Antimicrobial Effects of Cannabinoids

Subjects: Chemistry, Medicinal

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The development of new antibiotics is urgently needed to combat the threat of bacterial resistance. New classes of compounds that have novel properties are urgently needed for the development of effective antimicrobial agents. The extract of *Cannabis sativa* L. has been used to treat multiple ailments since ancient times. Its bioactivity is largely attributed to the cannabinoids found in its plant. Researchers are searching for new anti-infective agents that can treat various infections. Although its phytocannabinoid ingredients have a wide range of medical benefits beyond the treatment of infections, they are primarily associated to psychotropic effects. Different cannabinoids have been demonstrated to be helpful against harmful bacteria, including Gram-positive bacteria. Moreover, combination therapy involving the use of different antibiotics has shown synergism and broad-spectrum activity.

Cannabis sativa | phytocannabinoids | structure–activity relationships

1. Structures and Origin of Natural Cannabinoids from *Cannabis sativa*

C. sativa naturally produces cannabinoids, which typically have C21 and C22 terpenophenolic structures with varying oxidation patterns. There are currently approximately 120 phytocannabinoids discovered, and they can be divided into 11 different broad skeletal types [1][2]. The type of cannabinoids, such as delta-9-tetrahydrocannabinol (Δ^9-THC-type) (1) [3], contains a tricyclic 6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-1-o1 core structure, and its main representatives such as (-)-Δ^9-trans-tetrahydrocannabinolic acid (Δ^9-THCA) (2) and (-)-Δ^9-trans-tetrahydrocannabinol (Δ^9-THC) (1) include highly abundant cannabinoids of *C. sativa* [3][4]. Moreover, a class of cannabinoids such as Δ^8-THC-type contains isomers of class Δ^9-THC-type, demonstrating the same 6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-1-o1 core structure with a double bond [1]. The Δ^8-trans-tetrahydrocannabinol (Δ^8-THC) (5) is considered to be a major representative of this form and its concentration in plants is usually negligible due to the isomerisation of thermodynamically less stable double bond isomers such as Δ^9-THC (1) [3]. Cannabinoids of the cannabinol (CBN)-type share a similar core structure of 6H-benzo[c]chromen-1-ol with oxidised aromatic rings [1]. A comparatively minor constituent of *C. sativa* such as cannabinol (CBN) (7) is the primary representative of this class [3]. Although the content of CBN (7) increases in plant materials when oxidised, Δ^9-THC (1) is processed in the presence of oxygen [5]. High concentrations of the thermodynamically more stable cannabinoids CBN (7) and Δ^8-THC (5) can be found in processed cannabis products such as hashish and cannabis oil [5]. Additionally, the family of CBT-type cannabinoids, including cannabitriol (CBT) (9), clearly distinguishes itself from the Δ^9-THC-type cannabinoids by exhibiting a vicinal 9,10-trans-diol in the upper ring [1].

However, CBD-type cannabinoids show a tetrahydro-[1,1-biphenyl]-2,6-diol framework with large amount in *C. sativa* and can synthesise its dried extracts up to 40% [1][6]. CBD (10) is inherently instable and cyclises to Δ^9-THC (1) under acidic conditions [7]. The cyclisation process and oxidation of Δ^9-THC (1) to CBN (7) also occurs during pyrolysis [8]. The oxidative photocyclisation of CBD-type cannabinoids produced CBE-type cannabinoids, such as cannabielsoin (CBE) (14), that have a 5a,6,7,8,9,9a-hexahydrodibenzo[b,d]furan-1,6-diol framework [1][9]. The CBG-type cannabinoids, for instance, cannabigerol (CBG) (17), demonstrate a non-cyclised framework that are minor constituents in *C. sativa* that normally convert into Δ^9-THC-type cannabinoids during plant growth [1][10]. Moreover, cannabichromene (CBC) (19) is the most abundant CBC-type cannabinoids found in *C. sativa* [11]. The CBC (19) exposure to sunlight causes a [2 + 2]-photocycloaddition, forming cannabicyclol (CBL) (21) [12]. *C. sativa* can be split into a variety of other miscellaneous cannabinoids, such as dimeric cannabisol (23) or (-)-exo-*trans*-tetrahydrocannabinol (exo-THC) (22). Phytocannabinoids present a C22 and C19 terpenophenolic structure, including (-)-Δ^9-trans-tetrahydrocannabivarin (Δ^9–THCV) (3) or cannabidivarin (CBDV) (12), as shown in [13][14][15]. The terpenophenolic structure originates from the olivetolic acid (28) and monoterpene precursor geranyl diphosphate (GPP) that is synthesised via the olivetolic acid cyclase and a polyketide synthetase (PKS) [16][17]. The cannabigerolic acid synthetase (CBGAS) facilitates the catalysis of prenyl transfer by an electrophilic aromatic substitution, leading to the production of cannabigerolic acid (CBGA) (18) [18].

In contrary, the production of cannabidiolic acid (CBDA) (11) through an oxidation cyclisation is catalysed by cannabidiolic acid synthetase (CBDAS) [19]. Cannabichromenic acid (CBCA) (20) is also produced through oxidation cyclisation (13) and catalysed by the cannabichromenic acid synthetase (CBCAS) [20], although decarboxylation reactions also provide contributions to some extent during the smoking or baking of cannabis materials [21].

2. Structure–Activity Associations of Cannabinoids

It is widely known that cannabinoids have antimicrobial effects. Despite the various advantages of cannabinoids, their potential in helping combat antibiotic resistance is still largely untapped. Some studies have been conducted on the subject.

The researchers observed that both cannabinoids were active towards various types of bacteria, including those that are known to cause respiratory infections (*S. aureus* and *Streptococcus* species). Their results indicated that the compounds had an MIC of 1–5 µg/mL [22]. Although the binding of plasma proteins to the cannabinoids in the horse serum reduced its antibacterial activity against pathogenic bacteria, it did not impair the serum's ability to kill germs. In order to determine the properties of cannabichromene analogs, researchers tested the antimicrobial and anti-antibiotic properties of these substances [23]. The N-pentyl chain meta relative to the alcohol group is known to play a role in the development of antibiotic resistance against two bacterial species, namely *B. subtilis* and *S. aureus*. However, truncation to a methyl group is associated with an increase in antifungal activity. Moreover, the activity of isocannabichromenes was studied, but it was not as active as their analogs cannabichromene. This class of cannabinoids does not cause psychoactive effects, but it can improve its therapeutic potential. In one study, authors studied the performance of various cannabinoids on the development and maintenance of antibiotic

resistance against multidrug-resistant strains of *S. aureus* [24]. A study conducted on cannabidiolic acid revealed that it has good antimicrobial activity (MIC = 2 µg/mL).

It was also found that the presence of a carboxylate moiety did not affect its activity. According to the researchers, when it comes to treating various Gram-positive pathogens, CBD (10) has a MIC value of up to 1–2 µg/mL, which is significantly higher than CBDA (11) [25]. The inactive compounds in CBDA (11) were phenethyl and methyl. These could have been induced by the added hydrophobicity or by the steric bulk. The effects of acetylation and methylation on various hydroxyl groups were detrimental to the activity of microbial cells. The removal of the carboxylate increased the moderate activity of CBGA (18). Compared to CBND (16) and Δ^9-THC (1), Δ^9-THC acid exhibited a moderate bactericidal effect. Interestingly, the effects of switching to the N-pentyl group from the hydroxyl group were not significantly affect the antimicrobial activity. A study conducted on resorcinol not only revealed that it exhibited poor antimicrobial properties but also showed the importance of the hydrocarbons chain.

Currently, researchers evaluated the effects of endocannabinoid anandamide and arachidonyl serine on bacteria [26]. Despite their poor bactericidal activity against certain types of bacteria such as methicillin resistant *S. aureus* (MRSA), these compounds inhibited the formation of bacterial biofilms [26]. The changes induced by these compounds affected the cell aggregation, hydrophobicity, and membrane potential of various bacterial species. When combined with other antibiotics such as ampicillin, these agents can be used to treat MRSA-caused infections that recur [27]. It has been demonstrated that CBD (10) can improve the antibacterial effects of the peptide drug bacitracin against many bacteria, including *L. monocytogenes* and *E. faecalis* [28].

In another study, researchers evaluated various cannabinoid analogs against *E. coli* and MRSA [29]. Several common cannabinoids exhibited moderate to good activity when used in combination with other drugs. The increase in the minimum inhibitory concentration (MIC) values of various analogs, such as Δ^9-tetrahydrocannabivarin, due to the presence of a common n-propyl chain, which further highlighted the importance of this component in the membrane insertion process. Hydroxylation and carboxylation at position 11 of the Δ^9-THC (1) resulted in a loss of activity, which suggests that the presence of a lipophilicity in the prenyl tail may be important. CBG (17) was able to reduce the bacterial burden in the spleen in a mouse model of a systemic infection with MRSA by a factor of 2.8 $\log^{10}$ in colony-forming units. Although these analogs did not exhibit a bactericidal effect against *E. coli*, their consistent MIC values were over 128 µg/mL. In a study, CBG (17) was shown to be effective against Gram-negative bacteria by combining with polymyxin B. It is proposed that the polymyxins be added to the outer membrane of a Gram-negative pathogen to enable the CBG (17) to perform its functions. The study also revealed that cannabidiol can sensitise various antibiotics in combination with other drugs [30]. For various Gram-negative bacteria, CBD (10) was able to prevent the release of membrane-filled cargo containers. These containers play a vital role in inter-bacterial communication. When combined with other antibiotics, such as vancomycin, colistin, and erythromycin, CBD (10) was able to enhance the antimicrobial effect towards *E. coli*. The results of previous studies suggest that cannabinoids can potentially improve the efficacy of existing antibiotics.

3. Antimicrobial Activity of *Cannabis sativa*

The report about the antibacterial properties of cannabinoids was first published in the 1950s [31][32]. The bactericidal properties of cannabis were studied before the phytochemistry of the plant was fully established. This means that the antibacterial effects of *C. sativa* were not attributed to a specific component. In 1976, it was discovered that Δ^9-THC and CBD (10) can be used as bacteriostatic agents. They were also able to kill a panel of human pathogenic strains [22]. The antibacterial properties of the various *C. sativa* plant extracts have drawn significant attention, such as the oil and extract from the plant. Various methods have been used to isolate *C. sativa* extracts. Cold-pressing and solvent extraction techniques are commonly used to produce various products, such as cosmetics and food. However, new technologies are now being developed that allow them to generate superior results [33]. Pressurised liquid extractions are more efficient than filtration. They do not require filtration and have shorter processing times. On the other hand, ultra-sonic extraction techniques use less solvent and have improved yields. There are various methods that are commonly used for green extraction, such as supercritical fluid extraction and microwave-assisted extraction; however, up-scaling these processes is challenging [33].

Essential oils from five different cultivars of *C. sativa* were evaluated against a panel of Gram-negative and Gram-negative pathogens. The most common compounds found in oil samples were trans-β-ocimene, myrcene, and trans-caryophyllene, but they showed less antibacterial activities against *Brevibacterium linens* and *Acinetobacter calcoaceticus*. A comprehensive analysis of the various essential oils revealed that none of them had high levels of Δ^9-THC (1) and CBND (16). These compounds, which are known to be antimicrobials, could be utilised by *C. sativa* [34]. A study was conducted on the oil of the seeds, which were then extracted using petroleum and methanol. The agar diffusion method was used to extract antimicrobial properties from various extracts. It was shown that the extract exhibited effective responses towards different pathogenic strains. The lack of a comprehensive analysis of the plant's cannabinoid content is consistent with the findings of the study conducted by researchers [22]. There was no obvious antifungal activity observed. A small amount of petroleum ether extract was also observed to have beneficial effects against bacteria [35]. Inhibiting the development of harmful Gram-negative pathogens is also possible with the use of hot water and ethanol leaf extracts [36]. A study conducted on *C. sativa* shows that the plant's antioxidant and antimicrobial properties were compared after both aqueous and acetone extraction [37]. Compared to aqueous extracts, acetone extracts exhibited superior bactericidal properties. The effects of varying concentrations on the responses of different bacteria were studied. The most responsive species was the *V. cholera* bacterium, closely followed by the *P. aeruginosa*. The study revealed that *C. sativa* has antioxidant properties, which could be useful in treating various conditions. A study conducted by researchers revealed that the drug "Hashish" can kill harmful bacteria [38]. The results of the experiments revealed that cannabis extracts significantly inhibited the growth of *S. aureus* 25923. The results of the study support the idea that the antimicrobial properties of *C. sativa* plants grown in Vietnam are modest against Gram-positive bacteria. On the other hand, the extracts from cultivated strains of the plant exhibited less resistance to Gram-negative organisms [39]. The researchers also noted that the major components of the extracts exhibited moderate activity against Gram-positive pathogens [40].

Due to their low toxicity, hemp seed oil-based products are investigated for cosmetic and pharmaceutical applications. The antimicrobial properties of two different types of oil-based emulsions were determined. For instance, the activity of oil-based emulsions against *E. coli* was virtually zero. This might be a result of the higher

concentration of α-linolenic acid or, more likely, the removal of Δ^9-THC (1) during the refinement process [41]. The extract of *C. sativa* has been studied with respect to various types of antibiotic-resistant bacteria, such as MRSA, by using the disc diffusion method. The zone of inhibition of clinical isolates was observed as 9 to 15 mm. This was less than the diameter of vancomycin (13–24 mm). A combination of plant extracts, such as *Psidium guajava* and *Thuja orientalis*, exhibited a synergistic effect. Zone of inhibition diameters of up to 30 mm were observed in most cases. Flavonoids, such as quercetin, catechin, and gallic acid, were found in the leaf extract, but no traces of cannabinoids were detected [42]. In vitro studies conducted by scientists revealed that the extract of *C. sativa* inhibited the formation of *S. aureus* biofilms [43]. In a study conducted on dental plaque, researchers found that using cannabinoids can help decrease the bacterial colony count in the plaque. They also compared the effectiveness of these products with those from commercial brands such as Colgate [44].

Investigators are currently examining the commercial viability of Δ^9-THC-free essential oils from *C. sativa*, which could be used for various applications such as veterinary medicine and cosmetic products. The oil was evaluated against various strains of *S. aureus*, and it exhibited moderate antibiofilm activities and antibacterial effects. Moreover, antimicrobial activities were detected against *Helicobacter pylori* but not against other organisms. The study shows that the active compounds found in *C. sativa* are not only capable of acting as antimicrobial agents, but they also have biological properties [45]. A wide range of applications for hemp-seed hexane extracts has been studied, which exhibited that the oil extracted from this plant can also help in reducing acne-causing bacteria and can help prevent inflammation [46]. In order to better understand the properties of various essential oils, researchers conducted a comprehensive phytochemical analysis of 17 different types of hemp essential oil. A total of 71 compounds were identified, while some of these include terpene β-myrcene, trans-ocimene, and limonene. The inhibitory concentration of various oils was analysed against a group of Gram-positive bacteria, and they were able to show moderate antimicrobial activity. The effects of various cannabinoids, such as CBD (10) and terpene, on the development of the antibacterial effects were studied. Although activity was generally good to moderate against various types of bacteria, such as *Enterococcus* and *Salmonella*, it was lower against other types of bacteria. The antimicrobial effect of essential oils is likely caused by the presence of synergism between different compounds [47]. An interesting use for compounds derived from *C. sativa* is in water purification in order to isolate a combination of compounds, including terpene and cannabinoids; they immobilised them on a polyethersulfone hybrid membrane. The reduction in bacterial populations was observed for both Gram-negative and Gram-positive pathogens. Several different bacterial species, including common pathogens *E. coli* and *P. aeruginosa*, were found to have similar results. This aims to provide a cost-effective solution for the treatment of waterborne pathogens by using a combination of water filtration and purification [48]. The great potential of *C. sativa* for various applications in drug discovery is highlighted by its antimicrobial properties.

4. Antibacterial Mechanism of Action

Despite the lack of an effective mechanism of action for treating bacterial infections, recent advances have been made in the field of cannabinoids. Membrane permeability is one of the cannabis compounds' potential mechanisms of action. *L. monocytogenes'* cell integrity and wall structure were both disrupted by the terpene

limonene, which caused a leakage of several cell components [49]. Similar changes to those caused by β-caryophyllene were observed in the *Bacillus cereus* bacterium's membrane [50]. CBG (17) has shown that it can target the cytoplasmic membrane of Gram-positive bacteria. Gram-negative bacteria's inner membrane was permeabilised, enabling CBG (17) to perform in a manner comparable to that of Gram-positive bacteria [29]. A microscopic assessment of the efficiency of CBCA (20) on the development of *B. subtilis* showed that it induced a change in the bacterial membrane and nucleoid [51]. In vitro studies revealed that CBD (10) caused a depolarisation of the membrane of *S. aureus*, while this activity also disrupted the membrane potential of the bacterium. The combination of CBD (10) and bacitracin can cause various cell division defects and cell envelope abnormalities. It is believed that the abnormalities were caused by a loss of genes that regulates the division of cells [52].

Another mode of action of cannabinoids that can be used to alter cell communication is by blocking the release of membrane vesicles by bacteria. Although it was shown that CBD (10) can block the release of membrane vesicles from the pathogen, this effect was not significant in the presence of *S. aureus* [30]. Moreover, the effects of HU-210 on the bacterial communication system were studied, which showed that the drug can inhibit the quorum sensing (QS) system's ability to detect and respond to bacterial signals. It was also able to improve the swimming performance of *Vibrio harveyi* [53]. In one study, a radiolabeled synthesis test in *S. aureus* RN42200 revealed that various pathways that led to the synthesis of proteins, DNAs, and RNAs were significantly inhibited by concentrations near an MIC of 2–3 μg/mL^{-1} [54]. This suggests that rapid bactericidal action is carried out to shut down these pathways [55]. The reduction in lipid synthesis was observed at concentrations below the MIC, which supports the hypothesis that membrane-based effects were involved [24]. The presence of a membrane depolarisation in the presence of MRSA can provide additional evidence of membrane activity; however, this activity was not observed in *E. coli* [54]. A bacterial cytological profiling test performed on multiple antibiotics known to act through membrane permeabilisation showed that the results were consistent with previously published results [56][57]. The results of these studies suggest that CBD (10) can be very effective at disrupting bacterial membranes; however, it is not clear if this effect is caused by a specific molecular target.

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