DOI: 10.31276/VJSTE.64(4).70-78

Review Cannabis: A new strategy against methicillin-resistant *Staphylococcus aureus*

Emerson Joseph Addison*

Central Michigan University

Received 15 October 2021, accepted 7 January 2022

Abstract:

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global health concern. Many antibiotics are no longer effective at treating MRSA, which causes an increase in adverse patient outcomes. This has led to calls for new antibiotics and treatment strategies to combat the spread of MRSA and multidrug resistance (MDR). The antimicrobial secondary metabolites found in plants are a promising source for new antibiotics and treatment strategies. *Cannabis sativa* L. is especially promising, as it produces dozens of antimicrobial secondary metabolites that are active against *Staphylococcus aureus* (*S. aureus*) and MRSA strains. In addition to its antimicrobial properties against *S. aureus* and MRSA, cannabis has many other desirable properties for potential antibiotics. Cannabis secondary metabolites are active against a wide range of microorganisms, are generally safe, target multiple bacterial processes and structures, have antimicrobial synergies, have a low potential for resistance development, can be produced inexpensively and combined with existing antibiotics to further reduce costs, and contain secondary metabolites capable of penetrating a variety of in vivo environments. These characteristics make cannabis a potential resource against MRSA and MDR bacteria.

Keywords: **antimicrobial secondary metabolites, cannabinoids, cannabis, *cannabis sativa*, combination therapy, methicillin-resistant, MRSA, *S. aureus*.**

Classification numbers: **3.2, 3.3**

Introduction

S. aureus is a commensal bacterium that colonizes approximately 30% of the human population and acts as an opportunistic pathogen [1]. Typically, hosts are asymptomatic; however, infections are common and can range from mild skin infections and abscesses to invasive and life-threatening infections including bacteraemia, endocarditis, and pneumonia [1, 2]. In 2017, *S. aureus* bacteraemia was responsible for approximately 20,000 deaths and 120,000 infections in the United States [2].

S. aureus develops antibiotic resistance (AR) quickly and AR in *S. aureus* is widespread. MRSA is of particular concern as MRSA rates in World Health Organization (WHO) regions typically exceed 20%, which increases risks for patients and necessitates the use of second line, more toxic drugs [3]. In the past, vancomycin was considered the antibiotic of last resort for MRSA infections; however, due to the risk for adverse reactions and increasing rates of vancomycin resistance, newer antibiotics, such as linezolid, daptomycin, quinupristin/dalfopristin, and tigecycline are often used [4]. Unfortunately, these newer antibiotics can be expensive and have risks for adverse reactions [5]. Additionally, resistances, though rare, have already developed for linezolid, daptomycin, quinupristin/dalfopristin and tigecycline [4]. Thus, the search for new antibiotics that are effective against MRSA and other MDR bacteria continues.

This review will examine *Cannabis sativa* as a potential source for new antimicrobial compounds for the treatment of MRSA and other MDR bacteria. Cannabis is promising in this regard because it produces an abundance of antimicrobial secondary metabolites and has many other qualities that are desirable in antibiotic therapy and antibiotic development.

Cannabis sativa

Cannabis has been cultivated and used as medicine for thousands of years [6-11]. After millions of years of evolution, thousands of years of traditional cultivation and generations of modern selective breeding, there is considerable diversity among varieties of cannabis, with different strains having different medicinal properties [12].

As different cannabis cultivars can be easily cross-bred, and typical plant characteristics like height and leaflet width are insufficient distinctions between varieties, cannabis is often classified by "chemovar" according to biochemical characteristics [13-15]. Each chemovar boasts unique genetics and combinations of the various secondary metabolites found in cannabis [10-16]. As of the writing of this review, there are as many as 700 different chemovars of cannabis [9, 10, 15]. In addition to the influence of genetics on the chemical profile of each chemovar, the chemical profile of cannabis is further influenced by environmental and external factors including nutrition, humidity, temperature, age of plant, harvest time, plant stress, and plant organ and storage conditions [17-20].

Cannabis chemovars produce more than 500 natural secondary metabolites from 18 different chemical classes, including more than 100 cannabinoids and more than 200 terpenes [11, 12, 14, 19, 21-25].

*Email: emersonaddison@gmail.com

Cannabis is noteworthy for its production of cannabinoids, which are lipophilic molecules with low water solubility. Cannabinoids have been only rarely detected in non-cannabis plants [24]. The best-known cannabinoids are tetrahydrocannabinol (THC), the primary psychoactive compound, and cannabidiol (CBD), which is known for having a variety of medicinal properties and for being an antipsychotic. THC is a "weak partial agonist on CB1 and CB2 receptors," while CBD is a "negative allosteric modulator of CB1" [26].

In addition to terpenes and cannabinoids, hundreds of other compounds have been identified in cannabis, including 27 nitrogenous compounds, 18 amino acids, 3 proteins, 6 enzymes, 2 glycoproteins, 34 sugars and related compounds, 50 hydrocarbons, 7 simple alcohols, 12 simple aldehydes, 13 simple ketones, 20 simple acids, 23 fatty acids, 12 simple esters, 1 lactone, 11 steroids, 25 non-cannabinoid phenols, 23 flavonoids, 1 vitamin, 2 pigments, and 9 elements [27]. Cannabis resin, which is naturally produced in the trichomes, is rich in both cannabinoids and terpenes and is "valued for its psychoactive and medicinal properties" [11].

Antimicrobial activity vs S. aureus and MRSA

The essential oils and extracts from cannabis, as well as many of the individual cannabinoids, have antimicrobial properties and are active against various strains of *S. aureus* and MRSA [6-8, 17, 21, 28-50]. Antimicrobial activity against *S. aureus* and MRSA strains has been demonstrated by many *cannabinoids*, including cannabichromenic acid (CBCA) [32, 39, 40], cannabichromene (CBC) [6, 30, 40], cannabichromene-C_0 (CBC homolog) [6], cannabichromene-C_1 (CBC homolog) [6], isocannabichromene-C_0 [6], (±)-3″-hydroxy-$\Delta^{(4''5'')}$-cannabichromene [33], cannabidiolic acid (CBDA) [8, 30, 40], cannabidiol (CBD) [8, 30, 38-40, 44, 48-50], cannabidivarin methyl ester (CBDVM) [32], cannabigerol acid (CBGA) [30, 40], cannabigerol (CBG) [30, 39, 40], 4-acetoxy-2-geranyl-5-hydroxy-3-n-pentylphenol (CGB derivative) [33], 5-acetoxy-6-geranyl-3-n-pentyl-1,4-benzoquinone [46], 5-acetyl-4-hydroxycannabigerol [33], methylated cannabigerol [30], cannabinol (CBN) [30, 39, 40], 8-hydroxycannabinolic acid A [33], 1′ S-hydroxycannabinol [21], carmagerol [30], pre-Δ^9-tetrahydrocannabinol (Δ^9-THCA) [30, 40], Δ^9-tetrahydrocannabinol (Δ^9-THC) [30, 39, 40, 45, 50], cannabidivarin (CBDV) [40], cannabidivarinic acid (CBDVA) [40], Δ^8-tetrahydrocannabinol (Δ^8-THC) [40], tetrahydrocannabivarinic acid (THCVA) [40], Δ^9-tetrahydrocannabivarin (THCV) [40], exo-olefin THC [40], and +/- 11-OH Δ^9-THC [40]. Additionally, THC has been demonstrated to protect mice from acute respiratory distress syndrome (ARDS) and toxicity caused by the cytokine storm triggered by Staphylococcal enterotoxin B (SEB), which is a toxin produced by *S. aureus* [45].

Many of the non-cannabinoid phytocompounds in cannabis are also active against *S. aureus* and MRSA strains [30, 38, 41, 46, 51-68]. Antimicrobial activity against *S. aureus* and MRSA strains has been observed in α-bisabolol (levomenol) [51], carvacrol [65-68], eugenol [52], nerolidol [51], limonene [53], para-cymene (p-cymene) [53], myrcene (β-myrcene) [38, 53], olivetol [30], 1,8-cineole [54, 57, 58, 64], α-pinene [38, 52, 59, 60, 64], β-pinene [38, 52], α-terpineol [58, 60], α-terpinolene [38], terpinen-4-ol [58, 60], thymol [53, 65, 66], β-caryophyllene [38, 59], humulene (α-caryophyllene) [59], β-amyrin [61], cannflavin A [46], naringenin [41, 62, 63], caffeic acid [55], and linoleic acid [56].

Other antimicrobial activity

Antibiotic combinations that are effective against a variety of microorganisms are useful as empirical therapy for the treatment of unidentified pathogens [69]. In addition to being effective against MRSA, the antimicrobial properties of cannabis have been tested against other pathogenic microorganisms, including many species of gram-positive and gram-negative bacteria, a variety of clinically significant fungi, and Leishmania protozoa.

Cannabis essential oil and extracts are active against many species of gram-positive bacteria, including *Bacillus* cereus [38]; *Bacillus pumilus* [29]; *Bacillus subtilis* [7, 17, 29, 38]; *Brevibacterium linens* and *Brochothrix thermosphacta* [17]; *Clostridium tyrobutyricum*, *Clostridium bifermentans*, *Clostridium butyricum* and *Clostridium sporogenes* [70]; *Enterococcus faecalis* [35, 38, 47]; *Enterococcus faecium* and *Enterococcus hirae* [38, 70]; *Micrococcus flavus* [29]; *Listeria monocytogenes* and *Staphylococcus epidermidis* [38]; *Streptococcus salivarius* [70]; and the gram-positive to gram-variable *Micrococcus luteus* [17].

Cannabis essential oil and extracts are active against a variety of gram-negative bacteria, including *Acinetobacter calcoaceticus*, *Aeromonas hydrophyla* and *Beneckea natriegens* [17]; *Bordetella bronchiseptica* [29]; *Escherichia coli* [7, 17, 35, 47]; *Enterobacter aerogenes* [47]; *Flavobacterium suaveolens* [17]; *Helicobacter pylori* [41]; *Pectobacterium carotovorum* [70]; *Pseudomonas aeruginosa* [7, 35, 43]; *Pseudomonas campestris*, *Pseudomonas corrugata*, *Pseudomonas fluorescens*, *Pseudomonas savastanoi*, *Pseudomonas syringae*, and *Pseudomonas viridiflava* [70]; *Proteus vulgaris* [29]; *Salmonella typhimurium* [47]; and *Yersinia enterocolitica* [17].

Cannabis essential oil and extracts are active against a variety of fungi, including *Aspergillus niger* [29]; *Candida albicans* [7, 29, 43]; and *Candida sake*, *Kluyveromyces marxianus*, *Pichia membranaefaciens*, *Schizosaccharomyces pombe*, *Schizosaccharomyces japonicus*, *Torulaspora delbrueckii* and *Zygosaccharomyces bailii* [70]. Fractional cannabis distillations showed activity against *Candida glabrata*, *Candida krusei*, and *Cryptococcus neoformans*; cannabis extracts have also demonstrated activity against the protozoa *Leishmania donovani* [36].

In addition to being active against MRSA, many of the secondary metabolites found in cannabis, including many of the cannabinoids, have also been individually tested against other microorganisms. CBD is of particular interest as a potential antimicrobial, and in one study showed a consistent MIC (minimum inhibitory concentration) of 1-4 μg/ml against more than 20 types of gram-positive bacteria, including multiple strains of MRSA, MDR *Streptococcus pneumoniae*, *E. faecalis*, and the anaerobic bacteria *Clostridioides difficile* and *Cutibacterium* acnes [49]. CBD is also active against *L. mono-cytogenes*, *E. faecalis*, and methicillin-resistant *S. epidermidis* (MRSE) [44]. THC and CBD are active against *Streptococcus pyogenes*, *Streptococcus milleri*, and *Streptococcus faecalis* [50]. CBD and CBDA are active against *S. epidermis* [8]; carvacrol is also active against *S. epidermis* [65]. CBD, α-pinene, β-pinene, β-myrcene, α-terpinolene, and β-caryophyllene are active against *S. epidermidis*, *L. monocytogenes*, *E. faecalis*, *E. faecium*, *E. hirae*, *B.*

subtilis, and *B. cereus* [38]. Naringenin is active against *H. pylori* [41]. CBC and its homologs, analogues and isomers are active against several bacteria, including *B. subtilis*, and *M. smegmatis*; CBC is also active against *S. cerevisiae* and *Trichophyton mentagrophytes*; many CBC homologs and isomers are also active against *T. mentagrophytes*, *C. albicans*, and *S. cerevisiae* [6]. α-Humulene is active against *C. Neoformans*, *C. Glabrata*, *C. Krusei* and *L. Donovani* [36], and against *C. bifermentans*, *E. hirae*, *E. faecium* and *S. salivarius*, *P. viridiflava*, *P. membranaefaciens*, *S. cerevisiae*, *S. japonicus*, and *Z. bailii* [70]. α-Pinene is active against *S. salivarius*, *C. tyrobutyricum*, *C. bifermentans*, *C. butyricum*, *C. sporogenes*, *E. hirae*, *E. faecium*, *P. savastanoi*, *P. carotovorum*, *P. corrugata*, *P. fluorescens*, *P. syringae*, *P. viridiflava*, *P. campestris*, *C. sake*, *K. marxianus*, *P. membranaefaciens*, *S. cerevisiae*, *Schizosaccharomyces pombe*, *S. japonicus*, *T. delbrueckii*, and *Z. bailii* [70]. β-Pinene, myrcene and carmagnola are active against *C. bifermentans*, *E. hirae*, *E. faecium*, *P. corrugata*, *P. fluorescens*, *P. viridiflava*, *C. sake*, *K. marxianus*, *P. membranaefaciens*, *S. pombe*, and *S. japonicus* [70]. β-Caryophyllene shows weak activity against *C. neoformans* [36]. The compound 1′ S-hydroxycannabinol is active against *L. donovani* and *P. falciparum* [21]. Other compounds isolated from cannabis with antimicrobial properties include 5-acetoxy-6-geranyl-3-n-pentyl-1, which is active against *L. donovani* and *Plasmodium falciparum*; cannflavin A, cannflavin C, and β-acetyl cannabispiranol, which are active against *L. donovani*; and 6-prenylapigenin, which is active against *P. falciparum*, *L. donovani*, and *C. albicans* [46]. Other anti-microbial cannabinoids that were recently discovered include (±)-3″-hydroxy-$\Delta(^{4''},^{5''})$-cannabichromene, which is active against *C. albicans* and *C. krusei*; 4-acetoxy-2-geranyl-5-hydroxy-3-n-pentylphenol, active against C. krusei; 8-hydroxycannabinol, which is active against *C. albicans* and *M. intracellulare*; 8-hydroxycannabinolic acid A, which is active against *C. krusei* and *E. coli*; (±)-4-acetoxycannabichromene and 5-acetyl-4-hydroxycannabigerol, which are both active against *L. donovani* and *P. falciparum*; and (±)-3″-hydroxy-$\Delta(^{4''5''})$-cannabichromene and 4-acetoxy-2-geranyl-5-hydroxy-3-n-pentylphenol, which are both active against *L. donovani* [33].

The ability to target a wide range of pathogenic microorganisms must be contrasted against the potential to damage the microbiome, as "selective inhibition is of the utmost importance for the maintenance of healthy gut microbiota" [47]. Cannabis has promise in this regard, too. Cannabis extract displayed "no inhibitory effects on the growth of probiotic strains" *Lactobacillus paracasei*, *Lactobacillus reuteri*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Bifidobacterium bifidum*, *Bifidobacterium longum* and *Bifidobacterium breve* [47].

Mechanism of action and synergistic interactions

Cannabis is also desirable as a potential antibiotic source for its ability to engage multiple bacterial targets and synergistic interactions. Synergistic interactions that potentiate antimicrobial effects are an evolutionary strategy against microorganisms [69, 71]. Many successful antibiotics engage multiple bacterial targets, structures, or processes, and typically resistance takes longer to emerge when multiple targets are engaged [69]. Combination antibiotic therapies often utilize antibiotic synergies.

Plants produce an abundance of secondary metabolites that often rely on synergistic combinations [72]. Multi-target engagement is also common among plants, and the essential oils and secondary metabolites produced by plants target microorganisms in multiple ways that affect their pathological processes [73-76]. Essentially, plant secondary metabolites often work together synergistically and engage multiple targets using different mechanisms of action [74, 75].

Common mechanisms of action include the disruption of cytoplasmic membrane function and structure (including the efflux system), interaction with the membrane proteins (ATPases and others), interruption of DNA/RNA synthesis and function, destabilization of the proton motive force with leakage of ions, prevention of enzyme synthesis, induction of coagulation of cytoplasmic constituents, and interruption of normal cell communications (quorum sensing) [74, 75].

The high diversity of cannabis chemovars and antimicrobial metabolites increases the likelihood of antimicrobial synergies [38]. Additionally, cannabis produces many unique secondary metabolites that are known to attack multiple targets in *S. aureus* and MRSA. CBD is particularly notable for multiple target engagement in *S. aureus*. CBD sharply inhibits protein, DNA, RNA, peptidoglycan and lipid synthesis [49]. CBD is active against MRSA biofilms [40, 49] and causes depolarization of the cytoplasmic membrane [44, 49]. Additionally, CBD shows low resistance frequency and has a low propensity to induce resistance [49]. At high concentrations, many other cannabinoids are active against biofilms, including CBG, CBN, CBC, CBCA, Δ^9-THC, Δ^8-THC, exo-olefin THC, Δ^9-THCA, THCV, CBGA, CBDV, CBDA, and +/- 11-OH Δ^9-THC [40]. CBCA induces rapid degradation of the bacterial lipid membrane and bacterial nucleoid [32]. CBG targets the cytoplasmic membrane; represses biofilm formation and eradicates preformed biofilms; kills persisters by rapidly eradicating them to below detection thresholds within 30 minutes of treatment; and shows no resistance development after being challenged for spontaneous resistance mutations [39].

Many of the non-cannabinoid secondary metabolites common in cannabis also engage multiple targets in *S. aureus* and MRSA. Carvacrol affects the lipid bilayer of bacterial cytoplasmic membranes causing loss of integrity and collapse of proton motive force, which results in a leakage of cellular material [67], reduces [66, 67] and eradicates biofilms [66], and is active against dual-species biofilms [68]. Myrcene acts synergistically with many essential oil components against *S. aureus* [54]. 1,8-cineole, α-terpineol, and terpinen-4-ol are active on the cytoplasmic membrane causing predisposition to lysis, loss of 260 nm absorbing material, altered morphology, and loss of tolerance to NaCl [58]. Linoleic acid inhibits the efflux pump and is synergistic with erythromycin [56]. Naringenin inhibits the growth of *S. aureus*, disrupts the cytoplasmic membrane, and affects the expression of fatty-acid synthesizing genes [63]. At high levels, naringenin damages the cytoplasmic membrane and interacts with DNA by changing conformations and molecular morphology [62]. Levomenol and nerolidol enhance membrane permeability, thereby increasing susceptibility of *S. aureus* to many common antibiotics [51]. Thymol inhibits biofilm formation and eradicates biofilms [66]. Quercetin can decrease the proton-motive force [76]. Caffeic acid is active on efflux pumps by

inhibiting the MrsA pumps of the *S. aureus* strain RN-4220 and the NorA pump of *S. aureus* strain 1199B [55].

Antibiotic resistance

Resistance development is another consequence of conventional antibiotic therapies. The multitude of antimicrobial secondary metabolites in cannabis may help prevent or delay resistance. One of the benefits that is often argued of combination therapy is that the simultaneous use of multiple antibiotics can delay resistance development [77-80]. Increasing the number of drugs used in combinations could be an effective strategy as high-order combinations can potentially slow resistance development [81].

With dozens of antimicrobial secondary metabolites that are active against MRSA, cannabis is a potential source for high-order antimicrobial combinations. Moreover, the bioactivity of the antimicrobial secondary metabolites found in plants typically does not confer resistance, and the use of antimicrobial plant extracts is relatively effective at preventing and reducing resistance [72].

Recent studies suggest that resistance is unlikely to development to either CBD [49] or CBG [39]. Although some species of bacteria are capable of developing resistance to some essential oils, resistance development to essential oils is generally rare and the development of resistance may be dependent upon oil composition and species of bacteria [82].

To further prevent or counter resistance development, different chemovars, with different compositions of antimicrobial secondary metabolites, could be easily substituted in a manner similar to cycling and mixing strategies, which rely on switching antibiotic regimens on time intervals or a per-patient basis in order to reduce selective pressure for resistance development.

The entourage effect

The term "entourage effect" is sometimes used to describe the complex interactions and variety of effects that "inactive" compounds found in cannabis are thought to have on active compounds [9, 12-14, 18, 19]. Many studies suggest therapeutic synergies in cannabis, and it is often observed that the effects of the entire plant are greater than the effects of individual components [9, 12-14, 18, 19, 83, 84]. In addition to these studies, many consumers of cannabis attribute different physiological effects to different chemovars [9, 11, 12, 19].

Although some chemovars might be inappropriate for some patients, with more than 700 chemovars available, the diversity of chemovars makes it likely that a chemovar with an appropriate balance of secondary metabolites could be found or bred for specific conditions and most patients [12, 16, 34].

Cannabis could potentially be used to treat multiple conditions simultaneously. Although in vivo studies and clinical trials are needed to determine if cannabis or cannabis secondary metabolites are suitable for treating *S. aureus* and MRSA infections, cannabis is already recognized for its analgesic properties [9, 12, 31]. Should cannabis prove suitable for *S. aureus* and MRSA treatment, it could potentially be used to treat both the infection and associated pain, possibly eliminating the need for a separate analgesic. Clinical trials could also be conducted to determine if cannabis could replace multiple drugs when treating *S. aureus* infection presenting with SEB-induced ARDS, as THC is a potent anti-inflammatory that halts the cytokine storm caused by the overactive immune response to SEB [45].

Additionally, medicinal plants and plant-based antimicrobials are generally less expensive and easier to obtain than synthetic drugs, and can be combined with other antibiotics to reduce testing and development costs [72].

Pharmacokinetic profile

Pharmacokinetic considerations are an important factor of antimicrobial therapy. Penetration differences between antibiotics administered simultaneously can accelerate the development of MDR by allowing for the stepwise accumulation of mutations, which can lead to an increase in both the rate of mutation acquisition and the rate of selection for pre-existing mutations [85-87].

Cannabis has desirable pharmacokinetic properties. Many of the antimicrobial secondary metabolites of cannabis share similar penetration profiles, which could allow multiple antimicrobial compounds to penetrate many in vivo environments. Cannabinoids cross the blood-brain barrier and the placental barrier, are present in breast milk, reduce inflammation and can penetrate *S. aureus* biofilms [6, 12, 18, 23, 45, 83]. Terpenoids, flavonoids, anthocyanins, and many other secondary metabolites present in cannabis are known to reduce inflammation, cross the blood-brain barrier, and destroy *S. aureus* biofilms [12, 18, 19, 22, 66-68, 88].

Toxicity, drug-drug interactions

Cannabis generally poses a low risk for toxicity, which is another important consideration with antibiotic therapy. On a population scale, cannabis has as estimated margin of exposure, which is the ratio of toxicological threshold to estimated human intake, in excess of 10,000 [89] and many studies have concluded that it is nearly impossible to consume lethal quantities of either cannabis or THC [89-92]. Further evidence of the low toxicity of cannabis can be found in the fact that there are no reported deaths from cannabis overdose [9, 26, 93] despite the fact that cannabis is used globally by more than 100 million people [93]. The absence of overdose deaths from cannabis is likely due to the lack of CB1 receptors in the brainstem cardiorespiratory centres, causing minimal interaction in areas of the brain involved in respiration [9, 26, 31, 94]. CBD has low toxicity when tested against red blood cells and keratinocytes [8].

In addition to low toxicity, many of the adverse effects associated with cannabis tend to decrease with tolerance or can be mitigated by other constituents of the plant. Cardiovascular effects of cannabis, such as tachycardia and increased blood pressure, are minimal or transient, and subside with tolerance [92]. Tolerance to the psychoactive effects of cannabis typically develops over several days; however, tolerance generally does not develop to the medical benefits, allowing patients to maintain dose consistency for many years [26]. The side effects of THC are mitigated by other secondary metabolites present in cannabis, and natural cannabis causes fewer psychological side effects than synthetic THC [95]. Numerous studies demonstrate the antipsychotic properties of CBD and suggest that CBD can counter or mitigate many of the

adverse psychoactive effects of THC [16, 18, 25, 95-98]. CBD has been observed to reduce anxiety, tachycardia, hunger, and sedation [25]. CBD is also being studied as a potential treatment for psychosis and schizophrenia [18, 98-101]. Moreover, not all chemovars of cannabis contain THC. For example, hemp, which is grown primarily for CBD and industrial applications, has very low quantities of THC, usually less than 0.3% [17].

Cannabis generally does not decrease effectiveness of concomitant medications and significant drug interactions, though rare, are typically associated with concurrent use of depressants [26]. However, many cannabinoids are known to interact with enzymes [20, 23, 25] and drug-drug interactions due to cytochrome P450 (CYP450) inhibition could occur [20, 23, 25, 102]. CBD inhibition of CYP450 has been associated with adverse drug events and has the potential to cause pharmacokinetic and pharmacodynamic drug-drug interactions [102]. There is no evidence that cannabis increases overdose lethality from other drugs [92].

Conclusions

In vitro studies and animal model studies suggest cannabis and its secondary metabolites as a potential source for new antimicrobial compounds against *S. aureus* and MRSA strains. However, more research is needed to understand the complex pharmacokinetics and pharmacodynamics of cannabis and its secondary metabolites before effective antimicrobial therapies can be developed.

Specifically, in vivo studies are needed to determine penetration profiles, screen for drug-drug interactions, and to test suitability for treatment of systemic infection. Given the high number of cannabis secondary metabolites active against *S. aureus* and MRSA strains, the multiplicity of antimicrobial mechanisms of action, and the potential for synergistic interactions, cannabis secondary metabolites should also be studied as antibiotic combinations and as possible adjuvants to be administered alongside conventional antibiotics. In addition to possible use in antibiotic combination therapy, the low potential for overdose and the generally safe profile of cannabis could allow cannabis-based therapies to be administered at high doses. Furthermore, the diversity among cannabis chemovars could allow for other antibiotic strategies such as cycling and mixing. Further research is necessary.

Due to the number of antimicrobial secondary metabolites produced by cannabis and the diversity of cannabis chemovars, it could also be possible to cross-breed a chemovar that produces specific antimicrobial secondary metabolites that are active against a target pathogen. If the antibiotic potential of cannabis is confirmed upon further testing, cannabis could provide an inexpensive and abundant source of new antibiotics for the developing world.

Appendix

The following appendix contains a list of approximately 50 cannabis secondary metabolites and derivatives that demonstrate antimicrobial activity against *S. aureus* and MRSA strains (Table 1). This list could be useful in cross breeding a chemovar that produces dozens of antimicrobial secondary metabolites active against *S. aureus*. This chemovar could serve as the starting point for the production and extraction of a full-spectrum oil that contains specific antimicrobial secondary metabolites, in consistent proportions, that target *S. aureus* and MRSA strains. Such a combination of secondary metabolites could effectively emulate the high-order combination strategy that evolved in plants. Ultimately, the secondary metabolites of cannabis, used wisely and in the correct proportions, could provide a new treatment strategy for MRSA that improves patient outcomes and minimizes the development of new resistances.

Table 1. Antimicrobial activity of cannabis secondary metabolites against different strains of *S. aureus*.

Cannabis secondary metabolite	Description of activity
Cannabinoids	
Cannabichromenic acid (CBCA)	MIC of 2 µg/ml against MRSA USA 300 [39, 40]. Bactericidal against MRSA at 3.9 µM and MSSA 34397 at 7.8 µM [32].
Cannabichromene (CBC):	Anti-inflammatory; 24 and 48 h MIC of 1.56 µg/ml against *S. aureus* ATCC 6538 [6]. Represses biofilm formation of MRSA USA 300; MIC of 8 µg/ml against MRSA USA 300 [39, 40]. Inhibits SA-1199B, RN-4220, ATCC 25923, EMRSA-15, and EMRSA-16 at 2 µg/ml; inhibits XU-212 at 1 µg/ml [30].
Cannabichromene-C_0 (CBC homolog)	Anti-inflammatory; 24 and 48 h MIC of 12.5 µg/ml against *S. aureus* ATCC 6538 [6].
Cannabichromene-C_1 (CBC homolog)	Anti-inflammatory; 24 and 48 h MIC of 3.12 µg/ml against *S. aureus* ATCC 6538 [6].
Isocannabichromene-C_0	Anti-inflammatory; 24 and 48 h MIC of 12.5 µg/ml against *S. aureus* ATCC 6538 [6].
(±)-3″-hydroxy-$\Delta^{(4'',5'')}$-cannabichromene	IC_{50} against MRSA ATCC 35591 at 24.4 µM; IC_{50} against *S. aureus* ATCC 29213 29.6 µM [33].
Cannabidiolic acid (CBDA)	Inhibits SA-1199B, RN-4220, XU-212, ATCC 25923, EMRSA-15, and EMRSA-16 at 2 µg/ml [30]. MIC of 2 µg/ml against *S. aureus* ATCC 25923; MIC of 4 µg/ml against MRSA USA 300 [8]. Against MRSA USA 300, MIC of 16 µg/ml [39, 40]; inhibits biofilm formation [40].
Cannabidiol (CBD)	Engages multiple targets against *S. aureus*. Inhibits protein, DNA, RNA and peptidoglycan synthesis against *S. aureus* RN42200 at 2-3 µg/ml, rapidly shutting down synthesis pathways; reduces lipid synthesis at concentrations below MIC; membrane depolarization; MIC of 1-4 µg/ml against multiple strains MRSA, with similar MIC against VRSA; MIC90 against 132 MRSA and MSSA ATCC strains and Australian clinical isolates at 4 µg/ml; MIC50 and MIC_{90} of 1 µg/ml against an additional 50 MSSA and 50 MRSA USA-derived isolates; rapid bactericidal activity (<3 h) with minimum bactericidal concentration (MBC) of 2 µg/ml against MRSA ATCC 43300; able to penetrate and kill biofilms; minimum biofilm eradication concentration (MBEC) of 1-2 µg/ml against MSSA biofilms; MBEC of 2-4 µg/ml against MRSA biofilms; low innate resistance frequency value ($<3.78\times10^{-10}$) at 2x MIC against MRSA ATCC 43300); unlikely to induce resistance against MRSA ATCC 43300 (after 20 days of daily passage with 8 replications, 1.5-fold increase in MIC against CBD vs. 26-fold increase against daptomycin; non-toxic to human red blood cells, no signs of haemolysis up to 256 µg/ml; modest cytotoxicity against HEK-293 cells (human embryonic kidney), with CC_{50} around 200 µg/ml [49]. Causes depolarization of the cytoplasmic membrane against MRSA USA 300 at concentrations of 0.1 and 0.2 µg/ml; when combined with bacitracin, reduces MIC of BAC by 64-fold, and causes morphological changes including septa formations and membrane irregularities [44]. Bacteriostatic and bactericidal against *S. aureus* ATCC 6538 in nutrient broth agar between 1-5 µg/ml and in horse blood agar between 20-50 µg/ml [50]. Represses biofilm formation; MIC of 2 µg/ml against MRSA USA 300 [39, 40]. Inhibits SA-1199B, RN-4220, XU-212, EMRSA-15, and EMRSA-16 at 1 µg/ml; inhibits ATCC 25923 at 0.5 µg/ml [30]. MIC of 8 µg/ml against *S. aureus* ATCC 6538; MIC of 32 µg/ml against *S. aureus* 18As; MIC of 32 µg/ml against *S. aureus* 386 [38]. MIC against *S. aureus* ATCC 25923 and MRSA USA 300 at 1 µg/ml [8]. Against MRSA USA 300, a Canadian study published in 2021 reported CBD had an MIC value of 2.5 µg/ml and an MBC of 10 µg/ml; CBD powder had an inhibition zone of 11 mm and CBD oil had an inhibition zone of 9 mm [48].
Cannabidivarinic acid (CBDVA)	MIC of 32 µg/ml against MRSA USA 300 [40].
Cannabidivarin (CBDV)	MIC of 8 µg/ml against MRSA USA 300; inhibits biofilm formation [40].
Cannabidivarin methyl ester (CBDVM)	Bactericidal against MRSA at 15.6 µM [32].

Pre-Cannabigerol (Cannabigerolic-acid / CBGA)	Inhibits SA-1199B, XU-212, ATCC-25923, and EMRSA-16 at 4 µg/ml; inhibits RN-4220 and EMRSA-15 at 2 µg/ml [30]. MIC of 4 µg/ml against MRSA USA 300 [40].
Cannabigerol (CBG)	Active on the cytoplasmic membrane of gram-positive bacteria; represses biofilm formation of MRSA USA 300 by 50% at 0.5 µg/ml; MIC of 2 µg/ml against MRSA USA 300; eradicate preformed biofilms of MRSA USA 300 at 4 µg/ml; killed persisters in a concentration-dependent manner starting at 5 µg/ml; eradicated a population of ~108 CFU/ml MRSA persisters to below detection threshold within 30 minutes; MIC_{90} against 96 clinical isolates of MRSA ranged from 2-8 µg/ml, with one outlier isolate MIC_{90} of 0.0625; frequency of resistance less than 10^{-10} for MRSA; in vivo efficacy of CBG in systemic MRSA USA 300 mouse infection was comparable to vancomycin administered at a similar dose [39, 40]. Inhibits SA-1199B, RN-4220, XU-212, ATCC 25923, and EMRSA-16 at 1 µg/ml; inhibits EMRSA-15 at 2 µg/ml [30].
Cannabicyclol (CBL)	Represses biofilm formation against MRSA USA 300 [40].
4-Acetoxy-2-geranyl-5-hydroxy-3-n-pentylphenol (CGB - derivative)	IC_{50} against MRSA ATCC 35591 at 6.7 µM; IC50 against *S. aureus* ATCC 29213 12.2 µM [33].
5-Acetoxy-6-geranyl-3-n-pentyl-1,4-benzoquinone	IC_{50} against MRSA ATCC 43300 at 15 µg/ml [46].
5-Acetyl-4-hydroxycannabigerol	IC50 against MRSA ATCC 35591 at 53.4 µM [33].
+/- 11-OH Δ^9-THC	Represses biofilm formation of MRSA USA 300 [40].
Methylated Cannabigerol	Inhibits SA-1199B and XU-212 at 64 µg/ml [30].
Cannabinol (CBN)	Represses biofilm formation; MIC of 2 µg/ml against MRSA USA 300 [39, 40]. Inhibits SA-1199B, RN-4220, XU-212, ATCC-25923, and EMRSA-15 at 1 µg/ml [30].
8-Hydroxycannabinolic acid A	IC_{50} against *S. aureus* ATCC 29213 3.5 µM [33].
1′S-hydroxycannabinol	Active against MRSA ATCC 43300 at IC_{50} 10.0 µg/ml [21].
Carmagerol	Inhibits SA-1199B, RN-4220, and EMRSA-16 at 32 µg/ml; inhibits XU-212, ATCC 25923, and EMRSA-15 at 16 µg/ml [30].
(-)Δ^8-tetrahydrocannabinol (-Δ^8THC)	Against MRSA USA 300, MIC of 2 µg/ml; inhibits biofilm formation [39, 40].
Pre-Δ^9 -Tetrahydrocannabinol (Δ^9-THCA)	Inhibits SA-1199B, XU-212, and EMRSA-15 at 8 µg/ml; inhibits RN-4220, ATCC 25923, and EMRSA-16 at 4 µg/ml [30]. Inhibits biofilm formation against MRSA USA 300 [40].
Δ^9-tetrahydrocannabinolic acid A (THCAA)	MIC of 4 µg/ml against MRSA USA 300 [40].
Δ^9-tetrahydrocannabinol (Δ^9-THC)	Bacteriostatic and bactericidal against S. aureus ATCC 6538 in nutrient broth agar between 2-5 µg/ml and in horse blood agar between 20-50 µg/ml [50]. Represses biofilm formation; MIC of 2 µg/ml against MRSA USA 300 [39, 40]. Inhibits EMRSA-16 at 0.5 µg/ml; inhibits RN-4220, XU-212, and ATCC 25923 at 1 µg/ml; inhibits SA-1199B and EMRSA-15 at 2 µg/ml [30]. Protects mice from ARDS and toxicity post-SEB exposure by suppression of inflammatory cytokines and cessation of cytokine storm, attenuating SEB-mediated lung injury [45].
Tetrahydrocannabivarinic acid (THCVA)	MIC of 16 µg/ml against MRSA USA 300 [40].
Δ^9-tetrahydrocannabivarin (THCV)	MIC of 4 µg/ml against MRSA USA 300 [40].
Exo-olefin THC	Represses biofilm formation; MIC of 2 µg/ml against MRSA USA 300 [39, 40].
Terpenes and Terpenoids	
Alpha-bisabolol (α-bisabolol; levomenol)	Sesquiterpenoid. Disrupts bacterial cell membranes; increases susceptibility of *S. aureus* ATCC 6538 to many common antibiotics [51].
Carvacrol	A secondary terpene found in some cultivars. Targets the lipid bilayer of bacterial cytoplasmic membranes. MIC values against 25 strains of *S. aureus* range from 0.015-0.03% (v/v) [65]. Effective against *S. aureus* 6-ME, 810-CT, 815-CT, 808-CT, 5-ME, and 74-CCH: MIC of 0.015-0.031% (v/v); MBC of 0.062-0.125% (v/v); BIC (biofilm inhibitory concentration) of 0.031-0.125% (v/v); BEC (biofilm eradication concentration) of 0.125-0.5% (v/v) [66]. In both liquid and vapour forms, causes significant reduction in biofilm biomass and cultivable cell numbers of *S. aureus* 815 [67]. Interferes with formation of dual-species biofilms consisting of *S. aureus* NCTC 10788/ Salmonella enterica serovar Typhimurium NCTC 74; total inhibition of dual-species biofilm at high doses [68].
Eugenol	Inhibits growth and cell viability of a variety of *S. aureus* strains. MIC of 10 µg/ml against *S. aureus* ATCC 13150, *S. aureus* ATCC 6538, *S. aureus* ATCC 25923, and *S. aureus* ATCC LB 126 [52].
Nerolidol	Disrupts bacterial cell membranes; increases susceptibility of *S. aureus* ATCC 6538 to many common antibiotics [51].
Limonene	A cyclic monoterpene. MIC of about 80 µg/ml and MBC of about 110 µg/ml against MRSA ATCC 43300: [53].
Para-Cymene (p-cymene)	MIC of about 50 µg/ml and MBC of about 100 µg/ml against MRSA ATCC 43300 [53].
Myrcene (β-myrcene)	Synergizes the antibiotic potency of other essential oil components against *S. aureus* and a number of other bacteria [54]. MIC of 8 µg/ml against *S. aureus* ATCC 6538 and *S. aureus* 18As; MIC of 32 µg/ml against *S. aureus* 386 [38].
Olivetol	Inhibits SA-1199B, RN-4220, XU-212, EMRSA-15, and EMRSA-16 at 64 µg/ml; inhibits ATCC 25923 at 128 µg/ml [30].
1,8-Cineole	Bacteriostatic and bactericidal against *S. aureus* [54, 57]. Against *S. aureus* NCTC 6571: MIC of 0.5% (v/v); MBC of 1 % (v/v) [57]. Causes predisposition to lysis, loss of NaCl tolerance, loss of 260-nm-absorbing material on *S. aureus* ATCC 9144 [58]. MIC of 250 µg/ml against MRSA samples obtained from Eskisehir Osmangazi University [64].
α-Pinene	Inhibits growth and cell viability of a variety of *S. aureus* strains. Against *S. aureus* ATCC 25923, MIC_{20} of 13.6 µg/ml [59]. MIC of 1.25-2.5% (v/v) against *S. aureus* NCTC 9518 [60]. MIC of 20 µg/ml against *S. aureus* ATCC 13150, *S. aureus* ATCC 6538, and *S. aureus* ATCC 25923; MIC of 10 µg/ml against *S. aureus* ATCC LB 126 [52]. Against *S. aureus* ATCC 6538, MIC of 4 µg/ml; against *S. aureus* 18As and *S. aureus* 386, MIC of 16 µg/ml [38]. MIC of 1000 µg/ml against MRSA samples obtained from Eskisehir Osmangazi University [64].
β-Pinene	Inhibits growth and cell viability of a variety of *S. aureus* strains. MIC of 20 µg/ml against *S. aureus* ATCC 13150, *S. aureus* ATCC 6538, *S. aureus* ATCC 25923, and *S. aureus* ATCC LB 126 [52]. MIC against *S. aureus* ATCC 6538 at 4 µg/ml; MIC *S. aureus* 18As at 32 µg/ml; MIC *S. aureus* 386 at 8 µg/ml [38].
α-Terpineol	MIC between 0.16-0.31% (v/v) against *S. aureus* NCTC 9518 [60]. Causes predisposition to lysis, loss of NaCl tolerance, loss of 260-nm-absorbing material on *S. aureus* ATCC 9144 [58].
α-Terpinolene	MIC against *S. aureus* ATCC 6538 at 8 µg/ml; MIC *S. aureus* 18As and *S. aureus* 386 at 32 µg/ml [38].
Terpinen-4-ol	MIC between 0.31-0.63% (v/v) against *S. aureus* NCTC 9518 [60]. Causes predisposition to lysis, loss of NaCl tolerance, loss of 260-nm-absorbing material; electron microscopy showed formation of mesosomes and loss of cytoplasmic contents on *S. aureus* ATCC 9144 [58].
Thymol	A monoterpene. MIC values against 25 strains of *S. aureus* range from 0.03-0.06% (v/v) [65]. Effective against *S. aureus* 6-ME, 810-CT, 815-CT, 808-CT, 5-ME, and 74-CCH: MIC of 0.031-0.062% (v/v); MBC of 0.062-0.125% (v/v); BIC (biofilm inhibitory concentration) of 0.062-0.125% (v/v); BEC (biofilm eradication concentration) of 0.125-0.250% (v/v) [66]. MIC of about 80 µg/ml and MBC of about 110 µg/ml against MRSA ATCC 43300 [53].
β-Caryophyllene	Against *S. aureus* ATCC 25923 MIC20 of 5.1 µg/ml [59]. Against *S. aureus* ATCC 6538, MIC of 16 µg/ml; *S. aureus* 18As and *S. aureus* 386, MIC of 32 µg/ml [38].
Humulene (α-Caryophyllene)	Against *S. aureus* ATCC 25923, MIC_{20} of 2.6 µg/ml [59].
β-amyrin	MIC of 2.5 mg/ml against *S. aureus* NCTC 7447 [61].
Flavonoids	
Cannflavin A	IC_{50} against MRSA ATCC 43300 at 15 µg/ml [46].
Naringenin	Against *S. aureus* ATCC 6538, disrupts the cytoplasmic membrane at low levels; at high levels, damages cytoplasmic membrane, causing leakage of intracellular substances; DNA targeting effects; MIC of 1.84 mM (0.50 $g\ l^{-1}$) [62]. Significantly reduces growth rate of S. aureus cells in the concentration range of 0 to 2.20 mM, with no growth detected within 14 h when concentration was 2.20 mM; disrupts the cytoplasmic membrane, affects the expression of fatty-acid synthesizing genes [63]. MIC of 512 µg/ml and an MBEC corresponding to 2048 µg/ml against *S. aureus* 105 [41].
Acids	
Caffeic Acid	Caffeic acid is active on efflux pumps, inhibiting the MrsA pumps of the *S. aureus* strain RN-4220 and the NorA pump of the *S. aureus* strain 1199B [55].
Linoleic Acid	Efflux pump inhibition against MRSA RN-4220/pUL5054. At 16 µg/ml, linoleic acid displayed synergistic effects with erythromycin, reducing MIC value of erythromycin from 256 to 16 µg/m [56].

Other studies

A 1987 murine study showed that aqueous marijuana extract and marijuana smoke inhibited *S. aureus* NCTC 9789 [28].

A 1995 study at the University of Punjab found that cannabis extracts had strong inhibitory effect on *S. aureus* [29].

A 2001 study of 5 EOs from different cultivars of low-THC cannabis (SwissMix, Felina 34, Fedrina 74, Kompolti, and Secuemi) found inhibitory zones against *S. aureus* to be 7.1, 14.4, 10.0, 5.2 and 9.6 mm, respectively [17].

A 2008 study of native and naturalized plants in Minnesota and Wisconsin found that cannabis extracts had an inhibition zone of 25 mm against *S. aureus* ATCC 12600 [42].

A 2011 study of cannabis extracts found strong antimicrobial activity against *S. aureus*. Inhibition zone diameter was positively correlated with extraction time (at 2 h, 8 h, and 18 h) and extraction method (acetone extraction vs. methanol extraction). Acetone extraction inhibition zones at 2, 8 and 18 h of extraction time were 12, 16 and 20 mm, respectively; methanol extraction inhibition zones were 10, 14 and 20 mm, respectively [43].

A 2012 cannabis study from Chinese Medicine tested cannabis seed oil, and cannabis petroleum ether and methanol extracts of the whole plant against a number of microorganisms, including *S. aureus* 25923. Seed oil had an inhibition zone of 28 mm, while petroleum ether extract had an inhibition zone of 23 mm and methanol extracts had an inhibition zone of 12 mm. MIC value of methanol extract of seed oil was 25 µg/ml; methanol extract of whole plant was 50 µg/ml [7].

A 2014 study from Hazara University found in vitro activity of C. sativa leaf extracts against *S. aureus* ATCC 6538. The average inhibition zone of cannabis extract was 10.3 mm [35].

A study published in 2016 in the Records of Natural Products demonstrated antibacterial activity of a number of volatile fractions isolated from high potency *C. sativa* oil. IC50 values for *S. aureus* ATCC 29213 and MRSA ATCC 33591 were obtained. The volatile oil was active against *S. aureus* at MIC50 of 44.71 µg/ml, and against MRSA at MIC50 of 98.79 µg/ml. Six subfractions demonstrated potential antibacterial activity against both *S. aureus* and MRSA, with IC50 values between 0.93 µg/ml and 19.9 µg/ml against *S. aureus* and between 0.82 µg/ml and 17.34 µg/ml against MRSA [36].

A study published in 2018 in the Journal of Integrative Medicine evaluated the efficiency of ethanolic extracts of *C. sativa*, *T. orientalis*, and *P. guajava* against 20 MRSA strains. Cannabis extracts were effective individually at inhibiting MRSA strains; however, profound synergism was observed when cannabis extract was combined with *T. orientalis* extract [37].

A study published in 2018 in Molecules demonstrated antibacterial potential of cannabis essential oil and naringenin against several strains of *S. aureus* (*S. aureus* ATCC 29213, *S. aureus* 101 TV, *S. aureus* 104, and *S. aureus* 105). Essential oil was tested on all strains for MIC, MBC, and MBEC. Against *S. aureus* ATCC 29213, *S. aureus* 101 TV, and *S. aureus* 104, MIC, MBC, and MBEC values were identical, with MIC of 8 mg/ml, MBC of 16 mg/ml and MBEC of 24 mg/ml. Against *S. aureus* 105 TV, MIC and MBC values were also reported at 8 and 16 mg/ml, respectively; MBEC was 16 mg/ml [41].

A study of EOs from different strains of fibre-type cannabis, published in Molecules in 2019, revealed 4 strains that inhibited *S. aureus* ATCC 6538 at MIC ranging from 2 to 16 µg/ml; 3 strains inhibited *S. aureus* 18As at MIC ranging from 16 to 32 µg/ml; and 3 strains that inhibited *S. aureus* 386 at MIC ranging from 16 to 32 µg/ml [38].

In 2020 a study published in LWT - Food Science and Technology, it was found that hemp seed extract had an MIC of 1 mg/ml against *S. aureus* ATCC 35556 and *S. aureus* ATCC 25923. Complete biofilm inhibition of *S. aureus* ATCC 35556 occurred at concentrations of 0.5 mg/ml and 1 mg/ml [47].

COMPETING INTERESTS

The author declares that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] S. Tong, et al. (2015), "Staphylococcus aureus infections: Epidemiology, pathophysiology, clinical manifestations, and management", *Clinical Microbiology Reviews*, **28(3)**, pp.603-661.

[2] A.P. Kourtis, et al. (2019), "Vital signs: Epidemiology and recent trends in methicillin-resistant and in methicillin-susceptible Staphylococcus aureus bloodstream infections-United States", *Morbidity and Mortality Weekly Report*, **68(9)**, pp.214-219.

[3] World Health Organization (2014), *Antimicrobial resistance: Global report on surveillance*.

[4] A. Shariati, et al. (2020), "The global prevalence of Daptomycin, Tigecycline, Quinupristin/Dalfopristin, and Linezolid-resistant Staphylococcus aureus and coagulase-negative staphylococci strains: A systematic review and meta-analysis", *Antimicrobial Resistance & Infection Control*, **9(56)**, pp.1-20.

[5] M.S. Morehead, et al. (2018), "Emergence of global antibiotic resistance", *Prim Care*, **45(3)**, pp.467-484.

[6] C.E. Turner, et al. (1981), "Biological activity of cannabichromene, its homologs and isomers", *J. Clin Pharmacol*, **21(S1)**, pp.283S-291S.

[7] E.M.M. Ali, et al. (2012), "Antimicrobial activity of Cannabis sativa L.", *Chin. Med.*, **3(1)**, pp.61-64.

[8] L.D. Martinenghi, et al. (2020), "Isolation, purification, and antimicrobial characterization of cannabidiolic acid and cannabidiol from Cannabis sativa L.", *Biomolecules*, **10(6)**, DOI: 10.3390/biom10060900.

[9] L.E. Klumpers, et al. (2019), "A brief background on cannabis: From plant to medical indications", *Journal of AOAC International*, **102(2)**, pp.412-420.

[10] J. Sherma, et al. (2019), "Thin layer chromatography in the analysis of Cannabis and its components and synthetic cannabinoids", *Journal of Liquid Chromatography & Related Technologies*, **42(19-20)**, pp.613-628.

[11] J.K. Booth, et al. (2019), "Terpenes in Cannabis sativa-From plant genome to humans", *Plant Science*, **284**, pp.67-72.

[12] E.P. Baron (2018), "Medicinal properties of cannabinoids, terpenes, and flavonoids in cannabis, and benefits in migraine, headache, and pain: An update on current evidence and cannabis science", *Headache: The Journal of Head and Face Pain*, **58(7)**, pp.1139-1186.

[13] E.B. Russo (2019), "The case for the entourage effect and conventional breeding of clinical cannabis: No 'strain', no gain", *Frontiers in plant science*, **9**, DOI: 10.3389/fpls.2018.01969.

[14] M.A. Lewis, et al. (2018), "Pharmacological foundations of cannabis chemovars", *Planta medica*, **84(4)**, pp.225-233.

[15] A. Hazekamp, J.T. Fischedick (2012), "Cannabis from cultivar to chemovar", *Drug testing and analysis*, **4(7-8)**, pp.660-667.

[16] B.A. Whittle, et al. (2001), "Prospects for new cannabis-based prescription medicines", *Journal of Cannabis Therapeutics*, **1(3-4)**, pp.183-205.

[17] J. Novak, et al. (2001), "Essential oils of different cultivars of Cannabis sativa L. and their antimicrobial activity", *Flavour and fragrance journal*, **16(4)**, pp.259-262.

[18] K. Weston-Green (2018), "The united chemicals of cannabis: Beneficial effects of cannabis phytochemicals on the brain and cognition", In *Recent Advances in Cannabinoid Research,* DOI: 10.5772//intechopen.79266.

[19] G. Nahler, et al. (2019), "Cannabidiol and contributions of major hemp phytocompounds to the entourage effect, possible mechanisms", *J. Altern. Complementary Integr.* Med, **5(2)**, DOI: 10.24966/ACIM-7562/100070.

[20] C.M. Andre, et al. (2016), "Cannabis sativa: The plant of the thousand and one molecules", *Frontiers in plant science*, **7(19)**, DOI: 10.3389/fpls.2016.00019.

[21] S.A. Ahmed, et al. (2015), "Minor oxygenated cannabinoids from high potency Cannabis sativa L", *Phytochemistry*, **117**, pp.194-199.

[22] R. Gallily, et al. (2018), "The anti-inflammatory properties of terpenoids from cannabis", *Cannabis and Cannabinoid Research*, **3(1)**, pp.282-290.

[23] B.C. Foster, et al. (2019), "Cannabis and cannabinoids: Kinetics and interactions", *The American Journal of Medicine*, **132(11)**, pp.1266-1270.

[24] L.O. Hanuš, et al. (2016), "Phytocannabinoids: A unified critical inventory", *Natural product reports*, **33(12)**, pp.1357-1392.

[25] E.B. Russo, et al. (2017), "Cannabis pharmacology: The usual suspects and a few promising leads", *Advances in pharmacology*, **80**, pp.67-134.

[26] C.A. MacCallum, et al. (2018), "Practical considerations in medical cannabis administration and dosing", *European Journal of Internal Medicine*, **49**, pp.12-19.

[27] M.A. ElSohly, et al. (2005), "Chemical constituents of marijuana: The complex mixture of natural cannabinoids", *Life sciences*, **78(5)**, pp.539-548.

[28] M.K. Ashfaq, et al. (1987), "The effect of subacute marijuana smoke inhalation on experimentally induced dermonecrosis by *S. aureus* infection", *Immunopharmacology and Immunotoxicology,* **9(2-3)**, pp.319-331.

[29] K. Wasim, et al. (1995), "Antimicrobial studies of the leaf of cannabis sativa L.", *Pakistan Journal of Pharmaceutical Sciences*, **8(1)**, pp.29-38.

[30] G. Appendino, et al. (2008), "Antibacterial cannabinoids from Cannabis sativa: A structure-activity study", *Journal of Natural Products*, **71(8)**, pp.1427-1430.

[31] G. Appendino, et al. (2011), "Cannabinoids: Occurrence and medicinal chemistry", *Current Medicinal Chemistry*, **18(7),** pp.1085-1099.

[32] M. Galletta, et al. (2020), "Rapid antibacterial activity of cannabichromenic acid against methicillin-resistant staphylococcus aureus", *Antibiotics,* **9(8)**, DOI: 10.3390/antibiotics9080523.

[33] M.M. Radwan, et al. (2009), "Biologically active cannabinoids from high-potency Cannabis sativa", *Journal of Natural Products*, **72(5)**, pp.906-911.

[34] E.B. Russo (2011), "Taming THC: Potential cannabis synergy and phytocannabinoid terpenoid entourage effects", *British Journal of Pharmacology*, **163(7)**, pp.1344-1364.

[35] M. Naveed, et al. (2014), "In vitro antibacterial activity of Cannabis sativa leaf extracts to some selective pathogenicbacterial strains", *Int. J. Biosci.*,**4(4)**, pp.65-70.

[36] A.S. Wanas, et al. (2016), "Antifungal activity of the volatiles of high potency Cannabis sativa L. against Cryptococcus neoformans", *Records of Natural Products*, **10(2)**, pp.214-220.

[37] S. Chakraborty, et al. (2018), "Antimicrobial activity of Cannabis sativa, Thuja orientalis and Psidium guajava leaf extracts against methicillin-resistant Staphylococcus aureus", *Journal of Integrative Medicine*, **16(5)**, pp.350-357.

[38] R. Iseppi, et al. (2019), "Chemical characterization and evaluation of the antibacterial activity of essential oils from fibre-type Cannabis sativa L.(Hemp)", *Molecules*, **24(12)**, DOI: 10.3390/molecules24122302.

[39] M.A. Farha, et al. (2020), "Uncovering the hidden antibiotic potential of Cannabis", *ACS Infect Dis.,* **6(3)**, pp.338-346.

[40] M.A. Farha, et al. (2020), "Supplementary information for uncovering the hidden antibiotic potential of Cannabis", *ACS Infect Dis.,* **6(3)**,pp.1-21.

[41] G. Zengin, et al. (2018), "Chromatographic analyses, in vitro biological activities, and cytotoxicity of cannabis sativa l. Essential oil: A multidisciplinary study", *Molecules*, **23(12)**, DOI: 10.3390/molecules23123266.

[42] J.R. Borchardt, et al. (2008), "Antimicrobial activity of native and naturalized plants of Minnesota and Wisconsin", *J Med Plants Res.*, **2(5)**, pp.98-110.

[43] V.N. Mkpenie, et al. (2012), "Effect of extraction conditions on total polyphenol contents, antioxidant and antimicrobial activities of cannabis sativa l", *Electron J. Environ Agric. Food Chem.*, **11(04)**, pp.300-307.

[44] C.S. Wassmann, et al. (2020), "Cannabidiol is an effective helper compound in combination with bacitracin to kill Gram-positive bacteria", *Scientific Reports*, **10(1)**, DOI: 10.1038/s41598-020-60952-0.

[45] A. Mohammed, et al. (2020), "Administration of Δ^9 tetrahydrocannabinol (THC) post staphylococcal enterotoxin B exposure protects mice from acute respiratory distress syndrome and toxicity", *Frontiers in Pharmacology*, **11(893)**, DOI: 10.3389/fphar.2020.00893.

[46] M.M. Radwan, et al. (2008), "Non-cannabinoid constituents from a high potency Cannabis sativa variety", *Phytochemistry*, **69(14)**,pp.2627-2633.

[47] S. Frassinetti, et al. (2020), "Antimicrobial and antibiofilm activity of Cannabis sativa L. seeds extract against Staphylococcus aureus and growth effects on probiotic Lactobacillus spp.", *Lwt*, **124**, DOI: 10.1016/j.lwt.2020.109149109149.

[48] K.N. Tahsin, et al. (2021), "Antimicrobial studies of Cannabidiol as biomaterials against superbug MRSA", *CMBES Proceedings*, **44**, pp.1-10

[49] M.A.T. Blaskovich, et al. (2021), "The antimicrobial potential of cannabidiol", *Communications Biology*, **4(1)**, pp.1-18.

[50] B. Van Klingeren, et al. (1976), "Antibacterial activity of Δ^9-tetrahydrocannabinol and cannabidiol", *Antonie van Leeuwenhoek*, **42(1)**, pp.9-12.

[51] B.F. Brehm-Stecher, et al. (2003), "Sensitization of Staphylococcus aureus and Escherichia coli to antibiotics by the sesquiterpenoids nerolidol, farnesol, bisabolol, and apritone", *Antimicrobial Agents and Chemotherapy*, **47(10)**, pp.3357-3360.

[52] A.M. Leite, et al. (2007), "Inhibitory effect of beta-pinene, alpha-pinene and eugenol on the growth of potential infectious endocarditis causing Gram-positive bacteria", *Revista Brasileira de Ciências Farmacéuticas*, **43(1)**, pp.121-126.

[53] H. Li, et al. (2014), "Antibacterial activity and mechanism of action of Monarda punctata essential oil and its main components against common bacterial pathogens in respiratory tract", *International Journal of Clinical and Experimental Pathology*, **7(11)**, pp.7389-7398.

[54] F. Grotenhermen, et al. (2006), "Handbook of Cannabis Therapeutics: From Bench to Bedside*" Haworth Integrative Healing Press*, **1**, pp.1-49.

[55] J.F.S. Dos Santos, et al. (2018), "In vitro e in silico evaluation of the inhibition of Staphylococcus aureus efflux pumps by caffeic and gallic acid", *Comparative Immunology, Microbiology and Infectious Diseases*, **57**, pp.22-28.

[56] B.C.L. Chan, et al. (2015), "Combating against methicillin-resistant Staphylococcus aureus-two fatty acids from Purslane (Portulaca oleracea L.) exhibit synergistic effects with erythromycin", *Journal of Pharmacy and Pharmacology*, **67(1)**, pp.107-116.

[57] C.F. Carson, et al. (1995), "Antimicrobial activity of the major components of the essential oil of Melaleuca alternifolia", *Journal of Applied Bacteriology*, **78(3)**, pp.264-269.

[58] C.F. Carson, et al. (2002), "Mechanism of action of Melaleuca alternifolia (tea tree) oil on Staphylococcus aureus determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy", *Antimicrobial Agents and Chemotherapy*, **46(6)**, pp.1914-1920.

[59] A. Pichette, et al. (2006), "Composition and antibacterial activity of Abies balsamea essential oil", *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, **20(5)**, pp.371-373.

[60] A. Raman, et al. (1995), "Antimicrobial effects of tea tree oil and its major components on Staphylococcus aureus, Staph. epidermidis and Propionibacterium acnes", *Letters in Applied Microbiology*, **21(4)**, pp.242-245.

[61] N. Abdel-Raouf, et al. (2015), "Antibacterial β-amyrin isolated from Laurencia microcladia", *Arabian Journal of Chemistry*, **8(1)**, pp.32-37.

[62] L.H. Wang, et al. (2017), "Membrane and genomic DNA dual-targeting of citrus flavonoid naringenin against Staphylococcus aureus", *Integrative Biology*, **9(10)**, pp.820-829.

[63] L.H. Wang, et al. (2018), "Modification of membrane properties and fatty acids biosynthesis-related genes in Escherichia coli and Staphylococcus aureus: Implications for the antibacterial mechanism of naringenin", *Biochimica et Biophysica Acta (BBA)-Biomembranes*, **1860(2)**, pp.481-490.

[64] G. Özek, et al. (2010), "Gas chromatographic-mass spectrometric analysis of volatiles obtained by four different techniques from Salvia rosifolia Sm., and evaluation for biological activity", *Journal of Chromatography A*, **1217(5)**, pp.741-748.

[65] A. Nostro, et al. (2004), "Susceptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol", *FEMS Microbiology Letters*, **230(2)**, pp.191-195.

[66] A. Nostro, et al. (2007), "Effects of oregano, carvacrol and thymol on Staphylococcus aureus and Staphylococcus epidermidis biofilms", *Journal of Medical Microbiology*, **56(4)**, pp.519-523.

[67] A. Nostro, et al. (2009), "In vitro activity of carvacrol against staphylococcal preformed biofilm by liquid and vapour contact", *Journal of Medical Microbiology*, **58(6)**, pp.791-797.

[68] J.R. Knowles, et al. (2005), "Antimicrobial action of carvacrol at different stages of dual-species biofilm development by Staphylococcus aureus and Salmonella enterica serovar Typhimurium", *Applied and Environmental Microbiology*, **71(2)**, pp.797-803.

[69] M. Tyers, et al. (2019), "Drug combinations: A strategy to extend the life of antibiotics in the 21st century", *Nature Reviews Microbiology*, **17(3)**, pp.141-155.

[70] L. Nissen, et al. (2010), "Characterization and antimicrobial activity of essential oils of industrial hemp varieties (Cannabis sativa L.)", *Fitoterapia*, **81(5)**, pp.413-419.

[71] G.L. Challis, et al. (2003), "Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by Streptomyces species", *Proceedings of the National Academy of Sciences*, **100(2)**, pp.14555-14561.

[72] M.J. Cheesman, et al. (2017), "Developing new antimicrobial therapies: Are synergistic combinations of plant extracts/compounds with conventional antibiotics the solution?", *Pharmacognosy Reviews*, **11(22)**, pp.57-72.

[73] P.D. Gupta, et al. (2017), "Development of botanicals to combat antibiotic resistance", *Journal of Ayurveda and Integrative Medicine*, **8(4)**, pp.266-275.

[74] F. Nazzaro, et al. (2013), "Effect of essential oils on pathogenic bacteria", *Pharmaceuticals*, **6(12)**, pp.1451-1474.

[75] C.L. Gorlenko, et al. (2020), "Plant secondary metabolites in the battle of drugs and drug-resistant bacteria: New heroes or worse clones of antibiotics?", *Antibiotics*, **9(4)**, DOI: 10.3390/antibiotics9040170.

[76] I. Górniak, et al. (2019), "Comprehensive review of antimicrobial activities of plant flavonoids", *Phytochemistry Reviews*, **18(1)**, pp.241-272.

[77] G.J. Sullivan, et al. (2020), "How antibiotics work together: Molecular mechanisms behind combination therapy", *Current Opinion in Microbiology,* **57**, pp.31-40.

[78] P.R. Gonzales, et al. (2015), "Synergistic, collaterally sensitive β-lactam combinations suppress resistance in MRSA", *Nature Chemical Biology*, **11(11)**, pp.855-861.

[79] X. Zheng, et al. (2018), "Combination antibiotic exposure selectively alters the development of vancomycin intermediate resistance in Staphylococcus aureus", *Antimicrobial Agents and Chemotherapy*, **62(2)**, DOI: 10.1128/aac.02100-17.

[80] B. Raymond (2019), "Five rules for resistance management in the antibiotic apocalypse, a road map for integrated microbial management", *Evolutionary Applications*, **12**, pp.1079-1091.

[81] K. Yilancioglu, et al. (2019), "Design of high-order antibiotic combinations against M. tuberculosis by ranking and exclusion", *Scientific Reports*, **9**, DOI: 10.1038/s41598-019-48410-y.

[82] R. Becerril, et al. (2012), "Evaluation of bacterial resistance to essential oils and antibiotics after exposure to oregano and cinnamon essential oils", *Foodborne Pathogens and Disease*, **9(8)**, pp.699-705.

[83] J.M. McPartland, et al. (1999), "Side effects of pharmaceuticals not elicited by comparable herbal medicines: The case of tetrahydrocannabinol and marijuana", *Alternative Therapies in Health and Medicine*, **5(4)**, pp.57-62.

[84] J.W. Fairbairn, et al. (1981), "Activity of cannabis in relation to its delta' trans-tetrahydro-cannabinol content", *British Journal of Pharmacology*, **72(3)**, pp.401-409.

[85] S. Moreno-Gamez, et al. (2015), "Imperfect drug penetration leads to spatial monotherapy and rapid evolution of multidrug resistance", *Proceedings of the National Academy of Sciences*, **112(22)**, pp.E2874-E2883.

[86] Q. Zhang, et al. (2011), "Acceleration of emergence of bacterial antibiotic resistance in connected microenvironments", *Science*, **333(6050)**, pp.1764-1767.

[87] R. Hermsen, et al. (2012), "On the rapidity of antibiotic resistance evolution facilitated by a concentration gradient", *Proceedings of the National Academy of Sciences*, **109(27)**, pp.10775-10780.

[88] K.A. Youdim, et al. (2003), "Interaction between flavonoids and the blood-brain barrier: In vitro studies", *Journal of Neurochemistry*, **85(1)**, pp.180-192.

[89] D.W. Lachenmeier, et al. (2015), "Comparative risk assessment of alcohol, tobacco, cannabis and other illicit drugs using the margin of exposure approach", *Scientific Reports*, **5(8126)**, pp.1-7.

[90] P. Beaulieu (2005), "Toxic effects of cannabis and cannabinoids: Animal data", *Pain Research and Management*, **10(A)**, pp.23A-26A.

[91] R.S. Gable (2004), "Comparison of acute lethal toxicity of commonly abused psychoactive substances", *Addiction*, **99(6)**, pp.686-696.

[92] World Health Organization (2019), *WHO Expert Committee on Drug Dependence: Forty-first report.*

[93] E. Zahra, et al. (2020), "Rates, characteristics and manner of cannabis-related deaths in Australia 2000-2018", *Drug and Alcohol Dependence,* **212**, DOI: 10.1016/j.drugalcdep.2020.108028.

[94] M. Herkenham, et al. (1990), "Cannabinoid receptor localization in brain", *Proceedings of The National Academy of Sciences*, **87(5)**, pp.1932-1936.

[95] J.M. McPartland, et al. (2001), "Cannabis and cannabis extracts: Greater than the sum of their parts?", *Journal of Cannabis Therapeutics*, **1(3-4)**, pp.103-132.

[96] A. Englund, et al. (2013), "Cannabidiol inhibits THC-elicited paranoid symptoms and hippocampal-dependent memory impairment", *Journal of Psychopharmacology*, **27(1)**, pp.19-27.

[97] A.W. Zuardi, et al. (1982), "Action of cannabidiol on the anxiety and other effects produced by Δ^9-THC in normal subjects", *Psychopharmacology*, **76**, pp.245-250.

[98] A.W. Zuardi, et al. (2012), "A critical review of the antipsychotic effects of cannabidiol: 30 years of a translational investigation", *Curr. Pharm. Des.*, **18(32)**, pp.5131-5140.

[99] A. Batalla, et al. (2019), "The potential of cannabidiol as a treatment for psychosis and addiction: Who benefits most? A systematic review", *Journal of Clinical Medicine*, **8(7)**, DOI: 10.3390/jcm8071058.

[100] C. Davies, et al. (2019), "Cannabidiol as a potential treatment for psychosis", *Therapeutic Advances in Psychopharmacology,* **9**, DOI: 10.1177/2045125319881916.

[101] P. McGuire, et al. (2018), "Cannabidiol (CBD) as an adjunctive therapy in schizophrenia: A multicenter randomized controlled trial", *American Journal of Psychiatry*, **175(3)**, pp.225-231.

[102] J.D. Brown, et al. (2019), "Potential adverse drug events and drug-drug interactions with medical and consumer cannabidiol (CBD) use", *Journal of Clinical Medicine*, **8(7)**, DOI:10.3390/jcm8070989.