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Nanoencapsulation and antimicrobial activities of *Commiphora swynnertonii* resin against multidrug-resistant *Staphylococcus aureus* strains from mastitis cow patients

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Abstract

Bovine mastitis is an inflammatory disease of the mammary gland, largely caused by microbial infections and commonly managed through intramammary administration of antibiotics. However, the extensive and recurrent use of antibiotics has led to the emergence and spread of antibiotic-resistant pathogens, particularly multidrug-resistant (MDR) *Staphylococcus aureus*, posing significant veterinary and public health challenges. Herein, we investigated the potential of *Commiphora swynnertonii* resin-loaded nanoparticles as an antibiotic alternative. This study aimed to identify an effective nanocarrier platform for antimicrobial delivery of *C. swynnertonii* resin. To achieve this, different types of nanocarriers were explored: liposomes, alginate-based nanoparticles, chitosan-based nanoparticles (ChN), solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC), each with or without hyaluronic acid–stearylamine conjugate (HAC). Antimicrobial activity was assessed against 13 MDR *S. aureus* strains isolated from mastitis cow patients. Based on minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) assays, the tested nanocarriers were ranked in ascending order of antimicrobial coverage as follows: liposomes (0% inhibitory and 0% bactericidal), ChN (15.4% inhibitory and 0% bactericidal), NLC (23% inhibitory and 7.6% bactericidal), SLN (69% inhibitory and 46.2% bactericidal), and alginate nanoparticles (100% inhibitory and 53% bactericidal). HAC-containing alginate nanoparticles achieved the strongest activity, with MIC 26–417 µg/mL and MBC 35–417 µg/mL, followed by HAC-containing SLN with MIC 17–417 µg/mL and MBC 278–417 µg/mL. These findings highlight the potential of alginate-HAC nanoparticles as a promising platform for delivering *C. swynnertonii* resin constituents, offering a novel strategy to combat MDR mastitis pathogens through plant-based nanotherapeutics.

Keywords Antimicrobial resistance, *Commiphora swynnertonii*, Plant-derived antimicrobials, Nanoparticle drug delivery, *Staphylococcus aureus*, MDR mastitis, Nanotherapeutics



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1 Introduction

Antimicrobial resistance (AMR) has emerged as one of the most pressing global public health challenges, undermining the efficacy of infection prevention and treatment strategies [1]. In 2019, bacterial AMR was linked to an estimated 5 million deaths worldwide, and this burden is expected to rise sharply in the coming decades [2, 3]. The misuse and overuse of antibiotics across medical, agricultural, and veterinary sectors have accelerated the evolution and spread of resistant strains [4]. In livestock production systems, particularly the dairy industry, antibiotics remain the cornerstone of diseases management, with bovine mastitis representing one of the most treated conditions. Reports indicate that treated animals excrete up to 70–90% of administered antibiotics unmetabolized, introducing residues into soil and water ecosystems and contributing to environmental AMR dissemination [5].

Bovine mastitis (BM) is an inflammatory disorder of the mammary gland tissue caused predominantly by bacterial pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, and *Streptococcus uberis*. BM accounts for approximately 70–73% of antibiotic usage in the dairy industry [6, 7]. Among the causative pathogens, livestock-associated *S. aureus* (LA-*S. aureus*) is a major etiological agent of BM and constitutes a persistent reservoir of AMR within dairy herds [8]. These strains are highly adapted to the mammary gland environment, where intracellular survival, biofilm formation, and immune evasion promote chronic and recurrent infections [9]. Importantly, LA-*S. aureus*—including methicillin-resistant variants (e.g., CC398)—can be transmitted to humans via direct animal contact, contaminated milk, or environmental pathways, facilitating zoonotic infections and resistance gene dissemination [10]. LA-*S. aureus* in dairy cattle therefore underlines the reason mastitis management is associated with persistent challenges, such as the presence of antibiotic residues in milk, increased treatment failures, and heightened concerns for food safety and public health [11–13].

Growing concern over antibiotic overuse has accelerated research into alternative, sustainable strategies for BM management. Recent studies highlight the potential of plant-derived bioactives, probiotics, bacteriocins, antimicrobial peptides, bacteriophages, and nanoparticles as promising non-antibiotic interventions [14, 15]. Phytochemicals from natural products, particularly polyphenols and terpenoids, have shown broad-spectrum antimicrobial activity, reducing the infection burden while mitigating AMR risks [16, 17].

Among natural products, *Commiphora swynnertonii* (Burseraceae) is a resinous plant with documented use in traditional and ethnoveterinary medicine in East African pastoral communities, where its resin is applied to treat wounds, inflammatory conditions, and infectious diseases in humans and livestock [18]. Phytochemical studies have shown that this resin is rich in bioactive secondary metabolites—including terpenoids, flavonoids, steroids, tannins, coumarins, saponins, and anthraquinones—compound classes commonly associated with antimicrobial and anti-inflammatory activities [18, 19]. Importantly, antistaphylococcal activity of *C. swynnertonii* resin has been previously reported [20], and its terpenoid constituents have proven to be effective against LA-*S. aureus* [21], supporting the resin's relevance as a natural antimicrobial candidate for BM. However, the direct application of resin formulations in the mammary gland is hindered by poor solubility, rapid clearance during milking, and inconsistent delivery of active constituents in livestock. This provides a strong rationale for the present research

effort to encapsulate and evaluate the *C. swynnertonii* resin in nanocarriers against MDR LA-*S. aureus*.

Nanotechnology offers a promising platform to overcome these limitations by enhancing the solubility, bioavailability, and targeted delivery of plant-derived compounds. Nanocarriers can protect encapsulated phytochemicals from degradation, promote sustained release, and enable site-specific drug deposition while minimizing systemic exposure and adverse effects. Importantly, nanosystems can also circumvent classical mechanisms of bacterial resistance by enhancing intracellular penetration, disrupting biofilms, and reducing the need for systemic antibiotic exposure [22, 23]. By integrating natural antimicrobial agents within nanostructured delivery systems, it is possible to achieve dual benefits—enhanced antimicrobial potency and reduced AMR development. Despite this opportunity, no systematic comparative evaluation of nanocarrier platforms for encapsulating the phytochemically complex resin of *C. swynnertonii*—containing hydrophilic, hydrophobic, and amphiphilic metabolites— or their relative antimicrobial performance has been reported.

In this context, we investigated multiple nanocarrier systems—including liposomes, alginate-based nanoparticles, chitosan-based nanoparticles, solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC)—for the encapsulation of *C. swynnertonii* resin constituents. While previous studies have examined plant materials or nanoparticle carriers, no systematic comparison has been made across multiple nanopatforms for encapsulating *C. swynnertonii* resin, nor their relative antimicrobial activities against multidrug-resistant (MDR) *S. aureus* isolated from mastitis cases. To further explore the possibility of targeting and retention of nanoparticles within infected mammary tissues, we functionalized nanoparticles with hyaluronic acid (HA). HA is an endogenous ligand of the CD44 receptor, which is overexpressed on inflamed mammary epithelial cells and infiltrating immune cells during mastitis [24, 25]. The HA-mediated functionalization of nanoparticles is thought to enhance (i) selective targeting of inflamed tissues, (ii) mucoadhesion and prolonged intramammary retention, (iii) internalization into intracellular reservoirs where *S. aureus* can persist, and (iv) overall colloidal stability and biocompatibility [26].

The present work thus explores a hybrid strategy integrating natural plant therapeutics with nanotechnology-driven delivery systems to address the dual challenges of bovine mastitis and antimicrobial resistance. Different nano-formulations were systematically prepared with or without HA-surface functionalization, and characterized in terms of physicochemical properties, encapsulation efficiency, and phytochemical preservation. The antimicrobial activity of the resulting nanomaterials was then evaluated against MDR LA-*S. aureus* strains isolated from BM cases using standard in vitro assays. Through this comparative approach, the study aimed to identify nanocarrier systems capable of effectively encapsulating the complex phytochemical constituents of the resin while retaining antibacterial activity, thereby providing a foundation for further development of alternative strategies to tackle BM.

2 Materials and methods

2.1 Materials

All chemicals and reagents used in this study were of research or HPLC grade, and were employed without further purification unless otherwise stated. The materials utilized

for the synthesis of nanomaterials—including liposomes, nanostructured lipid carriers (NLC), solid lipid nanoparticles (SLN) and polymeric nanoparticles (alginate- and chitosan-based nanoparticles)—and for subsequent analytical assays are described below.

Plant material: *Commiphora swynnertonii* Burt (Burseraceae) resin was obtained from the Northern Tanzania district of Simanjiro (4° 0' 0" S, 36° 30' 0" E, 1360 above sea level). The plant material was taxonomically authenticated by a botanist at Tanzania National Herbarium in Arusha, Tanzania, and a voucher specimen (No. CK 6489) was deposited. No genetic barcoding was performed for this study, as botanical identification was based on established morphological criteria and herbarium comparison.

Nanomaterial synthesis reagents: Soy lecithin (General Chemical, Gujarat, India) and cholesterol (Merck, Hamburg, Germany) were used as lipid components for liposome formulation. The hyaluronic acid conjugate was synthesized using stearylamine (1-aminooctadecane, CAS No. 124-30-1), hyaluronic acid sodium salt (MW 15–18 kDa, 95–105% dried substance, CAS No. 9067-32-7), and the coupling agent 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, CAS No. 25952-53-8), all obtained from Sigma-Aldrich (St. Louis, MO, USA). Medium-molecular-weight chitosan and sodium alginate (Sigma-Aldrich, Hamburg, Germany) served as natural polymeric materials, while sodium tripolyphosphate (General Chemical Ltd., Gujarat, India) was used as the ionic cross-linker. Additional excipients included polyvinyl alcohol (PVA; Sigma-Aldrich, Hamburg, Germany), coconut oil (Costco Wholesale Canada Ltd., Toronto, Canada), beeswax (Labo Repharco BP Kin I, Kinshasa, Democratic Republic of Congo (DRC)), calcium phosphate (Minema Chemicals, Johannesburg, Republic of South Africa), Tween 80 (General Chemical Ltd., Gujarat, India), stearic acid (Sigma-Aldrich, Selangor, Malaysia), castor oil (Labo Repharco BP Kin I, Kinshasa, DRC), and polyvinylpyrrolidone (PVP; General Chemical Ltd., Gujarat, India).

Analytical reagents and solvents: Sodium tungstate and sodium molybdate (Unisource Chemicals Pvt. Ltd., India) were used in the preparation of the Folin-modified reagent for total phenol determination. Sodium carbonate (Merck, Hamburg, Germany), chloroform (Sigma-Aldrich, Louis, MO, USA), methanol (gradient grade for liquid chromatography, Merck, Hamburg, Germany), and Milli-Q water were employed as solvents and reagents for the analytical assays. All solvents used were compatible with chromatographic applications. These were procured from Merck (Germany) and VWR Chemicals Prolabo (Leuven, Belgium).

Reference standards and chromatographic materials: High-purity flavonoid standards—rutin and isoquercitrin ($\geq 99\%$) and quercetin (98.5%)—were obtained from Extrasynthese (Genay, France). Thin-layer chromatography (TLC) plates (Sigma-Aldrich, Saint Louis, USA) were used for phytochemical screening and qualitative profiling of nano-formulations to ensure reproducibility and high chromatographic performance across all analyses.

2.2 Synthesis of hyaluronic acid–stearylamine conjugate (HAC)

Given that bovine mastitis is characterized by a pronounced inflammatory response of the mammary gland, and that hyaluronic acid (HA) has been widely explored as a surface ligand for inflammation-associated applications [25, 26], we sought to incorporate HA into the prepared nanoparticles. However, native HA is highly hydrophilic and cannot be efficiently incorporated into lipidic or amphiphilic nanocarrier systems through direct

formulation. To address this limitation, HA was covalently conjugated to stearylamine to generate an amphiphilic structure suitable for stable integration at the nanocarrier interface. Stearylamine, a C18 alkyl amine, served as a hydrophobic anchoring moiety enabling covalent coupling with HA and facilitating its presentation at the nanoparticle surface. The resulting hyaluronic acid-stearylamine conjugate (HAC) was synthesized to provide a chemically defined means of surface decoration, allowing systematic comparison between conventional and surface-modified nanoparticles and assessment of the impact of surface functionalization on formulation performance.

HAC was synthesized following a protocol adapted from Toriyabe et al. [27], with modifications based on the coupling chemistry described by Prasad et al. [28]. The carbodiimide coupling agent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) was employed to activate the carboxyl groups of HA and promote covalent bonding with the primary amine of stearylamine (SA), yielding stable amide linkages. Briefly, 500 mg of HA (molecular weight 15–18 kDa) were dissolved in 100 mL of phosphate-buffered saline (PBS, pH 7.4) in a 500 mL Erlenmeyer flask equipped with a magnetic stir bar. The polymer solution was stirred for ten minutes to ensure complete dissolution. Subsequently, 100 mL of a stearylamine solution containing 315 mg of SA (molecular weight 269.5 g/mol) were added to the HA solution, and the mixture was stirred for an additional five minutes to facilitate initial electrostatic interactions between the two reactants. Next, 50 mL of an aqueous EDC·HCl solution (780 mg total mass) were added to activate the HA carboxyl groups, initiating amide bond formation between HA and SA. The reaction mixture was maintained under continuous magnetic stirring at room temperature (25 °C) for 24 h. The progress of the coupling reaction was monitored by thin-layer chromatography (TLC) at intervals of 2, 4, 6, and 24 h. Upon completion, the reaction mixture was transferred into a dialysis membrane and dialyzed against distilled water at room temperature for 48 h, with the external medium replaced every six hours to remove unreacted reagents and by-products. The purified conjugate solution was then freeze-dried (lyophilized) to obtain a dry, solid HAC product for solid-state characterizations.

As HAC represents a newly synthesized material (rather than a simple physical mixture), multiple molecular characterization techniques were employed to verify successful conjugation and material transformation. The obtained conjugate was characterized using thermogravimetric analysis (TGA), Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) as complementary techniques to confirm the formation of HAC and distinguish it from the individual starting materials. FTIR provides molecular-level evidence of covalent bond formation, TGA explores thermal degradation behavior, and XRD investigates changes in crystallinity and structural organization to establish the presence of a new molecular species.

TGA: Approximately 5–10 mg of each freeze-dried sample was placed in a platinum pan and heated under a nitrogen-saturated atmosphere using a PerkinElmer TGA-4000 instrument (USA). The temperature was ramped from 30 to 800 °C at a constant heating rate of 10 °C/min, with an inert nitrogen gas flow rate of 20 mL/min to prevent oxidative degradation. TGA thermograms and corresponding mass loss (DTG) curves were recorded throughout the heating process. Data were analyzed using Pyris TGA software (PerkinElmer) to determine the thermal decomposition profiles.

FTIR spectroscopy: FTIR spectra were recorded in Universal Attenuated Total Reflectance (UATR) mode using freeze-dried samples to monitor the functional group modifications occurring after the conjugation. Each spectrum was obtained by averaging 32 scans over the wavenumber range of 400–4000 cm^{-1} . The FTIR spectrum of HAC was compared with those of the raw materials to identify characteristic stretching vibration bands, confirming the formation of amide linkages between carboxyl groups of hyaluronic acid and amine groups of stearylamine.

XRD: To investigate the crystalline structure and phase characteristics of HAC in comparison with the raw materials, samples were carefully mounted on a flat sample holder and analyzed using a D2 Phaser® benchtop X-ray diffractometer (Bruker, Germany) equipped with an SSD160_2 (1 mode) detector. The system operated with a Cu K α radiation source ($\lambda = 1.54184 \text{ \AA}$) and graphite monochromator, under a fixed optics configuration at an accelerating voltage of 30 kV. Diffraction patterns were recorded over a 2θ range of 5° – 60° , and the resulting spectra were analyzed to assess crystallinity changes, phase transitions, and molecular reorganization indicative of successful conjugation between hyaluronic acid and stearylamine.

2.3 Preparation of nanoformulations

The preparation of nanoformulations is briefly described in dedicated subsections below and further details are presented in Table 1. All formulations were prepared in two sequential stages, resulting in the development of both conventional and surface-modified nanoparticles. The conventional nanomaterials comprised liposomes, polymeric nanoparticles (chitosan- and alginate-based), nanostructured lipid carriers, and solid lipid nanoparticles, all designed to encapsulate the constituents of *Commiphora swynnertonii* resin as the active therapeutic agent. The composition and preparation parameters of the different nanocarrier systems were primarily adopted from previously published protocols (Table 1). Because *C. swynnertonii* resin was encapsulated for the first time, the resin loading level and its mode of incorporation—using an equal distribution between organic and aqueous phases—were determined through preliminary exploratory trials to ensure process feasibility (data not shown). Initial exploratory evaluation of selected process variables (e.g., homogenization and sonication conditions) using a full factorial approach did not yield conclusive trends to define critical process parameters within the scope of this study. Consequently, all the key parameters were kept constant across formulations to enable a fair comparative evaluation of nanoformulations within a given category.

The surface-modified nanoformulations were subsequently generated by incorporating HAC in the corresponding conventional nanoparticles. The surface functionalization strategy was intended to enhance the physicochemical stability, biocompatibility, and site-specific targeting capacity of the nanocarriers, thereby improving their therapeutic efficacy against bovine mastitis and minimizing potential off-target effects.

2.3.1 Preparation of liposomes

Liposomes were prepared as blank, resin-loaded, and HA-functionalized formulations using the thin-film hydration method as previously described by Nkanga et al. [29] and Moyá et al. [30]. Briefly, lipid films were formed by solvent evaporation using a rotary evaporator (Büchi Rotavapor R-300, Switzerland), and subsequently hydrated, followed

Table 1 Summary of the formulations' composition and brief preparation steps

Nanoparticles type/coding	Components (mg/g)			Key preparation steps	References
	Resin	HAC	Core excipients		
Liposomes					
EL	0	0	Soy lecithin (750 mg) and cholesterol (250 mg)	Thin-film hydration method: lipid dissolution in chloroform → rotary evaporation → overnight drying → hydration at 70 °C → sonication (60 min) at 70 °C	[29, 30]
RL	100	0			
EL-HA	0	100			
RL-HA	100	100			
Alginate & chitosan nanoparticles (AN & ChN)					
EAN or EChN	0	0	Alginate or chitosan (750 mg); polyvinyl alcohol (125 mg); sodium tripolyphosphate (125 mg)	Nanoprecipitation: PVA dissolution and cooling → ingredients dispersion under stirring → sonication (40 min) at RT	[31, 32]
RAN or RChN	100	0			
EAN-HA or EChN-HA	0	100			
RAN-HA or RChN-HA	100	100			
Solid lipid nanoparticles					
ESN	0	0	Coconut oil (750 mg) beeswax (250 mg); polysorbate 80 (150 mg); calcium phosphate (25 mg)	Double hot emulsification: lipid melting → organic/aqueous phase homogenization → primary emulsion (w/o) → secondary emulsion (w/o/w) → sonication (40 min) at RT → cooling at 2–8 °C	[33, 34]
RSN	100	0			
ESN-HA	0	100			
RSN-HA	100	100			
Nanostructured lipid carrier					
ENC	0	0	Stearic acid (450 mg); castor oil (450 mg); polyvinylpyrrolidone (95 mg)	Same as for solid lipid nanoparticles	[34, 35]
RNC	100	0			
ENC-HA	0	100			
RNC-HA	100	100			

EL Empty Liposomes, *RL* Resin-loaded Liposomes, *EL-HA* Empty liposomes with hyaluronic acid, *RL-HA* Resin-loaded liposomes with hyaluronic acid, *ENC* Empty nanostructured lipid carriers, *RNC* Resin-loaded nanostructured lipid carriers, *ENC-HA* Empty nanostructured lipid carriers with hyaluronic acid, *RNC-HA* Resin-loaded nanostructured lipid carriers with hyaluronic acid, *ESN* Empty solid lipid nanoparticles, *RSN* Resin-loaded solid lipid nanoparticles, *ESN-HA* Empty solid lipid nanoparticles with hyaluronic acid, *RSN-HA* Resin-loaded solid lipid nanoparticles with hyaluronic acid, *EAN* Empty alginate nanoparticles, *RAN* Resin-loaded alginate nanoparticles, *EAN-HA* Empty alginate nanoparticles with hyaluronic acid, *RAN-HA* Resin-loaded alginate nanoparticles with hyaluronic acid, *EChN* Empty chitosan nanoparticles, *RChN* Resin-loaded chitosan nanoparticles, *EChN-HA* Empty chitosan nanoparticles with hyaluronic acid, *RChN-HA* Resin-loaded chitosan nanoparticles with hyaluronic acid, *w/o* water in oil emulsion, *w/o/w* water-in-oil-in water emulsion.

by sonication to obtain homogeneous liposomal dispersions. The resulting liposomal dispersions were stored at 4 °C until further treatments.

2.3.2 Preparation of alginate and chitosan-based polymeric nanoparticles

Alginate- and chitosan-based polymeric nanoparticles, including blank, resin-loaded and HAC-functionalized formulations, were prepared using nanoprecipitation technique described by Kharia et al. [31] and Pinheiro Machado et al. [32]. In brief, polymeric dispersions containing the resin and other relevant ingredients were subjected to controlled stirring and sonication to promote nanoparticle formation. The obtained

polymeric nanoparticle suspensions were subsequently stored at 4 °C until further processing.

2.3.3 Preparation of solid lipid nanoparticles and nanostructured lipid carriers

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) were each prepared as blank, resin-loaded and HAC-functionalized nanoparticles using a combined high-shear homogenization and double hot emulsification method, as previously described by Cheibani [33] and Hajjali [34]. Briefly, molten lipid mixtures and aqueous phases were emulsified at elevated temperature, followed by sonication and controlled cooling to obtain stable lipid-based nanoparticles [35].

2.4 Isolation and lyophilization of nanoparticles

Following synthesis, nanoparticles from all formulations were collected by centrifugation using a Beckman Coulter Allegra 64R centrifuge (Krefeld, Germany) at 15,000 rpm for 30 min at 4 °C. On one hand, the supernatants were carefully removed and saved for further encapsulation efficacy studies. On the other hand, the pellets were freeze-dried (lyophilized) using a BK-FD18T lyophilizer (Biobase, China). Nanoparticles were lyophilized to ensure stability and accurate mass-based analysis. This was motivated on the basis that nanoencapsulated *C. swynnertonii* resin represents a newly formulated active material with an unknown stability profile and storage behavior in aqueous suspensions; lyophilization was adopted as a conservative strategy to preserve nanoparticle structural integrity and prevent any degradation, or payload leakage. In addition, dry nanoparticles enabled precise redispersion at defined concentrations for polyphenols content determination and antimicrobial assays.

2.5 Characterization of nanomaterials

2.5.1 Dynamic light scattering

The particle size and Zeta potential of the nanoparticles were determined by dynamic light scattering (DLS) using a Zetasizer Malvern instrument (Zetasizer LAB-RED, model MAL1296930, Malvern Panalytical, UK). Aliquots of freshly prepared nanoparticle suspensions (collected after sonication) and redispersed lyophilized nanoparticles were gently diluted with Milli-Q water to obtain an optimal scattering intensity. Measurements were performed in triplicate at a scattering angle of 173° under controlled temperature conditions.

2.5.2 Transmission electron microscopy

For morphological characterization, the freeze-dried nanoparticle samples were dispersed in water and sonicated for 15–20 min to ensure homogenization. A carbon-coated Formvar copper grid was then carefully dipped into the suspension, allowing a thin film of the sample to adhere to the grid surface. The grid was subsequently air-dried at room temperature overnight prior to imaging. Transmission electron microscopy (TEM) images were acquired using a JEOL 2100 high-resolution transmission electron microscope operated under standard accelerating voltage and magnification settings to evaluate particle morphology, size, and dispersion uniformity.

2.6 Determination of encapsulation efficiency

The encapsulation efficiency (E.E.) of the prepared nanoparticles was determined based on the total polyphenol content, using unformulated *Commiphora swynnertonii* resin as a control for comparison. Given the complex and chemically diverse nature of the resin, which contains both phenolic and non-phenolic constituents, total polyphenols were used as a representative and reproducible surrogate marker to assess encapsulation efficiency across different nanocarrier platforms. Total polyphenol content (TPC) was determined using the Folin–Ciocalteu method. This method was selected as a widely applied colorimetric assay for plant-derived materials and well suited for comparative analysis of plant-based nanoparticle formulations [36]. The EE study quantified the proportion of encapsulated versus non-encapsulated (free) polyphenols; the latter was measured from the supernatants obtained after centrifugation of nanoparticles (see details in Sect. 2.4). The supernatants were expected to be free of resin constituents if encapsulation were 100% successful, and this assumption was verified by assessing the residual polyphenols in the supernatant.

For the control solution, 100 mg of resin were dissolved in 40 mL of methanol to serve as a reference solution to the supernatant; this is because the nanoformulations yielded an equivalent volume (40 mL) of supernatant following centrifugation, which enabled comparative analysis. To balance solvent composition between the supernatants and control solution, 2 mL of methanolic resin solution were mixed with 2 mL of distilled water, whereas 2 mL of each supernatant were mixed with 2 mL of methanol prior polyphenols analysis by colorimetry.

The TPC determination was done following a previously reported Folin–Ciocalteu method [36, 37]. Briefly, 500 μ L of each sample (supernatant or control) were added to 500 μ L of Folin–Ciocalteu reagent, mixed, and allowed to react for three minutes before the addition of 1000 μ L of 20% sodium carbonate solution. The mixtures were incubated in the dark for one hour at room temperature, and absorbance was recorded at 500 nm using a BK-UV 1800 spectrophotometer (Biobase, China). The % E.E. was calculated using the following equation (Eq. 1):

$$\text{E.E.} = \left(\frac{A_o - A_s}{A_o} \right) \times 100 \quad (1)$$

where A_o indicates the absorbance of control (resin alone without excipient); A_s indicates the absorbance of supernatant obtained following isolation of nanoparticles by centrifugation.

2.7 Determination of total polyphenols content in nanomaterials

Lyophilized nanoparticles were redispersed in methanol and subjected to sonication at 70 °C for one hour to ensure complete extraction of encapsulated polyphenols. After sonication, aliquots of each sample were collected and analyzed for TPC using the modified Folin–Ciocalteu. Quantification of TPC was achieved using a calibration curve derived from unformulated *Commiphora swynnertonii* resin with known polyphenol concentrations. The calibration relationship described the following linear regression equation (Eq. 2):

$$Y = 1.4878X + 0.0212 \quad (R^2 = 0.9955) \quad (2)$$

where Y represents the measured absorbance and X the corresponding polyphenol concentration (mg/mL). The calibration curve was established from three independent control curves, each prepared from serial dilutions of the resin solution in methanol, confirming the linearity of the method.

2.8 Phytochemical screening by thin layer chromatography

Thin-layer chromatography (TLC) was performed to screen and compare the phytochemical compositions of different nanoparticles loaded with the constituents of *Commiphora swynnertonii* resin. A methanolic solution of the resin (50 mg/3 mL) and extracts from the nanomaterials (600 mg/3 mL) were analyzed side-by-side to verify the presence of some resin metabolites in the synthesized nanoparticles. For each run, 20 μ L of resin extract, 30 μ L of nanoparticle extract, and reference standards (1 mg/mL) were carefully spotted onto silica gel F₂₅₄ TLC plates (Sigma-Aldrich, USA). Encapsulated phytochemicals were extracted from the nanoparticles by maceration in methanol followed by sonication at 50 °C for 30 min. The extracts were then filtered and used directly for chromatographic analysis. Elution of metabolites was carried out using solvent systems specific to different phytochemical classes, as described by Muipata et al. [38]: (i) Dichloromethane:formic acid:acetone (80:10:20, v/v)—for flavonoids and phenolic acids; (ii) Toluene:ethyl acetate (93:7, v/v)—for terpenoids; (iii) Ethyl acetate:methanol:water (25:3.375:2.5, v/v)—for anthraquinones; (iv) n-Butanol:water:glacial acetic acid (21:3.5:1.75, v/v)—for saponins; (v) Toluene:ether (1:1, v/v)—for coumarins; (vi) Dichloromethane:methanol:water (94:5:1, v/v)—for steroids; (vii) Ethyl acetate:methanol:water (40:8:5, v/v)—for tannins. Visualization of chromatograms was achieved either by direct observation under ultraviolet (UV) light at 254 and 366 nm or by spraying with selective detection reagents, including Neu's reagent, anisaldehyde–sulfuric acid, phosphovanillin, ethanolic KOH, and sulfuric acid. In some cases, plates were heated to 100 °C for ten minutes after reagent application to intensify characteristic color development of specific secondary metabolites. The resulting chromatograms were used to establish fingerprint profiles for the resin and the corresponding nanoparticle formulations, confirming the successful encapsulation and preservation of phytochemical constituents within the formulated nanoparticles.

2.9 Assessment of the antimicrobial efficacy of nanomaterials

2.9.1 Microbial strains and culture conditions

Bacterial Isolation: Multidrug-resistant *Staphylococcus aureus* strains used in this study were isolated from milk excreted by mastitis cow patients. A loopful of milk sample was inoculated onto Mannitol salt agar and Mac Conkey agar individually before being incubated at 37 °C for 24 h. A secondary culture was performed on blood agar and Mannitol salt agar until a pure culture was achieved. The colonial morphology was characterized by examining the macromorphological features of the specific bacteria.

Identification: Staphaurex® Plus latex agglutination test (Thermo Fisher Scientific, Oxoid Ltd., Basingstoke, UK) was used following the manufacturer's instructions to identify and differentiate *S. aureus* from other staphylococcal species. For *E. coli*, standard Gram staining, SIM test, IMViC tests, and TSI test were conducted. Molecular Tech such as PCR and sequencing was also used for identification.

Antimicrobial susceptibility test (AST): The AST was performed using the Kirby-Bauer Disk Diffusion method, and the antibiotics were chosen following the Clinical and Laboratory Standard Institute (CLSI) guidelines. Bacterial inoculum was prepared by suspending isolated colonies in sterile saline and adjusted to a 0.5 McFarland standard ($\sim 1.5 \times 10^8$ CFU/mL) using McFarland Densitometer. A sterile swab was used to inoculate Muller Hinton Agar (MHA) by streaking across the entire surface of the agar; this was done using the lawn culture method. Afterward, the antibiotic disks were placed on the agar, and the plate was incubated at 37 °C for 24 h. The zones of inhibition were measured using a vernier caliper and interpreted according to the 2025 CLSI Guideline. Additionally, a test for MRSA was conducted to identify Methicillin-resistant *S. aureus*.

Determination of multidrug-resistant (MDR) strains: Using Kirby-Bauer disk diffusion isolate strain with non-susceptibility to at least three or more antimicrobial groups of antibiotics was termed as MDR strain.

2.9.2 Agar well diffusion assay

The antimicrobial activity of the test compound was initially screened using the agar well diffusion method. Sterile Petri dishes containing MHA were inoculated with the prepared *Staphylococcus aureus* isolates suspension using a sterile cotton swab to achieve a uniform lawn. Wells of 6 mm diameter were aseptically punched into the agar using a sterile cork borer. Each well was filled with 100 μ L of the nanoformulation at the concentration of 1.25 μ g/mL. A positive control (standard antibiotic i.e. Gentamycin) and a negative control (solvent i.e. PBS) were included in each plate. Gentamicin was selected as the positive control due to its broad-spectrum bactericidal and anti-staphylococcal activity, and its ability to produce reproducible inhibition zones, enabling validation and benchmarking assays. Plates were pre-incubated at room temperature for 30 min to allow diffusion of the nanoformulation components, followed by incubation at 37 °C for 24 h for bacterial strains or at 28 °C for 48–72 h for resistant bacterial strains. Following incubation, the antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition around each well in millimeters (mm). Inhibition zone diameters were reported as numerical values to preserve quantitative resolution. As agar well diffusion is primarily a screening method rather than a standardized clinical susceptibility assay, categorical interpretation of inhibition zones was used only for comparative assessment of antimicrobial activity and not for definitive resistance classification.

2.9.3 Determination of minimum inhibitory concentration (MIC)

Antimicrobial activity was evaluated against 13 MDR *S. aureus* strains from bovine mastitis cases selected due to their clinical relevance and reduced susceptibility to common antibiotics, providing a stringent model for assessing the efficacy of the novel nanoformulations. The minimum inhibitory concentration (MIC) of the test compound was determined using the broth microdilution method, following the CLSI guidelines with minor modifications. Tenfold serial dilutions of the compound were prepared in Mueller–Hinton Broth (MHB) within sterile 96-well microtiter plates, resulting in final concentrations ranging from 1250 to 2.4 μ g/mL. Each well was inoculated with 100 μ L of a standardized microbial suspension to achieve a final cell density of approximately 1.5×10^8 CFU/mL (0.5 MacFarland standard). Appropriate controls were included on each plate: a growth control (medium plus microbial inoculum without the test

compound), a sterility control (medium plus test compound without inoculum), and a positive control (medium with microbial inoculum plus a reference antibiotic). Gentamicin was used as the positive control owing to its established bactericidal activity against *S. aureus* and its suitability for benchmarking antimicrobial performance. Plates were incubated at 37 °C for 24 h for bacterial isolates or at 28 °C for 48–72 h for bacterial strains. The MIC was defined as the lowest concentration of the test compound that completely inhibited visible microbial growth (i.e., absence of turbidity) in comparison to the growth control. For enhanced visualization of microbial viability, 20 µL of 0.2% concentration of 2,4,6-Trinitrotoluene dye was added to each well. A colorimetric change from the formation of colorless to pink was used as an indicator of metabolic activity and, consequently, microbial growth. All assays were performed in triplicate, and the MIC values were reported as the mean of the three independent experiment.

2.9.4 Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) was determined following standard microbiological procedures. After MIC determination, aliquots from wells showing no visible bacterial growth were aseptically subcultured onto Mueller–Hinton agar plates and incubated at 37 °C for 24 h. The lowest concentration at which no bacterial growth was observed on agar plates was recorded as the MBC, corresponding to ≥ 99.9% bacterial killing.

2.10 Data analysis

All data were analyzed using GraphPad Prism software, version 10.3.1 (Build 509) (GraphPad Software, San Diego, USA). Results were expressed as mean ± standard deviation (SD), and percentages were calculated where appropriate. Statistical comparisons between experimental groups were performed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc multiple comparisons test to determine significant differences. In cases involving pairwise comparisons, a Student's *t*-test was applied. At least *p*-value < 0.05 was considered statistically significant.

3 Results and discussion

3.1 Characterization of hyaluronic acid–stearylamine conjugate (HAC)

The synthesized hyaluronic acid–stearylamine conjugate (HAC) was obtained as a white, odorless solid with a cotton-like texture after lyophilization. The progress and completion of the conjugation reaction were initially monitored by thin-layer chromatography (TLC). Comparative analyses performed at 2, 4, 6, 12, 18, and 24 h revealed a gradual decrease in the intensity of the starting material spot corresponding to stearylamine, which was the only detectable starting material using ninhydrin reagent. The depletion of stearylamine as starting material was accompanied by the emergence of a new spot with a distinct retention factor (Figure S1), suggesting the formation of a new molecular species (HAC) in reaction medium. The resultant conjugate was purified by dialysis, freeze-dried and characterized using Fourier-transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and X-ray diffraction (XRD) to confirm successful conjugation and establish the final product's characteristics.

The FTIR spectra of the starting materials—hyaluronic acid (HA) and stearylamine (SA)—exhibited their characteristic absorption bands in the 4000–500 cm^{−1} region

(Fig. 1). The HA spectrum displayed intense O–H stretching vibrations (broad band around 3400 cm^{-1}) and prominent C=O stretching and COO^- asymmetric vibrations characteristic of carboxyl groups. In contrast, the SA spectrum showed distinct N–H stretching (around 3300 cm^{-1}) and C–H stretching bands (in the $2850\text{--}2950\text{ cm}^{-1}$ region), typical of long-chain amines. In the FTIR spectrum of the conjugate, notable spectral changes were observed relative to those of the individual components. The carboxyl stretching band of HA diminished in intensity, while new amide-related absorption bands appeared—particularly the amide I (C=O stretching, $\sim 1650\text{ cm}^{-1}$) and amide II (N–H bending, $\sim 1550\text{ cm}^{-1}$) vibrations. These spectral modifications indicate the formation of covalent amide linkages between the carboxyl groups of HA and the amine groups of SA, confirming successful conjugation. These results are consistent with previous findings reported by Toriyabe et al. [27] and Liu et al. [39], further validating that the conjugation reaction proceeded as intended.

TGA provided further evidence supporting the successful formation of HAC. The thermal decomposition profile of the conjugate differed markedly from those of the precursor materials (Fig. 2), indicating significant physicochemical modification at the molecular level. The TGA curve of hyaluronic acid (HA) displayed a characteristic two-step weight loss: an initial reduction below 150°C corresponding to moisture evaporation, followed by a major degradation phase attributed to depolymerization and decomposition of the polysaccharide backbone. In contrast, stearylamine (SA) exhibited a distinct thermal degradation pattern consistent with the breakdown of its long-chain crystalline fatty amine structure, showing greater stability at lower temperatures but rapid mass loss at higher ones. The HAC conjugate, however, presented a unique decomposition profile characterized by altered onset temperatures, shifted degradation peaks, and distinct multi-step weight-loss stages compared to either HA or SA alone. The derivative thermogravimetric (DTG) curve revealed the appearance of new thermal transitions, confirming the formation of a novel material with enhanced and distinct

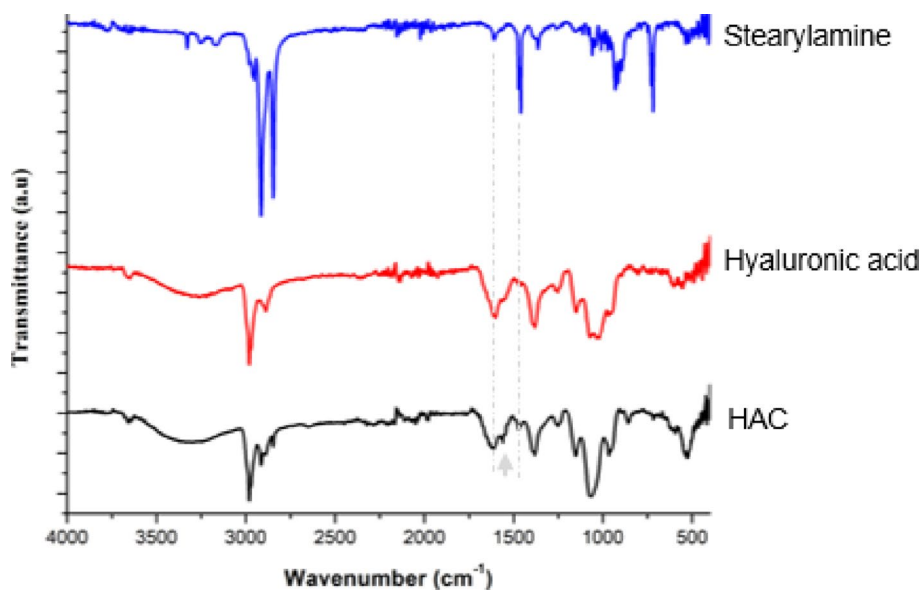


Fig. 1 FTIR spectra showing the characteristic functional groups of the hyaluronic acid-stearylamine conjugate (HAC) in comparison with the starting materials; the arrow indicates the newly formed amide C=O stretching, confirming successful conjugation of stearylamine to hyaluronic acid

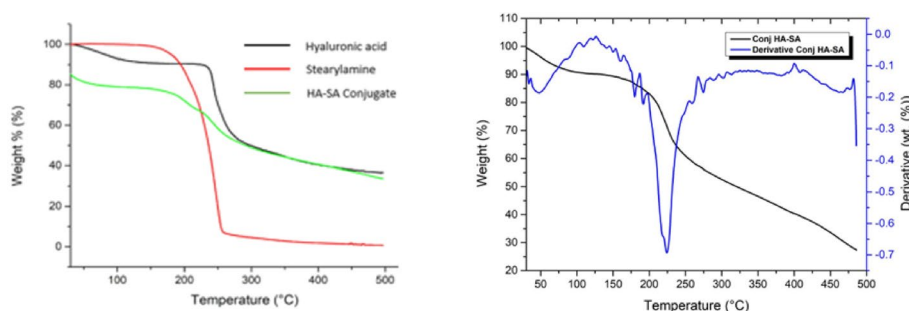


Fig. 2 Left graph: Comparison between TGA mass loss curves of raw materials versus conjugate, showing different thermal decomposition profiles. Right graph: Mass loss (black) and derivative thermogravimetric (blue) curves of the conjugate HAC, showing weight loss and decomposition stages as a function of temperature

thermal stability properties. These findings corroborate the FTIR results, further verifying that the conjugation reaction successfully produced a chemically and thermally distinct HA–stearylamine conjugate.

XRD analysis revealed pronounced structural differences between the parent materials and the hyaluronic acid–stearylamine conjugate (HAC) (Figure S2). These findings are consistent with previous reports on polymer–lipid conjugates by Liu et al. [39], further supporting the occurrence of successful chemical modification. The XRD pattern of hyaluronic acid (HA) exhibited a broad diffuse halo, characteristic of amorphous materials, confirming its non-crystalline polysaccharide nature. In contrast, stearylamine (SA) displayed sharp and intense diffraction peaks, indicative of a highly crystalline structure arising from the ordered packing of its long alkyl chains. The HAC conjugate, however, presented a distinct diffraction profile that differed from both precursors. Several crystalline peaks associated with stearylamine diminished or disappeared entirely, while new diffraction signals emerged at different 2θ angles. This transformation suggests that the conjugated polymer matrix exhibits partial ordering, resulting from the integration of stearylamine chains into the hyaluronic acid backbone. Overall, these structural modifications demonstrate the formation of a new semi-crystalline material, confirming successful conjugation and altered molecular organization within the HAC.

Taken together, the combined spectroscopic (FTIR), thermal (TGA), and structural (XRD) analyses suggest successful synthesis of the HAC. The disappearance of characteristic absorption bands from the starting materials, the emergence of new amide linkage signals, the distinct thermal degradation profile, and the altered crystallinity pattern collectively confirm the formation of a new chemically conjugated material with unique physicochemical properties. These findings demonstrate that the conjugation process effectively linked the carboxyl groups of hyaluronic acid with the amine groups of stearylamine, producing a hybrid polymer with distinctive stability pattern and modified structural organization. The synthesized HAC was subsequently incorporated in the nanoformulations, enabling the display of HA as a surface-functionalizing agent for all the various nanoparticles. The following sections present the results of nanoparticle formulation and characterization, and antimicrobial evaluation, highlighting the impact of HAC functionalization on nanoparticle behavior and antimicrobial efficacy.

3.2 Particulate characteristics of the prepared nanoformulations

The particle size, polydispersity index (PDI), and Zeta potential (ZP) of all nanoparticle formulations are summarized in Table 2. Overall, the physicochemical properties—including particle size, PDI, and surface charge—were found to vary depending on nanoparticle type and formulation compositions. However, statistical analyses comparing batches from a given type of nanoparticles revealed no significant differences between nanoformulations (>0.05), suggesting that the incorporation of *C. swynner-tonii* resin and/or HAC did not substantially alter the structural characteristics of the prepared nanoparticles. In general, particle sizes measurements from DLS suggested the presence of particles ranging from 61 to 1395 nm, with approximately 90% of nanoformulations falling within the nanometric range. According to the DLS size distribution graphs (Fig. 3 and 4), some of the prepared formulations exhibited particles sizes >1000 nm—notably, resin-loaded alginate nanoparticles (RAN) and empty alginate nanoparticles with HA (EAN-HA). While micro-particles formulations are still relevant for drug delivery applications [40], we further investigated all the formulations by TEM to confirm particle sizes and determine structural morphology.

As illustrated in Fig. 5, TEM micrographs revealed the presence of spherical particles and, unlike DLS data, none of the TEM samples exhibited particles with sizes >1000 nm, irrespective of the formulation. The discrepancies between TEM and DLS data are frequently observed, and are generally attributed to the nature of DLS as an intensity-based method. DLS measures the hydrodynamic diameter, which can take into account

Table 2 Particle size, polydispersity index (PDI) and Zeta potential of nanoformulations

Nanoparticle type	Composition code	Particle Size $\pm$ SD (nm)	PDI $\pm$ SD	Zeta potential $\pm$ SD (mV)
Liposomes	EL	133.1 $\pm$ 108.5	0.290 $\pm$ 0.080	− 62.70 $\pm$ 2.410
	RL	72.10 $\pm$ 21.60	0.330 $\pm$ 0.050	− 61.44 $\pm$ 4.590
	EL-HA	329.3 $\pm$ 331.3	0.420 $\pm$ 0.160	− 55.89 $\pm$ 1.970
	RL-HA	320.5 $\pm$ 423.5	0.410 $\pm$ 0.050	− 53.92 $\pm$ 2.340
Nanostructured Lipid carriers	ENC	115.0 $\pm$ 35.00	0.500 $\pm$ 0.190	− 35.00 $\pm$ 5.000
	RNC	130.0 $\pm$ 10.00	0.420 $\pm$ 0.160	− 24.00 $\pm$ 2.000
	ENC-HA	80.00 $\pm$ 0.000	0.900 $\pm$ 0.020	− 25.00 $\pm$ 1.400
	RNC-HA	81.00 $\pm$ 11.00	1.000 $\pm$ 0.000	− 30.00 $\pm$ 7.000
Solid Lipid Nanoparticles	ESN	125.0 $\pm$ 6.000	0.310 $\pm$ 0.500	− 23.00 $\pm$ 4.000
	RSN	132.0 $\pm$ 7.000	0.280 $\pm$ 0.070	− 29.00 $\pm$ 8.000
	ESN-HA	105.0 $\pm$ 40.00	0.700 $\pm$ 0.700	− 19.00 $\pm$ 26.00
	RSN-HA	207.0 $\pm$ 137.0	0.600 $\pm$ 0.120	− 13.00 $\pm$ 23.00
Alginate Nanoparticles	EAN	812.0 $\pm$ 47.00	0.660 $\pm$ 0.120	− 48.00 $\pm$ 4.160
	RAN	1819 $\pm$ 443.0	0.260 $\pm$ 0.17.00	− 57.00 $\pm$ 1.520
	EAN-HA	1395 $\pm$ 0.000	0.400 $\pm$ 0.000	− 53.00 $\pm$ 0.000
	RAN-HA	909.0 $\pm$ 298.4	0.520 $\pm$ 0.050	− 57.00 $\pm$ 0.700
Chitosan Nanoparticles	EChN	61.00 $\pm$ 83.10	0.770 $\pm$ 0.370	− 6.000 $\pm$ 2.640
	RChN	220.0 $\pm$ 93.60	0.360 $\pm$ 0.030	− 36.00 $\pm$ 7.210
	EChN-HA	740.0 $\pm$ 156.9	0.480 $\pm$ 0.180	− 3.000 $\pm$ 2.120
	RChN-HA	493.0 $\pm$ 68.12	0.320 $\pm$ 0.050	− 36.00 $\pm$ 5.850

EL Empty Liposomes, RL Resin-loaded Liposomes, EL-HA Empty liposomes with hyaluronic acid, RL-HA Resin-loaded liposomes with hyaluronic acid, ENC Empty nanostructured lipid carriers, RNC Resin-loaded nanostructured lipid carriers, ENC-HA Empty nanostructured lipid carriers with hyaluronic acid, RNC-HA Resin-loaded nanostructured lipid carriers with hyaluronic acid, ESN Empty solid lipid nanoparticles, RSN Resin-loaded solid lipid nanoparticles, ESN-HA Empty solid lipid nanoparticles with hyaluronic acid, RSN-HA Resin-loaded solid lipid nanoparticles with hyaluronic acid, EAN Empty alginate nanoparticles, RAN Resin-loaded alginate nanoparticles, EAN-HA Empty alginate nanoparticles with hyaluronic acid, RAN-HA Resin-loaded alginate nanoparticles with hyaluronic acid, EChN Empty chitosan nanoparticles, RChN Resin-loaded chitosan nanoparticles, EChN-HA Empty chitosan nanoparticles with hyaluronic acid, RChN-HA Resin-loaded chitosan nanoparticles with hyaluronic acid

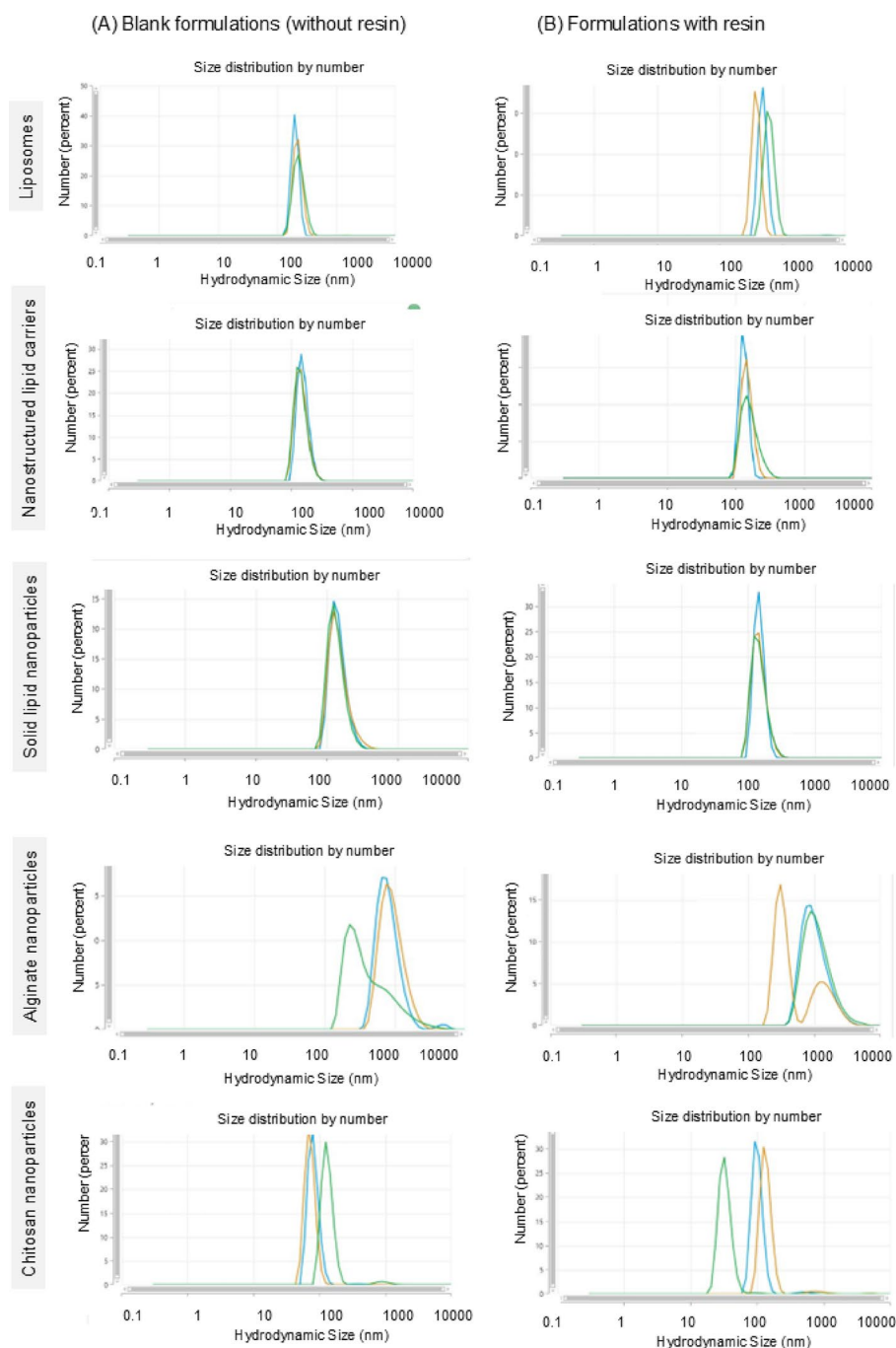


Fig. 3 DLS data showing particle size distribution by number for all nano-formulations (without HAC), suggesting negligible impact of resin on particle size distribution regardless of the type of nanoparticles

the solvation layers surrounding the particles, aggregates and any molecules adsorbed from the dispersion medium. The presence of aggregates observed in some of the samples could be a potential factor to explain the over estimation of average sizes from DLS, which needs to be supplemented by TEM data that established the nanometric nature of particles in the prepared formulations. Irregular morphologies and apparent aggregation are frequently observed in TEM images of polymeric and lipid-based nanoparticles and may result from sample preparation associated with conventional TEM analysis,

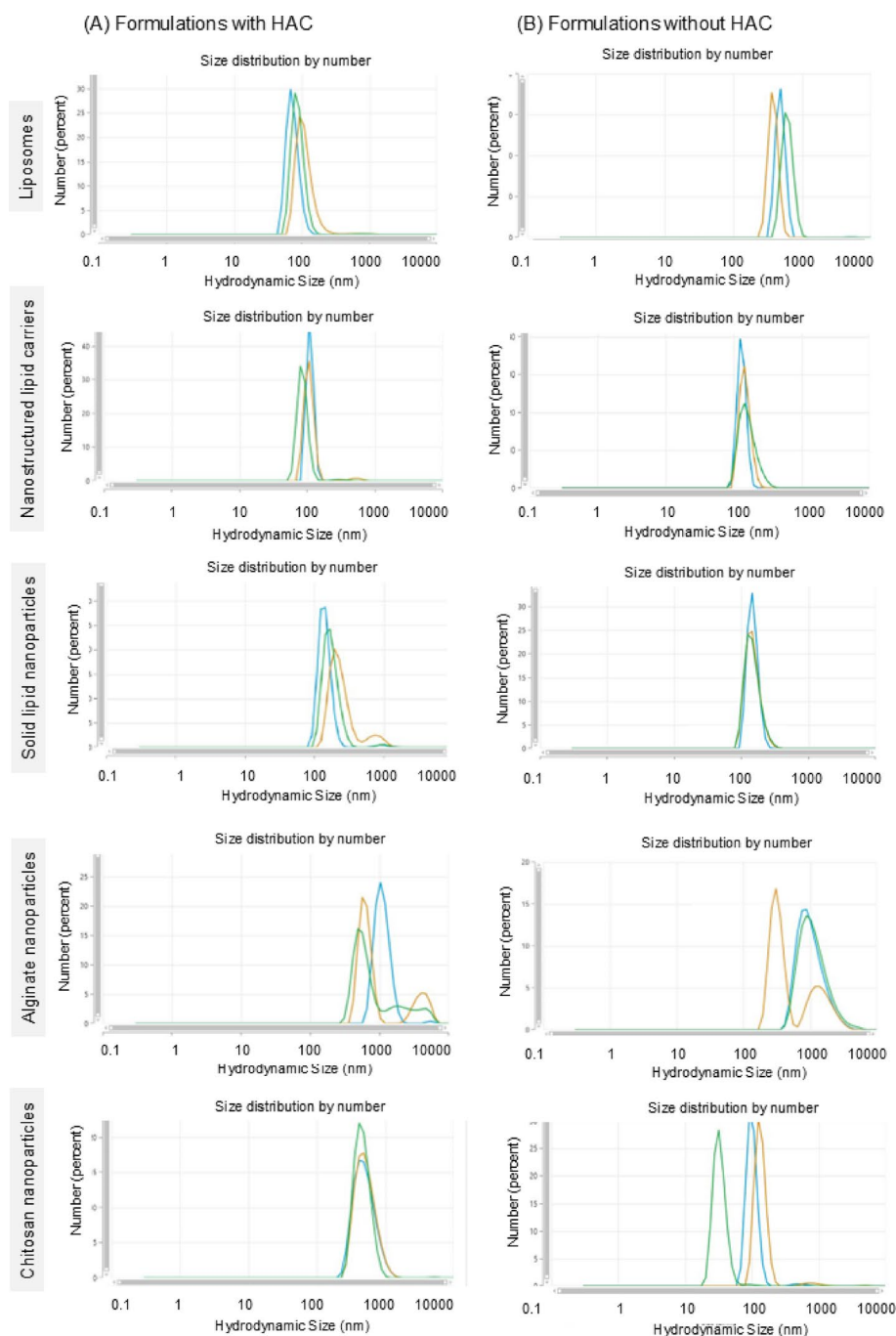


Fig. 4 DLS data showing particle size distribution by number for all resin-loaded nano-formulations, indicating negligible impact of hyaluronic acid addition on particle size distribution for the prepared nanoparticles

including solvent drying and grid adsorption [41]. Such dry-state features do not necessarily reflect colloidal behavior in aqueous suspension. Solution-state characterization by DLS indicated particle size distribution profiles with both submicron and larger particle populations; the former was mostly for lipid-based systems (liposomes, SLN and NLC), which seems to be paradoxical because these nanosystems exhibited more aggregation in TEM than alginate- and chitosan-based nanoparticles. No consistent effect of hyaluronic acid surface modification on particle morphology was observed.

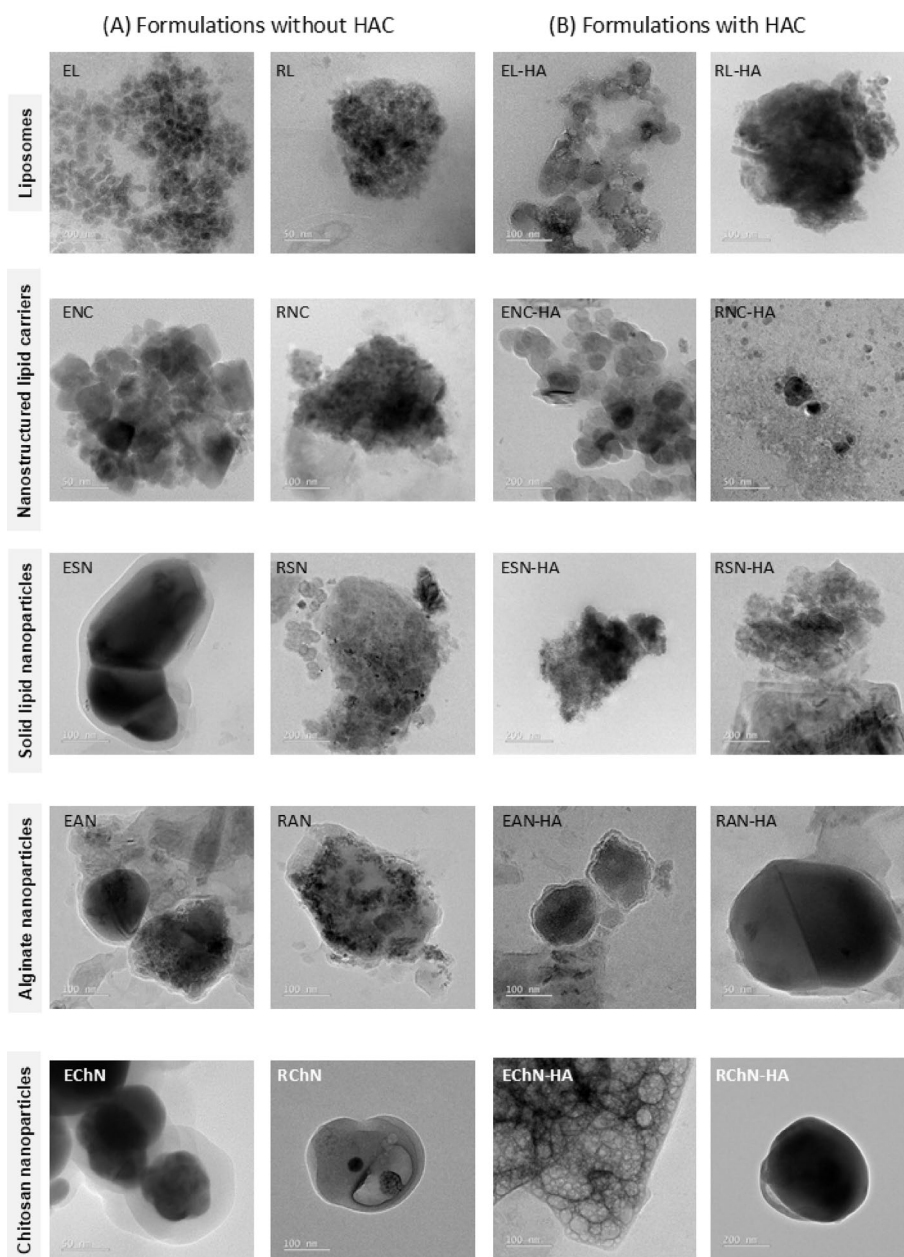


Fig. 5 TEM micrographs of resin-loaded and empty nanoformulations. *EL* Empty Liposomes; *RL* Resin-loaded Liposomes; *EL-HA* Empty liposomes with hyaluronic acid; *RL-HA* Resin-loaded liposomes with hyaluronic acid; *ENC* Empty nanostructured lipid carriers; *RNC* Resin-loaded nanostructured lipid carriers; *ENC-HA* Empty nanostructured lipid carriers with hyaluronic acid; *RNC-HA* Resin-loaded nanostructured lipid carriers with hyaluronic acid; *ESN* Empty solid lipid nanoparticles; *RSN* Resin-loaded solid lipid nanoparticles; *ESN-HA* Empty solid lipid nanoparticles with hyaluronic acid; *RSN-HA* Resin-loaded solid lipid nanoparticles with hyaluronic acid; *EAN* Empty alginate nanoparticles; *RAN* Resin-loaded alginate nanoparticles; *EAN-HA* Empty alginate nanoparticles with hyaluronic acid; *RAN-HA* Resin-loaded alginate nanoparticles with hyaluronic acid; *EChN* Empty chitosan nanoparticles; *RChN* Resin-loaded chitosan nanoparticles; *EChN-HA* Empty chitosan nanoparticles with hyaluronic acid; *RChN-HA* Resin-loaded chitosan nanoparticles with hyaluronic acid

PDI values for the prepared nanoformulations ranged from 0.2 to 1.0, indicating varying degrees of size distribution uniformity among formulations; however, size distribution data showed relatively narrowed size variations (Fig. 3 and 4). Nevertheless, most of the nanoformulations exhibited $PDI \leq 0.5$, indicating good homogeneity in sizes and

acceptable distribution profiles [42]. In common practice, low PDI values indicate uniform nanoparticle populations and are therefore desirable for reproducible biological behavior and consistent drug delivery performance. The higher PDI values observed for some formulations reflect size heterogeneity within batches. This variability is consistent with the exploratory nature of this study, which aimed to screen multiple nanocarrier platforms for their ability to encapsulate *C. swynnertonii* resin and retain antimicrobial activity rather than to fully optimize individual formulations. Preliminary factorial screening of selected process parameters did not reveal any consistent trends in PDI, particle size, or zeta potential (data not shown), suggesting that broader formulation-level optimization would be required, and this was beyond the scope of this comparative study. Given the chemical complexity and heterogeneity of the resin, as well as the diversity of carrier systems investigated, formulation-specific optimization was intentionally deferred to subsequent studies.

In addition to the variability in particle sizes, Zeta potential values were equally different across nanoparticles: the net surface charges of particles ranged between -3.00 ± 2.12 mV and -62.70 ± 2.41 mV, reflecting variable electrostatic stability among formulations depending on their composition. According to the literature, nanoparticles are expected to be colloidally stable when their surface charge lies between -20 mV and $+30$ mV [43]; and we were pleased to notice that all the nanoparticle batches produced in this study were within the indicated Zeta potential ranges, except blank chitosan-based NPs which showed very low density of surface charges.

3.3 Encapsulation efficiency of nanoparticles for resin's total polyphenols

The varying compositional ratios used in the formulation of liposomes, solid lipid nanoparticles, nanostructured lipid carriers, and polymeric nanoparticles enabled the formulation of nanoparticles with high encapsulation capacities, retaining no less than 70% of the total polyphenols from the *C. swynnertonii* resin (Fig. 6A). Notably, functionalization of nanoparticles with HAC further enhanced this encapsulation efficiency across all nanoparticle systems. This effective nanoencapsulation of polyphenols observed can be attributed to favorable physicochemical interactions between the polar functional groups of formulation components (e.g., phospholipids, polysaccharides, and fatty acids) and the phytochemical constituents of the resin. These interactions are likely promoted by hydrogen bonding and hydrophobic association, facilitating improved entrapment of both hydrophilic and hydrophobic phytochemicals within the nanocarrier matrices. In particular, the hyaluronic acid conjugate, being characterized by its linear polysaccharide backbone and intrinsic reactivity, played a key structural role. Its incorporation likely enhanced the stability and compactness of the nanocarriers, thereby increasing their encapsulation efficiency and ensuring better retention of total polyphenols. This observation aligns with previous findings by Sudha and Rose [44], who reported that the addition of HA derivatives to lipid–polymer systems improved drug encapsulation through enhanced matrix cohesion and molecular compatibility.

3.4 Total polyphenols contents in nanoparticles

The absorbance values obtained from the Folin–Ciocalteu assay were interpolated into the calibration curve, which represented the mean of three independently generated standard curves. The coefficient of determination ($R^2 = 0.9992$) obtained was sufficiently

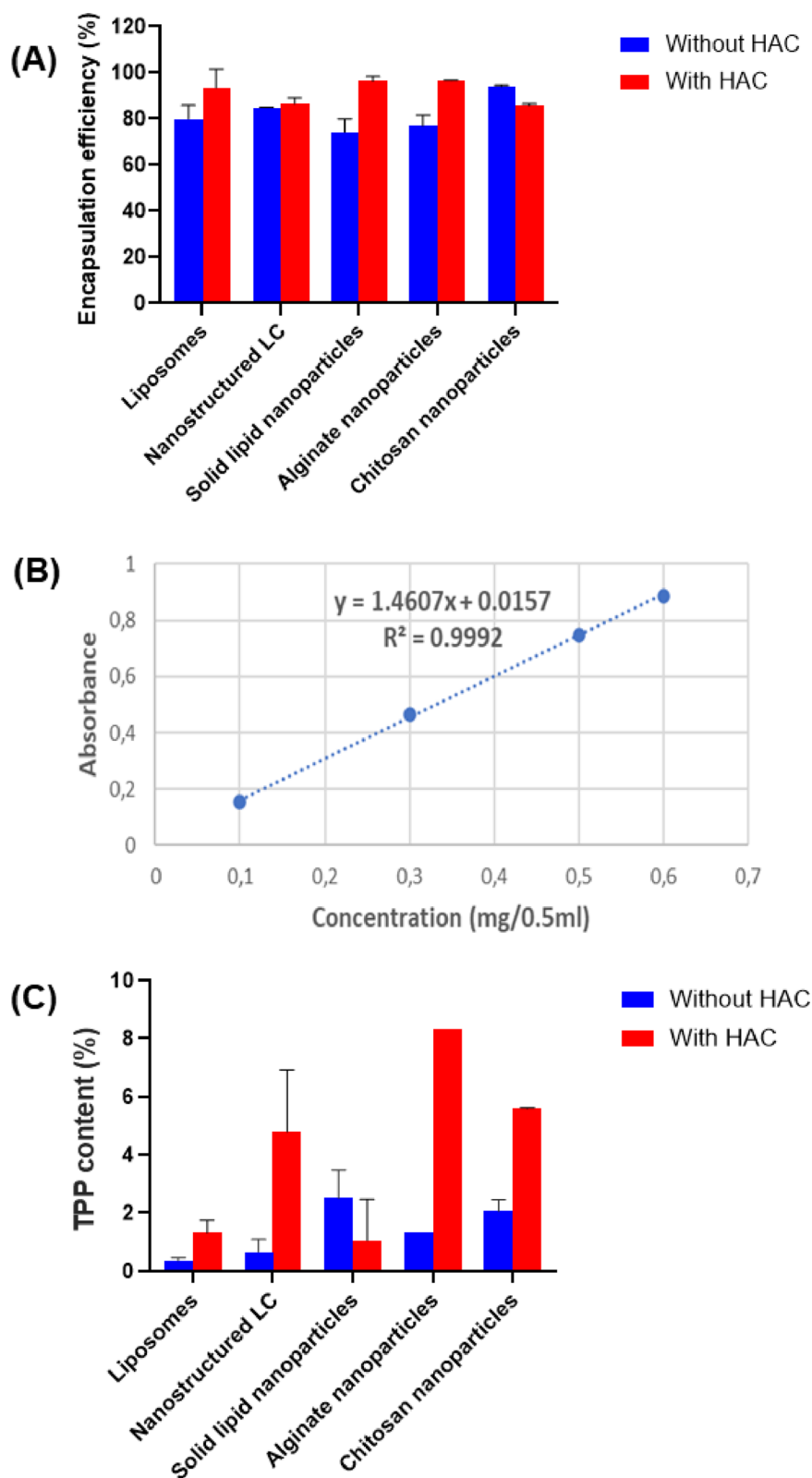


Fig. 6 Data showing the entrapment of resin's components in nanoparticles through total polyphenols (TPP) quantitation. **A** %EE of all nanoparticles for TPP. **B** Calibration curve showing linear variation in absorbance due to TPP as a function of the concentration of *C. swynnertonii* resin. **C** TPP contents indicating loading capacity of different nanoparticles. HAC hyaluronic acid-stearylamine conjugate

high to confirm good linearity and a strong correlation between absorbance values and polyphenol concentration in unformulated resin control (Fig. 6B), consistent with the analytical standards described by Stalikas and Sakkas [45]. Regardless of the type of nanoparticles, the formulation without hyaluronic acid conjugate encapsulated lower amounts of total polyphenols compared to their nanoparticles counterparts functionalized with HAC (Fig. 7C). In conjunction with the encapsulation efficiency data, this trend reaffirms the positive influence of HAC on enhancing encapsulation efficiency through improved molecular compatibility and structural reinforcement of the nanoparticle

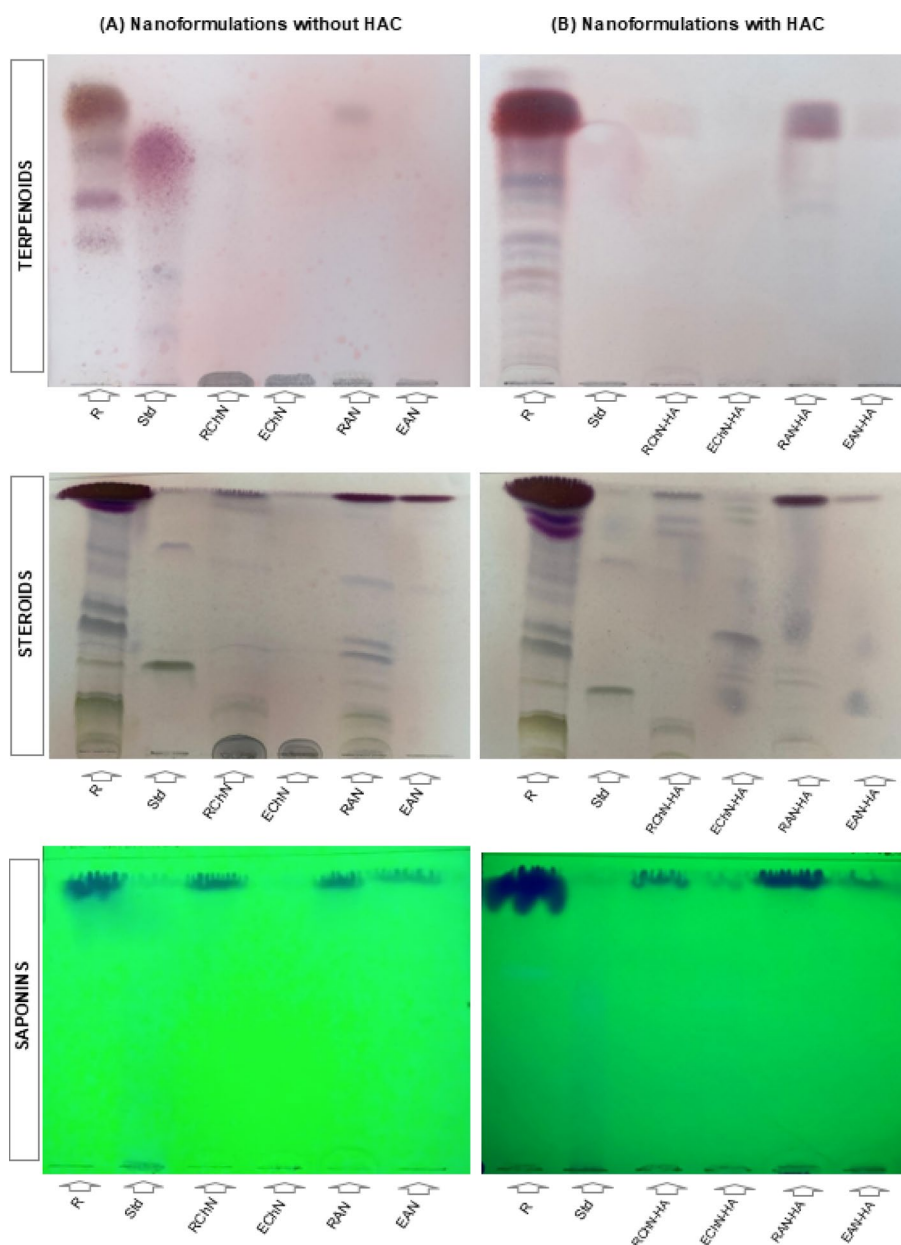


Fig. 7 Illustrative chromatograms showing phytochemical profiles of selected nanoparticles following the encapsulation of *C. swynnertonii* resin ("R"). Std standard; EAN Empty alginate nanoparticles; RAN Resin-loaded alginate nanoparticles; EAN-HA Empty alginate nanoparticles with hyaluronic acid; RAN-HA Resin-loaded alginate nanoparticles with hyaluronic acid; EChN Empty chitosan nanoparticles; RChN Resin-loaded chitosan nanoparticles; EChN-HA Empty chitosan nanoparticles with hyaluronic acid; RChN-HA Resin-loaded chitosan nanoparticles with hyaluronic acid

matrices. Among the different systems, liposomes demonstrated the smallest polyphenols contents: resin-loaded liposomes (RL) 0.500% and resin-loaded liposomes with HA (RL–HA) 1.440%, which may be attributed to their phospholipid bilayer's restricted loading capacity for polar phytochemicals. In contrast, polymeric nanoparticles—particularly alginate-based nanoformulations (RAN 1.310% and RAN–HA 8.320%)—showed the highest loading capacity, followed by resin-loaded solid lipid nanoparticles (RSN) 2.500% and resin-loaded solid lipid nanoparticles with HA (RSN–HA) 4.800%, both of which exhibited not only a strong affinity for polyphenolic compounds but also the best antimicrobial activities (as discussed later in Sect. 3.6). Overall, these results clearly demonstrate that functionalization of nanoparticles with hyaluronic acid conjugate significantly enhances polyphenol encapsulation efficiency, likely due to improved matrix stabilization, hydrogen bonding potential, and interaction between the conjugate's carbonyl and hydroxyl groups and the resin's polyphenolic constituents. Although polyphenols were quantified primarily as marker compounds to assess the encapsulation and loading capacity of nanoparticles for *C. swynnertonii* resin, the results of both %EE and polyphenol content analyses suggest successful entrapment of at least some of the resin's constituents within the prepared nanoparticles. However, given the possibility of molecular interactions between the resin's phytochemicals and the formulation components—interactions that could potentially alter or reduce bioactivity—a broader phytochemical screening was conducted. This analysis involved a comparative thin-layer chromatography (TLC) profiling of all nanoparticle formulations alongside the unformulated resin, with the objective of confirming the encapsulation of other secondary metabolites and assessing whether their molecular integrity and characteristic profiles were preserved following the nanoencapsulation processes.

3.5 Detection of phytochemicals encapsulated in nanoparticles

Phytochemical screening was carried out using TLC to identify the principal secondary metabolites present in *C. swynnertonii* resin and verify their encapsulation within the various nanomaterial formulations. This was done as a qualitative fingerprinting technique to compare the presence and preservation of major classes of secondary metabolites in the resin before and after nanoencapsulation. Data revealed that the resin contained a rich diversity of secondary metabolites, including terpenoids, steroids, anthraquinones, tannins, coumarins, saponins, and flavonoids, which is in agreement with previous studies that reported similar phytochemical profiles in *C. swynnertonii* resin [19, 46–49]. The abundance of these bioactive compounds explains the resin's broad pharmacological properties, such as antiviral, antimicrobial, and antitrypanosomal activities, as documented in previous work [18–20, 50, 51].

Given the complex chemical composition of the resin—comprising hydrophilic, hydrophobic, and amphiphilic compounds—the nanoparticle formulation strategy was designed to explore various nanostructures to accommodate multiple polarity ranges, enabling efficient encapsulation of the majority of phytochemical groups. As shown in Table 3, most metabolites identified in the crude resin were also detected in the resin-loaded nanoparticles, confirming successful encapsulation of a broad spectrum of secondary metabolites. Conversely, blank nanoparticles (without resin) exhibited no evident phytochemical signals (except cases where metabolites happened to be part of the composition—such as cholesterol in liposomes), confirming that the detected

Table 3 Results of phytochemical screening of resin compared to resin-loaded nanomaterials

Phytochemicals	Nanoformulations without HAC										
	R	RL	EL	RSN	ESN	RNC	ENC	RChN	EChN	RAN	EAN
Terpenoids	+	+	–	+	–	+	–	+	–	+	–
Steroids	+	+	–	+	–	+	–	+	–	+	–
Anthraquinones	+	+	–	+	–	+	–	+	–	+	–
Tannins	+	+	–	+	–	+	–	+	–	+	–
Coumarins	+	+	–	+	–	+	–	+	–	+	–
Saponins	+	+	–	+	–	+	–	+	–	+	–
Flavonoids	+	+	–	+	–	+	–	+	–	+	–
	Nanoformulations with HAC										
	R	RL-HA	EL-HA	RSN-HA	ESN-HA	RNC-HA	ENC-HA	RChN-HA	EChN-HA	RAN-HA	EAN-HA
Terpenoids	+	+	+	+	–	+	–	+	–	+	–
Steroids	+	+	+	+	–	+	–	+	–	+	–
Anthraquinones	+	+	+	+	–	+	–	+	–	+	–
Tannins	+	+	+	+	–	+	–	+	–	+	–
Coumarins	+	+	+	+	–	+	–	+	–	+	–
Saponins	+	+	+	+	–	+	–	+	–	+	–
Flavonoids	+	+	–	+	–	+	–	+	–	+	–

“+” Detected; “–” undetected. *R* Commiphora swynnertonii resin, *EL* Empty Liposomes, *RL* Resin-loaded Liposomes, *EL-HA* Empty liposomes with hyaluronic acid, *RL-HA* Resin-loaded liposomes with hyaluronic acid, *ENC* Empty nanostructured lipid carriers, *RNC* Resin-loaded nanostructured lipid carriers, *ENC-HA* Empty nanostructured lipid carriers with hyaluronic acid, *RNC-HA* Resin-loaded nanostructured lipid carriers with hyaluronic acid, *ESN* Empty solid lipid nanoparticles, *RSN* Resin-loaded solid lipid nanoparticles, *ESN-HA* Empty solid lipid nanoparticles with hyaluronic acid, *RSN-HA* Resin-loaded solid lipid nanoparticles with hyaluronic acid, *EAN* Empty alginate nanoparticles, *RAN* Resin-loaded alginate nanoparticles, *EAN-HA* Empty alginate nanoparticles with hyaluronic acid, *RAN-HA* Resin-loaded alginate nanoparticles with hyaluronic acid, *EChN* Empty chitosan nanoparticles, *RChN* Resin-loaded chitosan nanoparticles, *EChN-HA* Empty chitosan nanoparticles with hyaluronic acid, *RChN-HA* Resin-loaded chitosan nanoparticles with hyaluronic acid

compounds originated exclusively from the encapsulated resin’s constituents (Fig. 7; Figure S3). The assessment of nanoparticles decorated with hyaluronic acid enabled us to verify the impact of the incorporation of HAC on the encapsulation behavior and compound preservation between classical and HAC-containing formulations, demonstrating the influence of surface modification with HAC on the retention and stabilization of resin-derived phytochemicals [52]. Following functionalization with the HAC, the presence and diversity of phytochemical classes in the nanoparticles appeared to be largely preserved, but overall the phytochemical profile remained consistent with that of the unmodified formulations (Table 3). This indicates that the surface decoration process did not compromise the molecular integrity or stability of the encapsulated phytochemicals, although quantitative analysis (%EE and loading capacity) revealed positive impact of HAC on total polyphenols owing to potential beneficial interactions. Nevertheless, positive reactions were unexpectedly observed in the HAC-functionalized empty liposomes (EL–HA). These signals are likely false-positive responses arising from intrinsic chemical interactions between HAC and the TLC detection reagents rather than

genuine phytochemical content. In particular, hyaluronic acid is known to form coordination complexes with ferric ions (Fe^{3+}) used in the ferric chloride test for tannins, producing color changes that can mimic positive detection results. This phenomenon aligns with the findings of Mercê et al. [53], who demonstrated that HA forms stable Fe (III) coordination complexes in aqueous media, explaining the apparent discoloration in EL–HA samples.

Overall, *C. swynnertonii* resin exhibits a rich and diverse phytochemical composition, which remains well preserved following all the nanoencapsulation processes. TLC chromatograms confirmed this preservation, showing distinct, well-defined spots in all resin-containing formulations. Control samples remained negative, except for the EL–HA formulation, whose faint coloration is attributed to reagent complexation by HA rather than the presence of genuine metabolites. The successful preservation of *C. swynnertonii* resin's phytochemical constituents within all types of formulation (with/without HAC) provides a strong basis for evaluating their biological performance. Since the antimicrobial activity of this resin has been associated with its secondary metabolites—particularly terpenoids, flavonoids, and phenolic compounds—maintaining their integrity during nanoencapsulation was essential to ensure preservation of bioactivities. Therefore, the next stage of this study focused on evaluating the antimicrobial properties of the resin-loaded nanoparticles against *MDR* strains of *S. aureus*.

3.6 Antimicrobial activities

The antimicrobial activities of the developed nanoformulations were evaluated against 13 *MDR* strains of *Staphylococcus aureus* isolated from bovine mastitis milk samples. The choice of *S. aureus* as the target pathogen was guided by its predominant role in subclinical mastitis, its capacity to persist within mammary tissue, and its remarkable resistance to conventional antibiotic therapy [54]. The antimicrobial susceptibility profiles of all the 20 nanoparticle formulations were examined for their zones of inhibition, minimum inhibitory concentrations (MICs), and minimum bactericidal concentrations (MBCs). The unformulated *Commiphora swynnertonii* resin displayed potent antimicrobial activity, achieving 100% inhibition in both agar diffusion (inhibition zone 9–22 mm) and MIC assays (MIC 17–417 $\mu\text{g/mL}$), and 76.9% bactericidal activity in MBC tests (MBC 43–417 $\mu\text{g/mL}$). These findings are consistent with prior reports describing the anti-staphylococcal potential of the resin [19] and further underscore the relevance for its nanoencapsulation as an antimicrobial alternative to existing antibiotics. Among the nanoparticle formulations, the alginate-based nanoparticles functionalized with hyaluronic acid (RAN–HA) demonstrated the highest antibacterial performance, producing the largest inhibition zones (6–22 mm) and the lowest MIC (26–417 $\mu\text{g/mL}$) and MBC values (35–417 $\mu\text{g/mL}$) against *MDR S. aureus* (Tables 4, 5, 6). Additionally, RAN–HA outperformed the unformulated resin by achieving the broadest antimicrobial coverage across the 13 *MDR* strains, with 100% inhibition in MIC assays (Fig. 8). However, RAN–HA exhibited MBC coverage (53.8%) significantly smaller than the resin's (76.9%). This is not surprising because MIC and MBC quantify distinct antimicrobial endpoints: MIC reflects inhibition of visible growth under the assay conditions, whereas MBC reflects bacterial killing (typically defined as $\geq 99.9\%$ reduction in viable counts) and is therefore more stringent. Differences between MIC and MBC are often strain dependent and may reflect tolerance/persister subpopulations and physiological state [55, 56], where growth

inhibition can occur without bactericidal killing. In addition, several studies with plant-based nanoparticles revealed that formulation factors can contribute to MIC–MBC divergence [57, 58]: sustained exposure to inhibitory concentrations may be sufficient to suppress growth (favorable MIC), while bactericidal activity may require higher effective free concentrations, longer exposure, or more rapid release of active constituents to reach lethal thresholds in certain isolates. This suggests that RAN-HA behaves predominantly as a growth-inhibitory system against the tested MDR isolates (within the concentration range tested herein), while achieving bactericidal effects against only a subset of strains.

Nevertheless, when compared to other nanoformulations, the superior efficacy of the formulation RAN-HA may reflect a synergistic contribution of multiple factors, including (i) the bioactive resin encapsulated within the matrix; (ii) the intrinsic antimicrobial and anti-biofilm properties of alginate [59]; and (iii) the targeting and stabilizing effects conferred by HAC. Notably, nanoparticles containing HAC consistently exhibited higher encapsulation efficiency and greater polyphenol content compared to those without HAC, correlating with their improved antibacterial performance. This enhancement likely arises from the stronger matrix integrity and controlled-release properties imparted by HAC, which likely promoted better retention and gradual diffusion of bioactive compounds. The blank HAC-functionalized alginate nanoparticles (EAN–HA) exhibited some measurable activity while similar formulation without HAC did not exhibit any activities, indicating that HAC combined with alginate markedly contributed to antibacterial activities. These results align with findings by Athamneh et al. [60] and Gorroñogoitia et al. [61], who emphasized that combining alginate and hyaluronic acid generates hybrid nanostructures with enhanced mechanical stability, biocompatibility, and sustained-release behavior—which are attributes that likely benefited the present formulations. Resin-loaded solid lipid nanoparticles containing HA (RSN–HA) appears to be the second top performing nanoformulation, achieving 69% inhibitory and 46.2% bactericidal activity. In contrast, even in presence of HAC, liposomes, nanostructured lipid carriers and chitosan nanoparticles exhibited no/minimal antibacterial activity, potentially due to demonstrated lower encapsulation efficiency and reduced polyphenol loading. This suggests that these nano-systems had limited encapsulation capabilities for *C. swynnertonii* resin as a complex mixture of natural products. Taken together, these findings demonstrate that surface functionalization with hyaluronic acid conjugate not only improved encapsulation efficiency and polyphenols retention but also amplified the antimicrobial efficacy of the *C. swynnertonii* resin-loaded nanoparticles. The integration of HAC into the alginate nanosystems provided commendable structural, physicochemical, and biological advantages, establishing RAN–HA as the most promising candidate for targeted mastitis therapy among the tested nanomaterials.

4 Conclusion

This study aimed to encapsulate the bioactive compounds of *C. swynnertonii* resin in various nanocarriers and evaluate their antimicrobial activity against MDR *S. aureus* strains. Given the diversity in the chemical nature of the resin's compounds—comprising both polar and nonpolar phytochemicals—different types of nanoparticle systems were explored, including liposomes, alginate-based nanoparticles, chitosan-based nanoparticles, solid lipid nanoparticles, and nanostructured lipid carriers, each with or without

Table 4 Inhibition zones exhibited by nanoformulations against 13 MDR strains of *S. aureus*

[illegible]

Table 4 (continued)

Formulation	Inhibition zones $\pm$ SD (mm) for various MDR strains (n = 3)											
	S114	S538	S598	S580	S555	S1476	S1217	S2882	S237	S1331	S382	S238
<i>Control</i>												
Resin	22 $\pm$ 3	19 $\pm$ 1	18 $\pm$ 3	18 $\pm$ 2	18 $\pm$ 2	18 $\pm$ 3	15 $\pm$ 1	19 $\pm$ 1	14 $\pm$ 1	9 $\pm$ 1	13 $\pm$ 2	14 $\pm$ 2

Categorical interpretation: 6 mm = no inhibition; 7 to 10 mm = weak activity; 11 to 13 mm = moderate activity; and > 14 mm = strong activity. "—"Undetected.

Categorical interpretation: 6 mm = no inhibition; 7 to 10 mm = weak activity; 11 to 13 mm = moderate activity; and > 14 mm = strong activity. "–"Undetected.

Empty Liposomes, *EL-HA Empty liposomes* with hyaluronic acid, *RL Resin-loaded Liposomes*, *RL-HA Resin-loaded liposomes* with hyaluronic acid, *ENC Empty nanostructured lipid carriers*, *ENC-HA Empty nanostructured lipid carriers* with hyaluronic acid, *RNC-HA Resin-loaded nanostructured lipid carriers* with hyaluronic acid, *ESW Empty solid lipid nanoparticles*, *RSV Resin-loaded solid lipid nanoparticles*, *ESN-HA Empty solid lipid nanoparticles* with hyaluronic acid, *RSN-HA Resin-loaded solid lipid nanoparticles* with hyaluronic acid, *EAN Empty alginate nanoparticles*, *EAN-HA Empty alginate nanoparticles* with hyaluronic acid, *RAN-HA Resin-loaded alginate nanoparticles* with hyaluronic acid, *EChN Empty chitosan nanoparticles*, *EChN-HA Empty chitosan nanoparticles* with hyaluronic acid, *RChN-HA Resin-loaded chitosan nanoparticles* with hyaluronic acid

Table 5 Minimum inhibitory concentrations (MIC) of various nanoformulations against 13 different MDR strains of *S. aureus*

Formulation	MIC±SD (µg/mL) for various MDR strains (n=3)												
	S114	S538	S598	S580	S555	S1476	S1217	S2882	S228	S237	S1331	S382	S238
<i>Liposomes</i>													
EL	-	-	-	-	-	-	-	-	-	-	-	-	-
RL	-	-	-	-	-	-	-	-	-	-	-	-	-
EL-HA	-	-	-	-	-	-	-	-	-	-	-	-	-
RL-HA	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Nanostructured lipid carrier</i>													
ENC	-	-	-	-	-	-	-	-	-	-	-	-	-
RNC	-	278±241	-	-	-	-	-	-	-	-	-	35±15	-
ENC-HA	-	-	-	-	-	-	-	-	-	-	-	-	-
RNC-HA	-	417±0	-	417±0	-	-	-	-	-	-	-	43±15	-
<i>Solid lipid nanoparticles</i>													
ESN	-	-	-	-	-	-	-	-	-	-	-	-	-
RSN	-	-	-	-	-	-	-	-	35±15	-	-	-	-
ESN-HA	-	-	-	-	-	-	-	-	-	-	-	-	-
RSN-HA	17±8	417±0	17±8	417±0	-	417±0	-	417±0	417±0	417±0	-	-	417±0
<i>Alginate nanoparticles</i>													
EAN	-	-	-	-	-	-	-	-	-	-	-	-	-
RAN	-	-	-	-	-	-	-	-	-	-	-	-	-
EAN-HA	208±180	-	139±60	-	-	139±60	208.3±0	208.3±0	52±0	61±40	69±30	-	-
RAN-HA	35±15	52±9	43±15	26±23	417±0	139±60	104±2	278±120	208.3±0	139±60	278±120	208.3±0	139±60
<i>Chitosan nanoparticles</i>													
EChN	-	-	-	-	-	-	-	-	-	-	-	-	-
RChN	-	-	-	-	-	-	-	-	-	-	-	-	-
RChN-HA	-	-	-	-	-	-	-	-	-	-	-	-	-
RChN-HA	-	-	-	-	-	-	-	-	417±0	417±0	-	-	-

Table 5 (continued)

Formulation	MIC ± SD (µg/mL) for various MDR strains (n = 3)									
	S114	S538	S598	S580	S555	S1476	S1217	S2882	S228	S237
Positive control										
Resin	17 ± 8	35 ± 15	139 ± 60	174 ± 60	208 ± 0	417 ± 0	417 ± 0	347 ± 120	347 ± 120	417 ± 0
"n" Undetected										

EL Empty Liposomes, RL Resin-loaded Liposomes, EL-HA Empty liposomes with hyaluronic acid, RL-HA Resin-loaded liposomes with hyaluronic acid, ENC Empty nanostructured lipid carriers, RNC-HA Resin-loaded nanostructured lipid carriers with hyaluronic acid, ESN Empty solid lipid nanoparticles, RSN Resin-loaded solid lipid nanoparticles, ESN-HA Empty solid lipid nanoparticles with hyaluronic acid, RSN-HA Resin-loaded solid lipid nanoparticles with hyaluronic acid, EAN Empty alginate nanoparticles, RAN Resin-loaded alginate nanoparticles, EAN-HA Empty alginate nanoparticles with hyaluronic acid, RAN-HA Resin-loaded alginate nanoparticles with hyaluronic acid, EChN Empty chitosan nanoparticles, RChN Resin-loaded chitosan nanoparticles, EChN-HA Empty chitosan nanoparticles with hyaluronic acid, RChN-HA Resin-loaded chitosan nanoparticles with hyaluronic acid

[illegible]

Table 6 (continued)

Formulation	MBC ± SD (µg/mL) for various MDR strains (n = 3)												
	S114	S538	S598	S580	S555	S1476	S1217	S2882	S228	S237	S1331	S382	S238
Positive control													
Resin	43 ± 15	139 ± 60	208 ± 0	417 ± 0	417 ± 0	–	–	417 ± 0	417 ± 0	417 ± 0	–	417 ± 0	417 ± 0
n Undetected													

EL Empty Liposomes, RL Resin-loaded Liposomes, EL-HA Empty liposomes with hyaluronic acid, RL-HA Resin-loaded liposomes with hyaluronic acid, ENC Empty nanostructured lipid carriers, RNC-HA Resin-loaded nanostructured lipid carriers with hyaluronic acid, ESN Empty solid lipid nanoparticles, RSN Resin-loaded solid lipid nanoparticles, ESN-HA Empty solid lipid nanoparticles with hyaluronic acid, RSN-HA Resin-loaded solid lipid nanoparticles with hyaluronic acid, EAN Empty alginate nanoparticles, RAN Resin-loaded alginate nanoparticles, EAN-HA Empty alginate nanoparticles with hyaluronic acid, RAN-HA Resin-loaded alginate nanoparticles with hyaluronic acid, EChN Empty chitosan nanoparticles, RChN Resin-loaded chitosan nanoparticles, EChN-HA Empty chitosan nanoparticles with hyaluronic acid, RChN-HA Resin-loaded chitosan nanoparticles with hyaluronic acid

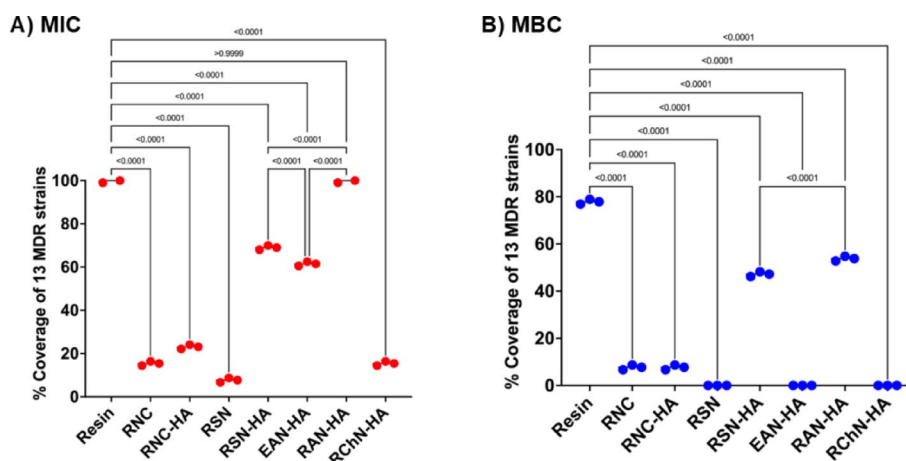


Fig. 8 Antimicrobial activity spectrum towards the 13 MDR strains of *S. aureus*. Data showing the proportion of the MDR strains that each antimicrobial nanoformulation covered, with minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) values >0 . The formulation RAN-HA and *C. swynnertonii* resin exhibited the broadest spectra of antibacterial inhibition and killing. RNC Resin-loaded nanostructured lipid carriers; RNC-HA Resin-loaded nanostructured lipid carriers with hyaluronic acid; RSN Resin-loaded solid lipid nanoparticles; RSN-HA Resin-loaded solid lipid nanoparticles with hyaluronic acid; EAN-HA Empty alginate nanoparticles with hyaluronic acid; RAN-HA Resin-loaded alginate nanoparticles with hyaluronic acid; RChN-HA Resin-loaded chitosan nanoparticles with hyaluronic acid. P-values were obtained from one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test

hyaluronic acid (HA) as a targeting ligand. The findings showed that HAC-functionalized formulations, particularly resin-loaded alginate nanoparticles with HA (RAN-HA) and resin-loaded solid lipid nanoparticles with HA (RSN-HA), exhibited enhanced encapsulation efficiencies, higher polyphenol content, and superior antimicrobial activity compared to their non-HA containing formulation counterparts. Thin-layer chromatography suggested that the investigated nanocarriers successfully encapsulated a wide range of phytochemicals—including terpenoids, steroids, anthraquinones, tannins, coumarins, saponins, and flavonoids—while preserving their molecular integrity. Among all formulations, RAN-HA demonstrated the most potent anti-staphylococcal activity, achieving 100% MIC coverage and 53.8% MBC coverage across the MDR isolates, followed by RSN-HA, which achieved 69% inhibitory and 46.2% bactericidal activity. This work provides the first comparative assessment of diverse nanocarrier systems for nanoencapsulation of *C. swynnertonii* resin and their antimicrobial performance against MDR mastitis-associated *S. aureus*.

Nevertheless, given the MIC-MBC divergence, future time-kill kinetics and extended-exposure assays will help delineate whether the reduced MBC coverage reflects slower killing dynamics, formulation-controlled release, or strain-specific tolerance phenotypes. In addition, since this study mainly focused on comparative performance across nanocarrier platforms, further research should conduct systematic optimization of formulation and process parameters using advanced design-of-experiments approaches to further refine product quality attributes. A further limitation of this study relates to the mass-to-volume dispersibility constraints of certain nanocarrier systems, which restricted the maximum achievable test concentrations. As a result, antimicrobial performance of some formulations may have been underestimated, as higher concentrations could not be reliably explored within the physicochemical stability limits of the suspensions. Therefore, further optimization of nanocarrier loading and stability may

enable higher effective concentrations and improved antimicrobial performance in future studies.

From a product development point of view, additional studies should include stability assessments, biocompatibility, pharmacokinetics and efficacy evaluation in mastitis animal models, as well as scale-up production trials for the most promising formulations—particularly RAN–HA and RSN–HA—to advance toward practical, sustainable applications in veterinary and translational medicine.

Supplementary Information

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Supplementary Material 1.

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In memoriam

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Author contributions

D.W.M., F.M.M., G.M.M., C.B., C.N. and B.K.W.-W.: Methodology, Investigation, Data curation, Formal analysis, Figures preparation, and Writing – original draft. M.M.M., P.K.M. and N.K.N.: Supervision, Project administration, Formal analysis, Validation, and Writing – review & editing. X.Z., G.G.B. and C.I.N.: Conceptualization, Funding acquisition, Project Management, Resources, Validation, and Writing – review & editing.

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Data availability

Data supporting the findings of this study are available within the paper and its Supplementary Information file. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publications

Not applicable.

Competing interests

The authors declare the following potential competing interests: Dr Christian I. Nkanga serves as the Chief Scientific Officer for Memsel Inc. The other authors declare no potential conflicts of interest.

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