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Review Article

Potential Applications of Cannabis Plant Extracts and Phytochemicals as Natural Antimicrobials

Hebah M.S. AL Ubeed[1*], Asgar Farahnaky[1], Emma L. Beckett[2,3], Momena Khandaker[4] and Christopher J. Pillidge[1]

[1]School of Science, STEM College, RMIT University, VIC, Australia; [2]School of Environmental and Life Sciences, University of Newcastle, NSW, Australia; [3]Hunter Medical Research Institute, New Lambton Heights, NSW, Australia; [4]School of Environmental and Rural Sciences, University of New England, NSW, Australia

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Abstract

Cannabis has been used to treat human diseases for millennia. However, scientific research on its properties has been relatively recent. The spread of antibiotic resistance among human and animal pathogens has stimulated a renewed search for alternative antimicrobial therapies from this and other plant sources. It is further possible that *Cannabis* extracts or purified cannabinoids could find their way into novel medical applications. Industrial hemp extracts could also be potentially used in food manufacturing (for example, to control biofilms or food packaging), for veterinary purposes, or for microbial control in cleaners and sanitizers. This review highlights the latest findings of *Cannabis* plant extracts and phytochemicals as new classes of potent antimicrobial agents and their mode of action against different microorganisms, including Gram-positive and Gram-negative bacteria. More importantly, the challenges of using cannabinoids as effective and affordable natural antimicrobial agents are reviewed. While antimicrobial and other applications of *Cannabis* extracts and phytochemicals (in general) look promising, there are also limitations, particularly around their possible toxic side effects. Accordingly, future research directions are proposed in this report. More research is needed to address the safety of these compounds, determine their activity *in vivo*, and define structural changes that influence their pharmacokinetic properties. Importantly, standardized tests will be essential to enable valid inter-laboratory comparisons. Future directions in research for developing novel broad-spectrum antibiotics based on *Cannabis* are discussed in this review article.

Keywords: *Cannabis*; Antibacterial; Antifungal; Antiviral; Natural phytochemicals; Medicinal Cannabis extract.

Abbreviations: Δ^8-THC, Delta-8-Tetrahydrocannabinol; ABTS, 2,2′-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt; ACE-2, Angiotensin-converting enzyme-2; $AgNP_S$, Silver nanoparticles; *B. cereus*, *Bacillus cereus*; *B. longum*, *Bifidobacterium longum*; *B. subtilis*, *Bacillus subtilis*; CBC, Cannabichromene; CBCA, Cannabichromene acid; CBD, Cannabidiol; CBDA, Cannabidiol acid; CBDV, Cannabidivarin; CBDVA, Cannabidivarin acid; CBG, Cannabigerol; CBGA, Cannabigerolic acid; CBL, Cannabicyclol; CBN, Cannabinol; CBNA, Cannabinol acid; CLSI, Clinical Laboratory and standards Institute; COVID-19, Human coronavirus; SARS-CoV2, severe acute respiratory syndrome coronavirus 2; DPPH, 2,2-diphenyl-1-picrylhydrazyl; *E. coli*, *Escherichia coli*; *E. faecalis*, *Enterococcus faecalis*; EOs, Essential Oils; GC-MS, Gas Chromatography Mass Spectrometry; GI, Gastrointestinal; GOT, Geranyl pyrophosphate, Olivetolate geranyltransferase; GPP, Geranyl pyrophosphate; HBV, Hepatitis B virus; HCV, Hepatitis C virus; *K. pneumoniae*, *Klebsiella pneumoniae*; KSHV, Kaposi's sarcoma-associated herpesvirus; *L. donovani*, *Leishmania donovani*; *M. smegmatis*, *Mycobacterium smegmatis*; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; MRSA, Methicillin-resistant *Staphylococcus aureus*; NCs, Nanocarriers; OLA, olivetol acid; *P. aeruginosa*, *Pseudomonas aeruginosa*; *P. falciparum*, *Plasmodium falciparum*; *P. guajava*, *Psidium guajava*; *S. aureus*, *Staphylococcus aureus*; *S. milleri*, *Streptococcus milleri*; *S. mutans*, *Streptococcus mutans*; *S. pyogenes*, *Streptococcus pyogenes*; *SFE-CO_2*, Supercritical fluid extraction with carbon dioxide; THC, Tetrahydrocannabinol; THCA, Tetrahydrocannabinolic acid; THCAA, Delta9-tetrahydrocannabinolic acid-A; THCAS, Tetrahydrocannabinolic acid synthase; THCV, Tetrahydrocannabivarin; THCVA, Tetrahydrocannabivarin acid; TMPRSS2, Transmembrane serine protease 2; $ZnNP_S$, Zinc Nanoparticles; Δ^9-THC, Delta-9-trans-Tetrahydrocannabinol.

***Correspondence to:** Hebah M.S. AL Ubeed, School of Science, STEM College, RMIT University, Bundoora, Melbourne, VIC 3083, Australia. ORCID: https://orcid.org/0000-0003-4344-9513. Tel: +61 3 9925 3347, Fax: RITM0806372, E-mail: hebah.al.ubeed@rmit.edu.au

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Introduction

Cannabis sativa L. or marijuana, is a flowering plant that has been used for millennia for food, drugs (both legal and illegal), textile (hemp), and religious purposes.[1] Selective breeding has resulted in numerous *C. sativa* strains (cultivars) with different properties. For example, hemp strains are fibrous and low in cannabinoids, while medicinal strains are highly flowering and contain both phytonutrients and phytochemicals.[2–4] Bioactive compounds can be extracted from oils or as aqueous phases from seeds, flowers, leaves, and stems using traditional techniques such as cold-pressing and solvent extraction or by contemporary procedures like ultrasound.[5] Supercritical fluid extraction with carbon dioxide (SFE-CO_2) is another technology used in industry to extract phytochemical com-

pounds.[6,7] This is no different from other plant products, which have been historically used as rich sources of natural products for human health.[8]

Potential therapeutic applications and bioactive mechanisms of crude *Cannabis* extracts and purified compounds derived from *C. sativa* have been investigated in various pharmacological scenarios, including their use as anti-convulsive, analgesic, anti-anxiety, and anti-emetic therapeutic drugs.[9] While much research has centered around the psychoactive properties of cannabinoids,[10] the antimicrobial properties of compounds extracted from *C. sativa* are now becoming of particular interest due to the emergence of antimicrobial resistance as a vital threat to human health globally.[11] Mitigating the human and economic impacts of this problem, and more broadly, the emergence of new microbial pathogens,[12] requires identifying and elucidating new antimicrobial therapies.

One possibility to combat such infections, apart from antibiotics, is to use phage-based therapies, including lysin therapy and engineered phage enzymes.[13,14] Another alternative is to use plant-derived compounds or extracts with antimicrobial properties (bacterial, fungal, and viral). Among these, *Cannabis* extracts and (more specifically) cannabinoids show colossal promise, especially towards multi-drug resistant microorganisms like MRSA (methicillin-resistant *Staphylococcus aureus*), which can be very difficult to treat.[15] While there is much anecdotal evidence on the efficacy of *Cannabis* compounds or extracts, controlled laboratory investigations of these novel antimicrobial agents, their mode of action, efficacy, and safety, together with the development of safe and approved disease treatment therapies, is certainly needed. *In vitro* antifungal, antibacterial, antimalarial, antileishmanial, and cytotoxic properties of *C. sativa* extracts and compounds have been investigated since the early 2000s.[13,16–19] However, significant challenges exist in assessing the efficacy of these agents (both impure phytochemical extracts and purified compounds), determining their safety, and implementing research findings. These challenges include high variability in cannabinoid content in different *C. sativa* strains; also for different parts of plants and in the extraction methods;[5] a lack of uniform methods for antimicrobial screening assays; diversity in microbial targets (strain variability); failure to consider the stability of extracts or the effects of additional compounds (impurities), both in cell culture experiments and animal trials. Furthermore, challenges regarding the negative perception of *Cannabis*, its safety and potential side effects, variable delivery methods, local and systemic effects, and synergistic effects need to be addressed.[20,21]

This review will describe the potential uses of agents found in medicinal *Cannabis* extracts as antimicrobials, the current knowledge on their mechanism of action, the challenges around phytochemicals' stability and bioavailability, optimized extraction protocols, and isolation of active antibiotic compounds. Also, the prospect of using new formulations containing 'old' antibiotics combined with therapeutic plant compounds to provide a synergistic killing effect is noted. Such combined treatments could more effectively kill or inhibit antibiotic-resistant bacteria causing local and systemic infections.

New classes of potent antimicrobial agents

It has been known for decades that *Cannabis* plant extracts can be effective antimicrobial agents.[22,23] More than 525 phytochemicals have been extracted and isolated in *C. sativa*.[24–26] The most important classes of *Cannabis* phytochemicals are the C21 terpenes, phenolics, and cannabinoids. The respective antimicrobial properties of these compounds will be discussed in this report.

There are many benefits for isolated cannabinoids, including antifungal, antibacterial, antimalarial, antileishmanial, and cytotoxic properties.[26] Active cannabinoids like CBG,[27,28] CBN, CBC,[29] and psychoactive cannabinoids delta-9-trans-tetrahydrocannabinol (Δ^9-THC)[17] (summarized in Table 1 and Fig. 1) and their precursors have a high level of antimicrobial activity.[16,26,30,31] Synthesis of cannabinoids is complex; essentially, they are derived from cannabigerolic acid (CBGA), which is the precursor of olivetol acid (OLA) and geranyl pyrophosphate (GPP), and is induced by the prenyltransferase geranyl pyrophosphate: olivetolategeranyltransferase (GOT).[32] There are some examples of co-enzymes involvement like tetrahydrocannabinolic acid synthase (THCAS), cannabidiolic acid synthase (CBDAS), or cannabichromeneacid synthase (CBCAS). With the assistance of the co-enzyme, CBGA is finally converted to tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and cannabichromenic acid (CBCA). Then, oxidation of THCA occurs to produce cannabinolic acid (CBNA) in the buds.[33,34] Cannabinoids Δ^9-THC, CBD, CBG, CBC, and CBN displayed anti-staphylococcal activity with even greater antibiotic effect than traditional antibiotics like norfloxacin, erythromycin, tetracycline, and oxacillin.[3] CBG exhibits antibacterial activities against *Streptococcus mutans (S.mutans)*.[35,36]

Both compounds Δ^9-THC and CBD showed significant antibacterial activity towards *S. aureus*, *Streptococcus pyogenes* (*S. pyogenes*), *Streptococcus milleri* (*S. milleri*), *Enterococcus faecalis* (*E. faecalis*), *E. coli*, *Salmonella typhi,* and *Proteus vulgaris*.[37] Farha, *et al.*[37] further described the antibacterial activity of specific cannabinoids CBC, CBCA, CBD, CBDV, CBDA, CBDVA, CBG, CBGA, CBN, CBL, THC, Δ^8-THC, exo-tetrahydrocannabinol (exo-THC), Δ^9-tetrahydrocannabinolic acid-A (THCAA), THCV, (±) 11-nor-9-carboxy-Δ^9 – THC, and (±) 11-hydroxy-Δ^9-THC against MRSA to inhibit its ability to form biofilms and stationary phase cells' resistance to antibiotics. CBD also showed potent activity against various MRSA strains.[38] A detailed paper on the antimicrobial activity of CBD, including mechanistic mode-of-action studies, was later provided by Blaskovich *et al*.[17] They showed that cannabidiol has a superior effect against biofilms. This cannabinoid could potentially treat Gram-negative bacteria infections, including *Neisseria gonorrhoeae* and Gram-positive bacterial infections. CBD was also shown to be a bigger inhibitor of membrane vesicle emission from *E. coli* (strain VCS257) compared to *S. aureus*. Whenever CBD was used in combination with selected antibiotics, it caused higher inhibition action against Gram-negative bacteria.[39] Similar to terpenes, cannabinoids show synergistic effects with antibiotics. Grassi *et al*.[40] demonstrated that when cannabinoids were combined with polymyxin B, there was an effective inhibition against Gram-negative bacteria.

Apart from cannabinoids, geranyl pyrophosphate is the precursor in synthesizing the terpenoids, leading to the creation of monoterpenoids in secretory cell plastids.[41] For instance, volatile oil fractions incorporate monoterpenoids (C10)[42] or sesquiterpenoids and triterpenoids in the cytoplasm.[43,44] Sesquiterpenes (C15) are major compounds in hemp extracts.[42] After harvest, *Cannabis* buds must be dried to remove the carboxylic acid functional group to extract high-purity CBD, CBC, and CBG. Then, oxidization of THC yields delta-8-tetrahydrocannabinol (Δ^8-THC, the main psychoactive compound) and CBN.[26,45,46]

Many factors affect the quality and consistency of these antibacterial extracts, including environmental and climatic conditions for the growth of leaves, flowers, and seeds. Also, Muscarà

Table 1. Cannabinoids from *Cannabis sativa L.* with antimicrobial activity

Active compound	Organism	Mode of action	References
CBD	*S. aureus*; *B. subtilis*	CBD is more active than CBDA due to exchanged positions of the one hydroxyl group and lipophilic side chain.	3,89,100
	Hepatitis C virus	CBD interacts with the CB_2 receptor and stimulates apoptosis in thymocytes and splenocytes, then inhibiting the proliferation of T-cells and macrophages as such mechanism cause to indirectly slows the pathogenic process of the HBV virus.	
	SARS-CoV-2	CBD additive or synergic effect with terpene control viral replication or clonal stable conformations with the binding of the transmembrane protease serine 2 (SARS-CoV-2) and angiotensin-converting enzyme-2 (ACE2).	
CBG	*Mycobacterium; Leishmania donovani; S. mutans; S. sanguis; S. sobrinus; S. salivarius;* MRSA	CBG alters membrane structures of treated bacteria and disorders of the cytoplasm activity.	70
CBC	*S.aureus; B.Subtils; C. albicans; Mycobacterium smegmatis; Saccharomyces cerevisiae; Trichophyton mentagrophytes*	The activity of CBC due to bearing the lipophilic side chain and one hydroxyl group in exchanged positions displayed similarly potent activity.	3,74
CBN	Anti-MRSA; antileishmanial; *Plasmodium falciparum*	Decreased or increased esterification or methylation of the carboxylic acid moieties were detrimental to the activity and additional hydroxy function in the lipophilic side chain.	3,76
Δ^9-THC	*S. aureus, Streptococcus pyogenes, Streptococcus. milleri, Enterococcus faecalis, E. coli, Salmonella typhi, Proteus vulgaris,* MRSA	Decreased or increased esterification or methylation of the carboxylic acid moieties were detrimental to the activity.	3,16

ACE-2, Angiotensin-converting enzyme-2; CBD, Cannabidiol; CBDA, Cannabidiol acid; CBG, Cannabigerol; CBN, Cannabinol; MRSA, Methicillin-resistant *Staphylococcus aureus*; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Δ^9-THC, Delta-9-trans-Tetrahydrocannabinol.

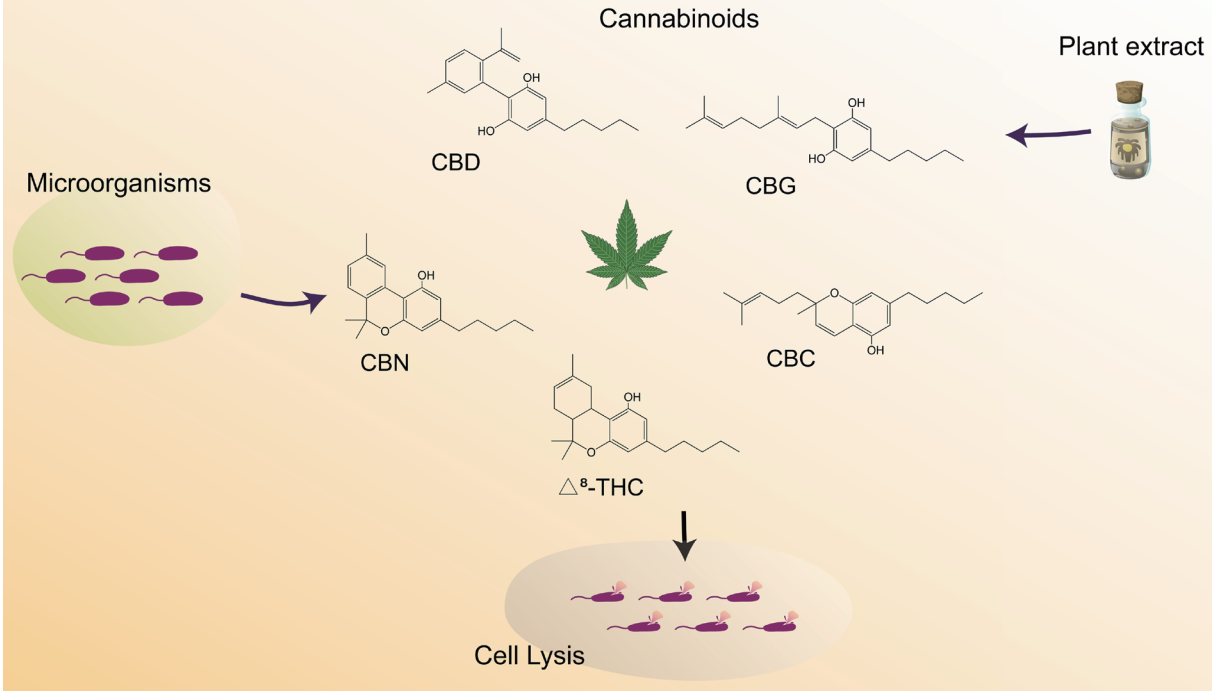

Fig. 1. Antimicrobial activity of cannabinoids like cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), cannabinol (CBN), and delta-8-tetrahydrocannabinol (Δ^8-THC).

et al.[47] studied two standardized hexane extracts for their phytonutrients and antibiotic activities. The first extract consisted of cannabidivarinic acid (CBDVA) and tetrahydrocannabivarinic acid (THCVA). The second extract contained cannabidivarin (CBDV) and tetrahydrocannabivarin (THCV) from a new Chinese *C.sativa* variety and other non-psychoactive strains. Both extracts showed extraordinary antioxidant activity and antimicrobial and antifungal properties against clinical strains of MRSA and other microorganisms.[28] Various methods have been applied to extract and isolate these and other antimicrobial agents, such as solvent extraction, physical methods, and supercritical fluid extraction, giving different results.[5,6]

Other *Cannabis* compounds with antimicrobial activity include terpenoids. These form a large percentage of essential oils.[48–51] Some 120 terpenoids have been found in marijuana and hemp,[52] but relatively few terpenes have been isolated, purified, and tested.[53] Hemp seed oil was also effective in controlling spoilage of food and phytopathogenic microorganisms.[16,54] An *in vitro* or cell culture study by Nostro *et al*.[55] considered a range of plant extracts, potentially inhibiting biofilms in food. They found that extracts were effective against *S. aureus*, including MRSA strains, *Escherichia coli* (*E. coli)*, and *Pseudomonas aeruginosa (P. aeruginosa*). Another study focused on linalool and α-Phellandrene, which were investigated due to their very low toxicity and ease of use and were registered as a potential new therapeutic.[47,56] Another type of active compound extracted from *Cannabis* seeds are the flavonoids, also effective against bacteria and yeast.[57]

Another active compound class that can be extracted from *Cannabis* and used as an antibiotic alone or in combination with other compounds is *Cannabis* alkaloids. Natural alkaloids accumulate to differing extents in particular parts of the plant, such as barks, roots, and leaves.[58,59] These alkaloids can be chemically modified to produce synthetic and semi-synthetic smart drugs. However, natural compounds are generally safer and cheaper than alternative synthetic compounds.[60] Currently, natural alkaloids are used as a therapeutic compound for human disease and as pesticides in agriculture.[61] Natural alkaloids and phenolic compounds have potential antimicrobial activity due to their ability to alter the structure of the bacterial wall.[62]

In summary, the most valuable compounds in *Cannabis,* besides cannabinoids, include its alkaloids, terpenoids, steroids, phenols, glycosides, and tannins.[63]

A novel mode of action

The most active compounds in *C. sativa* with antimicrobial potential are THC, CBD, CBG, and CBC.[56,64–66] These compounds may also effectively treat psychological and physiological disorders.[67,68] Medicinal *Cannabis* phytochemicals applied to specific medical devices were active against biofilms composed of Gram-positive and Gram-negative bacteria, and their mode of action was to alter and penetrate the bacteria cells[69] are summarized in Table 1. Various methods like scanning electron microscopy, transmission electron microscopy, Nile Red membrane staining, and laurdan membrane fluidity assays have shown that CBG inhibits bacteria via alterations to the bacterial cell structure by inducing membrane hyperpolarization and decreasing the membrane fluidity.[70] CBG exhibited moderate antimicrobial activity towards *Mycobacterium*[25] and *Leishmania donovani* (*L. donovani*).[3] Not only CBG but also its derivative iso-CBG-C1 showed potential activity against *S. aureus* and *Bacillus subtilis* (*B. subtilis*), and *Mycobacterium smegmatis* (*M. smegmatis*).[27]

Other studies found that CBD has a higher effect against Gram-positive bacteria than their acid form due to differences in lipophilicity.[27,71,72] Studies have shown that CBD and CBG have a higher antimicrobial effect than their precursors (acidic cannabinoids or raw material before drying) due to exchanged positions of a hydroxyl group and lipophilic side chain.[3]

While CBD and CBG are effective against Gram-positive bacteria, Krejci *et al.*[73] reported that both cannabinoids were inactive against Gram-negative bacteria. Moreover, compared to other natural cannabinoids and antibiotics like streptomycin, CBC has the highest activity against *S. aureus* and *B. subtilis*,[74] and similar effects were confirmed with iso-CBC due to connecting with the lipophilic side chain and a hydroxyl group in interchanged positions.[75] Furthermore, a cannabinol derivative CBN[76,77] showed moderate anti-MRSA, antileishmanial, and anti-*Plasmodium falciparum* (*P. falciparum*) activity. However, both Δ^9-THCVA and CBDVA showed lower anti-staphylococcal activity based on deleting a side chain compared to Δ^9-THCV and CBDV.[78,79] The primary action of CBG against MRSA is the disordering of the principal activity of the cytoplasm.[37] Recent structure-function studies have helped to determine the most effective cannabinoids.[80] Cannabinoids showed significant differences in antimicrobial activity due to a decrease or increase in methylation or esterification. The most active antimicrobial cannabinoids are Δ^9-THC, CBN, and CBG.[16,70]

No significant antifungal activity has been observed yet for cannabinoids.[19] However, the antiviral potential of CBD has received some attention.[81–86] CBD was effective against hepatitis C virus (HCV),[87–92] which causes liver inflammation.[93] In contrast, CBD was ineffective against the hepatitis B virus (HBV). CBD was more efficient than sofosbuvir and had a less cytotoxic effect.[94] Other research indirectly showed that CBD could effectively work and inhibit Kaposi's sarcoma-associated herpesvirus (KSHV).[95]

One key research target is control of the human coronavirus (COVID-19).[96–99] In a recent article, CBD in combination with terpenes showed antiviral effects against Human Coronavirus E229.[100] Also, in an earlier investigation, terpenes were found to show an antiviral effect on severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1).[101] However, while such compounds show promise, most of these antivirals have not been trialed on humans or undergone controlled clinical trials.[102,103] According to Chatow *et al*.,[100] the combined possibility of plant terpenes with CBD against a human coronavirus strain could be seen using a tissue cell culture model.

Although such studies are a good start, they cannot determine side effects or potential cytotoxicity as *in vivo* animal model studies. Recent studies showed phytochemicals have stable conformations with the binding enzyme of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has a vital role in viral replication, cloning, suppressing viral entry and activation, and down-regulating ACE2 receptor and TMPRSS2 enzyme.[104–106] Further investigation on developing new vaccines or antivirals will be needed to address strain variability, i.e., target all strains of COVID-19. This needs more intensive research in the future, together with studies giving mechanistic insights into their action.

Challenges in applications of cannabinoids as an antimicrobial agent

As noted earlier, the spread of antibiotic resistance genes is common among many pathogens, and with little or no pending discovery or development of new classes of antibiotics, the need to find

other agents for combating infections is vital.[37,107,108] Recently, the World Health Organization announced an archive of 12 microorganisms that have developed antibacterial resistance and are now considered a severe threat to health care. Eight of these pathogens are Gram-negative and are spectacularly hard to cure, while the other four Gram-positive microorganisms are the most significant threat.[109] The antimicrobial activity of *Cannabis* derives from essential oils (Eos) and purified cannabinoids, the latter receiving increased attention in recent years as possible therapeutic agents.[16,19,56,110] This section will first consider the antimicrobial effects of impure extracts and Eos, then discuss studies on purified cannabinoids.

The disc diffusion assay and the two-fold serial broth dilution assay, which generate a MIC value in a 96-well microtiter plate, are the most commonly used methods to measure antimicrobial effects.[111] The methodology for the MIC assay is standard and specified by the Clinical Laboratory and Standards Institute (CLSI).[112] However, a problem highlighted by Sadgrove and Jones[113] is that MIC values are normally quoted in µg/ml, which does not take into account molarity. The authors also noted that antimicrobial outcomes might be naively extrapolated beyond any reasonable systemic concentration level to show an effect. Even with method standardization, caution must be exercised when comparing inter-laboratory results to ensure that results cross-comparisons are valid. In one study, even though there was the bactericidal effect of both cannabinoids like THC and CBD against staphylococci and streptococci tested in broth media, the impact was much higher in horse blood agar or with 4% horse serum.[109]

Even though essential oils (Eos) can be extracted from low-THC *Cannabis* varieties cultivated without legal restrictions, relatively few studies have considered these Eos or extracts in terms of their antimicrobial activities. The use of *Cannabis* in this way strengthens the idea of growing hemp as a multi-use crop.[54] A fundamental challenge in medical *Cannabis* and hemp research requires production methods or post-harvesting processes that maintain the uniform quality of the product. Also, drying, extraction, isolation, and purification techniques must be optimized to obtain pure chemical composition and pharma grade for different products.[6,114–116]

One problem with assessing multiple studies is that *Cannabis* extracts vary enormously in composition and contain multiple different compounds, making result comparisons extremely difficult. Some key variables that make inter-laboratory comparisons difficult include the absence of standardized antimicrobial testing methodologies; microbiological media components in disk diffusion tests reacting with or modifying extracts; plant extracts containing unstable compounds making accurate quantitative analysis impossible; synergistic or antagonistic effects between multiple compounds; different cultivars or parts of plants used in different studies; and extraction protocols of varying efficiency leading to different outcomes. In one study, *C. sativa* and *Psidium guajava* extracts contained various active compounds like alkaloids, saponins, flavonoids, steroids, cardiac glycosides, terpenes, resins, tannins, and phenols. However, steroids, resins, and cardiac glycosides were absent in another medicinal plant, *Thuja orientalis*. Furthermore, hemp essential oils demonstrated high antimicrobial activity against Gram-positive bacteria.[20,54]

Some studies have focused on extracts of seeds of *Cannabis* plants.[117] Hemp seed extracts show good antimicrobial activity due to their composition of polyphenols, essentially caffeoyltyramine and cannabisin.[118] In another study,[117] *C. sativa L.* seed extracts inhibited biofilm formation by *S. aureus* ATCC 35556. In contrast, adding hemp seed extract to media stimulated the growth of Bifidobacterium and Lactobacillus probiotic strains.[117] The increased growth of *Bifidobacterium Longum* (*B. longum)* in the presence of hemp extract was due to protection from oxidative stress.[119]

Most studies on the effects of extracts use classical disc diffusion methods. However, recently, the study reported by Frassinetti *et al*.[117] was to determine the lowest concentrations with activity via MIC and a biofilm production and inhibition assay. A further study by Iseppi *et al.*[120] aimed to standardize the antibacterial method and provided better designs for experiments. Firstly, the separation of essential oils (EO) compounds was done by gas chromatography mass spectrometry (GC-MS). The EOs were tested via an agar well-diffusion assay, and MIC testing was done against multiple Gram-positive bacteria. The EOs and purified compounds were highly effective against *Enterococcus*, an opportunistic pathogen. These authors also found that two EOs and two purified compounds had lower MICs than amoxicillin or ampicillin against *Bacillus cereus* (*B. cereus)*.

Another active compound extracted from the seed was flavonoids; these were shown to be active against *Candida albicans*, *S. aureus*, and *P. aeruginosa*.[57] EOs of fiber-type hemp have also been applied to prevent food spoilage, incorporating antimicrobial extracts into food packaging. Further studies are needed on pure compounds.[28]

An exciting use of *Cannabis* extracts has been forming silver or zinc nanoparticles (Ag NPs and Zn NPs) with antimicrobial activity. Chouhan and Guleria,[18] Chauhan *et al.*[121] used *C. sativa* extracts to form stable nanoparticle emulsions with Ag-doped, Zn, and ZnO nanoparticles; AgNPs revealed an excellent antioxidant capacity and significant antibiotic activity against several human infection diseases by disc diffusion test, including *E. coli*, *Klebsiella pneumoniae* (*K. pneumoniae*), MRSA, *P. aeruginosa, S. typhi*, and *S.aureus,* and as an antifungal against *Fusarium* spp. *Rosellinia necatrix C. sativa* aqueous leaf extract (CSE) derived AgNPs also had antifungal and α-amylase inhibitory activity. There was minor activity against *Bacillus subtilis*, *S. aureus,* and *K. pneumoniae* tested by a well-diffusion assay with increasing concentrations of AgNPs. Other researchers[122] suggested a synergistic or symbiotic effect between terpenes, flavonoids, and cannabinoids in industry hemp strains to improve the effect of silver nanoparticles (AgNPs). Other studies showed the effective use of hemp extracts to produce nanoparticles effective against biofilms.[123] The main challenge with using nanoparticles based on *C. sativa* extracts is to develop suitable technologies for obtaining nanoparticles with specific properties used in pharmaceutical products. For recent reviews see.[124,125]

Synergistic effects between *Cannabis sativa* and plants such as *Allium sativum* (Garlic) have been shown.[126] Further research is required to study the impact of terpenes, in particular, as antimicrobial agents.[127] There may also be a synergistic effect when these compounds are applied with antibiotics simultaneously, as demonstrated for other plant extracts by Blesson *et al.*[128] In comparative testing, the presence of other compounds must be considered.

Several reviews have summarized the antimicrobial properties of the major cannabinoids against essential pathogenic microorganisms or viruses, including Gram-negative pathogens, MRSA, and SARS-CoV-2.[3,19,22,129,130] From this extensive compilation of data, there is a need for standardization of methodology and approaches so that valid comparisons and conclusions between laboratories can be made. This includes how *in vitro* results can be extrapolated to determine *in vivo* efficacy, determined (usually) by

using animal models.[37] However, further *in vivo* or animal model investigation is required to determine how the body reacts and responds to cannabinoids, including side effects.

Another challenge for using cannabinoids in terms of antimicrobial activity is to control their stability and bioavailability once introduced into the body. Cannabinoids have significant pharmacological activities but may show poor water solubility and become labile during processing and storage.[131] A commonly used method for the delivery of cannabinoids and improvements in bioavailability is using nanocarriers (NCs) to protect core materials from degradation during passage through the gastrointestinal (GI) tract.[132] Transmucosal oral routes of delivery offer distinct advantages.[5] Determining dosage forms for any medicinal product must consider product instability, such as at room temperature. In this regard, CBD is highly unstable and sensitive to oxidation.[133]

Future directions

For the potential of *Cannabis* plant extracts and phytochemicals to be applied as natural antimicrobials, further work is required to standardize and harmonize research. As is genuine for all-natural extracts, variability in natural bioactive contents between plants, parts of plants, and extracts needs to be addressed with assessment and clear transparent reporting of properties. Additional research to identify active compounds (in isolation and synergistic applications) will potentially circumvent these issues by refining test substances. However, challenges persist regarding stability, delivery, safety, non-uniform testing procedures, and negative perceptions of these products. A multi-disciplinary approach is required to facilitate the progress from promising potential antimicrobials to pharmacological success, with roles for biological sciences, pharmacology, chemistry, and social and other sciences.

Conclusions

The evolution of antibiotic-resistant bacteria and the emergence of new viruses such as COVID-19 have stimulated the search for non-traditional antimicrobial or antiviral treatments. In the case of bacteria, this includes alternatives to using antibiotics. While the use of *Cannabis* as a medicinal agent has been known for thousands of years, there is now great interest in discovering and analyzing potent antimicrobial agents derived from this plant, including its cannabinoids, terpenes, phenolics, and alkaloids. While this is welcome, inter-laboratory comparisons of results showing antimicrobial or antiviral activities are complex, as many variables affect results (such as plant-to-plant variations, differences in extraction protocols, synergistic/antagonistic effects of other compounds, and differences in antimicrobial assay protocols). There are also challenges for commercial applications, such as finding the best method for extraction and isolation of the active compound, not to mention understanding the stability and bioavailability of the cannabinoids and their pharmacokinetics once introduced into the body. Also, more study is necessary to determine the synergic effect of natural therapeutic plants in combination with *Cannabis*, to design functional nanoparticle delivery devices, and to test the safety and efficacy of an experimental new antibiotic in clinical trials.

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Author contributions

Conceptualization and design (HMSAU); writing original draft preparation, Abstract, New classes of potent antimicrobial agents, A novel mode of action, Conclusion (HMSAU); Introduction and future research directions (ELB); A novel mode of action (MK); Abstract, Introduction, Challenges on applications of Cannabinoids as an antimicrobial agent (CP); Figure design (HMSAU); writing a review, and editing (AF, CP, ELB, HMSAU). All authors have read and agreed to the published version of the manuscript.

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