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DEVELOPMENT, PHYSICOMECHANICAL, AND BIOCOMPATIBILITY EVALUATION OF ANTIMICROBIAL SURGICAL SUTURES COATED WITH LIPPIA SIDOIDES CHAM ESSENTIAL OIL

DESENVOLVIMENTO, AVALIAÇÃO FÍSICO-MECÂNICA E DE BIOCOMPATIBILIDADE DE SUTURAS CIRÚRGICAS ANTIMICROBIANAS REVESTIDAS COM ÓLEO ESSENCIAL DE *LIPPIA SIDOIDES* CHAM

DESARROLLO, EVALUACIÓN FISICOMECÁNICA Y DE BIOCOMPATIBILIDAD DE SUTURAS QUIRÚRGICAS ANTIMICROBIANAS RECUBIERTAS CON ACEITE ESENCIAL DE LIPPIA SIDOIDES CHAM

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ABSTRACT

Surgical site infections (SSIs) remain a complication associated with bacterial colonization of suture material. Although triclosan-coated sutures are used to reduce this risk, concerns regarding their safety and their role in resistance selection have prompted research into alternative approaches. This study evaluated polyglactin 910 sutures coated with Lippia sidoides Cham. essential oil (LSEO), rich in thymol, assessing their physicomechanical properties, in vitro antibiofilm activity against Staphylococcus aureus and Escherichia coli, and biocompatibility using the Hen's Egg Test–Chorioallantoic Membrane (HET-CAM) assay. Three suture groups were compared: uncoated polyglactin 910 (Vicryl_U), triclosan-coated polyglactin 910 (Vicryl_P2), and LSEO-coated polyglactin 910 (VLSEO). All groups met pharmacopoeial requirements for length, diameter, and tensile strength, with VLSEO exhibiting the highest mean tensile strength (13.76 ± 1.43 N), followed by Vicryl_P2 (13.07 ± 1.04 N) and Vicryl_U (12.43 ± 0.99 N). High-performance liquid chromatography confirmed effective thymol impregnation on the VLSEO surface (142 µg/cm), while scanning electron microscopy revealed uniform and intact surfaces. VLSEO exhibited antibiofilm activity comparable to Vicryl_P2, reducing bacterial cell survival to 18% for S. aureus and 30% for E. coli relative to the uncoated control. The HET-CAM assay classified both VLSEO and LSEO as non-irritant (0 ± 0). Thus, LSEO-coated sutures demonstrated adequate mechanical properties, antibiofilm activity, and biocompatibility, representing a promising alternative to triclosan-coated sutures for the prevention of SSIs.

KEYWORDS: *Lippia sidoides. Surgical suture. Biofilm. Surgical site infection. HET-CAM.*

RESUMO

As infecções do sítio cirúrgico (ISCs) permanecem uma complicação associada à colonização bacteriana do material de sutura. Embora suturas revestidas com triclosan sejam utilizadas para reduzir esse risco, preocupações relacionadas à segurança e à seleção de resistência têm estimulado a busca por alternativas.

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Este estudo avaliou suturas de poliglactina 910 revestidas com óleo essencial de *Lippia sidoides* Cham. (OELS), rico em timol, quanto às propriedades físico-mecânicas, atividade antibiofilme *in vitro* contra *Staphylococcus aureus* e *Escherichia coli*, e biocompatibilidade pelo ensaio Hen's Egg Test–Chorioallantoic Membrane (HET-CAM). Foram comparados três grupos de suturas: a poliglactina 910 não revestida (Vicryl_U), a poliglactina 910 revestida com triclosan (Vicryl_P2) e a poliglactina 910 revestida com OELS (VLSEO). Todos os grupos atenderam aos requisitos farmacopeicos quanto ao comprimento, diâmetro e resistência à tração, com maior resistência média à tração para VLSEO (13,76 ± 1,43 N), seguido de Vicryl_P2 (13,07 ± 1,04 N) e Vicryl_U (12,43 ± 0,99 N). A cromatografia líquida de alta eficiência confirmou a impregnação efetiva de timol na superfície da VLSEO (142 µg/cm), e a microscopia eletrônica de varredura revelou superfícies uniformes e íntegras. A VLSEO apresentou atividade antibiofilme comparável à do Vicryl_P2, reduzindo a sobrevivência bacteriana para 18% para *S. aureus* e 30% para *E. coli*, em relação ao controle não revestido. O ensaio HET-CAM classificou a VLSEO e o OELS como não irritantes (0 ± 0). Assim, suturas revestidas com OELS demonstraram propriedades mecânicas adequadas, ação antibiofilme e biocompatibilidade, configurando-se como uma alternativa promissora às suturas revestidas com triclosan para prevenção de ISCs.

PALAVRAS-CHAVE: Lippia sidoides. Sutura cirúrgica. Biofilme. Infecção do sítio cirúrgico. HET-CAM.

RESUMEN

Las infecciones del sitio quirúrgico (ISQ) siguen siendo una complicación asociada con la colonización bacteriana del material de sutura. Aunque las suturas recubiertas con triclosán se utilizan para reducir este riesgo, las preocupaciones relacionadas con su seguridad y la selección de resistencia han impulsado la búsqueda de alternativas. Este estudio evaluó suturas de poliglactina 910 recubiertas con aceite esencial de Lippia sidoides Cham. (AELS), rico en timol, considerando sus propiedades fisicomecánicas, actividad antibiofilm in vitro frente a Staphylococcus aureus y Escherichia coli y biocompatibilidad mediante el ensayo Hen's Egg Test–Chorioallantoic Membrane (HET-CAM). Se compararon tres grupos: poliglactina 910 sin recubrimiento (Vicryl_U), recubierta con triclosán (Vicryl_P2) y recubierta con AELS (VLSEO). Todos los grupos cumplieron los requisitos farmacopeicos de longitud, diámetro y resistencia a la tracción. VLSEO presentó la mayor resistencia media a la tracción (13,76 ± 1,43 N), seguida de Vicryl_P2 (13,07 ± 1,04 N) y Vicryl_U (12,43 ± 0,99 N). La cromatografía líquida de alta eficiencia confirmó la impregnación efectiva de timol en la superficie de VLSEO (142 µg/cm), mientras que la microscopía electrónica de barrido reveló superficies uniformes e íntegras. VLSEO presentó actividad antibiofilm comparable a Vicryl_P2, reduciendo la supervivencia bacteriana al 18% para S. aureus y al 30% para E. coli respecto al control sin recubrimiento. El ensayo HET-CAM clasificó VLSEO y AELS como no irritantes (0 ± 0). Estos resultados indican que las suturas recubiertas con AELS presentan propiedades mecánicas adecuadas, actividad antibiofilm y biocompatibilidad, constituyendo una alternativa prometedora al triclosán para prevenir las ISQ.

***PALABRAS CLAVE**: Lippia sidoides. Sutura quirúrgica. Biopelícula. Infección del sitio quirúrgico. HET-CAM.*

1. INTRODUCTION

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Surgical sutures provide mechanical support for tissue approximation and, once implanted, may act as a substrate for bacterial colonization, directly influencing the risk of surgical site infection (SSI) (Edlich *et al.*, 2010). SSIs remain among the most prevalent healthcare-associated infections worldwide, with incidence ranging from 3.0% to 15% and reaching up to 30.9% in low-resource settings (Wood *et al.*, 2025). Infections caused by multidrug-resistant pathogens further increase hospitalization time and healthcare costs (ECDC, 2025; Chipaga *et al.*, 2025).

Bacterial adhesion to suture material is a recognized determinant of SSI (Katz; Moderdechai; Mielman, 1981), particularly in braided sutures, whose multifilament structure favors microbial attachment and biofilm formation compared with monofilament materials (Fowler *et al.*, 2013; Masini *et al.*, 2011). *Staphylococcus aureus* and *Escherichia coli* are among the pathogens most frequently isolated from SSIs (Chipaga *et al.*, 2025; Ennab *et al.*, 2022).

Triclosan-coated polyglactin 910 sutures (Vicryl Plus®) were developed to address this issue and have shown consistent reductions in SSI risk (Belusic-Gobic *et al.*, 2007; Masini *et al.*, 2011; Urie *et al.*, 2021; Jalalzadeh *et al.*, 2025). However, concerns regarding triclosan's reproductive toxicity (Pelz; Tödtmann; Otten, 2015) and its potential role in antimicrobial resistance selection (Han *et al.*, 2016; Obermeier *et al.*, 2015; Wang *et al.*, 2015) have led regulatory agencies, including ANVISA, to restrict its use (ANVISA, 2021; Corrêa *et al.*, 2022).

Essential oils have emerged as promising alternatives. *Lippia sidoides* Cham., native to Brazil, produces an essential oil (LSEO) rich in thymol, with well-documented antibacterial and antifungal activity (Costa *et al.*, 2002; Fontenelle *et al.*, 2007), low cytotoxicity (De Medeiros *et al.*, 2011), and synergistic antibacterial effects between thymol and carvacrol (Swetha *et al.*, 2020). Its incorporation into controlled-release systems has shown efficient retention and gradual release of bioactive compounds (Fernandes *et al.*, 2008; Dantas *et al.*, 2025), supporting its potential in coated biomedical devices (Karakus; Sanli, 2025; Oliveira *et al.*, 2006).

This study aimed to evaluate polyglactin 910 sutures coated with *Lippia sidoides* essential oil and to characterize the material according to the Brazilian Pharmacopoeia, 6th edition. In vitro antibiofilm activity against *S. aureus* and *E. coli* was assessed, and the irritation potential of the coated sutures was evaluated using the Hen's Egg Test–Chorioallantoic Membrane (HET-CAM) assay to determine their biocompatibility profile.

2. METHODOLOGY

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2.1. *Lippia sidoides* Cham. Essential Oil

The essential oil of *Lippia sidoides* Cham. (LSEO) was obtained commercially from Laszlo® (Belo Horizonte, Minas Gerais, Brazil). Its chemical profile is predominantly phenolic, with thymol (2-isopropyl-5-methylphenol) accounting for 71% of the total composition and carvacrol (2-methyl-5-[1-methylethyl]phenol), also referred to as cymophenol, accounting for 1%, and p-cymene for 10.5%. At 20 °C, the oil has a density of 0.934 g/mL (Fernandes *et al.*, 2008).

2.2. Development of Surgical Suture Coating with *Lippia sidoides* Cham. (VLSEO)

Polyglactin 910 4-0 sutures were coated using a fatty acid-based technique adapted from the protocol proposed by Obermeier *et al.* (2015). Methanol (Sigma, St. Louis, Missouri, USA) was employed as the solvent for incorporating the essential oil of *Lippia sidoides* Cham. into the suture coating (VLSEO).

2.3. Scanning Electron Microscopy

Pieces of the resorbable sutures, with and without a coating, were placed on steel stubs and sputter-coated with a 15 nm layer of gold using a Leica™ ACE 200 gold metallizer. The samples were then observed and photographed along the entire length of the suture using a scanning electron microscope (SEM) (JSM-5600LV, Jeol, Peabody, MA, USA). The images were taken at ×400, ×190 and ×350 magnification at a voltage of 15 kV (Palhares Neto *et al.*, 2020).

2.4. High-Performance Liquid Chromatography (HPLC)

The identification and quantification of most of the compounds in the surgical sutures were performed by dipping 27.5 cm of the thread in 5 mL of P.A. ethanol, followed by incubation in an ultrasound bath for 10 minutes. Subsequently, 5 mL of deionized water was added to the solution, and the samples were placed in an ultrasound bath for an additional 10 minutes. After extraction of the thymol present on the threads, the solution was filtered and injected into a Shimadzu HPLC system with the following setup: a diode array detector (DAD) (SPDM20A), an automatic injector (SIL-20A), a multisolvent pump (LC-20AT), an NST® C8 column (250 mm × 3.9 mm × 5 µm), an oven operating temperature of 40 °C, a 6:4 methanol: NaH2PO4 0.03 M + 0.4% triethylamine mobile phase adjusted to pH 2.7 with phosphoric acid. The flow was 0.8 mL/min and the sample injection volume was 20 µL. An evaluation of the thymol peak was conducted at a wavelength of 275 nm (Muniz, 2018).

2.5. Physicomechanical Evaluation of the Suture Threads

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A set of standardized physicomechanical tests was performed on the absorbable suture threads, following the criteria established by the Brazilian Pharmacopoeia, 6th edition, for gauge 4-0 (metric size 1.5) surgical sutures. Depending on the parameter evaluated, assays were carried out either in triplicate or with ten replicates (n = 10), always under controlled laboratory conditions.

After the sterilization step, sutures were submitted to a storage validation protocol based on the guidelines of the Brazilian Pharmacopoeia (6th edition) and ABNT NBR 13904 (2003). Packaging followed a two-layer system, comprising an individual primary envelope enclosed within a secondary box-type package capable of holding one or more primary units.

Storage parameters were established to maintain the physicochemical and microbiological stability of the sutures for up to five years after sterilization. To this end, sutures were kept at 21 °C ± 4 °C under ambient humidity, inside a closed, ventilated environment shielded from light, dust, and outdoor weather conditions (ABNT, 2003). Such conditions were adopted to guarantee product safety, efficacy, and adherence to applicable technical and regulatory requirements.

Three experimental groups were evaluated in this study:

- **Vicryl_U:** uncoated polyglactin 910 sutures without any antimicrobial agent (Bioline® Fios Cirúrgicos Ltda, Anápolis-GO, Brazil);
- **Vicryl_P2**: commercial polyglactin 910 sutures coated with triclosan (Vicryl® Plus, Johnson & Johnson, USA);
- **VLSEO**: polyglactin 910 sutures coated with essential oil extracted from *Lippia sidoides* Cham.

2.6. Suture Thread Length and Diameter

The total length of each thread was measured without applying any tension or elongation, using a precision millimeter ruler. According to pharmacopoeial criteria, each sample must retain at least 95% of the nominal length indicated on the packaging.

Diameter analysis was conducted on ten suture samples (n = 10), which were photographed under standardized conditions using a professional-grade digital camera equipped with high-resolution optics (OS Rebel SL3, Canon, Taichung City, Taiwan). Images were captured under consistent magnification and lighting conditions, and subsequently calibrated using a micrometric ruler as a reference scale. Dimensional analysis was performed using ImageJ® software (National Institutes of Health, Bethesda, MD, USA), following validated morphometric protocols.

For each suture, a straight-line measurement was drawn across the widest transverse section to determine its diameter in millimeters. The resulting values were recorded and expressed as mean ± standard deviation for further analysis. According to the Brazilian Pharmacopoeia (6th edition), the acceptable diameter range for suture gauge 4-0 (metric 1.5) is 0.150 to 0.199 mm.

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2.7. Knot Tensile Strength and Evaluation of Needle Attachment Strength (Lf)

Tensile strength testing was performed on ten suture samples (n = 10) using a universal testing machine (Shimadzu AGS-X series Universal Testing Machine; Shimadzu, Tokyo, Japan). The tests were conducted under ambient laboratory conditions and followed the protocol described in the "Tensile Strength" section of the Brazilian Pharmacopoeia, 6th edition.

Each thread was positioned between two pneumatic grips specifically designed for delicate filaments, ensuring axial alignment and minimizing slippage. A uniaxial tensile force was applied at a constant crosshead speed until rupture. The maximum force (in Newtons) recorded prior to failure was considered the tensile strength of the sample.

The measured values were expressed as mean ± standard deviation for further analysis. According to the Brazilian Pharmacopoeia, the minimum required tensile strength for suture gauge 4-0 (metric 1.5) is 0.95 kgf (9.32 N).

The evaluation of Needle Attachment Strength (Lf) was previously conducted and certified by the original manufacturer, Bioline®, and was therefore not repeated in the present study.

2.8. Monospecies Biofilm Model with LSEO-Coated Sutures

Two independent monospecies biofilm assays were performed, one using *Staphylococcus aureus* (ATCC 15656) and another using *Escherichia coli* (ATCC 25922). For each assay, suture segments (2 cm) were immersed in 24-well cell culture plates containing 1.6 mL of BHI broth and 0.4 mL of the respective bacterial inoculum, standardized to 0.5 on the McFarland scale. The plates were incubated in a microbiological oven at 37 °C for 24 hours.

The surgical threads were first placed in a well containing saline solution to remove planktonic cells, then transferred to a test tube containing 2 mL of 0.9% saline solution and sonicated. Serial dilutions were subsequently performed, and 20 µL aliquots of the diluted solutions were plated on BHI agar medium. The plates were incubated at 37 °C for 24 hours, followed by counting of colony-forming units (CFUs). Commercial polyglactin 910 with triclosan (Vicryl® Plus) and polyglactin 910 without triclosan served as positive and negative controls, respectively (Iñiguez-Moreno *et al.*, 2018). All analyses were performed in triplicate.

2.9. Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM)

The skin irritation potential of the Vicryl surgical sutures and of the *Lippia sidoides* essential oil (LSEO) was evaluated using the Hen's Egg Test–Chorioallantoic Membrane (HET-CAM) assay. Fertilized hen eggs were transported under refrigeration (5–12 °C) and left at room temperature for

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approximately 20 minutes before being placed in the incubator. On day 0, the eggs were placed in the incubator with automatic rotation programmed every two hours, at a temperature of 37.8 °C and relative humidity of 50–60%. On the 9th day of incubation, rotation was stopped and the eggs were candled using a light source to verify the formation of the air chamber at the widest portion of the egg. Eggs without a visible embryo, without an air chamber, or with a displaced air chamber were discarded. Viable eggs were repositioned in the incubator with the wider end facing upward.

On the following day, each egg was individually removed and, with the aid of a light source, the region between the chorioallantoic membrane (CAM) and the air chamber was delimited. An opening was then made at the top of the egg using forceps, and the shell above the membrane insertion point was removed. The external membrane was moistened with 0.9% saline solution and carefully removed without damaging the CAM (Delago; Cordeiro; Valadares, 2023).

For the assay, Vicryl sutures with LSEO (VLSEO), Vicryl without antimicrobial agent (Vicryl_U), Vicryl Plus (Vicryl_P2) sutures, and LSEO alone were evaluated. After exposure of the CAM, the sample was applied and, following 20 seconds of contact, the surface was rinsed with saline solution to remove the sample (n = 4). The egg was then repositioned to its original orientation, and the membrane was observed for the occurrence of changes. As a negative control, 300 µL of 0.9% saline solution (NaCl) was used, and as a positive control, 300 µL of 0.1 M sodium hydroxide (NaOH) was used. Finally, the presence of hyperemia, hemorrhage, and coagulation was monitored for 5 minutes to determine the irritation index score of the samples and controls, Table 1 (Luepke, 1985; Nascimento Júnior *et al.*, 2025).

Table 1. Irritation score based on the occurrence of hemorrhage, coagulation and hyperemia.

Classification	**Score**
Non-irritant	0 – 0.9
Slightly irritant	1 – 4.9
Moderately irritant	5 – 8.9
Severely irritant	9 – 21

3. RESULTS

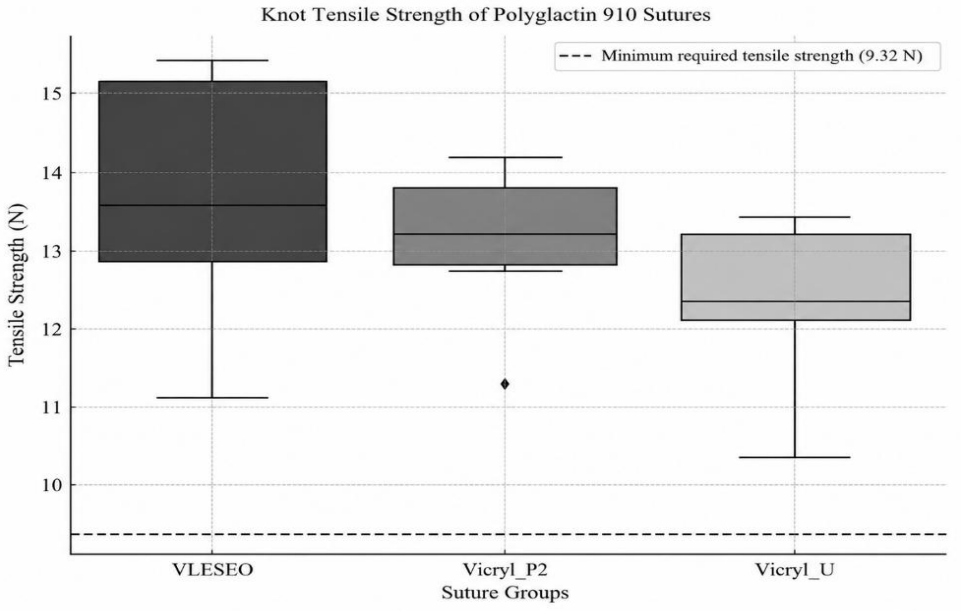
All suture threads evaluated complied with the specifications established by the Brazilian Pharmacopoeia, 6th edition, for gauge 4-0 sutures. Length measurements, performed without

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applied tension, showed that all samples retained more than 95% of their nominal length, satisfying pharmacopoeial requirements. Diameter analysis, conducted on ten samples per group (n = 10) using ImageJ® software, confirmed that Vicryl_U, Vicryl_P2, and VLSEO sutures presented transverse diameters consistently within the acceptable range of 0.150–0.199 mm, with mean values and standard deviations remaining within the upper and lower limits defined by the Brazilian standard for all three groups. These results indicate that the coating process, including LSEO incorporation, did not compromise the dimensional conformity of the sutures, confirming the structural integrity and pharmaceutical compliance required for surgical application.

Tensile strength testing, performed according to the Brazilian Pharmacopoeia (6th edition), which establishes a minimum knot tensile strength of 9.32 N for gauge 4-0 (metric 1.5) sutures, showed that all three groups exceeded this threshold. Mean maximum tensile strength was 13.76 ± 1.43 N for VLSEO, 13.07 ± 1.04 N for Vicryl_P2, and 12.43 ± 0.99 N for Vicryl_U. A Kruskal–Wallis test revealed no statistically significant differences among groups (H = 5.14; p = 0.076), although a trend toward lower values was observed for Vicryl_P2 (Fig. 1), suggesting that neither triclosan nor the LSEO coating adversely affected the mechanical performance of the sutures.

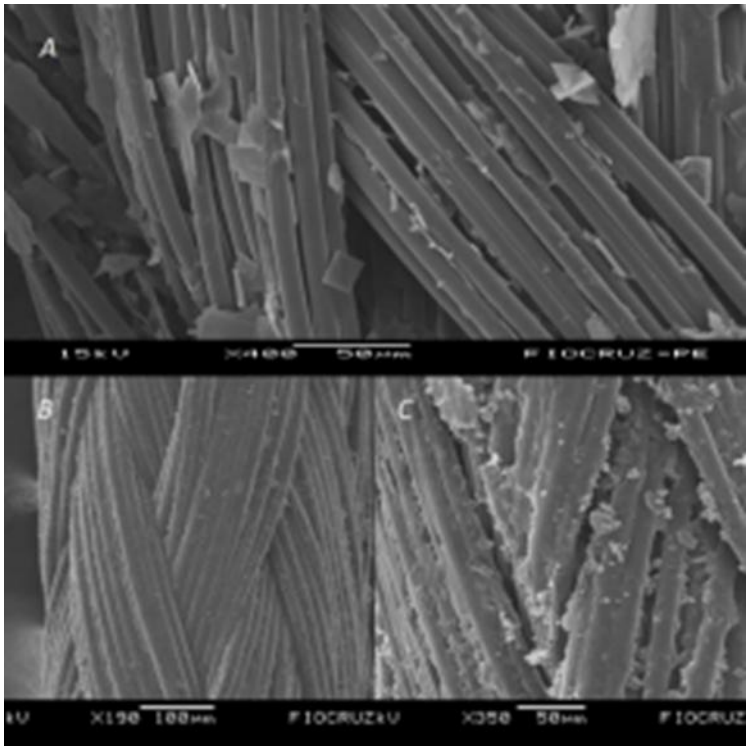
Figure 1. Knot tensile strength of polyglactin 910 sutures. The Kruskal–Wallis test was used for statistical analysis. VLSEO = suture coated with essential oil from Lippia sidoides Cham.; Vicryl_U = polyglactin 910 without coating; Vicryl_P2 = polyglactin 910 coated with triclosan



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HPLC analysis, performed to quantify thymol retained on the coated sutures, showed that VLSEO sutures analyzed one day after the coating procedure presented a thymol concentration of 142 µg/cm, confirming effective incorporation and short-term retention of the essential oil's main bioactive compound within the suture coating. Consistent with this finding, SEM analysis revealed uniform surface morphology across all suture groups, regardless of coating status: both coated (Vicryl_P2 and VLSEO) and uncoated (Vicryl_U) sutures exhibited preserved surface integrity, with no evidence of structural debris, cracking, or irregular deposition, indicating that the coating process did not alter the topographic properties of the suture surface (Fig. 2).

Figure 2. Photomicrographs of the threads studied. A: Scanning electron micrograph of a VLSEO suture showing the coating (x400); B: Scanning electron micrograph of the polyglactin 910 showing a multifilament structure (x190); C: Scanning electron micrograph of the polyglactin 910 with triclosan suture showing the coating (x350)



To approximate a clinically relevant model of bacterial attachment, comparable to conditions found in the oral cavity, monospecies biofilm assays were performed against *S. aureus* and *E. coli*. Sutures coated with LSEO (VLSEO) markedly reduced bacterial cell survival relative to controls, with mean values of 18% for *S. aureus* and 30% for *E. coli*, indicating relevant

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antibiofilm activity against both Gram-positive and Gram-negative pathogens commonly associated with surgical site infections.

Finally, the irritant potential of the coated sutures was assessed using the HET-CAM assay, an ethically favorable and sensitive alternative to traditional animal testing, widely employed to evaluate the irritant potential of synthetic and naturally derived substances. This method allowed the evaluation of vascular reactions on the chorioallantoic membrane (HET-CAM) of fertilized hen eggs, providing a predictive measure of the cutaneous and mucosal irritation potential of the tested materials (Vieira *et al.*, 2021). The effects observed on the HET-CAM following exposure to the surgical sutures and to the essential oil are presented in Fig. 3 and Table 2.

Figure 3. Photographs of the chorioallantoic membranes following exposure to Vicryl surgical sutures, with and without Lippia sidoides Cham. essential oil, to the isolated essential oil, and to the positive (0.1 M NaOH) and negative (0.9% NaCl) controls (n = 4)

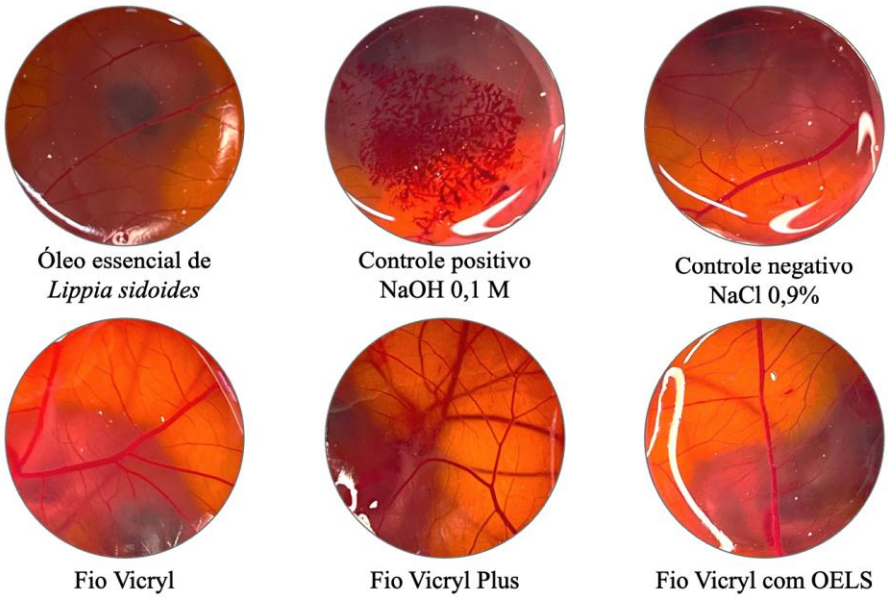


Table 2. Irritation results on the chorioallantoic membrane for Vicryl surgical sutures, with and without *Lippia sidoides* Cham. essential oil, for the essential oil applied directly to the chorioallantoic membrane, and for the positive (0.1 M NaOH) and negative (0.9% NaCl) controls, with their respective classifications based on the irritation score (mean ± standard deviation, n = 4)

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Substance	Irritation score	Classification
NaOH 0.1 M	20.76 ± 0.27	Severely irritant
NaCl 0.9%	0 ± 0	Non-irritant
Lippia sidoides essential oil	0 ± 0	Non-irritant
Vicryl_U	0 ± 0	Non-irritant
Vicryl_P2	0 ± 0	Non-irritant
VLSEO	0 ± 0	Non-irritant

4. DISCUSSION

Surgical site infections (SSIs) remain among the most frequent and costly complications of surgical care, with biofilm formation on suture material recognized as one of the principal mechanisms underlying their persistence and treatment failure (Seidelman; Mantyh; Anderson, 2023; Hrynyshyn; Simões; Borges, 2022). This burden is compounded by the increasing involvement of antimicrobial-resistant pathogens, which are independently associated with higher mortality and prolonged hospitalization in healthcare-associated infections (Budianu *et al.*, 2025; Chipaga *et al.*, 2025). Within this context, the development of antimicrobial suture coatings capable of preventing bacterial colonization without contributing to resistance selection remains a pressing clinical need, one that the present study directly addresses through the incorporation of Lippia sidoides Cham. essential oil (LSEO) into polyglactin 910 sutures (VLSEO).

The present findings confirm that the coating procedure successfully incorporated LSEO onto the suture surface, as demonstrated by scanning electron microscopy (SEM) and high-performance liquid chromatography (HPLC) analyses. SEM images revealed a uniform coating layer with preserved surface integrity, while HPLC quantification confirmed the effective impregnation of thymol, the major bioactive constituent of LSEO, within the suture matrix. These results are consistent with previous evidence indicating that essential oils can be efficiently incorporated into polymeric matrices without disrupting their structural properties, a principle well

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established in the field of active packaging and increasingly extended to biomedical applications (Ribeiro-Santos *et al.*, 2017).

Regarding mechanical performance, all suture groups met the minimum tensile strength required by the Brazilian Pharmacopoeia (6th edition) for gauge 4-0 (metric 1.5) sutures. Notably, VLSEO presented the highest mean tensile strength among the three groups evaluated, surpassing both the uncoated suture (Vicryl_U) and the commercial triclosan-coated suture (Vicryl_P2), although differences did not reach statistical significance (Kruskal–Wallis, $H = 5.14$; $p = 0.076$). In contrast, Vicryl_P2 exhibited a consistent trend toward lower mechanical performance despite its commercial antimicrobial coating. These findings indicate that the incorporation of LSEO not only preserved but may have enhanced the mechanical integrity of the suture thread, positioning VLSEO as a mechanically robust alternative to both uncoated and triclosan-coated sutures, and reinforcing the feasibility of essential oil-based coatings for surgical applications.

A biofilm represents a persistent form of microbial contamination that enables bacterial adhesion to the surface of biomaterials, constituting a major contributor to surgical site infection (Hrynyshyn; Simões; Borges, 2022). In the biofilm assays conducted in the present study, VLSEO sutures achieved antibiofilm efficacy comparable to that of the triclosan-coated suture (positive control), while the uncoated suture (negative control) exhibited significantly higher colony-forming unit (CFU) counts, 5.07-fold greater for S. aureus (ATCC 15656) and 3.3-fold greater for E. coli (ATCC 25922). This finding is consistent with a growing body of evidence demonstrating the in vitro antimicrobial efficacy of essential oils against clinically relevant pathogenic and nosocomial bacteria (Puvača *et al.*, 2021; Siugzdaite *et al.*, 2025), and supports the rationale for their use as active antimicrobial agents in medical device coatings. Importantly, unlike triclosan, whose antibacterial action depends on achieving specific minimum inhibitory and microbicidal concentrations against both susceptible and resistant strains of S. aureus and E. coli (Assadian *et al.*, 2011), LSEO exerts a broad-spectrum, multi-target mechanism of action attributed to its phenolic constituents, which is generally regarded as less prone to inducing selective pressure for resistance.

Triclosan-coated sutures have been extensively investigated and, in several studies, associated with reduced rates of bacterial colonization and SSI (Fowler *et al.*, 2013; Guo *et al.*, 2016), a benefit recently reaffirmed by a systematic review and meta-analysis (Jalalzadeh *et al.*, 2025). However, their clinical efficacy has been questioned in specific surgical contexts, such as oral surgery, where some studies report no significant advantage over uncoated sutures (Pelz; Tödtmann; Otten, 2015). Moreover, concerns regarding triclosan's safety profile have intensified,

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encompassing its potential to generate toxic by-products, contribute to environmental contamination, and promote the emergence of multidrug-resistant bacterial strains (Obermeier *et al.*, 2015), which is particularly concerning in Brazil, where antimicrobial resistance has been formally recognized as a public health priority requiring an integrated research response (Corrêa *et al.*, 2022). These concerns have prompted regulatory agencies, including ANVISA, to progressively restrict triclosan's use in healthcare and consumer products (ANVISA, 2021). Other antimicrobial strategies, such as silver-treated sutures, have also been proposed as alternatives to triclosan for the prevention of biofilm-associated SSIs (Paladini *et al.*, 2025); however, essential oil-based coatings offer the additional advantages of natural origin, biodegradability, and a favorable biocompatibility profile, positioning them as particularly attractive candidates for wound dressing and suture applications (Karakus; Sanli, 2025).

Consistent with these findings, the HET-CAM assay, performed according to a validated and internationally recognized protocol (Delago; Cordeiro; Valadares, 2023; Vieira *et al.*, 2021), using the irritation score criteria of Luepke (1985) and Nascimento Júnior *et al.* (2025), demonstrated that neither the coated sutures nor LSEO alone promoted vascular alterations on the chorioallantoic membrane, both being classified as non-irritant. The negative control (0.9% NaCl) similarly produced no vascular changes, whereas the positive control (0.1 M NaOH) induced severe hemorrhage, with an irritation score of 20.76 ± 0.27, validating the sensitivity and reliability of the assay. These results are in agreement with previous studies reporting adequate biocompatibility of surgical biomaterials combined with natural compounds at appropriate concentrations, particularly in topical applications intended for tissue repair (Wong, 2021). Although continuous topical use of essential oils has been associated with mild cutaneous inflammatory responses, primarily attributed to phenolic compounds such as thymol and carvacrol (Maia de Oliveira *et al.*, 2014), the absence of irritant effects observed in the present study may be explained by the concentration employed, the short exposure time, and the incorporation of the oil into the suture matrix, which likely favors a more controlled release of its active constituents rather than direct, unbound exposure of the tissue to the oil.

Nevertheless, this study presents limitations that should be acknowledged. First, the 24-hour evaluation period used in the biofilm assays is relatively short, and it remains unknown whether bacterial adhesion to the sutures would occur, or intensify, beyond this timeframe. Second, methicillin-resistant S. aureus (MRSA) strains were not included in the antimicrobial evaluation, which was restricted to standardized ATCC strains of E. coli and S. aureus; broader susceptibility testing against resistant clinical isolates, using current and emerging methodologies (Khan; Siddiqui; Park, 2019), would strengthen future characterization of LSEO's antimicrobial

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spectrum. Third, the biofilm model employed was monospecies rather than multispecies, limiting direct comparison with previous in situ and in vivo studies that more closely reproduce the polymicrobial nature of surgical wound infections. Future in vivo investigations are therefore warranted to elucidate the mechanisms of action of LSEO under clinically representative conditions and over extended time periods.

By combining mechanical reliability, antibiofilm efficacy comparable to a triclosan-based commercial product, and a favorable biocompatibility profile, the LSEO-coated suture developed in this study represents a technically sound and biologically safer alternative for surgical wound closure. Its adoption has the potential to benefit multiple stakeholders across the healthcare system: for surgeons and other healthcare professionals, it offers a reliable antimicrobial barrier without the efficacy limitations and safety concerns associated with triclosan; for patients, it may translate into a reduced risk of surgical site infection, shorter recovery times, and fewer postoperative complications; and for health institutions, it may contribute to lower costs associated with the treatment of infections caused by antimicrobial-resistant pathogens. Taken together, these findings support the continued development of Lippia sidoides essential oil-coated sutures as a promising, sustainable, and clinically relevant strategy for infection prevention in surgical practice.

5. FINAL CONSIDERATIONS

The incorporation of *Lippia sidoides* Cham. essential oil into polyglactin 910 sutures (VLSEO) proved to be a technically feasible and biologically safe strategy for surgical wound closure. The coated sutures fully complied with the physicomechanical requirements established by the Brazilian Pharmacopoeia, 6th edition, presenting tensile strength values numerically superior to both the uncoated and triclosan-coated sutures, without compromising dimensional conformity or surface integrity. HPLC analysis confirmed effective impregnation of thymol onto the suture surface, while biofilm assays demonstrated relevant antimicrobial activity against *S. aureus* and *E. coli*, comparable to that of the commercial triclosan-coated suture. Furthermore, the HET-CAM assay confirmed the non-irritant profile of both VLSEO and LSEO alone, reinforcing their biocompatibility. Taken together, these findings support VLSEO as a promising, biocompatible, and effective alternative to triclosan-coated sutures for the prevention of surgical site infections, warranting further in vivo investigation to validate its clinical applicability and potential adoption in surgical practice.

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