https://doi.org/10.17221/85/2025-CJAS

Antimicrobial properties of secondary metabolites of *Cannabis sativa*: A promising natural alternative for livestock health

Tereza Paulová[1,2], *Karel Novák*[3]*, *Eva Pěchoučková*[1,2]

[1]*Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*
[2]*Department of Nutritional Physiology and Animal Product Quality, Institute of Animal Science Prague (IAS), Prague, Czech Republic*
[3]*Department of Genetics and Breeding of Farm Animals, Institute of Animal Science Prague (IAS), Prague, Czech Republic*

**Corresponding author: novak.karel@vuzv.cz*

Citation: Paulová T., Novák K., Pěchoučková E. (2025): Antimicrobial properties of secondary metabolites of *Cannabis sativa*: A promising natural alternative for livestock health. Czech J. Anim. Sci., 70: 357–382.

Abstract: In addition to their practical importance as a medicinal plant, animal feed and a source of materials for the textile and construction industry, industrial varieties of *Cannabis sativa* L. (hemp in a wider sense) provide an alternative for controlling infectious diseases in livestock. Despite the genetic divergence between two primary groups of cannabis, i.e. medicinal cannabis and technical hemp, hemp plants also produce a wide spectrum of secondary metabolites. These include the main classes of cannabinoids and terpenoids, as well as representatives of flavonoids, stilbenoids, steroids, alkaloids, spiroindans, dihydrophenanthrenes, and lignanamides. Many of them exhibit antibiotic activity which can substitute or complement the use of traditional antibiotics in animal husbandry. For example, the cannabinoid fraction exhibits activity against the Gram-positive bacteria and some fungi. While the activity against Gram-negative bacteria is not characteristic of cannabinoids, these pathogens can still be affected by hemp terpenoids and flavonoids. The synergy among the secondary metabolite fractions or between the hemp metabolites and traditional antibiotics is also a favourable factor. The search for alternatives to traditional antibiotics is further driven by the increasing prevalence of genetically determined antibiotic resistance among veterinary pathogens, which poses the additional risk of transferring resistance traits to the human pathogens. The content of antibiotically active compounds in hemp can be enhanced through selection among existing genotypes, targeted breeding, cultivation conditions, and even by specific elicitation of secondary metabolites with the natural antibiotic function in the disease resistance of the plant. The switch to hemp metabolites is also supported by their compatibility as natural components of plant-based animal feed, and by favourable economic considerations.

Keywords: antibiotic; cannabidiol; cattle; hemp; poultry; *Staphylococcus*; THC

Supported by the project No. RO0723 for the long-term development of research organisation granted by The Ministry of Agriculture of the Czech Republic, and also by METROFOOD-CZ research infrastructure project (MEYS Grant No.: LM2023064).

https://doi.org/10.17221/85/2025-CJAS

INTRODUCTION

Hemp (*Cannabis sativa* L., of the family *Cannabaceae*) is widely used as a technological raw material and a fodder crop in agriculture, in parallel to its well-known applications in medicine and its abuse as a recreational drug. In response to diverse human uses, the originally wild-growing plant has differentiated into a range of specialised varieties. The species originates from Central and Northeast Asia and was initially used for medicinal and recreational purposes (Karas et al. 2020). Historical evidence suggests that hemp cultivation in China began approximately 6 000 years ago in the Yunnan province (Gao et al. 2020), as suggested by chloroplast DNA marker analyses (Osterberger et al. 2022). However, it seems that cannabis was used in parallel for its fibre as a textile raw material. Independent domestication in Europe may have occurred later, during the Copper or Bronze Age, as suggested by fossil pollen evidence (McPartland et al. 2018).

Following the incorporation of cannabis in agriculture, two basic groups of varieties emerged. The first group comprises the so-called medicinal varieties, denoted as drug-type ones, medicinal cannabis or marijuana, with a high content of secondary metabolites active in humans and animals. The biologically active compounds in medicinal varieties are represented by the class of cannabinoids in the first place, where the main and best-known substance with biological activity is Δ^9-tetrahydrocannabinol (THC). The second important cannabinoid is cannabidiol (CBD), also associated with the action against stress and anxiety, however, without pronounced occurrence among medicinal varieties (Brunetti et al. 2020). Nevertheless, a wide array of other active substances has been detected, including the second important group of terpenoids (Kaur et al. 2023). The primary medicinal effects include the treatment of various diseases and relief of symptoms such as pain, nausea and anxiety. Additionally, the immunological effects are primarily associated with the suppression of inflammation (Leung 2011).

In contrast, a range of varieties developed for their final use in agriculture, as a building material or in the textile industry have been created. This group of genotypes is denoted as technical hemp, fibre-type cannabis or industrial hemp (Kleinhenz et al. 2020). These varieties are characterised by a negligible content of psychoactive substances. In addition to the shoot matter as fodder, the seeds are used as a feed in both monogastric animals and ruminants. They are processed in the form of hemp seed cakes or hemp oil, as summarised by Papatzimos and Kasapidou (2024). The cannabis oil extracted from seeds is used both for food and technical purposes (Clarke and Merlin 2016).

The distinction between the cannabis groups is determined by the value of THC content in the plant dry mass. While the content in fibre-type plants is below 0.3%, the drug-type plants are supposed to contain more THC than this level. This value is reflected in the allowable THC threshold ≤0.3% for cannabis cultivation laid down in the European Union by the EU Regulation 2021/2115 (European Union 2021). The last classification reflecting major genes controlling the cannabinoid production is based on the THC and CBD ratios (Mandolino et al. 2003). While type I (mostly for recreational use) contains high THC vs low CBD, type II has a balanced THC/CBD ratio and is optimal for medical use, and type III is characterised by low THC and high CBD and includes industrial hemp. Unfortunately, the psychoactive properties of marijuana have contributed to its widespread misuse, making it the most commonly abused illicit drug in the world (Hurgobin et al. 2021).

Compared to other forage crops, hemp offers high yields of green matter and a very high adaptability to unfavourable natural conditions. That is why it is widely grown and used for animal production, mainly in arid Mediterranean and subtropical regions. Nevertheless, its cultivation range extends as far north as the Scandinavian countries and Northern Russia. For this reason, interest in its cultivation persists regardless of the periods of restrictive measures motivated by the necessity to maintain control over the misuse of the medicinal varieties. An example of such a trend is illustrated by developments in the USA in the 1970s (Welling et al. 2016).

Many secondary metabolites produced in hemp shoots, including cannabinoids and terpenoids, exhibit antimicrobial activity (Iseppi et al. 2019; Alfei et al. 2023). They may therefore influence the animal health by reducing levels of unfavourable microorganisms and enhancing their immune response. Even residual levels of psychoactive compounds from the cannabinoid group can affect the behav-

https://doi.org/10.17221/85/2025-CJAS

iour and physiological state of livestock animals. In this context, many studies have focused on the health effects of residual concentrations of cannabinoids, as well as on the identification of other products of secondary metabolism with antibacterial activity. The current understanding of the non-psychotropic effects of cannabinoids from cannabis has recently been examined by Alfei et al. (2023) and Sionov and Steinberg (2022).

The demand to replace traditional antibiotics in animal production with available phytochemicals, including those contained in hemp, is evident (Mala et al. 2021). The massive use of antibiotics in livestock production brings a real risk of generating and transmitting microbial genes coding for antimicrobial resistance (Kirchhelle 2018; Ahmed et al. 2024). Any possibility to circumvent this threat with global consequences should be considered. The renewed interest in hemp as a forage crop also depends on the ability to control the genetic structure of the cultivated cannabis varieties and to control the content of active substances (Sawler et al. 2015).

Legal measures prohibiting the preventive mass use of antibiotics seem to be a major factor forcing the search for alternative solutions. The danger of the uncontrolled spread of bacterial epizootics on large-scale farms is a limitation for further growth of the industry and even for the operation of the facilities of a current standard. The uncontrolled spread of salmonellosis or *E. coli* infection on a poultry farm might lead to the mortality of tens of percent. Therefore, any alternatives of the missing wide antibiotic pretreatment are extremely valuable. The infections caused by *Salmonella* can be taken as an example. Even not considering the huge economic losses caused by salmonellosis in poultry, its role in causing zoonoses is decisive by itself. The worldwide incidence of human salmonellosis is 1.3 billion, and approximately 3 million of patients die annually (Chimalizeni et al. 2010).

In the present review, we aim to summarise the current state of research on constitutively formed secondary substances with desirable as well as harmful activities in cannabis. Furthermore, this review summarises the antibacterial resistance related to diseases in livestock, primarily the gastrointestinal ones, followed by an overview of the reported antimicrobial activity of cannabis against the most commonly encountered bacteria. At the same time, strategies for enhancing the levels of beneficial compounds in hemp tissues are outlined. Finally, the review also summarises the prospects for the future use of cannabis in livestock production.

CLASSES OF SECONDARY METABOLITES DOCUMENTED FOR CANNABIS

In a classical survey of the phytochemical diversity of *C. sativa*, more than 480 compounds were detected, including 180 cannabinoids and abundant terpenes (Fischedick et al. 2010). Subsequently, among 554 identified compounds in *C. sativa* plants, 113 phytocannabinoids were identified and 120 terpenes (Calvi et al. 2018). Consistent with these findings, a recent survey of cannabis lines grown in the USA illustrates their rich chemical diversity according to the specific arrays of cannabinoids and terpenes formed (Smith et al. 2022).

In addition to the traditional division into marijuana types (for medicinal and general human consumption) and technical varieties (hemp), further clusters of chemical phenotypes (chemotypes) could be distinguished. Notably, the cannabinoid content in female inflorescences (*C. sativa* is a dioecious species) is decisive for the classification of cannabis varieties as this is the plant part used for narcotic purposes (Smith et al. 2022). However, the role of terpenes as active substances of cannabis is of comparable importance for distinguishing the chemotype (Iseppi et al. 2019).

Cannabinoids

The scheme of synthesis of common cannabinoids in the cannabis plant from geranyl-pyrophosphate (GPP) is illustrated in Figure 1.

Cannabinoids are biosynthesised as prenylated aromatic carboxylic acids. While neutral cannabinoids are almost absent in fresh plants, the acidic forms can be converted into their neutral homologues by spontaneous decarboxylation in the presence of light or heat. Moreover, cannabinoids can be oxidised, for example THC can be converted into cannabinol (CBN).

The first cannabinoid in the biosynthetic pathway is cannabigerolic acid (CBGA). It is sequen-

https://doi.org/10.17221/85/2025-CJAS

tially transformed into tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and cannabichromenic acid (CBCA), each by a particular synthase (Figure 1). The two major cannabinoids, which are best known for their therapeutic potential, are THC and CBD, i.e. the neutral homologues of THCA and CBDA, respectively. THC is the main psychoactive agent of *C. sativa* and it has anti-inflammatory, analgesic, appetite-stimulant, and antiemetic properties (Sionov and Steinberg 2022). In addition to cannabinoids, monoterpenes and sesquiterpenes are derived from different addition reactions of geranyl ($C_{10}H_{16}$) and farnesyl ($C_{15}H_{24}$) units, respectively.

The major component of the hemp plant, albeit without direct psychoactive properties, is cannabidiol (CBD), a small molecule of 314 Da (Blaskovich et al. 2021). A pentyl-substituted bisphenol aromatic group (pentylresorcinol) is linked to an alkyl-substituted cyclohexene terpene ring system according to Figure 1 (Mechoulam and Shvo 1963). Although CBD has no psychoactive properties (Wu et al. 2023), this substance can still modulate the euphoric effects of THC and it has antipsychotic, neuroprotective, anticancer, antidiabetic, and other positive effects, such as the ability to reduce tobacco addiction. CBD can easily be transformed to THC. Golombek et al. (2020) listed 16 different products of CBD conversion by a single reaction. A simple treatment with hydrochloric acid in an anhydrous environment of cyclohexane is sufficient for the intramolecular cyclization driven by the addition of a phenolic group to one of double bonds leading to Δ^9-THC and Δ^8-THC (trans-Δ^8-tetrahydrocannabinol). The possibility of CBD conversion *in vivo* in the acidic environment of gastric juice has been considered as well. The ease of chemical conversion makes CBD a substance prohibited in many countries in parallel to THC (Brunetti et al. 2020).

The second significant area of exploitation of cannabinoid compounds is their antimicrobial activity, which is summarised in Table 1. Zdenek Krejci (Krejci 1952) is likely a key personality in the

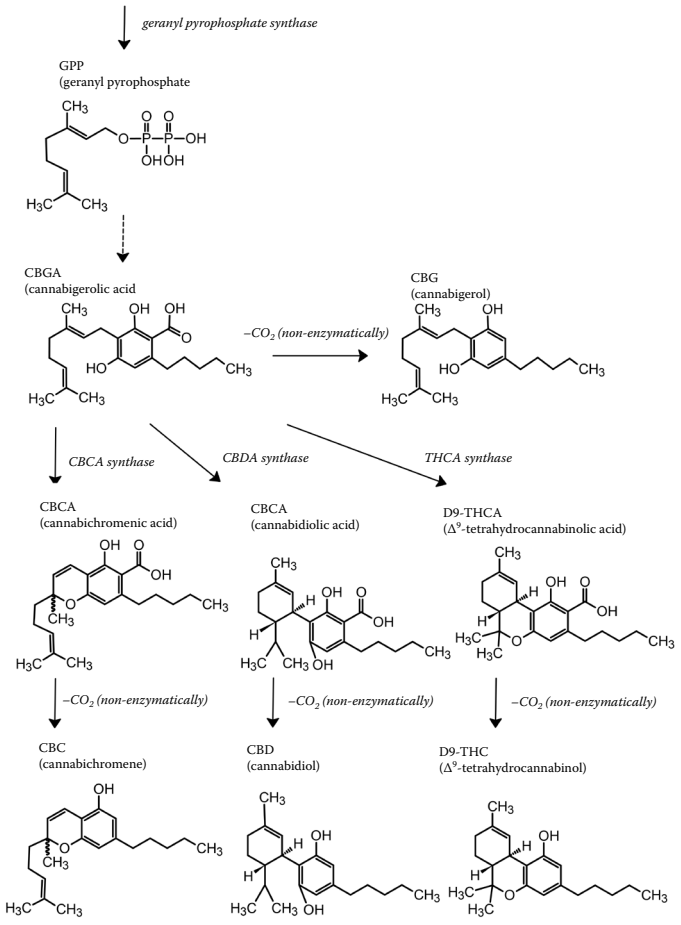

Figure 1. Synthesis of common cannabinoids in cannabis (https://x.com/smokereports 2008–2011)

https://doi.org/10.17221/85/2025-CJAS

Table 1. Representative anti-microbial compounds reported for cannabis

Substance	Occurrence and physiological activity	Antimicrobial activity	Reference
Cannabinoids			
Δ^9-tetrahydrocannabinol (D9-THC)	mainly in female inflorescences, primary psychoactive component of cannabis	MIC from 0.5 to 2.0 µg/ml towards 6 *S. aureus* MRSA strains	Appendino et al. (2008); Sionov and Steinberg (2022)
Cannabidiol (CBD)	major component of the hemp plant, mildly psychotropic, but also anti-psychotic effect, neuroprotective	MIC 0.5–2.0 µg/ml towards 6 strains MRSA	Appendino et al. (2008); Bloomfield et al. (2022)
CBD	–	generally MIC is 0.5–4.0 µg/ml in G^+ bacteria and as low as 0.25 µg/ml in some G^- genera, namely *Legionella*, *Neisseria*, and *Moraxella*	Blaskovich et al. (2021)
CBD	–	MIC = 100 µM against the model species *M. smegmatis* and 25 µM against *M. tuberculosis*, active also in infected macrophage cells, without cytotoxicity	Martinena et al. (2024)
CBD	–	MIC = 8 µg/ml for *S. aureus* ATCC 6538, 1 µg/ml for *L. monocytogenes* NCTC 10888, 1 µg/ml for *E. faecalis* ATCC 29212, and 8 µg/ml for *B. subtilis* ATCC 6633	Iseppi et al. (2019)
CBD	–	the MIC value for *S. typhimurium* is particularly low – 0.012 5 µg/ml, while the control antibiotic ampicillin had an MIC of 0.5 µg/ml	Gildea et al. (2022)
Cannabidiolic acid (CBDA)	–	antimicrobial activity reported	Krejci (1958); van Klingeren and Ten Ham (1976)
CBDA	–	MIC for *S. aureus* ATCC 25923 2 µg/ml, for MRSA USA300 4 µg/ml	Martinenghi et al. (2020)
Cannabigerolic acid (CBGA)	–	MIC 2–4 µg/ml for the strain MRSA ATCC 43300 and 1–2 µg/ml for *N. gonorrhoeae*	Blaskovich et al. (2021)
Cannabigerol (CBG)	–	MIC between 0.5 and 2.0 µg/ml in a set of 6 strains MRSA	Appendino et al. (2008)
CBG	–	4–8 µg/ml vs. the strain MRSA ATCC 43300 and 1–2 µg/ml towards *N. gonorrhoeae*	Blaskovich et al. (2021)
CBG	–	CBG modulated the quorum sensing system thus limiting bacterial infection	Aqawi et al. (2023)
Cannabichromene (CBC)	–	MIC between 0.5 and 2.0 µg/ml towards a set of 6 strains MRSA	Appendino et al. (2008)
Cannabichromenic acid (CBCA)	–	MIC = 1.41 µg/ml against MRSA	Galletta et al. (2020)
Cannabinol (CBN)	the first compound to be isolated from cannabis extract in the late 1800s, mildly psychoactive, agonist at both CB1 and CB2 receptors	MIC between 0.5 and 2.0 µg/ml towards 6 strains MRSA	Appendino et al. (2008); Iseger and Bossong (2015)

Czech Journal of Animal Science, 70, 2025 (9): 357–382

https://doi.org/10.17221/85/2025-CJAS

Table 1 to be continued

Substance	Occurrence and physiological activity	Antimicrobial activity	Reference
Terpenoids			
β-myrcene	in cannabis essential oil	MIC = 8 µg/ml for *S. aureus* ATCC 6538, 2 µg/ml for *L. monocytogenes* NCTC 10888, 1 µg/ml for *E. faecalis* ATCC 29212, and 32 µg/ml for *B. subtilis* ATCC 6633	Iseppi et al. (2019)
β-myrcene	–	MIC_{50} was 0.64 µg/ml for *E. coli*, 1.60 µg/ml for *S. enterica*, and 0.64 µg/ml for *S. aureus*	Wang et al. (2019)
β-pinene	in cannabis essential oil	MIC = 4 µg/ml for *S. aureus* ATCC 6538, 2 µg/ml for *L. monocytogenes* NCTC 10888, 0.5 µg/ml for *E. faecalis* ATCC 29212, and 4 µg/ml for *B. subtilis* ATCC 6633	Iseppi et al. (2019)
α-pinene,	in cannabis essential oil	MIC = 4 µg/ml for *S. aureus* ATCC 6538, 1 µg/ml for *L. monocytogenes* NCTC 10888, 2 µg/ml for *E. faecalis* ATCC 29212, and 8 µg/ml for *B. subtilis* ATCC 6633	Iseppi et al. (2019)
α-pinene,	–	MIC_{50} was 0.69 µg/ml for *E. coli*, 0.69 µg/ml for *S. enterica*, and 0.42 µg/ml for *S. aureus*	Wang et al. (2019)
Limonene	in cannabis essential oil	activity against *E. coli*, *P. aeruginosa*, *S. typhimurium*, *S. aureus*, *E. faecalis*, *L. monocytogenes*, and *A. brasiliensis*	Sovljanski et al. (2024)
Limonene	–	MIC_{50} was 0.42 µg/ml for *E. coli*, 0.42 µg/ml for *S. enterica*, and 0.42 µg/ml for *S. aureus*	Wang et al. (2019)
α-terpinolene	in cannabis essential oil	MIC = 8 µg/ml for *S. aureus* ATCC 6538, 2 µg/ml for *L. monocytogenes* NCTC 10888, 2 µg/ml for *E. faecalis* ATCC 29212, and 16 µg/ml for *B. subtilis* ATCC 6633	Iseppi et al. (2019)
trans-caryophyllene	–	MIC = 16 µg/ml for *S. aureus* ATCC 6538, 1 µg/ml for *L. monocytogenes* NCTC 10888, 1 µg/ml for *E. faecalis* ATCC 29212, and 1 µg/ml for *B. subtilis* ATCC 6633	Iseppi et al. (2019)
Flavonoids			
Naringenin	–	MIC for *S. aureus* strain 105 was 512 µg/ml	Zengin et al. (2018)
Naringenin	–	MIC for the biofilm formation in *S. mutans* was between 100 and 200 µg/ml	Yue et al. (2018)
Naringenin	–	modifications of cell-membrane fluidity and fatty-acid composition in *E. coli* ATCC 25922 and *S. aureus* ATCC 6538; the growth of *S. aureus* was inhibited at low concentrations	Wang et al. (2018)
Naringenin	–	MIC_{50} was 16 µg/ml for a set of *H. pylori* isolates and the reference strain ATCC 43629	Zengin et al. (2018)

https://doi.org/10.17221/85/2025-CJAS

Table 1 to be continued

Substance	Occurrence and physiological activity	Antimicrobial activity	Reference
Naringenin	–	inhibits quorum sensing in *V. harveyi*, inhibits biofilm formation in *V. harveyi* and *E. coli* O157:H7	Vikram et al. (2010)
Kaempferol	–	active against G^+ *S. aureus*, G^- *H. pylori*	Zengin et al. (2018)
Kaempferol	–	inhibits quorum sensing in *V. harveyi*, inhibits biofilm formation in *V. harveyi* and *E. coli* O157:H7	Vikram et al. (2010)
Quercetin	–	active against G^+ *S. aureus*, G^- *H. pylori*	Zengin et al. (2018)
Quercetin	–	inhibits quorum sensing in *V. harveyi*, inhibits biofilm formation by *V. harveyi* and *E. coli* O157:H7	Vikram et al. (2010)
Rutin	–	active against G^+ *S. aureus*, G^- *H. pylori*, has antioxidant properties	Zengin et al. (2018)

A. brasiliensis = *Aspergillus brasiliensis*; *B. subtilis* = *Bacillus subtilis*; *E. coli* = *Escherichia coli*; *E. faecalis* = *Enterococcus faecalis*; *H. pylori* = *Helicobacter pylori*; *L. monocytogenes* = *Listeria monocytogenes*; *M. smegmatis* = *Mycobacterium smegmatis*; *M. tuberculosis* = *Mycobacterium tuberculosis*; MIC = minimum inhibitory concentration; MRSA = methicillin-resistant *Staphylococcus aureus*; *N. gonorrhoeae* = *Neisseria gonorrhoeae*; *S. aureus* = *Staphylococcus aureus*; *S. enterica* = *Salmonella enterica*; *S. mutans* = *Streptococcus mutans*; *S. typhimurium* = *Salmonella typhimurium*; *V. harveyi* = *Vibrio harveyi*

field of modern medicine. He and his collaborators isolated cannabidiolic acid (CBDA) and demonstrated its antimicrobial activity (Krejci 1958). Following the subsequent work by van Klingeren and Ten Ham (1976), the next key study on the antimicrobial activity of CBD and related compounds was not published until recently (Appendino et al. 2008). In this work, five major cannabinoids, namely CBD, cannabichromene (CBC), cannabigerol (CBG), THC and cannabinol (CBN), were tested against a set of six methicillin-resistant *Staphylococcus aureus* (MRSA) strains, all of which were of clinical relevance. At the same time, all tested strains demonstrated very promising values of minimum inhibitory concentration (MIC) between 0.5 and 2.0 μg/ml with respect to the covered compounds.

These reports on the antimicrobial activity of CBD against Gram-positive bacteria species have been confirmed in subsequent studies, namely, on *S. aureus*, *Streptococcus pneumoniae*, and *Clostridioides difficile*. The activity against *S. aureus* is considered as critically important since this species is known for harbouring antibiotic resistance in MRSA strains. For this species, the MIC was observed at the traditionally recognised antimicrobial value of 1 μg/ml. In addition, the first observation of CBD activity in Gram-negative bacteria, including *Neisseria gonorrhoeae*, was reported (Blaskovich et al. 2021). Consistently with these works, the correlation between the cannabinoid content in the shoot of hydroponically grown hemp and the antimicrobial activity as determined against *S. aureus* has been documented (Malikova et al. 2024).

Concentrating on cannabis varieties without psychoactive cannabinoid compounds simplifies the approved legal use in animal production and veterinary science.

Terpenoids

The significant role of terpenes as active components in cannabis essential oil was demonstrated by Iseppi et al. (2019) through phytochemical characterization of 17 kinds of oils from different varieties. Among the 71 identified compounds, the terpenes β-myrcene, α-pinene, α-terpinolene, β-pinene, *trans*-ocimene and limonene prevailed (Figure 2).

https://doi.org/10.17221/85/2025-CJAS

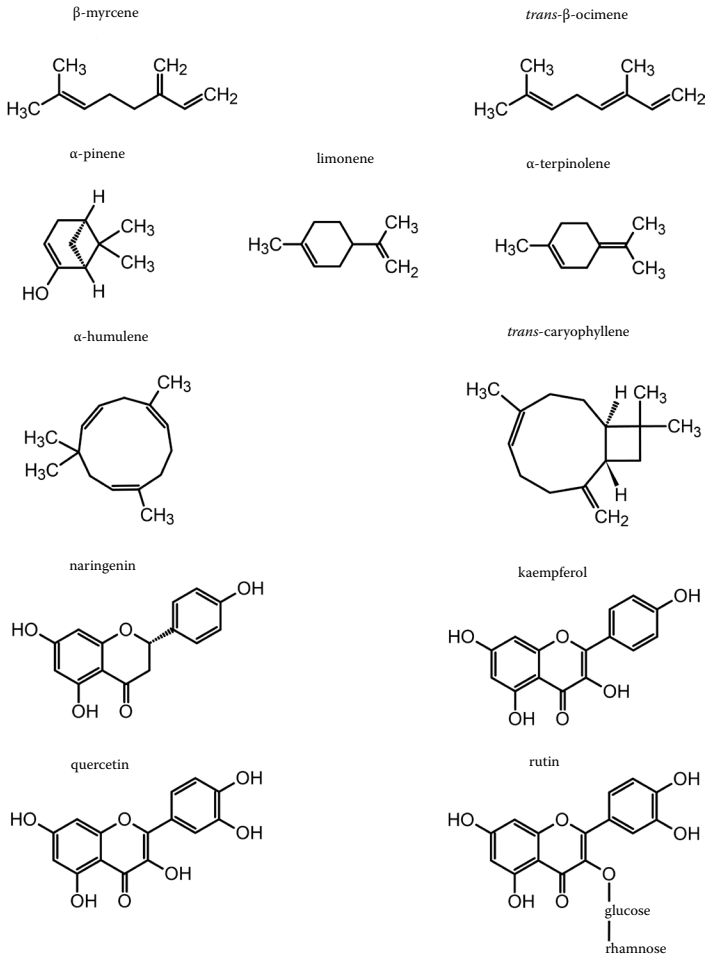

Figure 2. Basic terpenoids and flavonoids from cannabis extracts cited in the text

Consistently, 66 compounds were identified in the oil fraction and hydrolate from industrial hemp in a later study by Sovljanski et al. (2024). They formed classes of monoterpenes (9 species), oxygenated monoterpenes (24), sesquiterpenes (17), oxygenated sesquiterpenes (8) and eight non-classified compounds. Notably, their mixture demonstrated antimicrobial activity against nine model microorganisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *S. typhimurium*, *S. aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Saccharomyces cerevisiae*, *Candida albicans*, and *Aspergillus brasiliensis*.

The number of terpenes and terpenoids can even be extended (Kaur et al. 2023) using a two-dimensional gas chromatograph with a time-of-flight mass spectrometer (GC × GC-TOFMS). When the dried female flowers of six lines of *C. sativa* were surveyed, a total of 146 terpenes and terpenoids were detected. Twenty compounds were resolved additionally using enhanced separation in the second dimension. The identified compounds mostly belonged to the classes of monoterpenes, monoterpenoids, sesquiterpenes, and sesquiterpenoids. Four new terpenoids of cannabis were reported for the first time.

Other active compounds

As stated by Pollastro et al. (2018), the phytochemical analysis of cannabis demonstrated the presence of other classes of compounds with a potential for physiological activity in addition to cannabinoids and terpenoids. These compounds include flavonoids, spiroindans, dihydrostilbenes, dihydrophenanthrenes, lignanamides, steroids and alkaloids (Figure 2). The class of polyphenols includes prenylated flavonoids, stilbenoids and lignanamides. Consequently, the interaction of these compounds with the group of proper cannabinoids should be taken into account when esti-

https://doi.org/10.17221/85/2025-CJAS

mating or predicting the desirable effects of hemp products. However, a thorough survey of the range of compounds present in the hemp material is still conditional on the application of complementary extraction methods aimed at different groups of compounds (Brkljaca et al. 2023).

Recently, the gradual ethanolic extracts of hemp have been demonstrated to contain a number of flavonoids and terpenoids as major compounds in addition to cannabinoids, namely quercetin, kaempferol, rutin, chlorogenic acid, ferulic acid, CBG and alpha-tocopherol (Lanzoni et al. 2024a). This finding is consistent with the basic role of flavonoids in the formation of the antimicrobial resistance of plant hosts (Carlson and Dolphin 1981; Novak et al. 1995).

METHODS FOR TESTING BIOLOGICAL ACTIVITIES OF SECONDARY METABOLITES OCCURRING IN CANNABIS

The use of cannabis for medicinal purposes is well-documented as far back as 2 700 years BPE (Li 1974). The first studies dealing with the antibacterial activity of extracted cannabinoids appeared in the 1950's (Krejci 1952; Krejci 1958; Rabinovich et al. 1959). Starting from the 70's, more systemic tests were performed on the level of purified individual compounds. Both Δ^9-THC and CBD have partly been shown to be bacteriostatic and bactericidal towards Gram-positive bacteria (van Klingeren and Ten Ham 1976).

Various methods have been used for obtaining *C. sativa* extracts. However, the method of extraction of active compounds can be crucial in such tests. The concentrates include both essential oil and extracts obtained with petroleum ether, methanol, and hot water. Although the traditional techniques employ cold-pressing and solvent extraction, advanced technologies which generate analogical products are now expanding. They comprise pressurised liquid extraction without filtration and ultrasonic-assisted extraction. In this way, these methods reduce the processed volumes of solvent while preserving high yields of active compounds (Fathordoobady et al. 2019).

However, essential oils obtained without the extraction step are the main source of hemp terpenes used to test activity against both Gram-positive and Gram-negative pathogenic bacteria. It has been shown that the most abundant terpenoid compounds in hemp oil samples are α-pinene, myrcene, *trans*-β-ocimene, α-terpinolene, *trans*-caryophyllene and α-humulene (Novak et al. 2001). These types of hemp extracts, i.e. the essential oil and the hydrolate type, are almost free of cannabinoids, thus simplifying the test result interpretation. Consistently, extracts from seeds obtained either with non-polar petroleum ether or with methanol demonstrated an effect against Gram-positive pathogens in both cases, when the cup plate agar diffusion method was used for the activity assay. However, no antifungal activity was noticed in the same study.

Along with the cup plate agar diffusion method, disc diffusion experiments are the main sources of information about the antimicrobial properties of cannabis plant extracts or the fractioned mixture up to pure components. The diameter of the inhibition zone is a characteristic of the tested mixture/compound activity. Different bacterial species can be used in this simple system, as exemplified in a set of nosocomial infection agents *S. aureus*, MRSA, *E. coli*, *K. pneumoniae*, *P. aeruginosa* or *Acinetobacter baumannii* (Sarmadyan et al. 2014).

Alternatively, the MIC values can be determined for submerse bacterial cultures in suitable laboratory microtubes or other microplastics with a corresponding determination of optical density or dissipation. In the most rational variant, the plastic optical microplates are used for the serial dilution of the tested agent and cultivation. The inhibitory effect is then read on a standard microplate reader or on a real-time PCR device (e.g. Iseppi et al. 2019). This technique is recommended as the gold standard for antimicrobial susceptibility testing in the CLSI M100 manual (CLSI 2025).

The biofilm assay can also be used for the antimicrobial activity determination, as applied against *S. aureus* ATCC 25923 (MSSA) and *S. aureus* ATCC 43300 (MRSA) by Blaskovich et al. (2021) in microplates in combination with crystal violet staining. Similarly, this technique was used for comparing the antibiotic activity of ethanolic extracts of *C. sativa* and of other nine plant species. The extracts were tested against methicillin-resistant *S. aureus* (MRSA). *C. sativa* demonstrated the highest activity among the 10 plant species tested, where MIC of the dry extract was 0.25 mg/ml with 86% mortal-

https://doi.org/10.17221/85/2025-CJAS

ity. At a concentration of 0.125 mg/ml, the biofilm was reduced by 71% (Roshan et al. 2024). The major cannabinoids detected in this case of *C. sativa* extract were CBD and Δ^9-THC.

Variability in antimicrobial activity has also been documented among cannabis cultivars. In a recent work by Paulova et al. (2025), shoot ethanolic extracts from four cultivars of technical hemp (including cv. Bialobrzeskie, Santhica, Felina, and Futura) were tested against three bacterial pathogens, namely *S. aureus*, *Streptococcus agalactiae* and *Streptococcus dysgalactiae*. MICs were observed to range from 128 µg/ml to 2 048 µg/ml extract.

In another example reported, an acetone extract from hemp with a rich scale of isolated compounds displayed superior bactericidal properties (Lone and Lone 2012). Although a certain response was observed against all tested bacterial strains, *Vibrio cholerae* was the most sensitive bacterium, followed by *P. aeruginosa*. Antifungal activity was observed against *C. albicans* and *Candida neoformans* as well. This study also documented the antioxidant properties of *C. sativa* extract, thus widening its potential for clinical use.

On the contrary, no inhibition by cannabis was observed in Gram-negative bacterial species included in the above experiments reported by Blaskovich et al. (2021). This observation is consistent with the specific action of hemp extracts only against Gram-positive bacteria as compared to Gram-negative species, as documented by Sarmadyan et al. (2014). This opinion was also confirmed independently by Vu et al. (2016) and Lelario et al. (2018).

It should be taken into account that the antimicrobial effects of crude cannabis extracts can be at least partially due to other classes of compounds than cannabinoids themselves. This opinion is confirmed by the observation that the activities against the Gram-positive bacterial species are present even in the essential oil that has been made free of Δ^9-THC. Therefore, the modified version of essential oil is intended for use in medicinal, cosmetic, veterinary, agronomic and food applications. The flavanone naringenin has been linked to this residual antimicrobial activity, typically assessed against Gram-positive *S. aureus*. Although some activity of the cannabinoid-free fraction was also expressed against the Gram-negative bacterium *H. pylori*, no antifungal activity was observed like in the total extracts (Zengin et al. 2018).

In view of a parallel occurrence of antimicrobial activity both in the conventional and the terpenoid fractions, a synergistic effect cannot be ruled out. In any case, synergistic effects of cannabis extracts with extracts from other plant species have been reported. Combining 1 : 1 with other medicinal plant extracts such as *Psidium guajava* and *Thuja orientalis* demonstrated a synergistic effect, whereby the inhibition zone diameters ranged from 25 mm to 30 mm in most pathogens. However, the action on the cannabis side was ascribed solely to the presence of gallic acid, quercetin and other flavonoids such as catechin. These compounds were detected in the leaf extracts. No effect was ascribed to cannabinoids in this case, in contrast to the expectation (Chakraborty et al. 2018).

FACTORS AFFECTING PRODUCTION OF SECONDARY METABOLITES IN CANNABIS TISSUES

The main factors that might contribute to an increase in the content of antimicrobial compounds in cannabis plants exploited in agriculture are surveyed in Table 2.

Genetic factors

The differences in the chemical composition of the cannabis shoot fully reflect genetic factors. Although the draft genome of *C. sativa* was published already in 2011 (van Bakel et al. 2011), an assembly of sufficient quality was only accomplished upon a subsequent sequencing programme (Gao et al. 2020). PacBio single-molecule sequencing and Hi-C technology were used for obtaining a high-quality genomic sequence. The wild-type varieties of cannabis from Tibet as the original area of the species were intentionally chosen for establishing the reference genome of this key crop. The *C. sativa* reference genome is approximately 808 Mb long, however, the repetitive elements comprise 74.75% of the total sequence. Since *C. sativa* is a dioecious plant, and the Y chromosome is larger than the X chromosome, the female haploid genome is 818 Mb in size, in contrast to the male genome of 843 Mb (Sakamoto et al. 1998). The *C. sativa* genome codes for 38 828 protein products according to Gao et al. (2020). Cannabis DNA is di-

https://doi.org/10.17221/85/2025-CJAS

Table 2. Factors enhancing the content of secondary metabolites of cannabis with antimicrobial action

Type of a factor	Reference
Genetic factors	
Identification of genetic factors controlling production of secondary metabolites across the genome to be used in targeted breeding	Chen et al. (2022)
Traditional breeding based on the combining ability of the traditional germplasm	Cai et al. (2021); Osterberger et al. (2022)
Documentation and archiving of genetic diversity in the global centres of *C. sativa* genetic resources as a prerequisite for directed breeding	Torkamaneh and Jones (2022)
Growing conditions and agronomy	
Using the environmental factors including soil characteristics that affect the secondary metabolism of cannabis	Payment and Cvetkovska (2023)
The known effects of the controlled growing conditions including hydropony on the cannabinoid metabolism both in industrial hemp and medicinal cannabis	Sikora et al. (2011); Malik et al. (2021); Jemison (2024)
Exploiting the time course of accumulation of cannabinoids and terpenes in *C. sativa* plants	Aizpurua-Olaizola et al. (2016); Malikova et al. (2024)
The effect of UV irradiation on secondary metabolism both in the open ground and under controlled conditions can be exploited	Grisebach and Ebel (1978); Marti et al. (2014); Sirangelo et al. (2023); Timmons and Mattson (2023)
Elicitation of plant defence reactions	
Enhanced production of secondary metabolites upon the treatment with pathogenic factors in shoot, root and inflorescences, demonstrated, e.g. for *Golovinomyces* spp., *Fusarium* spp., *Botrytis cinerea* and *Pythium* spp.	Punja and Rodriguez (2018); Balthazar et al. (2020); Hurgobin et al. (2021); Trono (2024)
Non-specific elicitors of the plant defence system like salicylic acid, methyl jasmonate, γ-aminobutyric acid or chitosan can be applied in cannabis to enhance secondary metabolism	Matton et al. (1990); Mirzamohammad et al. (2021); Garrido et al. (2022); Beleggia et al. (2023)
Soil contaminated with inorganic elicitors like copper ions can increase secondary metabolism, including cannabinoids THC and CBD	Quagliata et al. (2021); Cahill et al. (2024)

vided into 10 chromosomes, where 3 + 1 genome duplication events were detected (Gao et al. 2020). Two duplication events are shared by the closely related plant *Trema (Parasponia) orientale* of the same family *Cannabaceae*.

The knowledge of the full cannabis genome has found utilisation in the identification of key genes that were under selection for the development of specific traits. It has been demonstrated for Chinese traditional cultivars that functional genes related to flowering time, seed germination, seed size, embryogenesis, growth, and stress responses have been selected during the process of cannabis domestication (Chen et al. 2022). For example, the adaptation of cultivated cannabis to different photoperiods along the latitude gradient occurs through the regulation of the expression of the FT-like gene (Flowering locus T-like).

The approach of further breeding aimed at increasing the content of biologically active substances in hemp is feasible. The directed breeding can be based both on the combining ability of the traditional germplasm and on the mutational approach.

However, the application of genetic manipulation techniques, albeit justified for pure research purposes, would inevitably lead to legal constraints and negative public opinion in most countries. The fact that the resources of *C. sativa* for traditional breeding have been concentrated in several global centres of plant genetic resources can be considered as an advantage (Cai et al. 2021; Osterberger et al. 2022), although the system of archiving and documentation of cannabis genetic diversity is still considered to be far from complete (Torkamaneh and Jones 2022).

https://doi.org/10.17221/85/2025-CJAS

Modern association studies including the advancement of the genomics of *C. sativa* should lead to the identification of genetic factors controlling the production of secondary metabolites across the genome. The paradigm of such an approach is provided by a comprehensive study on the factors controlling the traits of sex determination and flowering time in this species performed by Petit et al. (2020).

Cultivation conditions and agronomy

The methods of growing hemp plants can affect the content of different low-molecular-weight substances. The effect of environmental factors including soil properties on the activation of the secondary metabolism of cannabis has recently been summarised by Payment and Cvetkovska (2023). The effect of the growing conditions on the cannabinoid metabolism has been characterised many times, e.g. for medicinal cannabis by Jemison (2024) and for industrial hemp by Sikora et al. (2011).

Moreover, the effect of the vegetation phase on the cannabinoid spectrum and accumulation has been thoroughly described by Jemison (2024) in medicinal hemp and by Sikora et al. (2011) for industrial varieties. The time course of accumulation of cannabinoids and terpenes in three chemotypes of *C. sativa* plants has been characterised by Aizpurua-Olaizola et al. (2016) at one-week resolution when growing under controlled conditions. Flower and leaf samples were analysed for eight major cannabinoids using HPLC-DAD and 28 terpenes using GC-FID combined with GC-MS. The tetrahydrocannabinolic acid/cannabidiolic acid (THCA/CBDA) ratio was sufficient to stably distinguish chemotypes during growth. The dynamics of terpene content followed the division into chemotypes as well.

In the particular case of hydroponic cultivation using a nutrient solution according to Malik et al. (2021) and the expanded clay, i.e. keramzite, the effect of the growth phase on the cannabinoid range was described by Malikova et al. (2024), when the stage at 7–8 weeks from sowing was associated with a maximum in cannabinoid accumulation.

Consequently, the harvesting date must be considered as another important factor determining the content of active compounds. A shift in harvesting might even lead to a switch to a different legal class of production, e.g. a different category of legal classification of cannabis products according to the content of psychoactive compounds. Namely, the critical level is 0.3% of THC in the EU legislation as promoted by the European Union Drugs Agency – EUDA (until July 2, 2024 acting as EMCDDA – European Monitoring Centre for Drugs and Drug Addiction). Naturally, the EU legislation is harmonised with most of global legislation supported by the United Nations Office on Drugs and Crime (UNODC).

The comprehensive picture of chemical variability in cannabis on a time scale provides a framework for directed breeding for enhanced content of desirable chemical compounds. While standardised cultivation experiments are carried out in a fully controlled environment, including artificial light provided by fluorescent lamps in combination with wire bulbs, the plants grown in the open ground are often exposed to the effect of UV irradiation contained in sunlight. The UV component can affect the secondary metabolism in a pronounced way. This phenomenon is well known and exemplified for alpine plants (Grisebach and Ebel 1978). Therefore, the influence of UV light on the content of secondary compounds in the hemp aboveground parts was examined by Timmons and Mattson (2023). As a result of a short-time exposure for 15, 30, and 60 min at 7 W m^{-2} of UV-B radiation at the end of flowering, the cannabinoid content increased only slightly and below the significance threshold. Nevertheless, the influence of the elevated altitudes of growing sites should generally be considered when growing hemp for feed (Giupponi et al. 2020) and might contribute to the high activity of medicinal cannabis illegally grown and distributed in the mountain regions of Central Asia and Latin America (Trancoso et al. 2022).

Elicitation of plant defence reactions

This new approach has been taking shape over recent years. Although plant defence responses at the level of secondary metabolites with antimicrobial properties have been thoroughly described in many traditional species, starting from pea (Carlson and Dolphin 1981), cannabis seems to have escaped systematic study in this direction. Nevertheless, enhanced production of secondary metabolites upon the so-called elicitation process, i.e. after treatment

https://doi.org/10.17221/85/2025-CJAS

with stimuli mimicking the presence of pathogens, has been observed in a series of published works. Enhancing the accumulation of valuable bioactive compounds is potentially a way to increase the health effects of this crop.

A review by Sirangelo et al. (2023) attempted to summarise the current understanding of cannabis defence responses against common plant pathogens, including *Golovinomyces* spp. (a powdery mildew causal agent), *Fusarium* spp. (root and vessel pathogens), *Botrytis cinerea* (gray mould disease) and *Pythium* spp. (seedling damping-off), including the related disease-resistance genes of the host. The known defence-associated substances of cannabis have been summarised in the latest review by Trono (2024).

Additional studies have focused on the effects of elicitation in this expanding area of research on hemp. An effort to demonstrate cannabis plant immunisation at the seedling stage against the *Botrytis* mould infection using pre-treatment with *Pseudomonas* and *Bacillus* strains has not provided any positive evidence, however, the defence response to the *B. cinerea* inoculation by itself was recorded (Balthazar et al. 2020). On the basis of synthesis of the data for other plant groups and published observations in *C. sativa*, the set of host defence genes *ERF1*, *HEL*, *PAL*, *PR1*, and *PR2* was suggested to form the hemp response to pathogens and the resulting activation of secondary metabolism (Sirangelo et al. 2023). This paper is probably the first attempt to describe the cannabis defence response at the molecular level.

The consequences of plant defence responses are observable in the marijuana shoot as well as in the root tissues (Punja and Rodriguez 2018), and also in inflorescences (Hurgobin et al. 2021). Different kinds of known elicitors have been shown to be active in cannabis, which means the same as being recognised by the plant receptors. Salicylic acid (SA), which is known to be a non-specific elicitor of the plant defence system (Matton et al. 1990), acted in this species as well (Mirzamohammad et al. 2021; Garrido et al. 2022).

Another non-specific elicitor chitosan, a substance known to mimic the presence of pathogenic fungi, has been demonstrated to be active in cannabis upon foliar application (Beleggia et al. 2023). Differentially accumulated metabolites were monitored in the inflorescences of cv. Codimono treated with chitosan of three molecular weight fractions in solutions of 25 mg/l and 50 mg/l. The induced metabolites included cannabinoids, carotenoids, tocopherols, flavonoids and phenolic acid classes.

Similarly to the traditional plant species used in the studies of defence responses like tobacco, soybean or *Arabidopsis*, the suspension cell culture turned out to be a useful model system for *C. sativa* (Gabotti et al. 2019). In the case of industrial hemp (Futura variety), the well-known nonspecific elicitor methyl jasmonate (MeJa) increased both the activity and expression of the key enzymes of the isoprenoid synthesis pathway, namely phenylalanine ammonia-lyase (PAL) and tyrosine aminotransferase (TAT). As expected, the MeJa elicitation combined with tyrosine supplementation as an additional PAL substrate enhanced radical scavenging activity and antioxidant activity in a human erythrocyte model.

Another general factor associated with eliciting plant defence responses, UV irradiation, was tested by Marti et al. (2014) for the ability to induce secondary metabolites in cannabis. Clear modifications of phenylpropanoid metabolism were detected that led to an increased level of cinnamic acid amides, stilbene derivatives and other stilbene-related compounds. These changes induced by UV-C radiation were interpreted as induction of phytoalexins, i.e. plant antibiotics upregulated by infection (Carlson and Dolphin 1981). The analogy with the genus *Vitis* was likely considered since stilbenes are typical phytoalexins in grapevine.

To what extent the general defence response in cannabis involves the cannabinoid class of molecules was addressed by Garrido et al. (2022) in the context of medical cannabis. Signalling molecules known to be involved in the plant defence response, namely salicylic acid (SA), jasmonic acid (JA), and γ-aminobutyric acid (GABA), were tested by foliar application. SA, MeJA, and GABA produced changes in the accumulation of the two major cannabinoids, CBDA and THCA, in leaves and inflorescences of a medical cannabis variety. The combination of SA and MeJA at 0.1 mM increased CBDA and THCA accumulation in leaves by up to 57.3%.

Not surprisingly, growing hemp in the soil contaminated with copper, although feasible (Quagliata et al. 2021), has been demonstrated to cause an increase in secondary metabolites, including THC and CBD (Cahill et al. 2024). This observation fully corresponds to the known elicitor activity of cop-

https://doi.org/10.17221/85/2025-CJAS

per ions, as well as other heavy metal salts, as described in a model species like pea (Carlson and Dolphin 1981).

In summary, the presence of inducible compounds should also be taken into account when using hemp as feed. This principle of considering inducible secondary metabolites is already established in human dietology (Jeandet et al. 2014).

Taking into account the assumed origin of the increased level of cannabinoids in traditional and medicinal cultivars of cannabis as a consequence of genetic deregulation of the corresponding pathways, the effects of genetic mutations resulting in the spontaneous activation of defence metabolism are not excluded. On the other hand, such a mutational change might represent a way for obtaining a cannabis line with an improved antimicrobial effect.

ANTIMICROBIAL RESISTANCE

Mechanisms of cannabinoid action

It should be realised that two different types of cannabinoid action are considered while they should be associated with two different mechanisms. The one is psychotropic action. The other is antimicrobial action, which is also ascribed to this class of compounds. On the other hand, the terpenoid class of compounds and other substances of secondary metabolism are mostly treated only with respect to their antimicrobial action or for their role of synergetic enhancers of the primary action of cannabinoids.

Moreover, two levels of action must be considered in the case of antimicrobial compounds. While the psychotropic action is mediated only by the organism of humans or animals, the antimicrobial action may be expressed both on the microorganism level and on the level of the host cell. As stated by Sionov and Steinberg (2022): "while much is known about the cannabinoid targets in mammalians, little has been known about the microbial targets of these compounds so far". It is logical to postulate the existence of specific targets for this group of compounds in the microbes.

The perception of cannabinoids in the organism of animals, including humans, is mediated by cannabinoid receptors. They are denoted as CB1 and CB2 in mammals, and in a corresponding manner in the main livestock species including cattle. In addition to their role in affecting the neural system, cannabinoid receptors are also present in immune cells. Consequently, cannabinoids can modulate the immune system directly (Hernandez-Cervantes et al. 2017). It is an important feature of cannabinoids that both kinds of their action are mediated by the same receptor system, i.e. the same system of CB receptors is supposed to mediate the psychotropic and the antimicrobial action of cannabinoids (Karas et al. 2020; Wu et al. 2023).

The range of overlapping specific effects of individual cannabinoids in animals has been described by Aizpurua-Olaizola et al. (2016). For example, CBD is associated with the antipsychotic and neuroprotective action but it is considered to be nontoxic (Brunetti et al. 2020; Wu et al. 2023). On the other hand, Δ^9-THC acts as a partial agonist of the CB1 receptor and is considered toxic (Lambert and Fowler 2005). The action of cannabinoids also extends to the cardiovascular system: the adverse effect is ascribed to Δ^9-THC, which again acts via the CB1 receptor. On the other hand, CBD is supposed to exert a beneficial effect on the cardiovascular system (Pacher et al. 2018). A pathway of cannabinoid detoxification and catabolism is postulated, cytochrome P450 being the main member (Stout and Cimino 2014).

For understanding the psychotropic action of cannabinoids, the original physiological role of the perception system must be taken into account. The system of cannabinoid receptors evolved from the perception of the so-called endocannabinoid compounds. They are of endogenous origin, in contrast to cannabinoids in the true sense, and comprise, among others, the endogenous lipids 2-arachidonoyl glycerol (2-AG) and arachidonoyl ethanolamide (anandamide) (Lu and Mackie 2016). Their original physiological role consists in the psychological stabilisation of an individual and their disturbance leads to psychotic symptoms. However, the same receptor system is involved in the perception of cannabis metabolites and in the formation of their addictive effects.

The question remains as to what extent the second major effect of cannabinoids in addition to the psychotropic one, i.e. their role as antimicrobial agents, is mediated by the cannabinoid receptor system of animal hosts. Most of the reported antimicrobial effects of the cannabinoid fraction (Appendino et al. 2008; Malikova et al. 2024;

https://doi.org/10.17221/85/2025-CJAS

Paulova et al. 2025) reflect a direct interaction with microorganisms of both bacterial and fungal type, as implied by the *in vitro* assay systems. However, the beneficial effect of cannabinoids on infection resistance in eukaryotic organisms may also be just a consequence of the primary effect on the immune system since it is known that the activation of cannabinoid receptors CB1 and CB2 can enhance the immunity function (Hernandez-Cervantes et al. 2017).

An important factor in understanding the full scope of antimicrobial cannabinoid action is the synergism with other phytochemicals or even with traditional antibiotics. The differences in pharmaceutical properties of cannabis extracts from those expected for the particular chemotypes have been attributed to interactions, defined as "entourage effect", between cannabinoids and terpenes as a consequence of their synergism (Calvi et al. 2018). The reports on demonstrated synergies between individual cannabinoids and between cannabinoids and terpenoids have been summarised in detail by Chacon et al. (2022). However, most of the published effects can be related to the psychotropic activity of cannabinoids and/or cannabis terpenes. Information on the antimicrobial action as enhanced by additional hemp metabolites is still limited. It is expected that progress in this direction will be achieved.

The impact of antimicrobial resistance in livestock

The growing interest in natural crop-derived antimicrobials or in finding ways to enhance these substances in plants reflects the growing global problem of antimicrobial resistance (AMR), the related ineffectiveness of the available antibiotics, and the failure to find new antibiotics. The current state of the spread of AMR in bacteria pathogenic to farm animals and the consequences for the possibilities of treatment are summarised in Table 3.

Although antibiotics have essential applications in animal husbandry and livestock production (Williams-Nguyen et al. 2016), their increasing use and misuse in all sectors leads to the spread of AMR (Salam et al. 2023). This is partly because the antibiotics have long been administered to livestock not only to treat infections, but also to improve feed conversion efficiency and to prevent diseases. As a result, the bacteria have been subjected to incredible selective pressure, rapidly developing new survival strategies through mechanisms responsible for antibiotic resistance (Kirchhelle 2018). This is why AMR is now considered one of the most formidable global threats of the 21st century (Ahmed et al. 2024). The incidence and prevalence of infections caused by antimicrobial-resistant bacteria during the 21st century threaten the global public health as a silent pandemic and urgently require intervention (Padiyara et al. 2018; Salam et al. 2023; Ahmed et al. 2024).

Excessive use of antibiotics in livestock for treatment or growth promotion produces reservoirs of resistance. The current state with the main veterinary pathogens (*E. coli*, *S. typhimurium*, *C. perfringens*, *L. monocytogenes*, *Mycoplasma bovis*, *Campylobacter jejuni*, *S. aureus*, *C. difficile*, *K. pneumoniae*) has been reviewed by Doyle (2015), Halawa et al. (2023), Ahmed et al. (2024), by Robi et al. (2024) for cattle pathogens and by Kasanga et al. (2024) for *E. coli*. Resistant microbes have spread to the wider environment through fertilisers, contamination of waterways and consumer products.

In addition, other reservoirs of antibiotic residues which induce resistance in microbes on a global scale are foods of animal origin. These residues arise from the industrial use of antibiotics. Generally, the use of antibiotics should not be supported in the production of food for human consumption, basically because of the possible accumulation of residues in popular products such as meat, milk, eggs or honey. The levels of residues vary according to the geographical location, the level of development of the country, and the type of food analysed, with a higher risk in developing countries where no control measures or monitoring programs for antibiotic residues in food are implemented (Founou et al. 2016; Ghimpeteanu et al. 2022). This assumption is supported by a recent survey in Africa, in which higher residues in Nigeria (Ganiyat and Lateefat 2022), Tanzania (Kimera et al. 2015) or South Africa (Ramatla et al. 2017; Oladeji et al. 2025) have been reported. However, increased residue contents are also reported in recent studies from many other countries such as Italy, Argentina, Türkiye, Brazil and others (Nchima et al. 2017; Ghimpeteanu et al. 2022; Virto et al. 2022; Owusu-Doubreh et al. 2023; Adegbeye et al. 2024; Costa et al. 2024).

Czech Journal of Animal Science, 70, 2025 (9): 357–382

https://doi.org/10.17221/85/2025-CJAS

Table 3. Occurrence of main drug-resistant bacteria in livestock and consequences

Bacterial taxon	Host species of livestock	Resistance documented	Actually applicable treatment
Acinetobacter spp. (e.g. *A. baumannii*) (surveyed by Doyle et al. 2015)	sheep, goat, poultry, swine, cattle (Askari et al. 2019; Xu et al. 2022)	cortrimoxazol, tetracycline, trimethoprim, ampicillin-sulbactam, fluoroquinolones (ciprofloxacin, levofloxacin), aminoglycosides (gentamicin, tobramycin, amikacin, streptomycin), β-lactams (penicillin, carbapenems – imipenem, meropenem, cephalosporins – ceftazidime, cefepime), sulphonamides (Askari et al. 2019; Kyriakidis et al. 2021; Xu et al. 2022)	piperacillin-tazobactam, ampicillin-sulbactam, fluoroquinolones (ciprofloxacin), aminoglycosides, trimethoprim-sulfamethoxazol, β-lactams (including cephalosporins – ceftazidime, cefepime, and carbapenems – meropenem, imipenem-cilastatin) (Kanafani and Kanj 2023)
Campylobacter spp. (e.g. *C. jejuni, C. coli*) (surveyed by Pandey et al. 2024)	poultry, swine (Portes et al. 2023)	fluoroquinolones, β-lactams, tetracycline, macrolides (Enshaie et al. 2025)	macrolides (erythromycin), fluoroquinolones (Trott et al. 2021; Portes et al. 2023)
Clostridium spp. (e.g. *C. difficile, C. perfingens*) (surveyed by Doyle 2015)	cattle, poultry (Mora et al. 2020; Khan et al. 2021)	β-lactams, aminoglycosides (gentamicin, streptomycin, neomycin), tetracycline, sulphonamides, lincomycin amikacin, macrolides (erythromycin, spiramycin), rifampicin, chloramphenicol, spectinomycin, tylosin-fosfomycin, fluoroquinolone (ciprofloxacin, flumequine, norfloxacin, enrofloxacin), tetracycline (doxycycline, oxytetracycline), colistin, pefloxacin, trimethoprim-sulfamethoxazol (Osman and Elhariri 2013; Khan et al. 2021; Xu et al. 2022)	tetracycline, penicillin (amoxicillin, ampicillin), cefradine, fosfomycin, florfenicol, bacitracin, lincomycin (Osman and Elhariri 2013; Hargis and Swayne 2024; Enshaie et al. 2025)
Enterococcus spp. (e.g. *E. faecium, E. hirae, E. durans, E. gallinarum, E. casseliflavus, E. faecalis*) (surveyed by Doyle 2015)	swine poultry cattle sheep (Ekore et al. 2022; Xu et al. 2022)	erythromycin, tetracycline, rifampicin, vancomycin (Ekore et al. 2022; Xu et al. 2022)	tetracycline (Ekore et al. 2022)
Escherichia coli (surveyed by Pandey et al. 2024)	cattle, swine, poultry (Mann et al. 2011; Barros et al. 2023; Berchtold and Constable 2009)	penicillin (ampicillin), ceflasporines, colistin, carbapenems, β-lactams, aminoglycosides (gentamicin, streptomycin), tetracycline, sulphonamides, erythromycin, sulfamethoxazole/trimethoprim, chloramphenicol, kanamycin (Abdelwahab et al. 2022; Xu et al. 2022; Ahmed et al. 2024; Enshaie et al. 2025)	tetracycline, oxytetracycline, penicillin, ampicillin, amoxicillin, oxacillin, cefoxitin, gentamicin, sulfamethoxazole/trimethoprim (co-trimoxazole), enrofloxacin, ceftiofur (Abdelwahab et al. 2022; Enshaie et al. 2025)
Mycoplasma spp. (surveyed by Gautier-Bouchardon 2018; Enshaie et al. 2025)	ruminants, poultry (Gautier-Bouchardon 2018)	macrolides, fosfomycin, glycopeptides, β-lactams, sulphonamides, trimethoprim, polymyxin, rifampicin (Gautier-Bouchardon 2018; Enshaie et al. 2025)	macrolides (tylosin, tulathromycin, tilmicosin, erythromycin), tetracycline, lincosamides, fluoroquinolones, pleuromutilin, fenicol aminoglycosides (Gautier-Bouchardon 2018; Enshaie et al. 2025)

https://doi.org/10.17221/85/2025-CJAS

Table 3 to be continued

Bacterial taxon	Host species of livestock	Resistance documented	Actually applicable treatment
Salmonella spp. (surveyed by Pandey et al. 2024)	poultry, calves, swine, ruminants (Berchtold and Constable 2009; Soliani et al. 2023; Garcia-Diez et al. 2024)	aminoglycosides, cephalosporin, lincosamides, penicillin, fluoroquinolones, β-lactams, tetracycline, sulphonamides, ofloxacin, lincomycin, cloxacilin (Reygaert 2018; Xu et al. 2022; Enshaie et al. 2025)	macrolides, chlortetracycline, oxytetracycline, tetracycline, neomycin, azithromycin, cephalosporin, quinolone (Berchtold and Constable 2009; Trott et al. 2021; Garcia-Diez et al. 2024)
Staphylococcus spp. (e.g. *S. aureus*) (surveyed by Pandey et al. 2024)	cattle, ruminants, poultry, swine (Fitzgerald 2012; Islam et al. 2025)	methicillin, macroclides (azithromycin), linkosamides, streptogramin type B, fenicol, cycloserine/fusidic acid, vancomycin, colistin, penicillin (ampicillin, amoxicillin), tetracycline, chlortetracycline, kanamycin, gentamicin (Xu et al. 2022; Enshaie et al. 2025; Islam et al. 2025)	β-lactams, fluoroquinolones, tetracyclines, clindamycin, trimethoprim-sulfamethoxazole, vancomycin (Sato et al. 2018; Campbell et al. 2022; Zhang et al. 2023; Stefanetti et al. 2024)
Streptococcus spp. (*S. agalactiae*, *S. dysgalactiae*, *S. uberis*) (surveyed by Fenta et al. 2025)	cattle, swine (Dame-Korevaar et al. 2025; Islam et al. 2025)	tetracycline, macrolides (erythromycin), penicillin (ampicillin), oxytetracykline, streptomycin, enrofloxacin (Kabelitz et al. 2021; Fenta et al. 2025)	β-lactams, amoxicillin, ampicillin (Kabelitz et al. 2021; Dame-Korevaar et al. 2025)

In addition, monitoring data indicate an uneven distribution of antibiotics in animal tissues and products. Specifically in sheep, higher amounts of tetracycline were reported in kidney than in liver or muscle (Sallam et al. 2022). In terms of different products, tetracyclines are found at higher concentrations in cottage cheese or cheese than in products such as butter, buttermilk, whey or cream (Gajda et al. 2018). However, the distribution and concentration of individual antibiotic residues can be influenced by a wide range of factors including the class of antibiotics, food processing methods, the nature of specific antibiotics, the rate of elimination from the organism, the withdrawal period, the species and physiology of the animal, the -philic and -phobic nature of antibiotics, different metabolism in individual tissues, and last but not least drug-protein interactions (Hassan et al. 2021; Sallam et al. 2022; Virto et al. 2022; Adegbeye et al. 2024).

All these factors increase the risks of generation and transmission of multidrug resistant bacteria, for example *Campylobacter* or *Salmonella* (Doyle 2015). The impact of AMR in relation to livestock animals is particularly evident in the burden of foodborne pathogens, which is estimated to reach 600 million infections and 420 000 deaths each year (WHO 2015). As a result, treatment options for resistant pathogens become increasingly limited. The rising incidence of epidemics in livestock, in turn, often necessitates the culling of animals, which leads to significant economic losses and threats to food supplies (Ahmed et al. 2024). The administration of antimicrobial drugs in livestock is not generally distinct from their bulk use in agriculture, as the two systems are closely linked (Kirchhelle 2018).

Although livestock account for only about 2 percent of the global economy, their relative importance as a determinant of health and prosperity is greater, and especially so in the case of low and lower-middle income countries.

Therefore, the economic impacts of AMR on livestock production are more acutely felt in low-income countries. A report from the World Bank modelled an associated drop in livestock production of 3.1 to 11.1 percent in low-income countries and 3.1 to 8.9 percent in lower-middle income countries (Ahmed et al. 2017).

The growing body of scientific research in this area supports the implementation of measures to limit

https://doi.org/10.17221/85/2025-CJAS

the use of antimicrobial drugs and the application of antimicrobial surveillance programmes, which will lead to a reduction of resistance in animals (Patel et al. 2023). It is not surprising that the search for substances with antibiotic properties of plant, not microbial, origin started on a large scale, including the study of the promising group of medium-chain fatty acids from palm oils (Lalouckova et al. 2019). The quantification of the specific impacts of AMR on the productivity of the livestock production system is still limited in the framework of existing reports (Ahmed et al. 2024). Therefore, a reliable modelling of the actual burden of resistance is extremely useful. Part of the problem stems from uncertainty about the best ways to monitor AMR in humans, animals, and the environment (Wernli et al. 2017).

PROSPECTS FOR THE USE OF HEMP ANTIMICROBIAL AGENTS IN ANIMAL PRODUCTION

The data collected on the effects of hemp inclusion in the diet of monogastric animals has recently been summarised by Lanzoni et al. (2024b). The promising results of investigations on the application of hemp or hemp-derived compounds in animals published to date cover a range of animal species. The accumulated data supports the policy of the European Food Safety Authority FEEDAP panel as claimed in the 2011 report (EFSA 2011).

In the general approach, crude ethanol extract from the inflorescences of *C. sativa* cv. Futura 75 (a technical hemp variety) was tested directly against the bacterial pathogens *L. monocytogenes*, *S. typhimurium*, *Escherichia coli* and *Staphylococcus* spp. on the nutrient agar plates with CBD as a reference compound. When spotted on agar, all products were efficient only against Gram-positive bacteria. Specifically, the extracts corresponding to 0.017 and 0.3 mg/ml CBD were effective against *L. monocytogenes* and *Staphylococcus* spp., respectively. However, all bacteria including the Gram-negative family of *Enterobacteriaceae* and coliforms were suppressed at least during meat storage (Pasquali et al. 2020).

Nevertheless, the beneficial effect of a hemp diet on the health of domestic species has been reported independently of any targeted phytochemical research. For example, a study of the effect of hemp seed oil supplementation on the plasma lipid profile, liver function, milk fatty acid, cholesterol, and vitamin A content in goats was carried out (Cozma et al. 2015).

The practical application of cannabinoids has also been tested in the model of cattle. The *in vitro* effect of CBD on ruminal microflora was estimated by incubating the rumen fluid on agarised amino-acid medium for 24 h in the presence of CBD. Although the highest concentration of CBD caused a reduction in produced ammonia by 10–15 mM at the highest tested concentration (860 µg CBD/ml), this approach is not yet considered as ready for use in practice (Lakes et al. 2024).

The same approach in poultry can be illustrated by the specific case of laying hens. The addition of 9% of hemp co-product had a documented positive effect on egg production and on yolk quality (Lanzoni et al. 2025). In an effort to employ CBD against *Clostridium perfringens* in broiler chickens, 30 g CBD/1 000 g of feed was administered following a challenge with *C. perfringens* bacteria and lipopolysaccharide added to feed. The CBD treatment helped to maintain blood biochemical and antioxidant parameters and finally it increased the body weight (Bien et al. 2024). To identify the mechanism of CBD effect on *C. perfringens* in broiler chickens, CBD was applied in combination with selenium nanoparticles. As a result, the upregulation of genes for the gut barrier function was observed, in parallel to shifts in the gut bacterial enzyme activity towards the increased energy uptake. Collagenase was upregulated as well (Konieczka et al. 2020).

Some positive effects can be indirectly caused by hemp products just by stimulating the capacity of the immune system. Accordingly, the inclusion of dried *Cannabis sativa* leaves in the feed mixture of broiler chickens increased the CD4(+) and CD8(+) lymphocyte subpopulations and it also increased the CD4(+) : CD8(+) cell ratios (Balenovic et al. 2024). Consistently, different levels of hemp seeds in the diet of broiler chickens led to a significant reduction in serum lipids and total cholesterol. In parallel, coliform counts in the caecum and jejunum were reduced linearly with the hemp seed percentage (Vispute et al. 2019).

In horses, CBDA or the combination of CBG/CBD were tested for the alleviation of chronic osteoarthritis. The ethanolic leaf extracts of *C. sativa* enabled pain modulation and welfare improvement.

https://doi.org/10.17221/85/2025-CJAS

In the used extract, the major cannabinoids detected were CBD and Δ^9-THC (Aragona et al. 2024).

Cannabis products may also find application in such unusual disease models as scrapie in sheep, a specific case of transmissible spongiform encephalopathy. Application of CBD inhibited the neurotoxicity of protease-resistant prion protein and also affected the microglial cell migration (Dirikoc et al. 2007).

There is a general assumption that cannabis-derived compounds can possess antimicrobial activity based on a different mode of action from traditional antibiotics. They can thus avoid the already established microbial resistance and do not impose any additional selection pressure on promoting new genetic resistance factors in pathogens. Genetic recombination in the environment and in the hosts leads to the spread of multidrug-resistant bacteria (MDR), which can disrupt even well-organised medical care. To illustrate the seriousness of the situation, it suffices to note that only four new classes of antibiotics have been developed and approved during the last four decades (Homer et al. 2025). This trend has been a source of growing concern since 2011 (Cooper and Shlaes 2011).

The presence of antimicrobial compounds in the shoot of cannabis is fully compatible with its use in animal forage. This automatically solves the problem of delivery, which is perceived with traditional antibiotics that have to be artificially combined with feed or administered by injection. These promising prospects for cannabis as an antimicrobial agent were discussed in the review by Karas et al. (2020).

CONCLUSION

Although the antimicrobial activity of cannabis has been known since the early 1950's, interest in its potential use for livestock production and veterinary solutions has surged in recent years. The main reason for this turn is undoubtedly the spread of genetic determinants of resistance among veterinary pathogens and further. This process in its turn has led to massive restrictions on the exploitation of traditional antibiotics in agriculture and veterinary practice.

The possibility of replacing traditional antibiotics with antimicrobial substances from fodder plants has prompted intensive research focused on this goal in cannabis. This direction brought almost exhaustive data on the array of secondary metabolites in cannabis and on their associated antimicrobial activities. Moreover, strategies for enhancing or modifying the composition of cannabis secondary metabolites are taking shape, whether through the selection of genotype, by cultivation conditions and technology, or the targeted elicitation of hemp metabolites involved in the natural response to the microbial pathogens of plants (phytoalexins). Using the synergy between the cannabis metabolites and the traditional antibiotics is taking shape as well. The cannabinoid group of compounds remains the first choice due to the combination of the antimicrobial activity with positive effects on animal physiology and their advanced characterisation.

Although the collected research data allow considering a large-scale use of cannabis feed or other products for reducing the veterinary infections, the testing under production conditions still has to be pursued on a large scale. Nevertheless, support to this type of projects on a production or pilot scale can be expected given the research data collected to date.

Acknowledgement

We thank our colleagues for critical reading and corrections to the manuscript and the agency MERIVA collaborators for the amendments to the text.

REFERENCES

Abdelwahab GE, Ishag HZA, Al Hammadi ZM, Al Yammahi SMS, Mohd Yusof MFB, Al Yassi MSY, Al Neyadi SSA, Al Mansoori AMA, Al Hamadi FHA, Al Hamadi IAS, Hosani MAAA, Mohammed Al Muhairi SS. Antibiotics resistance in Escherichia coli isolated from livestock in the Emirate of Abu Dhabi, UAE, 2014–2019. Int J Microbiol. 2022 Apr 26;2022:3411560.

Adegbeye MJ, Adetuyi BO, Igirigi AI, Adisa A, Palangi V, Aiyedun S, Alvarado-Ramirez ER, Elghandour MMMY, Molina OM, Oladipo AA, Salem AZM. Comprehensive insights into antibiotic residues in livestock products: Distribution, factors, challenges, opportunities, and implications for food safety and public health. Food Control. 2024 Sep;163:110545.

Ahmed SA, Baris E, Go DS, Lofgren H, Osorio-Rodarte I, Thierfelder K. Assessing the global economic and poverty

https://doi.org/10.17221/85/2025-CJAS

effects of antimicrobial resistance. Policy research working paper. World Bank Group; 2017 Jun: 8133.

Ahmed SK, Hussein S, Qurbani K, Ibrahim RH, Fareeq A, Mahmood KA, Mohamed MG. Antimicrobial resistance: Impacts, challenges, and future prospects. J Med Surg Public Health. 2024 Apr;19(2):100081.

Aizpurua-Olaizola O, Soydaner U, Ozturk E, Schibano D, Simsir Y, Navarro P, Etxebarria N, Usobiaga A. Evolution of the cannabinoid and terpene content during the growth of Cannabis sativa plants from different chemotypes. J Nat Prod. 2016 Feb 26;79(2):324-31.

Alfei S, Schito GC, Schito AM. Synthetic pathways to non-psychotropic phytocannabinoids as promising molecules to develop novel antibiotics: A review. Pharmaceutics. 2023 Jul 5;15(7):1889.

Appendino G, Gibbons S, Giana A, Pagani A, Grassi G, Stavri M, Smith E, Rahman M. Antibacterial cannabinoids from Cannabis sativa: A structure – Activity study. J Nat Products. 2008 Aug;71(8):1427-30.

Aqawi M, Sionov RV, Friedman M, Steinberg D. The antibacterial effect of cannabigerol toward Streptococcus mutans is influenced by the autoinducers 21-CSP and AI-2. Biomedicines. 2023 Feb 22;11(3):668.

Aragona F, Tabbì M, Gugliandolo E, Giannetto C, D'Angelo F, Fazio F, Interlandi C. Role of cannabidiolic acid or the combination of cannabigerol/cannabidiol in pain modulation and welfare improvement in horses with chronic osteoarthritis. Front Vet Sci. 2024 Dec 10;11:1496473.

Askari N, Momtaz H, Tajbakhsh E. Acinetobacter baumannii in sheep, goat, and camel raw meat: Virulence and antibiotic resistance pattern. AIMS Microbiol. 2019 Sep 23; 5(3):272-84.

Balenovic M, Janjecic Z, Savic V, Kasap A, Popovic M, Simpraga B, Sokolovic M, Bedekovic D, Kis G, Zglavnik T, Spoljaric D, Krstulovic F, Listes I, Zelenika TA. Immunostimulatory and antibacterial effects of Cannabis sativa L. leaves on broilers. Animals (Basel). 2024 Apr 11; 14(8):1159.

Balthazar C, Cantin G, Novinscak A, Joly DL, Filion M. Expression of putative defense responses in Cannabis primed by Pseudomonas and/or Bacillus strains and infected by Botrytis cinerea. Front Plant Sci. 2020 Nov;11:572112.

Barros MM, Castro J, Araujo D, Campos AM, Oliveira R, Silva S, Outor-Monteiro D, Almeida C. Swine colibacillosis: Global epidemiologic and antimicrobial scenario. Antibiotics (Basel). 2023 Mar 30;12(4):682.

Beleggia R, Iannucci A, Menga V, Quitadamo F, Suriano S, Citti C, Pecchioni N, Trono D. Impact of chitosan-based foliar application on the phytochemical content and the antioxidant activity in hemp (Cannabis sativa L.) inflorescences. Plants (Basel). 2023 Oct 26;12(21):3692.

Berchtold JF, Constable PD. Antibiotic treatment of diarrhea in preweaned calves. In: Anderson DE, Rings M, Abrahamsen EJ, editors. Food animal practice. St. Louis: Saunders Elsevier; 2009. p. 520-5.

Bien D, Michalczuk M, Jozwik A, Matuszewski A, Konieczka P. Effects of Cannabis sativa extract on growth performance, meat physicochemical properties, and oxidative status in chickens challenged with Clostridium perfringens and lipopolysaccharide. Anim Sci Pap Rep. 2024 Mar 1;42(1):81-108.

Blaskovich MAT, Kavanagh AM, Elliott AG, Zhang B, Ramu S, Amado M, Lowe GJ, Hinton AO, Pham DMT, Zuegg J, Beare N, Quach D, Sharp MD, Pogliano J, Rogers AP, Lyras D, Tan L, West NP, Crawford DW, Peterson ML, Callahan M, Thurn M. The antimicrobial potential of cannabidiol. Commun Biol. 2021 Jan 19;4(1):7.

Bloomfield MAP, Yamamori Y, Hindocha C, Jones APM, Yim JLL, Walker HR, Statton B, Wall MB, Lees RH, Howes OD, Curran VH, Roiser JP, Freeman TP. The acute effects of cannabidiol on emotional processing and anxiety: A neurocognitive imaging study. Psychopharmacology (Berl). 2022 May;239(5):1539-49.

Brkljaca N, Durovic S, Milosevic S, Gasic U, Pankovic D, Zekovic Z, Pavlic B. Sequential extraction approach for sustainable recovery of various hemp (Cannabis sativa L.) bioactive compounds. Sustain Chem Pharm. 2023 Oct; 35:101213.

Brunetti P, Lo Faro AF, Pirani F, Berretta P, Pacifici R, Pichini S, Busardo FP. Pharmacology and legal status of cannabidiol. Ann Ist Super Sanita. 2020 Jul-Sep;56(3):285-91.

Cahill MS, Arsenault T, Bui TH, Zuverza-Mena N, Bharadwaj A, Prapayotin-Riveros K, White JC, Dimkpa CO. Copper stimulation of tetrahydrocannabinol and cannabidiol production in hemp (Cannabis sativa L) is copper-type, dose, and cultivar dependent. J Agric Food Chem. 2024 Apr 3;72(13):6921-30.

Cai S, Zhang Z, Huang S, Bai X, Huang Z, Zhang YJ, Huang L, Tang W, Haughn G, You S, Liu Y. CannabisGDB: A comprehensive genomic database for Cannabis Sativa L. Plant Biotechnol J. 2021 May;19(5):857-9.

Calvi L, Pentimalli D, Panseri S, Giupponi L, Gelmini F, Beretta G, Vitali D, Bruno M, Zilio E, Pavlovic R, Giorgi A. Comprehensive quality evaluation of medical Cannabis sativa L. inflorescence and macerated oils based on HS-SPME coupled to GC-MS and LC-HRMS (q-exactive orbitrap®) approach. J Pharm Biomed Anal. 2018 Feb 20; 150:208-19.

Campbell AJ, Dotel R, Braddick M, Britton PN, Eisen DP, Francis JR, Lynar S, McMullan B, Meagher N, Nelson J, O'Sullivan MVN, Price DJ, Robinson JO, Whelan A, Tong SYC, Bowen AC, Davis JS. Clindamycin adjunctive ther-

https://doi.org/10.17221/85/2025-CJAS

apy for severe Staphylococcus aureus treatment evaluation (CASSETTE)-an open-labelled pilot randomized controlled trial. JAC Antimicrob Resist. 2022 Feb 17; 4(1):dlac014.

Carlson RE, Dolphin DH. Chromatographic analysis of isoflavonoid accumulation in stressed Pisum sativum. Phytochemistry. 1981;20(9):2281-4.

Chacon FT, Raup-Konsavage WM, Vrana KE, Kellogg JJ. Secondary terpenes in Cannabis sativa L.: Synthesis and synergy. Biomedicines. 2022 Dec 6;10(12):3142.

Chakraborty S, Afaq N, Singh N, Majumdar S. Antimicrobial activity of Cannabis sativa, Thuja orientalis and Psidium guajava leaf extracts against methicillin-resistant Staphylococcus aureus. J Integr Med. 2018 Sep;16(5):350-7.

Chen X, Guo HY, Zhang QY, Wang L, Guo R, Zhan YX, Lv P, Xu YP, Guo MB, Zhang Y, Zhang K, Liu YH, Yang M. Whole-genome resequencing of wild and cultivated cannabis reveals the genetic structure and adaptive selection of important traits. BMC Plant Biol. 2022 Jul 27;22(1):371.

Chimalizeni HY, Kawaza K, Molyneux E. The epidemiology and management of non typhoidal salmonella infections. In: Finn A, Curtis N, Pollard AJ, editors. Hot topics in infection and immunity in children VI. Springer: New York, NY, USA; 2010. p. 33–46.

Clarke RC, Merlin MD. Cannabis domestication, breeding history, present day genetic diversity, and future prospects. Crit Rev Plant Sci. 2016;35(5-6):293-327.

CLSI – Clinical and Laboratory Standards Institute. CLSI M100 – Performance standards for antimicrobial susceptibility testing. 35th ed. Wayne, PA: CLSI; 2025.

Cooper MA, Shlaes D. Fix the antibiotics pipeline. Nature. 2011 Apr 7;472(7341):32.

Costa LV, Gebara C, Zacaroni OF, Freitas NE, Silva AND, Prado CS, Nunes IA, Cavicchioli VQ, Duarte FOS, Lage ME, Alencar FR, Machado BAS, Hodel KVS, Minafra C. Antibiotic residues in raw cow's milk: A systematic review of the last decade. Foods. 2024 Nov 24;13(23):3758.

Cozma A, Andrei S, Pintea A, Miere D, Filip L, Loghin F, Ferlay A. Effect of hemp seed oil supplementation on plasma lipid profile, liver function, milk fatty acid, cholesterol, and vitamin A concentrations in Carpathian goats. Czech J Anim Sci. 2015 Jul;60(7):289-301.

Dame-Korevaar A, Gielen C, van Hout J, Bouwknegt M, Faba L, Vrieling M. Quantification of antibiotic usage against Streptococcus suis in weaner pigs in the Netherlands between 2017 and 2021. Prev Vet Med. 2025 Feb;235:106400.

Dirikoc S, Priola SA, Marella M, Zsurger N, Chabry J. Nonpsychoactive cannabidiol prevents prion accumulation and protects neurons against prion toxicity. J Neurosci. 2007 Sep 5;27(36):9537-44.

Doyle ME. Multidrug-resistant pathogens in the food supply. Foodborne Pathog Dis. 2015 Apr;12(4):261-79.

EFSA – European Food Safety Authority, Panel on additives and products or substances used in animal feed (FEEDAP). Scientific opinion on the safety of hemp (Cannabis genus) for use as animal feed. EFSA J. 2011 Mar 14;9(3):2011.

Ekore DO, Onanga R, Nguema PPM, Lozano C, Kumulungui BS. The antibiotics used in livestock and their impact on resistance in Enterococcus faecium and Enterococcus hirae on farms in Gabon. Antibiotics (Basel). 2022 Feb 10; 11(2):224.

Enshaie E, Nigam S, Patel S, Rai V. Livestock antibiotics use and antimicrobial resistance. Antibiotics. 2025 Jun 19; 11(6):621.

European Union. Regulation (EU) 2021/2115 of the European Parliament and of the Council establishing rules on support for strategic plans... Eur Union Regul. 2021 Dec 2;2021/2115.

Fathordoobady F, Singh A, Kitts DD, Singh AP. Hemp (Cannabis sativa L) extract: Anti-microbial properties, methods of extraction, and potential oral delivery. Food Rev Int. 2019 Apr 22;35:7:664-84.

Fenta MD, Feleke MG, Feleke G, Mebratu AS, Mengistu BA, Demessie Y. Streptococcal infection and its antimicrobial resistance profile associated with bovine mastitis in Ethiopia: A systematic review and meta-analysis. Front Vet Sci. 2025 Mar 12;12:1503904.

Fischedick JT, Hazekamp A, Erkelens T, Choi YH, Verpoorte R. Metabolic fingerprinting of Cannabis sativa L., cannabinoids and terpenoids for chemotaxonomic and drug standardization purposes. Phytochemistry. 2010 Dec; 71(17-18):2058-73.

Fitzgerald JR. Livestock-associated Staphylococcus aureus: Origin, evolution and public health threat. Trends Microbiol. 2012 Mar 1;20(4):192-8.

Founou LL, Founou RC, Essack SY. Antibiotic resistance in the food chain: A developing country-perspective. Front Microbiol. 2016 Nov 23;7:1881.

Gabotti D, Locatelli F, Cusano E, Baldoni E, Genga A, Pucci L, Consonni R, Mattana M. Cell suspensions of Cannabis sativa (var Futura): Effect of elicitation on metabolite content and antioxidant activity. Molecules. 2019 Nov 9;24(22):4056.

Gajda A, Nowacka-Kozak E, Gbylik-Sikorska M, Posyniak A. Tetracycline antibiotics transfer from contaminated milk to dairy products and the effect of the skimming step and pasteurisation process on residue concentrations. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2018 Jan;35(1):66-76.

Galletta M., Reekie TA, Nagalingam G, Bottomley AL, Harry EJ, Kassiou M, Triccas JA. Rapid antibacterial ac-

https://doi.org/10.17221/85/2025-CJAS

tivity of cannabichromenic acid against methicillin-resistant Staphylococcus aureus. Antibiotics (Basel). 2020 Aug 16;9(8):523.

Ganiyat KO, Lateefat OB. Chemical composition, antioxidant and antimicrobial activities of volatile oils of Periscopsis laxiflora leaf and stem. Bull Chem Soc Ethiopia. 2022;36(3):717-25.

Gao S, Wang B, Xie S, Xu X, Zhang J, Pei L, Yu Y, Yang W, Zhang Y. A high-quality reference genome of wild Cannabis sativa. Hortic Res. 2020 May 2;7(1):73.

Garcia-Diez J, Moura D, Grispoldi L, Cenci-Goga B, Saraiva S, Silva F, Saraiva C, Ausina J. Salmonella spp. in domestic ruminants, evaluation of antimicrobial resistance based on the one health approach – A systematic review and meta-analysis. Vet Sci. 2024 Jul 14;11(7):315.

Garrido J, Rico S, Corral C, Sanchez C, Vidal N, Martinez-Quesada JJ, Ferreiro-Vera C. Exogenous application of stress-related signaling molecules affect growth and cannabinoid accumulation in medical cannabis (Cannabis sativa L.). Front Plant Sci. 2022 Dec 20;13:1082554.

Gautier-Bouchardon AV. Antimicrobial resistance in Mycoplasma spp. Microbiol Spectr. 2018 Jul;6(4):10.1128/microbiolspec.arba-0030-2018.

Ghimpeteanu OM, Pogurschi EN, Popa DC, Dragomir N, Dragotoiu T, Mihai OD, Petcu CD. Antibiotic use in livestock and residues in food – A public health threat: A review. Foods. 2022 May 16;11(10):1430.

Gildea L, Ayariga JA, Ajayi OS, Xu J, Villafane R, Samuel-Foo M. Cannabis sativa CBD extract shows promising antibacterial activity against Salmonella typhimurium and S. newington. Molecules. 2022 Apr 21;27(9):2669.

Giupponi L, Leoni V, Pavlovic R, Giorgi A. Influence of altitude on phytochemical composition of hemp inflorescence: A metabolomic approach. Molecules. 2020 Mar 18;25(6):1381.

Golombek P, Müller M, Barthlott I, Sproll C, Lachenmeier DW. Conversion of cannabidiol (CBD) into psychotropic cannabinoids including tetrahydrocannabinol (THC): A controversy in the scientific literature. Toxics. 2020 Jun 3;8(2):41.

Grisebach H, Ebel J. Phytoalexins, chemical defense substances of higher-plants. Angew Chem Int Ed. 1978 Sep;17(9):635-47.

Halawa EM, Fadel M, Al-Rabia MW, Behairy A, Nouh NA, Abdo M, Olga R, Fericean L, Atwa AM, El-Nablaway M, Abdeen A. Antibiotic action and resistance: updated review of mechanisms, spread, influencing factors, and alternative approaches for combating resistance. Front Pharmacol. 2023 Jan 12;14:1305294.

Hargis BM, Swayne DE. Necrotic enteritis in poultry. MSD Vet Manual. 2024.

Hassan MM, El Zowalaty ME, Lundkvist A, Jarhult JD, Khan Nayem MR, Tanzin AZ, Badsha MR, Khan SA, Ashour HM. Residual antimicrobial agents in food originating from animals. Trends Food Sci Technol. 2021 May;111:141-50.

Hernandez-Cervantes R, Mendez-Diaz M, Prospero-Garcia O, Morales-Montor J. Immunoregulatory role of cannabinoids during infectious disease. Neuroimmunomodulation. 2017;24:183-99.

Homer JA, Johnson RM, Koelln RA, Moorhouse AD, Moses JE. Strategic re-engineering of antibiotics. Nat Rev Bioeng. 2025 Mar;3(3):213-29.

Hurgobin B, Tamiru-Oli M, Welling MT, Doblin MS, Bacic A, Whelan J, Lewsey MG. Recent advances in Cannabis sativa genomics research. New Phytol. 2021 Apr;230(1):73-89.

Iseger TA, Bossong MG. A systematic review of the antipsychotic properties of cannabidiol in humans. Schizophr Res. 2015 Mar;162(1-3):153-61.

Iseppi R, Brighenti V, Licata M, Lambertini A, Sabia C, Messi P, Pellati F, Benvenuti S. Chemical characterization and evaluation of the antibacterial activity of essential oils from fibre-type Cannabis sativa L. (Hemp). Molecules. 2019 Jun 21;24(12):2302.

Islam M, Hossain I, Islam S, Azam G, Sultana S. Prevalence, antibiotic resistance patterns, and virulence factors of Staphylococcus aureus isolates associated with bovine mastitis in northern Bangladesh. Heliyon. 2025 Jan 16;11(2):e42107.

Jeandet P, Hebrard C, Deville MA, Cordelier S, Dorey S, Aziz A, Crouzet J. Deciphering the role of phytoalexins in plant-microorganism interactions and human health. Molecules. 2014 Nov 6;19(11):18033-56.

Jemison JM, Jr. Soil and weed management effects on floral hemp (Cannabis sativa L.) production and cannabinoid content. Agron J. 2024;116:1758-74.

Kabelitz T, Aubry E, van Vorst K, Amon T, Fulde M. The role of Streptococcus spp. in bovine mastitis. Microorganisms. 2021 Jul 14;9(7):1497.

Kanafani ZA, Kanj SS. Acinetobacter infection: Treatment and prevention [Internet]. UpToDate. 2023 Nov 8; [cited 2025 Feb 5]. Available from: https://www.uptodate.com/contents/acinetobacter-infection-treatment-and-prevention/print.

Karas JA, Labell JM, Wong LJM, Paulin OKA, Mazeh AC, Hussein MH, Li J, Velkov T. The antimicrobial activity of cannabinoids. Antibiotics (Basel). 2020 Jul 13;9(7):406.

Kasanga M, Shempela DM, Daka V, Mwikisa MJ, Sikalima J, Chanda D, Mudenda S. Antimicrobial resistance profiles of Escherichia coli isolated from clinical and environmental samples: findings and implications. JAC Antimicrob Resist. 2024 Apr 23;6(2):dlae061.

https://doi.org/10.17221/85/2025-CJAS

Kaur J, Sun N, Hill JE. Comprehensive profiling of terpenes and terpenoids in different Cannabis strains using GC × GC-TOFMS. Separations. 2023 Sep 13;10(9):500.

Khan MUZ, Humza M, Yang SL, Iqbal MZ, Xu X, Cai JP. Evaluation and optimization of antibiotics resistance profile against Clostridium perfringens from buffalo and cattle in Pakistan. Antibiotics (Basel). 2021 Jan 11;10(1):59.

Kimera ZI, Mdegela RH, Mhaiki CJN, Karimuribo ED, Mabiki F, Nonga HE, Mwesongo J. Determination of oxytetracycline residues in cattle meat marketed in the Kilosa district, Tanzania. Onderstepoort J Vet Res. 2015 Nov 27; 82(1):911.

Kirchhelle C. Patchwork regulation won't stop antimicrobial resistance. Nature. 2018;563:473.

Kleinhenz MD, Magnin G, Lin Z, Griffin J, Kleinhenz KE, Montgomery S, Curtis A, Martin M, Coetzee JF. Plasma concentrations of eleven cannabinoids in cattle following oral administration of industrial hemp (Cannabis sativa). Sci Rep. 2020 Jul 29;10:12753.

Konieczka P, Szkopek D, Kinsner M, Fotschki B, Juskiewicz J, Banach J. Cannabis-derived cannabidiol and nanoselenium improve gut barrier function and affect bacterial enzyme activity in chickens subjected to C. perfringens challenge. Vet Res. 2020 Nov 23;51:141.

Krejci Z. Antibakterialni ucinek Cannabis indica [Antibacterial action of Cannabis indica]. Lékařské listy. 1952; 7:500-3. Czech.

Krejci Z. Hemp (Cannabis sativa) – Antibiotic drugs. II. Method and results of bacteriological experiments and preliminary clinical experience. Pharmazie. 1958 Mar; 13(3):155-66.

Kyriakidis I, Vasileiou E, Pana ZD, Tragiannidis A. Acinetobacter baumannii antibiotic resistance mechanisms. Pathogens. 2021 Mar 19;10(3):373.

Lakes JE, Davis BE, Flythe MD. Activity of cannabidiol on ex vivo amino acid fermentation by bovine rumen microbiota. Fermentation. 2024 May 21;10(6):267.

Lalouckova K, Mala L, Slanickova P, Skrivanova E. In vitro antimicrobial effect of palm oils rich in medium-chain fatty acids against mastitis-causing Gram-positive bacteria. Czech J Anim Sci. 2019 Aug 31;64(8):325-31.

Lambert DM, Fowler CJ. The endocannabinoid system: Drug targets, lead compounds, and potential therapeutic applications. J Med Chem. 2005 Jul 19;48(16):5059-87.

Lanzoni D, Skrivan M, Englmaierova M, Petrosillo E, Marchetti L, Skrivanova V, Bontempo V, Rebucci R, Baldi A, Giromini C. Effects of dietary hemp co-product inclusion on laying hens performances and on egg nutritional and functional profile. Ital J Anim Sci. 2025;24(1):248-65.

Lanzoni D, Skrivanova E, Pinotti L, Rebucci R, Baldi A, Giromini C. Review: Nutritional aspects of hemp-based products and their effects on health and performance of monogastric animals. Animal. 2024a Feb;18(2):101058.

Lanzoni L, Pinotti L, Baldi A, Cheli F, Dell'Orto V, Rossi L, Savoini G, Trevisi E, Bontempo V. Phenolic profile and antioxidant activity of hemp co-products following green chemical extraction and ex vivo digestion. Ital J Anim Sci. 2024b May 14;23:651-663.

Lelario F, Scrano L, De Franchi S, Bonomo MG, Salzano G, Milan S, Milella L, Bufo SA. Identification and antimicrobial activity of most representative secondary metabolites from different plant species. Chem Biol Technol Agric. 2018;5:13.

Leung L. Cannabis and its derivatives: review of medical use. J Am Board Fam Med. 2011 Jul-Aug;24(4):452-62.

Li HL. The origin and use of cannabis in Eastern Asia: linguistic-cultural implications. Econ Bot. 1974 Jul;28: 293-301.

Lone TA, Lone RA. Extraction of cannabinoids from Cannabis sativa L plant and its potential antimicrobial activity. Universal J Med Dent. 2012;1(4):51-5.

Lu HC, Mackie K. An introduction to the endogenous cannabinoid system. Biol Psychiatry. 2016 Apr 1;79(7):516-25.

Mala L, Lalouckova K, Skrivanova E. Bacterial skin infections in livestock and plant-based alternatives to their antibiotic treatment. Animals (Basel). 2021 Aug 23; 11(8):2473.

Malik M, Velechovsky J, Tlustos P. The overview of existing knowledge on medical cannabis plants growing. Plant Soil Environ. 2021 Aug 31;67(8):425-442.

Malikova L, Malik M, Pavlik J, Ulman M, Pechouckova E, Skrivan M, Kokoska L, Tlustos P. Anti-staphylococcal activity of soilless cultivated cannabis across the whole vegetation cycle under various nutritional treatments in relation to cannabinoid content. Sci Rep. 2024 Feb 9; 14:4343.

Mandolino G, Bagatta M, Carboni A, Ranalli P, de Meijer E. Qualitative and quantitative aspects of the inheritance of chemical phenotype in Cannabis. J Ind Hemp. 2003; 8(2):51-72.

Mann S, Siler JD, Jordan D, Warnick LD. Antimicrobial susceptibility of fecal Escherichia coli isolates in dairy cows following systemic treatment with ceftiofur or penicillin. Foodborne Pathog Dis. 2011 Aug;8(8):861-7.

Marti G, Schnee S, Andrey Y, Simoes-Pires C, Carrupt P-A, Wolfender J-L, Gindro K. Study of leaf metabolome modifications induced by UV-C radiations in representative Vitis, Cissus and Cannabis species by LC-MS based metabolomics and antioxidant assays. Molecules. 2014 Sep 5; 19(9):14004-21.

Martinena CB, Corleto M, Martinez MMB, Amiano NO, Garcia VE, Maffia PC, Tateosian NL. Antimicrobial effect

https://doi.org/10.17221/85/2025-CJAS

of cannabidiol on intracellular Mycobacterium tuberculosis. Cannabis Cannabinoid Res. 2024 Apr 1;9(2):464-9.

Martinenghi LD, Jonsson R, Lund T, Jenssen H. Isolation, purification, and antimicrobial characterization of cannabidiolic acid and cannabidiol from Cannabis sativa L. Biomolecules. 2020 Jun 12;10(6):900.

Matton DP, Constabel P, Brisson N. Alcohol-dehydrogenase gene-expression in potato following elicitor and stress treatment. Plant Mol Biol. 1990 May;14(5):775-83.

McPartland JM, Guy GW, Hegman W. Cannabis is indigenous to Europe and cultivation began during the Copper or Bronze age: a probabilistic synthesis of fossil pollen studies. Veget Hist Archaeobot. 2018 May 5;27:635-48.

Mechoulam R, Shvo Y. Hashish. 1. Structure of cannabidiol. Tetrahedron. 1963 Dec;19(12):2073-8.

Mirzamohammad E, Alirezalu A, Alirezalu K, Norozi A, Ansari A. Improvement of the antioxidant activity, phytochemicals, and cannabinoid compounds of Cannabis sativa by salicylic acid elicitor. Food Sci Nutr. 2021 Dec 2; 9(12):6873-81.

Mora ZV-dl, Macias-Rodriguez ME, Arratia-Quijada J, Gonzalez-Torres YS, Nuno K, Villarruel-Lopez A. Clostridium perfringens as foodborne pathogen in broiler production: Pathophysiology and potential strategies for controlling necrotic enteritis. Animals (Basel). 2020 Sep 22;10(9):1718.

Nchima G, Choongo K, Muzandu K, Nalubamba K, Muma J, Bumbangi F, Monga G, Kangwa H. Determination of oxytetracycline and sulphamethazine residues in marketed beef from selected parts of Zambia to assess compliance with maximum residual limits. Am J Res Commun. 2017; 5:42-64.

Novak J, Zitterl-Eglseer K, Deans SG, Franz CM. Essential oils of different cultivars of Cannabis sativa L and their antimicrobial activity. Flavour Fragr J. 2001;16:259-62.

Novak K, Kropacova M, Havlicek V, Skrdleta V. Isoflavonoid phytoalexin pisatin is not recognized by the flavonoid receptor NodD of Rhizobium leguminosarum bv viciae. Folia Microbiol. 1995 Oct;40(5):535-40.

Oladeji OM, Mugivhisa LL, Olowoyo JO. Antibiotic residues in animal products from some African countries and their possible impact on human health. Antibiotics (Basel). 2025 Jan 14;14(1):90.

Osman KM, Elhariri M. Antibiotic resistance of Clostridium perfringens isolates from broiler chickens in Egypt. Rev Sci Tech. 2013;32(3):841-50.

Osterberger E, Lohwasser U, Jovanovic D, Ruzicka J, Novak J. The origin of the genus Cannabis. Genet Resour Crop Evol. 2022;69:1439-49.

Owusu-Doubreh B, Appaw WO, Abe-Inge V. Antibiotic residues in poultry eggs and its implications on public health: A review. Sci Afr. 2023;19:e01456.

Pacher P, Steffens S, Hasko G, Schindler TH, Kunos G. Cardiovascular effects of marijuana and synthetic cannabinoids: The good, the bad, and the ugly. Nat Rev Cardiol. 2018;15:151-66.

Padiyara P, Inoue H, Sprenger M. Global governance mechanisms to address antimicrobial resistance. Infect Dis (Auckl). 2018 Apr 11;11:1178633718767887.

Pandey S, Doo H, Keum GB, Kim ES, Kwak J, Ryu S, Choi Y, Kang JY, Kim S, Lee NR, Oh KK, Lee JH, Kim HB. Antibiotic resistance in livestock, environment and humans: One Health perspective. J Anim Sci Technol. 2024 Mar 31; 66(2):266-78.

Papatzimos G, Kasapidou E. Review of hemp components as functional feed and food ingredients: impact on animal product quality traits and nutritional value. Explor Foods Foodomics. 2024 Nov 11;2(6):626-50.

Pasquali F, Schinzari M, Lucchi A, Mandrioli M, Toschi TG, De Cesare A, Manfreda G. Preliminary data on the antimicrobial effect of Cannabis sativa L. variety Futura 75 against food-borne pathogens in vitro as well as against naturally occurring microbial populations on minced meat during storage. Ital J Food Saf. 2020;9(2):8581.

Patel J, Harant A, Fernandes G, Mwamelo AJ, Hein W, Dekker D, Sridhar D. Measuring the global response to antimicrobial resistance, 2020-21: a systematic governance analysis of 114 countries. Lancet Infect Dis. 2023;23(6): 706-18.

Paulova T, Malikova L, Lanzoni D, Taubner T, Malik M, Houdkova M, Pechouckova E. Inhibitory potential of Cannabis biomass extracts on livestock-associated staphylococcal and streptococcal pathogens. Microorganisms. 2025;13(2):432.

Payment J, Cvetkovska M. The responses of Cannabis sativa to environmental stress: a balancing act. Botany. 2023;101: 318-32.

Petit J, Salentijn EMJ, Paulo M-J, Denneboom C, Trindade LM. Genetic architecture of flowering time and sex determination in hemp (Cannabis sativa L): A genome-wide association study. Front Plant Sci. 2020 Nov 4;11:569958.

Pollastro F, Minassi A, Fresu LG. Cannabis phenolics and their bioactivities. Curr Med Chem. 2018;25(10):1160-85.

Portes AB, Panzenhagen P, dos Santos AMP, Conte CA Jr. Antibiotic resistance in Campylobacter: A systematic review of South American isolates. Antibiotics (Basel). 2023 Mar 9;12(3):548.

Punja ZK, Rodriguez G. Fusarium and Pythium species infecting roots of hydroponically grown marijuana (Cannabis sativa L.) plants. Can J Plant Pathol. 2018;40:498-513.

Quagliata G, Celletti S, Coppa E, Mimmo T, Cesco S, Astolfi S. Potential use of copper-contaminated soils for copper (Cannabis sativa L) cultivation. Environments. 2021;8(11):111.

https://doi.org/10.17221/85/2025-CJAS

Rabinovich AS, BIu A, Zelepukha SI. Isolation and investigation of antibacterial properties of preparations from wild hemp (Cannabis ruderalis) growing in the Ukraine. Mikrobiolohichnyi Zhurnal. 1959;21:40-8.

Ramatla T, Ngoma L, Adetunji M, Mwanza M. Evaluation of antibiotic residues in raw meat using different analytical methods. Antibiotics (Basel). 2017;6(4):34.

Reygaert WC. An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol. 2018 Jun 26; 4(3):482-501.

Robi DT, Mossie T, Temteme S. A comprehensive review of the common bacterial infections in dairy calves and advanced strategies for health management. Vet Med (Auckl). 2024 Jan 24;15:1-14.

Roshan M, Singh I, Vats A, Behera M, Singh DP, Gautam D, Rajput S, Tarak J, Packirisamy G, De S. Antimicrobial and antibiofilm effect of cannabinoids from Cannabis sativa against methicillin-resistant Staphylococcus aureus (MRSA) causing bovine mastitis. Int Microbiol. 2024 Dec;27(6):1839-1852.

Sakamoto K, Akiyama Y, Fukui K, Kamada H, Satoh S. Characterization, genome sizes and morphology of sex chromosomes in hemp (Cannabis sativa L). Cytologia. 1998 Dec;63(4):459-64.

Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, Alqumber MAA. Antimicrobial resistance: A growing serious threat for global public health. Healthcare (Basel). 2023 Jul 5;11(13):1946.

Sallam NA, Peterson CS, Borgland SL. Sex differences in the metabolic outcomes of high-fat diet in perinatal cannabis-exposed mice. J Psychiatry Neurosci. 2022;47(3):S14-5.

Sarmadyan H, Solhi H, Hajimir T, Najarian-Araghi N, Ghaznavi-Rad E. Determination of the antimicrobial effects of hydro-alcoholic extract of Cannabis sativa on multiple drug resistant bacteria isolated from nosocomial infections. Iran J Toxicol. 2014;7:967-72.

Sato T, Kawamura M, Furukawa E, Fujimura S. Screening method for trimethoprim/sulfamethoxazole-resistant small colony variants of Staphylococcus aureus. J Glob Antimicrob Resist. 2018 Dec;15:1-5.

Sawler J, Stout JM, Gardner KM, Hudson D, Vidmar J, Butler L, Page JE, Myles S. The genetic structure of marijuana and hemp. PLoS One. 2015 Aug 26;10(8):e0133292.

Sikora V, Berenji J, Latkovic D. Influence of agroclimatic conditions on content of main cannabinoids in industrial hemp (Cannabis sativa L). Genetika. 2011 Aug;43(3): 449-56.

Sionov RV, Steinberg D. Anti-microbial activity of phytocannabinoids and endocannabinoids in the light of their physiological and pathophysiological roles. Biomedicines. 2022 Mar 9;10(3):631.

Sirangelo TM, Ludlow RA, Spadafora ND. Molecular mechanisms underlying potential pathogen resistance in Cannabis sativa. Plants (Basel). 2023 Jul 25;12(15):2764.

Smith CJ, Vergara D, Keegan B, Jikomes N. The phytochemical diversity of commercial Cannabis in the United States. PLoS One. 2022 May 19;17(5):e0267498.

Soliani L, Rugna G, Prosperi A, Chiapponi C, Luppi A. Salmonella infection in pigs: Disease, prevalence, and a link between swine and human health. Pathogens. 2023 Oct 21; 12(10):1267.

Sovljanski O, Acimovic M, Sikora V, Koren A, Saveljic A, Tomic A, Tesevic V. Exploring (un)covered potentials of industrial hemp (Cannabis sativa L) essential oil and hydrolate: From chemical characterization to biological activities. Nat Prod Commun. 2024;19(1):1-12.

Stefanetti V, Passamonti F, Rampacci E. Antimicrobial strategies proposed for the treatment of S. pseudintermedius and other dermato-pathogenic Staphylococcus spp. In Companion Animals: A Narrative Review. Vet Sci. 2024 Jul 11;11(7):311.

Stout SM, Cimino NM. Exogenous cannabinoids as substrates, inhibitors, and inducers of human drug metabolizing enzymes: A systematic review. Drug Metab Rev. 2014;46:86-95.

Timmons B, Mattson N. Effect of end of cycle supplemental UV-B radiation on yield and cannabinoid quality of two hemp cultivars. HortScience. 2023;58:S247.

Torkamaneh D, Jones AMP. Cannabis, the multibillion dollar plant that no genebank wanted. Genome. 2022 Jan; 65(1):1-5.

Trancoso I, de Souza GAR, dos Santos PR, dos Santos KD, de Miranda RMDN, da Silva ALPM, Santos DZ, Garcia-Tejero IF, Campostrini E. Cannabis sativa L.: Crop management and abiotic factors that affect phytocannabinoid production. Agronomy. 2022 Jun 22;12(7):1492.

Trono D. Elicitation as a tool to improve the accumulation of secondary metabolites in Cannabis sativa. Phytochem Rev. 2024 Sep 17;24:3119-55.

Trott DJ, Turnidge J, Kovac JH, Simjee S, Wilson D, Watts J. Comparative macrolide use in humans and animals: should macrolides be moved off the World Health Organisation's critically important antimicrobial list? J Antimicrob Chemother. 2021 Jul 15;76(8):1955-61.

van Bakel H, Stout JM, Cote AG, Tallon CM, Sharpe AG, Hughes TR, Page JE. The draft genome and transcriptome of Cannabis sativa. Genome Biol. 2011 Oct 20;12(10):R102.

van Klingeren B, Ten Ham M. Antibacterial activity of Δ^9-tetrahydrocannabinol and cannabidiol. Anton Leeuw Int J G. 1976 Mar;42:9-12.

Vikram A, Jayaprakasha GK, Jesudhasan PR, Pillai SD, Patil BS. Suppression of bacterial cell-cell signalling, biofilm

https://doi.org/10.17221/85/2025-CJAS

formation and type III secretion system by citrus flavonoids. J Appl Microbiol. 2010 Aug;109(2):515-27.

Virto M, Santamarina-Garcia G, Amores G, Hernandez I. Antibiotics in dairy production: Where is the problem? Dairy. 2022 Aug 1;3(3):541-64.

Vispute MM, Sharma D, Mandal AB, Rokade JJ, Tyagi PK, Yadav AS. Effect of dietary supplementation of hemp (Cannabis sativa) and dill seed (Anethum graveolens) on performance, serum biochemicals and gut health of broiler chickens. J Anim Physiol Anim Nutr (Berl). 2019 Mar;103(2):525-33.

Vu TT, Kim H, Tran VK, Le Dang Q, Nguyen HT, Kim H, Kim IS, Choi GJ, Kim JC. In vitro antibacterial activity of selected medicinal plants traditionally used in Vietnam against human pathogenic bacteria. BMC Complement Altern Med. 2016 Jan 27;16:32.

Wang CY, Chen YW, Hou CY. Antioxidant and antibacterial activity of seven predominant terpenoids. Int J Food Prop. 2019 Geb 22;22(1):230-8.

Wang LH, Zeng XA, Wang MS, Brennan CS, Gong D. Modification of membrane properties and fatty acids biosynthesis-related genes in Escherichia coli and Staphylococcus aureus: Implications for the antibacterial mechanism of naringenin. Biochim Biophys Acta Biomembr. 2018 Feb;1860(2):481-90.

Welling MT, Shapter T, Rose TJ, Liu L, Stanger R, King GJ. A belated green revolution for cannabis: Virtual genetic resources to fast-track cultivar development. Front Plant Sci. 2016 Jul 29;7:1113.

Wernli D, Jørgensen PS, Morel CM, Carroll S, Harbarth S, Levrat N, Pittet D. Mapping global policy discourse on antimicrobial resistance. BMJ Glob Health. 2017 Jul 13; 2(2):e000378.

WHO. Foodborne Disease Burden Epidemiology Reference Group. WHO estimates of the global burden of foodborne diseases 2007–2015. WHO. 2015.

Williams-Nguyen J, Sallach JB, Bartelt-Hunt S, Boxall AB, Durso LM, McLain JE, Singer RS, Snow DD, Zilles JL. Antibiotics and antibiotic resistance in agroecosystems: State of the science. J Envir Qual. 2016 Mar;45(2):394-406.

Wu Q, Guo M, Zou L, Wang Q, Xia Y. 8,9-dihydrocannabidiol, an alternative of cannabidiol, its preparation, antibacterial and antioxidant ability. Molecules. 2023 Jan 3; 28(1):445.

Xu CM, Kong LQ, Gao HF, Cheng XY, Wang XM. A review of current bacterial resistance to antibiotics in food animals. Front Microbiol. 2022 May 12;13:822689.

Yue J, Yang H, Liu S, Song F, Guo J, Huang C. Influence of naringenin on the biofilm formation of Streptococcus mutans. J Dent. 2018 Sep;76:24-31.

Zengin G, Menghini L, Di Sotto A, Mancinelli R, Sisto F, Carradori S, Cesa S, Fraschetti C, Filippi A, Angiolella L, Locatelli M, Mannina L, Ingallina C, Puca V, D'Antonio M, Grande R. Chromatographic analyses, in vitro biological activities, and cytotoxicity of Cannabis sativa L essential oil: A multidisciplinary study. Molecules. 2018 Dec 10; 23(12):3266.

Zhang G, Zhang N, Xu J, Yang T, Yin H, Cai Y. Efficacy and safety of vancomycin for the treatment of Staphylococcus aureus bacteraemia: A systematic review and meta-analysis. Int J Antimicrob Agents. 2023 Oct;62(4):106946.

Received: June 18, 2025
Accepted: August 13, 2025
Published online: September 29, 2025