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In vitro antibacterial activity of hemp (*Cannabis sativa* L.) extract seed oil against multidrug resistant bacterial pathogens in small animal veterinary dermatology

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ABSTRACT

There is an urgent need for alternative antimicrobial therapies in veterinary small animal dermatology due to the limited therapeutic options available for treatment of infections caused by multidrug-resistant bacteria. This study aimed to evaluate the potential of hemp (*Cannabis sativa* L.) seed oil for topical treatment of localized infections of the skin, such as otitis externa. Antimicrobial activity was determined by broth microdilution using a strain collection of bacterial pathogens associated with skin infections, including *Staphylococcus pseudintermedius* (n = 120), *Staphylococcus aureus* (n = 48), and *Pseudomonas aeruginosa* (n = 26). Checkerboard dilution tests were used to assess the interaction of hemp seed oil with two antimicrobials used for management of otitis externa, gentamicin and enrofloxacin, while *in vitro* cytotoxicity was evaluated by the cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay on mouse fibroblast cell line L929. Minimum inhibitory concentrations (MICs) in staphylococci (0.025–0.2 % v/v) were markedly lower than in *P. aeruginosa* (>0.4 % v/v). Within *S. pseudintermedius*, methicillin-resistant strains displayed lower susceptibility compared to susceptible strains. Hemp seed oil showed synergy with gentamicin (Fractional Inhibitory Concentration Index < 0.5), reducing the MIC of gentamicin-resistant *S. pseudintermedius* strains (≥16 μg/ml) below the clinical susceptibility breakpoint (≤4 μg/ml). No changes in cell viability were observed at concentrations below 2 % v/v. These findings suggest that hemp seed oil could be an effective and safe alternative or adjuvant to conventional antimicrobials for managing otitis externa and other skin focal infections caused by staphylococci, including methicillin-resistant strains.

1. Introduction

The rise of multidrug-resistant (MDR) bacterial pathogens in veterinary small animal dermatology, coupled with the shortage of new antimicrobials, underscores the urgent need for new treatment strategies (Martins et al., 2022). Management of MDR bacterial infections has become particularly challenging in the EU after the recent ban of antibiotics that are not authorized for animal use (Schmerold et al., 2023). The European Food Safety Authority (EFSA) Panel on Animal Health and Welfare has identified *S. pseudintermedius* and *P. aeruginosa* as the most relevant antimicrobial-resistant bacteria in small animals across the EU (Nielsen et al., 2021). Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is a major concern due to the typical MDR profiles of certain epidemic lineages, such as clonal complexes 71 and 45 (Dos Santos et al., 2016).

This bacterial species is the most prevalent cause of canine bacterial infections in dogs, especially pyoderma, otitis externa and wound infection (Bannoehr & Guardabassi, 2012). Among Gram-negative pathogens, *Pseudomonas aeruginosa* is a major contributor to canine otitis externa, exacerbated by the increasing prevalence of MDR strains

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(De Martino et al., 2016). While dogs are not typically colonized by *Staphylococcus aureus*, skin, post-surgical and wound infections caused by methicillin-resistant strains (MRSA) are sporadically reported, posing significant treatment challenges (Pantosti, 2012; Haag et al., 2019).

As the demand for alternatives to conventional antibiotics grows, the scientific community is placing greater emphasis on the antimicrobial properties of natural substances (Helmy et al., 2023). Plants and their derivatives have shown considerable antimicrobial potential, with numerous studies demonstrating the *in vitro* efficacy of plant extracts against MRSA (Hammer et al., 1999; Meroni et al., 2020). Among plant-derived substances, hemp and its extracts have attracted significant attention(Nissen et al., 2010a, 2010b). Of the various hemp varieties, *Cannabis sativa* L. is the most extensively studied in medicine due to its adaptability and ease of cultivation in different climates (Montserrat-De La Paz et al., 2014; Mikulcová et al., 2017). *Cannabis sativa* L. has demonstrated multiple pharmacological properties, including antibacterial activity against MRSA and MRSP, which is attributed to the presence of cannabinoids (Chiong et al., 2024).

Hemp extracts contain a broad range of bioactive compounds that act synergistically, making it more difficult for bacteria to develop resistance (Martinenghi et al., 2020; Luz-Veiga et al., 2023).

The antimicrobial effects of hemp extracts are often attributed to the presence of cannabinoids like THC and CBD (Appendino et al., 2008). Although the exact mechanism behind the antimicrobial action of hemp extracts remains unclear, a study suggests that cannabidiol may be associated with the rapid disruption of bacterial cytoplasmic membranes, though it is uncertain whether this effect involves a specific molecular target (Blaskovich et al., 2021). Unlike hemp extracts such as essential oils, hemp seed oil, obtained by cold pressing hemp seeds, is free of cannabinoids, including THC and CBD. Any detectable traces of these compounds in products like the oil are probably due to accidental contamination from the plant's floral part (Montserrat-De La Paz et al., 2014; Mikulcová et al., 2017). It is interesting to investigate whether the hemp seed oil could still exert an antimicrobial effect, as it might reveal other potential bactericidal molecules.

Topical therapy with cannabidiol is a promising option in dermatology due to its effectiveness against biofilms, low propensity to induce resistance, and demonstrated *in vivo* efficacy (Blaskovich et al., 2021). Additionally, topical cannabinoids have shown beneficial effects on the skin, including anti-inflammatory, anti-itching, analgesics, wound healing and anti-proliferative properties (Filipiuc et al., 2023). In this study, the potential of hemp extract seed oil in veterinary dermatology was investigated by assessing its antibacterial activity against MDR strains of bacterial pathogens associated with skin infections in dogs. The synergistic effect of hemp seed oil when combined with gentamicin and enrofloxacin, two antimicrobial agents used in veterinary practice for topical treatment of otitis externa, were explored.

2. Materials and methods

2.1. Bacterial strains

A total of 198 bacterial strains, including 124 clinical *S. pseudintermedius* isolates (70 MRSP and 54 MSSP), 48 clinical *S. aureus* isolates (19 MRSA and 29 MSSA) and 26 *P. aeruginosa* isolates, collected from dogs between 2008 and 2023 as part of routine diagnostics at the Department of Veterinary Medicine at the University of Copenhagen (Denmark) and at the Department of Veterinary Medicine at the University of Perugia (Italy) were included in this study (Table S1, see Supplementary Materials).

2.2. Hemp extract seed oil

The hemp extract seed oil was provided by BioAgrigea Company (Padova, Italy) and was obtained by the cold extraction method from the hemp variety FUTURA 75. Cannabinoid titration was performed by ultra-high performance liquid chromatography-MS/MS (UHPLC-MS/MS) with an analyte limit of detection of 0.01 mg/ml. Prior to testing, the oil was diluted in DMSO to a maximum concentration of 6.4 % v/v.

2.3. MIC testing

Hemp seed oil minimal inhibitory concentration (MIC) was determined in the range 0.4–0.007 % v/v via the broth microdilution method according to CLSI guidelines (Clinical and Laboratory Standards Institute CLSI, 2018). *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853 were used as quality control strains. The effect of high concentration of DMSO on bacterial growth was assessed by growth kinetics on representative strains. Statistical analysis was performed to assess the significance of differences between groups. Results were interpreted using the Student's T test to compare means between methicillin-resistant and susceptible strains groups. A p value of ≤ 0.05 was considered statistically significant. All statistical analyses were conducted using GraphPad Prism version 8 (GraphPad Software, San Diego, USA).

2.4. Checkerboard assay

After testing the gentamicin and enrofloxacin MICs by broth microdilution (range 128–0.25 µg/ml), checkerboard assays were performed to understand interactions of these antimicrobials with hemp extract seed oil as previously described (Sopirala et al., 2010) with some modifications. Briefly, antimicrobials were serially diluted 2-fold in the rows in a 96-well microtiter plate, while the hemp seed oil was serially diluted 2-fold in the columns to create a matrix in which each well contained a combination of both agents at different concentrations. Fifty microliters of the bacterial suspension were inoculated into each well at a final concentration of 5×10^5 CFU/ml. The plates were incubated aerobically at 37 °C for 20 h. After reading the optical turbidity of the wells, the Fractional Inhibitory Concentration Index (FICI) was calculated according to the following formula: $FICI = [MIC_{A(A+B)}/MIC_A + MIC_{B(A+B)}/MIC_B]$, where $MIC_{A(A+B)}$ and $MICB_{(A+B)}$ represent the concentrations of compounds A and B, respectively, in the combination, while MIC_A and MIC_B represent the MIC of each compound individually. The interaction of the two compounds was interpreted as synergy, antagonism or indifference for FICI values of ≤ 0.5, > 4.0 and > 0.5–4.0, respectively.

2.5. Cell cytotoxicity assay

MTT test was performed on L929 cell line exposed to different concentrations of hemp extract seed oil (2 %, 1 %, 0.5 %, 0.25 %, 0.125 % v/v) or DMSO, which was tested as the same concentration as vehicle control. The L929 cell line was cultivated in low glucose DMEM supplemented with 10 % FBS, penicillin (100 IU/ml) and streptomycin (100 µg/ml) at 37°C and 5 % CO_2. L929 were seeded in 96-well plates at 10^4 cells/cm^2 and incubated for 24 h. The medium was then replaced with 100 µl of complete cell medium supplemented hemp seed oil or DMSO. After 24 h, 10 µl of MTT 5 mg/ml were added to each well and incubated for 4 h. At the end of the incubation, the cell medium was removed, and the formazan precipitates was solubilized with 100 µl DMSO and the absorbance was measured at 570 and 630 nm. The relative cell viability was calculated by comparing the absorbance value of the treated cells with that of the untreated cells. The analysis was performed in three independent experiments and each dilution was performed in triplicate, and results were compared using one-way analysis of variance (ANOVA) with post-hoc Tukey's HSD and Bonferroni correction in GraphPad Prism version 8 (GraphPad Software, San Diego, USA). P values ≤ 0.05 were considered significant.

3. Results

3.1. Hemp seed oil cannabinoid concentrations

Prior to testing, the cannabinoid concentration of the hemp extract seed oil was determined by UHPLC-MS/MS. Cannabidiolic acid showed the highest concentration (1.3 mg/ml), followed by CBD (0.29 mg/ml) and delta-9-tetrahydrocannabinolic acid (0.04 mg/ml). Cannabinol and Δ^9-THC concentrations were under the limit of detection (< 0.01 mg/ml).

3.2. Antimicrobial activity of hemp seed oil

Growth kinetics analysis of representative bacteria strains in the presence of DMSO showed that concentrations below 1.6 % had no effect on the growth dynamics of the bacteria compared to the untreated control (Fig. S1, see Supplementary Materials). This concentration was selected as the highest concentration tested in MIC assay. MIC values of hemp seed oil on 124 clinical *S. pseudintermedius* strains ranged between 0.025 to > 0.4 % (v/v) (Fig. 1A). MRSP strains generally displayed higher MIC values (above 0.1 %) compared to susceptible strains (Fig. 1A), with a significant difference observed between MRSP and MSSP (Fig. 1C; $p < 0.001$). In contrast, no significant difference was noted between methicillin-resistant and susceptible *S. aureus* strains (Fig. 1D). All *P. aeruginosa* strains displayed MIC values greater than 0.4 % (v/v).

3.3. Synergistic activity of hemp seed oil in combination with gentamicin

Thirteen gentamicin-resistant MRSP strains were selected for checkerboard assay. These exhibited a gentamicin MIC of 64–128 µg/ml (Table 1). The hemp seed oil showed strong synergistic interactions (FICI < 0.5) with gentamicin for all isolates tested with at least 4-fold decrease of gentamicin MIC (Table 1).

The hemp seed oil did not show any synergistic interactions (0, 5 > FICI > 4.0) with enrofloxacin for all MRSP strains tested (n = 13) and with both gentamicin and enrofloxacin for the *P. aeruginosa* strains tested (n = 7). Hemp sensitized gentamicin-resistant MRSP strains (R≥16 µg/ml) to MIC below the CLSI susceptibility breakpoint (S≤4 µg/ml).

3.4. Effect of hemp seed oil on L929 cell line

The cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

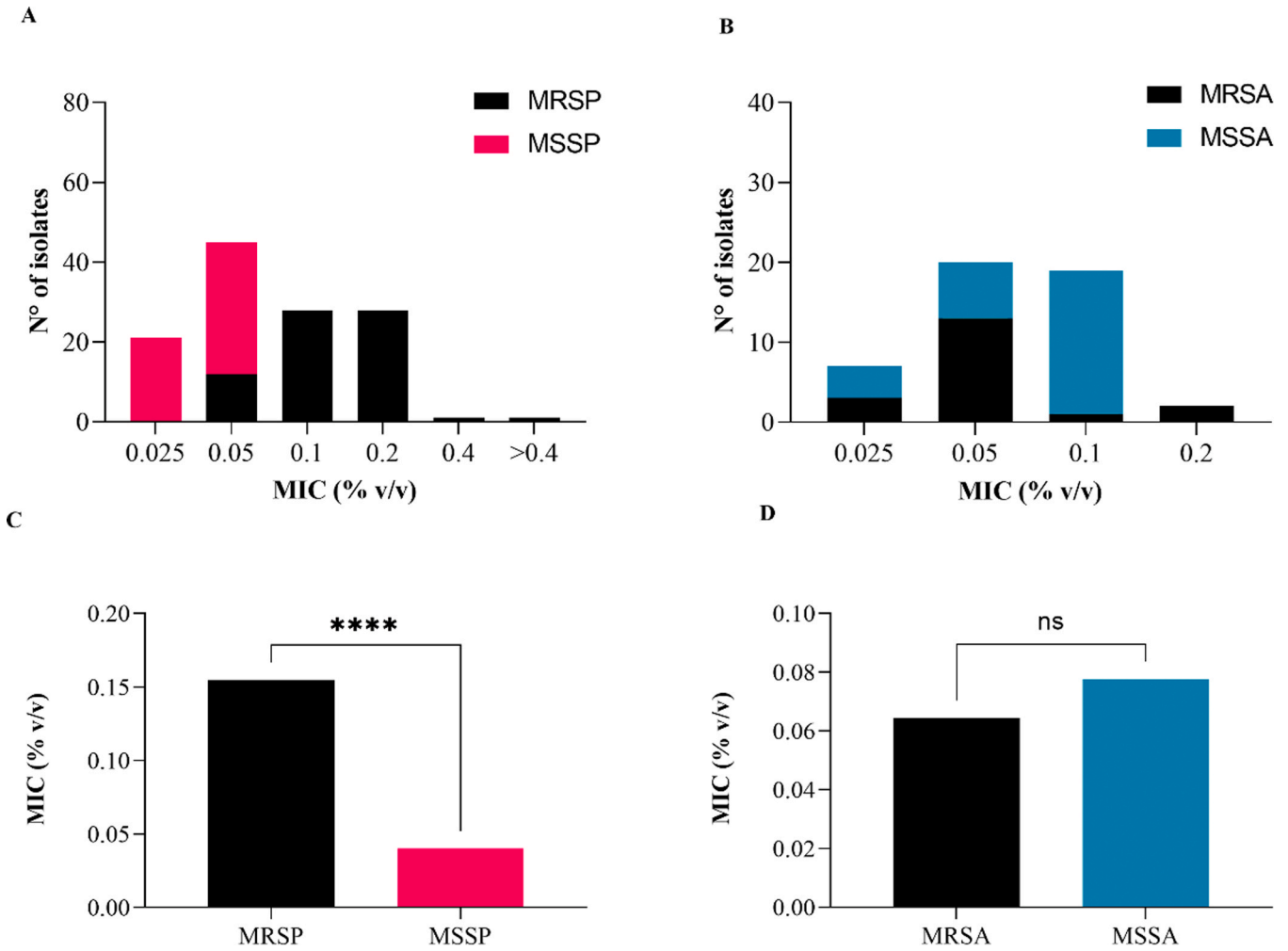

Fig. 1. Distribution of minimum inhibitory concentration (MIC) values (% v/v) of hemp seed oil for *Staphylococcus* isolates. (A) Mic distribution for methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) and methicillin-susceptible *Staphylococcus pseudintermedius* (MSSP), showing higher susceptibility of MSSP isolates at lower MIC values. (B) Mic distribution for methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA), with MSSA isolates demonstrating greater sensitivity to lower concentrations of hemp oil. (C) Results show a statistically significant difference ($p < 0.001$) in MIC values between MRSP and MSSP isolates, indicating that MSSP strains are more susceptible to hemp seed oil. (D) No statistically significant difference in MIC values is observed between MRSA and MSSA isolates, suggesting similar levels of susceptibility to hemp seed oil between these two groups.

Table 1
Checkerboard results for gentamicin and hemp seed oil combination in 13 clinical MRSP strains.

Isolate ID	MIC_A [a]	$MIC_{A(A+B)}$ [b]	MIC_B [c]	$MIC_{B(A+B)}$ [d]	FICI [e]
37711–3	128	1	0.1	0.05	0.25
45250–1	64	2	0.1	0.05	0.25
45250–2	64	4	0.05	0.025	0.375
46507	64	4	0.05	0.025	0.375
41484	128	2	0.2	0.1	0.187
42654	128	2	0.1	0.05	0.188
27150	64	4	0.1	0.05	0.137
32908	64	2	0.1	0.05	0.137
31369	64	2	0.1	0.05	0.137
30655	64	1	0.1	0.05	0.075
30538	64	1	0.2	0.05	0.131
30511	64	1	0.2	0.05	0.131
28522	64	2	0.2	0.025	0.069

[a] MIC_A, MIC (μg/ml) of gentamicin alone.
[b] $MIC_{A(A+B)}$, MIC of gentamicin in combination with Hemp seed oil.
[c] MIC_B, MIC (v/v) of Hemp seed oil alone.
[d] $MIC_{B(A+B)}$, MIC of Hemp seed oil in combination with gentamicin.
[e] FICI is given for the combination with the highest degree of synergy.

bromide (MTT) reduction assay MTT assay was performed on mouse fibroblast L929 cells to evaluate the cytotoxicity effect of the hemp extract seed oil. Incubation of cells with hemp seed oil concentrations of 1 % v/v or below did not lead to changes in cell viability (Fig. 2). However, at 2 % v/v of hemp seed oil a marked decrease in cell viability was observed (relative viability 4 %). DMSO did not show any reduction in cell viability at any of the tested concentrations (Fig. 2).

4. Discussion

The antimicrobial activity of hemp extract seed oil against pathogenic bacteria in small animal veterinary dermatology has been investigated. To date, the focus has primarily been on the essential oil of hemp or on specific cannabinoids such as CBD and THC (Nissen et al., 2010a, 2010b; Puškárová et al., 2017; Iseppi et al., 2019; Nocera et al., 2020). To the best of our knowledge, this is the first study to evaluate the antimicrobial activity of hemp extract seed oil against MDR pathogens isolated from dogs using a quantitative approach. In all previous studies on hemp seed oil, the antimicrobial effect was assessed using semi-quantitative methods, such as disk diffusion, targeting the bacteria primarily responsible for spoilage, including gram-positive *Bacillus subtilis*, *Bacillus cereus*, *Enterococcus faecalis* and *Staphylococcus aureus* and the Gram-negative *Citrobacter freundii*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Serratia marcescens* (Nissen et al., 2010a, 2010b; Mikulcová et al., 2017;). To allow a quantitative approach, scalar dilutions of the seed oil were prepared using diluted

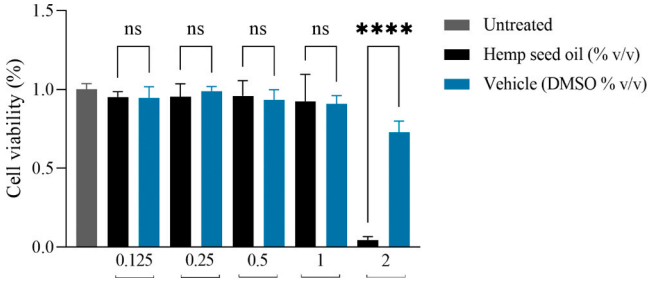

Fig. 2. Relative viability of L929 cell line treated with increasing concentrations of hemp seed oil, as compared to the vehicle control (DMSO), after 24 h of exposure, measured by MTT assay. No statistically significant impact on cell viability was observed for both hemp seed oil and DMSO at concentrations ≤ 1 %. A significant decrease in cell viability ($p < 0.001$) was detected at 2 % vol/vol between hemp seed oil and vehicle control. Data are presented as mean ± SD of three independent experiments, each comprising three replicates.

DMSO which is widely used to solubilize oils without interfering with their biological effects when used at subinhibitory concentrations (Modrzyński et al., 2019; Tunçer & Gurbanov, 2023). The highest seed oil concentration that could be solubilized was 0.4 % with 1.6 % DMSO, and it was demonstrated that this concentration does not interfere with bacterial growth.

Results indicated that hemp seed oil can exert strong antimicrobial activity against staphylococci, including MRSP and MRSA (Fig. 1). Direct comparison with existing literature is not feasible, as no similar studies have been reported. Additionally, comparing the antimicrobial activity of hemp extracts from different studies can be problematic and misleading due to variations in evaluation methods and various factors that influence the chemical composition of these extracts, such as the plant organ used for extraction (e.g. leaves, flowers or seeds), as well as plant's maturity and harvesting stage (Ostapczuk et al., 2021). Interestingly, MRSP strains displayed higher MICs compared to susceptible strains, a trend not observed for MRSA (Fig. 1). This suggests that the structural or functional changes in the cell wall associated with methicillin resistance in *S. pseudintermedius*, but not in *S. aureus*, may interfere with penetration and/or activity of hemp seed oil.

While investigating the reason behind this result goes beyond the scope of this study, further research is warranted to elucidate the potential relationships between methicillin resistance and susceptibility to hemp seed oil.

It was not surprising that hemp seed oil showed lower antimicrobial activity against *P. aeruginosa* since no studies on seed oil or essential oils, including those from hemp, have demonstrated a good efficacy against this Gram-negative species. According to de Sousa et al. (2023), this could be due to the presence of liposaccharides in Gram-negative bacteria, which prevent the attachment of seed oil to the cell membrane.

As widely reported in the literature, the antimicrobial properties of hemp extracts are related to the presence of cannabinoids such as THC and CBD (Baswan et al., 2020; Martinenghi et al., 2020; Blaskovich et al., 2021; Robaina Cabrera et al., 2021; Luz-Veiga et al., 2023). However, some studies suggest that minor constituents of hemp extract, particularly terpenes like β-caryophyllene and limonene, may also play a role in the overall antimicrobial activity (Ostapczuk et al., 2021; Schofs et al., 2021). It should be noted that the hemp seed oil used in this study was THC-free and had a very low CBD content (0.285 mg/ml), detectable only as an impurity during the extraction processes. These levels are too low to exhibit antibacterial activity. Hemp seed oil has a relatively complex macro composition, and therefore, numerous compounds beyond cannabinoids, whether alone or in combination, have the potential to exert antimicrobial activity (Leizer et al., 2000).

In current veterinary dermatology, topical treatment is recommended over systemic treatment for managing otitis externa and localized skin infections because it reduces the risk of side effects and lowers the likelihood of selecting resistant strains at other body sites, such as the intestinal tract, where most bacteria reside(Bajwa, 2019). Here, it has been demonstrated a synergistic effect of hemp seed oil with gentamicin, resulting in a significant reduction in the MIC below the CLSI clinical susceptibility breakpoint (S ≤4 μg/ml) in *S. pseudintermedius*. Studies on various plant species have demonstrated that natural phytochemicals, including flavonoids and phenolic acids such as protocatechuic acid and chlorogenic acid (Chai et al., 2019), as well as punicalagin (Kiran et al., 2024), can exert synergistic effects with conventional antibiotics, particularly against Gram-positive pathogens. Although the specific mechanisms underlying this synergy were not directly investigated, potential mechanisms may involve disruption of bacterial membrane integrity, inhibition of efflux pumps, and interference with quorum sensing pathways (Frassinetti et al., 2020). Our findings suggest that hemp seed oil could be a promising option for managing localized skin and ear infections caused by Gram-positive strains. In addition, it may enhance the efficacy of gentamicin in treating otitis externa, particularly in cases where high levels of exudate in the ear canal can compromise gentamicin stability and effectiveness

(Bajwa, 2019; Carlotti, 1991).

To support the future application of hemp seed oil as a topical treatment, the cytotoxic effect was evaluated on the fibroblast cell line L929, which was chosen because it is widely used for testing the cytotoxicity of natural products. As demonstrated by the MTT assay results, hemp extract seed oil at concentrations corresponding to the MIC values recorded for the bacterial strains included in the study does not produce any toxic effects on the cellular growth, indicating that it is safe for use in topical applications.

Despite its merits, the present study has its limitations. First, this study focused on the analysis of a single hemp extract, specifically seed oil, which composition is influenced to external factor, such as climatic conditions. Consequently, compositional variations across different production years may impact its antimicrobial effectiveness. Secondly, a detailed characterization of the hemp seed oil was not performed, and the mechanism behind its antimicrobial properties needs further investigations. Finally, aadditionally in-vivo experiments are needed to confirm the safety of the oil for animal treatment.

5. Conclusions

This work demonstrates the antibacterial activity of hemp seed oil against staphylococci associated with skin infections in dogs at concentrations that are not cytotoxic. The antimicrobial activity against MRSP and MRSA, along with the synergistic effect observed when combined with gentamicin, underscores the potential of hemp seed oil as an alternative or adjunctive topical antimicrobial therapy for otitis externa and other focal infections in small animal veterinary dermatology. Future studies are warranted to investigate the safety of the oil under in-vivo conditions and the mechanisms behind the decreased susceptibility in MRSP but not MRSA strains.

CRediT authorship contribution statement

Luca Guardabassi: Writing – review & editing, Writing – original draft, Methodology. **Marina De Benedetti:** Resources. **Alessandra Di Salvo:** Writing – review & editing. **Giorgia della Rocca:** Writing – review & editing. **Patrizia Casagrande Proietti:** Writing – review & editing, Writing – original draft, Conceptualization. **Valeria Toppi:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Laura Musa:** Investigation. **Gabriele Scattini:** Methodology, Conceptualization. **Elisa Rampacci:** Data curation, Conceptualization. **Mattia Pirolo:** Writing – review & editing, Methodology, Investigation, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2025.106392.

References

Appendino, G., Gibbons, S., Giana, A., Pagani, A., Grassi, G., Stavri, M., Smith, E., Rahman, M.M., 2008. Antibacterial cannabinoids from cannabis sativa: a structure-activity study. Journal of Natural Products 71 (8), 1427–1430. https://doi.org/10.1021/np8002673.

Bajwa, J., 2019. Canine otitis externa - treatment and complications. *canadian.* Veterinary Journal 60 (1), 97–99.

Bannoehr, J., Guardabassi, L., 2012. Staphylococcus pseudintermedius in the dog: taxonomy, diagnostics, ecology, epidemiology and pathogenicity. Veterinary Dermatology 23 (4). https://doi.org/10.1111/j.1365-3164.2012.01046.x.

Baswan, S.M., Klosner, A.E., Glynn, K., Rajgopal, A., Malik, K., Yim, S., Stern, N., 2020. Therapeutic potential of cannabidiol (CBD) for skin health and disorders. Clinical Cosmetic and Investigational Dermatology 13, 927–942. https://doi.org/10.2147/CCID.S286411.

Blaskovich, M.A.T., Kavanagh, A.M., Elliott, A.G., Zhang, B., Ramu, S., Amado, M., Lowe, G.J., Hinton, A.O., Pham, D.M.T., Zuegg, J., Beare, N., Quach, D., Sharp, M.D., Pogliano, J., Rogers, A.P., Lyras, D., Tan, L., West, N.P., Crawford, D.W., Thurn, M., 2021. The antimicrobial potential of cannabidiol. Communications Biology 4 (1). https://doi.org/10.1038/s42003-020-01530-y.

Carlotti, D.N., 1991. Diagnosis and medical treatment of otitis externa in dogs and cats. Journal of Small Animal Practice 32 (8), 394–400. https://doi.org/10.1111/j.1748-5827.1991.tb00963.x.

Chai, B., Jiang, W.J., Hu, M., Wu, Y., Si, H., 2019. In vitro synergistic interactions of protocatechuic acid and chlorogenic acid in combination with antibiotics against animal pathogens. Synergy 9, 100055. https://doi.org/10.1016/j.synres.2019.100055.

Chiong, F., Tsai, D., Ahmad, D., Samarawickrema, I., & Sinnollareddy, M. (2024). *Cannabinoids as Antibacterial Agents: A Systematic and Critical Review of In Vitro Efficacy Against Streptococcus and Staphylococcus*.

Clinical and Laboratory Standards Institute (CLSI), 2018. M07: clinical and laboratory standards institute methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Clinical and Laboratory Standards Institute (CLSI) 91.

De Martino, L., Nocera, F.P., Mallardo, K., Nizza, S., Masturzo, E., Fiorito, F., Iovane, G., Catalanotti, P., 2016. An update on microbiological causes of canine otitis externa in campania region, Italy. Asian Pacific Journal of Tropical Biomedicine 6 (5), 384–389. https://doi.org/10.1016/j.apjtb.2015.11.012.

de Sousa, T., Garcês, A., Silva, A., Lopes, R., Alegria, N., Hébraud, M., Igrejas, G., Poeta, P., 2023. The impact of the virulence of pseudomonas aeruginosa isolated from dogs. Veterinary Sciences 10 (5). https://doi.org/10.3390/vetsci10050343.

Dos Santos, T.P., Damborg, P., Moodley, A., Guardabassi, L., 2016. Systematic review on global epidemiology of methicillin-resistant staphylococcus pseudintermedius: inference of population structure from multilocus sequence typing data. Frontiers in Microbiology 7 (OCT), 1–12. https://doi.org/10.3389/fmicb.2016.01599.

Filipiuc, S.I., Neagu, A.N., Uritu, C.M., Tamba, B.I., Filipiuc, L.E., Tudorancea, I.M., Boca, A.N., Hâncu, M.F., Porumb, V., Bild, W., 2023. The skin and natural Cannabinoids–Topical and transdermal applications. Pharmaceuticals 16 (7), 1–35. https://doi.org/10.3390/ph16071049.

Frassinetti, S., Gabriele, M., Moccia, E., Longo, V., Di Gioia, D., 2020. Antimicrobial and antibiofilm activity of cannabis sativa L. Seeds extract against staphylococcus aureus and growth effects on probiotic lactobacillus spp. Lwt 124 (February), 109149. https://doi.org/10.1016/j.lwt.2020.109149.

Haag, A.F., Ross Fitzgerald, J., Penadés, J.R., 2019. Staphylococcus aureus in animals. GramPositive Pathogens 1, 731–746. https://doi.org/10.1128/9781683670131.ch46.

Hammer, K.A., Carson, C.F., Riley, T.V., 1999. Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology 86 (6), 985–990. https://doi.org/10.1046/j.1365-2672.1999.00780.x.

Helmy, Y.A., Taha-Abdelaziz, K., Hawwas, H.A.E.H., Ghosh, S., AlKafaas, S.S., Moawad, M.M.M., Saied, E.M., Kassem, I.I., Mawad, A.M.M., 2023. Antimicrobial resistance and recent alternatives to antibiotics for the control of bacterial pathogens with an emphasis on foodborne pathogens. In: Antibiotics, 12. MDPI. https://doi.org/10.3390/antibiotics12020274.

Iseppi, R., Brighenti, V., Licata, M., Lambertini, A., Sabia, C., Messi, P., Pellati, F., Benvenuti, S., 2019. Chemical characterization and evaluation of the antibacterial activity of essential oils from fibre-type cannabis sativa L. (Hemp). Molecules 24 (12). https://doi.org/10.3390/molecules24122302.

Kiran, S., Tariq, A., Iqbal, S., Naseem, Z., Siddique, W., Jabeen, S., Bashir, R., Hussain, A., Rahman, M., Habib, F.E., Rauf, W., Ali, A., Sarwar, Y., Jander, G., Iqbal, M., 2024. Punicalagin, a pomegranate polyphenol sensitizes the activity of antibiotics against three MDR pathogens of the enterobacteriaceae. BMC Complementary Medicine and Therapies 24 (1), 1–22. https://doi.org/10.1186/s12906-024-04376-7.

Leizer, C., Ribnicky, D.M., Poulev, A., Dushenkov, D., Raskin, I., 2000. The composition of hemp seed oil and its potential as an important source of nutrition. Journal of Nutraceuticals Functional and Medical Foods 2 (4), 35–53. https://doi.org/10.1300/J133v02n04_04.

Luz-Veiga, M., Amorim, M., Pinto-Ribeiro, I., Oliveira, A.L.S., Silva, S., Pimentel, L.L., Rodríguez-Alcalá, L.M., Madureira, R., Pintado, M., Azevedo-Silva, J., Fernandes, J., 2023. Cannabidiol and cannabigerol exert antimicrobial activity without compromising skin microbiota. International Journal of Molecular Sciences 24 (3). https://doi.org/10.3390/ijms24032389.

Martinenghi, L.D., Jønsson, R., Lund, T., Jenssen, H., 2020. Isolation, purification, and antimicrobial characterization of cannabidiolic acid and cannabidiol from cannabis sativa l. Biomolecules 10 (6), 1–16. https://doi.org/10.3390/biom10060900.

Martins, E., Maboni, G., Battisti, R., da Costa, L., Selva, H.L., Levitzki, E.D., Gressler, L.T., 2022. High rates of multidrug resistance in bacteria associated with small animal otitis: a study of cumulative microbiological culture and antimicrobial susceptibility. Microbial Pathogenesis 165 (October 2021). https://doi.org/10.1016/j.micpath.2022.105399.

Meroni, G., Cardin, E., Rendina, C., Millar, V.R.H., Filipe, J.F.S., Martino, P.A., 2020. In vitro efficacy of essential oils from melaleuca alternifolia and rosmarinus officinalis, manuka honey-based gel, and propolis as antibacterial agents against canine staphylococcus pseudintermedius strains. Antibiotics 9 (6), 1–14. https://doi.org/10.3390/antibiotics9060344.

Mikulcová, V., Kašpárková, V., Humpolíček, P., Buňková, L., 2017. Formulation, characterization and properties of hemp seed oil and its emulsions. Molecules 22 (5). https://doi.org/10.3390/molecules22050700.

Modrzyński, J.J., Christensen, J.H., Brandt, K.K., 2019. Evaluation of dimethyl sulfoxide (DMSO) as a co-solvent for toxicity testing of hydrophobic organic compounds. Ecotoxicology 28 (9), 1136–1141. https://doi.org/10.1007/s10646-019-02107-0.

Montserrat-De La Paz, S., Marín-Aguilar, F., García-Giménez, M.D., Fernández-Arche, M. A., 2014. Hemp (Cannabis sativa L.) seed oil: analytical and phytochemical characterization of the unsaponifiable fraction. Journal of Agricultural and Food Chemistry 62 (5), 1105–1110. https://doi.org/10.1021/jf404278q.

Nielsen, S.S., Bicout, D.J., Calistri, P., Canali, E., Drewe, J.A., Garin-Bastuji, B., Gonzales Rojas, J.L., Gortazar Schmidt, C., Herskin, M., Michel, V., Miranda Chueca, M.A., Padalino, B., Pasquali, P., Roberts, H.C., Sihvonen, L.H., Spoolder, H., Stahl, K., Velarde, A., Viltrop, A., Alvarez, J., 2021. Assessment of animal diseases caused by bacteria resistant to antimicrobials: dogs and cats. EFSA Journal 19 (6). https://doi.org/10.2903/j.efsa.2021.6680.

Nissen, L., Zatta, A., Stefanini, I., Grandi, S., Sgorbati, B., Biavati, B., Monti, A., 2010a. Characterization and antimicrobial activity of essential oils of industrial hemp varieties (Cannabis sativa L.). Fitoterapia 81 (5), 413–419. https://doi.org/10.1016/j.fitote.2009.11.010.

Nissen, L., Zatta, A., Stefanini, I., Grandi, S., Sgorbati, B., Biavati, B., Monti, A., 2010b. Characterization and antimicrobial activity of essential oils of industrial hemp varieties (Cannabis sativa L.). Fitoterapia 81 (5), 413–419. https://doi.org/10.1016/j.fitote.2009.11.010.

Nocera, F.P., Mancini, S., Najar, B., Bertelloni, F., Pistelli, L., Filippis, A.De, Fiorito, F., De Martino, L., Fratini, F., 2020. Antimicrobial activity of some essential oils against methicillin-susceptible and methicillin-resistant staphylococcus pseudintermedius-associated pyoderma in dogs. Animals 10 (10), 1–12. https://doi.org/10.3390/ani10101782.

Ostapczuk, K., Apori, S.O., Estrada, G., Tian, F., 2021. Hemp growth factors and extraction methods effect on antimicrobial activity of hemp seed oil: a systematic review. Separations 8 (10). https://doi.org/10.3390/separations8100183.

Pantosti, A., 2012. Methicillin-resistant staphylococcus aureus associated with animals and its relevance to human health. Frontiers in Microbiology 3 (APR), 1–12. https://doi.org/10.3389/fmicb.2012.00127.

Pušká rová, A., Bučková, M., Kraková, L., Pangallo, D., Kozics, K., 2017. The antibacterial and antifungal activity of six essential oils and their cyto/genotoxicity to human HEL 12469 cells. Scientific Reports 7 (1). https://doi.org/10.1038/s41598-017-08673-9.

Robaina Cabrera, C.L., Keir-Rudman, S., Horniman, N., Clarkson, N., Page, C., 2021. The anti-inflammatory effects of cannabidiol and cannabigerol alone, and in combination. Pulmonary Pharmacology and Therapeutics 69 (January), 102047. https://doi.org/10.1016/j.pupt.2021.102047.

Schmerold, I., van Geijlswijk, I., Gehring, R., 2023. European regulations on the use of antibiotics in veterinary Medicine. European Journal of Pharmaceutical Sciences 189 (April), 106473. https://doi.org/10.1016/j.ejps.2023.106473.

Schofs, L., Sparo, M.D., Sánchez Bruni, S.F., 2021. The antimicrobial effect behind cannabis sativa. Pharmacology Research and Perspectives 9 (2), 1–17. https://doi.org/10.1002/prp2.761.

Sopirala, M.M., Mangino, J.E., Gebreyes, W.A., Biller, B., Bannerman, T., Balada-Llasat, J.M., Pancholi, P., 2010. Synergy testing by etest, microdilution checkerboard, and time-kill methods for pan-drug-resistant acinetobacter baumannii. Antimicrobial Agents and Chemotherapy 54 (11), 4678–4683. https://doi.org/10.1128/AAC.00497-10.

Tunçer, S., Gurbanov, R., 2023. Non-growth inhibitory doses of dimethyl sulfoxide alter gene expression and epigenetic pattern of bacteria. Applied Microbiology and Biotechnology 107 (1), 299–312. https://doi.org/10.1007/s00253-022-12296-0.