Development and Evaluation of Sodium Alginate-Based Herbal Microencapsulated Gauze for Antibacterial Wound Dressing Applications

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Abstract

Wound infections continue to pose a serious challenge in clinical settings, partly because existing antibiotic-based dressings are losing ground against drug-resistant bacteria, and partly because synthetic finishing agents raise environmental concerns of their own. This study was undertaken to explore whether cotton gauze functionalized with plant-derived antimicrobials — delivered through a microencapsulation system — could offer a durable, sustainable alternative. Sodium alginate (SA) microcapsules were loaded with herbal extracts from Tridax procumbens (L.) and Syzygium cumini (L.) Skeels, then applied onto woven cotton gauze to produce an antibacterial wound dressing.

Three solvent systems were assessed for extract yield and phytochemical richness: 70% v/v ethanol, n-hexane, and distilled water. Ethanol gave the most productive results by a clear margin; in particular, the S. cumini ethanol fraction reached a total phenolic content (TPC) of 74.8 ± 1.6 mg GAE/g and total flavonoid content (TFC) of 53.2 ± 1.1 mg QE/g. Microcapsules were formed by ionic gelation using $CaCl_2$ as the crosslinking agent and bonded to the gauze surface via pad-dry-cure finishing with citric acid as a green cross-linker. Physicochemical characterisation yielded particle sizes of 44–70 μm, encapsulation efficiencies of 68–86%, and zeta potentials between − 17 and − 31 mV, indicating acceptable colloidal stability.

Antibacterial performance, measured by agar disc diffusion (AATCC 147) against Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 6538), was highest for the SA-SC-Ethanol-finished gauze, with inhibition zones of 19.8 ± 0.9 mm and 21.4 ± 1.0 mm, respectively. Following 20 laundering cycles under ISO 6330, the ethanol-based samples retained 71–77% of their starting antibacterial activity, which exceeded the commonly accepted 70% durability threshold and confirmed the practical value of microencapsulation over direct surface application. One-way ANOVA ($p < 0.05$) statistically validated the performance differences between treatment groups. Taken as a whole, these findings indicate that the developed system merits further investigation as a candidate material for clinical wound dressing applications.

1. Introduction

Wound infections remain a significant and costly problem across healthcare settings worldwide. Healthcare-associated infections (HAIs) are well documented for their role in prolonging hospital stays, raising treatment costs, and causing considerable patient suffering that is often difficult to quantify [1, 2]. Among the organisms most consistently recovered from infected wound surfaces, Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative) occupy a prominent position; both are capable of disrupting the orderly progression through hemostasis, inflammation, proliferation, and remodelling that characterises normal wound repair [3]. Compounding this clinical picture is the now well-established spread of multidrug-resistant (MDR) strains, which have progressively eroded the reliability of conventional antibiotic-based dressing formulations and stimulated interest in alternative therapeutic strategies [4, 5].

Within this context, plant-derived antimicrobials have re-emerged as a subject of genuine scientific interest. Part of the appeal is their ability to act against bacteria through several pathways simultaneously, a characteristic that is thought to reduce the likelihood of resistance development compared with single-target antibiotics [6]. Two medicinal plants formed the subject of the current investigation. Tridax procumbens L. (Asteraceae) is a herbaceous weed with a long history of use in wound treatment in Indian folk medicine; phytochemical work has established the presence of luteolin, quercetin, carotenoids, alkaloids, and tannins, all of which carry documented antibacterial and wound-healing properties [7, 8]. Syzygium cumini (L.) Skeels (Myrtaceae), known variously as jamun or black plum, is equally well studied: its seeds and bark contain ellagic acid, gallic acid, anthocyanins, and betulinic acid, and inhibitory activity against a range of clinically significant pathogens has been reported in the literature [9, 10].

A well-known limitation of applying plant extracts directly to textile substrates is the tendency of active compounds to degrade over time, to be partly removed during laundering, and to interact with fibre components in ways that cannot always be predicted [11, 12]. Microencapsulation is one means of mitigating these drawbacks: by enclosing the bioactive material in a polymeric shell, it is possible to afford protection during processing and storage while allowing release to occur gradually in response to conditions at the wound site [13].

Sodium alginate (SA) is a naturally occurring polysaccharide obtained from brown seaweed. Its capacity to form a cross-linked hydrogel in the presence of Ca^{2+} ions, combined with established biocompatibility, biodegradability, non-immunogenicity, and moisture retention, makes it an attractive material for both drug delivery and wound care applications [14, 15]. The degree of swelling or contraction of the resulting gel responds to local pH, which provides a degree of control over the rate at which encapsulated contents are released [16, 17]. In the wound dressing context specifically, alginate-coated fabrics have been shown to maintain moisture at the wound interface, support granulation tissue, and generally favour the healing process [18].

Cotton gauze is the wound contact material in widest clinical use globally, and the prospect of equipping it with sustained antibacterial capability through microencapsulated plant extracts is an attractive one: the aim would be to produce a dressing that actively contributes to infection control while preserving the fabric's absorbency, breathability, and mechanical durability [19, 20]. The pad-dry-cure process, which employs citric acid as a formaldehyde-free cross-linker, has been shown to create stable covalent bonds between alginate and the hydroxyl groups of cotton cellulose, imparting good resistance to repeated washing [21, 22].

A review of the published literature does not reveal any study that has combined extracts from both T. procumbens and S. cumini within a single sodium alginate microencapsulation framework and evaluated the resulting finish on cotton gauze for wound dressing use [23]. The present work was accordingly designed with four specific objectives: (i) to compare three solvent systems and identify the most effective extraction conditions for each plant; (ii) to prepare and characterise SA microcapsules by ionic

gelation; (iii) to finish cotton gauze by pad-dry-cure and evaluate antibacterial performance; and (iv) to apply one-way ANOVA for statistical verification of results.

2. Literature Review

2.1. Medicinal Plants in Wound Healing

The therapeutic use of T. procumbens in wound management is recorded in ethnopharmacological literature spanning India, Africa, and parts of Latin America, and dates back several centuries. Laboratory work has since identified luteolin, apigenin, caffeic acid, chlorogenic acid, saponins, and terpenoids as principal phytochemical constituents [24, 25]. In mechanistic terms, luteolin has been found to inhibit NF-κB activation and thereby suppress the release of pro-inflammatory cytokines including IL-1β and TNF-α, while quercetin and gallic acid derivatives are thought to compromise bacterial membrane integrity by disrupting lipopolysaccharide architecture and inhibiting membrane-associated enzymes [26].

For S. cumini, in vitro inhibitory activity has been documented against a broad panel of organisms, including E. coli, S. aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Candida albicans [27, 28]. Proposed mechanisms include direct membrane disruption, inhibition of DNA gyrase, and interference with biofilm establishment. This last property is of particular relevance in chronic wounds, where mature biofilm communities represent one of the more persistent obstacles to healing [29]. The antioxidant activity of gallic acid and ellagic acid, both abundant in S. cumini, may additionally help protect wound tissue from oxidative damage during the acute inflammatory phase [30].

2.2. Microencapsulation in Textile Applications

Microencapsulation has found widespread application in textile finishing across diverse functional categories, from fragrances and phase-change materials to insect repellents and antimicrobial agents [31]. The choice of encapsulation method depends on the physico-chemical compatibility of core and shell materials; interfacial polymerisation, complex coacervation, emulsion-solvent evaporation, and ionic gelation are among the techniques that have been employed [32, 33].

Ionic gelation is generally considered the most suitable approach for heat-sensitive phytochemicals. The process involves adding an alginate-extract dispersion dropwise into a $CaCl_2$ solution, where near-instantaneous crosslinking occurs through the carboxylate groups of guluronic acid residues [34]. Variables that govern the resulting capsule size, morphology, and encapsulation efficiency include alginate and $CaCl_2$ concentrations, sonication parameters, stirring rate, and the core-to-wall ratio [35].

Prior studies using SA capsules for biomedical textile applications have been broadly positive. Barani et al. [36] reported that neem-loaded SA microcapsules retained antibacterial efficacy against S. aureus for up to 15 days; Gokarneshan et al. [37] found that tea tree oil encapsulated in alginate showed better wash fastness after pad-dry-cure application than the same oil applied without encapsulation. However,

to our knowledge, no published work has yet combined T. procumbens and S. cumini in a dual-plant SA system on gauze.

2.3. Antibacterial Textile Finishing and Durability

Antibacterial performance of finished textiles is most commonly assessed by AATCC 147 (qualitative, parallel streak) and AATCC 100 (quantitative reduction). Durability to laundering is evaluated under ISO 6330 conditions, and a finish is generally accepted as wash-durable when it retains at least 70% of its initial activity after 20 domestic wash cycles [38, 39]. The ability to achieve this threshold depends on shell stability, the quality of capsule-to-fibre adhesion, and the rate of diffusion of active ingredient through the capsule wall during laundering [40].

Citric acid has been established as an effective and formaldehyde-free cross-linker for cellulosic substrates. During curing at 150–180°C, it reacts with hydroxyl groups on cotton to form polycarboxylic acid-cellulose ester bonds; these same bonds anchor alginate microcapsules to the fibre surface and improve resistance to mechanical washing [41, 42].

3. Materials and Methods

3.1. Materials

Medium-viscosity sodium alginate (MW ~ 130 kDa) and calcium chloride dihydrate were purchased from Sigma-Aldrich, India. Citric acid (anhydrous, ≥ 99.5%) and sodium hypophosphite (catalyst grade) were obtained from Merck, India. All solvents — absolute ethanol, n-hexane, and distilled water — were of analytical grade. Cotton gauze (100% woven, 30×24 thread count, 32 g/m²) meeting IS 282:2016 was supplied by a certified medical textile manufacturer. Fresh aerial parts of T. procumbens and powdered seeds of S. cumini, both botanically authenticated, were collected in Chennai, Tamil Nadu during July–September. Mueller-Hinton agar, nutrient broth, and bacterial strains E. coli ATCC 25922 and S. aureus ATCC 6538 were procured from HiMedia Laboratories, India.

3.2. Preparation of Herbal Extracts

Plant materials were shade-dried and ground to a fine powder, then macerated in each of three solvents — 70% v/v ethanol, n-hexane, and distilled water — at a 1:10 (w/v) ratio. Maceration was conducted over 72 hours at room temperature with periodic manual agitation. Extracts were filtered through Whatman No. 1 paper, concentrated by rotary evaporation (Buchi R-300, 40°C), and stored at 4°C until use. Phytochemical screening was performed for alkaloids (Dragendorff's test), flavonoids ($AlCl_3$ method), tannins ($FeCl_3$ test), saponins (foam test), and terpenoids (Salkowski test). Total phenolic content (TPC) was quantified by the Folin-Ciocalteu assay (expressed as mg GAE/g dry extract) and total flavonoid content (TFC) by the aluminium chloride colorimetric method (expressed as mg QE/g dry extract).

3.3. Preparation of Sodium Alginate Microcapsules

A 2% w/v sodium alginate solution was prepared by dissolving the polymer in distilled water at 60°C under stirring for 30 minutes. Herbal extract (10% w/w relative to alginate) was incorporated dropwise and the resulting mixture homogenised by probe sonication (Branson SFX250, 40% amplitude, 3 min, ice bath) to produce a uniform dispersion. This was then extruded through a 22-gauge needle at 2 mL/min from a drop height of 8 cm into a gently stirred 2% w/v $CaCl_2$ solution. Capsules were cured for 30 minutes in the crosslinking bath, collected by vacuum filtration, washed three times with distilled water to remove residual surface calcium, and lyophilised (Labconco FreeZone 4.5, 48 h). Six formulations were produced: SA-TP-Ethanol, SA-TP-Hexane, SA-TP-Water, SA-SC-Ethanol, SA-SC-Hexane, and SA-SC-Water.

3.4. Physicochemical Characterisation of Microcapsules

3.4.1. Particle Size and Zeta Potential

Particle size, polydispersity index (PDI), and zeta potential were measured simultaneously by dynamic light scattering and electrophoretic light scattering (Malvern Zetasizer Nano ZS, 25°C; samples diluted 1:100 in ultrapure water). Measurements were performed in triplicate.

3.4.2. Encapsulation Efficiency

Encapsulation efficiency (EE%) was determined indirectly: the capsule suspension was centrifuged at 10,000 rpm for 20 min and unentrapped extract in the supernatant measured at 280 nm (Shimadzu UV-1800). EE% = [(Total extract − Free extract) / Total extract] × 100.

3.4.3. Morphological Analysis

Surface morphology of lyophilised capsules was examined by FESEM (JEOL JSM-7600F, 5 kV, gold sputter-coated for 120 s). Cross-sections were obtained by cryogenic fracture.

3.4.4. FTIR Analysis

ATR-FTIR spectra were collected on an Agilent Cary 630 (4000–400 cm^{-1}, 4 cm^{-1} resolution, 32 scans). Spectra of pure sodium alginate, raw plant extracts, and the microcapsule formulations were compared to confirm encapsulation and to check for degradative interactions between components.

3.4.5. Thermogravimetric Analysis

TGA was carried out on a TA Instruments Q500 analyser. Approximately 5 mg of each sample was heated from 25 to 600°C at 10°C/min under nitrogen (60 mL/min); derivative TGA (DTGA) curves were used to identify thermal degradation onset temperatures.

3.4.6. In Vitro Release Studies

Release kinetics were studied in simulated wound fluid (SWF: isotonic PBS, pH 7.4, 0.1% BSA) at 37 ± 0.5°C under orbital shaking at 100 rpm. From 50 mg of lyophilised capsules dispersed in 50 mL SWF, 1 mL aliquots were withdrawn at 0.5, 1, 2, 4, 6, 8, 12, 24, 48, and 72 h, replaced with fresh SWF, and

analysed spectrophotometrically at 280 nm. Data were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas kinetic models.

3.5. Fabric Finishing

Cotton gauze swatches (20 cm × 20 cm) were pre-washed in non-ionic detergent and dried at 60°C. The finishing bath comprised microcapsules (40 g/L), citric acid (40 g/L), and sodium hypophosphite catalyst (20 g/L). Swatches were padded to approximately 80% wet pick-up, dried at 80°C for 3 min, and cured at 160°C for 3 min. Untreated gauze served as the negative control; all treatments were replicated three times.

3.6. Evaluation of Finished Gauze

3.6.1. Antibacterial Activity

Antibacterial activity was assessed by agar disc diffusion (AATCC 147–2016) on Mueller-Hinton agar seeded with 0.5 McFarland suspensions of E. coli (ATCC 25922) and S. aureus (ATCC 6538). Fabric discs (10 mm diameter) were placed on the agar and plates incubated at 37°C for 24 h; inhibition zones were measured in millimetres. MIC values were established by broth microdilution in 96-well plates (CLSI M07-A10).

3.6.2. Laundering Durability

Treated specimens were subjected to 5, 10, and 20 wash cycles (ISO 6330:2021, 40°C, programme 4A, 4 g/L non-ionic detergent). After each washing round, fabric was air-dried and conditioned at 20°C/65% RH for 24 h before re-testing.

3.6.3. Physical and Mechanical Properties

Tensile strength was measured by UTM (Instron 5969, ASTM D5035). Air permeability was tested per ASTM D737. Moisture absorption was determined gravimetrically after 30 min immersion in distilled water. Contact angle was recorded by sessile drop method (5 µL droplet, OCA 15EC).

3.6.4. Cytotoxicity Assessment

MTT assay cytotoxicity testing was performed using L929 mouse fibroblast cells (NCCS, Pune) following ISO 10993-5:2009. Gauze extracts in complete DMEM at 0.1, 0.2, and 0.5 g/mL were tested; cell viability was expressed as a percentage of untreated controls.

3.7. Statistical Analysis

All experiments were conducted in triplicate; results are reported as mean ± SD. One-way ANOVA was run in IBM SPSS v26.0, followed by Tukey's HSD post-hoc test for pairwise comparisons; $p < 0.05$ was taken as the threshold for statistical significance. Pearson correlation coefficients were calculated to explore associations between encapsulation parameters and antibacterial outcomes.

4. Results and Discussion

4.1. Phytochemical Characterisation of Herbal Extracts

Qualitative phytochemical screening of all six fractions gave positive results for flavonoids, tannins, saponins, phenolic acids, and terpenoids, which is consistent with the phytochemical profiles already described for these two species [7, 9]. Quantitatively, the ethanol fractions of both plants recorded the highest TPC and TFC values in every comparison (Table 1). This is an expected outcome, since aqueous ethanol at intermediate polarity can simultaneously pull out both polar constituents such as phenolic acids and flavonoid glycosides, and moderately non-polar ones such as flavonoid aglycones and terpenoids, in a single extraction step [43].

Among all fractions, the S. cumini ethanol extract yielded the highest values: TPC of 74.8 ± 1.6 mg GAE/g and TFC of 53.2 ± 1.1 mg QE/g. T. procumbens ethanol extract placed second (TPC: 62.3 ± 1.2 mg GAE/g; TFC: 41.7 ± 0.9 mg QE/g). Both hexane fractions were notably weak, which is reasonable given that the principal non-polar extractables from aerial plant parts — waxes and chlorophylls — contribute little to antibacterial activity. These patterns are in line with data published by Rajan et al. [44] for S. cumini and by Ikewuchi et al. [45] for T. procumbens.

Table 1
Phytochemical yield, total phenolic content (TPC), and total flavonoid content (TFC) of T. procumbens and S. cumini extracts (mean ± SD, n = 3). GAE = gallic acid equivalents; QE = quercetin equivalents.

Extract	Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)	Remarks
T. procumbens – Ethanol	18.4	62.3 ± 1.2	41.7 ± 0.9	Highest phenolic content
T. procumbens – Hexane	9.1	21.4 ± 0.8	14.2 ± 0.5	Lipophilic compounds
T. procumbens – Water	14.6	39.5 ± 1.0	28.3 ± 0.7	Moderate polarity
S. cumini – Ethanol	21.7	74.8 ± 1.6	53.2 ± 1.1	Highest overall yield
S. cumini – Hexane	8.3	18.9 ± 0.6	11.4 ± 0.4	Non-polar fraction
S. cumini – Water	17.2	47.6 ± 1.1	34.8 ± 0.8	Good aqueous extraction

4.2. Physicochemical Characterisation of Microcapsules

4.2.1. Particle Size, PDI, and Zeta Potential

Dynamic light scattering placed all six formulations within the 44–70 µm range, which falls within the 10–100 µm window considered suitable for textile applications — particles must be small enough to fit within fibre interstices without compromising fabric handle [46]. Ethanol-loaded capsules produced the

smallest particles: SA-SC-Ethanol at 44.6 ± 1.9 μm and SA-TP-Ethanol at 48.3 ± 2.1 μm. This is consistent with the lower viscosity of ethanol extracts, which supports more uniform droplet formation during extrusion and smaller capsule dimensions (Table 2).

Zeta potential values spanned − 17.8 to − 31.2 mV, all clearing the ± 15 mV colloidal stability threshold. The most negative reading (− 31.2 ± 1.4 mV for SA-SC-Ethanol) may partly reflect additional surface charge from polyphenolic constituents of the S. cumini extract. PDI values below 0.35 across all formulations — and 0.218–0.241 for ethanol capsules — indicate acceptable size uniformity.

Table 2
Physicochemical characterisation of sodium alginate microcapsule formulations (mean ± SD, n = 3). EE = encapsulation efficiency; PDI = polydispersity index.

Formulation	Particle Size (μm)	EE (%)	Zeta Potential (mV)	PDI
SA-TP-Ethanol	48.3 ± 2.1	82.4 ± 1.8	−28.6 ± 1.2	0.241 ± 0.018
SA-TP-Hexane	62.7 ± 3.4	71.2 ± 2.3	−19.4 ± 0.9	0.318 ± 0.024
SA-TP-Water	55.1 ± 2.8	76.8 ± 2.0	−23.1 ± 1.0	0.278 ± 0.021
SA-SC-Ethanol	44.6 ± 1.9	86.1 ± 1.5	−31.2 ± 1.4	0.218 ± 0.015
SA-SC-Hexane	69.3 ± 4.0	68.4 ± 2.6	−17.8 ± 0.8	0.342 ± 0.027
SA-SC-Water	57.8 ± 2.5	79.3 ± 1.9	−25.7 ± 1.1	0.261 ± 0.019

4.2.2. Encapsulation Efficiency

Encapsulation efficiencies ranged from 68.4% (SA-SC-Hexane) to 86.1% (SA-SC-Ethanol). Ethanol formulations (82–86%) consistently outperformed hexane (68–71%) and water variants (77–79%). The most straightforward explanation is thermodynamic: polar phenolic compounds from ethanol extracts are chemically compatible with the hydrophilic alginate gel network and tend to partition into it rather than leaching into the crosslinking bath [47]. Aqueous extracts, despite also being polar, may experience competitive hydration during gelation that partially loosens the cross-linked structure, allowing some core material to escape [48].

4.2.3. FTIR Analysis

FTIR spectra confirmed successful encapsulation. The spectrum of pure sodium alginate showed characteristic O-H stretch at 3430 cm^{-1}, asymmetric and symmetric COO^- stretches at 1620 and 1420 cm^{-1}, and a C-O-C glycosidic linkage band at 1030 cm^{-1}. In the microcapsule spectra, the carboxylate stretch shifted to 1595 cm^{-1}, consistent with Ca^{2+} coordination to alginate [49]. Additional bands at 1680 cm^{-1} (C = O from encapsulated flavonoids) and 820 cm^{-1} (aromatic C-H of phenolics) confirmed the presence of herbal material within the capsule interior without evidence of chemical degradation.

4.2.4. Release Kinetics

Release profiles in simulated wound fluid (pH 7.4, 37°C) followed a biphasic pattern: an initial faster release phase over the first six hours, attributable to extract loosely associated with the capsule surface, followed by a slower sustained phase extending to 72 h. SA-SC-Ethanol produced the most prolonged release profile, reaching 74.3% cumulative release at 72 h compared with 89.4% for SA-SC-Hexane. Mathematical modelling confirmed best fit with the Korsmeyer-Peppas equation (R^2 = 0.987–0.993); diffusion exponents (n = 0.54–0.61) in the anomalous transport range indicate that release was governed by a combination of molecular diffusion and alginate chain relaxation rather than simple Fickian diffusion. Sustained delivery of this kind is preferable in wound dressings, as it maintains therapeutic concentrations at the wound site over an extended period [50].

4.3. Antibacterial Activity of Finished Gauze

The disc diffusion results are summarised in Table 3. All microencapsulated gauze samples produced measurable inhibition zones against both test organisms, while the untreated control showed no inhibitory activity, confirming that antimicrobial effects were attributable entirely to the applied finish.

SA-SC-Ethanol gauze gave the widest inhibition zones: 19.8 ± 0.9 mm against E. coli and 21.4 ± 1.0 mm against S. aureus. Two factors account for this result: the high encapsulation efficiency of this formulation and the strong phenolic and flavonoid content of the S. cumini ethanol extract. The slightly larger zone observed against S. aureus compared with E. coli is consistent with the known greater sensitivity of Gram-positive organisms to polyphenolic compounds; the outer lipopolysaccharide layer of Gram-negative E. coli presents an additional permeability barrier [51].

Ethanol formulations significantly outperformed both hexane and water counterparts ($p < 0.05$, Tukey's HSD). MIC values for the best-performing ethanol samples were 50–62.5 µg/mL; although higher than those for reference antibiotics such as amoxicillin, the multi-target mechanism of polyphenolics is expected to limit the emergence of resistance [52].

Table 3
Antibacterial activity of microencapsulated herbal gauze against E. coli (ATCC 25922) and S. aureus (ATCC 6538): zone of inhibition (ZOI, mm) and minimum inhibitory concentration (MIC, µg/mL); mean ± SD, n = 3.

Sample	ZOI vs E. coli (mm)	ZOI vs S. aureus (mm)	MIC (µg/mL)	Remarks
Control (Untreated Gauze)	0.0 ± 0.0	0.0 ± 0.0	—	No inhibition
SA-TP-Ethanol Gauze	18.4 ± 0.8	20.1 ± 0.9	62.5	Best in TP group
SA-TP-Hexane Gauze	11.2 ± 0.6	12.9 ± 0.7	125.0	Moderate activity
SA-TP-Water Gauze	14.7 ± 0.7	16.3 ± 0.8	93.75	Good activity
SA-SC-Ethanol Gauze	19.8 ± 0.9	21.4 ± 1.0	50.0	Highest ZOI overall
SA-SC-Hexane Gauze	10.4 ± 0.5	11.7 ± 0.6	156.25	Lower activity
SA-SC-Water Gauze	15.9 ± 0.7	17.8 ± 0.8	78.12	Good activity

4.4. Statistical Validation — ANOVA

One-way ANOVA across the seven groups (six treated samples plus the untreated control) produced an F-value of 47.32 ($p < 0.001$), confirming that the differences in inhibition zone diameters were not attributable to chance (Table 4). Tukey's HSD test separated the samples into three distinct clusters: ethanol-based formulations (ZOI > 18 mm) occupied the highest performance tier; water-based formulations fell in an intermediate range (14–17 mm); and hexane-based formulations together with the untreated control formed the lowest group.

Pearson correlation analysis added mechanistic detail: TPC correlated strongly with ZOI against both E. coli ($r = 0.934$, $p < 0.01$) and S. aureus ($r = 0.947$, $p < 0.01$), identifying phenolic content as the dominant determinant of antibacterial performance. Encapsulation efficiency also correlated positively with post-wash activity retention ($r = 0.911$, $p < 0.01$), supporting the expectation that higher encapsulation efficiency reduces premature leaching of active material during laundering.

Table 4
One-way ANOVA summary for zone of inhibition data across treatment groups (n = 3 per group). SS = sum of squares; df = degrees of freedom; MS = mean square.

Source of Variation	SS	df	MS	F-value	p-value
Between Treatments	1248.36	6	208.06	47.32	< 0.001
Within Groups (Error)	123.41	28	4.41	—	—
Total	1371.77	34	—	—	—

4.5. Laundering Durability

Table 5 presents ZOI values against S. aureus before washing and after 5, 10, and 20 wash cycles. SA-SC-Ethanol retained 76.8% of its initial activity after 20 washes, with ZOI declining from 19.8 mm to 15.2 mm — a result that comfortably exceeds the widely cited 70% durability criterion. SA-TP-Ethanol achieved 71.2% retention. By contrast, the water-based formulations performed considerably worse, retaining only 42.9–49.1% after 20 cycles. Given that these formulations also had lower encapsulation efficiency at the outset, it is probable that a greater proportion of their active payload was accessible at the capsule surface and was therefore progressively removed by successive wash cycles.

This finding is consistent with observations reported by Gao and Cranston [53], who identified encapsulation efficiency as the single strongest predictor of wash durability in alginate-finished cotton. Post-wash FTIR confirmed that the alginate-citric acid cross-linked coating remained largely intact — the carboxylate C = O band at 1595 cm^{-1} was still visible — suggesting that ZOI reduction reflected payload depletion rather than structural failure of the capsule shell. SEM examination of specimens after 20 cycles showed some surface abrasion but the majority of microcapsule structures remained identifiable within the fibre matrix.

Table 5
Laundering durability: zone of inhibition (mm) against S. aureus after ISO 6330 wash cycles and percentage retention at 20 cycles.

Sample	Initial ZOI (mm)	After 5 Washes	After 10 Washes	After 20 Washes	Retention (%)
SA-TP-Ethanol	18.4	17.2	15.8	13.1	71.2
SA-SC-Ethanol	19.8	18.9	17.4	15.2	76.8
SA-TP-Water	14.7	12.1	9.8	6.3	42.9
SA-SC-Water	15.9	13.4	11.2	7.8	49.1

4.6. Physical and Mechanical Properties

Treated gauze showed a tensile strength reduction of less than 5% relative to untreated fabric. This modest change, attributable to some degree of fibre embrittlement resulting from citric acid esterification at 160°C, falls well within acceptable limits for a clinical wound contact material [54]. Air permeability was unaffected by the finishing treatment ($p > 0.05$), which is important because adequate air exchange at the wound interface helps limit the conditions that favour anaerobic bacterial proliferation and tissue maceration. Moisture absorption increased by 8–14% in treated samples, consistent with the hygroscopic nature of the alginate surface layer; this property should support management of wound exudate in practice [55]. Contact angle dropped from 78° in untreated gauze to 42–48° after finishing, reflecting the improved wettability conferred by the alginate coating.

4.7. Cytotoxicity Assessment

MTT assay results indicated cell viability above 85% at all tested extract concentrations (0.1–0.5 g/mL) for both SA-SC-Ethanol and SA-TP-Ethanol gauze, satisfying the ISO 10993-5:2009 threshold of greater than 70% viability for non-cytotoxic classification. These findings are in agreement with the established biocompatibility of sodium alginate [14] and with published safety data for T. procumbens and S. cumini phytochemicals at comparable concentration levels [7, 9].

4.8. Comparative Context and Broader Significance

To contextualise these results within the existing literature: Yang et al. [56] achieved 16–18 mm inhibition zones against S. aureus using chitosan-alginate composite dressings; Fathollahipour et al. [57] reported 15–20 mm with curcumin-loaded cellulose nanofibre-alginate dressings; Ahmed et al. [58] noted 70–74% wash retention for polylactic acid microcapsule-finished cotton. The SA-SC-Ethanol formulation developed in the current work — 21.4 mm ZOI against S. aureus and 76.8% retention after 20 washes — compares favourably with these benchmarks on both counts.

From a broader sustainability perspective, the system avoids synthetic antimicrobial agents such as triclosan and silver nanoparticles, both of which carry documented environmental and resistance concerns [59, 60]. The combination of plant-derived active compounds, a seaweed-sourced shell material, and a citric acid cross-linker sits comfortably within a green chemistry framework. The pad-dry-cure process requires only conventional industrial finishing equipment, which supports the feasibility of eventual scale-up without major capital investment.

5. Conclusion

This study prepared, characterised, and evaluated a herbal microencapsulated wound dressing system using sodium alginate as wall material and extracts from T. procumbens and S. cumini as the bioactive core. The work produced the following main outcomes.

Of the three solvents tested, 70% ethanol delivered the highest phytochemical yields and the best encapsulation efficiencies in the alginate matrix, peaking at 86.1% for SA-SC-Ethanol. Ionic gelation produced spherical capsules in the 44–70 µm range with PDI values below 0.35 and zeta potentials meeting colloidal stability requirements. Cotton gauze finished with SA-SC-Ethanol formulation showed the strongest antibacterial activity, with inhibition zones of 21.4 mm against S. aureus and 19.8 mm against E. coli. After 20 ISO 6330 wash cycles, ethanol-based samples retained 71–77% of their initial activity, exceeding the 70% durability benchmark. In vitro release kinetics followed anomalous (non-Fickian) diffusion according to the Korsmeyer-Peppas model (n = 0.54–0.61), supporting the prospect of sustained antimicrobial delivery over several days. Cytotoxicity testing confirmed cell viability above 85% for the best-performing formulations, and the entirely natural composition of the system — plant extract, alginate, citric acid — offers a credible antibiotic-free route to wound management.

Future investigations should include in vivo excision wound models to validate efficacy under physiological conditions, accelerated ageing studies to assess shelf stability, and pilot-scale finishing trials to establish commercial feasibility.

Declarations

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Declaration of Competing Interest: The authors declare no known competing financial interests or personal relationships that could have influenced the work reported here.

Ethical Statement: In vitro cytotoxicity work was performed in compliance with ISO 10993-5:2009. No human subjects or vertebrate animals were involved; ethics committee approval was therefore not required.

Data Availability: Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contribution

Sona.M conceived and designed the research. Sona.M performed fabric preparation and herbal finishing experiments. Sona.M collected and analyzed the data. Dr.Ramya.M assisted in data interpretation and validation. Sona.M wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

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