









Research article

Antibacterial and antioxidant activities of aqueous extracts of medicinal plants against canine *Staphylococcus* isolates: Potential for veterinary dermatology applications

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Abstract

Alternative therapeutic approaches, including antimicrobial peptides, bacteriophage therapy, autovaccines, and natural products such as honey and medicinal plants, have emerged as promising strategies for managing canine skin infections in the context of increasing antimicrobial resistance in veterinary medicine. With the global rise in antimicrobial resistance, particularly among canine *Staphylococcus* species, plant-based therapies represent a viable alternative to conventional antimicrobials. This study investigated the bioactive properties of aqueous extracts from *Croton mubango*, *Tephrosia vogelii*, *Ipomoea batatas*, and *Prunus persica* leaves collected in Huambo, Angola, to assess its potential for veterinary dermatology applications. The extracts were evaluated in vitro for antibacterial, antioxidant, and hemolytic activities, as well as the effects of storage time and temperature on antibacterial capacity, and the potential of a guar gum (GG) biogel as a vehicle for topical application of the extracts. Data were analyzed using mixed linear and logistic models, followed by Tukey's test ($p < 0.05$). All extracts demonstrated high antioxidant activity, with *P. persica* exhibiting the highest antioxidant potential in the DPPH assay and *C. mubango* in the ABTS and FRAP assays. Also, *P. persica* extract showed the lowest hemolytic activity (10.69%) and the highest antibacterial activity against *Staphylococcus* species isolated from dogs, which persisted after three months of storage. The GG biogel modulated the antibacterial activity of the extracts, enhancing the inhibitory effect of the *T. vogelii* extract. These findings underscore the significance of African medicinal plants and identify *P. persica* as a promising source of natural antibacterial and antioxidant compounds to be used in veterinary medicine for the control of canine skin infections.

Keywords: Antibacterial, Antioxidant, Dogs, Plant extracts, Skin infections, *Staphylococci*

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Introduction

Plants are recognized as significant sources of medicinal practices to treat diseases and to active ingredients for medical applications. restore or enhance immune function (Aslam et al., 2016). Historically, they have been utilized in traditional Plants synthesize and accumulate

secondary metabolites, with concentrations that may fluctuate depending on environmental conditions and developmental stage (Angelini, 2024). Leaves typically contain the highest concentrations of bioactive compounds and are generally preferred for therapeutic use (Woo et al., 2023). Given the global increase in bacterial resistance in both hospital and community settings, investigating plant-based alternatives as novel antibacterial agents has become increasingly important (Oliveira et al., 2022; WHO, 2022).

Croton mubango, a member of the Euphorbiaceae family, is notable among traditional medicinal plants for its diverse therapeutic applications (Van Ee et al., 2011). It is used in traditional medicine to treat infectious, inflammatory, and metabolic diseases (Salatino et al., 2007; Alfred Maroyi, 2018). In Angola, oral infusions are prepared from the leaves, bark, and roots of *C. mubango*. However, clinical and laboratory validation of their use remains necessary, and the antioxidant, antimicrobial activities, and potential toxicity of *C. mubango* infusions require further investigation (Alfred Maroyi, 2018).

Tephrosia vogelii is traditionally employed to manage fungal and bacterial diseases and represents a potential source of antimicrobial agents (Mlozi et al., 2020). It is widely utilized in traditional veterinary and ethnomedical practices throughout tropical Africa. Mlozi et al. (2020) demonstrated that *T. vogelii* extracts may be effective in controlling bacterial infections caused by *Staphylococcus aureus*, *Salmonella Typhi*, and *Klebsiella pneumoniae*.

Ipomoea batatas is a medicinal plant with reported anticancer, antidiabetic, and anti-inflammatory properties (Alam, 2021). Its leaves contain bioactive compounds, including flavonoids, alkaloids, and phenolic acids (Mohanraj and Sivasankar, 2014), and exhibit strong inhibitory activity against both Gram-positive and Gram-negative bacteria (Islam, 2008).

Prunus persica is widely utilized for both medicinal and nutritional purposes, due to its rich composition in bioactive compounds (Kumar and Chaudhary, 2017), associated with antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities. Notably, *P. persica* leaves are traditionally used to treat a variety of skin conditions (Bhat et al., 2020).

Given the therapeutic potential of plant extracts, the development of suitable delivery systems for in vivo application is essential. Innovative herbal drug delivery systems can address limitations related to solubility, stability, and bioavailability of plant bioactive compounds, thereby enhancing therapeutic efficacy and minimizing adverse effects (Sarangi and Padhi, 2018). Effective delivery vehicles are particularly important for topical therapeutics. Guar gum (GG) has been identified as a stable and effective delivery system for the antimicrobial peptide nisin, enabling it to maintain its activity against *S. aureus* isolated from diabetic foot ulcers, and supporting its application in novel topical antimicrobial formulations (Santos et al., 2016).

In Africa, *Staphylococci* are of significant concern in veterinary medicine due to their roles as opportunistic pathogens and reservoirs of antimicrobial resistance determinants (Da Costa et al., 2025). Reports of *Staphylococcal* species isolated from companion dogs in Angola exhibiting high resistance and virulence emphasize the importance of these microorganisms in infection development and resistance transmission between animals and humans (Da Costa et al., 2025). These findings underscore the necessity for effective control and therapeutic strategies tailored to the African context and consistent with the One Health approach.

This study evaluated the antioxidant and hemolytic properties of aqueous leaf extracts from *C. mubango*, *T. vogelii*, *I. batatas*, and *P. persica*, as well as their antibacterial potential against a collection of *Staphylococcus* isolates obtained from dogs in Angola. The effects of storage on the inhibitory activity of the extracts and the potential of a GG biogel for their topical delivery were also assessed. Collectively, these findings support the potential application of plant-based topical formulations in veterinary medicine, namely as alternative treatments for skin infections in dogs.

Material and methods

Bacterial collection

This study used a collection of 56 staphylococcal isolates obtained from companion dogs in Angola, as previously characterized by da Costa et al. (2025). Swab samples were collected from the skin, oral cavity, and nasal mucosa of 32 dogs, presented for routine clinical consultations

at a veterinary clinic in Huambo province (Angola, Africa). Both healthy and clinically affected animals were included, irrespective of age, breed, or sex, with none having received antimicrobial treatment for at least two weeks before sampling. From 96 swab samples, 115 presumptive staphylococcal isolates were initially obtained. Of these, 56 representative isolates were selected based on molecular fingerprinting for further studies. The collection includes isolates belonging to the species *Mammaliicoccus sciuri*, *Staphylococcus xylosus*, *Staphylococcus equorum*, *Mammaliicoccus vitulinus*, *Mammaliicoccus lentus*, *Staphylococcus aureus*, and *Staphylococcus* spp., and representing a range of antimicrobial and virulence profiles, including multidrug-resistant strains, thereby reflecting the clinical and epidemiological diversity of *Staphylococci* in companion animals.

Plant leaf collection

Identification was performed by experienced technicians using taxonomic descriptions from the [Royal Botanic Gardens \(2024\)](#), based on macromorphological characteristics, including leaf features, growth form, and distinctive structures such as roots and fruits. Scientific names and taxonomic authorities were provided in accordance with the International Code of Nomenclature for algae, fungi, and plants.

The leaves of *C. mubango* [*Croton mubango* Müll. Arg; Family Euphorbiaceae], *T. vogelii* [*Tephrosia vogelii* (Hook. f.) Kuntze; Family Fabaceae], *I. batatas* [*Ipomoea batatas* (L.) Lam., family Convolvulaceae] and *P. persica* [*Prunus persica* (L.) Batsch, Family Rosaceae] were collected in January and March 2024, in Huambo, Angola. Voucher specimens of *T. vogelii* (Hb74) and *C. mubango* (Hb89) were deposited at the “Centro Nacional de Botânica”, Luanda (Angola). Voucher specimens for the remaining species were not deposited ([Figure 1](#)).



Figure 1: Plant species used in the study: 1. *C. mubango*; 2. *T. vogelii*; 3. *I. batatas*; 4. *P. persica* (originals).

Preparation of aqueous extracts from the leaves of the plants

Leaves of *C. mubango*, *T. vogelii*, *I. batatas*, and *P. persica* were collected in the morning and air-dried in the dark at room temperature for four days. The dried leaves were ground into a fine powder using a mortar and pestle and stored at room temperature. For extraction, 2 g of powder from each plant was mixed with 20 mL of sterile distilled water. The suspension was boiled for 5 min, centrifuged at 4,000 x g for 5 min, and the supernatant was collected and filtered. The resulting filtrates (extracts) were stored at 4°C. Extracts were tested directly to replicate traditional application methods used in Angola. Suspension concentrations were standardized to the initial plant-to-solvent ratio.

Antioxidant tests

Antioxidant activity of the extracts was evaluated using three in vitro assays: DPPH (1,1-diphenyl 2-picrylhydrazyl), ABTS (2,2-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid), and FRAP (ferric reducing antioxidant power). Absorbance values for all assays were measured using a FLUOstar OPTIMA microplate reader (BMG LABTECH, Offenburg, Germany) and recorded with BMG LABTECH OPTIMA software (version 2.20 R14). DPPH assay results were expressed as percentage radical scavenging activity (%RSA), while ABTS and FRAP results were quantified using Trolox and Fe²⁺ standard calibration curves, respectively.

DPPH (1,1-diphenyl 2-picrylhydrazyl) assay

The DPPH assay was conducted following the method described by [Sidiropoulou et al. \(2022\)](#). In summary, 20 µL of each plant leaf extract was combined with 20 µL of a 0.1 mM methanolic DPPH solution (D9132-1G, Sigma-Aldrich, Darmstadt, Germany). Trolox (Invitrogen, Vienna, Austria) served as the positive control, and distilled water as the negative control. Absorbance at 540 nm was measured for each sample at 20 min (t1) and 60 min (t2). The percentage of radical scavenging activity (%RSA) was calculated as follows:

$$\%RSA = [(Ab - As) / Ab] \times 100$$

where Ab is the absorbance of the blank sample (containing water instead of extracts) and As is the absorbance of each extract.

ABTS (2,2-azino-bis 3-ethylbenzothiazoline-6-

sulfonic acid) assay.

The ABTS assay was conducted using the Antioxidant Assay Kit (CS0790, Sigma-Aldrich, St. Louis, MO, USA) in accordance with the manufacturer's instructions. Trolox was used as the positive control, and the reaction mixture without extract served as the negative control. Results were determined using a Trolox standard curve and expressed as Trolox equivalents (mM), representing the total antioxidant capacity of the samples.

FRAP (Ferric Reducing Antioxidant Power assay)

FRAP was assessed using the colorimetric FRAP Assay Kit (MAK369, Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. The FRAP Positive Control was used as the positive control, and wells containing only the reaction mixture without extract served as negative controls. Results were calculated using the standard curve and expressed as ferrous equivalents (mM Fe²⁺), representing the total reducing capacity of the samples.

Hemolytic activity of plant leaf extracts

Hemolytic activity of the extracts was assessed following the method of [Mendonça et al. \(2021\)](#). Canine blood samples were obtained during routine veterinary procedures with owners' consent, collected in EDTA tubes, and stored at 4°C until use. To isolate canine red blood cells (cRBCs), 1 mL of each sample was centrifuged at 4000 rpm for 5 min, and the pellet was washed three times in 1× phosphate saline buffer (PBS; 150 mM NaCl, 5 mM Na₂HPO₄, and 1.7 mM KH₂PO₄; pH 7.4). The supernatant was discarded, and the pellet was resuspended in PBS (100 µL of cRBCs in 20 mL). Test wells of a 96-well microplate were filled with 100 µL of each plant extract and 100 µL of the cRBCs suspension. The positive control well contained 100 µL of Triton (1%) (Sigma-Aldrich, St. Louis, MO, USA) and 100 µL of cRBCs suspension, while the negative control well contained 200 µL of PBS. Microplates were incubated at 37°C for 1 h with agitation at 100 rpm, then centrifuged at 4000 rpm at 4°C for 5 min. Supernatants were transferred to a new microplate, and hemolysis was quantified by measuring hemoglobin (Hb) release at 450 nm. The percentage of hemolysis was determined as follows:

$$\text{Haemolysis (\%)} = \frac{(\text{Abs Treated} - \text{Abs Extract})}{(\text{Abs Triton} - \text{Abs Buffer})} \times 10$$

where Abs treated corresponds to the absorbance of cRBCs treated with the extracts, Abs extract to the absorbance of each extract without cRBCs, Abs Triton to the absorbance of cRBCs treated with Triton, and Abs buffer to the absorbance of PBS.

Antibacterial activity studies

Spot-on lawn test of the leaf extracts

Antibacterial activity of the extracts was evaluated using the spot-on-lawn method adapted from Adib Lesaux et al. (2025) and Mourão et al. (2024). Each extract was tested individually against a collection of 56 *Staphylococci* isolated from dogs in Angola, previously identified at the species level (Da Costa et al., 2025).

Bacterial isolates were inoculated in Brain Heart Infusion (BHI) agar (Oxoid, Hampshire, UK) and incubated overnight at 37°C. Standardized bacterial suspensions with a turbidity of 0.5 McFarland (1.5×10^8 CFU/mL) were then prepared in sterile saline and evenly spread onto Mueller–Hinton (MH) agar plates (Merck, Darmstadt, Germany). Subsequently, 10 µL of each extract was applied to the agar surface, and plates were incubated at 37°C for 72 h. Plates were visually inspected at 24, 48, and 72 h to assess bacterial growth inhibition, which was classified as absent, weak, intermediate, or strong based on the transparency of the inhibition spot. Completely transparent spots indicated strong inhibition, intermediate transparency indicated intermediate inhibition, and low transparency indicated weak inhibition. Absence of inhibition was classified as no antibacterial potential. The reference strain *Staphylococcus aureus* ATCC 25923 (Manassas, VA, USA) was used as a quality control, and Ampicillin (Oxoid, Hampshire, UK) served as the positive control.

Effect of storage on the antibacterial activity of the extracts

The effect of storage on the antibacterial activity of each plant extract was evaluated by storing the extracts at -20°C, 4°C, room temperature, 27°C, and 37°C for 90 days (Cunha et al., 2020; Soares et al., 2020). Antibacterial activity assays were conducted at day 0, 30, and 90 using the previously described spot-on-lawn assay. Six

selected isolates from the staphylococcal collection were used in these assays.

Potential of a Guar gum (GG) biogel as a delivery system for the extracts

A 1.5% GG biogel (w/v) suspension was prepared by dissolving 0.75 g of Guar gum (Sigma-Aldrich, St. Louis, MO, USA) in 50 mL of distilled water, followed by heat sterilization (Cunha et al., 2018). Each extract was combined with the GG biogel in a 1:1 (v/v) ratio. The resulting formulations were stored at -20°C, 4°C, room temperature, 27°C, and 37°C, and their antibacterial activity was evaluated as previously described.

Statistical analyses

Statistical analyses were conducted using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). FRAP and ABTS results were compared using ANOVA (PROC GLM) with plant leaf extracts as a fixed effect, and adjusted means were compared using Tukey's test ($p < 0.05$). DPPH was evaluated at two time points (20 and 60 min) and analyzed using repeated-measures ANOVA (PROC MIXED), with plant leaf extracts, time point, and their interaction as fixed effects. Hemolytic activity was analyzed using a linear mixed effects model (PROC MIXED), considering plant leaf extracts as a fixed effect and including experiment and replication within experiment as random effects. Adjusted means were compared using Tukey's test ($p < 0.05$). Antibacterial activity was analyzed using a cumulative ordinal mixed-effects logistic regression model implemented in PROC GLIMMIX. The fixed-effects structure included plant leaf extracts (*C. mubango*, *I. batatas*, *P. persica*, *T. vogelii*), incorporation in GG (incorporated or not incorporated), condition (27°C, 37°C, 4°C, room temperature, -20°C), and day of evaluation (0, 30, and 90), as well as biologically relevant two-way interactions (extract \times GG biogel, plant leaf extracts \times day, and plant leaf extracts \times condition). A random intercept for isolate was included to account for variability among isolates. Model parameters were estimated by maximum likelihood using the Laplace approximation, and statistical significance was assessed using Type III tests of fixed effects. Odds ratios (OR) were interpreted as follows: OR < 1 indicates a higher probability of intermediate or strong inhibition, whereas OR > 1 indicates a higher probability of absent or weak inhibition. Differences were considered statistically

significant at $p \leq 0.05$, and tendencies were noted when $0.05 < p \leq 0.10$. Least square means (LSMeans) are presented throughout the study, and the standard error of the mean (\pm SEM) was derived from a pooled estimate of residual variance within each model. Identical SEM values may be observed across treatments within the same assay, reflecting the common variance structure assumed in the statistical models rather than a lack of variability in the data. All experiments were performed in triplicate on independent days.

Results

Antioxidant tests

In the DPPH assay, the *C. mubango* extract exhibited a %RSA of $89.05\% \pm 16.44$ at 20 minutes and of $56.63\% \pm 16.44$ at 60 minutes. The *I. batatas* extract showed a %RSA of $96.79\% \pm 16.44$ at 20 minutes and of $71.44\% \pm 16.44$ at 60 minutes. The *P. persica* extract demonstrated a %RSA of $104.47\% \pm 16.44$ at 20 minutes and of $70.33\% \pm 16.44$ at 60 minutes. The *T. vogelii* extract displayed a %RSA of $104.16\% \pm 16.44$ at both 20 and 60 minutes (Figure 2A). No significant differences were observed between extracts. All extracts exhibited higher %RSA at 20 minutes (89.05–104.47%) than at 60 minutes (56.63–104.16%). Antioxidant activity was significantly influenced by time, with lower %RSA values at 60 minutes compared to 20 minutes ($p = 0.0002$). RSA values exceeding 100% reflect LSMean estimates derived from the mixed-effects model and experimental variability relative to the Trolox reference, rather than absolute stoichiometric scavenging.

The ABTS assay results are shown in Figure 2B as follows: $0.52 \text{ mM} \pm 0.004$ for *C. mubango*, $0.47 \text{ mM} \pm 0.004$ for *I. batatas*, $0.42 \text{ mM} \pm 0.004$ for *P. persica*, and $0.51 \text{ mM} \pm 0.004$ for *T. vogelii*. Significant differences were observed among the extracts. *P. persica* demonstrated significantly lower antioxidant activity when compared to *C. mubango*, *I. batatas*, and *T. vogelii* ($p < 0.001$). No significant difference was detected between *C. mubango* and *T. vogelii* ($p > 0.05$). *I. batatas* exhibited intermediate antioxidant activity, which differed significantly from all other extracts ($p < 0.05$).

The concentration of iron equivalents

determined by the FRAP assay is shown in Figure 2C. The results were $1.38 \text{ mM} \pm 0.15$ for *C. mubango*, $1.18 \text{ mM} \pm 0.15$ for *I. batatas*, $1.15 \text{ mM} \pm 0.15$ for *P. persica*, and $1.32 \text{ mM} \pm 0.15$ for *T. vogelii*. No significant differences were observed among the extracts ($p > 0.05$).

Hemolytic activity of the plant leaf extracts

The quantitative hemolysis assay revealed mean hemolysis values (Means \pm SEM) of $48.09\% \pm 6.54$ for *C. mubango*, $99.69\% \pm 6.54$ for *I. batatas*, $10.69\% \pm 6.54$ for *P. persica*, and $12.81\% \pm 6.54$ for *T. vogelii*. Statistical analysis indicated significant differences in hemolytic activity among the extracts ($p < 0.05$). *I. batatas* demonstrated the highest hemolytic activity, whereas *C. mubango* showed an intermediate effect. *P. persica* and *T. vogelii* did not differ significantly from each other but exhibited substantially lower hemolytic activity compared to *I. batatas* and *C. mubango* (Figure 3).

Antibacterial activity studies

The inhibitory capacity varied among the extracts (Figure 4). *P. persica* exhibited the highest inhibitory effect, followed by *I. batatas* and *C. mubango*. *T. vogelii* demonstrated the lowest inhibitory effect (Table 2).

Multiple comparisons were conducted among the extracts. Statistically significant differences in antibacterial activity were identified for the following pairs: *C. mubango* versus *P. persica* ($p = 0.0386$), *I. batatas* versus *P. persica* ($p = 0.0442$), *C. mubango* versus *T. vogelii* ($p = 0.0012$), *I. batatas* versus *T. vogelii* ($p < 0.0001$), and *T. vogelii* versus *P. persica* ($p = 0.0222$). No significant difference was observed between *C. mubango* and *I. batatas* ($p = 0.3992$).

These findings indicate that the antibacterial potential of the *T. vogelii* extract differs significantly from all other extracts, and that *P. persica* also differs from both *C. mubango* and *I. batatas* (Table 3).

Table 4 summarizes the comparisons of results observed at different time points (24, 48, and 72 hours). The difference in antibacterial activity between 24 and 48 hours was significant ($p = 0.0064$). However, differences between 24 and 72 hours and between 48 and 72 hours were not significant ($p > 0.05$), suggesting that the inhibitory effect remains stable after 48 hours.

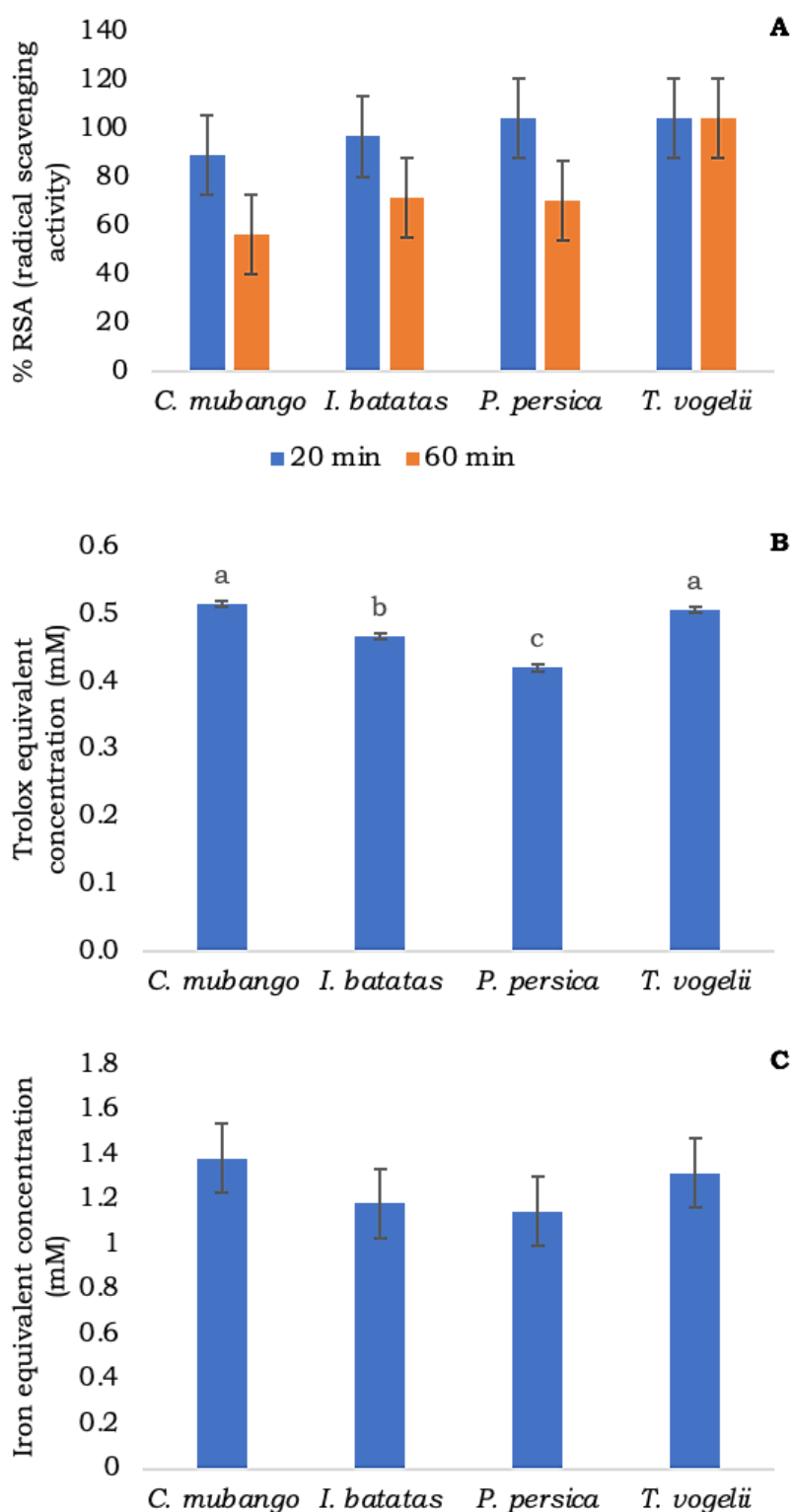


Figure 2: Graphical representation of the antioxidant capacity of aqueous extracts from *C. mubango*, *T. vogelii*, *I. batatas*, and *P. persica*. (A) Antioxidant activity of the leaf extracts evaluated by the DPPH assay, expressed as percentage of radical scavenging activity (%RSA), at 20 and 60 minutes (means \pm standard error of the mean); (B) Antioxidant activity of the leaf extracts evaluated by the ABTS assay, expressed as Trolox equivalent concentration (mM) (least-squares means \pm standard error of the mean). Bars sharing the same letter do not differ significantly ($p > 0.05$). (C) Antioxidant activity of the leaf extracts evaluated by the FRAP assay, expressed as iron equivalent concentration (mM) (least-squares means \pm standard error of the mean). % - Percentage; mM - millimolar.

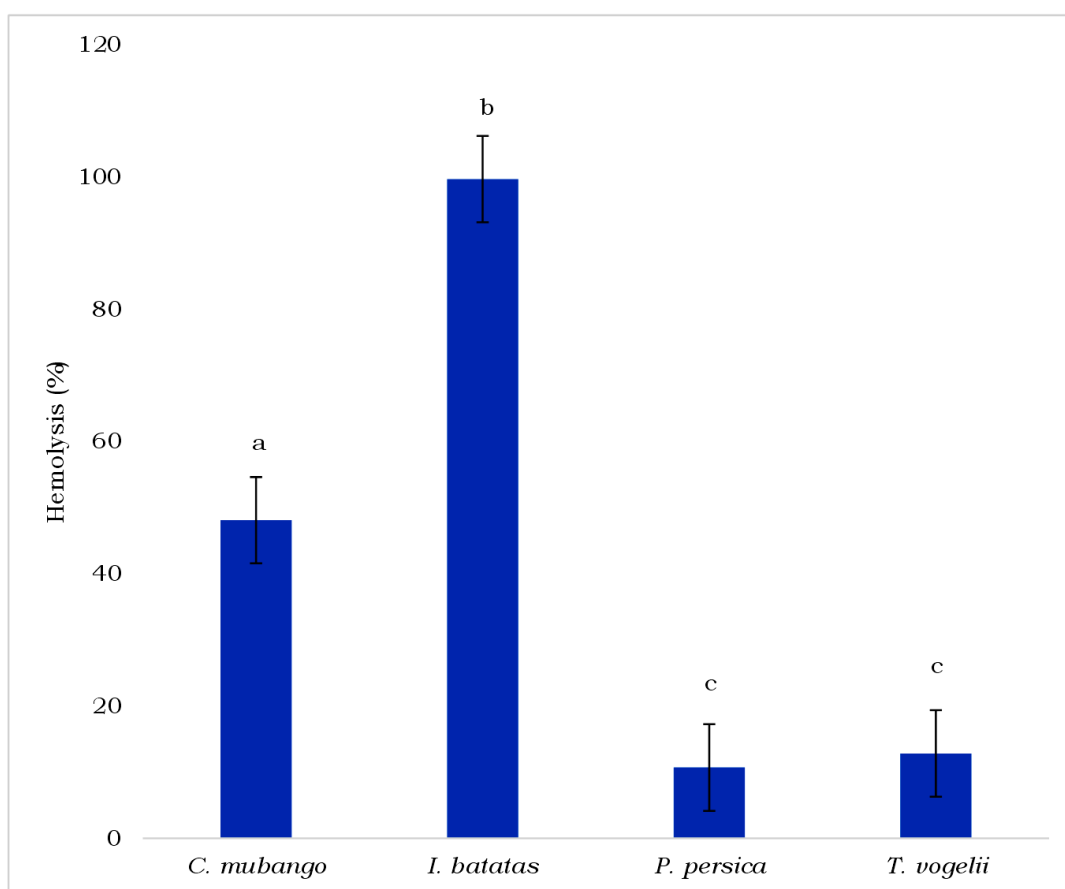


Figure 3: Percentage (%) of hemolysis promoted by the plant leaf extracts under study (Least square means \pm Standard Error of the Mean). Bars sharing the same letter correspond to non-significant differences ($p > 0.05$).

Table 2: Inhibitory activity of plant extracts against canine staphylococcal isolates observed at 24, 48, and 72h, expressed as number (n) and percentage (%) of isolates per inhibition category.

Extract	Time (h)	No inhibitory activity n (%)	Mild n (%)	Intermediate n (%)	Strong n (%)
<i>C. mubango</i>	24	46 (82.1%)	10 (17.9%)	0 (0.0%)	0 (0.0%)
	48	41 (73.2%)	14 (25.0%)	1 (1.8%)	0 (0.0%)
	72	39 (69.6%)	14 (25.0%)	3 (5.4%)	0 (0.0%)
<i>I. batatas</i>	24	43 (76.8%)	12 (21.4%)	1 (1.8%)	0 (0.0%)
	48	40 (71.4%)	12 (21.4%)	4 (7.1%)	0 (0.0%)
	72	38 (67.9%)	10 (17.9%)	8 (14.3%)	0 (0.0%)
<i>T. vogelii</i>	24	54 (96.4%)	2 (3.6%)	0 (0.0%)	0 (0.0%)
	48	53 (94.6%)	3 (5.4%)	0 (0.0%)	0 (0.0%)
	72	53 (94.6%)	3 (5.4%)	0 (0.0%)	0 (0.0%)
<i>P. persica</i>	24	0 (0.0%)	0 (0.0%)	0 (0.0%)	56 (100.0%)
	48	0 (0.0%)	0 (0.0%)	0 (0.0%)	56 (100.0%)
	72	0 (0.0%)	0 (0.0%)	0 (0.0%)	56 (100.0%)

Note: Results are expressed as number (n) and percentage (%) of isolates (n=56) per inhibition category for each extract and timepoint. Final classification for each isolate was determined based on the predominant result (mode) across three independent assays.

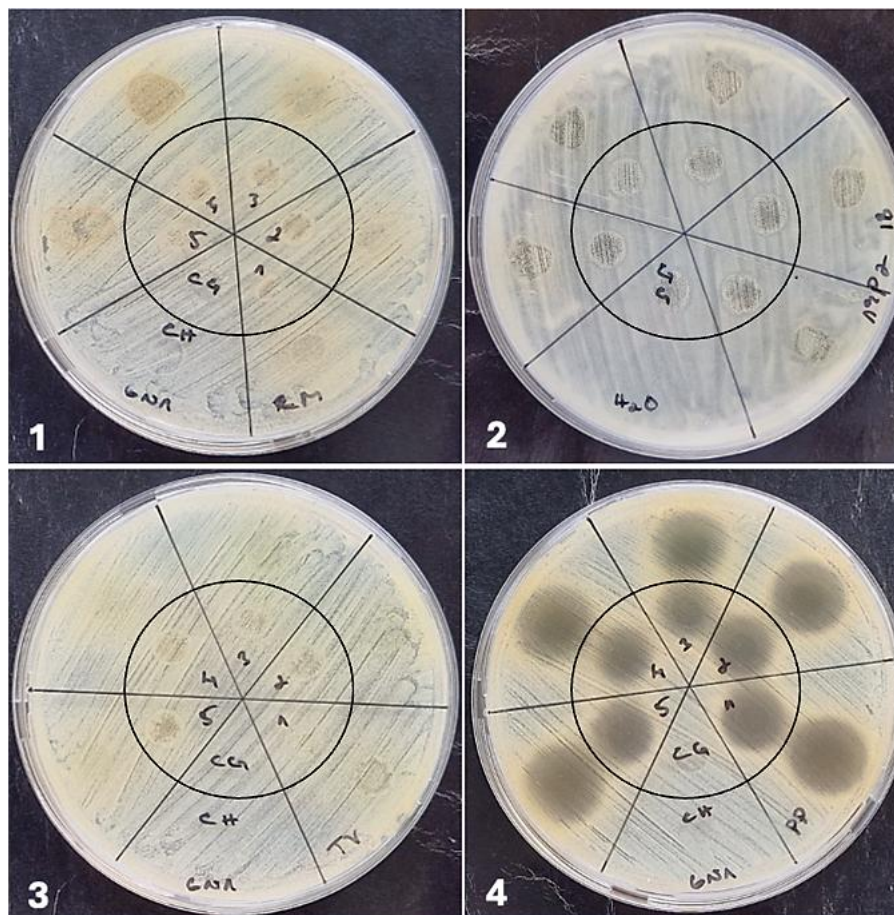


Figure 4: Spot-on-lawn assay showing the antibacterial activity of the different plant leaf extracts incorporated or not in the GG biogel against the *Staphylococcus* spp. isolates: 1. *C. mubango* extract; 2. *I. batatas* extract; 3. *T. vogelii* extract; 4. *P. persica* extract. Inside the circle: extract incorporated in the GG biogel; outside the circle: extract not incorporated in the GG biogel.

Table 3: Statistical comparisons between the antibacterial activity of the different plant leaf extracts under evaluation: estimated differences, Odds Ratio, Confidence Interval (IC 95%) and statistical significance (p-value).

Comparisons	Est. difference	Odds Ratio	IC 95%	p-value
<i>C. mubango</i> vs <i>I. batatas</i>	0.7233	2.0611	(-0.9641 - 2.4106)	0.3992 ^{ns}
<i>C. mubango</i> vs <i>P. persica</i>	25.8227	1.639E11	(1.3701 - 50.2753)	0.0386
<i>C. mubango</i> vs <i>T. vogelii</i>	-2.7562	0.06353	(-4.4165 - 1.0960)	0.0012
<i>I. batatas</i> vs <i>P. persica</i>	25.0994	7.953E10	(0.6621 - 49.5367)	0.0442
<i>I. batatas</i> vs <i>T. vogelii</i>	-3.4795	0.03082	(-5.1317 - -1.8273)	<0.0001
<i>T. vogelii</i> vs <i>P. persica</i>	28.5789	2.58E12	(61.9059 - 1.075E23)	0.0222

Note: (ns) not significant ($p \geq 0.05$).

Table 4: Multiple comparisons among experimental time points (24, 48, and 72h).

Comparisons	Est. difference	Odds Ratio	IC 95%	p-value
24 vs 48	0.8188	2.2679	(0.2304 - 1.4073)	0.0064
24 vs 72	2.9573	19.2454	(-7.9240 - 13.8385)	0.5941 ^{ns}
48 vs 72	2.1384	8.4861	(-8.7449 - 13.0218)	0.7000 ^{ns}

Note: Odds Ratio, Confidence Interval (IC 95%). (ns) not significant ($p \geq 0.05$).

Effect of storage on the antibacterial activity of the extracts

The main effect analysis of plant leaf extracts inhibition demonstrated significant overall differences among the extracts ($p < 0.0001$). Significant interactions were also identified for plant leaf extracts by days ($p < 0.0001$), plant leaf extracts by GG biogel ($p < 0.0001$), and plant leaf extracts by condition ($p < 0.0001$). These findings indicate that the magnitude and direction of these effects are context-dependent. Consequently, pairwise comparisons of the main effect should be interpreted as global average effects across all experimental conditions, including time and storage temperature.

P. persica extract consistently resulted in stronger inhibition spots compared to all other extracts (*C. mubango* vs *P. persica*, $p < 0.0001$; *I. batatas* vs *P. persica*, $p < 0.0001$; *P. persica* vs *T. vogelii*, $p < 0.0001$). Conversely, *T. vogelii* extract produced lower inhibition spots than both *C.*

mubango and *I. batatas* (*C. mubango* vs *T. vogelii*, $p < 0.0001$; *I. batatas* vs *T. vogelii*, $p < 0.0001$).

Analysis of temporal dynamics showed that the extracts by days interaction resulted in significant variation in inhibition across timepoints for all extracts except for *P. persica* (*C. mubango*, $p < 0.0001$; *I. batatas*, $p = 0.0002$; *P. persica*, $p = 0.0831$; *T. vogelii*, $p = 0.0014$). *P. persica* extract was the only one whose inhibition classification remained stable over time. Inhibition by *T. vogelii* and *C. mubango* extracts increased from day 0 to day 90 (*T. vogelii*: 0 vs 90, $p = 0.0012$; *C. mubango*: 0 vs 90, $p = 0.0001$), whereas inhibition by *I. batatas* decreased (0 vs 90, $p = 0.0004$). For the extracts by condition interaction ($p < 0.0001$), statistically significant differences within extracts were observed only in comparison with the control (without extract). This indicates that differences between extracts incubated at different storage temperatures were not statistically significant (Table 5).

Table 5: Effects of storage conditions on the antibacterial activity of the plant leaf extracts under study.

Factor / Interaction	Result summary	Biological interpretation	p-value
Extract (main effect)	Significant differences among extracts	<i>P. persica</i> showed the highest activity; <i>T. vogelii</i> lowest	<0.0001 *
Global comparisons	<i>P. persica</i> > <i>C. mubango</i> ≈ <i>I. batatas</i> > <i>T. vogelii</i>	<i>P. persica</i> has superior antibacterial potential	<0.0001 *
Extract × Days	Effect of time depends on extract	Temporal stability varies across extracts	<0.0001 *
<i>C. mubango</i>	Activity increases over time	Storage enhances antibacterial activity	<0.0001 *
<i>I. batatas</i>	Activity decreases over time	Loss of antibacterial activity during storage	0.0002*
<i>P. persica</i>	No change over time	Stable antibacterial activity	0.0831
<i>T. vogelii</i>	Activity increases over time	Storage improves the antibacterial effect	0.0014*
Extract × Condition	Differences only vs control	No meaningful effect of storage temperature on antibacterial activity	<0.0001 *
Within-extract contrasts	Significant only vs control	Storage conditions behave similarly	—

Note: * Statistically significant ($p \leq 0.05$).

Potential of a GG biogel to act as a delivery system for the extracts

The incorporation of the plant leaf extracts in the GG biogel revealed extract-specific responses ($p < 0.0001$). For the *T. vogelii* and *C. mubango* extracts, the incorporation in the GG biogel was associated with a shift towards higher inhibition potential, reflected by a reduced probability of absent or weak inhibition spots (*T. vogelii*: incorporated vs not incorporated, $p < 0.0001$; *C. mubango*: incorporated vs not incorporated, $p = 0.0003$). In contrast, for the *P. persica* extract,

incorporation into the GG biogel was associated with a shift towards lower inhibition, increasing the probability of absent or weak inhibition spots (*P. persica*: incorporated vs not incorporated, $p < 0.0001$). The *I. batatas* extract showed no significant change after incorporation in the GG biogel, indicating that its antibacterial activity was not affected (*I. batatas*: incorporated vs not incorporated, $p = 0.3354$). No significant effect of storage temperature on the antibacterial activity of the extracts incorporated in the GG biogel was observed (Table 6).

Table 6: Effect of the incorporation in the Guar gum biogel on the antibacterial activity of the plant leaf extracts under study.

Extract	Direction of effect (incorporated vs not incorporated)	Interpretation	p-value
<i>C. mubango</i>	↑ (incorporated > not incorporated)	Incorporation in GG enhances antibacterial activity	0.0003
<i>I. batatas</i>	—	Incorporation in GG has no effect	0.3354
<i>P. persica</i>	↓ (incorporated < not incorporated)	Incorporation in GG reduces antibacterial activity	<0.0001
<i>T. vogelii</i>	↑ (incorporated > not incorporated)	Incorporation in GG enhances antibacterial activity	<0.0001

Note: (incorporated) extract incorporated in the GG biogel; (not incorporated) extract not incorporated in the GG biogel. Direction of effect refers to shifts in inhibition categories. (↑) increased activity; (↓) decreased.

Discussion

Alternative therapeutic approaches for managing canine skin infections, especially those caused by multidrug-resistant *Staphylococci*, have received increasing attention in recent years. Antimicrobial peptides, bacteriophage therapy, autovaccines, and natural compounds have been extensively investigated in this context (Guardabassi et al., 2018; Squires, 2021; Alaoui Mdarhri et al., 2022; Mourão et al., 2024). Natural products, including honey and medicinal plants, exhibit antimicrobial, antibiofilm, and wound-healing properties, positioning them as promising complementary treatments for skin infections (Maddocks and Jenkins, 2013; Tresch et al., 2019). These strategies are particularly significant in veterinary dermatology, where topical and complementary therapies are essential, and they support the One Health approach by reducing antimicrobial use, thereby limiting resistance dissemination.

Specifically, in Africa, plant-based medicines are widely used despite limited validation, with many species showing relevant antioxidant and antibacterial properties (Chaves et al., 2020; Fadda et al., 2014; Przygodzka et al., 2014; Sidiropoulou et al., 2022).

In this study, all plant extracts tested demonstrated antioxidant activity across all assays. In the DPPH assay, extracts exhibited high initial activity that declined over time, likely due to compound depletion or instability (Fadda et al., 2014; Sidiropoulou et al., 2022). *P. persica* and *T. vogelii* displayed the highest initial activity, while *C. mubango* had the lowest activity at 60 minutes; however, no significant differences were detected, and *T. vogelii* maintained a more stable activity. The ABTS assay further confirmed the antioxidant capacity of all extracts (Przygodzka et al., 2014), indicating

their ability to neutralize free radicals through electron or hydrogen transfer (Munteanu and Apetrei, 2021). Pairwise comparisons revealed lower activity for *P. persica*, whereas *C. mubango* and *T. vogelii* exhibited similar values, potentially due to comparable phenolic and flavonoid content. The FRAP assay, which assesses the reduction of ferric ions by antioxidants (Payne et al., 2013), showed no significant differences among extracts, suggesting similar reducing power.

Regarding hemolytic potential, the *I. batatas* extract exhibited high hemolytic activity, consistent with previous findings (Amin and Dannenfelser, 2006), potentially due to the presence of saponins that disrupt erythrocyte membranes (Osuntokun et al., 2020; Sparg et al., 2004). In contrast, *P. persica* and *T. vogelii* extracts demonstrated low to moderate hemolysis, indicating greater suitability for biological applications.

The antibacterial activity of the extracts was assessed using staphylococcal isolates from Angolan dogs, representing microorganisms naturally associated with this host and relevant to local veterinary practice (Bannoehr and Guardabassi, 2012). The use of local strains enabled the evaluation of extract efficacy under biologically and geographically pertinent conditions, thereby advancing antibacterial research tailored to the regional context (Loeffler and Lloyd, 2018). All extracts demonstrated antibacterial activity, with *P. persica* consistently producing stronger inhibition spots and *T. vogelii* producing weaker spots. The inhibitory activity of *T. vogelii* differed significantly from the other extracts, and increased following incorporation into the GG biogel, indicating extract-specific responses to GG biogel incorporation. Temporal analysis showed a significant change in antibacterial activity between 24 and 48 hours,

with no difference between 48 and 72 hours, suggesting stabilization of the antibacterial effect after 48 hours. These findings align with previous reports that natural extracts often exhibit a strong initial antibacterial effect, followed by sustained activity over time (Abera et al., 2024; Sultana et al., 2024).

Distinct differences in antibacterial activity were observed among the extracts. The *P. persica* extract demonstrated the highest overall antibacterial efficacy, likely attributable to its higher phenolic and flavonoid content, which can be responsible for the damage bacterial membranes and inhibition of essential enzymes (Oulahal and Degraeve, 2022). In contrast, *I. batatas* and *C. mubango* extracts showed intermediate activity, possibly due to a lower content of bioactive compounds or polarity differences (Da S Rocha et al., 2021; Sultana et al., 2024). *T. vogelii* extract exhibited the lowest antibacterial activity, potentially resulting from reduced levels of active compounds or limited capacity to penetrate the bacterial cell wall (Mlozi et al., 2020). These results are consistent with previous studies reporting variability in antibacterial efficacy among plant extracts (Srikacha and Ratananikom, 2020; Zouine et al., 2024). Overall, *P. persica* emerges as the most promising extract for controlling resistant microorganisms.

Evaluating the effects of storage time and temperature on the antibacterial activity of extracts is critical for determining their stability and efficacy, as chemical and structural changes may occur during storage (Arabshahi-D et al., 2007; Postružnik et al., 2024). Understanding these variations informs on storage standardization, shelf-life determination, and application safety (Arabshahi-D et al., 2007; Postružnik et al., 2024). In this study, the impact of storage time was extract-dependent: *P. persica* maintained stable activity over 90 days, *C. mubango* and *T. vogelii* exhibited increased activity over time, and *I. batatas* showed decreased activity, possibly due to compositional changes. Antibacterial activity of the extracts remained stable across all tested storage temperatures, indicating no temperature-dependent effects, consistent with previous findings for *Prunus* and *Ipomoea* extracts (Chen et al., 2019; Mlozi et al., 2023) and *Croton* extracts stored under refrigeration (Rath et al., 2011).

Developing effective delivery systems is crucial

to optimizing the therapeutic efficacy of new antimicrobials (Kashyap et al., 2015). Many natural compounds exhibit low solubility, chemical instability, or limited bioavailability, which diminishes their *in vivo* effectiveness. Efficient delivery systems are therefore necessary to convert promising compounds into safe, stable, and clinically viable therapies (Zhao et al., 2024). The natural polysaccharide GG has been shown to be an effective and stable delivery system for topical formulations targeting bacterial skin infections (Santos et al., 2016). The impact of the incorporation of extracts into the GG biogel on their antibacterial activity varied by extract, supporting the hypothesis that matrix-phytochemical interactions influence bioavailability and efficacy. The antibacterial activity of *P. persica* extract decreased after incorporation into the GG biogel, but remained higher when compared to the other extracts. This reduction may result from interactions between the extract and the GG biogel matrix, affecting the release, diffusion, or availability of bioactive compounds (Dangi et al., 2022). Conversely, incorporation of *T. vogelii* and *C. mubango* extracts into the GG biogel increased their activity, possibly due to improved dispersion, protection, or controlled release of bioactive compounds within the galactomannan matrix (Chen et al., 2024; Khan et al., 2021), which may also explain the increased activity over time. *I. batatas* extract was not significantly affected by GG biogel incorporation, consistent with the overall decline in its antibacterial activity during storage, potentially due to component instability. Overall, these results indicate that GG biogel incorporation can modulate antibacterial activity depending on the phytochemical composition of the extracts.

This study has some limitations that should be considered. Only aqueous leaf extracts, obtained by a single extraction method, were used, and no chemical characterization of the bioactive substances was performed. This prevents the identification of the compounds responsible for the observed effects and excludes the characterization of the bioactive properties of the lipophilic fraction. The antioxidant assays were performed using fixed volumes/concentrations of the extracts, and the antibacterial evaluation was restricted to *in vitro* qualitative assays. Quantitative approaches, such as broth microdilution to determine inhibitory and bactericidal concentrations,

should be used in future studies. The specific mechanisms underlying the observed antibacterial effects also require further investigation.

Despite these limitations, *P. persica* emerged as the most promising plant extract for potential application in veterinary medicine to control canine skin infections, warranting further characterization and broader antibacterial evaluation.

Conclusions

Research into alternative therapies, including medicinal plants, offers a valuable complementary approach to conventional antimicrobials for the effective and sustainable management of canine skin infections within a One Health framework. In this study, aqueous extracts of *P. persica*, *C. mubango*, *I. batatas*, and *T. vogelii* demonstrated strong antioxidant capacity. Among these, *P. persica* extract exhibited the most consistent antibacterial activity and favorable safety profile, whereas *I. batatas* showed greater hemolytic effects. Incorporation into GG biogel altered antimicrobial performance, notably enhancing the activity of the *T. vogelii* extract. Although antibacterial efficacy varied with storage duration, it remained stable across different temperatures. *P. persica* was identified as the most promising plant extract for the control of skin staphylococcal infections, warranting further investigation. Overall, these findings identify *P. persica* as a promising source of bioactive compounds for pharmaceutical and biotechnological applications, particularly for the control of pathogenic microorganisms in veterinary medicine.

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