



FORMULATION AND EVALUATION OF CHITOSAN-BASED WOUND HEALING FILM CONTAINING CROTON BONPLANDIANUM EXTRACT

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ABSTRACT:

Wound healing is a coordinated biological process involving haemostasis and inflammation, proliferation, and tissue remodeling. An effective wound dressing should provide a protective barrier, maintain a suitable environment for healing, possess adequate flexibility and mechanical strength, and ideally provide antimicrobial activity. The present study focused on the formulation and evaluation of a herbal wound-healing film containing *Croton bonplandianum* leaf extract using chitosan as the main film-forming polymer. *Croton bonplandianum* was selected because the submitted report describes its traditional use for cuts, wounds, skin disorders and infections and reports wound-healing, antimicrobial, anti-inflammatory and antioxidant activities [1,2,3,4,5,6]. The leaves were shade dried, powdered and extracted using ethanol: water (7:3). Preliminary phytochemical screening and IR spectroscopic analysis were performed. The extract was incorporated into chitosan/PVP films with glycerol as plasticizer by the solvent-casting method. Four formulations (F1–F4) were prepared by varying chitosan and PVP concentrations. F4 showed the most satisfactory physical characteristics and the highest zone of inhibition among the formulations, measuring 2.8 cm against *Staphylococcus aureus*, compared with 3.0 cm for the tetracycline pellet. The optimized film showed a thickness of 0.290 mm, surface pH 5.9, folding endurance >500 folds, 23% elongation at break and 100% flatness. The findings support the potential of *Croton bonplandianum* extract in a chitosan-based herbal wound dressing, while further safety, efficacy, release and clinical investigations are required.

1. Introduction:

Herbal medicines have been used since ancient times and remain important sources of bioactive compounds. The submitted report describes a continuing interest in plant-based medicines because of their availability, affordability and potential pharmacological actions. Medicinal plants contain constituents such as alkaloids, flavonoids, glycosides, tannins, terpenoids and phenolic compounds that may

contribute to antimicrobial, antioxidant and anti-inflammatory effects [17,18].

Wound healing restores damaged tissue through overlapping phases of haemostasis/inflammation, proliferation and remodeling. Disturbance of these phases may lead to infection or chronic non-healing wounds. An ideal dressing should be biocompatible, provide a physical barrier against microorganisms,

permit gas exchange, maintain appropriate hydration, accommodate wound movement and possess adequate mechanical strength and flexibility [11].

Film dressings provide a thin protective layer over superficial wounds and can offer visibility, flexibility, barrier protection and a moist healing environment [11,14]. Chitosan is particularly attractive as a wound-dressing polymer because the project report attributes antimicrobial, tissue-regenerative and haemostatic functions to it [10,15]. PVP was used to improve film flexibility and adhesion, while glycerol acted as a plasticizer [13]. Croton bonplandianum extract was incorporated as the herbal active component.

Croton bonplandianum Baill. (Euphorbiaceae), a common weed traditionally known to arrest bleeding from fresh cuts, was selected for the present study because of its well-documented antimicrobial, anti-inflammatory, and wound-healing properties [1,2,3,4,5]. Its extract was combined with chitosan, a biocompatible, haemostatic, and antimicrobial biopolymer [10,15], to develop a low-cost herbal wound-healing film, with polyvinylpyrrolidone (PVP) and glycerol used to impart flexibility [13].

Croton bonplandianum Baill., commonly known as Ban Tulasi or railway weed, is an erect annual herb of the family Euphorbiaceae, native to South America and widely naturalised along roadsides and wastelands in India [1,2]. The plant grows 30–60 cm tall with pubescent, branched stems, alternate lanceolate leaves, and terminal racemes bearing minute unisexual flowers; injury to any part releases a milky latex that is traditionally applied to stop bleeding from cuts and wounds [1,2]. Ethnomedicinally, the leaves and latex are used for wounds, skin infections, and inflammation, while the roots and seeds are used for gastrointestinal and hepatic disorders [1,2]. Phytochemical studies report alkaloids, flavonoids, tannins, saponins, triterpenoids (ursolic and oleanolic acids), and β -sitosterol in its leaves, stem, and latex - constituents that underlie its antimicrobial, anti-inflammatory, and antioxidant activity and justify its selection for the present formulation [2,6,7,8,9,19].

The present study aimed to formulate and evaluate a chitosan-based wound-healing film incorporated with Croton bonplandianum leaf extract. The study involved the collection and extraction of Croton bonplandianum leaves, preliminary phytochemical

screening and IR characterisation of the extract, and formulation of biodegradable wound-healing films using chitosan and PVP as film-forming polymers with glycerol as a plasticiser by the solvent-casting method. The prepared films were evaluated for their physicochemical and mechanical properties, including thickness, surface pH, folding endurance, flatness, and percentage elongation at break. The antimicrobial activity of the plant extract and formulated films against Staphylococcus aureus was also assessed, and the formulations were compared to identify the optimised formulation for wound-healing application.

2 Materials and Methods:

Croton bonplandianum Baill plant extract used as drug sample. It is collected from Parikallpattu(village), Tiruvannamalai (Dt). It is authenticated by Dr. J. Suresh Kumar, Associate Professor in department of botany at Kalaignar Karunanidhi Govt. of arts and science college, Tiruvannamalai-606 603. The chemicals used in the study includes Acetic acid (1%), Chitosan, PVP (Poly-vinyl-pyrrolidone), Ethanol, Glycerol and Distilled water.

Methods:

Preparation of plant extract:

Fresh leaves were collected, cleaned and shade dried at room temperature for several days. The dried leaves were mechanically powdered. Fifty grams of powdered leaf material were soaked in 500 mL of ethanol:water (7:3) for 5–7 days at room temperature with periodic shaking. The extract was filtered through Whatman No. 41 filter paper and ethanol was evaporated on a hot plate to obtain the plant extract [17,18].

Phytochemical screening and IR analysis:

Preliminary screening was performed for alkaloids, glycosides, flavonoids, phenolic compounds, tannins, saponins and triterpenoids using the tests described in the project report [17,18]. IR analysis was used to identify characteristic functional groups. The reported bands included 3200–3500 cm^{-1} for O–H stretching associated with phenolic/tannin constituents, 3000–2850 cm^{-1} for C–H stretching associated with alkanes/terpenoids, 3250–3400 cm^{-1} for N–H stretching associated with alkaloids, and 1000–1320 cm^{-1} for C–O stretching associated with ether/flavonoid groups.

Preparation of chitosan solution:

A 1% v/v acetic acid solution was prepared by measuring 0.1 mL acetic acid and making the volume up to 10 mL with distilled water. Chitosan was added slowly with continuous stirring to prevent lump formation. The solution was stirred for 4–6 hours until a clear homogeneous viscous solution was obtained; mild heating at 40–50°C was used to enhance dissolution [10,13,15].

Preparation of wound healing film by casting method:

The measured chitosan solution was placed in a clean beaker. PVP was separately dissolved in distilled water and added slowly to the chitosan solution under continuous stirring. Four drops of glycerol were added as plasticizer. Croton bonplandianum extract (0.400 g) was separately dissolved in 1 mL distilled water and added slowly to the polymer mixture. The final volume was adjusted to 20 mL with distilled water. The bubble-free solution was cast onto a clean, levelled Petri dish and dried at room temperature or in a hot-air oven at 40–45°C [14,20]. The dried film was peeled carefully and stored in a desiccator.

Antimicrobial assay:

Antimicrobial activity was evaluated by agar well/disc diffusion against the selected test organism, with *Staphylococcus aureus* reported in the results [16]. The clear inhibition zone surrounding the standard, plant extract and formulations was measured in centimeters. Percentage inhibition of the plant extract was also estimated from absorbance values using the control and test absorbance.

Serial dilution:

Blank: Add 1.5 g of Beef extract, 2.5 g of Peptone, 2.5 g of Sodium chloride and make up for 500ml with distilled water. Autoclave the medium at 121°C for 15 minutes under psi pressure then cool down the medium at 45-50 °C. Then incubate at 37 °C for 48 hours & Measure absorbance at 254nm.

Control: Add 10ml of nutrient broth, 0.5ml of Micro-organism (*staphylococcus*). Then incubate at 37 °C for 48 hours and measure the absorbance at 254nm.

Standard: Add 10 ml of nutrient broth solution, 0.5 ml of micro-organism (*staphylococcus*), 1ml of 10mcg

tetracycline solution. Then incubate at 37 °C for 24-48 hours and measure the absorbance at 254nm.

Test: Add 10 ml of nutrient broth solution, 0.5 ml of micro-organism (*staphylococcus*), 100mcg of plant extract. Then incubate at 37 °C for 24-48 hours and measure the absorbance at 254nm. This procedure can be conducted to all other various concentrations (100mcg, 200mcg, 300mcg, 400mcg, 500mcg, 600mcg, 700mcg, 800mcg, 900mcg, 1000mcg).[16]

Calculation:

Percentage (%) inhibition of anti-microbial activity by using the plant extract of *Croton bonplandianum* Baill. can be calculated by [16]

$$\% \text{ Inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100$$

Film evaluation:

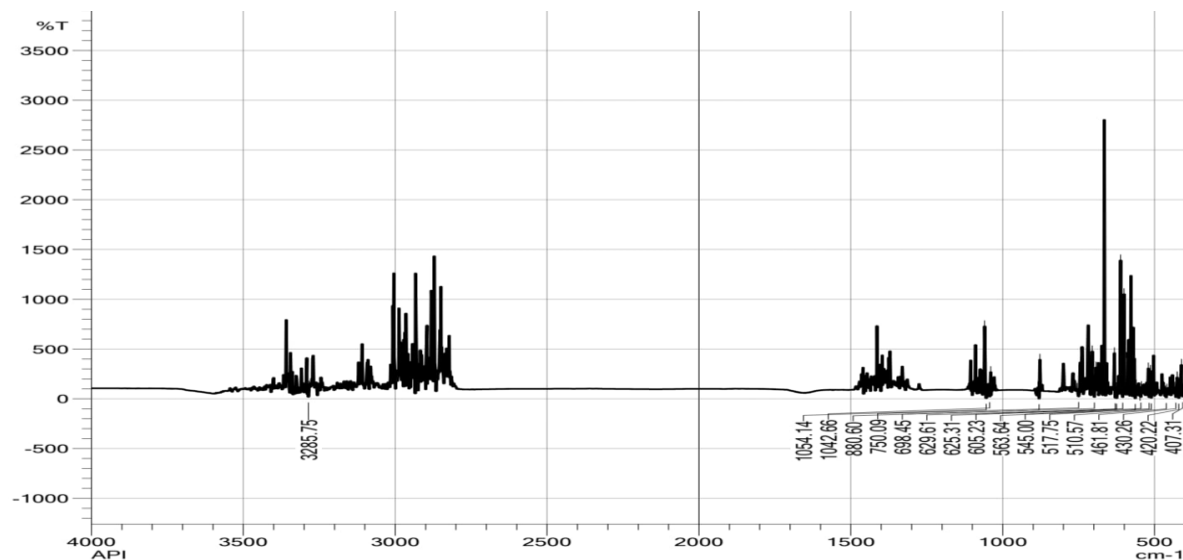
Physical appearance was assessed for shape, elasticity, flexibility, stickiness, smoothness and visible imperfections [12,13]. **Thickness** was measured at different positions and averaged [12,14]. **Surface pH** was determined after equilibration of the film in distilled water for one hour at 25°C [12,14]. **Folding endurance** was determined by repeatedly folding a 2 × 2 cm strip at the same position until breakage [12,14,15]. **Flatness** was assessed using longitudinal strips cut from different sections of the film. Initial and final lengths were measured, and percentage constriction was calculated. The report defines 100% flatness as zero percentage constriction [12,14]. **Percentage elongation at break** was determined by stretching a film strip until break and recording the change in length [12,14].

3. Results and Discussion:**Phytochemical screening:**

The positive reactions indicate the presence of several classes of phytoconstituents in the *Croton bonplandianum* extract. The report discusses these constituents as possible contributors to the observed antioxidant, antimicrobial and anti-inflammatory potential [2,6,7,8,9,19].

Table 01: Phytochemical screening

Constituent	Tests reported	Inference
Alkaloids	Dragondroff's; picric acid	Positive
Flavonoids	Lead acetate; ferric chloride	Positive
Glycosides	Legal's; aqueous NaOH	Positive
Phenolic compounds	Gelatin; lead acetate	Positive
Tannins	Bromine water; lead subacetate	Positive
Saponins	Foam test	Positive
Triterpenoids	Salkowski's test	Positive

IR spectroscopic method:**Fig 01: The IR spectrum of Croton bonplandianum. Baill plant was taken.****Table 02: IR interpretation**

S.no	Frequency	Mode	Functional group	Phytoconstituents
01	3200-3500	OH stretch	Phenol	Tannins
02	3000-2850	C-H stretch	Alkane	Terpenoids
03	3250-3400	N-H stretch	Amine	Alkaloids
04	1000-1320	C-O stretch	Ether	Flavonoids

Formulation table:**Table 03: Composition formulation developed in four batches [F1-F4]**

Ingredient	F1	F2	F3	F4
Croton bonplandianum extract	0.4 g	0.4 g	0.4 g	0.4 g
Chitosan	0.09 g	0.09 g	0.27 g	0.27 g
PVP	0.8 g	1.0 g	0.8 g	1.0 g
Glycerol	1.8 mL	1.8 mL	1.8 mL	1.8 mL
Water	q.s.	q.s.	q.s.	q.s.

Fig 02: Formulation 1



Fig 03: Formulation 2

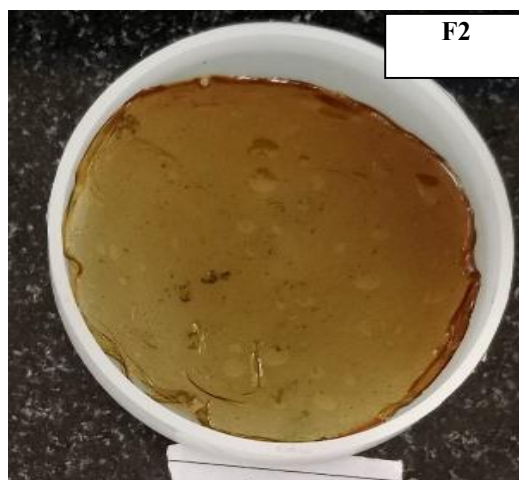


Fig 04: Formulation 3

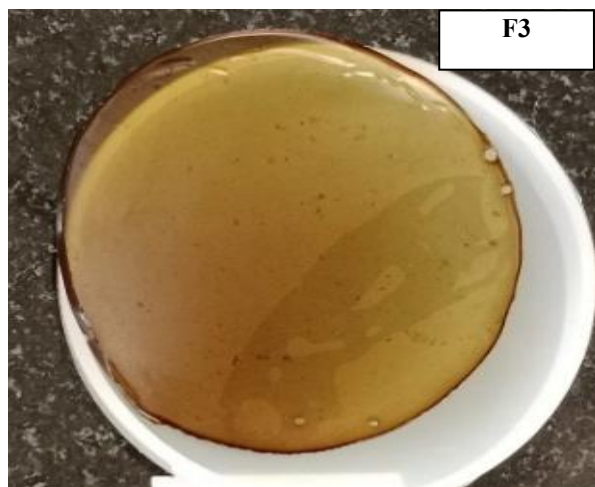
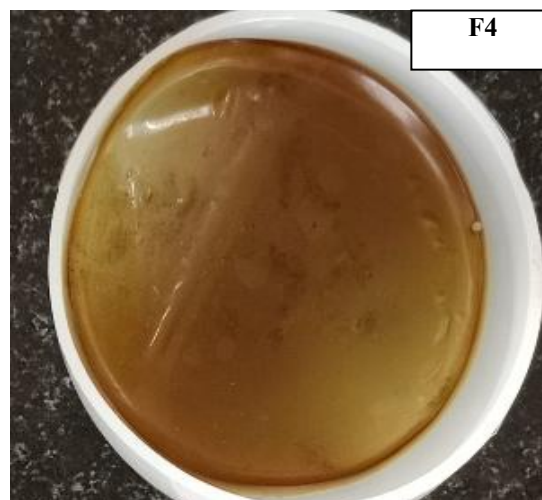


Fig 05: Formulation 4



Antimicrobial activity of formulations:

Table 04: Zone of inhibition interpretation.

Sample	Zone of inhibition (cm)	Interpretation
Tetracycline pellet	3.0	Strong activity
Standard solution	2.6	Moderate activity
Plant extract solution	1.5	Weak activity
F1	0	No activity
F2	0	No activity
F3	2.0	Moderate activity
F4	2.8	Strong activity

Among the four film formulations, F4 produced the highest inhibition zone (2.8 cm), approaching the tetracycline pellet (3.0 cm). F1 and F2 showed no measurable inhibition, whereas F3 showed moderate activity. The results were interpreted in the report as indicating F4 as the optimized formulation for antimicrobial performance.

Fig 06: standard zone of inhibition

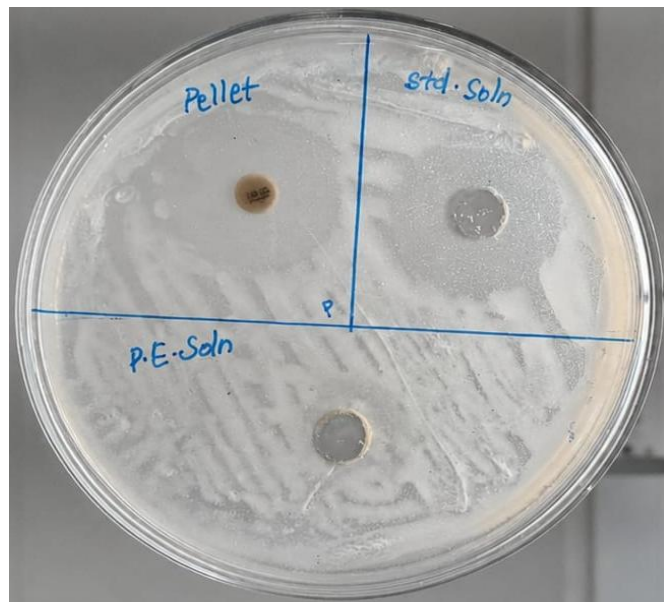
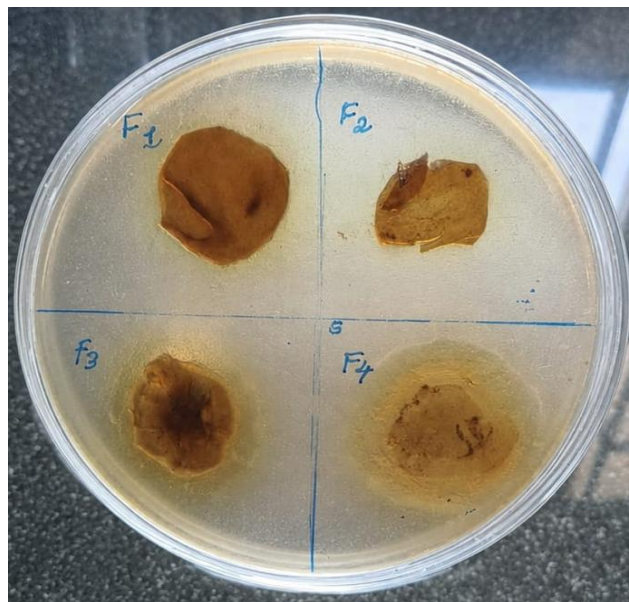


Fig 07: zone of inhibition of formulated films



Concentration-dependent plant extract activity:

Table 05: percentage inhibition analysis

Concentration (μg)	Absorbance	% Inhibition
100	0.3980	11.31
200	0.3091	13.02
300	0.3078	13.39
400	0.2520	29.09
500	0.2448	31.11
600	0.2164	39.11
700	0.2006	43.55
800	0.1126	68.31
900	0.0810	77.20
1000	0.0104	97.07

The plant extract demonstrated an increasing percentage inhibition with increasing concentration in the reported serial-dilution experiment. The highest tested concentration, 1000 μg , showed 97.07% inhibition. This concentration dependent upon supports the presence of biologically active constituents in the extract.

Film evaluation:

1.Physical evaluation:

Table 06: physical appearance parameters.

Parameter	F1	F2	F3	F4
Shape	Irregular	Regular	Regular	Regular
Elasticity	More elastic	Normal	Normal	Normal
Flexibility	No flexibility	Normal	Normal	Normal
Stickiness	More sticky	Normal	Normal	Normal

The formulations differed in their physical characteristics. F1 was irregular, excessively elastic, lacked flexibility and was stickier. F2, F3 and F4 showed regular shape, normal elasticity, normal flexibility and normal stickiness. Considering physical characteristics together with antimicrobial performance, F4 was selected as the optimized formulation.

2. Film evaluation parameters:

Table 07: evaluation test results

Evaluation parameter	Result
Thickness	0.290 mm
Surface pH	5.9
Folding endurance	>500 folds
Percentage elongation at break	23%
Flatness	100%

The optimized film had a thickness of 0.290 mm, indicating a uniform polymeric matrix in the reported evaluation. A surface pH of 5.9 was reported, within the skin-compatible range stated in the herbal wound healing film [12,14]. Folding endurance greater than 500 folds indicated high flexibility and resistance to repeated folding [15]. Elongation at break of 23% indicated useful elasticity, while 100% flatness indicated absence of measurable constriction.

4. Discussion:

The results suggest that the combined polymer system provided a workable film matrix and that changing chitosan and PVP concentrations influenced the physical characteristics of the films [13,15,20]. Increasing chitosan in F3 and F4 was associated with improved physical performance compared with F1. The higher PVP level in F4 was accompanied by the best overall physical and antimicrobial profile among the tested formulations. The antimicrobial activity of F4 may reflect the combined contribution of the Croton bonplandianum extract and the film matrix, but the present study does not separately quantify the contribution of each component.

5. Conclusion:

The present study successfully developed a chitosan-based herbal wound-healing film containing Croton bonplandianum leaf extract using the solvent-casting technique. The plant was selected based on its traditional use in wound and skin disorders and the pharmacological activities described in the literature review. The optimized formulation exhibited good physical appearance, uniformity, flexibility and satisfactory film characteristics. The evaluation studies showed positive results, indicating acceptable thickness, folding endurance, swelling and moisture properties. [1,2,3,4,5,6,7,8,9] The formulation also demonstrated promising antimicrobial activity, with the optimized formulation showing a clear zone of inhibition. Phytochemical studies confirmed the

presence of bioactive constituents such as flavonoids, phenolics, tannins and terpenoids. Overall, the results suggest that the developed Croton bonplandianum loaded chitosan film has good potential as a topical wound dressing for wound healing applications.

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7. References:

1. Lodhi R, Mohanty PK. Traditional Uses, Phytochemistry and Pharmacology of Croton bonplandianum—A Review. *Int J Ayurvedic Med*. 2021;12(2):185-189.
2. Ghosh T, Biswas MK, Roy P, Guin C. A review on traditional and pharmacological uses of Croton bonplandianum with special reference to phytochemical aspect. *Eur J Med Plants*. 2018;22(4):1-10.
3. Vennila V, Udayakumar R. Antibacterial activity of Croton bonplandianum against some bacterial isolates from infected wounds. *Br Microbiol Res J*. 2014;5(1):8.
4. Thenmozhi M, Vasuki K, Dhanalakshmi M, Devi KM. Evaluation of anti-inflammatory potential of Croton bonplandianum Baill leaf. *Int J Pharm Biol Sci*. 2013;7(1):9.
5. Vennila V, Udayakumar R. Wound healing efficacy of herbal preparations of Croton bonplandianum Baill. on excision wound in experimental rats. *Curr Tradit Med*. 2017;3(1):38-50.

6. Divya S, et al. Study on wound healing and antioxidant activity of *Croton bonplandianum* leaf extract. 2011.
7. Basak G, Chowdhury T, Karmakar A, et al. Antibacterial activity of *Croton bonplandianum* leaf extracts and isolation of stigmasterol. 2020.
8. Rao YV, Raju M, et al. Antioxidant, protease, catalase and peroxidase activities of *Croton bonplandianum* latex. 2018.
9. Meera Kumari, Siddiqui MA, et al. Evaluation of immune modulation activity, antioxidant activity, and phytochemical screening of *Croton bonplandianum* Baill. 2024.
10. Franco PB, Almeida LA, Marques RF, et al. Chitosan associated with the extract of unripe banana peel for potential wound dressing application. *Int J Polym Sci.* 2017;2017:9761047.
11. Almadani YH, Vorstenbosch J, Davison PG, Murphy AM. Wound healing: a comprehensive review. *Semin Plast Surg.* 2021;35(3):141-144.
12. Seshadri S, Sundaraganapathy HD, Gopathy S, et al. Wound healing property of herbal dressing film using chitosan and peel extracts of *Citrus limetta*.
13. Aldalbahi A, Thamer BM, Abdulhameed MM, El-Newehy MH. Fabrication of biodegradable and antibacterial films of chitosan/polyvinylpyrrolidone containing *Eucalyptus citriodora* extracts. *Int J Biol Macromol.* 2024;266:131001.
14. Borbolla-Jiménez FV, Peña-Corona SI, Farah SJ, et al. Films for wound healing fabricated using a solvent casting technique. *Pharmaceutics.* 2023;15(7):1914.
15. Thirupathi K, Raorane CJ, Ramkumar V, et al. Update on chitosan-based hydrogels: preparation, characterization, and antimicrobial and antibiofilm applications. *Gels.* 2022;9(1):35.
16. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: a review. *J Pharm Anal.* 2016;6(2):71-79.
17. Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines—a review. *Int J Biodivers Conserv.* 2012;4(3):101-112.
18. Kumari R, Kotecha M. A review on the standardization of herbal medicines. *Int J Pharm Sci Res.* 2016;7(2):97-106.
19. Bar G, Bar M. Antibacterial efficiency of *Croton bonplandianum* plant extract treated cotton fabric. *Curr Trends Fash Technol Text Eng.* 2020;7(1):5-9.
20. Chelminiak-Dudkiewicz D, Smolarkiewicz-Wyczachowski A, Mylkie K, et al. Chitosan-based films with cannabis oil as a base material for wound dressing application. *Sci Rep.* 2022;12:18658.