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Development and characterisation of biodegradable chitosan-herbal composite films for wound dressing applications

P Ganesan[1,a], T Karthik[1]& J Hema[2]

[1]Department of Textile Technology, [2]Department of Biotechnology, PSG College of Technology, Coimbatore 641 004, India

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This study aims to develop a novel herbal curative film for wound dressing by holding a combination of herbs (*Eugenia jambolana*, *Aerva lanata*), and chitosan, acting as a drug-releasing agent on the wound surface. The films are formed using a simple film-casting technique with citric acid as the solvent and glycerol as a plasticiser. Surface morphology is carried out using scanning electron microscopy, and the chemical composition is confirmed through Energy Dispersive X-ray Analysis (EDAX). The physical properties of the film are assessed using standard methods. Bioevaluation characteristics like antimicrobial efficacy are measured in terms of zone of inhibition against *Bacillus subtilis* and *Pseudomonas aeruginosa*. Additionally, microbial penetration, drug release activity, cytotoxicity, and contact dermatitis are analysed as per standard protocols. The results demonstrate that the developed films exhibit promising performance and potential in biodegradable wound dressing applications with desirable wound healing properties.

Keyword: Antimicrobial, Chitosan, Film, Herbs, Polymer, Wound dressing

1 Introduction

The rise of antibiotic-resistant bacteria continues to drive the expansion of the wound care market. In response, specialised wound dressings are being created through interdisciplinary research tailored to specific wound types[1]. The emergence of biomaterials has paved the way for new directions in the research on wound care and wound dressing materials.

Biopolymers have become a focal point in wound dressing research due to their natural origin and versatile properties. These materials are derived from a range of biological sources, including plants (e.g., pectins), animals (e.g., collagen, hyaluronic acid, and chitosan), fungi (e.g., chitin), bacteria (e.g., xanthan and gellan gum), and algae (e.g., alginate). Due to their natural origins, biopolymers exhibit drawbacks related to variations from batch to batch, potentially impacting the physicochemical properties and even the shelf life of the material. These discrepancies are contingent upon factors like the species from which the biopolymers are derived, environmental conditions (e.g., weather and soil conditions), the time of year they are harvested, and the methods employed for extraction and purification. Nonetheless, polymers derived from nature serve as significant and adaptable alternatives as raw materials for manufacturing wound dressings with diverse properties and functions. They occasionally add value to natural resources that would otherwise be discarded, such as the byproducts of marine food production, predominantly crustacean shells, which are utilised for chitosan production[2-5].

Chitin, an abundant and renewable biopolymer found in the shells of crabs, prawns and many crustaceans, can be enzymatically or chemically deacetylated to produce chitosan. Chitosan is a copolymer of deacetylated (D-glucosamine) and acetylated (N-acetyl-D-glucosamine) units of glucosamine linked by β-(1,4) glycosidic bonds[6-7]. Owing to its biocompatibility, low cytotoxicity, excellent biodegradability and antimicrobial activity, chitosan is widely used in the development of wound care products[8-12].

Eugenia jambolana (*syn. Syzigium cumini (L.)*), black plum or jamun, is widely used in traditional Indian medicine. Various plant parts, particularly the seeds are rich in phytochemicals, including acids, tannins and anthocyanins, glycosides, fats, resins, albumin, chlorophyll, and a range of phenolic and polyphenolic compounds including jambosine, gallic acid, ellagic acid, corilagin, etc., Dry seeds have been reported to contain 11.67 % alcohol soluble extractive, 3.397 % inorganic 40 % of water-soluble gummy fibre

[a]Corresponding author.
E-mail: ganeshg007@gmail.com

and 15 % of water-insoluble neutral detergent fibres[13-14]. In Ayurveda, jamun seed paste is applied on the burn wounds.

Aerva lanata (Mountain Knot grass, Local name – sirupulay) is another plant of medicinal significance. The plant contains various alkaloids, flavonoids and other phytochemicals like methyl grevillate, lupeol, lupeol acetate, benzoic acid, β-sitosteryl acetate and tannic acid[15-16].

Recent studies suggest that combining chitosan and herbal extracts can accelerate wound healing. The use of biofilms to cover or treat wounds has a long-standing history and remains an essential area within medical textiles. Textile materials and products that have been engineered to meet particular needs in medical applications should have a combination of properties pertaining to strength, flexibility, biocompatibility, biodegradability, non-toxic, antimicrobial, oxygen permeable and controlled drug delivery[17-22].

In this study, a biodegradable wound dressing is developed based on chitosan blended with extracts of *E. jambolana* and *A. lanata*. The objective is to create biofilms that facilitate sustained drug release at the wound site, thereby enhancing healing outcomes.

2 Materials and Methods

Medium molecular weight chitosan, with a degree of deacetylation (DA) greater than 75 %, was procured from Sigma-Aldrich Chemicals Ltd. (Bangalore, India). *E. jambolana* and *A. lanata* were sourced from natural organic suppliers. Citric acid, glycerol and polystyrene petri dishes were purchased from Sigma-Aldrich.

2.1 Preparation of Solution

A 1 % chitosan solution (CS) was prepared by dissolving 1 g of chitosan powder 100 mL of 2 % (w/v) citric acid. The solution was heated at 60–70 °C for 30 min with continuous stirring, then allowed to cool to room temperature (25°–28° C) prior to use[22].

Herbal extracts were prepared by mixing 10 g of finely ground *E. jambolana* and *A. lanata* powder with 100 mL of distilled water and allowed to stand for 24 h. The supernatant was collected and stored at 4°C for further use.

2.2 Preparation of CS/Herbal Blended Film

A simple film casting technique was used to prepare the biodegradable blended films. 100 mL of 1 % (w/v) chitosan and herbal solutions were mixed homogeneously. The film casting solutions were poured on the 10 × 2 cm polystyrene Petri dish before drying in an oven at 40°C for 24 h to obtain blended films. The films were carefully removed from the plate and stored in desiccators for future use.

2.3 Physical Characterisation

The thickness of the chitosan-herbal blended film was measured using a micrometre (least count of 0.001mm) at five locations (centre and four corners) according to the ASTM D-882 standard. The mean thickness was recorded. Five samples (2.0 x 2.0 cm) from each film were weighed as per the ASTM D-882 on electronic balance. The mean weight was calculated.

For swelling percentage, the chitosan-herbal blended film was cut into 1.0 x 1.0 cm samples and dried in a vacuum at 25°–28°C for a week. The weight of the samples was measured, and then they were soaked in 50 mL of distilled water at 37°C for 72 h. The swollen films were gently blotted and reweighed.

$$\text{Swelling \%} = [(W_S - W_D) / W_D] \times 100$$

where W_S and W_D are the weights of the swollen and dry films (g), respectively.

The flexibility of the film was essential to handle the film easily and for the comfortable, secured application of film on the wound. The Folding endurance test was performed to determine flexibility. It was determined by repeatedly folding a film sampleat the same place until it broke or reached 300 folds manually. The number of folds without breaking was recorded as the folding endurance[23].

2.3.1 Degradation Properties

Dried samples were weighed (W_o) before the experiment. Then, the samples were immersed in methanol at a film area-to-volume ratio of 0.1 cm^{-1} for set durations, washed with deionised water, and dried at 40 °C. Final weights (W_f) were used to calculate the degradation index (D_i) based on the mass loss using the below equation (Eq. 1):

$$\%\ Degradation\ (D_i) = \frac{(W_o - W_f)}{W_f} \times 100$$

Where W_O and W_f are the initial and final weights of the films (g).

2.3.2 Porosity

The porosity (P) of porous films was determined by dissolving the films in ethanol at 25–28°C. The cut-out

porous film was placed in a scaled test tube with the media (ethanol) until the sample was fully immersed without air bubbles on the surface. The porosity of porous films was calculated following the Eq. 2:

$$P = (V1 - V3 \,/\, V2 - V3)X100$$

where P is porosity; V1, initial volume of ethanol; V2, volume of ethanol after film immersion; V3, volume of ethanol after the film removal.

2.3.3 Water Vapor Transmission Rate

The desiccant method was used to measure the water vapor transmission rate as per the standard ASTM E 96-95. The test specimens were cut and placed on top of open bottles containing 5 g of silica gel. These bottles were held in place with a screw lid (test area: 4.9 cm^2). The bottles were conditioned in the desiccator containing silica gel for 12 h. Then, the bottles were placed in a desiccator containing NaCl at 30° C and 75% relative humidity. The bottles were weighed at 6, 12 and 24 h, and the water vapor penetration rate was calculated as per the following equation:

$$WVTR\ in\ g/m^2/hour = \frac{G}{T \times A}$$

where G is change in silica gel weight (g); A, test area (m^2); T, time (h) during which G occurred.

2.4 Surface Characterisation

2.4.1 Scanning Electron Microscopy

The surface morphology of the chitosan-herbal extract blended film was examined by Scanning Electron Microscope (JEOL, JSEM-6390LV, Japan). Dry chitosan-herbal extract blended film were cut into pieces and used as samples. Samples were gold coated by mounting on the SEM sample stab with the help of a double-sided sticking tape. Then, samples were observed under an accelerating voltage of1.2 kV, and photomicrographs of suitable magnifications were obtained.

2.4.2 Energy Disperse Spectroscopy

Energy Disperse Spectroscopy (EDAX) provides energy disperse X-ray spectroscopy microanalysis, electron backscatter diffraction and micro X-ray fluorescent systems. Energy Disperse Analysis Spectroscopy is an analytical technique used for elemental composition analysis based on X-rays emitted by the sample when an electron beam is bombarded on it.

2.5 Bio Evaluation of Film

2.5.1 Antimicrobial Activity (Agar Diffusion Method, AATC 100 Quantitative Method)

50 bacterial cultures (*Pseudomonas aeruginosa* and *Bacillus subtilis*) were prepared in nutrient broth and incubated overnight at 37 °C. Nutrient agar plates were inoculated with each bacterium. Control and test samples (18 mm diameter) were placed on the plates, followed by incubation at 37 °C for 24 h. Zones of inhibition were measured.

2.5.2 Contact Dermatitis Test

To determine the allergenic property of the film, a contact dermatitis patch test was performed. The sensitivity and specificity of the test were 70-80 %. The samples (25 cm x 25 cm) were placed on the patch of hairless skin of the outer upper arm of human subjects for 24 h after obtaining their consent. A patch and prick test were evaluated after 20 min of application to avoid adverse effects. The samples were considered allergenic if they caused itching or rashes. The results were observed and reported as per the International Contact Dermatitis Research Group (ICDRG) given in Table 1.

2.5.3 In Vitro Drug Release Test

Film samples were placed in dialysis tubes and immersed in 100 mL deionised water. Samples were stirred continuously using a magnetic stirrer, and 5 mL aliquots were withdrawn at 10 min intervals, replacing it with fresh water. The percentage release of drugs was estimated using a UV-Vis spectrophotometer at 280 nm. All tests were performedin triplicates with different areas of films to find the content uniformity.

2.5.4 In Vitro Cytotoxicity Test

In vitro cytotoxicity test was performed as per ISO 10993-5 using the direct contact method on L929 cells. After 24 h incubation, morphological changes were assessed microscopically in both test samples

Table 1 — Grade notation attributes of contact dermatitis test

Grade	Attributes
Extreme positive (+++)	Coalescing vesicles bullous reaction
Strong positive (++)	Erythema, papules, infiltration
Weak positive (+)	Erythema, infiltration, discrete papules
Irritant (IR)	Discrete, patchy follicular or homogeneous erythema with no infiltration
Doubtful (?)	Faint macular or homogeneous erythema with no infiltration
Negative (-)	No signs of irritation or erythema

and control. The supernatant culture medium was discarded, and as per qualitative means of screening, the sample and control were examined using cytochemical staining. The changes in vacuolization, detachment, general morphology, and membrane integrity and cell lysis were assessed. The report was depicted as per grades 0, 1, 2, 3, and 4 based on reactivity like none, slight, mild, moderate, and severe, respectively.

2.5.5 Microbial Penetration Test

The ability of the film to prevent microbial penetration was tested using this method. The films were placed on 10 mL open vials. The vials contained 5 mL of nutrient broth and held in place with a screw lid. Along with the sample in one vial, a vial closed with a tightly packed cotton ball was used as a negative control and an open vial was used as a positive control. All the tested vials were placed in an open environment for one week. The cloudiness of the nutrient broth was recorded as microbial contamination.

3 Results and Discussion

3.1 Physical Characterisation

The physical properties of chitosan-herbal extract blended films are presented in Table 2. The thickness of the prepared film ranges from 0.21 to 0.34 mm, with a maximum thickness of 0.34 mm observed for the CS/*E. jambolana* (2:1) formulation. A notable increase in thickness is observed due to the addition of herbal extracts, indicating that the addition of herbal extracts contributes to structural density. Using the same glass plate and an equal volume of the prepared solutions ensured uniformity in film formation and minimises deviation in thickness.

The mass per unit area of the film lies between 0.21 to 0.28 g. Similar to thickness, this parameter increases with herbal extract content. Chitosan-herbal extract blended films exhibited a mass per unit area ranging between 0.23 to 0.28 g/sq. cm, respectively. The consistency in film preparation methods implies that any variation in mass is directly attributable to the compositional ratio of chitosan and herbal extracts.

The highest folding endurance (516 folds) is observed in CS/*E. jambolana* (1:1) films. This indicates that the addition of glycerol (plasticiser) significantly increases folding endurance and improves the flexibility of the film. Flexibility is an essential property of wound dressing as it influences the ability to conform to the dressing. So, the film integrity with skin folding is considered very good when applied to the wound surface[24].

3.1.1 Degradation Properties

Degradation studies indicate that all films show rapid mass loss within the first 30 min of immersion in organic solvent, followed by a slower rate. CS/*A. Lanata* (1:1) blended films exhibit the highest degradation percentage (85 %), whereas the pure chitosan film films degrade by 43 %. The higher herbal content appears to facilitate breakdown, potentially due to increased porosity and decreased crosslinking density.

3.1.2 Porosity

The porosity of the blended films increases with herbal content. CS/*A. lanata* (1:1) blended films show the highest porosity (33%), which correlates with the observed increase in degradation (Table 2). The herbal content may play a role in developing the pore gaps in the film surface and core level. This could be the reason for the higher degradation % observed in these samples.

3.2 Surface Morphology of Chitosan-Herbal Blended Films

SEM analysis reveals that pure chitosan films possess a smooth and uniform surface (Fig. 1a). In contrast, CS/*E. jambolana* and CS/*A. lanata* blended films (Figs 1 b & c) show rough surfaces with irregular bulges and embedded materials, indicating the presence of herbal contents.

Table 2 — Physical properties of CS/Herbal blended films

Film type	Thickness, mm	Mass per unit area, g/sq. cm	Folding endurance	Degradation, %	Porosity, %
Chitosan	0.24 ± 0.03	0.20 ± 0.013	585 ± 5.8	43 ± 0.96	13 ± 0.74
CS/*E. jambolana* (1:1)	0.28 ± 0.04	0.23 ± 0.016	516 ± 6.4	68 ± 0.89	15 ± 0.68
CS/*E. jambolana* (1:2)	0.29 ± 0.03	0.27 ± 0.015	312 ± 4.9	54 ± 0.73	18 ± 0.59
CS/*E. jambolana* (2:1)	0.34 ± 0.02	0.28 ± 0.012	132 ± 5.9	45 ± 0.88	20 ± 0.66
CS/*A. lanata* (1:1)	0.25 ± 0.05	0.21 ± 0.018	154 ± 6.2	85 ± 0.92	33 ± 0.52
CS/*A. lanata* (1:2)	0.29 ± 0.03	0.26 ± 0.016	147 ± 7.1	83 ± 0.95	30 ± 0.65
CS/*A. lanata* (2:1)	0.30 ± 0.03	0.27 ± 0.016	120 ± 5.9	80 ± 0.91	28 ± 0.78

** Values indicate mean* ± SD

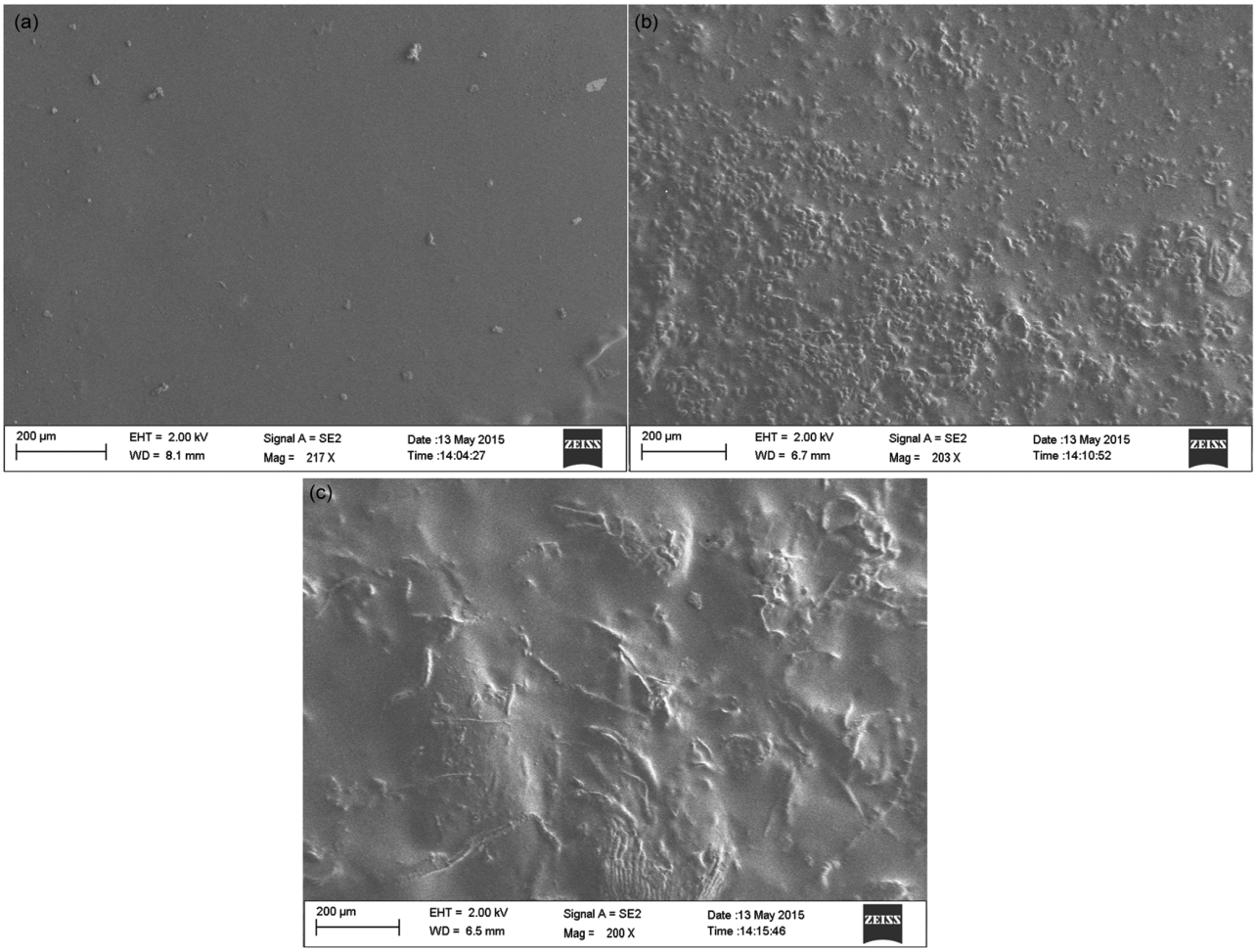

Fig. 1 — SEM images of (a) chitosan film, (b) CS/*E. jambolana* film and (c) CS/*A. lanata* film

3.3 Energy Dispersion Analysis of Bio Films (EDAX)

Fig. 2 shows the EDAX spectrum confirming the elemental composition of the films. The spectrum of the chitosan film (Fig. 2a) shows carbon and oxygen (C-Carbon and O-Oxygen) peaks, consistent with the polymer structure. CS/*E. jambolana* films (Fig. 2b) contain additional elements such as calcium and sodium (Ca – Calcium and Na – sodium), while CS/*A. lanata* films (Fig. 2c) exhibit potassium, magnesium, silicon, and chlorine (K-Potassium, Mg-Magnesium, Si-Silicon and Cl-chlorine). These reflect the herbal constituents incorporated into the films.

3.4 Fluid Handling Properties

3.4.1 Swelling Percentage

The water absorption capacity of the film is of utmost importance when they are used for wound healing applications. All chitosan-herbal blended films exhibit improved swelling (20 – 34 %) compared to chitosan alone (Table 3, Fig. 3). Chitosan has good moisture absorption ability; it absorbs water and continuously vaporises it through the film to the environment[25-26].

3.4.2 Water Vapour Transmission Rate

WVTR is measured under steady-state conditions, considering the contribution of the moisture absorbed by the film as negligible. As stated in the literature[27], water vapour transmission varies inversely with thickness due to the mass difference. The water vapour transmission for chitosan herbal film ranges between 1600 g/m^2/day – 2000 g/m^2/day (Table 3), which is comparable to the commercial wound dressing. WVTR of chitosan and CS/*E. jambolana* 2:1 film shows WVTR of 2000g/m^2/day and 1850 g/m^2/day respectively. It is also observed that WVTR increases faster between 6-12 h than between 12- 24 h.

Water vapour transmissions in chitosan film are attributed to many factors. Due to the addition of glycerol as a plasticizer, inter-chain spacing may be

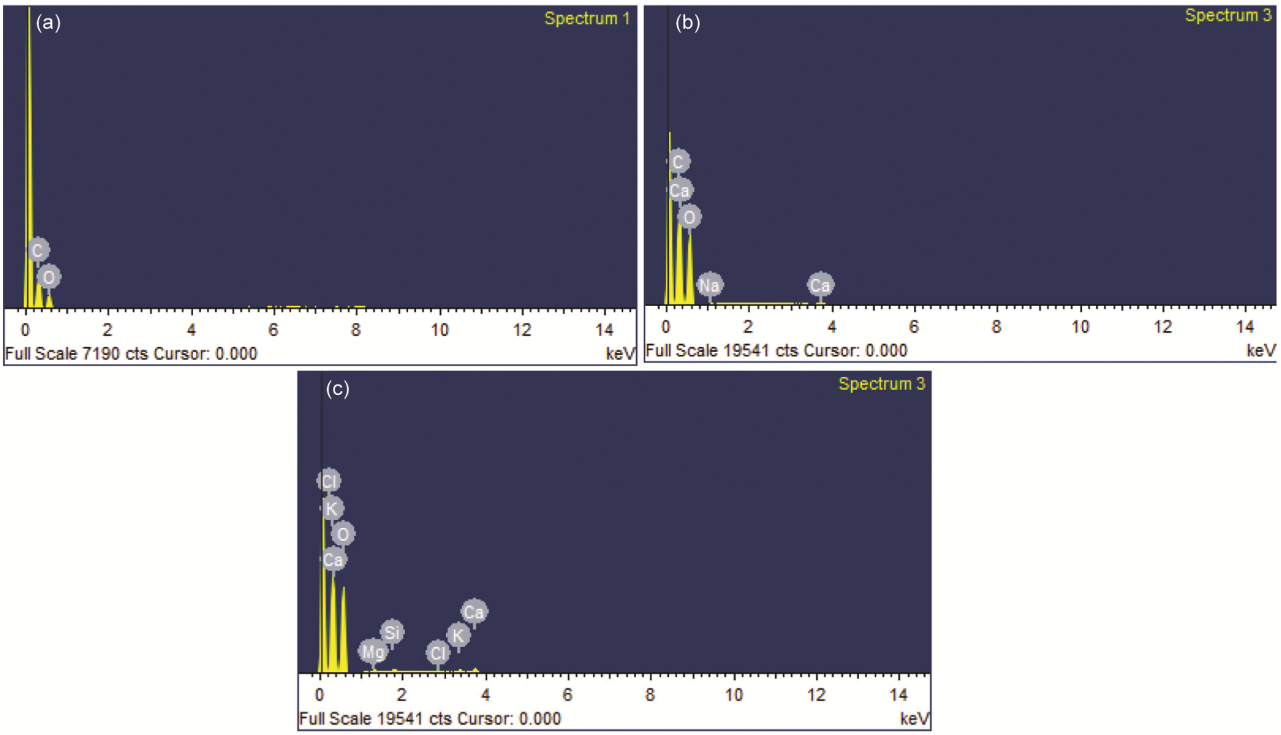

Fig. 2 — (a) EDAX spectra of (a) chitosan, (b) CS/*E. jambolana* and (c) CS/*A. lanata* films

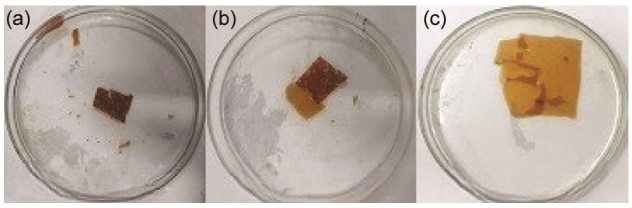

Fig. 3 — Swelling performance of chitosan-herbal blended films (a) 1 h, (b) 2h and (c) 12 h

Table 3 — Fluid handling properties of chitosan-herbal blendedfilm

Type of film	Swelling ratio, %	Water vapour transmission rate, g/m^3/day
Chitosan	12	2000
CS/*E. jambolana* (1:1)	25	1700
CS/*E. jambolana* (1:2)	30	1800
CS/*E. jambolana* (2:1)	34	1850
CS/*A. lanata* (1:1)	20	1600
CS/*A. lanata* (1:2)	23	1600
CS/*A. lanata* (2:1)	24	1700

increased. This reduces the intermolecular interactions when the glycerol molecules are added to the polymer network. Hence, water molecules diffuse through the film while glycerol acts as a humectant and enhances the water-holding capacity of the polymer matrix[28]. Chitosan has good moisture absorption ability, as it absorbs water and vaporises it through the film to the environment continuously.

Table 4 — Antimicrobial activity of chitosan-herbal blended films

Type of film	Zone of inhibition, mm	
	B. subtilis	*P. aeruginosa*
Chitosan	21	20
CS/*E. jambolana* (1:1)	23	23
CS/*E. jambolana* (1:2)	26	21
CS/*E. jambolana* (2:1)	29	22
CS/*A. lanata* (1:1)	22	21
CS/*A. lanata* (1:2)	25	23
CS/*A. lanata* (2:1)	27	22

3.5 Microbiological Evaluation

3.5.1 Antimicrobial Activity

The agar diffusion test confirms that all blended films exhibit antimicrobial activity against *B. subtilis* and *P. aeruginosa* (Table 4). The CS/*E. jambolana* 2:1 blended film demonstrates the highest zone of inhibition of 29 mm against *B. subtilis*. The CS/*E. jambolana* (1:1) and CS/*A. lanata* (1:2) blended films show maximum zone of inhibition of 23 mm against *P. aeruginosa* (Table 4).

3.5.2 Contact Dermatitis Test

Inferences of contact dermatitis tests are tabulated in Table 5. No allergic reaction is indicated by negative (-), mild reaction is indicated by doubtful (?), and moderate allergic reaction is indicated by (IR).

Table 5 — Contact dermatitis test of chitosan-herbal blended films

Subject	Sample					
	CS/*E. jambolana* 1:1	CS/*E. jambolana* 1:2	CS/*E. jambolana* 2:1	CS/*A. lanata* 1:1	CS/*A. lanata* 1:2	CS/*A. lanata* 2:1
01 (Male, 25 Y)	-	-	-	-	-	-
02 (Male, 23 Y)	-	-	-	?	?	?
03 (Male, 23 Y)	IR	-	-	IR	-	-
04 (Male, 19 Y)	-	-	-	-	-	-
05 (Male, 20 Y)	-	-	-	-	-	-
06 (Male, 19 Y)	IR	-	-	-	-	?
07 (Male, 21 Y)	-	-	?	-	?	-
08 (Male, 19 Y)	-	-	-	-	-	-
09 (Male, 20 Y)	-	-	-	-	-	-
10 (Male, 21 Y)	?	-	?	-		

(*– = No reaction, ? = Doubtful, IR = Irritant reaction*)

The results reveal that one subject experienced an allergic irritant reaction to three films, and the conclusion may be that the subject's skin is too sensitive. In contrast, the films having citric acid as a solvent show allergic irritant reactions and doubtful in the case of the three subjects. This might be because of the residual traces of citric acid in the film even after thorough washing. The irritant reaction subsided and became doubtful with the addition of herbal components. The allergy diminishes with increased herbal content, suggesting that herbal extracts may modulate skin irritation. It can be interpreted that citric acid may cause skin allergies in people with sensitive skin[29,30].

3.5.3 *In Vitro Drug Release Test*

The release of the drug from the chitosan-herbal extract films is shown in Figure 4. It shows a continuous but slow release of the active herbal constituents from the film, indicating that the blending of chitosan does not interfere with drug release. This controlled release profile supports the suitability of the films for prolonged wound coverage and sustained antimicrobial action.

3.5.4 *In Vitro Cytotoxicity Analysis*

As per ISO 10993:5, achieving a numerical grade of more than 2 is considered a cytotoxicity effect. Figure 5 shows the cytotoxicity test before and after the staining of developed films.

The control films (chitosan film) show no cytotoxic reactivity to fibroblast cells, while the chitosan-herbal blended films exhibit slight cytotoxic reactivity after 24 h of contact. Since all the blended films achieve a numerical grade not greater than 2, the samples are considered non-cytotoxic. As expected, the control samples showed no cytotoxic response[31-34].

Table 6 — Microbial penetration test

Type of film	Microbial contamination	
	Positive control	Negative control
Chitosan (Control sample)	Yes	No
CS/*E. jambolana* (1:1)	Yes	No
CS/*E. jambolana* (1:2)	Yes	No
CS/*E. jambolana* (2:1)	Yes	No
CS/*A. lanata* (1:1)	Yes	No
CS/*A. lanata* (1:2)	Yes	No
CS/*A. lanata* (2:1)	Yes	No

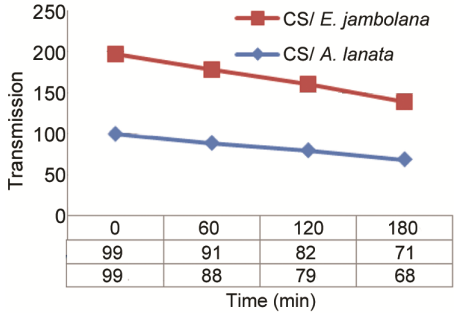

Fig. 4 — *In vitro* drug release profile of chitosan-herbal blended films

3.5.5 *Microbial Penetration Test*

Microbial penetration tests (Table 6) show no microbial contamination in the test sample or the negative control tubes. Only positive controls exhibit turbidity, indicating that the films effectively prevent microbial infiltration and thus possess good potential to be used in wound dressings because of their ability to bind the negatively charged bacteria to the positively charged amino groups of the chitosan polymer and herbs by reducing the primary wound contamination. Hence, the protection of a wound from secondary bacterial infection can be achieved[35].

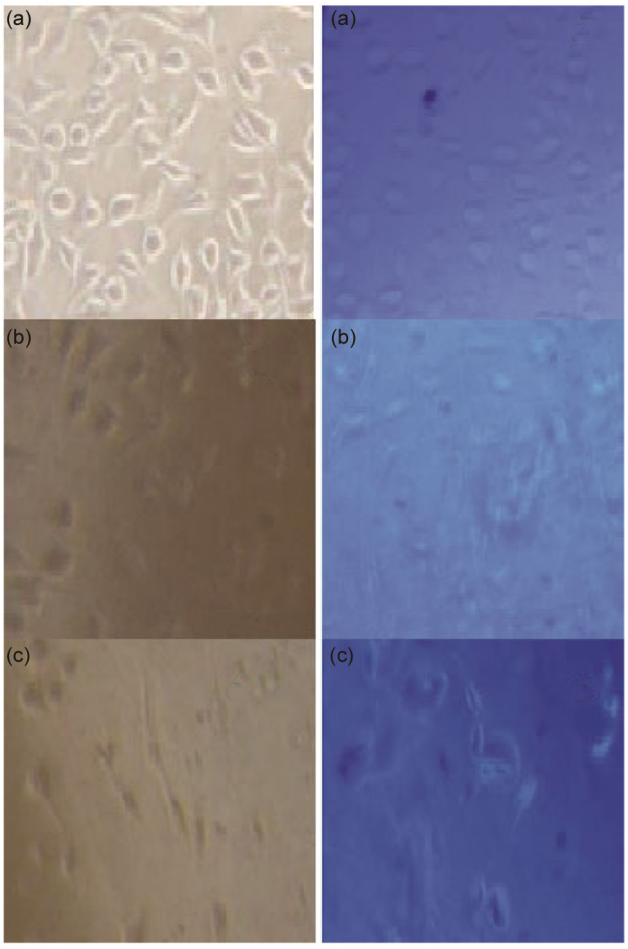

Fig. 5 — Cytotoxicity test before (left panel) and after (right panel) staining (a) control, (b) CS/*E. jambolana*and (c) CS/*A. lanata*

4 Conclusion

This study demonstrates the successful development of biodegradable chitosan–herbal blended films incorporating *E. jambolana* and *A. lanata* extracts for potential wound dressing applications. The films exhibit desirable physical and functional properties, including adequate thickness, flexibility, porosity, and degradation behaviour. Surface morphology and EDAX analysis confirm the presence of chitosan and herbal groups in the biofilms. The films show effective antimicrobial activity against *B. subtilis* and *P. aeruginosa*, with no significant cytotoxicity observed against fibroblast cells. Contact dermatitis tests indicate minimal to no allergenic response, while microbial penetration tests confirm the films barrier function. Additionally, sustained drug release from the films supports their potential for controlled topical delivery. This indicates that the developed composite films are promising for safe and effective wound dressings.

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