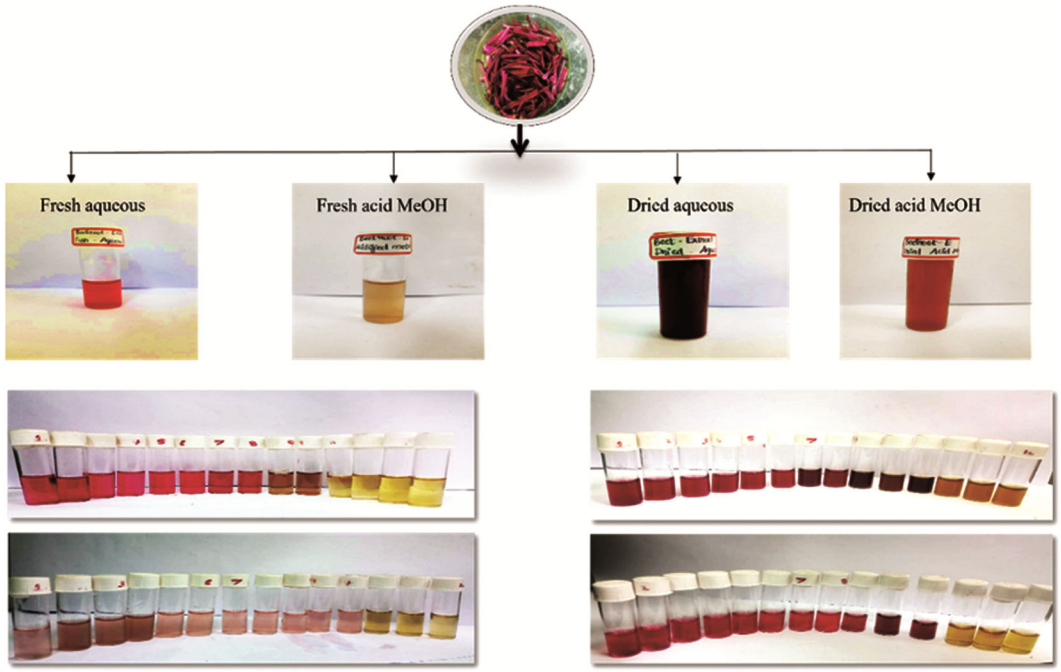

Fig 1 — Aqueous extraction of crude anthocyanin from beetroot stalk and evaluation of the pH stability of the film

Estimation of the Total Anthocyanin Content (TAC)

The monomeric anthocyanin and total anthocyanin content were determined using a pH differential approach. A pH gradient spanning pH 1-14 was created in a buffer series, and the anthocyanin extract was added to determine the color changes dependent on pH[11].

The standard pH differential was used to perform a quantitative analysis of Total Anthocyanin Content (TAC)[12]. A tenfold dilution of the anthocyanin extract was performed individually in two buffer components of 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer. The absorbance of each solution was determined at 700 and 510 nm using a UV-vis-spectrophotometer. The TAC could then be evaluated as portrayed in the following equation[10],

$$TAC=(A\times MW\times 100)/MA$$

Were,

$A= (A_{510} - A_{700})$ pH 1$- (A_{510} - A_{700})$ pH 4.5

MW= Molecular weight (449.2g/mol)

MA=Molar absorption of cyanidin-3-glucoside (26900)

Sample preparation and extraction of macroalgae

The *Gracilaria acerosa* was collected from the coast of Rameswaram (mandapam), Tamilnadu, India (Lat: 91632.56 N, Long: 79075.3 E)[12]. It is a good source for bioactive applications because it is a species with a high biomass proliferation rate (7–10-fold in 2–3 months). The biomass was washed in distilled water, dried in an oven at 50°C and ground [13]. The extraction was done by using distilled water (1:10, w/v) under magnetic stirring for 40 minutes and then filtered to get the aqueous extract[13].

Phytochemical analysis of the anthocyanin and *G.acerosa* extract

Total phenolic content (TPC)

Phenolic compounds in macroalgae are important for their radical scavenging ability due to their high antioxidant activity[14]. Total phenolic content (TPC) was measured by Folin–Ciocalteu method with gallic acid (1 mg/mL) as calibration standard in the crude extracts. A standard solution of gallic acid was first prepare. Next, 0.5mL of the macroalgal extract and 2.5mL of 10% Folin–Ciocalteu reagent and 2mL of 2% (w/v) sodium carbonate solution[15] were combined. The mixture was incubated for 15 min at 45 °C for development of chromophore. The absorbance was then determined at 765 nm in a UV–Vis spectrophotometer[14].

Total flavonoid content (TFC)

The total flavonoids content was estimated by the aluminium chloride colorimetric method with quercetin (1 mg/mL) as the standard[16]. In brief, 1 mL of the extract was added to methanol, 10% aluminium chloride and potassium acetate, and then distilled water[17]. The absorbance was measured after a 30-min incubation at room temperature with a UV–Vis

spectrophotometer at 415 nm[16]. The results were reported in mg of quercetin equivalents per gram of sample (mg QE/g).

Characterization of the extracts

UV-Vis spectroscopy

Buffer solutions with pH values of 1, 3, 7, 9, 12, and 14 were prepared to determine the pH-specific action of the anthocyanin extract. To guarantee consistency in all samples, an extract: buffer ratio was used to mix the extract with each of the buffers[18]. The absorbance spectra of aqueous solutions of UV-Vis were created over a wavelength range of 200 to 900 nm by UV-Vis spectrophotometer[11]. The wavelength of the maximum absorption (lambda max) was determined under each pH condition to observe color intensity and changes in the spectrum. This analysis provided important information regarding the stability and modification of anthocyanin structures at different pH values, based on the peak data.

Fourier transform infrared spectroscopy

Infrared (IR) spectroscopy was used to detect the chemical functional groups in the crude extract of the sample. IR spectra were measured in the 4000-400 cm -1 range. The application of infrared radiation to the extract caused typical vibrational transitions in the molecular bonds, leading to the appearance of actual absorption peaks[19]. These absorption bands are related to different functional groups and constitute a molecular fingerprint of the sample. The acquired spectra were analyzed to determine the functional groups in the extract, as described by[19].

Total Antioxidant activity of the extracts

The antioxidant activity of the extracts was assessed by DPPH free radical scavenging assay[20]. In brief, 1 mM of DPPH solution was prepared in ethanol while ascorbic acid was used as a calibration standard (1 mg/L)[21]. One mL of the standard or sample extract was transferred together with 1 mL of the DPPH solution into darkness at room temperature for 30 min. After that, the absorbance was read at 520 nm in a UV–Vis spectrophotometer[20]. Ethanol was served as a blank, and radical scavenging activity was determined.

$$\% \text{ of Inhibition} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right) \text{X100}$$

Antimicrobial activity of the *G.acerosa* extract

G. acerosa extract has antimicrobial effects by inhibiting the reproduction of wound-connected disease-causing agents. Antimicrobial properties are the capacity of a substance to inhibit or kill microbes[22]. Typical methods used to measure the antimicrobial potential of natural compounds are agar well diffusion, disc diffusion, and agar-broth dilution. In this study, antimicrobial activity was assessed using the agar well diffusion assay. Agar plates were prepared correctly, and at the same dose the subculture of *Staphylococcus epidermidis* and *Staphylococcus aureus* was swabbed fully on the agar plate[23]. *G. acerosa* extract at varying concentrations (200, 400, 600, 800, and 1000 µg mL^{-1}) was added to different wells on the agar. After 24 h of incubation at 37°C, the plates were observed to determine the zones of inhibition, which were measured to determine the antimicrobial power of the extract.

Preparation of anthocyanin-incorporated gauze cloth and estimation of its pH stability

The anthocyanin solution was diluted with distilled water in different proportions to facilitate the incorporation of anthocyanins into the gauze fabric. Sterile gauze (2 × 2 cm) samples were immersed in the diluted solutions of anthocyanins and orbital shaking at 30 min was conducive to the penetration of the dyes on the cloth[11]. The air-dried samples were incubated at ambient temperature after treatment according to a protocol described by[12]. The PH sensitivity and structural stability of the anthocyanin-treated gauze were determined using the pH differential method. Buffer solutions of different pHs (1-14) were provided, and the gauze with the infused anthocyanin was subjected to the conditions at each pH value[11]. Colour transition under different environments was noted visually to assess the response of anthocyanins chromatically to varying degrees of pH. The spectrum of colour found in the infused fabric was evident and sensitive to pH as it was red in harsher acidic medium, purple under close to neutral pH and blue under alkaline medium. These findings align with the findings reported by[12] and confirm the applicability of anthocyanins as bio chromic agents that might be potentially used in pH-responsive textiles and wound monitoring systems.

Preparation of the film infused with *G.acerosa* extract

The carboxymethyl cellulose (CMC) was dissolved in distilled water to different concentrations (1%, 2%, 3%, 4%, and 5% w/v) and stirred continuously under

50°C temperature at 30 minutes. According to the preliminary test based on visual inspection and the initial evaluation, the solution containing 2% of CMC proved ideal film-forming characteristics and moisture content suitable enough[24]. Hence, the films that were prepared using CMC as 2% were chosen to continue analysis. The films were then soaked in a range of epichlorohydrin concentrations (v/v) as a crosslinking agent and then subjected to a physically-based characterization[25]. Some of the films contained the crosslinking agent with an antimicrobial seaweed extract that had been previously characterized as a potent source of antimicrobial activity to add bioactivity to the final composite film.

Characterization of the G.acerosa extract infused film

Moisture content

The manipulation of the moisture of the film was done by slicing the film into small equal pieces that were 2 x 2 cm. The weight of a sample was measured in such a way that the initial wet weight was measured on exact balance[26]. The samples were then transferred into hot air oven using a temperature of 50° C with duration of 2 hours to enable total drying. It then was allowed to dry and the resulting weight of the film was obtained. The moisture percentage was found out by the following equation:

$$\text{Moisture Content (\%)} = \text{Initial Weight} - \text{Final Weight} \times 100$$

This method was adapted from previously reported protocols[27].

Swelling ratio

The swelling ratio of the film was derived according to an altered procedure depending on methods that have been published[27]. Samples of film were squared up to result in the same dimensions of 2X2 cm. The wet weight of each sample was measured first. The films were subsequently put in a hot air oven at 50 °C for 4 hours to come up with the dry weight. The films were dried and then placed in a 50 mL of distilled water in a beaker and left to swell at ambient temperature without disturbance for half an hour[28]. The films were then removed gently, any water present on the surface was dried by using dry tissues of paper and the swollen weight was noted. The ratio between swelling and mass was used as follows:

$$\%\text{ of Swelling index} = \frac{\text{Swollen Weight of the film} - \text{Initial dry weight of the film}}{\text{Initial dry weight of the film}} \times 100$$

Solubility

The solubility of the film was determined by dividing the film into regular pieces of 2 cm x 2 cm. The initial wet weight of each sample was taken, and then it was dried in a hot air oven at 50 °C for a 4-hour duration to get the initial dry weight[28]. These then dried films were rehydrated by incubating in 50 mL distilled water and agitated on a shaker at room temperature for 2 h to enable solubilization[29]. After incubation, the undissolved film portion was removed, again dried in a hot air oven, and the final dry weight was recorded. Solubility of the film was determined by a formula that is as follows:

$$\text{Solubility\%} = \frac{\text{Initial dried weight} - \text{Final dried Weight}}{\text{Initial dried weight}} \times 100$$

Acid solubility

The acid solubility of the film was determined by cutting the film to a size of 2 x 2 cm, and its initial wet weight was recorded. Further, all samples were dried in a hot air oven at 50° C to give the initial dry weight a total of 4 hours[28]. The obtained dried films were then incubated in a 50 mL 1 M solution of hydrochloric acid (HCl) and shaken at room temperature within 2 hours[29]. The films were removed, after their incubation period, carefully, rinsed where necessary in order to remove the surface acid, and re-dried again in a hot air oven to measure the final dry weight. The acid solubility of the film was determined as stated in the formula below:

$$\text{Acid Solubility\%} = \frac{\text{Initial dried weight} - \text{Final dried Weight}}{\text{Initial dried weight}} \times 100$$

pH

The pH of the film was measured after cutting out a 2 x 2 cm piece of the film and dissolving it in distilled water; the mixture was stirred so that it could be homogenized. A type of pH/ORP benchtop meter (HI 6221-02, Hanna Instruments, Thailand) was used to test the pH of the resultant solution.

Thickness

The dried film thickness (diameter: 90mm) was obtained by screw a digital micrometer (QuantuMikeIP65, Mitutoyo, Japan) in accordance with the technique used by[28]. Ten points were measured on the film surface, and the average of those measurements was captured and noted as a final thickness.

Tensile strength

The dried film thickness (diameter: 90mm) was obtained by screw a digital micrometer (QuantuMike

IP65, Mitutoyo, Japan) in accordance with the technique used by[27]. Ten points were measured on the film surface, and the average of those measurements was captured and noted as a final thickness.

Fabrication of smart wound dressing

Using the macroalgae extract as an active ingredient, the smart wound dressings were made with carboxymethyl cellulose (CMC)-based film-forming solution. Stirring of the sample was done to have an even distribution of the extract throughout the polymer matrix[30]. The solution thus prepared was then applied uniformly on gauze fabric, which had been pre-infused with anthocyanin[31]. To form the complete wound dressing functional material, the composite was then dried out using a hot air oven at 50°C.

Scanning Electron Microscopy (SEM)

The surface morphologies of the film were studied by Scanning Electron Microscopy (SEM). The method entails a focused beam of electrons being scanned across the solid sample surface to produce high-resolution images that offer in-depth understandings of the microstructure and surface properties of the film[31].

Evaluate the pH stability of the smart wound dressing

The pH stability of the smart wound dressing was determined based on the pH differential test to evaluate the integrity of the structural composition and its color change responsiveness to the embedded anthocyanins as part of the smart wound dressing material[31]. A range of buffer solutions of pH 1 to 14 was prepared, and a few drops of the buffer were placed directly upon the surface of the wound dressing[28]. The entire area of the corresponding color changes was observed and noted to get a reading on whether the material is pH-responsive or not. At the acidic condition, the dressing turned red colored; at neutral pH, it changed to purple; and in alkaline condition, it changed to green. The pH-responsive color changes are expected given the behavior of anthocyanins observed by[29], therefore, establishing the bio chromic capabilities of the smart dressing wound dressing.

Analyze the color properties of the smart wound dressing

The color characteristics of the smart wound dressing were determined through the color space system of CIELAB, which was defined by the International Commission on Illumination in 1976. In this system, the characterization of color is done using three parameters: L* (lightness), a* (red-green axis), and b* (blue-yellow axis). The film samples were trimmed to a smaller size, and colorimeter measurements on the surfaces were performed with a spectrophotometer (CM-5, Konica Minolta, Japan). L*, a*, and b* values were noted as given in[32].

Evaluate the Antimicrobial activity of the smart wound dressing

Antimicrobial activity of the film was determined through the agar well diffusion method. Agar plates were prepared as follows: sterile agar media were poured into Petri dishes and cooled off in a sterile environment. After solidification, bacterial suspensions of *Staphylococcus* aureus and *Staphylococcus epidermidis* were inoculated on the plates, the surfaces of which were swabbed evenly. Precisely laid-down film samples (test and control) inoculated agar surfaces were incubated at 32 °C for a 24-hour period[31]. Antimicrobial activity was achieved, as evidenced by the presence of clear zones of inhibition around film samples. To assess the antimicrobial property, the diameters of the inhibition zones were recorded with the help of a digital caliper, as explained in the article by (Bodea, I. M *et al* 2022).

Biodegradability analysis of the smart bio chromic therapeutic bandage

To assess bio-degradability of the smart bio chromic bandage, pieces of film 2X2cm in size were buried in soil. As explained by[29] the samples that were put in a box were fully exposed to natural soil under normal laboratory conditions. `Initial dry weight of each sample was taken before burial. Samples were also thoroughly collected at regular intervals every day, washed adhering soil by gentle technique, dried using a hot air oven and then weighed[32]. The biodegradation was then calculated by the percentage of weight loss in the following formula:

$$B\ (\%) = ((W_0 - W_1)/W_0) \times 100$$

B= Biodegradability efficiency

W_0- Initial weight of Film

W_1- Final weight of Film

Results and discussion

Extraction of anthocyanin from beetroot stalk

The aqueous extraction method was found to be efficient and eco-friendly for recovery of pigments. Beetroot stalk water extract (BSE) was prepared using vortex-assisted extraction (1:10 w/v), centrifugation and repeated extraction process, and it was found that the BSE contained high level of betalain (456.82 ±

18.60 mg/L) with significant levels of phenolics (139.87 ± 0.45 mg/g) and flavonoids (31.17 ± 2.24 mg/g) [30]. It is noteworthy that pigment recovery (0.512 ± 0.01 mg D3G/g equivalent) was higher in water than in acidified methanol, suggesting that there was a better preservation of chromophore integrity in water. The extract showed very strong colour changes depending on the pH. Near neutral pH (5–6) caused less red–purple coloration, and acidic pH (pH ~3) caused less or yellowish tones. When the pH value was greater than 7, the pigments shifted towards darker/greenish colors, because of structural changes. These variations are related to the functional modifications like glycosylation, methoxylation which increases the stability[31]. Moreover, variation of pigment expression between different plant tissues indicates the need to carefully select sources for biochromic applications[32].

Investigating pH-Sensitive colour change of crude anthocyanin solution with UV-Vis:

The UV-Vis analysis of crude anthocyanin solution indicated that the peak absorbance is 528 nm, which is observed in highly acidic conditions, and thus it proves the predominance of flavylium cation (Fig. 2). At pH 3-7, absorbance declined with a small hypochromic alteration, representing transformation to carbinol pseudo base and chalcone derivatives to give light pink colours[32]. In pH 9-14, the visible absorbance further lowered and a loose band of about 615 nm appeared, which is attributed to the formation of quinoidal bases and greenish colour. These findings indicate that anthocyanins may serve as sensitive pH biosensors, a defining characteristic of real-time wound-based sensors, because chronically wounded patients display an alkaline microclimate[31].

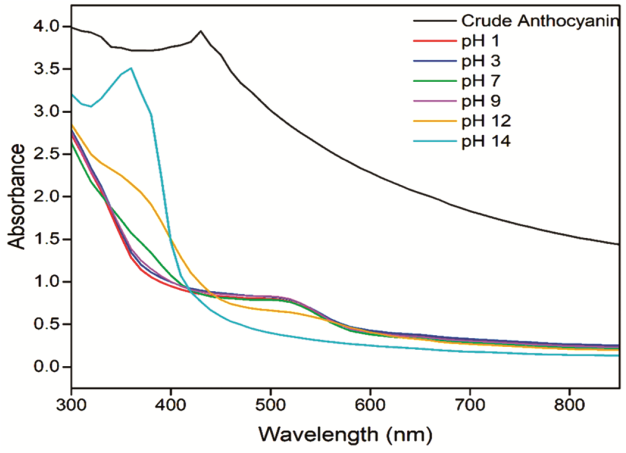

Fig 2 — pH-Sensitive colour changes of crude anthocyanin solution with UV-Vis

Extraction and Phytochemical Characterization of *Gelidium acerosa*

The water soluble bioactives namely phenolics and flavonoids were detected in aqueous extract of Gelidium acerosa collected from the coast at Rameswaram[13]. The total phenolic content (TPC) and total flavonoid content (TFC) of the extract were 0.255 mg GAE/g and 0.364 mg/mL, respectively. Conversely, the beetroot stalk extract (BSE) initially measured was found to have lower values (TPC: 0.111mg GAE/g; TFC: 0.206mg/mL). The literature, however, shows much higher phytochemical contents for aqueous BSE, which included phenolics (139.87 ± 0.45 mg/g) and flavonoids (31.17 ± 2.24 mg/g) suggesting a high dependency of extraction conditions on the yield (Fig. 3)[33].

Total anti-oxidant activity by DPPH

The DPPH Radical Scavenging Activity was carried out using 2,2-Diphenyl-1-Picryl Hydrazine. The antioxidant properties of the beetroot stalk extract (BSE) and Gracilaria acerosa extract (GAE) were determined by the DPPH assay, compared with ascorbic acid[20]. In all samples, there was a significant increase in radical scavenging activity with increasing concentration (20–100 µg/mL) (Fig. 4). The maximum inhibition (82%) was observed with ascorbic acid at 20 µg/mL while 99% inhibition was recorded at 100 µg/mL. The extracts were compared for their activity and BSE proved to be the most potent with its activity range of 48% to 94% which is close to the standard while GAE showed comparatively low activity range of 45% to 79%[21]. The richness of BSE in betalains (456.82 ± 18.60 mg/L), phenolics (139.87 ± 0.45 mg/g) and flavonoids with significant contributions from chlorogenic acid and ferulic acid, which are two well-known antioxidants with effective

Fig 3 — Stepwise processing of G. acerosa for aqueous extract preparation. Freshly collected G. acerosa (left) was washed, dried, and ground into a fine powder (middle), which was then subjected to aqueous extraction, yielding a light brown extract (right) for further analysis

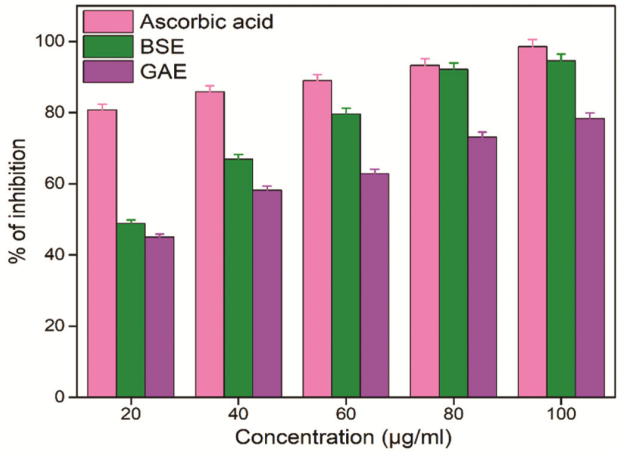

Fig 4 — DPPH radical scavenging activity of beet stalk extract (BSE), Gracilaria acerosa extract (GAE), and ascorbic acid at varying concentrations (20–100 µg/mL). Results are expressed as percentage inhibition of DPPH radicals. Ascorbic acid served as the positive control

radical scavenging activity, confirms the high antioxidant potential. The result of reported IC_{50} value (207.33 ± 4.19 µL/mL) also supports its high DPPH scavenging efficiency, as seen in the previous studies[34].

FTIR analysis of the extracts

FT-IR spectra validated the presence of essential functional groups in both, anthocyanin extract and Gelidium acerosa extract (Fig. 5 & Fig. 6). The O–H stretching band (around 3447 cm–1) and the C=O stretching band (around 1637 cm–1) were observed, which support the presence of hydroxyl groups of phenolics and C=O stretching of flavonoid structures in the anthocyanin extract[35]. In addition, the C=C aromatic vibrating band (around 1416 cm–1) was observed. Other bands indicated aliphatic C–H and substituted aromatic C–C vibrations.

Likewise, bands of G. acerosa extract around 3459 cm-1 are due to the presence of hydroxyl groups which are characteristic of compounds with hydroxyl groups, and bands around 2890 cm-1 (CH_2 stretching) are characteristic of carbohydrates and lipids[36]. The bands around ~1634 cm^{-1} were attributed to carbonyl groups and those around ~717 cm^{-1} were assigned to aromatic C–H bending and polysaccharide groups like alginates.

Anti-microbial activity of the extracts

Agar diffusion analysis indicated selective antimicrobial activity with Staphylococcus aureus inhibited by beetroot stalk extract (BSE) and G. acerosa at 1000µg/mL (Beetroot, BSE; 8±0.3mm;

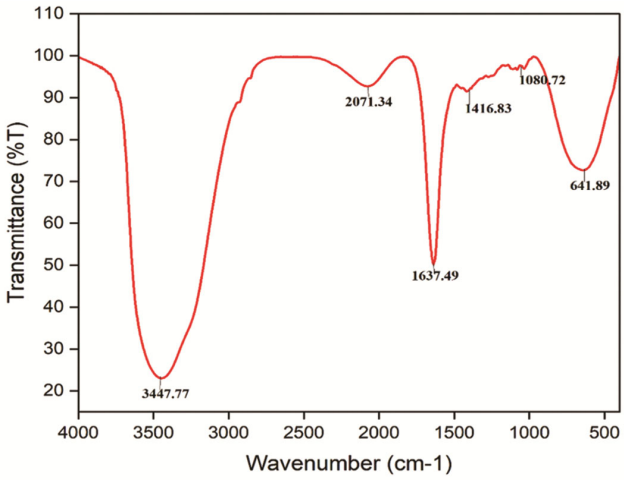

Fig 5 — FT-IR spectrum of the anthocyanin extract (BSE)

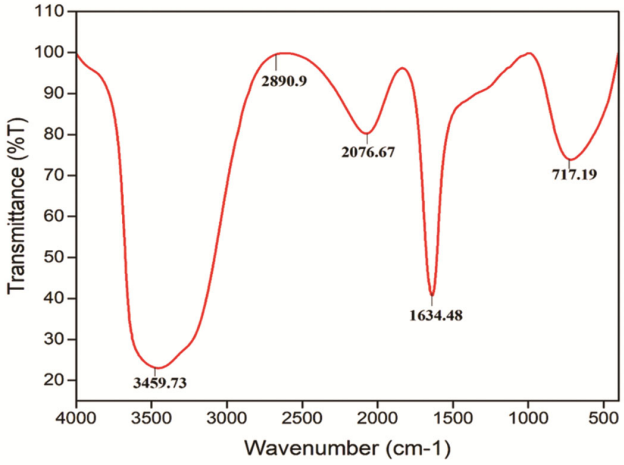

Fig 6 — FT-IR spectrum of Gelidium acerosa extract

Table 1 — Antimicrobial activity of BSA

ANTIMICROBIAL ACTIVITY OF ANTHOCYANIN SOLUTION		
ORGANISM	CONCENTRATION (µg/ml)	ZONE OF INHIBITION (mm)
Staphylococcus aureus	Control (Amoxicillin)	13 ±0.3
	200	4±0.3
	400	6±0.3
	600	6.5±0.3
	800	7.5±0.3
	1000	8±0.3
Bacillus subtilis	Control (Amoxicillin)	6±0.3
	800	0.5±0.3
	1000	2±0.3

G. acerosa, 9±0.3mm). Very little activity was seen against *Bacillus subtilis* and *S epidermidis* (Table 1). However, antimicrobial activities of BSE are stronger in the literature, especially against Salmonella senftenberg (19.80 mm), and moderate against

Escherichia coli (14.70 mm) and S. aureus (13.10 mm), which indicates that there are variations depending on the conditions of the extraction and testing[37]. The antimicrobial activity is mainly due to phenolic and flavonoid compounds that cause cell wall damage and interfere with microbial metabolism[15]. Besides, the use of anthocyanin-based systems has also demonstrated improved efficiency when associated to polymer-based systems, such as composite films (gelatin–carboxymethyl cellulose with red cabbage anthocyanins) that inhibited B. subtilis by up to 90.54%. The results indicate the promising application of these bioactive extracts in the field of antimicrobial and wound healing properties.

Development of Anthocyanin-Infused Gauze and CMC-GAE Films

Anthocyanin solutions with different proportions were added to gauze; this offered the best colour distribution and colour change that was sharp with respect to pH (Fig. 7). CMC film containing *G. acerosa* extract (2% CMC with different crosslinkers) was characterized by moisture content (29.4%), swelling index (7.9%), water solubility index (30.3%), and acid solubility index (29%), showing that it could retain moisture and release bioactive (Table 2)[37].

Characterization of the GAE infused CMC film as base for smart bandage

From the 1–5% CMC formulations, 2% CMC film was chosen for the subsequent analysis because it had an optimal flexibility and structural integrity[37]. Different percentages of crosslinker were added to the films (1-10%) and tested for moisture content, swelling index, water solubility index (WSI), acid solubility index (ASI), and pH (Table 3). The water retention property was observed as moisture content was high with crosslinker concentration (1-4%) and maximum moisture content was 35.4% for corn. It was observed that with the increase in the amount of crosslinker (1-4%), the moisture content was increased, for corn the moisture content was as high as 35.4%, which indicated that there was good water retention property. However, it fell down to ~28% beyond 4%, indicating limited numbers of hydrophilic sites as a result of crosslinking[35]. Likewise, the swelling index reached a maximum for 0.4% crosslinker, and decreased with further increase of the crosslinker concentration because of greater network density, which would not allow the polymers to expand. The WSI and ASI values showed a wide variation with crosslinker concentration, with the highest value of WSI being 55.5% at 8% crosslinker concentration and the highest value of ASI being 82.5% at 10% crosslinker concentration[37] which could be attributed to polymer matrix restructuring and exposure of polar groups. The pH stability (7.2–7.31) showed that no adverse effects were done to the film environment by the crosslinking process[31].

Mechanical and barrier properties (Table 4) indicated that tear strength, tear force and elongation

Table 2 — Antimicrobial activity of GAE

ANTIMICROBIAL ACTIVITY OF *GELIDIUM ACEROSA*		
ORGANISM	CONCENTRATION (µg/ml)	ZONE OF INHIBITION (mm)
Staphylococcus aureus	Control (Erythromycin)	14±0.3
	600	4±0.3
	800	8±0.3
	1000	9±0.3
Staphylococcus epidermidis	Control (Erythromycin)	5±0.3
	1000	2±0.3

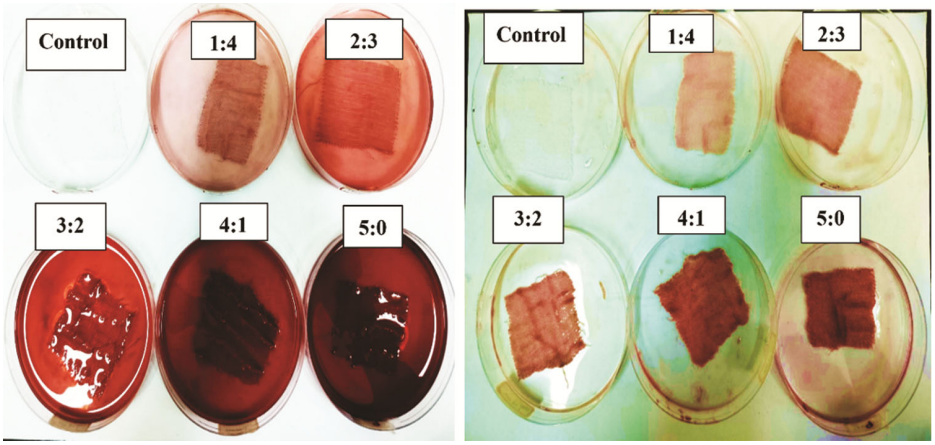

Fig 7 — Optimization of Anthocyanin extract incorporation into Gauze cloth

Table 3 — Physical characteristics of GAE extract infused 2% CMC films with crosslinker

2%CMC FILM WITH VARYING CROSSLINKER	MOISTURE CONTENT [%]	SWELLING INDEX [%]	WATER SOLUBILITY INDEX [%]	ACID SOLUBILITY INDEX [%]	Ph
1%	29.4	7.9	30.3	29.0	7.07
2%	33.9	6.6	33.3	36.2	7.02
4%	35.4	0.4	36.3	40.4	7.13
6%	34.2	0.7	46.6	48.0	7.26
8%	28.0	0.2	55.5	54.7	7.29
10%	31.7	1.1	55.0	82.5	7.31

Table 4 — physiochemical Characteristic of the film

SAMPLE	THICKNESS (mm)	TEAR FORCE (N)	TEAR STRENGTH (Mpa)	TENSILE STRENGTH (Mpa)	ELONGATION AT BREAK (%)	OTR (g/ m^2 × 24 h)	WVTR (g/ m^2 ×24 h)
CMC film	0.09	7.71	5.36	4.82	9.63	130.2	1496.1
CMC + Algal extract	0.07	12.21	8.56	7.7	29.71	32.7	1538.5

at break of pure CMC film (0.09 mm) were 5.36 MPa, 7.71 N and 9.63, respectively. The incorporation of algal extract showed better performance with the composite film (0.07 mm), which had higher tear force (12.21 N), tear strength (8.56 MPa), tensile strength (7.7 MPa), and elongation at break (29.71), showing that the film was more flexible and tough[32]. The barrier analysis showed a dramatic decrease in OTR from 130.2 to 32.7 g/m2 x 24 h, which meant that it had better oxygen barrier properties. The incorporation of algal extract, however, resulted in an increase of WVTR from 1496.1 to 1538.5 (g/m2×24 h) indicating enhanced hydrophilicity[31]. In general, algal extract showed improvements in mechanical strength and oxygen barrier properties and decreases in water vapor resistance.

Formulation of Smart wound dressing

CMC–Gelidium acerosa (GAE) hydrogel was coated onto anthocyanin-impregnated gauze and dried at 50 °C to prepare an anthocyanin-containing multifunctional dressing. CMC was highly soluble and had high surface area which could interact with the anthocyanins and resulted in a uniform immobilization on the gauze[38]. This integration is essential for fast and reversible pH-responsive color changes which reflect the status of wounds. This made a uniform coating on the resulting dressing, while maintaining the porosity of the dressing for exudate absorption and breathability (Fig. 8)[39]. They provide the following features that facilitate a moist healing environment conducive to regeneration of tissue[40]. Moreover, the immobilization of anthocyanin in the hydrogel made it more colorimetrically sensitive to allow real-time pH monitoring. The bioactivity of GAE and visual sensing ability allow

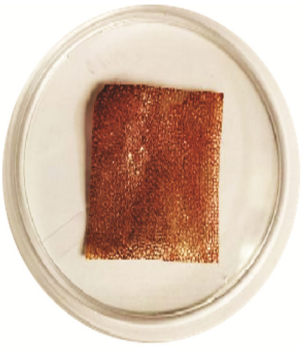

Fig. 8 — Smart Biochromic bandage

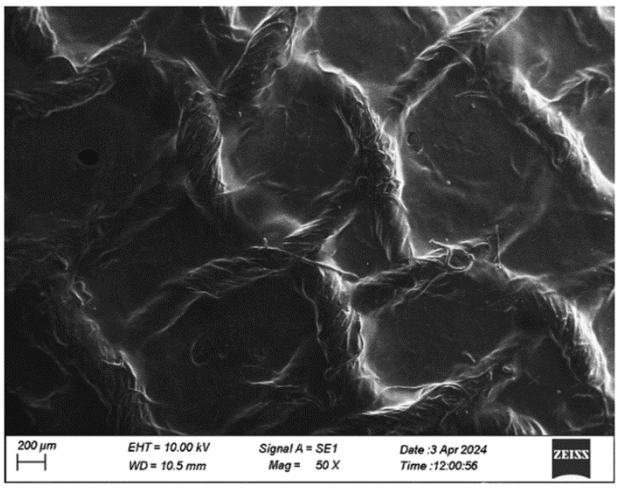

Fig 9 — SEM Images of Bio chromic Smart dressing

the dressing to be a multifunctional wound care system, providing a therapeutic support and non-invasive monitoring of wound healing and infection[39].

pH stability of smart biochromic therapeutic bandage

The sensitivity of anthocyanin-CMC-GAE dressing to pH was assessed by being immersed in buffer ranging between pH 1-14 (Fig. 9). The dressing

revealed different and gradual colour transformation throughout this range (Fig. 10). Under the most acidic conditions (pH 1-4), a bright pink colour was observed and revealed to a yellow-brown color under alkaline conditions (pH 9-14)[41].

Such strong colorimetric variations indicate not only the structural stability of anthocyanins placed in the gel matrix but also indicate the extreme sensitivity of anthocyanins to pH variations. This type of responsiveness is specifically important to wound monitoring since most wounds, which are chronic or infected, typically have an alkaline microenvironment because bacteria multiply and inflammatory responses occur[39]. This dressing can be a non-invasive diagnostic instrument because it allows showing a visual, real-time evidence of local pH variations, which will allow providing timely interventions and more effective monitoring of the healing process[35]. The embedding of anthocyanins in the hydrogel framework guarantees the colour changes are repeatable and long-term, and strengthens the application of the dressing as a versatile therapy/ monitoring unit.

Colorimetric Behavior of CMC–GAE Biochromic Dressing

A CMC-*Gelidium acerosa* (GAE) hydrogel dressing exhibited inherent dyeability, in the absence of a mordant, as a result of anthocyanin interactions with the amide- and carboxylic acid-rich matrix functionalities[35]. The dressing appeared dark reddish-purple at neutral pH (7), which is also expected of the flavylium cation with its neutral species. In acidic solution (pH 1.5), the anthocyanin molecules were protonated, which stabilized the flavylium cation and a bright pink-red colour was formed[39]. On the other hand, the presence of alkaline conditions brought about structural changes to the quinonoidal and chalcone analogs to give a greenish-yellow coloration (Table 5).

These visual observations were confirmed by the quantitative analysis based on the CIELAB colour system. Lightness (L *3) was maximum (25.39) at pH 8 and declined with increased alkalinity indicating a lower percentage of pigments being saturated, perhaps through structure degradation[42]. The a -parameter (red-green axis) reached its maximum value at pH 6 and changed gradually to negative values at pH greater than 8, which was the typical green shift at alkaline pH. Likewise, the b, parameter (yellow-blue axis) was positive in acidic media, but became negative at higher pH, to indicate a transition to bluish-yellow colours[41].

Evaluation of antimicrobial activity of the smart bio chromic dressing

The antimicrobial performance of the anthocyanin - CMC -*Gelidium acerosa* (GAE) dressing was compared against Staphylococcus epidermidis and Staphylococcus aureus by using the agar well diffusion technique (Fig. 11). The untreated gauze (control) did not have any inhibitory zones hence proving the lack of inbuilt antimicrobial property[38]. Conversely, the biochromic dressing had distinct areas of inhibition with a 4 mm diameter of *S. epidermidis* and a diameter of 6.5 mm of *S. aureus* (Table 6).

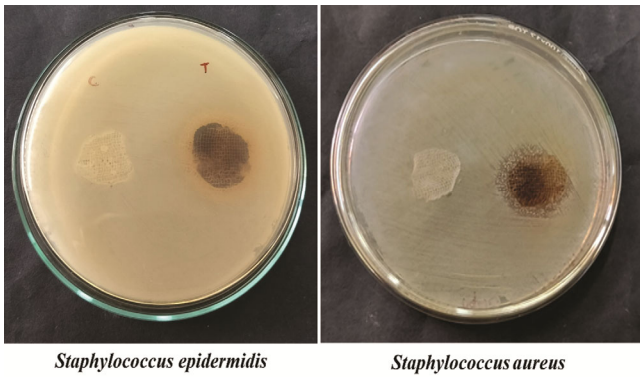

Fig 11 — Antimicrobial activity of the smart bio chromic dressing

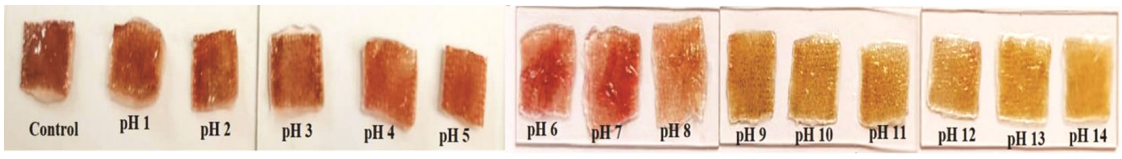

Fig 10 — pH stability of the smart bio chromic bandage

Table 5 — Colour studies of biochromic dressing

pH	1	2	3	4	5	6	7	8	9	10	11	12	13	14
L*	12.77	14.38	16.87	18.99	22.05	23.29	24.89	25.39	19.73	17.08	17.78	18.88	19.64	21.21
A*	2.09	0.89	5.34	6.58	6.73	9.46	4.87	-0.11	-0.135	-0.15	-0.25	-0.62	-0.76	-0.76
B*	0.59	0.98	1.33	1.69	2.09	3.01	2.12	1.02	0.69	-0.05	-0.08	-0.13	-0.21	-0.24

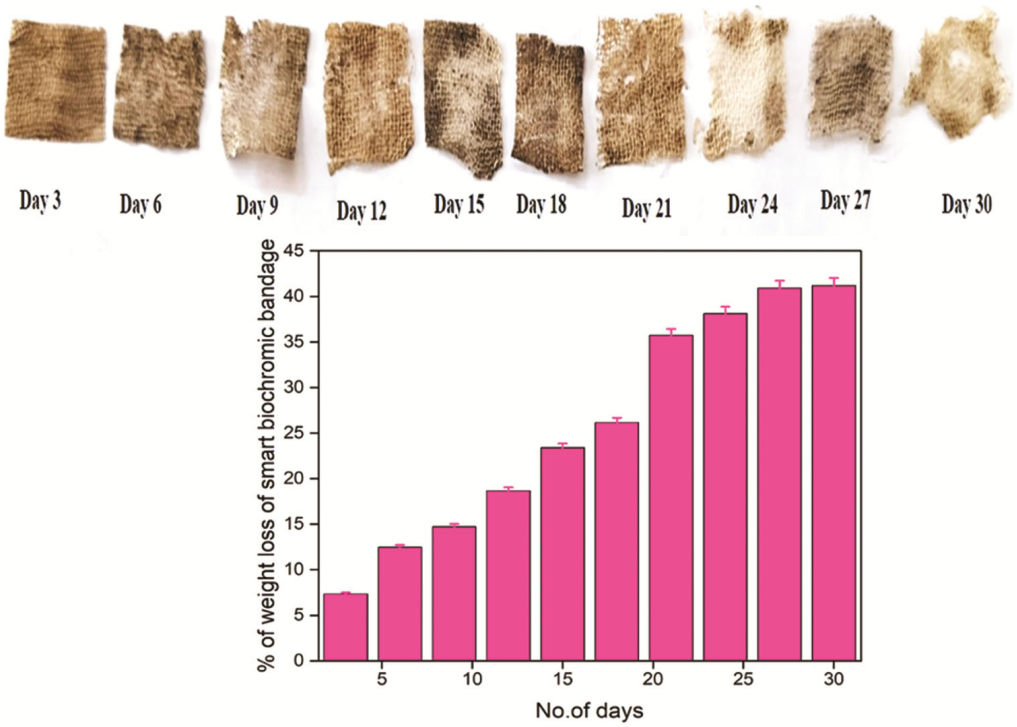

Fig 12 — Biodegradability of the smart biochromic dressing over 30 days, showing progressive degradation and ~41% weight loss by Day 30

Table 6 — Antimicrobial evaluation of the smart bio chromic dressing

ORGANISM	CONCENTRATION (µg/ml)	ZONE OF INHIBITION (mm)
Staphylococcus epidermidis	Control (Gauze cloth)	0
	Biochromic dressing	±4
Staphylococcus aureus	Control (Gauze cloth)	0
	Biochromic dressing	±6.5

The antimicrobial effect observed can be as a result of the presence of phenolic and flavonoid compounds in beetroot stalk anthocyanins and GAE bio actives. These agents are reported to destabilize bacterial cell membranes, disrupt metabolic activities and suppress bacterial proliferation, especially Gram-positive strains[36]. The increase in the level of activity toward *S. aureus* indicates that the dressing has potential to prevent infections of clinically relevant pathogens.

Notably, this two-fold purpose, antimicrobial effect and pH-responsive colorimetric monitoring, places the dressing in a position of multifunctional wound care material[40]. It not only offers visual confirmation of wound status but also vigorously mitigates the risk of bacterial colonization, therefore, enhancing safer and more active wound healing[43].

Biodegradability Evaluation of the Smart Bio chromic Therapeutic Dressing

Environmental confer ability of the anthocyanin-CMC-*Gelidium acerosa* (GAE) dressing was determined by soil burial tests as a measure of biodegradation. With the study, progressive loss of weight in the dressing was observed, with its maximum change of about 40% observed after 30 days[43]. The trend pattern of degradation is constant and monotonic, which shows that the breakdown process is stable and predictable, which is the feature of the eco-friendly character of the hydrogel-based dressing[44].

Such biodegradability is attributed to the natural polymeric matrix (CMC) and plant/marine-derived bio actives, which are per se vulnerable to microbial and environmental degradation (Fig. 12). The results of this research point to the possibility of the dressing to be used sustainably in wound care when the issue of safe disposal is of paramount importance[44]. It is through this fusion of therapeutic functionality, pH-responsive monitoring, and environmentally friendly degradation such that this biochromic dressing offers a holistic solution that is consistent with both clinical efficacy and a green footprint[45].

Conclusion

The present research has managed to reveal the valorization of beetroot stalk and *Gracilaria acerosa*

as renewable and sustainable sources to be used in the creation of a multifunctional smart bio chromic wound dressing. Anthocyanins in beetroot stalks were extracted by means of water, which maintained the consistency and bioactivity of the extract and gave high quantities of bioactive substances of *G. acerosa* in the absence of toxically solvents. The two extracts had pronounced antioxidant and antimicrobial potentials, and beetroot stalk extract had a high level of pH sensitivity and radical activity. FTIR examination established the existence of functional groups that are pertinent in wound healing and bioactive interaction. Anthocyanins embedding into gauze and CMC-GAE hydrogel films yielded dressings with a strong pH-dependent colorimetric response, and enabled visual real-time analysis of wound conditions. The dressings retained important physical characteristics, such as porosity, water retention, and mechanical stability, as well as exhibited antimicrobial activity, especially against Staphylococcus aureus. The biodegradability of the dressing was also proven through soil burial studies and thus illustrates that it is eco-friendly. Generally, the paper fulfilled its goal of creating a natural, sustainable, and multifunctional wound dressing incorporating bioactivity, pH-sensing, and environmental eco-friendliness. This intelligent dressing has the potential to be a next-generation wound care product to in-situ monitoring and sustained biomedical purposes.

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References

1 Sindhu PE, Taj T, Fakrudheen F, Sahana TG & Ahmed MG, Introduction to wound healing: Factors affecting, phases, and principles of wound healing. In: *Nanomaterials for Wound Healing*, CRC Press (2025) 1.

2 Moradifar F, Sepahdoost N, Tavakoli P & Mirzapoor A, Multi-functional dressings for recovery and screenable treatment of wounds: A review. *Heliyon*, 11 (2025) 1.

3 Yang B, Xu J, Liu S, Wu C, Li Y, Lv M et al., Applications of bioactive herbal extracts in dressing materials for skin wound repair: Ingredients, mechanisms and innovations. (2025).

4 Alberts A, Bratu AG, Niculescu AG & Grumezescu AM, Collagen-based wound dressings: Innovations, mechanisms, and clinical applications. *Gels*, 11 (2025) 271.

5 Li Y, Song S, Song J, Gong R & Abbas G, Electrochemical pH sensor incorporated wearables for state-of-the-art wound care. *ACS Sens*, 10 (2025) 1690.

6 Xu Z, Yu X, Liu W, Cheng J & Xiong G, pH-responsive indicator films contained composite anthocyanins for monitoring meat freshness. *Food Chem X*, (2025) 102487.

7 Cadar E, Popescu A, Dragan AML, Pesterau AM, Pascale C, Anuta V et al., Bioactive compounds of marine algae and their potential health and nutraceutical applications: A review. *Mar Drugs*, 23 (2025) 152.

8 Tamanna, Sharma T, Mittal R & Kaushik P, Advances in polymer-based scaffolding for enhanced wound healing: A comprehensive review. *Int J Polym Mater Polym Biomater*, 74 (2025) 684.

9 Saleha FM, Aboelhaggagb RM & Abo-Zaida EM, Healthy sorbet with eggplant peel and beet stalk-leaves extracts as natural colorants. (2023).

10 Taghavi T, Patel H, Akande OE & Galam DCA, Total anthocyanin content of strawberry and the profile changes by extraction methods and sample processing. *Foods*, 11 (2022) 1072.

11 Lee J, Durst RW, Wrolstad RE et al., Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants and wines by the pH differential method. *J AOAC Int*, 88 (2005) 1269.

12 Khoo HE, Azlan A, Tang ST & Lim SM, Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr Res*, 61 (2017) 1361779.

13 Nur MG, Rahman M, Dip TM, Hossain MH, Hossain NB, Baratchi S et al., Recent advances in bioactive wound dressings. *Wound Repair Regen*, 33 (2025) e13233.

14 Torres P, Osaki S, Silveira E, dos Santos DY & Chow F, Comprehensive evaluation of Folin–Ciocalteu assay for total phenolic quantification in algae. *Algal Res*, 80 (2024) 103503.

15 Genwali GR, Acharya PP & Rajbhandari M, Isolation of gallic acid and estimation of total phenolic content in some medicinal plants and their antioxidant activity. *Nepal J Sci Technol*, 14 (2013) 95.

16 Pallab K, Tapan B, Tapas P & Ramen K, Estimation of total flavonoids content and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum*. *J Drug Deliv Ther*, 3 (2013) 33.

17 Nayeem N, Imran M, Asdaq SMB, Rabbani SI, Alanazi FA, Alamri AS et al., Total phenolic, flavonoid contents and biological activities of *Astragalus spinosus*. *Saudi J Biol Sci*, 29 (2022) 1277.

18 Albert NW, Two novel MYB transcriptional activators regulate anthocyanin pigmentation patterns in Petunia. (Doctoral thesis) Massey University (2009).

19 D'Souza L, Devi P, Shridhar MP & Naik CG, Use of FTIR spectroscopy to study cadmium-induced changes in algae. (2008).

20 Sharma M, Saklani K, Dhingra GK, Purohit S & Chandra S, Total phenolic and flavonoid contents and antioxidant activity of *Dactylorhiza hatagirea*. *Curr Biotechnol*, (2025).

21 Suryani N, Dewi YK & Hidayanti BR, Evaluation of bioactive phytochemicals and antioxidant activity of *Ficus racemosa* as herbal tea. *J Penelit Pendidik IPA*, 11 (2025) 1078.

22 Tsakni A, Kyriakopoulou E, Letsiou S, Halvatsiotis P, Rigopoulos H, Vassilaki N et al., In vitro determination of antimicrobial, antioxidant and antiviral properties of plant extracts. *Microorganisms*, 13 (2025) 177.

23 Kadalmani S, Razack SA, Ramakrishnan G & Sahadevan R, Extraction and bioevaluation of phycoerythrin from *Halymenia refugium*. *Indian J Biochem Biophys*, 62 (2025) 1277.

24 Rasheed HA, Adeleke AA, Nzerem P, Olosho AI, Ogedengbe TS & Jesuloluwa S, Isolation and optimization of cellulose from agricultural wastes. *Sci Rep*, 14 (2024) 14310.

25 Zhu J, Zhang Z, Wen Y, Song X, Tan WK, Ong CN & Li J, Advances in superabsorbent hydrogels from agro waste. *J Agric Food Chem*, 72 (2024) 22399.

26 AboulFotouh K, Southard B, Dao HM, Xu H, Moon C, Williams RO & Cui Z, Effect of lipid composition on nanoparticle properties. *Int J Pharm*, 650 (2024) 123688.

27 Kong I & Nyam KL, Development of biopolymer edible film with essential oil nanoemulsion. *J Food Sci Technol*, 62 (2025) 1472.

28 Zhou M, Han Y, McClements DJ, Cheng C & Chen S, Co-encapsulation of anthocyanin and cinnamaldehyde. (2024).

29 Biswal AK, Chakraborty S, Saha J, Panda PK, Pradhan SK, Behera PK et al., Fabrication and characterization of starch-based biodegradable film. *ACS Food Sci Technol*, 4 (2024) 1844.

30 Abdo EM, Mansour HM, Darwish AMG, El-Sohaimy SA, Gomaa MA, Shaltout OE & Allam MG, Beetroot stalk extract as functional colorant in yogurt beverages. *Fermentation*, 9 (2023) 878.

31 Olán-Jiménez KA, Cruz-Rodríguez RI, Couder-García BDC, Jacobo-Herrera N, Ruiz-Lau N, Hernández-Cruz MDC et al., Characterization of CMC and alginate-based dressings. *Polysaccharides*, 6 (2025) 75.

32 Melnyk H, Havryliuk O, Zaets I, Sergeyeva T, Zubova G, Korovina V et al., Red cabbage anthocyanin-loaded hydrogel for wound healing. *Polymers*, 17 (2025) 2116.

33 Subasini S, Bhavadharani D, Devi SG, Kanagalakshmi M & Pius A, Halochromic CMC strips for food quality detection. *Food Anal Methods*, (2025).

34 Bodea IM, Cătunescu GM, Pop CR, Fiț NI, David AP, Dudescu MC et al., Antimicrobial properties of cellulose films with herbal extracts. *Polymers*, 14 (2022) 1435.

35 Sendri N & Bhandari P, Anthocyanins: Biosynthesis and applications. *Phytochem Rev*, 23 (2024) 1913.

36 Tarmizi E, Putri AZE, Nusandari MA, Husnil YA, Saragih R & Lalasari LH, Identification of anthocyanin structure by FTIR. (2019).

37 Akbar SA & Hasan M, Evaluation of bioactive composition of macroalgae from Banda Aceh coast. *Sci Technol Asia*, (2024) 194.

38 Kaewprachu P, Jaisan C, Rawdkuen S, Tongdeesoontorn W, Karbowiak T, Degraeve P et al., Properties of CMC/anthocyanin films for food monitoring. *Carbohydr Polym Technol Appl*, (2025) 100877.

39 Soleimani Z, Baharifar H, Najmoddin N & Khoshnevisan K, CMC/gelatin hydrogel dressing for wound healing. *Gels*, 11 (2025) 38.

40 Rosales-Murillo SS, Sánchez-Bodón J, Hernández Olmos SL, Ibarra-Vázquez MF, Guerrero-Ramírez LG, Pérez-Álvarez L et al., Anthocyanin-loaded polymers as bioactive materials. *Polymers*, 16 (2024) 163.

41 Cristea AG, Lisă EL, Iacob S, Dragostin I, Ștefan CS, Fulga I et al., Antimicrobial smart dressings for wound care. *Pharmaceuticals*, 18 (2025) 825.

42 Alaysuy O, Snari RM, Alfi AA, Aldawsari AM, Abu-Melha S, Khalifa ME & El-Metwaly NM, Development of smart biochromic bandage using red cabbage extract. *Int J Biol Macromol*, 211 (2022) 390.

43 Basu P, Banerjee A, Okoro PD, Masoumi A, Kanjilal B, Akbari M et al., Integration of polymers and biosensors in wound healing. *Adv Healthc Mater*, 13 (2024) 2401461.

44 Moeinipour A, Afkhami A & Madrakian T, Development of smart polymer film for sensing applications. *Sci Rep*, 15 (2025) 31816.

45 Naser M, Nasr MM & Shehata LH, Smart wound dressings for advanced biomedical applications. (2024).