Australian Journal of Crop Science
AJCS
Aust J Crop Sci. 20(04):311-317 (2026) | ISSN:1835-2707
https://doi.org/10.21475/ajcs.26.20.04.pne144

Evaluation of the action of *Tetradenia riparia* essential oil against *Staphylococcus* spp. isolated from surfaces and instruments in urological procedures

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Submitted: 02/10/2025

Revised: 10/11/2025

Accepted: 20/03/2026

Abstract: Bacterial resistance represents one of the greatest challenges to global public health, particularly in healthcare environments, where the intensive and sometimes inappropriate use of antimicrobials favors the selection of multidrug-resistant strains. In this context, essential oils derived from medicinal plants have attracted growing interest from the scientific community for their potential as natural therapeutic alternatives. Thus, this study evaluated the in vitro antimicrobial activity of essential oil obtained from *Tetradenia riparia* leaves against 14 clinical isolates of *Staphylococcus* spp. isolated from surfaces and instruments used in urological procedures. The essential oil was extracted by hydrodistillation and its activity was analyzed using the broth microdilution method to determine the Minimum Inhibitory Concentration (MIC). The bacterial isolates were previously identified and their susceptibility to conventional antibiotics, including oxacillin and vancomycin, was tested. The oil showed inhibitory activity against 100% of the isolates tested, with MICs ranging from 0.6 µg/mL up to 20 µg/mL. Most strains were inhibited by 1.2 µg/mL. Although oxacillin exhibited the lowest mean MIC among the antimicrobials evaluated in this study, the essential oil was effective even against isolates resistant to conventional antimicrobials. Antimicrobial action is attributed to the presence of compounds such as monoterpenes and sesquiterpenes, which are capable of promoting the disruption of the bacterial cell membrane and the interruption of essential metabolic processes. These results reinforce the potential of *T. riparia* essential oil as a natural antimicrobial agent, with promising applicability as an adjuvant in the control of healthcare-associated infections.

Keywords: Medicinal plants; Natural antimicrobials; Nosocomial infections; Contaminated surfaces; Microbial resistance.

Introduction

Antimicrobial resistance (AMR) represents one of the greatest threats to global health today. According to the World Health Organization (WHO), it is estimated that by 2050, infections caused by resistant bacteria could surpass cancer deaths (WHO, 2023). *Staphylococcus* spp., such as methicillin-resistant *Staphylococcus aureus* (MRSA), which is frequently involved in severe nosocomial infections. This scenario challenges the efficacy of conventional antimicrobials and drives the search for new therapeutic strategies, including the use of plant-derived substances with antimicrobial action (Murray et al., 2022; Silva et al., 2025).

Growing interest in medicinal plants as an alternative or complement to conventional antimicrobial therapy stems from public health policies and advances in scientific research. Essential oils, volatile compounds extracted from various plant species, have demonstrated remarkable antimicrobial properties. Their mechanism of action includes destabilization of the cell membrane, inhibition of essential enzymes, and alteration of microbial metabolism, which confers efficacy against resistant strains and potentially lowers the risk of resistance development (Sena et al., 2024). Furthermore, their low

toxicity and action on multiple targets make these compounds promising options for combating healthcare-associated infections (HAIs).
Among the plants with recognized antimicrobial potential, *Tetradenia riparia* stands out. It is popularly known as "incense stick" or "fog plume'. Originating from Southern Africa, the species is widely used in traditional medicine and has been studied for its therapeutic properties, particularly those associated with its constituents, such as monoterpenes, sesquiterpenes, and abietane-type diterpenes (Mabona et al., 2013; Gazim et al., 2014). Studies demonstrate its activity against *Staphylococcus aureus*, including resistant strains; however, studies evaluating its effect on bacteria isolated from specific clinical environments, such as urological instruments, are scarce.
The strategy of using essential oils as adjuvants in the control of HAIs holds significant potential, especially in high-risk environments such as urological settings, where catheters and invasive procedures are routinely used. The use of actual bacterial isolates from clinical surfaces, rather than standard laboratory strains, offers a more representative approach to the hospital environment, allowing for a reliable assessment of the effectiveness of the natural compounds tested (Endo et al., 2018; Hamilton-Amachree et al., 2024).
In view of the above, the present study aimed to evaluate the *in vitro* antimicrobial activity of *Tetradenia riparia* essential oil, vancomycin, and oxacillin on *Staphylococcus* spp. strains isolated from urological healthcare environments.

Results

Regarding the *Tetradenia riparia* essential oil's MIC, approximately 6.25% (1/16) of the isolates exhibited the lowest inhibitory concentration observed in this study (0.6 μg/mL), while 56.25% (9/16) of the isolates had an MIC of 1.2 μg/mL. Two isolates (12.5%; 2/16) had MICs greater than 20 μg/mL (the maximum concentration evaluated) and were therefore excluded from the analyses presented in Table 1.
Regarding conventional antibiotic susceptibility, only one isolate from, recovered from a hospital kidney tank (before autoclave sterilization), was resistant to oxacillin (MIC >2 μg/mL). All other tested isolates were susceptible to oxacillin (≤2 μg/mL) (Table 2).
Comparing the antibacterial potential of oxacillin, vancomycin, and *Tetradenia riparia* essential oil, analyses demonstrated a statistically significant difference between the MICs of the three treatments ($p<0.05$; $p = 0.00002$). Figure 1 shows that oxacillin exhibited significantly lower MICs than both vancomycin ($p=0.003$) and *Tetradenia riparia* essential oil ($p=0.003$). These findings indicate that oxacillin is more effective in inhibiting the bacterial growth of *Staphylococcus* spp. environmental isolates.

Table 1. Relative and absolute frequency (%) of *Staphylococcus* spp. inhibited by the essential oil of *Tetradenia riparia* leaves.

***Tetradenia riparia* leaf essential oil (μg/mL)**	**Number of inhibited isolates (MIC)***	**Percentage (%) of inhibited isolates**
20	01	7.14
10	01	7.14
05	01	7.14
2.5	01	7.14
1.2	09	64.28
0.6	01	7.14
0.3	00	0.00
0.1	00	0.00
Total	14	100.00

* Minimum inhibitory concentration. The mean percentage of inhibited isolates was 12.5 (standard deviation ± 21.171). The MIC of the positive control (*Staphylococcus aureus* ATCC 29214) was 0.6 μg/mL.

Discussion

The assessment of the antimicrobial activity of *Tetradenia riparia* essential oil against *Staphylococcus* spp. isolates from urological environments yielded significant results, with inhibition observed in 100% (14/14) of the isolates included in the MIC determination. The most frequent MIC was 1.2 μg/mL, inhibiting 56.25% of the total strains, indicating a promising potential of the essential oil as an antimicrobial agent. Comparison with vancomycin and oxacillin, revealed that, although the antibiotics exhibited lower MICs, the oil demonstrated complementary effectiveness, suggesting its potential application as a therapeutic adjuvant.
Susceptibility varied among the isolates, with those recovered from surgical surfaces exhibiting greater susceptibility than those collected from outpatient equipment. This variation may be related to the type of surface and previous exposure to cleaning agents and antibiotics—a phenomenon already described in the literature as a modulating factor of bacterial resistance development (Scanavacca et al., 2023). When compared to vancomycin and oxacillin, the oil demonstrated a comparable action, thereby confirming its antimicrobial efficacy.

Table 2. Minimum inhibitory concentration (MIC) of oxacillin and vancomycin against *Staphylococcus* spp. isolates.

Sample		**Oxacillin MIC (μg/mL)**	**Vancomycin MIC (μg/mL)**
1	Clinical cautery generator	0.50	0.75
2	Clinical instrument table	0.50	1.50
3	Clinical faucet	0.50	1.50
4	Clinical procedure room bench	0.19	1.00
5	Clinical video tower TV	0.19	0.75
6	Clinical video camera head	0.38	1.50
7	Purge bench H.	0.75	2.00
8	Autoclave handle 1 H.	0.38	1.50
9	Autoclave handle 2 H.	0.50	1.00
10	Purge faucet H.	0.50	1.50
11	Double J clamp	0.38	0.75
12	Purge instrument bench H.	0.00	1.00
13	Patient stretcher H.	0.38	1.00
14	Procedure room faucet H.	0.38	1.00
15	Kidney basin H.	3.00	1.00
16	Surgical light H.	0.38	1.50
ATCC		0.38	1.00
Mean±standard deviation		0.56±0.673	1.2±0.367

Legend: MIC=Minimum inhibitory concentration. To calculate the mean and standard deviation of each antimicrobial, the positive control (*Staphylococcus aureus* ATCC 29214) was not included.

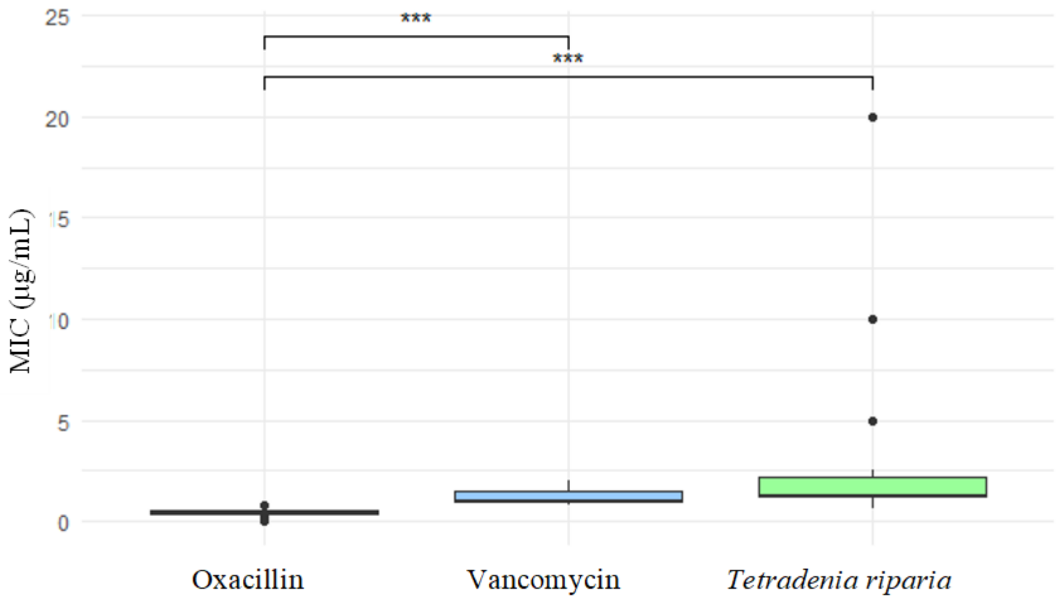

Figure 1. Comparison of the mean (mean±standard deviation) minimum inhibitory concentrations (MICs) of the antimicrobials oxacillin (0.557µg/mL±0.673), vancomycin (1.203 µg/mL±0.368), and essential oil of *Tetradenia riparia* leaves (3.493 µg/mL ±5.362) against environmental isolates of *Staphylococcus* spp., from a hospital and a clinic in Umuarama, Paraná. ***Statistically significant difference between treatments.

Previous studies on *T. riparia* essential oil focus mainly on leaf extracts obtained by hydrodistillation and provide valuable comparative data. Sena et al. (2024) analyzed leaf essential oil collected in Southern Brazil and identified antiviral, antioxidant, and antiproliferative activities, highlighting the presence of oxy-sesquiterpenes and diterpenes. Crucially, they did not quantify the antimicrobial effect against human clinical strains. Laginestra et al. (2024) tested *T. riparia* leaf essential oil against bacteria isolated from bovine mastitis (contaminated milk samples) and observed reduced bacterial growth but

did not detail MIC values. Quantitatively, Melo et al. (2015) reported MICs ranging from 31.2 to 500 μg/mL against *Streptococcus* spp., also utilizing essential oil from the leaves.
To date, no studies have been found evaluating *T. riparia* essential oil against human clinical or hospital isolates of *Staphylococcus* spp. This gap reinforces the relevance of the present study, which evaluates isolates obtained from surfaces and instruments in clinical and surgical urological environments, contributing novel data to literature. Furthermore, the inhibitory effects reported by Sena et al. (2024) and Laginestra et al. (2024) were qualitative (observing reduced microbial growth), lacking the precise quantification of antimicrobial activity provided by the MIC approach used herein.
The antimicrobial activity of *T. riparia* essential oil is associated with the presence of major compounds—identified via gas chromatography coupled to mass spectrometry (GC-MS) (Laginestra et al., 2024) - such as spathulenol, fenchone, aromadendrene oxide, (E,E)-farnesol, and dronabinol. These substances primarily belong to the classes of oxygenated sesquiterpenes and monoterpenes, which are known to destabilize the bacterial plasma membrane, thereby interfering with its structural and functional integrity. Studies show that these compounds can increase membrane permeability, lead to the leakage of cytoplasmic contents, and inhibit enzymes essential for microbial metabolism (Munive Núñez, 2023). Furthermore, the presence of multiple bioactive constituents suggests a synergistic action that enhances the oil's antimicrobial effects, even against strains with relevant resistance profiles. Therefore, *T. riparia* essential oil represents a promising natural alternative for inhibiting pathogenic bacteria, particularly in clinical contexts requiring new therapeutic strategies.
The therapeutic relevance of *T. riparia* has been demonstrated in recent studies that points to its anti-inflammatory activity and toxicological safety, especially with the use of hydroalcoholic extracts. In an *in vivo* model, Ndayambaje et al. (2025) reported the extract from the plant leaves did not present acute or subacute toxicity even at high doses (up to 5000 mg/kg), without relevant histopathological, biochemical, or hematological changes. Furthermore, the extract promoted significant anti-inflammatory effects, with 75.25% inhibition in vascular permeability and 49.96% in nitric oxide production, in addition to reducing inflammatory edema in mice. These effects were attributed to the presence of phenolic and flavonoid compounds with recognized antioxidant activity.
The few studies on the toxicity of *T. riparia* essential oil indicate that it has a relatively favorable safety profile. In a study conducted by Oliveira et al. (2022), *T. riparia* essential oil was evaluated for toxicity against target and non-target organisms. The results demonstrated significant larvicidal activity against the vectors *Anopheles gambiae* and *Aedes aegypti*, with LC_{50} values of 44.8 μg/mL and 50.2 μg/mL, respectively. For the non-target organisms evaluated, LC_{50} of 142.9 μg/mL was observed for the fish *Poecilia reticulata* and 153.9 μg/mL for the dragonfly *Crocothemis erythraea (*Oliveira et al., 2022).
These data indicate that, although the oil presents toxicity to the vectors of interest, its action on non-target species occurs at higher concentrations, providing initial evidence of the compound's selectivity. However, caution is needed when extrapolating these results to topical or environmental use in humans, as studies characterizing dermal toxicity, irritation, sensitization, or cumulative effects of *T. riparia* essential oil are still lacking.
Although the present study used the plant's essential oil, and not the extract, it is important to highlight that both share some bioactive constituents, such as terpenes and volatile compounds. However, the chemical profile and mechanisms of action may differ, especially regarding toxicity, concentration, and lipophilicity. Therefore, the need for specific investigations with the essential oil of *T. riparia* for toxicological characterization is reinforced, even though the findings with the extract support the therapeutic potential of the species and serve as an indication of preliminary safety.
The main mechanisms of action attributed to *T. riparia* essential oil involve changes in the structural integrity of the bacterial cell membrane, with consequent increase in permeability, loss of ions and intracellular macromolecules and inhibition of enzymes essential to microbial metabolism. These effects are the result of the synergistic action of compounds such as spathulenol, fenchone, aromadendrene oxide, (E,E)-farnesol, and dronabinol, which are major components of the plant's oil composition (Laginestra et al., 2024). These terpenoids, especially the oxygenated ones, have high lipophilicity, which favors their penetration into the lipid bilayer of the bacterial membrane, promoting structural disorganization and cell rupture (Laginestra et al., 2024). The literature indicates that this multi-target action reduces the likelihood of resistance development, as it reaches multiple bacterial targets simultaneously (Burt, 2004; Rufa'I et al., 2017).
Furthermore, there is robust evidence that (E,E)-farnesol acts directly on energy metabolism and cell membrane integrity in *Staphylococcus*. For example, Inoue et al. (2016) demonstrated that farnesol causes potassium ion leakage in *Staphylococcus aureus* by disrupting the plasma membrane, confirming physical damage to the cellular structure. In another study, Jabra-Rizk et al. (2006) demonstrated that farnesol, in addition to inhibiting biofilm formation by *S. aureus*, causes membrane permeabilization, evidenced by increased ethidium bromide uptake, and potentiates the action of antibiotics, providing a basis for the inhibition mechanism observed in our research. It was also observed that farnesol interferes with cell wall biosynthesis, impairing the transport of peptidoglycan precursors, according to Inoue et al. (2016).
These therapeutic results, associated with the profile of major compounds in *T. riparia* oil, such as (E,E)-farnesol, strengthen the plausibility of the antimicrobial effects observed in *Staphylococcus* spp. isolates in this study. The use of *T. riparia* essential oil as an antimicrobial agent brings important implications for clinical practice, especially in hospital environments where surface contamination represents a constant risk of pathogen dissemination. Considering the challenges associated with bacterial resistance in healthcare facilities, the use of essential oils in topical formulations, disinfectant sprays, or hospital cleaning products may represent an effective and low-cost alternative (Muthaiyan et al., 2012), particularly regarding *T. riparia* due to the promising activity presented in this research. However, practical feasibility depends on factors such as standardization of chemical composition, toxicological evaluation, and regulatory approval. Nevertheless, this study reinforces the idea that natural resources can be integrated into infection prevention and control strategies.

A key limitation of the study is the absence of a detailed phytochemical analysis of the essential oil used, which would strengthen the correlation between composition and antimicrobial activity. Furthermore, the evaluation was restricted to in vitro tests, which, although essential as an initial step, do not allow for the direct inference of efficacy in complex biological systems. Future studies should explore aspects such as oil stability, interaction with surface materials, toxicological evaluation in animal models, and synergy with conventional antimicrobials. Testing bacterial biofilms would also be relevant, given their role in persistent healthcare-associated infections.
Another relevant point is that the focus was exclusively on the *Staphylococcus* genus, without exploring the oil's broader spectrum of action against other hospital microorganisms, such as *Pseudomonas aeruginosa* and *Enterococcus faecalis*, which have mentioned in previous studies (Gazim et al., 2010). Future research should include *in vivo* testing, antibiotic synergism analyses, and evaluation of the effect on bacterial biofilms.
It is therefore concluded that the essential oil of *Tetradenia riparia* demonstrated significant inhibitory activity against strains of *Staphylococcus* spp. strains isolated from clinical and surgical urological environments. These findings reinforce the potential of this plant as an alternative source of antimicrobial compounds, with future application in surface sanitation, healthcare-associated infection control, and topical formulations. However, to consolidate its practical use, more in-depth studies on toxicity, mechanisms of action, and effectiveness *in vivo* models are needed.

Materials and methods

Ethical considerations
Ethical approval for the research was granted by the management of the involved institutions, under the assurance of confidentiality regarding the identity of participating units and the provision of a technical report upon study completion.

Sample origin
The *Staphylococcus* spp. isolates used in this research were obtained from environmental samples collected at a clinic and a hospital, both specializing in urology care. Sampling was performed on furniture, equipment, and instruments utilized in both outpatient and surgical procedures, using sterile swabs with appropriate transport medium.
The samples were then sent to the Preventive Veterinary Medicine Laboratory at the Universidade Paranaense (UNIPAR) for subsequent microbiological processing.

Sample processing and isolate identification
The collected samples were inoculated in Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hours. The cultures were then subcultured onto blood agar plates and incubated under the same conditions to achieve aerobic bacterial isolation. Representative colonies from each sample were selected and purified by successive subculturing.
Phenotypic identification was performed via morphological analysis of colonies, Gram staining, and conventional biochemical tests, as described by Koneman et al. (2008). Subsequently, aliquots of the isolates were stored in BHI broth supplemented with 15% glycerol at -20°C until further testing with antimicrobials and essential oil.

Determination of the minimum inhibitory concentration of vancomycin and oxacillin
The determination of the minimum inhibitory concentration (MIC) was determined for 14 *Staphylococcus* spp. isolates using the agar diffusion method with concentration gradient strips (Etest® bioMérieux) for oxacillin and vancomycin. These antibiotics were chosen due to their clinical relevance in treating infections caused by this microorganism.
The susceptibility test was performed on Mueller-Hinton agar plates. To prepare the inoculum, recent bacterial colonies were suspended in sterile saline solution, and the turbidity was adjusted using to the 0.5 McFarland scale. The bacterial suspension was then uniformly spread across the entire agar surface using a sterile swab. Following plate preparation, the Etest strip was carefully positioned on the surface of the medium. MIC readings were performed after incubation at 37°C for 24 h, considering the point at which the inhibition ellipse intersected the concentration gradient scale on the strip.

Plant material collection and essential oil extraction
The leaves of *Tetradenia riparia* were collected at the Medicinal Garden of the Universidade Paranaense (UNIPAR), Umuarama-PR, in the Northwest region of Paraná State, Brazil. Geographic coordinates were 23° 46.225' S and 53° 16.730' W, at an altitude of 391 m.
The collection was carried out in June 2025. Botanical identification was performed, and an exsiccata is deposited in the Educational Herbarium of the Universidade Paranaense under accession number 2502. The species is registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under access number AD97496.
Essential oil was obtained by hydrodistillation in a modified Clevenger apparatus for 4 hours. The oil layer was separated using hexane and dried by filtering with anhydrous sodium sulfate. The solvent was allowed to evaporate completely under refrigeration at 4°C (Sena et al., 2024).

Evaluation of the antibacterial activity of Tetradenia riparia essential oil
The antibacterial assay was performed using the microdilution method (CLSI, 2015) to determine the inhibitory activity of *Tetradenia riparia* leaf essential oil against 14 *Staphylococcus* spp. clinical isolates and the reference strain, *Staphylococcus aureus* ATCC 29214.

Ninety-six-well U-bottom polystyrene plates were used. Serial dilutions of the *T. riparia* essential oil were prepared in Mueller Hinton Broth (MHB) supplemented with 0.02% (v/v) Tween 80 (used as a surfactant) to yield the following eight final concentrations: 20 µg/mL, 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.2 µg/mL, 0.6 µg/mL, 0.3 µg/mL, and 0.1 µg/mL.
Subsequently, a standardized inoculum was added to each well to achieve a final concentration of 10^5 colony-forming units (CFU) per mL. Plates were then incubated at 35°C for 24 h. The following controls were included: (i) Growth Control (MHB + inoculum), (ii) Sterility Control (MHB only), and (iii) Solvent Control (MHB + Tween 80 + inoculum).
Following incubation, visual readings were performed. Inhibition was subsequently confirmed by adding 10 µL of a 10% 2,3,5-triphenyltetrazolium chloride indicator solution, followed by additional incubation for 30 minutes. Bacterial growth was evidenced by the appearance of a pink color in the wells.
The MIC was defined as the lowest concentration of the essential oil that completely inhibited visible bacterial growth. All assays were performed in triplicate.

Statistical analysis
Absolute and relative frequencies (number and percentage of isolates inhibited by each concentration of *T. riparia* leaf essential oil) were calculated. Mean and standard deviation were subsequently calculated for the observed MIC values.
To compare the mean MICs of oxacillin, vancomycin, and *Tetradenia riparia* essential oil against environmental *Staphylococcus* spp. isolates, data normality was assessed using the Shapiro-Wilk test. This test indicated that none of the treatment groups followed a normal distribution. Consequently, the non-parametric Friedman's Test for related samples was employed to evaluate the overall difference in MICs among the three antimicrobials. The test yielded a significant difference ($p<0.05$). Paired comparisons were subsequently performed using the Wilcoxon Signed-Rank Test with Bonferroni correction (Ceballos-Garzon et al., 2025).
Statistical analyses were performed using R software (version 4.4.1).

Conclusions

Based on the proposed objectives and the results obtained, this study demonstrated that *Tetradenia riparia* essential oil exhibits relevant antimicrobial activity against environmental strains of *Staphylococcus* spp. isolated from surfaces and instruments used in clinical and surgical urological environments. Inhibition of bacterial growth reinforces its potential as a natural alternative in the control of healthcare-associated infections. Although the oil's efficacy was inferior to that of conventional antibiotics in terms of minimum inhibitory concentration, its action was consistent and relevant, especially considering the complexity of the current bacterial resistance scenario. The low toxicity reported in the literature, coupled with its efficacy against resistant microorganisms, indicates that *T. riparia* can be considered a promising candidate for topical formulations or hospital hygiene products. However, it is necessary to advance with *in vivo* studies, in addition to phytochemical analyses and stability tests, to consolidate its safe and effective use in clinical practice. This work contributes to expanding knowledge about the use of herbal medicines in combating bacterial resistance and highlights the importance of research that prioritizes microorganisms isolated from real environments, bringing science closer to practical application in public health.

Acknowledgments

The authors would like to thank Universidade Paranaense (UNIPAR) for the financial support provided for this study and the healthcare institutions for granting permission to conduct the research.

Authors' contributions

Conceptualization LNB. and DDG, methodology, FAPF, FEPF, CD, JFA, FGCB, SAR, HMSO, and ZCG, Validation, LNB, FAPF, DDG, and ZCG, writing–original draft, LNB and FAPF, writing review and editing, LNB, FAPF, and DDG, Supervision and project leader, LNB.

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