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Review Article

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Cannabis as antivirals

Yi Lin Sea[1], Yee Jing Gee[1], Sunil Kumar Lal[1,2], Wee Sim Choo[1,*]

[1] School of Science, Monash University Malaysia, 47500 Bandar Sunway, Selangor, Malaysia
[2] Tropical Medicine and Biology Platform, Monash University Malaysia, 47500 Bandar Sunway, Selangor, Malaysia

*Corresponding author: School of Science, Monash University Malaysia, 47500 Bandar Sunway, Selangor, Malaysia; E-mail: choo.wee.sim@monash.edu

Abstract

Cannabis is a plant notorious for its psychoactive effect, but when used correctly, it provides a plethora of medicinal benefits. With more than 400 active compounds that have therapeutic properties, cannabis has been accepted widely as a medical treatment and for recreational purposes in several countries. The compounds exhibit various clinical benefits, which include, but are not limited to, anticancer, antimicrobial, and antioxidant properties. Among the vast range of compounds, multiple research papers have shown that cannabinoids, such as cannabidiol and delta-9-tetrahydrocannabinol, have antiviral effects. Recently, scientists found that both compounds can reduce severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) viral infection by downregulating ACE2 transcript levels and by exerting anti-inflammatory properties. These compounds also act as the SARS-CoV-2 main protease inhibitors that block viral replication. Apart from cannabinoids, terpenes in cannabis plants have also been widely explored for their antiviral properties. With particular emphasis on four different viruses, SARS-CoV-2, human immunodeficiency virus, hepatitis C virus, and herpes simplex virus-1, this review discussed the role of cannabis compounds in combating viral infections and the potential of both cannabinoids and terpenes as novel antiviral therapeutics.

Keywords: cannabinoid, terpenes, SARS-CoV-2, human immunodeficiency virus, hepatitis C virus, herpes simplex virus

Introduction

The word ‘cannabis’ is often associated with ‘marijuana’; however, both do not mean the same. Cannabis covers all products derived from plants such as *Cannabis sativa*, *C. ruderalis*, and *C. indica*, while marijuana refers to the products from these plants containing tetrahydrocannabinol (THC) [National Centre for Complementary and Integrative Health (NIH) 2019]. Cannabis is an illicit drug due to its psychoactive effects, resulting in several psychological and physical health problems. The side effects of extensive use of cannabis include impaired memory and body movement, altered mental state, euphoria, difficulty focusing, and an increased appetite. Physical effects such as dyspnoea, nausea, increased heart rate, and short-term symptoms like conjunctivitis may also occur (Crippa et al. 2009). On the other hand, cannabis compounds are used off-license for medical purposes to treat several diseases and alleviate symptoms such as chronic pain, nausea, and many more in several countries. Cannabis used for medical purposes can be administered orally or through a medical vaporizing device (Freeman et al. 2019). Cannabis is known to be involved in human physiological activities such as blood pressure, appetite stimulation, pain regulation, and immune response, as well as pathological conditions such as anti-carcinogenesis. Hence, drugs containing cannabis are found to be helpful in treating epilepsy, nausea, and vomiting caused by cancer chemotherapy, and improving the appetite of the human immunodeficiency virus (HIV) infected patients. Besides, cannabis compounds have also shown a promising effect as analgesics for neuropathic pain (Fraguas-Sánchez and Torres-Suárez 2018).

More than 445 chemical substances are found in the cannabis plant, and about 120 of these are cannabinoid compounds (Peng and Shahidi 2021). In the cannabis plant, the cannabinoid compounds are usually stored as acids, but after the cannabis products are heated and processed, the acid gradually turns into psychoactive constituents, such as THC, cannabinol, cannabidiol (CBD), cannabicyclol, cannabitriol, cannabigerol (CBG), cannabielsoin, and cannabinodiol (Fig. 1). Among them, delta-9-tetrahydrocannabinol (Δ-9-THC) and CBD are the main cannabinoids (Atakan 2012). Initially, CBD was thought to be the metabolic parent to the cannabinoid Δ-9-THC, but later it was found that it was synthesized as cannabidiol acid and tetrahydrocannabinol acid, respectively, from a cannabigerol acid precursor based on genetically determined ratios, and followed by decarboxylation to produce CBD and Δ-9-THC (Russo and Guy 2006). The opposing effects of these two compounds were noticed in some research studies even though both of them have similar chemical structures (Fig. 1). When both drugs were given simultaneously, CBD was capable of blocking the effects of Δ-9-THC (Gunasakera et al. 2021).

About 400 chemical substances found in cannabis plants are terpenes and phenolic compounds (Andre et al. 2016). The cannabis plant produces pungent smelling terpenes to attract pollinators and repel pests. More recently, scientists have observed that terpenes actually have antioxidants and anti-inflammatory properties (Marques et al. 2019). Besides, terpenes are reported to have promising antimicrobial properties against methicillin-resistant *Staphylococcus aureus*, a Gram-positive bacterium (Karas et al. 2020).

In the past few years, there is some scientific evidence showing that these cannabis-derived compounds could have antiviral properties from their chemical groups and structures. They can reduce morbidities and slow down the progression of various types of viral illnesses. This review focuses on the therapeutic effects of cannabis compounds such as THC, CBD, and terpenes in the diseases caused by viral pathogens, including

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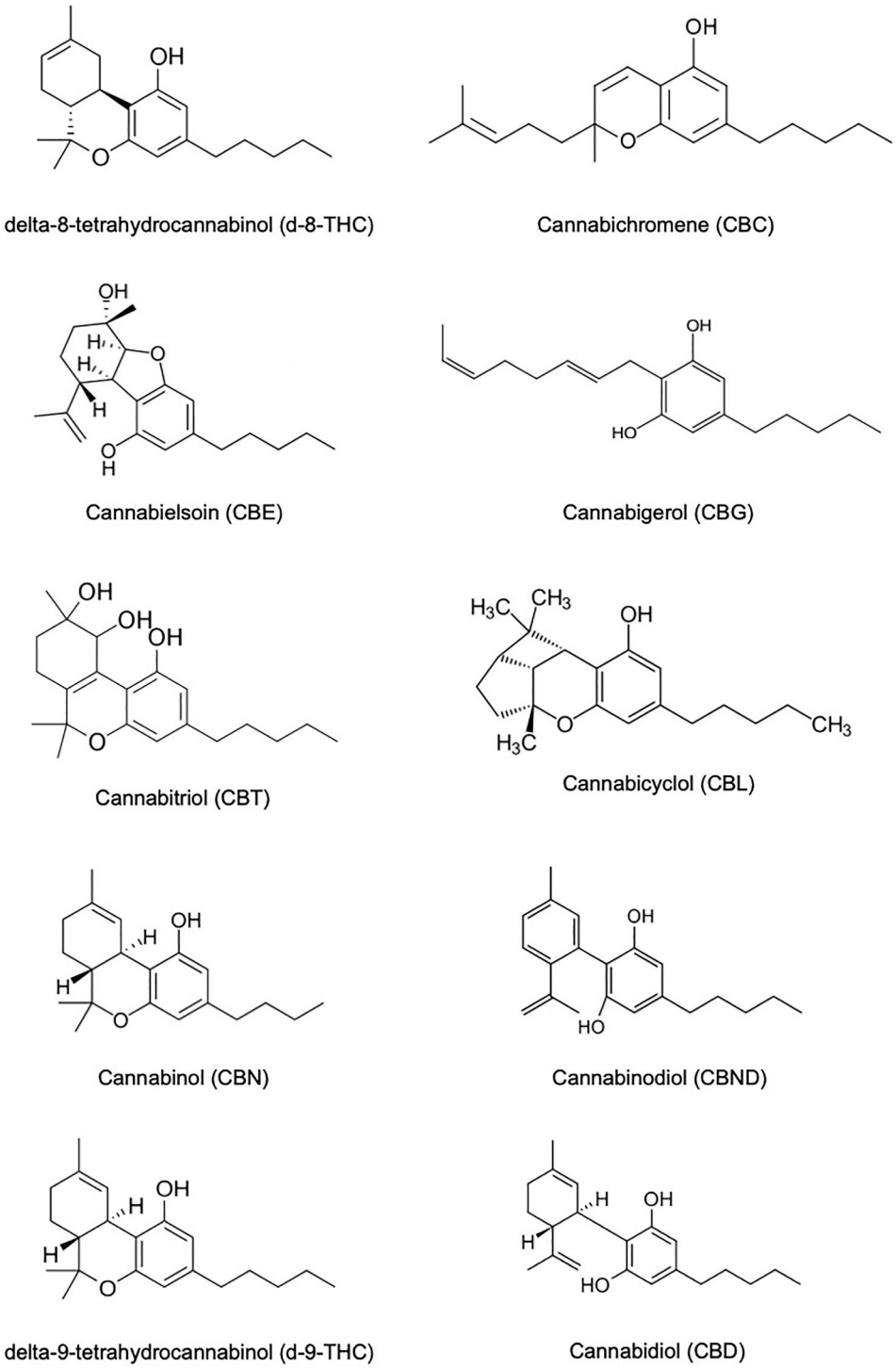

Figure 1. Structural overview of various cannabinoids.

severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), HIV, hepatitis C virus (HCV), and herpes simplex virus-1 (HSV-1).

Cannabis compounds and viral infections

The human body has different cell-signalling pathways, and the endocannabinoid system is one of the biological systems identified in the 1990s. It comprises endocannabinoids such as anandamide, 2-arachidonoylglycerol, and *N*-arachidonoyl dopamine (Paland et al. 2021). These compounds serve as endogenous lipid neurotransmitters to activate cannabinoid receptors, cannabinoid 1 receptor (CB1) and cannabinoid 2 receptor (CB2), respectively. Although the role of endocannabinoid is similar to that of the neurotransmitter dopamine, it still differs in various ways. In particular, endocannabinoids work as retrograde signals as they travel backward from the postsynaptic side to the presynaptic side (Castillo et al. 2012). Some early studies have reported that Δ-9-THC resembles the en-

docannabinoid and acts as an exogenous partial agonist that attaches to the cannabinoid receptors (Gertsch et al. 2010).

CB1 receptors are found mainly in the brain and peripheral nervous system, including the liver, uterus, testicular tissue, and thyroid, while CB2 receptors are expressed predominantly in the immune cells, especially the macrophages lineage, and gastrointestinal system. The major role of CB1 receptors is to block the release of neurotransmitters by inhibiting the voltage-sensitive calcium channels and adenylyl cyclase. Several studies have found that when the presynaptic CB1 is activated, it leads to the reduction of neurotransmission (Hoffman and Lupica 2000). On the other hand, CB2 receptors were reported to control the functions of immune cells in inflammatory disease models. Animal models lacking CB2 receptors have shown an exacerbated inflammatory response due to an increase in immune cell function. Hence, the administration of CB2 receptor agonists such as Δ-9-THC is able to treat inflammation (Newton et al. 2009), which has been proven in numerous studies (Yuan et al. 2002, Eisenstein et al. 2007).

In the past few decades, human civilization has encountered many pathogen outbreaks. Despite the Spanish flu, which infected one-third of the human population in 1918, there was no large-scale virus infection until the emergence of a new virus called SARS-CoV-2 in 2019. It is a new strain of coronavirus that causes coronavirus disease 2019 (COVID-19), a type of respiratory disease responsible for the ongoing pandemic. The general public and scientists are eager to discover a treatment option for COVID-19. Although the U.S. Food and Drug Administration (FDA) has approved an antiviral drug called remdesivir and authorized the emergency use of Pfizer Paxlovid to treat COVID-19, there could be serious side effects while using these medications (U.S. FDA 2022). There are various potential antiviral agents being investigated for their therapeutic effects, including cannabinoids and terpenes from cannabis plants. The anti-inflammatory effect of CBD and Δ-9-THC might be beneficial in treating viral illnesses.

When a virus infects the host, the virus genome is incorporated into the host cell DNA, thereby dominating the host's function. It can control the cells to produce viral nucleic acid and proteins, resulting in the formation of new virus particles. Hundreds of virus offspring are then released through cell lysis and continue to infect the surrounding healthy host cells. Concurrently, innate and adaptive immunity in our body are activated to fight against the viruses. An inflammatory response is crucial for the assembly of immune cells to the site of infection. The immune cells, including macrophages, lymphocytes, and neutrophils, control the virus production, while the secretion of inflammatory mediators such as cytokines, chemokines, and complement components helps to limit the spread of the virus and promote cellular repair and recovery (Cohen 2016).

Antiviral effects of cannabinoids

Although cannabis plants contain many cannabinoid compounds, the two significant phytocannabinoids are CBD and Δ-9-THC. Many research studies have been performed to investigate their biological effects. It is well documented that Δ-9-THC is a partial agonist to both endocannabinoid receptors. It can produce psychotomimetic effects by activating the CB1 receptor and being involved in the immune system by binding to CB2 receptors. On the other hand, CBD was ascertained to regulate immune responses and it actually has few to no psychoactive effects (Boggs et al. 2016). It acts as an antagonist to CB1 receptors and an agonist to CB2 receptors, which is slightly different from the compound Δ-9-THC (Thomas et al. 2007). The diverse and negating effects of the two main cannabinoids were observed in some early studies. CBD was discovered to reduce many adverse effects associated with anxiety but enhance the analgesic effects of THC in an animal study conducted earlier (Karniol and Carlini 1973). Many other studies also provide credence that CBD can minimize the psychomimetic outcome of THC (Boggs et al. 2016). In general, the effect of cannabinoids could be detrimental or beneficial depending on the type of virus infection and the concentration used. For instance, the adverse effects of Δ-9-THC include the impairment of inflammatory myeloid cell response to influenza infection (Karmaus et al. 2013). Besides the negative effects, it is purported that Δ-9-THC possesses analgesic properties, and anti-emetic effects along with immune-modulating actions. CBD is also commonly used to treat patients with anxiety or insomnia. Therefore, prescribed drugs such as nabilone, dronabinol, and oromucosal spray nabiximols containing both THC and CBD were approved by the United States and several other countries such as Canada, Mexico, and parts of Europe (Whiting et al. 2015). The anti-inflammatory and associated antiviral properties of cannabinoids are discussed further in this review and summarized in Table 1.

Severe acute respiratory syndrome coronavirus-2

Inflammation is a two-edged sword. A normal inflammatory response, which is under control, is beneficial to the host as it can augment host immunity against viruses. Whereas, several types of viruses such as SARS-CoV-2 may induce hyper inflammation, resulting from production of excessive cytokines known as a cytokine storm. This is a dangerous immune reaction as the overpowering inflammation also causes damage to healthy cells, resulting in various symptoms that range from fever to organ failure and may eventually cause a life-threatening situation. In fact, most of the COVID-19 symptoms and mortality are associated with uncontrolled proinflammatory responses (Mishra et al. 2020).

There is limited evidence that demonstrates the therapeutic effects of cannabinoids in viral infections. Some scientists believe that Δ-9-THC may be advantageous in viral illnesses, when the inflammatory response of the host is pathogenic. The anti-inflammatory properties of cannabinoids were found to be associated with the treatment of COVID-19, the most worrying virus infection at this point. When a person is infected with SARS-CoV-2, the virus enters the host by angiotensin-converting enzyme II (ACE2) present in several tissues, including oral and nasal membrane lining, lung tissues, renal tissue, and gastrointestinal tract. The expression of ACE2 in the nasal epithelium is age-dependent, providing the reason why the young generation has a lower prevalence of COVID-19, since they have lower nasal gene expression of ACE2. Conversely, in elderly people or men, there is more ACE2 expressed so it would provide more viral entry points for the virus to infect the cells. Thus, there is a higher risk of severe clinical manifestations in older adults compared with the younger population (Beyerstedt et al. 2021). Cannabinoids that contain CBD and Δ-9-THC can modulate the ACE2 protein level; it may, therefore decrease disease susceptibility. EpiOral, EpiAirway, and EpiIntestinal tissues were treated with proinflammatory cytokines at first to examine the anti-inflammatory effects of

Table 1. Effects of cannabinoids in viral infections

Viral pathogen	Types of experiments	Compound/antiviral potency	Biochemical assay(s)	Model	Observations	Reference
SARS-CoV-2	*In vitro*	Cannabinoids: Δ-9-THC and CBD (0.01 mg ml^{-1})	RNA-seq, western blotting	Human 3D models: EpiAirway tissues, EpiOral tissues, and EpiIntestinal tissues	Downregulated the ACE2 transcript levels and exerted anti-inflammatory properties in EpiOral, EpiAirway, and EpiIntestinal tissues	Wang et al. (2020)
	In vitro/In silico	Δ-9-THC and CBD50% viral inhibitory concentration (IC_{50}): 10.25 μM, and 7.91 μM)	Immunofluorescence	Vero cells from *Chlorocebus*	Inhibited SARS-CoV-2 M^{pro} and reduced proinflammatory cytokine levels	Raj et al. (2020)
	In vitro	CBD (Median effective concentration (EC_{50}): 0.6–1.8 μM)	SARS-CoV-2 infection assay	A549-ACE2 cells	CBD significantly inhibited SARS-CoV-2 replication at an EC_{50} of 0.6–1.8 μM	Nguyen et al. (2022)
	In vitro	CBGA and CBDA (IC_{50}: 24 and 37 μg ml^{-1})	Pseudovirus neutralization assay, focus forming assay	Vero E6 cells	Blocked the entry of SARS-CoV-2 to the cells	van-Breeman et al. (2022)
HIV	*In vitro*	CB2 receptor agonists: JWH-133 (100 nM)	Quantitation of viral membrane fusion	Human	Decreased F-actin in $CD4^+$ T cells	Costantino et al. (2012)
	In vivo	Δ-9-THC (10 mg kg^{-1})	Mouse cytokine enzyme-linked immunosorbent assay	Male Swiss mice	Acute treatment (1 h): significant decrease of interleukin-2 (IL-2) production. Chronic treatment (7–14 days): persistent reduction in IL-2 and interferon- γ (IFN-γ)	Massi et al. (1998)
	In vitro	Δ-9-THC (1, 5, and 10 μM)	Flow cytometry and real-time PCR	Human	Suppressed IFN-α and IL-7-mediated stimulation of T cells, as shown by reduced IFNR2 expression and IFN-α-induced STAT1 phosphorylation in T cells	Henriquez et al. (2018)
HCV	*In vitro*	CB1 receptor antagonist: AM251 (1, 5, 7.5, or 10 μM)	$TCID_{50}$ assay, qPCR, and western blot densitometry	Human	Inhibited intracellular RNA replication, viral protein translation, and virus infectivity in a dose-dependent manner, with reduction up to 70% for HCV RNA, 80% for HCV core protein, and 90% for viral infectivity at 10 μM compared with DMSO controls	Shahidi et al. (2014)
	In vitro	CBD (10 μM, EC_{50} 3.163 μM)	Anti-hepatitis C assay	Human	Inhibited HCV replication 84.6% at a dose of 10 μM, with EC_{50} of 3.163 μM	Lowe et al. (2017)

cannabis extracts. The extracts from *C. sativa*, especially CBD resulted in downregulation of ACE2 gene expression. ACE2 protein modulation is particularly important as it is involved in disease progression. Thus, further research studies could be conducted based on this novel finding to develop prevention approaches for COVID-19 as well as other viral infections which exploit ACE2 receptors as a molecular gateway (Wang et al. 2020).

Raj et al. (2020) have suggested that Δ-9-THC and CBD are possible drugs against SARS-CoV-2. The experiments were performed *in vitro* and *in silico* to observe interactions between cannabinoids and the SARS-CoV-2 main protease (M^{pro}). SARS-CoV-2 M^{pro} has an essential role in the life cycle of the virus itself as it is responsible for the processing of translated viral RNA polyproteins. This is why SARS-CoV-2 M^{pro} is considered as one of the most suitable targets to block viral replication. The two cannabinoids, Δ-9-THC and CBD, are found to form stable binding with M^{pro}, inhibiting M^{pro}-mediated translation, and protein maturation. From the dose-response curve analysed by immunofluorescence, Δ-9-THC and CBD with the 50% viral inhibitory concentration (IC_{50}) of 10.25 μmol L^{-1} and 7.91 μmol L^{-1}, respectively, were found to be stronger antiviral compounds against human coronavirus compared with the reference antiviral drugs Remdesivir, Chloroquine and Lopinavir (Raj et al. 2020). Although Remdesivir could reduce the death rate of COVID-19 patients, subpleural inflammatory infiltrates in the lungs can still occur (Williamson et al. 2020). A reduction of lung function can continue even after recovery. Hence, two major cannabinoids, especially CBD, the one that has no psychoactive effects, could be added as an adjunct to the antiviral drugs against SARS-CoV-2 to inhibit the SARS-CoV-2 M^{pro}, which leads to blocking the translation process and also act as agonists of CB-2 receptor to reduce proinflammatory cytokine levels in lung cells (Raj et al. 2020).

Besides, SARS-CoV-2 has developed strategies to modulate host's signalling pathways, such as the interferon pathway, to be in favour of its own replication (Znaidia and Demeret 2022). Nguyen and team have found that CBD can block SARS-CoV-2 replication by activating host ER stress and induce the antiviral interferon (Nguyen et al. 2022), with a median effective concentration (EC_{50}) of 0.6–1.8 μmol L^{-1}. CBD increases the accumulation of reactive oxygen species (ROS) in the cells and potently induces ER stress. In response to this change, the unfolded protein response (UPR) is activated via the inositol-requiring enzyme-1α (IRE1α) pathway to ensure correct folding of proteins and restoration of homeostasis. Under stress, oligomerization and autophosphorylation of IRE1α occurs, which subsequently activates its RNase that is involved in splicing of X-box binding protein 1 (XBP1) mRNA (Hetz et al. 2020). In SARS-CoV-2 infection, the induction of the RNase activity of IRE1α by CBD contributes to its antiviral activity by degrading viral RNA. The fragment RNA potentially accounts for further stimulation of the antiviral interferons. Besides, the team has found that the interferon (IFN)-stimulated gene expression has been upregulated in the presence of CBD, suggesting involvement of the interferon pathway in the antiviral activity of CBD (Fig. 2). For example, upregulation of 2′-5′-oligoadenylate synthetase 1 (OAS1), an IFN-induced gene that activates the RNase L, was induced by CBD, resulting in viral RNA degradation (Nguyen et al. 2022).

In addition, when a person is infected with COVID-19, it can trigger the production of excessive levels of proinflammatory cytokines, including interleukins, interferons as well as tumor necrosis factor-α (TNF-α) in an event called 'cytokine storm'. The host immune system then becomes hyperactive, resulting in an aggressive inflammatory response (Tang et al. 2020). In the later stages of SARS-CoV-2 infection, it was found that CBD and Δ-9-THC significantly restricted the release of cytokines induced by the virus and reduced the recruitment of immune cells to the tissues, thus suppressing the production of cytokines that could cause the cytokine storm (Raj et al. 2020, Nguyen et al. 2022). As a CB2 agonist, CBD inhibited the toll-like receptor 4/nuclear factor-κB (TLR4/NF-κB) signalling pathways that are main mediators of the proinflammatory cytokine expression (Vallée 2022). Therefore, CBD is a potential antiviral agent that can act at early infection stages by hindering viral entry and viral replication, but also suppress the exuberant immune reaction in later stages (Byrareddy and Mohan 2020). The mechanism of action of cannabinoids in SARS-CoV-2 inhibition is illustrated in Fig. 2.

Although vaccines have been developed, due to their limited availability and the rate of virus mutation, SARS-CoV-2 infections are unlikely to end in the near future. The constant mutation of viruses has led to the creation of new variants. From the first variant named alpha (first detected in the United Kingdom), till the latest variant, omicron (first identified in South Africa), there are new variants emerging continuously [Centers of Disease Control and Prevention (CDC) 2022]. The genetic changes in SARS-CoV-2 happen randomly thereby resulting in variations in virus immunogenicity and pathophysiology. Hence, scientists are eager to develop therapeutic agents against SARS-CoV-2 to minimize infections and hospitalizations. There is a recent study indicating that the biosynthetic precursors of Δ-9-THC and CBD called cannabigerolic acid (CBGA) and cannabidiolic acid (CBDA) could prevent the entry of SARS-CoV-2 into cells with IC50 values of 24 and 37 μg ml^{-1}, respectively. These two cannabinoids were shown to have the highest binding affinities to the spike protein on the virus, thereby blocking the virus infection (van-Breeman et al. 2022).

Human immunodeficiency virus

HIV is a retrovirus that causes acquired immunodeficiency syndrome (AIDS), an infection where the virus attacks the immune cells and renders the patient more vulnerable to other pathogens and diseases. It is usually transmitted through direct contact of HIV patient's body fluids, unprotected sexual contact, or from mother to child during pregnancy. Currently there is no cure or an effective HIV vaccine, but treatment with highly active antiretroviral therapy (HAART) is able to slow the disease progression. Despite consistent antiretroviral therapy, which may suppress the virus replication, individuals with HIV have a greater risk of developing comorbidities such as diabetes, cardiovascular events, cancer, hepatic, and renal diseases (Costiniuk et al. 2019). Moreover, patients who have gone through HAART are expected to have a shorter life expectancy. The increased morbidity is found to be associated with chronic immune response and inflammation. HIV patients, with or without HAART, experience higher levels of T cell activation compared with uninfected individuals, leading to T cell depletion either directly by apoptosis, or indirectly by an inflammatory response (Hunt et al. 2003). Hence, novel

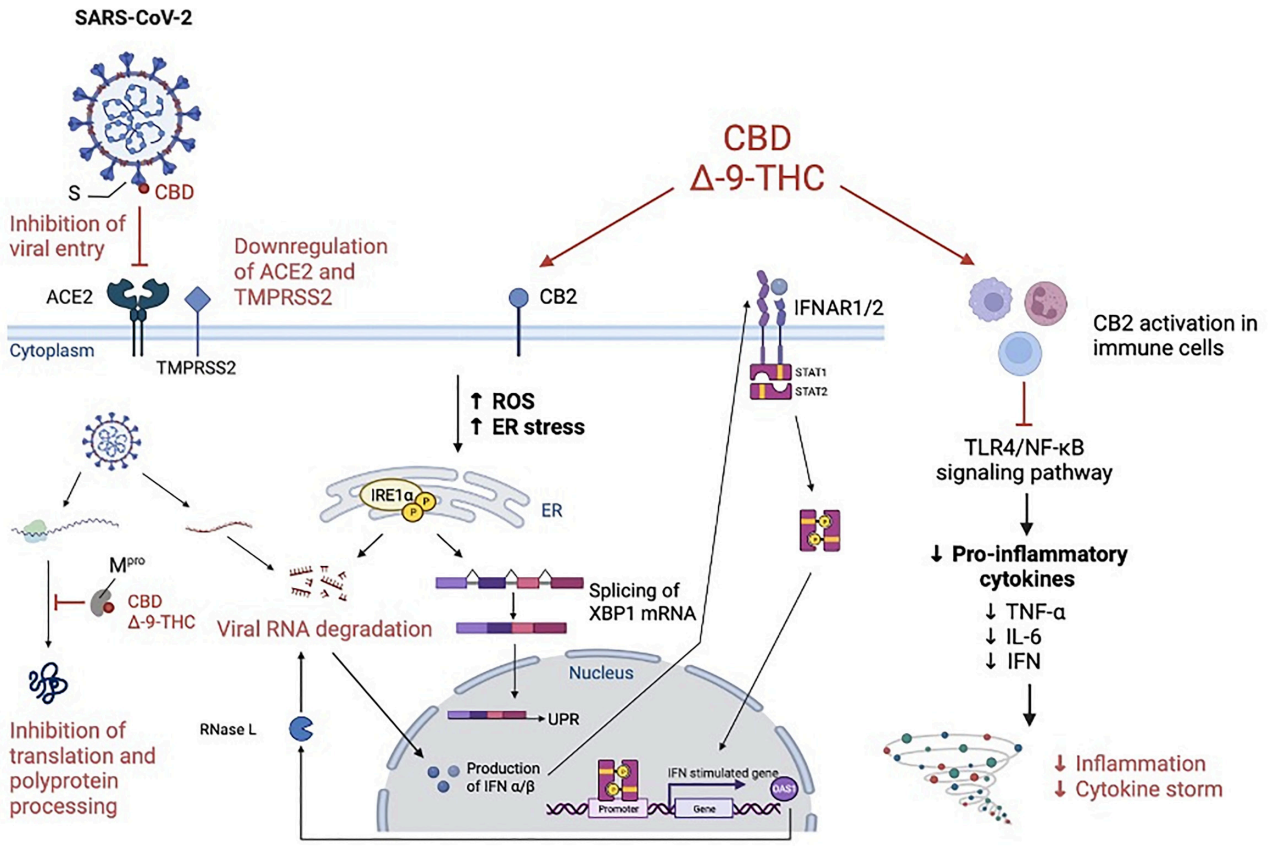

Figure 2. Mechanism of action of cannabinoids (cannabidiol, CBD and Δ-9-tetrahydrocannabinol, Δ-9-THC) in SARS-CoV-2 infection. Adapted from Hetz et al. (2020), Nguyen et al. (2022) and Raj et al. (2020). CBD binds to the viral spike (S) protein with high affinity, blocking its binding to the host receptor, angiotensin converting enzyme 2 (ACE2), causing inhibition of viral entry. CBD also downregulates the expression of the ACE2 receptor and the transmembrane protease serine 2 (TMPRSS2) that is crucial for viral S protein priming, further limiting viral entry. CBD and Δ-9-THC also bind stably to the viral protease, M pro, inhibiting viral translation, and protein processing. CBD and Δ-9-THC is also a potent ER stress inducer by increasing the reactive oxygen species (ROS). Oligomerization and phosphorylation of the inositol-requiring enzyme-1α (IRE1α) under stress activates its allosteric RNase activity, which is responsible in splicing of the X-box binding protein 1 (XBP1) mRNA and accounts for digestion of viral RNA. The spliced mRNA activates the unfolded protein response (UPR) to restore homeostasis, whereas the viral RNA fragments stimulate production of type 1 IFN. By autocrine or paracrine signalling, the IFNs binds to the interferon-α/β receptor (IFNAR) and activates the signalling pathway that results in expression of IFN stimulated genes, such as the 2′-5′-oligoadenylate synthetase 1 (OAS1), which in turns activates RNase L that contributes to the degradation of viral RNA. As CB2 receptor agonists, CBD and Δ-9-THC are also able to inhibit the toll-like receptor 4/nuclear factor-êB (TLR4/NF-êB) signalling pathway that effectively reduces the release of proinflammatory cytokines from the immune cells. This prevents the fatal inflammatory condition known as cytokine storm that is commonly found in SARS-CoV-2 infection. (figure drawn with Biorender)

treatments that work cooperatively with HAART are required to reduce immune activation and inflammation.

Having both anti-inflammatory and immunomodulatory effects, cannabinoids may assist in the treatment of AIDS. CCR5 and CXCR-4 are chemokine co-receptors that HIV can use to infect $CD4^+$ T cells. In an earlier study, the activation of CB2 receptors *in vitro* decreased the primary infection in $CD4^+$ T cells with CXCR4-tropic virus. CB2 receptor agonism decreased the activity of CXCR4-activation mediated G-protein and mitogen-activated protein kinase (MAPK) phosphorylation. It can also modify the structure of resting $CD4^+$ T cells by reducing F-actin levels, which is a key cytoskeletal component for cellular function and motility (Constantino et al. 2012). This will prevent the movement of the nucleoprotein complex of HIV to the cell nucleus. Thus, the clinical application of CB2 receptor agonists such as THC and CBD in the treatment of AIDS may offer several therapeutic benefits against CXCR4-tropic HIV in the late infection stage.

Furthermore, Massi et al. (1998) conducted an *in vivo* study to investigate the outcome of acute and chronic treatment of Δ-9-THC on respective immune system components of male mice. For the acute regimen, a dose of 10 mg kg^{-1} was injected into the mice. For the chronic treatment, the same dose of Δ-9-THC was injected into the mice twice a day. The mice were then killed an hour after the last injection of Δ-9-THC. The spleen was removed aseptically from the mice and the splenocytes were seeded in micro-culture plates to observe the proliferation. The activity of natural killer (NK) cells and the cytokines level was measured. In this experiment, results have shown that in acute treatment, NK cells activity was not inhibited, but there was a substantial decline in interleukin-2 (IL-2) production. On the other hand, in the chronic regimen, which was performed for 14 days, NK cells activity was depressed and the level of IL-2 and IFN-γ was reduced by 40%. In conclusion, these research findings suggest that *in vivo* exposure of Δ-9-THC has profound effects on immune parameters.

Medicinal cannabinoids are used widely in several countries to treat neuropathic pain and anxiety in HIV-infected patients. From some early studies, Δ-9-THC was proven to have significant results in suppressing T cell responses and de-

creasing cytokine production (Eisenstein and Meissler 2015). Interferon-alpha (IFN-α), a cytokine produced during viral infection, is responsible for $CD8^+$ T cell activation, and promotes IL-7 responsiveness. It is reported that IFN-α induced IFN-stimulated genes (ISGs) can also suppress HIV replication (George and Mattapallil 2018). One of the objectives in this experiment was to examine the effects of THC on T cell response stimulated by IFN-α and IL-7 in HIV-infected individuals. These results have shown that THC could suppress the effects of IL-7 on the proliferation of T cells, by inhibiting the IL-7 induced STAT5 phosphorylation. There was also a decreased response in $CD8^+$ T cells to signal transducer and activator of transcription-1 (STAT1) of HIV patients compared with healthy individuals, while $CD4^+$ T cells from both donors were equivalent (Henriquez et al. 2018). Since the peripheral immune activation of $CD8^+$ T cells is associated with the development of inflammatory response in HIV patients, therefore, less inflammatory response was observed in cannabis users. The outcome has implied that the use of cannabinoids may be advantageous to HIV patients with HAART, where THC can help to suppress stimulation of T cells associated with neural inflammation while keeping $CD4^+$ T cells unchanged.

Hepatitis C virus

HCV causes hepatitis C liver disease. Hepatitis C can be categorized into acute and chronic hepatitis, ranging from mild to severe lifelong illness. It is one of the major factors that causes liver cirrhosis and hepatocellular carcinoma (HCC). HCV is a bloodborne virus as it spreads by direct exposure to blood, for example, through unsafe injection practices or unprotected sexual intercourse. HCV infection is involved in the development of other metabolic disorders, including insulin resistance, lymphoma, steatosis, and atherosclerosis. Current antiviral drugs such as sofosbuvir and daclatasvir can clear most of the viruses in HCV-infected patients, but hepatic fibrosis may persist [Centres for Disease Control and Prevention (CDC) 2021]. Thus, novel therapeutic strategies are needed to reduce fibrogenesis and reverse HCV disease progression.

Oral cannabinoids were found to be effective in managing symptoms caused by HCV treatment that led to weight loss (Costiniuk et al. 2008). Subsequently, scientists discovered that the CB1 receptor might be associated with the pathogenesis of HCV infection. Cannabinoid receptors are poorly expressed in healthy liver tissue, but they are highly expressed in HCV patients. HCV triggers the activation of the CB1 receptor and contributes to liver fibrogenesis, steatosis, and cirrhosis via cannabinoid signalling pathways. Conversely, activation of the CB2 receptor in HCV patients exhibited antifibrogenic and hepatoprotective effects (Toyoda et al. 2011). In chronic hepatitis C patients, CB1 receptors were shown to be upregulated in the liver, and the increased expression of CB1 receptors will promote the virus replication. Hence, this suggests the potential of inhibition of CB1 signalling by antagonists as a new regimen against HCV. An example of CB1 antagonist is *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251), which has been found to effectively inhibit the replication of HCV RNA, expression of the viral proteins, production of viral progeny, and virus infectivity at an optimal dose of 10 μM (Shahidi et al. 2014).

Lowe et al. (2017) evaluated the antiviral effects of CBD in hepatitis B and hepatitis C. CBD was not active against HBV infection *in vitro* but exerted a cytotoxicity effect on the liver cell line, which was used to culture the virus. Meanwhile, in an HCV assay, CBD successfully inhibited the virus by 84.5% with minimal cytotoxicity effect. The direct antiviral activity of CBD towards HCV has demonstrated that the compound has an effect on hepatitis, which is caused by activated T cells and macrophages. As CBD plays a role in interacting with the CB2 receptor, it can induce apoptosis in splenocytes and thymocytes, which will then prevent the proliferation of T cells and macrophages, stopping them from attacking liver cells and the release of proinflammatory cytokines. Thus, CBD is suggested to be a potential antiviral drug for viral hepatitis infection, possibly as an accompanying remedy with existing therapies.

In addition, a review stated that the use of cannabis might also alleviate the risk of diabetes (Sidney 2016). Therefore, Barré et al. (2020) conducted a statistical analysis among chronic HCV patients selected from the French national, multicentre, observational ANRS CO22 Hepather cohort. The objective of this research was to examine whether the use of cannabis is associated with the risk of getting diabetes in HCV patients. By performing a cross-sectional study in 9000 HCV patients, current cannabis users were found to have a 51% lower chance of getting insulin resistance and diabetes. Moreover, Adejumo et al. (2018) also reported that the use of cannabis is related to a lower incidence of liver cirrhosis in chronic HCV patients, but it has no difference in mortality and the risk of getting liver carcinoma between cannabis users and non-cannabis users. Yet, further longitudinal studies are needed to ensure the effects of cannabis compounds on diabetes and liver cirrhosis.

Amount of cannabinoids for medical purposes

Although there is no standard dose of consumption for cannabis, the recommended dose for Δ-9-THC in adults is 2.5–10 mg (Lile et al. 2011), whereas 5 mg has been established by the National Institute on Drug Abuse as the standard measure for research studies (NIDA 2021). This is corroborated by an *in vivo* study that established that a dose of 10 mg kg^{-1} of Δ-9-THC has marked suppression of immunological effects in mice (Massi et al. 1998). However, it is essential to note that Δ-9-THC exerts psychoactive side effects such as anxiety, delusions, and disorganised thinking, thus coadministration with CBD is a very promising treatment as it counteracts the side effects of Δ-9-THC.

CBD is a better alternative as therapeutic treatment as it has no psychoactive effects. It has been found to control immunity in HIV infection at 10–20 mg kg^{-1} per day and in Ebola virus disease at 1.7–10 mg kg^{-1} per day (Reznik et al. 2016, Costiniuk et al. 2019). Apart from viral infections, 1 mg kg^{-1} per day of CBD has been recommended for pain treatment, whereas CBD has been found effective in Crohn's disease at 0.3 mg kg^{-1} per day (Fasinu et al. 2016). In fact, the dosing of CBD ranged from < 1 to 50 mg kg^{-1} per day in humans has shown improvement in a variety of medical conditions (Millar et al. 2019). When referring to single administration, an oral dose of 100–900 mg CBD or a combination of 10.8 mg THC and 10 mg CBD has been found to be safe in healthy adults (Larsen and Shahinas 2020; Qian et al. 2019). Moreover, most of the data obtained from above-discussed studies on SARS-CoV-2, HIV, HSV-1, and HCV infections are *in vitro* studies, in which they showed effective concentrations of CBD

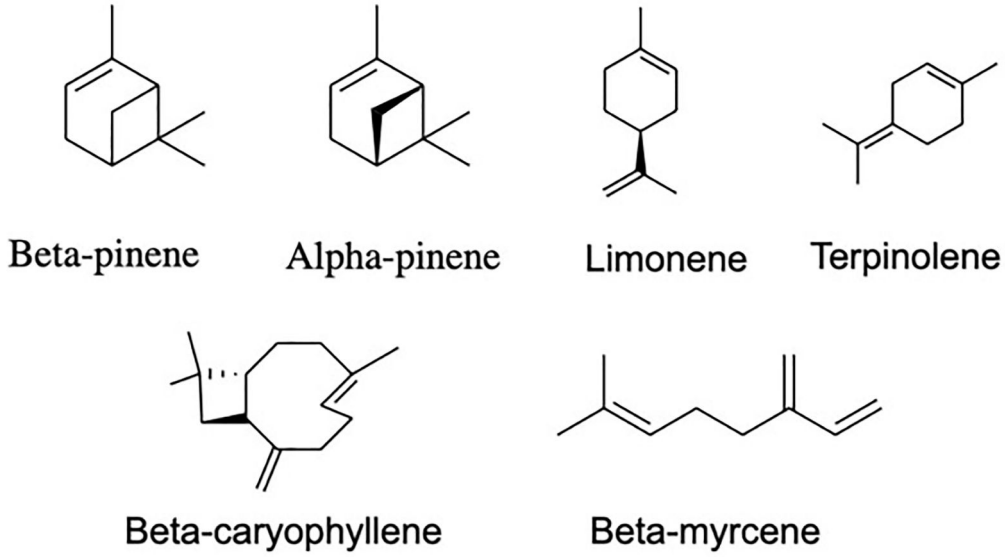

Figure 3. Chemical structure of the major monoterpenes isolated from plant *C. sativa*.

that ranged from 1–30 μmol L^{-1} of THC or CBD. Therefore, there is still a lack of data on the effective dose for viral infections in humans. Due to the wide range of CBD activity, further studies and clinical trials are still required to identify the therapeutic dose of CBD in various viral infections discussed in this review.

In addition, as patients with seizures commonly use 100 mg ml^{-1} oral solution of CBD as treatment, Nguyen et al. (2022) compared the rate of SARS-Cov-2 infection between patients with seizure-related history who took CBD medication and those that did not. They found that patients with 100 mg ml^{-1} CBD medication have a significantly lower COVID-19 positivity. This finding may serve as a good reference in finding the effective dose of CBD in SARS-CoV-2 infection treatment.

Antiviral effects of terpenes

Medicinal plants produce an assortment of chemical compounds with the potential of blocking viral replication and infection. It was commonly accepted that cannabinoids from cannabis plants are merely bioactive molecules. Only in the past few years, chemists started to pay attention to other cannabis compounds, for example, terpenes. They have discovered that the unique medicinal role of terpenes may exhibit some therapeutic properties. Terpenes are aromatic essential oils secreted from the same glands of the cannabis plant that produce cannabinoids such as THC and CBD. They can be categorized into four main groups: monoterpenes, sesquiterpenes, diterpenes, and triterpenes. To date, >200 terpenes are reported from different cannabis plant genotypes, where 58 and 38 of them are monoterpenes and sesquiterpenes, respectively. Among others, the major monoterpenes compounds are β-pinene, α-pinene, limonene, terpinolene, β-caryophyllene, and β-myrcene (Fig. 3) (Sommano et al. 2020).

Terpenes have diverse biological and pharmacological activities such as anticancer, antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory (Hanuš and Hod 2020). They have been employed in phytomedicine, showing the ability to inhibit viral replication and decrease plaque formation. It is impossible to describe every therapeutic effect of terpenes, but in this review paper, the role of terpenes in SARS-CoV-2 and HSV infection is discussed.

Severe acute respiratory syndrome coronavirus-2

Besides cannabinoids from cannabis plants, terpenes may be potential therapeutic compounds to COVID-19 (Diniz et al. 2021). At first, glycyrrhizin, a triterpenes from licorice roots, was the most active compound in blocking the replication of SARS-associated coronavirus. The mechanism of how glycyrrhizin alters SARS coronavirus is unclear, but it can affect cellular signalling pathways such as protein kinase C, casein kinase II, and transcription factors such as activator protein 1. It was also reported that glycyrrhizin has been used previously to treat chronic hepatitis C and HIV patients (Cinatl et al. 2003).

Spike protein receptor-binding domain (S-RBD) is a vital protein located on the SARS-CoV-2 spike domain. It acts as a dock to body receptor for the entry and binding of SARS-CoV-2 to host cells. Therefore, it has emerged as a primary antiviral drug target for COVID-19 prevention and treatment. In this study, terpenes were used to inhibit S-RBD on SARS-COV-2 to prevent attachment to the human ACE2 receptor. The methods used in this study were high-throughput screening (HTS) along with multiple computational techniques for the identification of novel therapeutics. After screening, three candidates among the terpene compounds, NPACT01552, NPACT01557, and NPACT00631 showed the highest binding energies when interacting with S-RBD. The stable conformations of these three terpenes candidates suggested that they could serve as potent antiviral drugs against SARS-CoV-2 (Muhseen et al. 2020) (Table 2).

Till date, there are seven human coronaviruses (HCoV) identified. Among them, the HCoV-229E strain was used in this preliminary research because it is a good alternative to the highly contagious SARS-CoV-2. The aim of this research was to investigate the antiviral characteristic of a patented terpene formulation called NT-VRL-1 against HCoV-229E, with or without the presence of CBD. NT-VRL-1 formulation generally consisted of thirty types of terpenes from cannabis

Table 2. Effects of terpenes in viral infections.

Viral pathogen	Types of experiment	Treatment	Model	Observations	Reference
SARS-CoV-2	*In silico*	Terpenes: NPACT01552, NPACT01557 and NPACT00631	-	NPACT01552, NPACT01557 and NPACT00631 showed the highest binding energies to S-RBD.	(Muhseen et al. 2020)
	In vitro	NT-VRL-1 terpenes formulation and CBD	Human lung fibroblast (MRC-5)	Both CBD and NT-VRL-1 exhibited antiviral effects, resulting the highest cell viability ($P < 0.001$).	(Chatow et al. 2021)
HSV-1	*In vitro*	Essential oils which include terpenes from various plants	Vero cells from African green monkey	Showed virucidal action by inhibiting the virus replication.	(Duschatzky et al. 2005)
	In vitro	Essential oils consisted terpenes such as α-pinene, and β-pinene	Vero cells	Exhibited antiviral activity with a IC_{50} value of 200 mg ml^{-1} and a SI of 5.	(Loizzo et al. 2008)
	In vitro	Phenylpropanoids and sesquiterpenes: β-caryophyllene	African green monkey kidney cells (RC-37)	Suppressed HSV-1 infection by 40%–98%.	(Astani *et al.* 2011)
	In vitro	Monoterpenes: β–pinene and limonene	African green monkey kidney cells (RC-37)	Reduced viral infectivity by 100% when monoterpenes directly interact with free virus particles.	(Astani and Schnitzler 2014)

plants, as well as other plants. The major components are β-caryophyllene, citral, and eucalyptol. In this study, the mixture of 10 g ml^{-1} of NT-VRL-1 and 1 g ml^{-1} of CBD produced the most effective treatment with the highest cell viability. The combination of NT-VRL-1 with CBD enhanced the antiviral effects, indicating the additive or synergistic effect between CBD and terpenes. Collectively, NT-VRL-1 with CBD has amplified the outcome, suggesting the combination of both compounds may be a potential antiviral agent against COVID-19 disease (Chatow et al. 2021) (Table 2). However, the mechanism still remains uncertain and further research needs to be conducted.

Herpes simplex virus

HSV is the common viral pathogen that causes herpes. There are two types of HSV, HSV-1, and HSV-2. HSV-1 primarily causes oral herpes, which is responsible for fever blisters and cold sores normally occurring around the face and mouth. It is usually transmitted through common interactions such as sharing lipstick, using the same eating utensils, or even kissing. Meanwhile, HSV-2 is responsible for genital herpes. It is usually spread by sexual contact or transmitted to an infant during childbirth. The major symptoms of herpes simplex include blistering sores, pain when urinating, itching, or flu-like symptoms such as fever, malaise, and swollen lymph nodes [World Health Organization (WHO) 2021]. People who have multiple sex partners or have a weak immune system are at a higher risk of acquiring HSV. Acyclovir, an antiviral medication, is an available treatment for HSV infection. It inhibits virus replication by interfering with the DNA polymerase in the cell. It can then reduce the severity of HSV clinical manifestations and control the virus itself (Whitley and Roizman 2001). However, it may produce serious side effects and the emergence of new strains of HSV will be resistant to the commonly used antiherpetic drugs. Thus, scientists nowadays are putting more efforts into discovering novel antiherpetic drugs without adverse effects of vaccines to treat and prevent HSV infection (Wijesinghe et al. 2021).

In some earlier studies, the essential oils extracted from Southern American aromatic plants were screened for their cytotoxicity and viral inhibitory effect against HSV-1, dengue virus type 2 (DENV-2), and Junin virus (JNV). The oils that showed antiviral properties consist primarily of monoterpenes, carvone, carveol, limonene, α and β-pinene, and caryophyllene. (Duschatzky et al. 2005) (Table 2). A similar study also reported that the essential oils from seven Lebanon plants, including *Laurus nobilis*, *Thuja orientalis*, *Juniperus oxycedrus* ssp. *oxycedrus*, *Cupressus sempervirens* ssp. *Pyramidalis*, *Salvia officinalis*, *Pistacia palaestina*, and *Satureja thymbra* can inhibit the replication of SARS-CoV and HSV-1. Among the essential oils, β-myrcene and α-pinene present also in the cannabis plant, are the main constituents of *Juniperus oxycedrus* berry oil. The oxycedrus oil with IC_{50} value of 200 μg ml^{-1} and a selective index (SI) of 5, has exhibited high antiviral activity against HSV-1 (Loizzo et al. 2008) (Table 2).

Astani et al. (2011) also showed that essential oils that contain terpenes have antiviral activity against HSV-1, including acyclovir-resistant HSV-1. Considering that the essential oils are able to inhibit drug resistant isolates, the mechanism of acyclovir and terpenes must be different (Fig. 4). HSV-1 was incubated with six phenylpropanoids and sesquiterpenes for antiviral assays. Pretreatment of HSV-1 with β-caryophyllene exhibited a 98% reduction of virus infectivity at maximum noncytotoxicity concentration. It has been revealed that monoterpenes and sesquiterpenes can decrease plaque formation of HSV-1 by about 60%–90%. In addition, β-caryophyllene also showed a high selectivity index (SI) value of 140, which indicates that β-caryophyllene is preferable for antiviral treatment. Astani and Schnitzler (2014) discovered that monoterpenes, i.e. beta-pinene and limonene can reduce HSV-1 infectivity by 100% when interacting directly with free virus particles. Yet, when these drugs were added to host cells prior to infection or after entry of HSV-1, only moderate effect of antiviral activity was revealed (Astani and Schnitzler 2014) (Table 2). Since the major composition of terpenes in

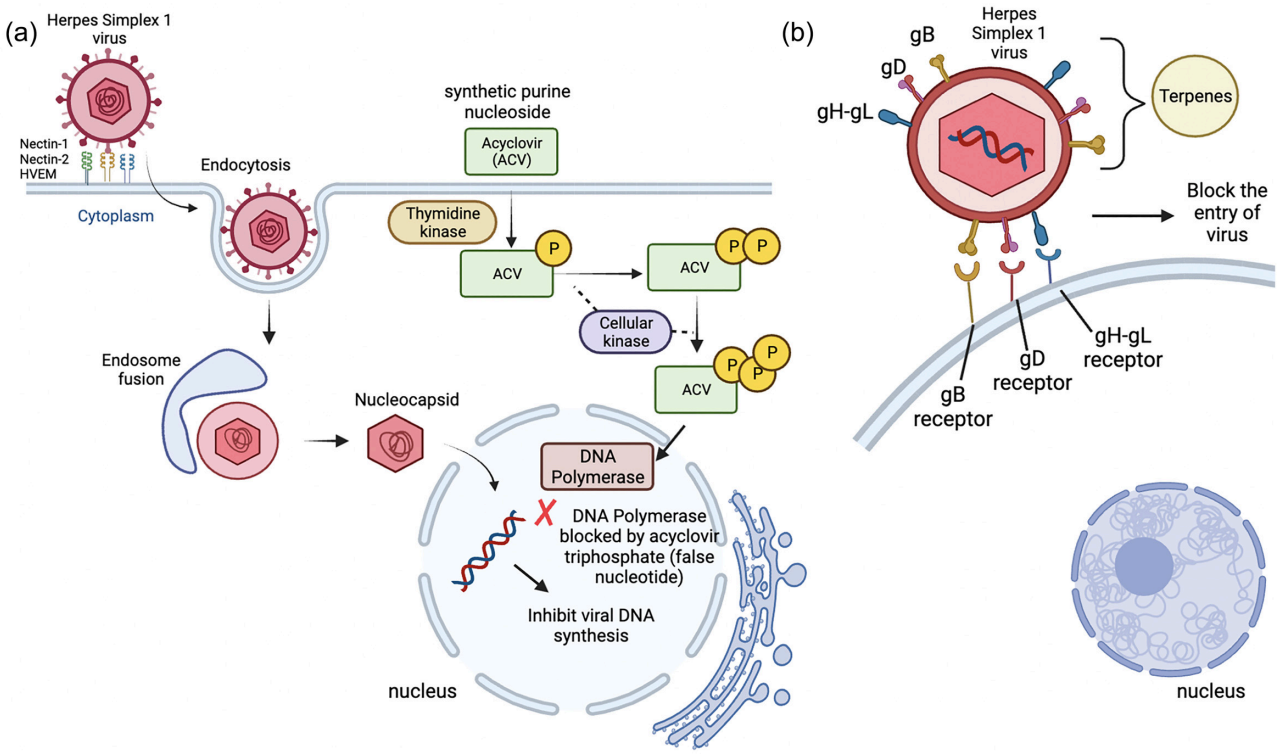

Figure 4. Mechanism of acyclovir and terpenes (monoterpenes) in HSV-1 infection. Adapted from Mostafa et al. (2018) and Mieres-Castro et al. (2021). (a) Acyclovir is a synthetic purine nucleoside analogue with antiviral properties against human herpes virus, including HSV-1. Thymidine kinase (TK) is an enzyme that combines phosphate with nucleosides to form nucleotides, which are then incorporated into DNA. The antiviral activity of acyclovir is highly selective against the virus as the TK of uninfected cells will not bind to acyclovir as a substrate. Therefore, the cell cytotoxicity of acyclovir is low. However, TK encoded by HSV-1 will be altered and acyclovir will then be converted into a false nucleotide, resulting in the inhibition of the viral DNA synthesis. (b) Terpenes, including β-pinene and limonene, were examined for their inhibitory activity against HSV-1 *in vivo*. It was shown that monoterpenes probably inactivated HSV-1 before it entered the cell on account of the drug incubation time frame. When herpes virus was incubated prior to host cell infection, the essential oils exhibited high antiviral activity. The monoterpenes bind to the glycoproteins, i.e. gB, gD, and gH-gL, and block the binding of the virus to its receptor as well as the fusion with the lipid bilayer envelope of the host cell. (figure drawn with Biorender)

the plants mentioned earlier is identical to the terpenes from cannabis plants, they might exhibit the same antiviral properties. However, there is limited evidence for the antiviral effects of terpenes from cannabis plants. Further studies using terpenes from cannabis should be investigated to provide scientific verification on the antiviral activity.

Other foods containing terpenes

Besides *C. sativa*, terpenes are also found in several other plant sources such as citrus fruits, eucalyptus, thyme, and tea tree (*Melaleuca alternifolia*) (Cox-Georgian et al. 2019). Scientists have evaluated the antiviral properties of terpenes, where monoterpenes showed good results. For instance, D-limonene, which is the major composition discovered in the essential oils that go through the cold pressing process, is derived mainly from citrus fruits, including oranges, lemons, and limes (Mahajan et al. 2017). Due to the emergence of COVID-19, phytochemicals, such as limonene, were proposed for their potential to be candidate drugs. It was shown to inhibit inflammatory mediators and regulate several signalling pathways. As mentioned earlier, the pathogenesis of COVID-19 was hypothesized to involve inflammation; thus limonene may help in reducing the severity of the disease, resulting from its antiviral, anti-inflammatory, and immunomodulatory properties (Meeran et al. 2021).

Moreover, essential oils from eucalyptus, tea tree, and thyme were also found to exhibit antiviral activity against HSV-1 *in vitro*. These essential oils include α-terpinene, γ-terpinene, α-pinene, p-cymene, terpinen-4-ol, α-terpineol, thymol, citral, and 1,8-cineole. Among these chemical compounds, the antiviral properties of monoterpene hydrocarbons were moderately better than monoterpene alcohol, where α-pinene and α-terpineol showed the highest SI (Astani et al. 2010).

Although terpenes are present in a wide range of plants, the composition of each terpene highly varies in different plant sources. Despite these variances, it has been suggested that the concentration of terpenes in cannabis extract has to be >0.05% (v/w) to modulate biological functions (Somanno et al. 2020). *In vivo* rodent studies on mental health also suggested effective concentration of terpenes such as α/β-pinene, which has been tested in mice at concentrations of 5–100 mg kg^{-1} (Guzmán-Gutiérrez et al. 2012, Weston-Green et al. 2021, Yang et al. 2016). Hence, more preclinical and clinical trials are crucial to identify the optimal dosing of terpenes for antiviral purposes. Besides, most of the discussed studies that focus on the antiviral effects of terpenes are *in vitro* studies. These studies explored the IC_{50} values of various essential oils that have terpenes as their main constituents. The IC_{50} of the essential oils covers a wide range, suggesting wide active

concentrations of terpenes in different biological pathways in viral infections. For example, the IC_{50} values of tea tree oil and *oxycedrus* oil in HSV-1 infection were 2 μg ml^{-1} and 200 mg ml^{-1}, respectively (Loizzo et al. 2008, Astani et al. 2010). Loizzo et al. (2008) has also reported that the IC_{50} of *Laurus nobilis* oil in SARS-CoV is 120 mg ml^{-1}. Thus, these findings may serve as a stepping stone in the search for effective dose of terpenes in treatment of various viral infections, including COVID-19.

Conclusion and future perspectives

Cannabis is the most widely abused illicit drug around the world as it can cause mental and physical health problems among abusers. Recently, scientists have discovered the potential medical roles of cannabis compounds in viral diseases. Cannabinoids such as CBD and Δ-9-THC, as well as essential oil such as terpenes extracted from the cannabis plants, were reported to have therapeutic effects in several virus infections such as SARS-CoV-2, HIV, HCV, and HSV.

Cannabinoids were found to downregulate the ACE2 gene expression and exhibit anti-inflammatory effects to reduce hyper-inflammation in COVID-19 patients. CBD and Δ-9-THC can bind to the main protease of SARS-CoV-2 and activate RNase to prevent viral inhibition. Besides, cannabinoids can also decrease proinflammatory cytokine production such as interleukin 2 and interferon alpha in HIV patients. CB2 receptor agonists such as THC and CBD can decrease F-actin in CD4$^+$ T cells, leading to a reduction in HIV replication. In addition, the CB1 receptor was found to be associated with HCV infection. Hence, CBD, which is a CB1 receptor antagonist, can inhibit virus replication and viral protein translation.

On the other hand, terpenes extracted from cannabis plants possess antiviral properties against SARS-CoV-2. The combination of CBD and terpenes was shown to be effective in inhibiting the virus replication. In addition, multiple studies have shown that terpenes extracted from various plants have potential roles in treating herpes caused by HSV. These findings suggest that terpenes from cannabis plants may possess similar antiviral effects against HSV.

There is very limited *in vivo* investigation of the antiviral effect of these compounds. Despite the therapeutic effect, cannabis is an illicit drug that can be consumed in a harmful and abusive manner. Thus, preclinical and clinical trials in humans are very restricted due to the legalization of cannabis compounds in a few countries. Studies on the effects of the compound containing both CBD and THC are also limited. Besides, there is still a gap in revealing the exact mechanism of how cannabinoids and terpenes help in reducing replication of various viruses. Moreover, due to the wide range of activities of cannabinoids and terpenes , further *in vivo* and clinical studies are essential to determine the effective dose of the cannabis compounds to maximize their therapeutic benefits in viral infections. In short, we are still very far from the level of evidence required to consider cannabis compounds as a regimen for viral illnesses. Hence, it is necessary to explore further the mechanisms of cannabinoids and terpenes in viral infection. Further research studies need to be conducted to provide sufficient scientific evidence on the antiviral effects of cannabis described in this review; however preliminary research described in this review points toward many new putative antiviral strategies.

Conflict of interest

The authors have no conflict of interest to declare.

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Author contributions

Y.L.S. and Y.J.G. contributed to the conception of the work, interpreting the relevant literature, and drafting of the manuscript. Critical revision and final approval of the article were done by W.S.C. and S.K.L.

Data availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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