

Article

Saponin-Enriched Fraction of *Sarcomphalus joazeiro*: Chemical Characterization, Silver Nanoparticle Synthesis, and Their Mutual Antibiotic-Modifying Potential

Natália Kelly Gomes de Carvalho ¹, Mariana Pereira da Silva ¹, Débora Odília Duarte Leite ¹ ,
Fazia Fernandes Galvão Rodrigues ¹, Joice Barbosa do Nascimento ¹ , Milena Lima Guimarães ² ,
Helinando Pequeno de Oliveira ² , Lucicleia Barros de Vasconcelos ³ , Maryana Melo Frota ³ ,
Josean Fechine Tavares ⁴ , Thiago Araújo de Medeiros Brito ⁴ , Fabiola Fernandes Galvão Rodrigues ¹
and José Galberto Martins da Costa ^{1,*} 

- ¹ Natural Products Research Laboratory, Department of Biological Chemistry, Universidade Regional do Cariri, Rua Coronel Antônio Luíz, 1161-Pimenta, Crato 63105-010, CE, Brazil; nataliakellygc@gmail.com (N.K.G.d.C.); mariana.pereira@urca.br (M.P.d.S.); biodeboraleite@yahoo.com.br (D.O.D.L.); faziagalvao@gmail.com (F.F.G.R.); joicenascimento2010@live.com (J.B.d.N.); fabiolafer@gmail.com (F.F.G.R.)
- ² Materials Science Research Institute, Federal University of the São Francisco Valley, Av. Antonio Carlos Magalhães, 510-Santo Antônio, Juazeiro 48902-300, BA, Brazil; milenalimaguiaraes@gmail.com (M.L.G.); helinando@gmail.com (H.P.d.O.)
- ³ Department of Food Engineering, Federal University of Ceará, Av. Mister Hull, s/n-Pici, Fortaleza 60455-760, CE, Brazil; lucicleia_barros@ufc.br (L.B.d.V.); mary.m.frota@gmail.com (M.M.F.)
- ⁴ Multi-User Laboratory for Characterization and Analysis, Program of Natural and Synthetic Bioactive Products, Health Sciences Center, Federal University of Paraíba, João Pessoa 58051-900, PB, Brazil; josean@lft.ufpb.br (J.F.T.); thiagobrito@lft.ufpb.br (T.A.d.M.B.)
- * Correspondence: galberto.martins@gmail.com

Abstract

Antibiotic resistance has emerged as a major global health challenge, underscoring the urgent need for alternative therapeutic strategies capable of enhancing the efficacy of existing antibiotics. In this context, saponin-based nanomaterials have attracted considerable attention due to their potential as antibiotic-modulating systems. This study investigated a saponin-enriched fraction obtained from the bark of *Sarcomphalus joazeiro* Mart. (SEF-4), its application in the green synthesis of silver nanoparticles, and the antibiotic-modulating potential of the resulting nanoformulation. SEF-4 was obtained from the ethanolic bark extract through liquid–liquid partitioning (52% yield), followed by column chromatographic purification and chemical characterization using LC-ESI-QTOF-MS. The purified fraction was subsequently employed as both a reducing and stabilizing agent for the synthesis of silver nanoparticles (putative AgNP-SEF-4), which were physicochemically characterized. Antibacterial activity and antibiotic-modulating effects were evaluated using the broth microdilution method against standard and multidrug-resistant bacterial strains. LC-ESI-QTOF-MS analysis enabled the putative identification of five jujubogenin-type triterpenoid saponins bearing tetra-, penta-, and hexasaccharide moieties with distinct glycosylation profiles; however, the precise sugar sequence, monosaccharide composition, and glycosidic linkage positions remain to be confirmed through complementary NMR and hydrolysis studies. Although neither SEF-4 nor putative AgNP-SEF-4 displayed clinically relevant intrinsic antibacterial activity, the nanoformulation significantly enhanced the activity of aminoglycoside antibiotics. The most pronounced modulatory effects were observed against *Klebsiella pneumoniae* ATCC 1705 in combination with amikacin and against both standard and multidrug-resistant *Escherichia coli* strains when combined with gentamicin or amikacin. These findings highlight the potential of putative AgNP-SEF-4 as an an-



Academic Editor: George Grant

Received: 18 May 2026

Revised: 24 June 2026

Accepted: 25 June 2026

Published: 1 July 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article

distributed under the terms and

conditions of the [Creative Commons](https://creativecommons.org/licenses/by/4.0/)

[Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

tibiotic adjuvant capable of potentiating aminoglycoside efficacy and increasing bacterial susceptibility, including in multidrug-resistant strains.

Keywords: *Sarcomphalus joazeiro*; triterpenic saponins; silver nanoparticles; antibiotic resistance; antibiotic modulation

1. Introduction

Antibiotic resistance represents one of the most critical threats to global public health and is projected to account for millions of deaths annually in the coming decades [1,2]. This phenomenon results from several mechanisms, including drug efflux, target-site modification, enzymatic inactivation, and biofilm formation, and is further exacerbated by the inappropriate and excessive use of antimicrobial agents [3,4]. Among the most clinically relevant multidrug-resistant pathogens are *Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and *Escherichia coli*, which are associated with a broad spectrum of difficult-to-treat infections and elevated mortality rates [4,5].

Conventional antibacterial agents used to treat these infections are frequently associated with adverse effects and declining therapeutic efficacy, emphasizing the need for alternative antimicrobial strategies. In this context, natural products have been extensively investigated as sources of bioactive compounds with therapeutic potential. Recent studies have demonstrated that saponins exert antibacterial effects through multiple mechanisms. Due to their amphiphilic nature, these metabolites can interact with bacterial membrane lipids, disrupting membrane integrity, increasing permeability, and promoting the leakage of intracellular constituents, ultimately leading to cell death. In addition, increasing evidence indicates that saponins and other triterpenoids may inhibit biofilm formation, interfere with quorum-sensing pathways, and enhance bacterial susceptibility to conventional antibiotics. Consequently, these compounds have attracted attention as potential adjuvants for combating infections caused by multidrug-resistant pathogens [6,7]. Collectively, these findings support the potential of saponins as promising scaffolds for the development of novel antimicrobial agents.

In parallel, the green synthesis of silver nanoparticles (AgNPs) has emerged as a sustainable and environmentally friendly strategy for producing antibacterial nanomaterials. Such nanoformulations can improve the stability, bioavailability, and pharmacokinetic properties of bioactive compounds while enhancing or modulating their biological activities [8–11]. Moreover, saponins can act as both reducing and stabilizing agents during the green synthesis of metallic nanoparticles, enabling the combination of their intrinsic biological properties with the physicochemical advantages conferred by nanotechnology [12].

Sarcomphalus joazeiro Mart. (Rhamnaceae), formerly classified as *Ziziphus joazeiro* Mart., is a species endemic to the Caatinga biome and widely distributed throughout northeastern Brazil. Commonly known as “joazeiro”, “juá-babão”, “juazeiro”, and “juá”, this species has long been used in traditional medicine. Its leaves and bark are traditionally employed as antiseptic and expectorant remedies and for the treatment of oral and dental disorders, among other applications [13,14]. The bark is particularly recognized for its bactericidal properties, which have largely been attributed to its high content of triterpenoid saponins. The saponins reported in *S. joazeiro* are predominantly jujubogenin-type derivatives containing oligosaccharide chains attached to the C-3 position of the aglycone. These compounds differ mainly in the composition and length of their glycosidic moieties (Figure 1) [15].

In this context, the present study investigates, for the first time, the use of a saponin-enriched fraction obtained from the bark of *S. joazeiro* as both a reducing and stabilizing agent for the green synthesis of silver nanoparticles. Furthermore, the antibacterial and antibiotic-

modulating activities of the resulting nanoformulation were evaluated against standard and multidrug-resistant bacterial strains. By integrating bioactive natural products with nanotechnology, this study contributes to the understanding of the biotechnological potential of *S. joazeiro*-derived saponins and highlights their applicability in the development of sustainable nanostructured systems as adjuvant approaches for combating antimicrobial resistance.

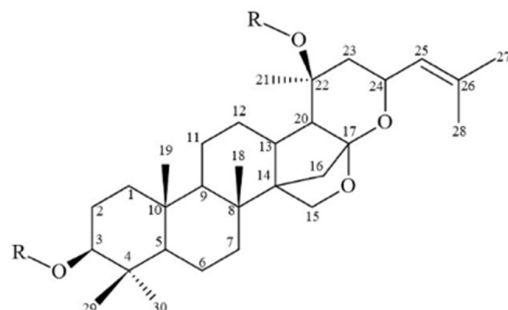


Figure 1. Structural representation of the aglycone jujubogenin commonly found in *S. joazeiro*. Source: Authors' own work (2026).

2. Materials and Methods

2.1. Collection and Extraction of Plant Material

The bark of *S. joazeiro* (980 g) was collected in January 2023 in the district of Tabocas, Municipality of Exu, Pernambuco, Brazil. A voucher specimen was deposited in the Dárdano de Andrade Lima Herbarium of the Regional University of Cariri (URCA) under accession number 13.346 and identified as *S. joazeiro* Mart.

The plant material was ground and subjected to cold maceration for 72 h at room temperature, initially with hexane for defatting and subsequently with ethanol to obtain the crude extract. The organic solvents were removed under reduced pressure using a rotary evaporator. The yield of the ethanolic bark extract of *S. joazeiro* (EEBSJ) was 4.52%.

2.2. Obtaining the Saponin-Enriched Fraction

EEBSJ (5.0 g) was subjected to selective precipitation using a methanol–acetone solution (4:1, *v/v*) at 40 °C. After complete solubilization, the solution was cooled for 10 min, followed by the addition of acetone to promote the precipitation of less polar compounds. The mixture was then centrifuged at 3500 rpm for 5 min. The resulting precipitate, corresponding to the saponin-enriched fraction (SEF), afforded a yield of 52.0% after drying.

2.3. Purification of the Saponin-Enriched Fraction and Identification Test

SEF was purified by column chromatography using mixtures of ethyl acetate and methanol in different proportions, as well as each solvent individually. Among the fractions obtained, fraction 4 (SEF-4), eluted with methanol–ethyl acetate (8:2, *v/v*), exhibited an *R_f* value of 0.603 and a yield of 6.0%, and was therefore selected for subsequent biological assays.

Fraction monitoring was performed by thin-layer chromatography (TLC) using methanol–ethyl acetate (1:1, *v/v*) as the mobile phase. The chromatographic plates were sprayed with vanillin reagent (vanillin/acetic acid/sulfuric acid) and heated to visualize the spots.

The presence of saponins was assessed using the foam test. Briefly, 3 mg of SEF-4 was dissolved in distilled water (20 mL), vigorously shaken for 2 min, and allowed to stand for 30 min. Persistent foam formation was considered indicative of the presence of saponins.

2.4. LC-ESI-Q-TOF-MS Analysis

A 5 µL aliquot of SEF-4 solution (200 µg/mL) was injected into an LC-40D X3 system (Shimadzu, Kyoto, Japan) equipped with CBM-40, DGU-40S, SIL-40C X3, and CTD-40S

modules and coupled to an LCMS-9050 mass spectrometer (Shimadzu, Kyoto, Japan) fitted with an electrospray ionization (ESI) source and a quadrupole time-of-flight (QTOF) analyzer (Shimadzu, Kyoto, Japan).

Chromatographic separation was achieved on a C18 column (Ascentis, 150 × 4.6 mm, 2.7 µm) using a mobile phase consisting of 0.1% formic acid in water (A) and methanol (B). Elution was performed using a linear gradient from 60% to 100% B over 20 min, followed by an isocratic step at 100% B for 7 min. The flow rate was maintained at 0.3 mL·min^{−1} and the column temperature at 40 °C.

Mass spectrometric conditions included a capillary voltage of 2.0 kV, a nebulizing gas flow rate of 3.0 L·min^{−1}, a drying gas flow rate of 10 mL·min^{−1}, and an interface temperature of 300 °C. Data were acquired in positive ionization mode over an *m/z* range of 300–1400. Fragmentation experiments were performed using argon as the collision gas at collision energies ranging from 5 to 55 eV, while product ions were monitored over an *m/z* range of 50–1400. Data acquisition was carried out using a data-independent acquisition (DIA) method comprising 32 fragmentation events and a loop time of 1.188 s.

2.5. Green Synthesis of the Silver Nanoformulation

Silver nanoparticles incorporating SEF-4 (putative AgNP-SEF-4) were synthesized according to the method described by ([16]), with minor modifications. The green synthesis was carried out using a 1 mM aqueous AgNO₃ solution maintained at 95–98 °C under constant stirring and protected from light. Subsequently, 4 mL of SEF-4 solution (500 µg/mL) was added dropwise and the reaction mixture was maintained under stirring for 60 min. Putative AgNP-SEF-4 were obtained under neutral conditions, with the pH adjusted using 0.1 M NaOH [16].

Zeta Potential, Particle Size, and Polydispersity Index

The average hydrodynamic diameter (Z-average), polydispersity index (PDI), and zeta potential (ζ) of putative AgNP-SEF-4 suspensions were determined by dynamic light scattering (DLS) and electrophoretic mobility measurements. In addition, the colloidal stability of the nanoformulation was evaluated after one month of storage. All analyses were performed using a Zetasizer Nano Series instrument (Malvern Instruments, Worcestershire, UK) at 25 °C.

Prior to analysis, the colloidal suspensions were diluted in deionized water (1:50, *v/v*) to ensure adequate dispersion and minimize multiple-scattering effects. Particle size was expressed as the mean hydrodynamic diameter, whereas the PDI was used to evaluate the homogeneity of the particle size distribution.

2.6. Antibacterial Activity

2.6.1. Minimum Inhibitory Concentration (MIC)

The antibacterial assays were performed using bacterial strains obtained from the Oswaldo Cruz Foundation (FIOCRUZ, Rio de Janeiro, Brazil), including the standard strains *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 1705, and *Escherichia coli* ATCC 25922, as well as the multidrug-resistant strains *S. aureus* 10, *P. aeruginosa* 027, *K. pneumoniae* KPC, and *E. coli* 06.

The minimum inhibitory concentration (MIC) was determined using the broth microdilution method in 96-well microplates according to CLSI document M100 [17]. Samples were initially prepared at 500 µg/mL and subjected to serial twofold dilutions, resulting in final concentrations ranging from 250 to 4 µg/mL. Each well received 100 µL of the bacterial inoculum. The plates were incubated aerobically at 35 ± 2 °C for 24 h.

Bacterial growth was assessed colorimetrically following the addition of 25 μL of resazurin solution (0.01%, *w/v*). The MIC was defined as the lowest sample concentration capable of completely inhibiting visible bacterial growth.

2.6.2. Antibiotic-Modifying Activity

The antibiotic-modulating activity of SEF-4 and putative AgNP-SEF-4 was evaluated in combination with amikacin and gentamicin according to ([18]). The samples were tested at subinhibitory concentrations corresponding to MIC/8.

For each assay, wells received 100 μL of a solution containing BHI broth (10%) and 100 μL of bacterial inoculum supplemented with the test sample. Serial twofold dilutions of the antibiotics were then prepared, resulting in final concentrations ranging from 500 to 0.25 $\mu\text{g/mL}$. The plates were incubated aerobically at $35 \pm 2^\circ\text{C}$ for 24 h. Antibacterial activity was determined colorimetrically after the addition of 25 μL of resazurin solution (0.01%, *w/v*) [18] (Figure 2).

SCHEME FOR ADMINISTRATION OF ANTIBIOTICS AND NANOPARTICLES

Single coadministration at the beginning of the assay

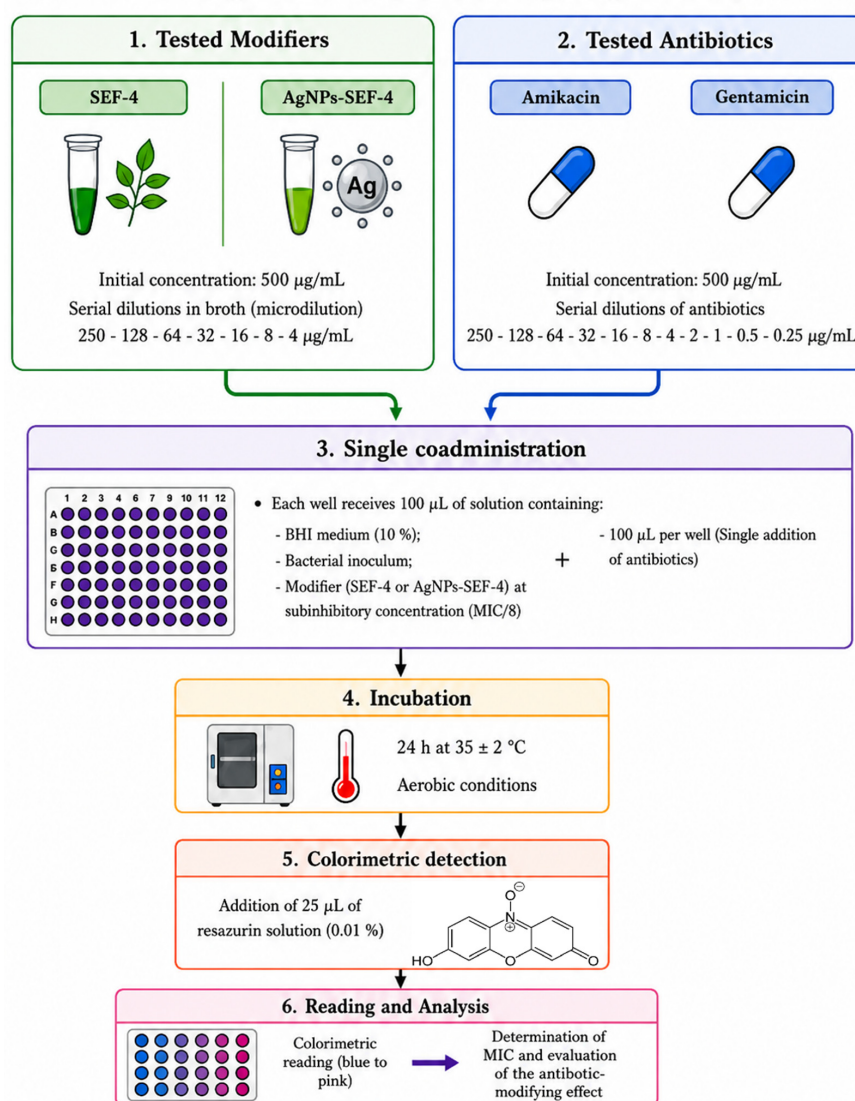


Figure 2. Flowchart of the minimum inhibitory concentration (MIC) assay and antibiotic modifying potential evaluating the saponin-enriched fraction (SEF-4) and silver nanoparticles (putative AgNP-sef-4). Generated using ChatGPT (GPT-5.5, OpenAI) image generation tools (OpenAI) and adapted by the authors.

2.7. Statistical Analysis

Statistical analyses were performed using two-way analysis of variance (two-way ANOVA) followed by Bonferroni's multiple-comparison test using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). MIC values were expressed as geometric means because MIC determinations are based on serial twofold dilutions and therefore represent logarithmically distributed data. The use of geometric means is widely accepted for the analysis of MIC values and provides a more appropriate basis for comparisons among treatment groups. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Chemical Characterization

3.1.1. Chromatographic Profile of the Saponin-Enriched Fraction

The saponin-enriched fraction obtained from the ethanolic bark extract of *S. joazeiro* (EEBSJ) was analyzed by LC-ESI-QTOF-MS in positive ionization mode. The total ion chromatogram (TIC) (Figure 3) revealed five major peaks with retention times ranging from 22.18 to 28.34 min. These peaks displayed distinct relative abundances, with one chromatographic signal predominating over the others (Table S1).

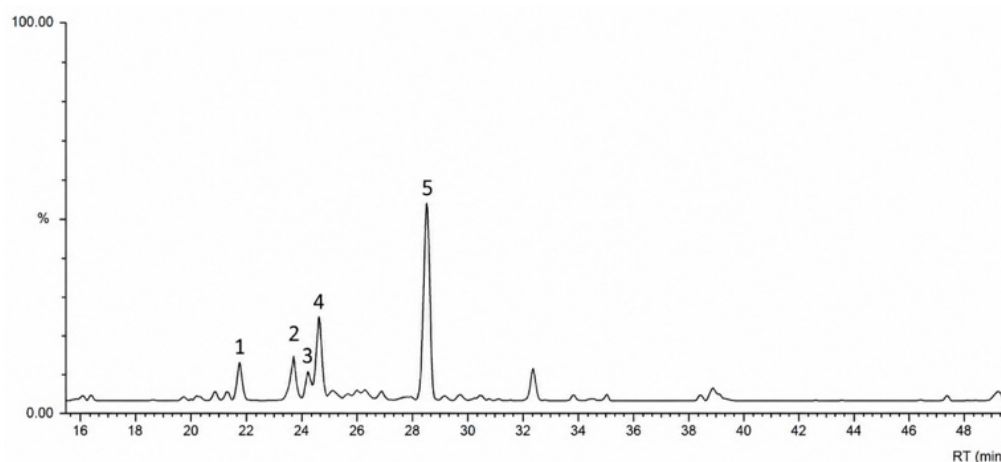


Figure 3. Total ion chromatogram (TIC) obtained by LC-ESI-QTOF-MS of the saponin-enriched fraction of *S. joazeiro* bark (SEF-4) showing the main metabolites detected (compounds 1–5).

3.1.2. Putative Identification of Metabolites by LC-HRMS/MS

The interpretation of the LC-ESI-QTOF-MS/MS data was based on accurate mass measurements, characteristic neutral losses of sugar residues, and fragmentation patterns commonly reported for triterpenoid saponins in the literature [19–22].

The five major chromatographic peaks exhibited protonated molecular ions ($[M + H]^+$) at m/z 1101.536, 1145.563, 1145.563, 1115.552, and 1371.219, corresponding to compounds 1–5 (Figure S1). These ions are consistent with high-molecular-weight glycosylated metabolites. Based on accurate mass measurements (mass error < 5 ppm), molecular formulas compatible with highly oxygenated triterpenoid saponins were proposed (Table 1).

Table 1. Chromatographic data, exact masses, and MS/MS fragmentation of compounds 1–5 detected in the saponin-enriched fraction (SEF-4).

Compound	RT (min)	m/z Observed [M + H] ⁺	m/z Calculated	Error (ppm)	Proposed Molecular Formula *	Main Fragments (m/z)	Interpretation
1	22.18	1101.536	1101.535	0.9	C ₅₄ H ₈₄ O ₂₃	911, 779, 617, 455	Sequential losses of saccharide units; fragment at m/z 455 attributed to triterpene aglycone.
2	23.84	1145.563	1145.561	1.7	C ₅₆ H ₈₈ O ₂₄	935, 811, 649, 487	Successive losses of saccharide units (mainly hexoses); intermediate fragments containing part of the aglycone.
3	24.25	1145.563	1145.561	1.7	C ₅₆ H ₈₈ O ₂₄	811, 649, 487	Fragmentation pattern similar to compound 2; probable glycosidic isomer with successive losses of hexoses.
4	24.85	1115.552	1115.551	0.9	C ₅₅ H ₈₆ O ₂₃	781, 649, 487	Initial loss of two saccharide units followed by additional sugar losses.
5	28.34	1371.219	1371.217	1.5	C ₆₆ H ₁₀₄ O ₃₀	1209, 1047, 921, 587, 455	Successive losses of saccharide units indicating a more extensive oligosaccharide chain.

* Proposed Molecular Formula.

3.1.3. Structural Elucidation and Proposal of Putative Structures for the Detected Compounds

Compound 1 ([M + H]⁺, m/z 1101.536) generated fragment ions at m/z 911, 779, 617, and 455, reflecting successive neutral losses commonly associated with sugar residues. The fragment ion at m/z 455 is consistent with a triterpenoid aglycone and may be related to jujubogenin-type sapogenins previously reported for species of the genus *Sarcomphalus* (*Ziziphus*) [20,21]. Based on its molecular mass and fragmentation profile, compound 1 was tentatively assigned as a tetraglycosylated triterpenoid saponin.

Compounds 2 and 3 ([M + H]⁺, m/z 1145.563) exhibited highly similar MS/MS spectra, producing fragment ions at m/z 811, 649, and 487. These fragmentation patterns suggest structurally related pentaglycosylated saponins, possibly representing isomeric compounds that differ in sugar composition, sequence, or glycosidic linkage positions.

Compound 4 ([M + H]⁺, m/z 1115.552) generated fragment ions at m/z 781, 649, and 487. The observed fragmentation pattern indicates sequential losses of sugar residues and suggests a tetraglycosylated saponin structurally related to compound 1.

Compound 5 ([M + H]⁺, m/z 1371.219) produced fragment ions at m/z 1209, 1047, 921, and 587, consistent with successive losses of glycosidic units. Its higher molecular mass suggests the presence of a more extended oligosaccharide chain compared with the other detected metabolites. Accordingly, compound 5 was tentatively assigned as a highly glycosylated triterpenoid saponin.

Overall, the fragmentation profiles of compounds 1–5 were characterized predominantly by neutral losses of 162 and 146 Da, which are commonly attributed to hexose and deoxyhexose residues, respectively [23]. This behavior is consistent with the fragmentation pattern typically observed for triterpenoid saponins. The occurrence of the ion at m/z 455 in compounds 1 and 5 suggests a common aglycone core, whereas the overall

similarity among the MS/MS spectra indicates that these metabolites belong to a related structural series differing primarily in the number and composition of their glycosidic residues (Figure 4; Table 2).

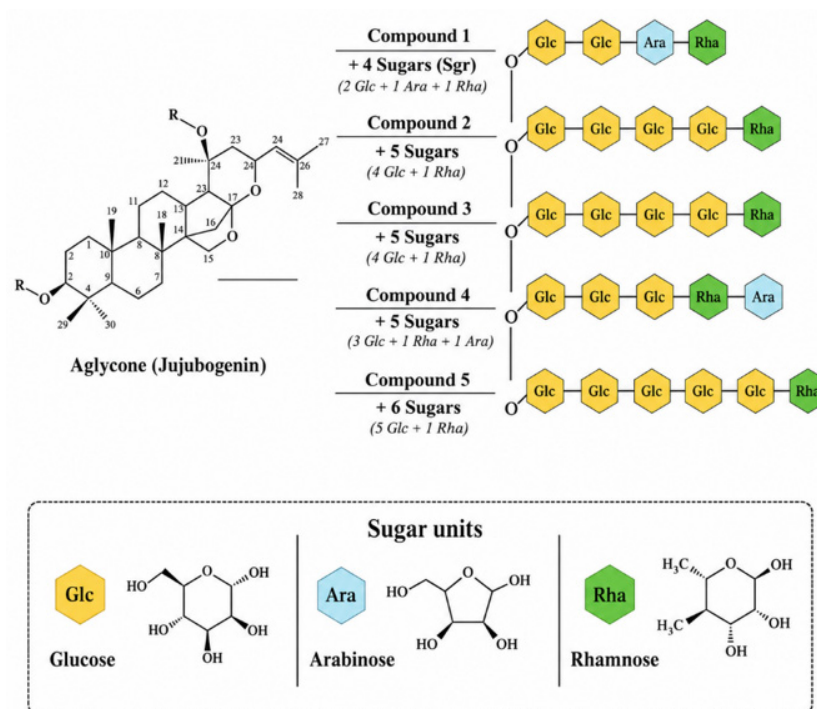


Figure 4. Structure of the proposed triterpenic aglycone (juzubogenin) and schematic representation of the putative glycosidic chains * of compounds 1 to 5, detected by LC-HRMS/MS in the enriched fraction of *S. joazeiro*. * The structures differ mainly in the number and composition of the saccharide units linked to the C-3 position.

Table 2. Comparison between saponins described for the genus *Sarcomphalus* (*Ziziphus*) and the compounds detected in this study.

Compound	m/z [M + H] ⁺	Sugar Units	Suggested Composition	Comparison with Literature	Reference
1	1101	4	Glc ₂ Ara Rha	Compatible with described tetraglycoside saponins	[20–22]
2	1145	5	Glc ₄ Rha	Similar to pentaglycosidic saponins	[20–22]
3	1145	5	Glc ₄ Rha	Probable structural isomer	[20–22]
4	1115	4–5	Glc ₃ Ara Rha	Composition similar to reported saponins	[20–22]
5	1371	6	Glc ₅ Rha	Greater mass than most saponins described	[20–22]

It should be emphasized that the proposed structures are tentative and were inferred exclusively from high-resolution mass spectrometric data. Definitive structural elucidation, including the determination of sugar composition, sequence, linkage positions, and stereochemistry, will require complementary analyses such as nuclear magnetic resonance (NMR) spectroscopy and hydrolysis studies.

3.1.4. Biosynthetic Series of Progressive Glycosylation and Structural Diversity of the Detected Saponins

The LC-HRMS/MS data suggest that the detected metabolites constitute a structurally related series of triterpenoid saponins derived from a common aglycone scaffold and differ-

entiated mainly by the number and composition of attached sugar residues. Similar patterns have been reported for triterpenoid saponins in several plant species and are generally attributed to the sequential action of glycosyltransferases during biosynthesis [20–22].

The biosynthesis of triterpenoid saponins involves the cyclization of 2,3-oxidosqualene to generate the triterpenoid backbone, followed by oxidation and glycosylation reactions catalyzed by UDP-glycosyltransferases (UGTs), producing metabolites with progressively increasing degrees of glycosylation [23]. This biosynthetic model is consistent with the occurrence of compounds displaying closely related molecular masses and fragmentation profiles.

Compounds 1–5 exhibited molecular masses and fragmentation patterns compatible with saponins containing different degrees of glycosylation, suggesting a putative biosynthetic series of structurally related metabolites. Within this series, compound 1 may represent a less glycosylated derivative, compounds 2–4 intermediate glycosylation forms, and compound 5 the most highly glycosylated metabolite detected. The proposed distribution of glycosidic residues is summarized in Table 2 and compared with structurally related saponins previously reported for species of the genus *Sarcomphalus* (*Ziziphus*).

The mass distribution profile (Figure S2) further indicates that compounds 1–4 fall within the molecular-weight range commonly reported for saponins from this genus, whereas compound 5 is located near the upper limit of this distribution, suggesting a more highly glycosylated and potentially less frequently reported structural variant.

3.2. Silver Nanoparticles

The synthesis of silver nanoparticles using SEF-4 resulted in a visible color change from colorless to yellow, a phenomenon commonly associated with the reduction of Ag^+ ions and the formation of silver nanoparticles. Considering the chemical profile of SEF-4, the saponins and other phytochemical constituents present in the fraction likely contributed to the reduction and stabilization processes involved in nanoparticle formation.

The synthesis was further monitored by UV–Vis spectrophotometry, which showed the appearance of an absorption band attributed to localized surface plasmon resonance (LSPR), a characteristic optical property of silver nanoparticles arising from the collective oscillation of conduction-band electrons (Figure 5). The position and intensity of this band are known to be influenced by nanoparticle size, morphology, and aggregation state [24,25].

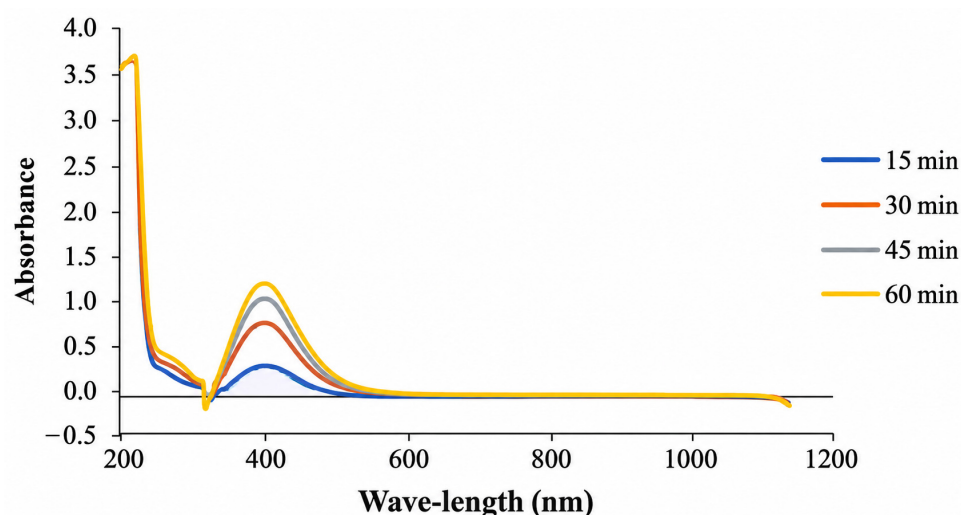


Figure 5. Monitoring of UV–Vis wavelength (nm) spectra versus absorbance of the formation kinetics of silver nanoparticles (putative AgNP-SEF-4) at a concentration of 500 ppm during a period of 15 min to 60 min.

Intensity-based particle size distribution analysis revealed a predominantly monomodal profile, with a maximum peak centered at approximately 100 nm (Figure 6). This distribution pattern suggests the formation of a relatively uniform nanoparticle population, corroborating the successful synthesis of the putative AgNP-SEF-4 nanoformulation.

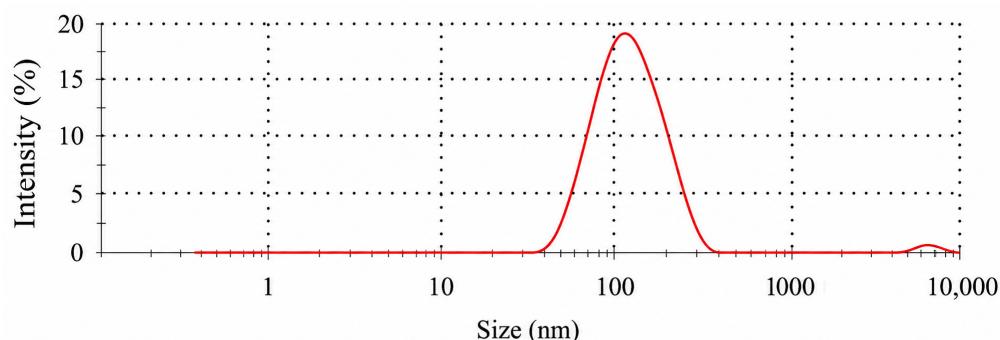


Figure 6. Size distribution (nm) versus intensity (%) of putative AgNP-SEF-4.

The average size, polydispersity index, and zeta potential characteristics are illustrated in Table 3.

Table 3. Particle size (nm), polydispersity index (PDI) and zeta potential (mV) of the silver nanoparticle stabilized with the saponin-enriched fraction (putative AgNP-SEF-4) and stability after one month of storage.

Sample	(nm)	PDI	ZP (mV)
Putative AgNP-SEF-4	114.46	0.147	-23.6 ± 1.90
Putative AgNP-SEF-4 stability after one month of storage	167.76	0.221	-26.8 ± 6.38

3.3. Antibacterial Activity

The minimum inhibitory concentration (MIC) values against multidrug-resistant strains and standards are illustrated in Table 4.

Table 4. Minimum inhibitory concentration (MIC) of the saponin-enriched fraction (4) (SEF-4) and silver nanoparticles stabilized with the saponin-enriched fraction (4) (putative AgNP-SEF-4) and 1 mM AgNO₃ on the strains *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 1705, *E. coli* ATCC 25922, *S. aureus* 10, *P. aeruginosa* 027, *K. pneumoniae* KPC, and *E. coli* 06.

Strains	(μg/mL)		
	SEF-4	Putative AgNP-SEF-4	AgNO ₃
Standard			
<i>S. aureus</i> ATCC 25923	500	125	250
<i>E. coli</i> ATCC 25922	500	62.5	250
<i>K. pneumoniae</i> ATCC 1705	500	62.5	250
<i>P. aeruginosa</i> ATCC 27853	500	500	500
Multi-resistant			
<i>S. aureus</i> 10	500	62.5	250
<i>E. coli</i> 06	500	125	250
<i>K. pneumoniae</i> KPC	500	62.5	250
<i>P. aeruginosa</i> 027	500	62.5	250

To evaluate the modulatory effect, aminoglycosides (gentamicin and amikacin) were used, whose activity depends on cellular permeability and are therefore more susceptible

to modulation by natural metabolites, as evidenced by the resistance profile presented in Table 5 [26–28].

Table 5. Antibiotic resistance profile of the tested bacterial strains.

Strain	Number	Resistance Profile
Standard		
<i>S. aureus</i>	ATCC 25923	Penicillin
<i>E. coli</i>	ATCC 25922	Ampicillin
<i>K. pneumoniae</i>	ATCC 1705	Ampicillin
<i>P. aeruginosa</i>	ATCC 27853	Ampicillin, ceftriaxone, chloramphenicol, tetracycline
Multi-resistant		
<i>S. aureus</i>	10	Oxacillin, penicillins, cephalosporins, erythromycin, ciprofloxacin
<i>E. coli</i>	06	Ampicillin, ceftriaxone, ceftazidime, cefotaxime, ciprofloxacin
<i>K. pneumoniae</i>	KPC	Ampicillin, cephalosporins, carbapenems (imipenem, meropenem), ciprofloxacin
<i>P. aeruginosa</i>	027	Ceftriaxone, ciprofloxacin, imipenem (variable), tetracycline

Modulatory Activity

The results of the antibiotic-modifying potential of SEF-4 and putative AgNP-SEF-4 are presented in Figure 7A–D. Overall, putative AgNP-SEF-4 was more effective than SEF-4 alone, particularly against Gram-negative strains.

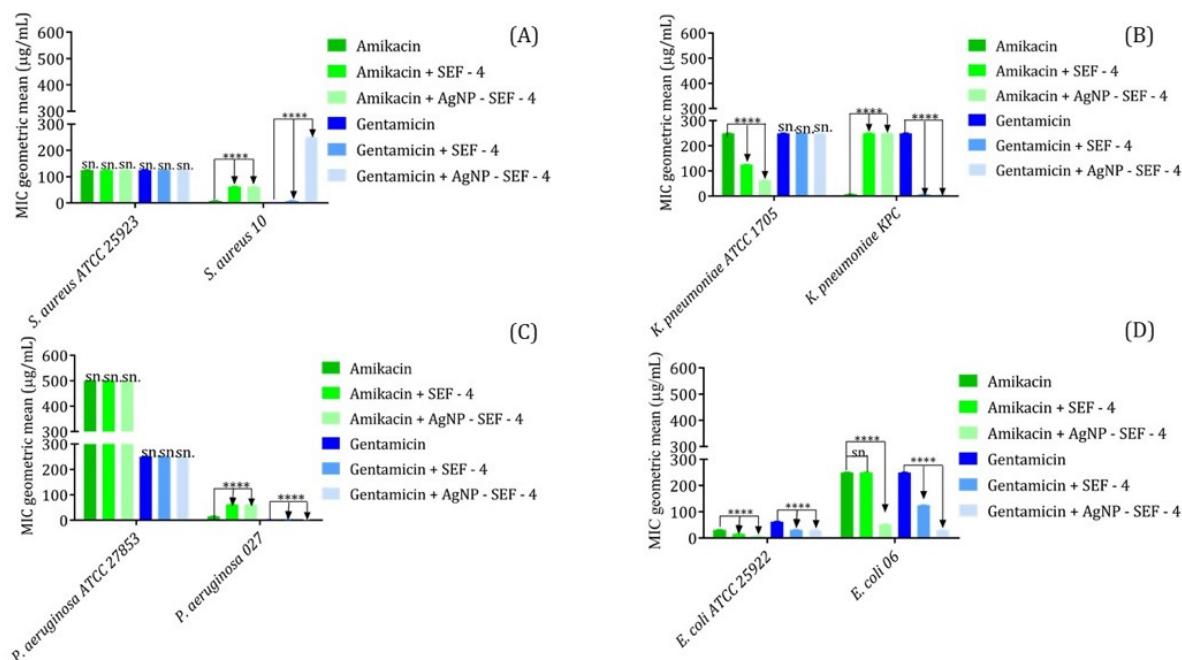


Figure 7. (A) Potential modulator of antibiotic activity of the saponin-enriched fraction (SEF-4) of the ethanolic extract of *S. joazeiro* bark (EEBSJ) in combination with aminoglycosides (amikacin and gentamicin) against bacterial strains *S. aureus* ATCC 25923 and *S. aureus* 10, (B) *K. pneumoniae* ATCC 1705 and *K. pneumoniae* KPC, (C) *P. aeruginosa* ATCC 27853 and *P. aeruginosa* 027, (D) *E. coli* ATCC 25922 and *E. coli* 06. Statistical analysis was performed using two-way ANOVA followed by the Bonferroni post hoc test with GraphPad Prism 8.0 software. **** $p < 0.05$ indicates a statistically significant difference between the groups.

4. Discussion

LC-ESI-QTOF-MS analysis of SEF-4 revealed a series of high-molecular-weight ions with closely related retention times, a chromatographic profile commonly observed for triterpenoid saponins. These metabolites often differ mainly in the composition, sequence, and length of their glycosidic chains and therefore exhibit similar retention behavior under reversed-phase chromatographic conditions [20,21].

The detection of protonated molecular ions with m/z values above 1000 Da further supports the occurrence of highly glycosylated metabolites, which are frequently reported in species of the genus *Sarcomphalus* and related taxa [29]. Moreover, previous phytochemical studies have demonstrated that the bark of *S. joazeiro* is particularly rich in triterpenoid saponins [30], supporting the enrichment of this metabolite class in the SEF-4 fraction.

The MS/MS spectra exhibited characteristic neutral losses of approximately 162 and 146 Da, commonly attributed to hexose and deoxyhexose residues, respectively. Such fragmentation behavior is typical of triterpenoid saponins, in which collision-induced dissociation preferentially promotes the cleavage of glycosidic bonds prior to fragmentation of the aglycone moiety [20,31,32]. Collectively, the accurate mass measurements and fragmentation profiles are consistent with high-molecular-weight glycosylated triterpenoids containing multiple sugar residues.

The detected metabolites are compatible with oligoglycosylated triterpenoid saponins containing hexose and deoxyhexose units, a structural feature frequently reported as a chemotaxonomic characteristic of the genus *Sarcomphalus* [29]. Although definitive structural elucidation requires isolation and complementary spectroscopic analyses, particularly NMR experiments, the LC-HRMS/MS data strongly support the tentative assignment of these metabolites as jujubogenin-type triterpenoid saponins.

Previous phytochemical investigations have reported numerous triterpenoid saponins in species of the genus *Sarcomphalus*, typically containing glucose, rhamnose, and arabinose residues arranged in oligosaccharide chains linked predominantly to the C-3 position of the aglycone. Variations in sugar composition, sequence, and chain length contribute substantially to the structural diversity observed within this class of metabolites [21,29]. The putative structures proposed herein are consistent with these previously reported biosynthetic and structural patterns.

Among the detected metabolites, compound 5 (m/z 1371.219) was noteworthy because of its comparatively high molecular mass and fragmentation profile, which suggest the presence of a more extensively glycosylated structure. Metabolites within this mass range are less frequently reported for the genus and may represent a less common structural variant. However, confirmation of this hypothesis will require additional structural studies.

The fragmentation behavior observed for compound 5 is also compatible with a putative biosynthetic series involving progressive glycosylation of a common triterpenoid aglycone. Such a pattern is consistent with the biosynthetic pathways described for triterpenoid saponins, in which increasing glycosylation results in progressively higher molecular masses, frequently ranging from approximately 1000 to 1400 Da [20,33].

The UV-Vis spectra of the putative AgNP-SEF-4 formulation exhibited a localized surface plasmon resonance (LSPR) band centered at 416 nm after completion of the reaction. During the initial stages of synthesis (15–45 min), a red shift toward 420–440 nm was observed, suggesting dynamic changes associated with nanoparticle nucleation and growth during the reduction of Ag^+ ions to metallic silver (Ag^0) [25].

Similar optical characteristics have been reported for silver-containing nanostructures synthesized using extracts of *S. joazeiro* and related species, including *Ziziphus jujuba* and *Ziziphus nummularia*, which commonly exhibit LSPR bands between 400 and 460 nm [11,16,34].

Freshly prepared putative AgNP-SEF-4 exhibited an average hydrodynamic diameter of 114.46 nm, a zeta potential of -23.6 ± 1.90 mV, and a PDI of 0.147 (Table 3). These values indicate a negatively charged colloidal system with a relatively narrow particle-size distribution. The negative surface charge contributes to electrostatic repulsion between particles, whereas the low PDI value suggests satisfactory size homogeneity, both of which are desirable characteristics for colloidal nanoformulations [11].

After one month of storage, the average hydrodynamic diameter increased to 167.76 nm and the PDI increased from 0.147 to 0.221, suggesting partial aggregation of the dispersed particles over time. Nevertheless, the PDI remained below 0.3, indicating that the system retained a relatively homogeneous size distribution [23]. The zeta potential remained negative (-26.8 ± 6.38 mV), suggesting the maintenance of moderate colloidal stability during storage.

It is important to note that the antibacterial assays were performed using freshly synthesized putative AgNP-SEF-4. Therefore, the physicochemical changes observed after one month of storage did not influence the biological activity results reported in this study.

The pH of the reaction medium likely contributed to nanoparticle formation and stability. Near-neutral conditions favor a balance between reduction and stabilization processes, whereas highly acidic conditions may promote aggregation, and highly alkaline conditions may increase particle heterogeneity. Accordingly, the neutral pH employed in the present study may have favored the formation of relatively homogeneous colloidal dispersions [16].

Overall, LC-ESI-QTOF-MS analysis enabled the characterization of the predominant saponins present in SEF-4, while DLS, PDI, zeta potential, and UV-Vis analyses provided evidence consistent with the formation of stable silver-containing nanostructures. However, although the obtained data support nanoparticle formation, they do not unequivocally exclude the possibility of silver-organic complexes or other silver-containing assemblies. For this reason, the designation “putative AgNP-SEF-4” was adopted throughout this study.

Future investigations incorporating transmission and/or scanning electron microscopy (TEM and SEM), energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), and complementary spectroscopic analyses will be necessary to confirm the morphology, elemental composition, crystalline structure, and nanoscale organization of the synthesized material. Likewise, NMR-based studies will be required for definitive structural elucidation of the detected saponins.

In the antibacterial assays, SEF-4 did not exhibit clinically relevant activity against the tested reference strains, with MIC values ≥ 500 $\mu\text{g/mL}$. In contrast, putative AgNP-SEF-4 displayed enhanced antibacterial activity against *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 1705, with MIC values of 62.5 $\mu\text{g/mL}$, whereas no improvement was observed against *P. aeruginosa* ATCC 27853 (MIC ≥ 500 $\mu\text{g/mL}$). For *S. aureus*, putative AgNP-SEF-4 reduced the MIC to 125 $\mu\text{g/mL}$ for the reference strain and 62.5 $\mu\text{g/mL}$ for the multidrug-resistant isolate. Silver nitrate (AgNO_3 , 1 mM) exhibited MIC values of 250 $\mu\text{g/mL}$ against all tested strains.

A similar trend was observed for the multidrug-resistant isolates. Whereas SEF-4 maintained MIC values ≥ 500 $\mu\text{g/mL}$, putative AgNP-SEF-4 exhibited MIC values of 62.5 $\mu\text{g/mL}$ against *S. aureus* 10, *P. aeruginosa* 027, and *K. pneumoniae* KPC, and 125 $\mu\text{g/mL}$ against *E. coli* 06. AgNO_3 maintained an MIC of 250 $\mu\text{g/mL}$ against all multidrug-resistant strains (Table 4).

Previous studies have reported MIC values ≥ 1024 $\mu\text{g/mL}$ for extracts and fractions obtained from *S. joazeiro* [35,36]. The improved antibacterial activity observed for putative AgNP-SEF-4 may be associated with both the enrichment of saponins in SEF-4 and the

formation of silver-containing nanostructures, which may favor interactions with bacterial cell envelopes and enhance the availability of bioactive constituents at microbial targets [14].

Evaluation of antibiotic-modulating activity revealed strain-dependent interaction profiles. Against *S. aureus* ATCC 25923, neither SEF-4 nor putative AgNP-SEF-4 significantly altered the activity of the tested aminoglycosides, indicating the absence of a detectable modulatory effect. In contrast, both treatments increased antibiotic MIC values against the multidrug-resistant strain *S. aureus* 10, suggesting an antagonistic interaction (Figure 7A).

Among the Gram-negative bacteria, antagonism was also observed against *P. aeruginosa* 027, whereas no statistically significant interaction was detected for *P. aeruginosa* ATCC 27853 (Figure 7C).

For *K. pneumoniae* KPC, the combination with amikacin resulted in an antagonistic effect, whereas a marked potentiating effect was observed with gentamicin, reducing its MIC from 125 to 7.81 µg/mL. In *K. pneumoniae* ATCC 1705, the combination with amikacin enhanced antibacterial activity, reducing the MIC from 250 to 125 µg/mL in the presence of SEF-4 and to 62.5 µg/mL in the presence of putative AgNP-SEF-4. No significant effect was observed for gentamicin in this strain (Figure 7B).

In *E. coli* 06, the combination with gentamicin substantially enhanced the antibacterial activity, reducing the MIC from 250 to 125 µg/mL in the presence of SEF-4 and to 31.25 µg/mL in the presence of putative AgNP-SEF-4. For amikacin, only putative AgNP-SEF-4 exhibited a modulatory effect, decreasing the MIC from 250 to 62.5 µg/mL. Likewise, in *E. coli* ATCC 25922, both samples reduced the MIC values of gentamicin and amikacin, with the most pronounced effect observed for putative AgNP-SEF-4 (Figure 7D).

The observed reductions in MIC values indicate that both SEF-4 and putative AgNP-SEF-4 can influence bacterial susceptibility to aminoglycosides, supporting their potential application as antibiotic adjuvants capable of enhancing antimicrobial efficacy under specific conditions.

Silva et al. (2024) reported that a saponin-enriched fraction obtained from *S. joazeiro* potentiated the activity of gentamicin, particularly against *E. coli*, reducing the MIC from 40 to 4 µg/mL [35]. These findings are consistent with the results obtained in the present study and support the hypothesis that saponins may contribute to the modulation of aminoglycoside activity, although the underlying molecular mechanisms remain incompletely understood.

Recent studies have demonstrated that silver nanoparticles synthesized using species of the genus *Ziziphus* frequently exhibit greater activity against Gram-negative bacteria. This behavior has been attributed to structural features of the Gram-negative cell envelope, particularly the presence of an outer membrane rich in lipopolysaccharides, which may influence nanoparticle adhesion, penetration, and cellular interactions [7,11].

SEF-4 and its corresponding nanoformulation contain putative jujubogenin-type saponins bearing oligosaccharide chains. The amphiphilic nature of these metabolites may facilitate interactions with biological membranes and contribute to changes in membrane permeability, especially in Gram-negative bacteria [7,37–39].

Furthermore, several studies have suggested that highly glycosylated triterpenoid saponins may exhibit enhanced biological activity due to their amphiphilic properties and increased capacity for intermolecular interactions [37–40]. The metabolites tentatively identified in the present study are consistent with this structural profile.

The hydrophobic triterpenoid aglycone may interact with phospholipid bilayers, potentially promoting membrane destabilization, increased permeability, and leakage of intracellular constituents, thereby contributing to antibacterial effects [6,41]. However, the precise mechanisms responsible for the observed biological activities remain to be elucidated.

Putative AgNP-SEF-4 exhibited greater antibacterial and antibiotic-modulating activity than the isolated fraction, suggesting that nanoformulation may improve the biological performance of the saponin-enriched fraction. The average hydrodynamic diameter (~114 nm) and relatively narrow particle-size distribution may favor interactions with bacterial cells, whereas synthesis under near-neutral pH conditions likely contributed to colloidal stability and dispersion homogeneity [16,42,43].

Although putative AgNP-SEF-4 exhibited a negative zeta potential (-23.6 ± 1.90 mV), interactions with bacterial cells may still occur through multiple physicochemical mechanisms, including van der Waals forces, hydrophobic interactions, ionic shielding effects, and processes mediated by the organic corona surrounding the nanoparticles [9,42–44].

Following contact with bacterial cells, silver-containing nanostructures may release Ag^+ ions capable of interacting with thiol-containing biomolecules, promoting oxidative stress and affecting essential cellular components, including proteins, lipids, and nucleic acids [7,44,45]. Nevertheless, these mechanisms were not directly investigated in the present study and should therefore be regarded as plausible hypotheses based on previous reports.

The antagonistic interactions observed in multidrug-resistant strains, particularly *S. aureus* 10, *P. aeruginosa* 027, and *K. pneumoniae* KPC, highlight the complexity of bacterial resistance mechanisms. Factors such as efflux pumps, reduced membrane permeability, enzymatic inactivation, adaptive stress responses, and potential physicochemical interactions among saponins, nanoparticles, and antibiotics may influence the outcome of these combinations [46,47].

Overall, the results suggest that putative AgNP-SEF-4 has the potential to enhance aminoglycoside activity, particularly against Gram-negative bacteria. However, the occurrence of antagonistic interactions in certain multidrug-resistant strains underscores the need for further mechanistic studies to clarify the molecular basis of these effects and to support the future development of this nanoformulation as an antimicrobial adjuvant.

5. Conclusions

The saponin-enriched fraction obtained from *S. joazeiro* (SEF-4) was tentatively characterized as containing five jujubogenin-type triterpenoid saponins bearing tetra-, penta-, and hexaglycosylated structures. This amphiphilic chemical profile may have contributed to the reducing and stabilizing properties of the fraction, enabling its application in the green synthesis of silver-containing nanostructures (putative AgNP-SEF-4). The resulting nanoformulation exhibited physicochemical characteristics compatible with biological applications, including an average hydrodynamic diameter within the nanoscale range, low polydispersity, and satisfactory colloidal stability.

From a biological perspective, although antagonistic interactions were observed in certain multidrug-resistant strains, putative AgNP-SEF-4 demonstrated the ability to modulate aminoglycoside activity, resulting in substantial reductions in MIC values, particularly against *E. coli* and *K. pneumoniae*. These findings suggest that nanoformulation improved the biological performance of the saponin-enriched fraction and may have contributed to enhanced bacterial susceptibility through mechanisms involving membrane interactions and the antimicrobial properties commonly attributed to silver-containing nanomaterials.

Overall, the results indicate that putative AgNP-SEF-4 represents a promising nanostructured system for enhancing aminoglycoside efficacy and modulating bacterial susceptibility, especially in Gram-negative bacteria. However, the occurrence of antagonistic interactions in some multidrug-resistant phenotypes highlights the complexity of nanoparticle–antibiotic–bacteria interactions and underscores the need for further investigation.

Future studies should focus on elucidating the molecular mechanisms underlying the observed modulatory effects, evaluating the toxicological and pharmacokinetic profiles of

the nanoformulation, and validating its efficacy in relevant in vivo models. In addition, complementary analytical approaches, including NMR spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD), will be important for further corroborating metabolite structural assignments and confirming the morphology, elemental composition, and crystalline characteristics of the synthesized material. Such investigations will be essential to advance the potential application of putative AgNP-SEF-4 as an antibiotic adjuvant for combating antimicrobial-resistant pathogens.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/chemistry8070092/s1>, Figure S1: Full scan mass spectra of the compounds putatively identified in the saponin-enriched fraction (SEF-4) (A) Compound 1; (B) Compound 2; (C) Compound 3; (D) Compound 4; (E) Compound 5; Figure S2. Comparative distribution of the molecular masses of compounds 1–5 detected by LC-ESI-QTOF-MS in the saponin-enriched fraction of *Sarcomphalus joazeiro* (SEF-4). Dashed lines indicate the typical range of triterpenic saponin masses described in the literature for species of the genus; Table S1: Main peaks observed in the TIC chromatogram of the analyzed fraction and their respective relative abundances (%).

Author Contributions: N.K.G.d.C.: Article writing and formatting. J.F.T. and T.A.d.M.B.: Chemical analysis. M.P.d.S., D.O.D.L., F.F.G.R. (Fázia Fernandes Galvão Rodrigues) and J.B.d.N.: Biological tests. H.P.d.O. and M.L.G.: Synthesized the nanoparticles. L.B.d.V. and M.M.F.: Characterization of the nanoparticles. F.F.G.R. (Fabiola Fernandes Galvão Rodrigues) and J.G.M.d.C.: Came up with the idea for the article and critically reviewed the work. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data are contained within the article.

Acknowledgments: This work was carried out at the Laboratory for Research on Natural Products (LPPN) of the Department of Biological Chemistry at the Regional University of Cariri (URCA), with support from the Coordination for the Improvement of Higher Education Personnel (CAPES), the National Council for Scientific and Technological Development (CNPq), the Funding Authority for Studies and Projects (FINEP), the Ceará Foundation for the Support of Scientific and Technological Development (FUNCAP), and the National Institute of Science and Technology—Food (INCT-ALIM).

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

AgNPs	Silver nanoparticles
SEF-4	Saponin-enriched fraction
MIC	Minimum inhibitory concentration

References

1. Giono-Cerezo, S.; Santos-Preciado, J.I.; Morfín-Otero, M.R.; Torres-López, F.J.; Alcántar-Curiel, M.D. Resistencia antimicrobiana. Importancia y esfuerzos por contenerla. *Gac. Médica México* **2020**, *156*, 171–178. [[CrossRef](#)]
2. Tran, T.D.; Olsson, M.A.; Choudhury, M.A.; McMillan, D.J.; Cullen, J.K.; Parsons, P.G.; Bernhardt, P.V.; Reddell, P.W.; Ogbourne, S.M. Antibacterial 5 α -Spirostane Saponins from the Fruit of *Cordyline allamanda*. *J. Nat. Prod.* **2019**, *82*, 2809–2817. [[CrossRef](#)] [[PubMed](#)]
3. Girma, A.; Alamnie, G.; Bekele, T.; Mebratie, G.; Mekuye, B.; Abera, B.; Workineh, D.; Tabor, A.; Jufar, D. Green-Synthesised Silver Nanoparticles: Antibacterial Activity and Alternative Mechanisms of Action to Combat Multidrug-Resistant Bacterial Pathogens: A Systematic Literature Review. *Green Chem. Lett. Rev.* **2024**, *17*, 2412601.
4. Langendorf, C.; Bossard, C.; Nackers, F.; Comelli, A.; Berghmans, M.; Molay, G.; Gomez, F.G.; Dicko, A.; Bangwen, E.; Kalwangila, T.; et al. Community and Hospital-Acquired Invasive Bacterial Infections in Hospitalised Patients with Advanced HIV Disease: A Prospective Study in Kinshasa, DRC. *Int. J. Infect. Dis.* **2026**, *164*, 108383. [[CrossRef](#)] [[PubMed](#)]

5. Murray, C.J.L.; Ikuta, K.S.; Sharara, F.; Swetschinski, L.; Aguilar, G.R.; Gray, A.; Han, C.; Bisignano, C.; Rao, P.; Wool, E.; et al. Global Burden of Bacterial Antimicrobial Resistance in 2019: A Systematic Analysis. *Lancet* **2022**, *399*, 629–655. [\[CrossRef\]](#)
6. Khan, M.I.; Ahhmed, A.; Shin, J.H.; Baek, J.S.; Kim, M.Y.; Kim, J.D. Green Tea Seed Isolated Saponins Exerts Antibacterial Effects against Various Strains of Gram Positive and Gram Negative Bacteria, a Comprehensive Study in Vitro and In Vivo. *Evid.-Based Complement. Altern. Med.* **2018**, *2018*, 3486106.
7. Li, J.; Monje-Galvan, V. Effect of Glycone Diversity on the Interaction of Triterpenoid Saponins and Lipid Bilayers. *ACS Appl. Bio Mater.* **2024**, *7*, 553–563. [\[PubMed\]](#)
8. Dada, A.O.; Adekolab, F.A.; Dada, F.E.; Adelani-Akande, A.T.; Bello, M.O.; Okonkwo, C.R.; Inyinbor, A.A.; Oluyori, A.P.; Olayanju, A.; Ajanaku, K.O.; et al. Silver Nanoparticle Synthesis by *Acalypha wilkesiana* Extract: Phytochemical Screening, Characterization, Influence of Operational Parameters, and Preliminary Antibacterial Testing. *Heliyon* **2019**, *5*, e02517. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Platania, V.; Kaldeli-Kerou, A.; Karamanidou, T.; Kouki, M.; Tsouknidas, A.; Chatzinikolaidou, M. Antibacterial Effect of Colloidal Suspensions Varying in Silver Nanoparticles and Ions Concentrations. *Nanomaterials* **2022**, *12*, 31.
10. Dagher, W.; Alassod, A.; Al Hinnawi, M.F.; Alghoraibi, I.; Taher, A.; Alnhlaoui, M. Characterization and Antibacterial Effect of Green-Synthesised Silver Nanoparticles Using Different Extraction Methods from *Ziziphus spina-christi* (Sidr) Leaf Extract Collected from Syria. *RSC Adv.* **2025**, *15*, 35642–35659. [\[PubMed\]](#)
11. Padalia, H.; Chanda, S. Synthesis of Silver Nanoparticles Using *Ziziphus nummularia* Leaf Extract and Evaluation of Their Antimicrobial, Antioxidant, Cytotoxic and Genotoxic Potential (4-in-1 System). *Artif. Cells Nanomed. Biotechnol.* **2021**, *49*, 354–366. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Guimarães, M.L.; Ferraz, R.F.; Oliveira, R.A.P.; Costa, J.G.M.; Leite, D.O.D.; Costa, M.M.; Oliveira, H.P. Light-Mediated Antibacterial Activity of Composites of Polypyrrole and Green Zinc Oxide Nanoparticles Synthesized Using *Sarcomphalus joazeiro* Extract. *ACS Omega* **2025**, *10*, 43591–43606. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Andrade, F.S.; Figueirêdo, R.M.F.d.; Queiroz, A.J.d.M.; Albuquerque Junior, N.d.M.; Silva, A.P.d.F.; Amadeu, L.T.S.; Carvalho, R.d.O.; Silva, W.P.d.; Saraiva, M.M.T.; Hamawand, I. General Aspects and Applications of Juazeiro (*Ziziphus joazeiro* Mart.): Bioactive Compounds, Antioxidant Activity, and Antimicrobial Potential. *Processes* **2025**, *13*, 3352. [\[CrossRef\]](#)
14. Carvalho, N.K.G.; Silva, M.P.; Leite, D.O.D.; Rodrigues, F.F.G.; Costa, J.G.M. Bioactive Compounds and Biological Activities of *Sarcomphalus joazeiro* (Mart.) Hauenschild. *Chem. Biodivers.* **2025**, *22*, e202500700.
15. Andrade, J.C.; Silva, A.R.P.; Santos, A.T.L.; Freitas, M.A.; Matos, Y.M.L.S.; Braga, M.F.B.M.; Bezerra, C.F.; Gonçalo, M.I.P.; Gomez, M.C.V.; Rolóm, M.; et al. Chemical Composition, Antiparasitic and Cytotoxic Activities of Aqueous Extracts of *Ziziphus joazeiro* Mart. *Asian Pac. J. Trop. Biomed.* **2019**, *9*, 222–226. [\[CrossRef\]](#)
16. Guimarães, M.L.; Silva, F.A.G.; Costa, M.M.; Oliveira, H.P. Green Synthesis of Silver Nanoparticles Using *Ziziphus joazeiro* Leaf Extract for Production of Antibacterial Agents. *Appl. Nanosci.* **2020**, *10*, 1073–1081.
17. Weinstein, M.P.; Patel, J.B.; Campeau, S.; Eliopoulos, G.M.; Galas, M.F.; Humphries, R.M.; Jenkins, S.G.; Lewis, J.S., II; Limbago, B.; Mathers, A.J. *Performance Standards for Antimicrobial Susceptibility Testing*, 28th ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2018.
18. Rodrigues, F.F.G.; Boligon, A.A.; Menezes, I.R.A.; Galvão-Rodrigues, F.F.; Salazar, G.J.T.; Nonato, C.F.A.; Braga, N.T.T.M.; Correia, F.M.A.; Caldas, G.F.R.; Coutinho, H.D.M.; et al. HPLC/DAD, Antibacterial and Antioxidant Activities of *Plectranthus* Species (Lamiaceae) Combined with Chemometric Calculations. *Molecules* **2021**, *26*, 7665. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Domon, B.; Costello, C.E. A Systematic Nomenclature for Carbohydrate Fragmentations in FAB-MS/MS Spectra of Glycoconjugates. *Glycoconj. J.* **1988**, *5*, 397–409.
20. Vincken, J.P.; Heng, L.; de Groot, A.; Gruppen, H. Saponins, Classification and Occurrence in the Plant Kingdom. *Phytochemistry* **2007**, *68*, 275–297. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Guo, S.; Duan, J.-A.; Tang, Y.-P.; Yang, N.-Y.; Qian, D.-W.; Su, S.-L.; Shang, E.-X. Characterization of triterpenic acids in fruits of *Ziziphus* species by HPLC-ELSD-MS. *J. Agric. Food Chem.* **2010**, *58*, 6285–6289. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Augustin, J.M.; Kuzina, V.; Andersen, S.B.; Bak, S. Molecular Activities, Biosynthesis and Evolution of Triterpenoid Saponins. *Phytochemistry* **2011**, *72*, 435–457. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Moses, T.; Papadopoulou, K.K.; Osbourn, A. Metabolic and Functional Diversity of Saponins, Biosynthetic Intermediates and Semi-Synthetic Derivatives. *Crit. Rev. Biochem. Mol. Biol.* **2014**, *49*, 439–462. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Moreno-Martin, G.; León-González, M.E.; Madrid, Y. Simultaneous Determination of the Size and Concentration of AgNPs in Water Samples by UV-Vis Spectrophotometry and Chemometrics Tools. *Talanta* **2018**, *188*, 393–403. [\[PubMed\]](#)
25. Zaheer, Z.; Bawazir, W.A.; Basaleh, A.S.; Alhogbi, B.G.; Albukhari, S.M. Effects of Anionic and Cationic Surfactants on the Surface Plasmon Resonance Intensity of Biogenic Silver Nanoparticles: Stability and Position of Optical Band. *J. Mol. Liq.* **2023**, *385*, 122363. [\[CrossRef\]](#)
26. Blair, J.M.A.; Webber, M.A.; Baylay, A.J.; Ogbolu, D.O.; Piddock, L.J.V. Molecular Mechanisms of Antibiotic Resistance. *Nat. Rev. Microbiol.* **2015**, *13*, 42–51. [\[PubMed\]](#)

27. Coates, A.R.M.; Hu, Y.; Holt, J.; Yeh, P. Antibiotic Combination Therapy against Resistant Bacterial Infections: Synergy, Rejuvenation and Resistance Reduction. *Expert Rev. Anti-Infect. Ther.* **2020**, *18*, 5–15. [[CrossRef](#)] [[PubMed](#)]
28. Farhadi, F.; Khameneh, B.; Iranshahi, M.; Iranshahy, M. Antibacterial Activity of Flavonoids and Their Structure–Activity Relationship: An Update Review. *Phytother. Res.* **2019**, *33*, 13–40. [[PubMed](#)]
29. Pan, F.; Zhao, X.; Liu, F.; Luo, Z.; Chen, S.; Liu, Z.; Zhao, Z.; Liu, M.; Wang, L. Triterpenoids in Jujube: A Review of Composition, Content Diversity, Pharmacological Effects, Synthetic Pathway, and Variation during Domestication. *Plants* **2023**, *12*, 1501. [[CrossRef](#)] [[PubMed](#)]
30. Nascimento, A.M.; Moreira, L.O.; Fonseca, T.S.; Marques, C.A.; Leitão, G.G.; Tavares, E.S.; Simas, R.C.; Leitão, S.G.; Campos, M.F.; Mendonça, S.C. *Ziziphus joazeiro*, a saponin-rich Brazilian medicinal plant: Pharmacognostic characterization of bark and leaves. *Rev. Bras. Farmacogn.* **2020**, *30*, 756–764. [[CrossRef](#)]
31. Madl, T.; Sterk, H.; Mittelbach, M.; Rechberger, G.N. Tandem Mass Spectrometric Analysis of a Complex Triterpene Saponin Mixture of *Chenopodium quinoa*. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 795–806. [[CrossRef](#)] [[PubMed](#)]
32. Sun, X.; Yang, X.; Xue, P.; Zhang, Z.; Ren, G. Improved Antibacterial Effects of Alkali-Transformed Saponin from Quinoa Husks against Halitosis-Related Bacteria. *BMC Complement. Altern. Med.* **2019**, *19*, 46. [[PubMed](#)]
33. Khakimov, B.; Tseng, L.H.; Godejohann, M.; Bak, S.; Engelsens, S.B. Screening for Triterpenoid Saponins in Plants Using Hyphenated Analytical Platforms. *Molecules* **2016**, *21*, 1614. [[CrossRef](#)] [[PubMed](#)]
34. Preet, S.; Tomar, R.S. Anthelmintic Effect of Biofabricated Silver Nanoparticles Using *Ziziphus jujuba* Leaf Extract on Nutritional Status of *Haemonchus contortus*. *Small Rumin. Res.* **2017**, *154*, 45–51. [[CrossRef](#)]
35. Silva, A.R.P.; Costa, M.S.; Araujo, N.J.S.; Alencar, G.G.; Ribeiro, P.R.V.; Tavares, J.F.; Pinheiro, J.C.A.; Coutinho, H.D.M. UPLC-QTOF-MS/MS characterization of a fraction enriched in saponins from *Sarcomphalus joazeiro* species and evaluation of the antibacterial activity against multidrug-resistant bacteria. *S. Afr. J. Bot.* **2024**, *165*, 324–330.
36. Andrade, J.C.; Silvaa, A.R.P.; Santos, A.T.L.; Freitas, M.A.; Carneiro, J.N.P.; Gonçalves, M.I.P.; Souza, A.; Freitas, T.S.; Ribeiro, P.R.V.; Brito, E.S.; et al. UPLC-MS-ESI-QTOF Characterization and Evaluation of the Antibacterial and Modulatory Antibiotic Activity of *Ziziphus joazeiro* Mart. Aqueous Extracts. *S. Afr. J. Bot.* **2019**, *123*, 105–112.
37. Patra, A.K. *Dietary Phytochemicals and Microbes*; Springer: Dordrecht, The Netherlands, 2012.
38. Smulek, W.; Rojewska, M.; Pacholak, A.; Machrowicz, O.; Prochaska, K.; Kaczorek, E. Co-Interaction of Nitrofurantoin and Saponins Surfactants with Biomembrane Leads to an Increase in Antibiotic's Antibacterial Activity. *J. Mol. Liq.* **2022**, *364*, 120070.
39. Wei, M.P.; Yu, H.; Guo, Y.H.; Cheng, Y.L.; Xie, Y.F.; Yao, W.R. Antibacterial Activity of Sapindus Saponins against Microorganisms Related to Food Hygiene and the Synergistic Action Mode of Sapindoside A and B against *Micrococcus luteus* In Vitro. *Food Control.* **2021**, *130*, 108337. [[CrossRef](#)]
40. Sparg, S.G.; Light, M.E.; Van Staden, J. Biological Activities and Distribution of Plant Saponins. *J. Ethnopharmacol.* **2004**, *94*, 219–243. [[CrossRef](#)] [[PubMed](#)]
41. Nugraha, A.P.; Sibero, M.T.; Nugraha, A.P.; Puspitaningrum, M.S.; Rizqianti, Y.; Rahmadhani, D.; Kharisma, V.D.; Ramadhani, N.F.; Ridwan, R.D.; Noor, T.N.E.T.A.; et al. Anti-periodontopathogenic ability of mangrove leaves (*Aegiceras corniculatum*) ethanol extract: In silico and in vitro study. *Eur. J. Dent.* **2023**, *17*, 46–56. [[PubMed](#)]
42. Franco, D.; Calabrese, G.; Guglielmino, S.P.P.; Conoci, S. Metal-Based Nanoparticles: Antibacterial Mechanisms and Biomedical Application. *Microorganisms* **2022**, *10*, 1778. [[CrossRef](#)] [[PubMed](#)]
43. Sharaf, E.M.; Hassan, A.; AL-Salmi, F.A.; Albalwe, F.M.; Albalawi, H.M.R.; Darwish, D.B.; Fayad, E. Synergistic Antibacterial Activity of Compact Silver/Magnetite Core-Shell Nanoparticles against Gram-Negative Foodborne Pathogens. *Front. Microbiol.* **2022**, *13*, 929491. [[PubMed](#)]
44. Raj, M.; Singh, M.; Kumar, V.; Sharma, A.K.; Sharma, V.; Yadav, M.; Sharma, J.; Sherawat, N. An updated overview of nanostructured silver as a novel class of biomedical agent. *Lett. Appl. Nanobiosci.* **2024**, *13*, 198. [[CrossRef](#)]
45. Walczewska, A.; Grzywacz, D.; Bednarczyk, D.; Dawgul, M.; Nowacki, A.; Kamysz, W.; Liberek, B.; Myszka, H. N-alkyl derivatives of diosgenyl 2-amino-2-deoxy- β -D-glucopyranoside: Synthesis and antimicrobial activity. *Beilstein J. Org. Chem.* **2015**, *11*, 869–874. [[PubMed](#)]
46. Noumedem, J.A.K.; Mihasan, M.; Kuate, J.R.; Stefan, M.; Cojocar, D.; Dzoyem, J.P.; Kuete, V. In vitro antibacterial and antibiotic-potential activities of four edible plants against multidrug-resistant Gram-negative species. *BMC Complement. Altern. Med.* **2013**, *13*, 190. [[PubMed](#)]
47. Costa, M.S.; Barbosa Filho, J.M.; Silva, A.R.P.; Tavares, J.F.; Araújo, N.J.S.; Freitas, T.S.; Pereira, F.N., Jr.; Maia, F.P.A.; Sousa, E.O.; Vasconcelos, J.E.L.; et al. Evaluation of the antibacterial and inhibitory activity of NorA and MepA efflux pumps from *Staphylococcus aureus* by diosgenin. *Life Sci.* **2022**, *308*, 120978. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.