

THE POTENTIAL OF HEMP BIOMASS AS A SOURCE OF ANTIMICROBIAL AGENTS AGAINST STAPHYLOCOCCAL AND STREPTOCOCCAL INFECTIONS IN ANIMALS

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ABSTRACT

Bacterial infections caused by staphylococci and streptococci pose a serious burden on animal production. The growing antimicrobial resistance of these pathogens significantly limits treatment options and underscores the need to search for alternative antimicrobial agents. In this context, *Cannabis sativa* L. is one of the most intensively studied plants due to its high content of secondary metabolites, particularly cannabinoids and terpenoids.

The aim of this study was to compare the *in vitro* antimicrobial activity of ethanol extracts from five industrial hemp cultivars (Bialobrzeskie, Felina 32, Futura 75, mixed, and Santhica 27) against strains of *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus dysgalactiae*, including both antibiotic-resistant and susceptible forms. Antibacterial activity was determined using the broth microdilution method and expressed as the minimum inhibitory concentration (MIC).

All five tested extracts exhibited inhibitory activity against all tested strains, with MIC values ranging from 128 to 2048 µg/ml. The most effective was the extract from the Santhica 27 variety (MIC 128 µg/ml against *Str. agalactiae* and clinical isolates of *S. aureus*), followed by the Bialobrzieskie and mixed varieties. Extracts from the Felina 32 and Futura 75 varieties exhibited only weak activity. The highest total cannabinoid content was determined in the extract from the Bialobrzieskie variety (21,740.94 µg/g), and the lowest in the Futura 75 extract (4,713.11 µg/g). Higher antibacterial efficacy correlated with higher cannabinoid content, and the observed effect likely results from the synergistic action of the entire complex of active compounds.

The Santhica 27, Bialobrzieskie, and mixed varieties appear to be the most promising for further research. Follow-up *in vivo* experiments are necessary to verify their therapeutic potential.

Keywords: antibacterial; bacteria; cannabinoids; cannabis; pathogen;

INTRODUCTION

Bacterial infections pose a serious burden on livestock production. According to estimates by the World Organisation for Animal Health, approximately 20% of livestock production is lost annually due to disease, resulting in financial losses estimated at \$358.4 billion per year (Health for Animals 2023). Among the most significant causative agents of bacterial diseases in livestock are *Staphylococcus aureus* and streptococcal species, particularly *Streptococcus agalactiae* and *Streptococcus dysgalactiae*, which are the main causes of mastitis, the most common and economically significant disease of the mammary gland in dairy cows, with estimated costs of approximately \$ 147 per cow per year (Mesquita et al. 2019; Cheng & Han 2020).

The situation is further complicated by the growing antimicrobial resistance (AMR) of these pathogens. In *S. aureus* isolated from pigs, 100 % resistance to tetracycline, 88 % to penicillin, and 64% to clindamycin were reported, with methicillin resistance (MRSA) posing a serious problem in both veterinary and human medicine (Rao et al. 2022). For streptococcal species, increasing resistance to clindamycin, erythromycin, and tetracycline has been documented (Guy et al. 2023). The excessive use of antibiotics in animal production creates reservoirs of resistance and contributes to the spread of AMR encoding genes into the environment, which has global implications for public health (Kirchhelle, 2018; Ahmed et al. 2024).

In response to this situation, interest in natural alternatives to traditional antibiotics is growing. *Cannabis sativa* L. is one of the most intensively studied plants in this context. Industrial hemp varieties produce a broad spectrum of biologically active secondary metabolites, including cannabinoids, terpenoids, flavonoids, and other compounds with proven antimicrobial activity (Paulová et al.

2025a; Sionov & Steinberg 2022). Cannabinoids exhibit activity primarily against Gram-positive bacteria, but also against their biofilms (Appendino et al. 2008; Blaskovich et al. 2021). Importantly, industrial varieties containing $\leq 0.2\%$ THC are permitted for use in animal feed within the EU (European Food Safety Authority (EFSA) 2011), which is a prerequisite for their use in subsequent *in vivo* studies.

However, research to date has focused primarily on the activity of isolated cannabinoids or extracts from medicinal cannabis strains with high cannabinoid content. To date, there has been a lack of comparative studies on the antibacterial potential of ethanol extracts from individual industrial hemp varieties against pathogens relevant to bovine mastitis. The aim of this study was therefore to compare the *in vitro* antibacterial activity of ethanol extracts from five industrial hemp varieties (Bialobrzeskie, Felina 32, Futura 75, mixed, and Santhica 27) against strains of *S. aureus*, *Str. agalactiae*, and *Str. dysgalactiae*, including antibiotic resistant and susceptible clinical and standard isolates.

MATERIAL AND METHODS

Plant materials

Four varieties of industrial hemp biomass (Bialobrzeskie, Felina 32, Futura 75, and Santhica 27) obtained from the Czech Hemp Institute (Chrašnice, CZ) were tested. The Bialobrzeskie, Santhica 27, and Felina 32 varieties were harvested in October 2021, while the Felina 32 variety was harvested again in September 2022. In addition, a mixed variant was tested, prepared by combining the Bialobrzeskie, Santhica 27, and Felina 32.

Preparation of hemp biomass extracts

The dried samples were homogenized and ground into a powder using a grinding bowl, and 0.3 g of each sample was added to 10 mL of 96% ethanol (Penta, Prague, CZ). The resulting mixture was vigorously shaken for 60 minutes on a shaker (IKA KS 130 Basic, Staufen, DE) (800 rpm). The mixture was then filtered under low pressure. The residue from the filter was transferred to a flask, and 10 mL of ethanol was added. The same extraction procedure was repeated twice. After three extractions, the total volume of 30 mL was collected in a single 50-mL plastic tube (VWR, Stříbrná Skalice, CZ).

Quantification of cannabinoid content in extracts

For analysis, 0.5 mL of the ethanol extract was filtered through a nylon filter (0.22 μm ; Labicom, Olomouc, CZ) and diluted 100-fold. After transferring the sample to an HPLC vial, 20 μL was injected into the chromatographic system. Chromatographic separation of 14 cannabinoids (CBDVA – cannabidivarinic acid, CBDV – cannabidivarin, CBDA – cannabidiolic acid, CBGA – cannabigerolic acid, CBG – cannabigerol, CBD – cannabidiol, THCV – tetrahydrocannabidivarin, THCVA – tetrahydrocannabidivarinic acid, CBN – cannabinol, Δ^9 -THC – Δ^9 -tetrahydrocannabinol,

Δ 8-THC – Δ 8-tetrahydrocannabinol, CBC – cannabichromene, THCA – tetrahydrocannabinolic acid, CBCA – cannabichromenic acid) was performed according to Czauderna et al. (2024) using a NexLeaf guard column (5 × 4.6 mm), a Shim-Pak column (Shimadzu, 2.2 μ m, 75 × 3 mm), and two Phenomenex C18 columns (Synergi 2.5 μ m, Hydro RP, 100 Å, 100 × 3 mm) on a high-performance liquid chromatography system (Shimadzu, Kyoto, JP) equipped with an SPD-M20A detector. The column temperature was maintained at 40 °C. All samples were analyzed using a linear gradient of 99.9% HPLC-grade acetonitrile (Chem-Lab NV, Zedelgem, BE) with 0.1% formic acid (Lachner, Neratovice, CZ) (v/v; solvent B) and 99.8% HPLC-grade purity methanol (Chem-Lab NV, Zedelgem, BE) (v/v; solvent C). The maximum system pressure was 24, MPA.

Bacterial strains and growth medium

Four strains of *S. aureus* and two strains of the genus *Streptococcus* were used in this study. The standard *S. aureus* strains ATCC 29213 and ATCC 43300 were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Clinical isolates SA1 and SA3 were provided by Motol University Hospital (Prague, CZ) and identified by MALDI-TOF-MS according to Rondevaldová et al. (2018). *Str. agalactiae* strain DSM 2134 and *Str. dysgalactiae* strain DSM 20662 were obtained from the German Resource Centre for Biological Material (DSM, Braunschweig, GE). Cation-modified Mueller-Hinton broth (Oxoid, Basingstoke, UK) was used for the cultivation of *S. aureus* strains; streptococcal strains were cultured in tryptone soy broth (Oxoid, Basingstoke, UK). All culture media were adjusted to pH 7.6 using Trizma base (Sigma-Aldrich, St. Louis, MO, USA). The tested strains included the resistant reference strain *S. aureus* ATCC 43300 (MRSA – methicillin-resistant *S. aureus*, resistant to methicillin and oxacillin) and the susceptible control strain *S. aureus* ATCC 29213 (MSSA – methicillin-susceptible *S. aureus*).

Broth microdilution method

The antibacterial potential of the extracts was determined using the broth microdilution method in 96-well microtiter plates according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2018), with minor modifications based on Cos et al. (2006) for a more effective evaluation of the antimicrobial potential of natural substances. Serial dilutions of industrial hemp extracts in the range of 16–2048 μ g/mL were prepared in the appropriate culture medium using a manual multichannel pipette. Plates were inoculated with a bacterial suspension at a final concentration of 5×10^5 CFU/mL, adjusted using a McFarland densitometer, and incubated at 37 °C for 24 h under aerobic conditions. The growth of *S. aureus* strains was assessed at 405 nm using an Infinite 200 PRO microplate reader (Tecan, Männedorf, CH). The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the extract that inhibited bacterial growth by $\geq 80\%$ compared to the control without the test substance. An uninoculated well with broth served as the sterile control, and an inoculated well without extract

served as the negative (growth) control. Ethanol used as a solvent showed no inhibitory effect against any of the tested strains. The susceptibility of *S. aureus*, *Str. agalactiae*, and *Str. dysgalactiae* strains to antibiotic controls (oxacillin, penicillin) was verified. All samples were tested in three independent experiments, each performed in triplicate, and the results are expressed as modal MIC values.

Data analysis

Microsoft Excel was used to analyse the data obtained from the spectrophotometer. The calculations were performed in Excel, and the resulting MIC values are presented as the median or mode.

RESULTS AND DISCUSSION

Results

Antimicrobial potential of hemp extract

The results of *in vitro* inhibitory effects of hemp extracts on the tested bacterial strains of *S. aureus*, *Str. agalactiae*, and *Str. dysgalactiae*, are summarized in Table 1.

The lowest MIC value (128 µg/mL) was recorded for the Santhica 27 cultivar against *Str. agalactiae* and both clinical isolates of *S. aureus* (SA1 and SA3). The same inhibitory activity against *Str. agalactiae* was also achieved by extracts of the Bialobrzeskie cultivar and the mixed cultivar. Against standard *S. aureus* strains (ATCC 29213 and ATCC 43300), the Santhica 27 cultivar exhibited a slightly higher MIC (256 µg/mL), followed by the Bialobrzeskie cultivars (256 and 512 µg/mL) and the mixed cultivar (512 µg/mL). An MIC value of 256 µg/mL was similarly observed for the Bialobrzeskie cultivar and the mixed extract against clinical isolates SA1 and SA3, as well as for the Felina 32 and Futura 75 cultivars (MIC 256 and 512 µg/mL) against *Str. agalactiae* and clinical isolates of *S. aureus*. The Felina 32

Tab. 1: Antimicrobial potential of hemp extracts on gram positive tested pathogens (Paulová et al. 2025b)

Bacterium	Strain	Hemp extracts/MIC (µg/ml)				
		Bialobrzesikie	Felina 32	Futura 75	Mixed	Santhica 27
S. aureus	ATCC 29213	512	1024	1024	512	256
	ATCC 43300	256	1024	1024	512	256
	SA1	256	512	512	256	128
	SA3	256	512	512	256	128
S. agalactiae	DSM 2134	128	256	256	128	128
S. dysgalactiae	DSM 20662	1024	2048	2048	1024	1024

Abbreviations: *S. aureus* – *Staphylococcus aureus*; ATCC – American Type Culture Collection; SA1, SA3 – clinical isolates *Staphylococcus aureus*; *S. agalactiae* – *Streptococcus agalactiae*; *S. dysgalactiae* – *Streptococcus dysgalactiae*; DSM – German Resource Centre for Biological Material; MIC – minimum inhibitory concentration (expressed as an average of three independent experiments performed in triplicate)

and Futura 75 cultivars exhibited only weak inhibitory activity against standard *S. aureus* strains (MIC 1024 µg/mL). The most resistant strain tested was *Str. dysgalactiae*, for which all five cultivars showed uniformly weak inhibitory activity with an MIC of 2048 µg/mL. Slight differences compared to previously published data can be attributed to different methodological approaches and differences in the bacterial strains used.

The content of monitored cannabinoids in hemp extracts

Differences in cannabinoid content were observed among the five extracts tested. The total content of the analyzed cannabinoids is shown in Figure 1, and the content of individual cannabinoids is listed in Table 2.

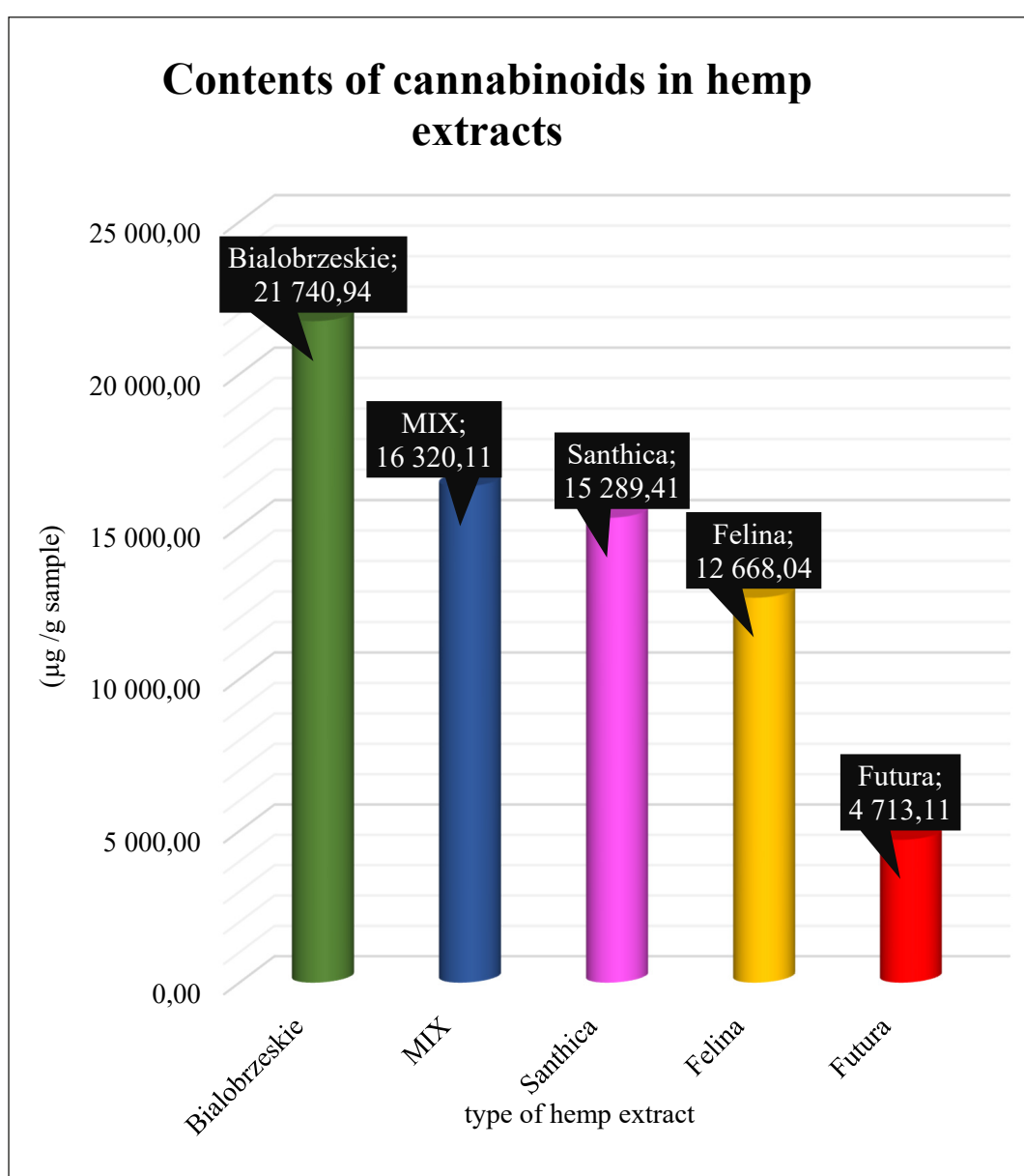


Fig.1: Contents of cannabinoids in hemp extracts (Paulová et al. 2025b)

Tab. 2: Contents of cannabinoids in hemp extracts (Paulová et al. 2025b)

Observed cannabinoids	Contents of cannabinoids in hemp extracts (µg/g vzorku)				
	Bialobrzeskie	Felina 32	Futura 75	Mixed	Santhica 27
CBDVA	360,58	144,94	63,82	258,58	ND
CBDV	ND	8,65	14,9	ND	ND
CBDA	15 208,79	7 404,60	2 776,69	10 206,44	2 650,84
CBGA	471,21	2 727,43	213,81	2 215,25	10 450,78
CBG	89,78	269,63	46 373	232,44	1163,19
CBD	2 839,14	521,72	1257,34	1590,11	370,69
THCV	ND	ND	ND	ND	ND
THCVA	ND	ND	ND	ND	ND
CBN	ND	ND	15,6	ND	ND
Δ9-THC	513,27	ND	40,61	261,37	59,36
Δ8-THC	ND	ND	ND	ND	ND
CBC	ND	301,71	105,28	ND	ND
THCA	1 350,45	314,58	53,32	746,26	194,74
CBCA	907,73	974,79	154,62	809,65	399,81
Total value	21 740,94	12 668,04	4713,11	16 320,11	15 289,41

Abbreviations: CBDVA – cannabidivarinic acid, CBDV – cannabidivarin, CBDA – cannabidiolic acid, CBGA – cannabigerolic acid, CBG – cannabigerol, CBD – cannabidiol, THCV – tetrahydrocannabidivarin, THCVA – tetrahydrocannabidivarinic acid, CBN – cannabinol, Δ9-THC – Δ9-tetrahydrocannabinol, Δ8-THC – Δ8-tetrahydrocannabinol, CBC – cannabichromene, THCA – tetrahydrocannabinolic, CBCA – cannabichromenic acid, ND – not detected.

The most abundant cannabinoid in all analyzed extracts was CBDA, followed by CBD, THCA, and CBCA. In contrast, THCV, THCVA, and Δ⁸-THC were not detected in any of the extracts. CBDV, CBN, and CBC were recorded only in low concentrations. The highest total cannabinoid content was determined in the extract of the Bialobrzeskie cultivar (21,740.94 µg/g), followed by the mixed extract (16,320.11 µg/g) and the extract of the Santhica 27 cultivar (15,289.41 µg/g). A lower cannabinoid content was recorded in the extract of the Felina 32 cultivar (12,668.04 µg/g), and the lowest value was found in the Futura 75 cultivar (4,713.11 µg/g).

Discussion

All tested ethanol extracts from hemp demonstrated some degree of *in vitro* antimicrobial activity against all tested bacterial strains, including both antibiotic-resistant and susceptible strains. However, their efficacy varied significantly—MIC values ranged from 128 to 1024 µg/ml. Although these values are higher than those reported in the literature for individual cannabinoids, our results are partially consistent with recent studies demonstrating the inhibitory effects of crude cannabis extracts on *Staphylococcus* strains, with MICs ranging from 8 to 8,552 µg/ml (Ali et al. 2012; Kaur et al. 2015; Skala et al. 2022; Giselle et al. 2023). Lower MIC values (8 µg/ml) for the ethanol extract against *S. aureus* (ATCC 29213) were

published by Skala et al. (2022). However, these results are difficult to directly compare with our study, as the total cannabinoid content in the extracts by Skala et al. (2022) was substantially higher (245,940 and 329,100 µg/g) than in the extracts from our study, with the contents of individual cannabinoids—particularly Δ9-THC, CBC, and CBG—being up to an order of magnitude higher in their extracts. These differences may explain the lower MIC values, as the antibacterial activity of these cannabinoids is well documented (Sionov & Steinberg 2022). Furthermore, Skala et al. (2022) worked with a medicinal cannabis strain with a relatively high cannabinoid content, whereas our study utilized industrial cannabis varieties with a THC content $\leq 0.2\%$ (EFSA 2011), which are permitted for use in livestock and whose application is therefore relevant for potential subsequent *in vivo* experiments. Giselle et al. (2023) also report values comparable to those in our study for *S. aureus* (ATCC 29213): MIC of the inflorescence extract 115.25 µg/ml and MIC of the root extract 8552.38 µg/ml. For streptococci, MIC values for individual cannabinoids have been determined for various species like *Streptococcus mutans* (MIC 2.5 µg/ml), *Streptococcus sanguis* (MIC 1 µg/ml), *Streptococcus pyogenes* (2–5 µg/ml), and *Streptococcus pneumoniae* (1–2 µg/ml) (van Klinger- en & ten Ham 1976; Aqawi et al. 2021; Blaskovich et al. 2021). The MIC values from our study are higher, which can be attributed to the complex composition of whole extracts, in which individual components may interact with one another or exhibit different mechanisms of action. For comparison, Skala et al. (2022) report an MIC of 128 µg/ml for the ethanol extract against *Str. pyogenes*, which corresponds to the values from our study.

The cannabinoid content in *Cannabis sativa* is influenced by a number of biotic and abiotic factors, such as nutrient availability, growth stage, storage conditions, light intensity, and temperature (Glivar et al. 2020). In our study, differences in cannabinoid content were observed among the five extracts tested, which likely influenced their antimicrobial activity. Higher antimicrobial activity was observed in extracts with higher cannabinoid content (Bialobrzeskie, mixed, and Santhica 27 varieties), while lower activity was recorded in extracts with lower content (Felina 32 and Futura 75 varieties). These findings suggest that cannabinoid content could be one of the key factors influencing the antibacterial efficacy of cannabis extracts. However, it is important to emphasize that the observed activity cannot be attributed solely to the cannabinoids themselves, but rather to the synergistic action of the entire complex of substances contained in cannabis (Giselle et al. 2023).

CONCLUSION

The present study evaluated the *in vitro* antimicrobial activity of ethanol extracts from five industrial hemp varieties (Felina 32, Futura 75, mixed, Santhica 27, and Bialobrzeskie) against strains of *S. aureus*, *Str. agalactiae* and *Str. dysgalactiae*, including both antibiotic-resistant and susceptible forms. All five tested ethanol

extracts exhibited some degree of inhibitory activity against the tested bacterial strains. The extract from the Santhica 27 variety proved to be the most effective, followed by the mixed and Bialobrzeskie varieties. Extracts from the Felina 32 and Futura 75 varieties exhibited only weak antibacterial activity. The results obtained demonstrate that the composition of hemp extracts varies greatly by variety, which is directly reflected in the differing degrees of growth inhibition of individual bacterial species. This variability underscores the need for further research focused on the relationship between the chemical composition of the extracts and their antibacterial effect. In the context of increasing antimicrobial resistance of pathogens and the restriction of preventive antibiotic use in animal production, hemp represents a promising source of natural antimicrobial substances. The Santhica 27, Bialobrzeskie, and mixed varieties appear to be the most promising candidates for further research. However, to verify the therapeutic potential of cannabis extracts in the treatment of staphylococcal and streptococcal infections in livestock, it will be necessary to conduct follow-up in vivo experiments.

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