MEDWIN PUBLISHERS
Committed to Create Value for Researchers

International Journal of Pharmacognosy & Chinese Medicine
ISSN: 2576-4772

Narrative Review: Phytocannabinoids and their Potential Use as a Phytochemotherapy

Suttithumsatid W[1] and Panichayupakaranant P[1,2]*

[1]Department of Pharmacognosy and Pharmaceutical Botany, Prince of Songkla University, Thailand

[2]Phytomedicine and Pharmaceutical Biotechnology Excellence Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand

Review Article

Volume 4 Issue 3

Received Date: November 15, 2020

Published Date: November 30, 2020

DOI: 10.23880/ipcm-16000209

***Corresponding author:** Pharkphoom Panichayupakaranant, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai 90112, Thailand, Tel: +66-74-288980; Email: pharkphoom.p@psu.ac.th

Abstract

Cannabis sativa L. has been used as an herbal medicine for centuries. This plant is a natural source of cannabinoids, including Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), the two major phytocannabinoids that have been a recently interested research topic on their therapeutic properties. The human endocannabinoid system generally consists of receptors, endogenous ligands, and metabolizing enzymes, and plays an important role in various physiological and pathological effects. Based on cancer therapy, phytocannabinoids have mainly been used for palliative care treatment, i.e. relieving nausea and vomiting caused by chemotherapy and stimulating appetite. Additionally, in many preclinical experiments, including *in vitro* and *in vivo* studies, cannabinoids have exhibited anticancer effects against numerous cancer cell lines through various mechanisms. For example, THC induced apoptosis of cancer cells *via* cannabinoid receptors by a stimulation of ceramide synthesis led to an activation of the endoplasmic reticulum stress-related signaling pathway, and the induction of autophagy through a calmodulin-activated kinase kinase β. In contrast, the anticancer activity of CBD is related to other types of receptors, i.e. orphan G-protein coupled receptor 55 (GPR55), transient receptor potential vanilloid receptor 1 (TRPV1), transient receptor potential melastatin 8 (TRPM8), which mainly relied on the stimulation of reactive oxygen species (ROS) production, leading to induced cancer cell death. Although, there are many reports on anticancer properties of phytocannabinoids, *in vitr*o and *in vivo*, high quality clinical trials concerning their efficacy and safety are still essential to approve their potential use as a phytochemotherapy.

Keywords: *Cannabis sativa;* Cannabinoid; Cancer; Tetrahydrocannabinol; Cannabidiol ; HPLC

Introduction

Cancer is a generic term for the uncontrolled growth of abnormal cells that affect the human body. Cancer is a serious global public health problem and the second leading cause of death that estimated 9.6 million deaths in 2018. The most common causes of cancer death were lung cancer (1.76 million deaths); colorectal cancer (862,000 deaths); liver cancer (828,945 deaths); stomach cancer (783,000 deaths) and breast cancer (627,000 deaths), respectively. The number of new cancer cases per year is expected to rise to 23.6 million by 2030 [1]. Currently, conventional medicine plays an important role in cancer treatment. There are many routes of cancer treatment depending on types of cancer, the levels of invasion and the performance status of patients [2]. However, most patients commonly need a combination of treatments, such as surgery with chemotherapy and/or radiation therapy. In addition, immunotherapy, targeted

therapy, and hormone therapy also have benefits in cancer treatment [2,3]. Moreover, side effects of cancer treatment, especially from systemic chemotherapy, are often severe, such as cardiotoxicity, bladder toxicity, neurotoxicity, renal toxicity, gastrointestinal toxicity, hepatotoxicity, and hematopoietic system injury. Subsequently, these adverse effects may be a major cause of therapy discontinuation [4,5].

Currently, potential strategies to manage adverse effect from cancer treatment focus on alternative treatments, especially the use of natural products including crude herbal extracts and pure phytochemicals [4]. Many plant-derived anticancer agents are being clinically used to treat or prevent development of cancer. These plants-derived anticancer agents can be divided into 4 groups, namely *Vinca* alkaloids (from *Catharanthus roseus*), *Taxus* diterpenes (from *Taxus brevifolia*), *Podophyllum* lignans (from *Podophyllum peltatum* and *P. hexandrum*), and *Camptotheca* alkaloids (from *Camptotheca acuminate*) [6]. *Vinca* alkaloids, i.e. vinblastine and vincristine, are common allopathic medicine used for treatment of Hodgkin's disease and acute lymphoblastic leukemia in children by inhibiting the dynamics of microtubules by binding to β-tubulin in the mitotic spindle [7,8]. Paclitaxel (Taxol®) is a *Taxus* diterpene widely used as the microtubule disruptors for treatment of ovarian, breast, lung, head, and neck cancers [9,10]. Podophyllotoxin is a podophyllum lignan that has been used for treatment of numerous cancers including testicular, breast, pancreatic, lung, stomach, and ovarian cancers. A mechanism of action of podophyllotoxin is based on binding with tubulin, thus preventing cell division [11]. Camptothecin, a *Camptotheca* alkaloid that has been used as a broad-spectrum anticancer agent for different types of cancer patient, such as small-cell and non-small cell lung, breast and liver cancers [12]. Camptothecin binds to the topoisomerase I and DNA complex, thus causing DNA damage and resulting in cell apoptosis [13,14].

Cannabis sativa has been used as an herbal medicine for centuries. It is a natural source of natural cannabinoids, including Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), which have been investigated for their pharmacological properties [15]. Therefore, medical use of *C. sativa* has recently been increasing with the rise of scientific investigation and publication. The endocannabinoid system consisting of receptors, endogenous ligands, and metabolizing enzymes, makes important contributions in physiological and pathological processes of the human body [16]. Currently, there are many preclinical and clinical studies indicating that cannabinoids have therapeutic potential in many diseases, such as nausea and vomiting associated with cancer chemotherapy, intractable childhood epilepsy, muscle spasms in patients with multiple sclerosis, and they even display some anticancer properties. In cancer therapy, cannabinoids have recently been used for palliative care, i.e. relieving nausea and vomiting caused by chemotherapy and stimulating appetite [17]. However, many preclinical experiments including *in vitro* and *in vivo* studies have revealed that cannabinoids possessed anti-tumor effects against numerous cancer cell lines through various mechanisms [18,19]. Nevertheless, anticancer effects of cannabinoids appear to be dependent on the type of cancer and concentration of cannabinoids [20]. This review aims to summarize the scientific studies on anticancer potentials of THC and CBD. Moreover, important information, including botanical sources, physicochemical properties, biogenesis, quantitative HPLC analysis, and toxicity assessment are described.

Retrieval Strategies and Methods

Based on this narrative review, the keywords of *Cannabis sativa*, cannabinoid, endocannabinoid, phytocannabinoid, anticancer, antitumor, tetrahydrocannabinol, and cannabidiol were searched in the online search engines, including PubMed (https://pubmed.ncbi.nlm.nih.gov/advanced), Science Direct (https://www.sciencedirect.com/search) and Google (https://www.google.com) to obtain 100 selected scientific articles. Most of them have been published between 2006-2020.

Cannabinoids

Endocannabinoids: Basically, cannabinoids can be divided into 3 groups, namely endocannabinoids, phytocannabinoids and synthetic cannabinoids [20]. Endocannabinoids are the endogenous ligands that are produced in the human body and bind to the cannabinoid receptors. Two major endocannabinoids, anandamide (*N*-arachidonoylethanolamine; AEA) and 2-arachidonoyl glycerol (2-AG) have been identified as arachidonic acid derivatives (Figure 1), which are produced on demand in response to increase intracellular Ca^{2+} concentration and/or to activate Gq/11-coupled receptor [21,22]. Endocannabinoids are produced from postsynaptic terminals following neuronal activation and mediated retrograde signaling systems in the brain [23]. AEA acts as a partial agonist of cannabinoid receptors type 1 (CB1) with higher affinity and is almost inactive against cannabinoid receptors type 2 (CB2). AEA also activates transient receptor potential vanilloid receptor 1 (TRPV1) and the peroxisome proliferator-activated receptor (PPAR). AEA is catalyzed from N-acyl-phosphatidylethanolamine (NAPE) by NAPE-specific phospholipase D and degraded by fatty acid amide hydrolase into arachidonic acid and ethanolamine whereas 2-AG acts as a full agonist on both CB1 and CB2 with moderate to low affinity. 2-AG is produced from diacylglycerol (DAG) by either DAG lipase-alpha or -beta, and mostly hydrolyzed by

monoacylglycerol lipase into arachidonic acid and glycerol [22,24].

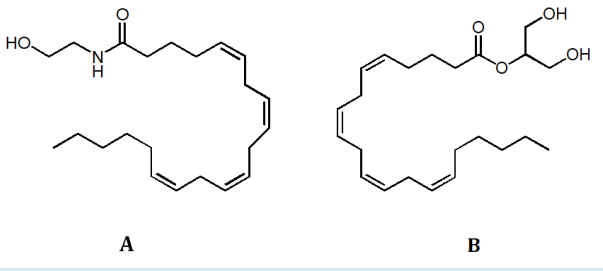

Figure 1: Chemical structures of anandamide (A) and 2-arachidonoylglycerol (B).

Phytocannabinoids: Phytocannabinoids are a group of plant-derived secondary metabolites. Phytocannabinoids have been identified in several plant species, including *Echinacea purpurea*, *E. angustifolia*, *E. pallida*, *Acmella oleracea*, *Helichrysum umbraculigerum*, *Radula marginata* and are mostly found in *Cannabis sativa* [25]. These cannabinoids have an affinity to interact with the cannabinoid receptors. The chemical structures of phytocannabinoids consist of lipid structure coupled with alkylresorcinol and monoterpene moieties. Currently, more than 100 phytocannabinoids have been identified in *C. sativa*. *Cannabis* cannabinoids are produced and accumulated as cannabinoid acids, i.e. cannabigerolic acid (CBGA) and cannabigerovaric acid (CBGVA), and then enzymatically converted into their active metabolites, such as THC, CBD, cannabigerol (CBG), cannabichromene (CBC), cannabicyclol (CBL), cannabinodiol (CBND), cannabinol (CBN), Δ9-tetrahydrocannabivarin (THCV), cannabivarin (CBV), and cannabidivarin (CBDV) by cannabinoid synthase [26].

Cannabis sativa*:* Cannabis or hemp is plants in Cannabaceae family native to Central Asia. Cannabis is generally divided into 3 species, including *C. sativa*, *C. indica*, and *C. ruderalis* [27]. However, to date the common scientific emphasis is that cannabis is monotypic and consists only of a single species as *C. sativa* [28]. *C. sativa* is an annual dioeciously flowering plant that has long been used as a source of fibers, food, oil, and traditional medicine. The history of Cannabis in traditional medicines has been reported for 5000 years and is recommended for eczema, psoriasis, and inflammatory diseases. Recently, it has been suggested for new therapeutic uses, including cancer disease [29,30].

C. sativa contains several chemical compounds, such as cannabinoids, terpenoids, flavonoids, and alkaloids. Among these, at least 100 are cannabinoids, a group of compounds that have unique chemical structures [29]. The plant produces all cannabinoids in their acidic forms and subsequently decarboxylated into their neutral forms. The cannabinoids are produced in the plant's glandular trichomes that are located on the surface of the entire plant especially on female flowers [29].

Physicochemical Properties of THC and CBD: THC (6a,7,8,10a-tetrahydro-6,6,9-trimethyl-3-pentyl-6H-dibenzo[b,d]pyran-1-ol) is a major psychoactive cannabinoid of *C. sativa*. THC has a molecular formula of $C_{21}H_{30}O_2$ with a molecular weight of 314.5 g/mol (Figure 2). THC is a colorless oil that has good solubility in several solvents, such as ethanol, acetone, dimethyl sulfoxide (DMSO), chloroform, benzyl alcohol and sesame oil, but has a very low solubility in water [31,32]. THC has a high boiling point at 157° C and a melting point less than 25° C [33,34].

CBD (2-[1R-3-methyl-6R-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol) has a low psychoactive effect due to its low affinity to the cannabinoid receptors. CBD has a molecular formula of $C_{21}H_{30}O_2$ and a molecular weight of 314.5 g/mol, like THC (Figure 2). CBD is crystalline solid and soluble in DMSO and ethanol [35,35]. The boiling points of CBD are not clear, but are in the range of 160-180° C, and the melting point is 67.5°C [33,36].

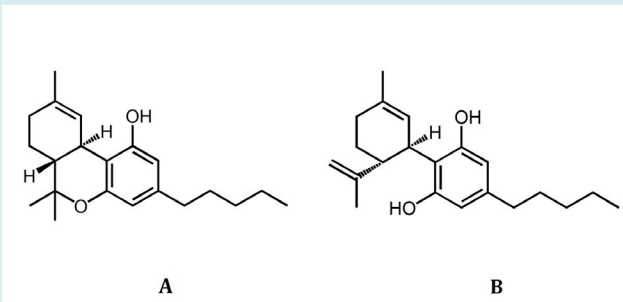

Figure 2: Chemical structures of Δ-9-tetrahydrocannabinol (A) and cannabidiol (B).

Biogenetic Pathway of Cannabinoids: Cannabinoids is a group of terpenophenolics that is comprised of two important parts, namely a diphenol (resorcin) carrying an alkyl chain, olivetolic acid and a monoterpene moiety, geranyl pyrophosphate (GPP) [37]. Although there are more than 60 cannabinoids already found this review focuses on the pathway that leads to the biosynthesis of THC and CBD [38]. Biosynthesis of olivetolic acid is started from a conversion of pyruvate, a product from oxidation of glucose in the glycolysis pathway, to acetyl-CoA by pyruvate dehydrogenase (PDH) or PDH bypass (Figure 3). Acetyl-CoA is subsequently converted into malonyl-CoA using acetyl-CoA carboxylase [37]. Another precursor in this pathway is hexanoyl-CoA, a medium-chain fatty acyl-CoA produced by two steps. Firstly, *n*-hexanol is generated by a breakdown of

C18 unsaturated fatty acids *via* the lipoxygenase pathway or obtained by an early termination of the fatty acid biosynthesis. Then, the conversion of *n*-hexanol to hexanoyl-CoA is catalyzed by acyl-CoA synthetase [39]. Subsequently, the condensation of malonyl-CoA with hexanoyl-CoA using 3,5,7-trioxododecanoyl-CoA synthase or tetraketide synthase (TKS) to produce 3,5,7-trioxododecanoyl-CoA. Finally, olivetolic acid is formed *via* aldol cyclization of 3,5,7-trioxododecanoyl-CoA using olivetolic acid cyclase [37,40].

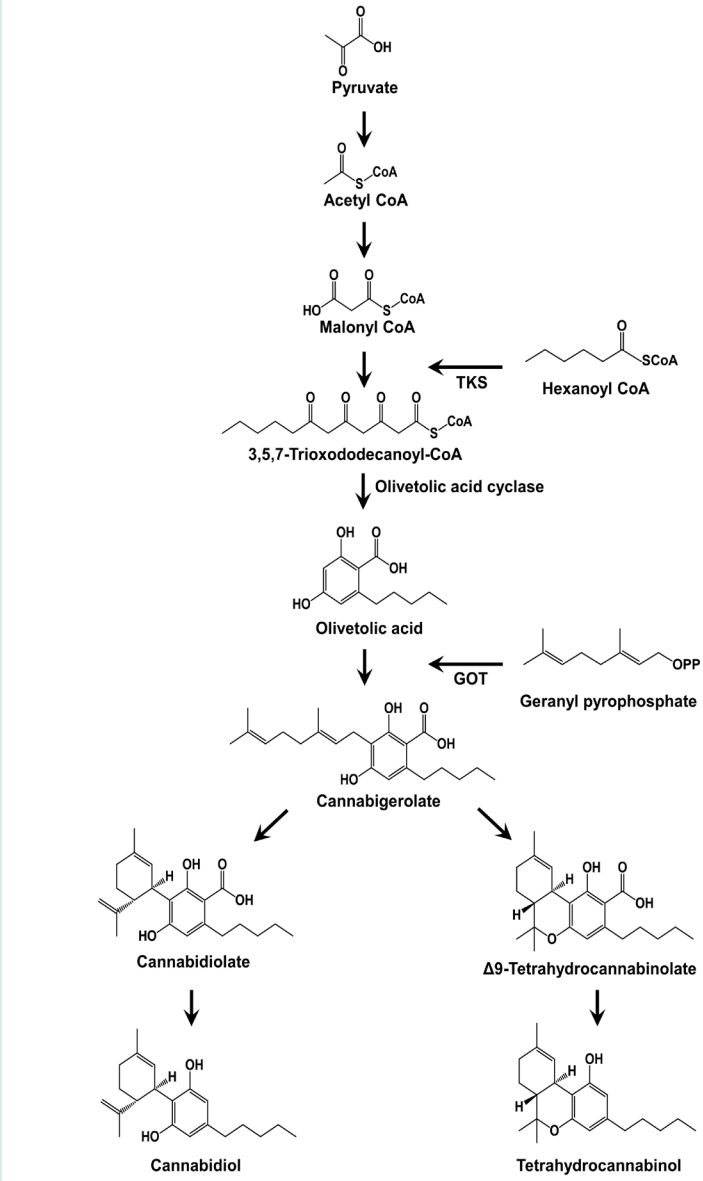

Figure 3: Biosynthesis of Δ-9-tetrahydrocannabinol and cannabidiol.

Isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) are two important precursors used in biosynthesis of geranyl pyrophosphate (GPP) *via* the 2C-methyl-d-erythritol-4-phosphate (MEP) pathway. The head-to-tail condensation of IPP and DMAPP to form GPP is catalyzed by geranyl diphosphate synthase [41].

Cannabigerolic acid (CBGA), the central precursor for cannabinoid biosynthesis is produced *via* the condensation of GPP and olivetolic acid using geranyl pyrophosphate: olivetolate geranyltransferase (GOT). CBGA is subsequently converted into Δ-9-tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) by THCA synthase and

cannabidiolic acid synthase, respectively. Finally, THCA and CBDA are decarboxylated to produce THC and CBD, respectively [29,37].

Quantitative HPLC Analysis of Cannabinoids

A simple reverse phase high performance liquid chromatography (HPLC) has been developed for the determination of THC and CBD in an oromucosal spray made from cannabis extract. This method involved the use of a reverse phase Zorbax C-18 column (4.6 mm × 100 mm, 3.5 µm) isocatically eluted by a mixture of methanol and water (85:15 v/v) with a flow rate of 1.0 ml/min and a quantitative determination wavelength of 220 nm. Both THC and CBD were eluted within 10 minutes. The method was validated through linearity, accuracy, precision, limits of detection and quantitation and specificity parameters, and it was found that the method exhibited good linearity, accuracy, precision, sensitivity, specificity, and quantitation limits. This method shows a good separation and short run time (10 min) for determination of an amount of THC and CBD [42].

In addition, a simple isocratic HPLC method for analyzing THC, CBD and other cannabinoids in *C. sativa* and *C. indica* plant powders and their extracts has been reported. Analyses are performed using a Luna C18 column (octadecyl silica, 150 mm × 3 mm i.d., 3 µm) at 37°C, and sample vials have been set at 10°C. The mobile phase consisted of water/acetonitrile at a ratio of 9:31 (v/v), with 0.1% formic acid (v/v) and 10 mM ammonium formate. The detection wavelength was set at 275 nm. This method needed a relatively short analysis time (8.5 min) and also exhibited a good analytical performance, including accuracy, precision, sensitivity, linearity, and stability [43].

Moreover, Mandrioli, et al. have reported a reversed-phase HPLC method for detection of ten cannabinoids from the inflorescences of *C. sativa*. This method is performed using a reverse phase column, Nex-Leaf CBX Potency (150 × 4.6 mm, 2.7 µm with a guard column Nex-Leaf CBX 5 × 4.6 mm, 2.7 µm) at 35° C, the autosampler set at 4° C, and the UV detection at 220 nm. The mobile phase A consisted of water and 0.085% phosphoric acid, while the mobile phase B composed of acetonitrile and 0.085% phosphoric acid under gradient elution. The method has been validated and exhibited good results of validation parameters including repeatability, reproducibility, recovery value, linearity, and sensitivity [44].

Another reversed phase HPLC method has been developed for determination of eight cannabinoids, including CBDV, CBDA, CBGA, CBG, CBD, CBN, THC, and THCA in *C. sativa* samples. The method was performed using a Phenomenex Kinetex XB-C18 column (150 × 4.6 mm, 2.6 µm) with a gradient elution, using 0.1% formic acid in water and acetonitrile (with 0.1% formic acid). A UV detection of all cannabinoids was set at 220 nm. The method has been validated using the parameters, including recovery, bias, intraday- and interday-precision [45].

Brighenti et al. have reported another HPLC method using a gradient elution system for analysis of four non-psychoactive cannabinoids, namely CBDA, CBGA, CBG and CBD in *C. sativa* extracts. The analysis was performed using a reversed phase HPLC method coupled with diode array (UV/DAD) detection. The method involved the use of an Ascentis Express C18 column (150 mm × 3.0 mm, 2.7 µm) eluted with a mixture of 0.1% formic acid in both water and acetonitrile, under a gradient elution system, with running time of 30 minutes. The chromatograms were detected at a wavelength of 210 nm for CBG and CBD, and at 220 nm for CBDA and CBGA. The method has been successfully validated for linearity, sensitivity, precision, accuracy, and specificity [46].

Anticancer Effects of Cannabinoids

Receptors and Mechanisms of Anticancer

The biological effects of cannabinoids are regulated by cannabinoid receptors. Recently, two major cannabinoid-specific receptors, namely cannabinoid receptors type 1 (CB1) and cannabinoid receptors type 2 (CB2) have been described. Both CB1 and CB2 are G protein-coupled receptors family [22,47]. Although CB1 is mainly located at various locations in the central nervous system, expression of CB1 receptor is not limited to the central nervous system. It is widely expressed in many different locations of body, including peripheral nervous system, liver, skeletal muscle, pancreas, and adipocyte. In contrast, CB2 is located particularly in immune and blood cell systems, including macrophage, spleen, tonsils, thymus, and leukocyte [22,48]. Action of THC depends on ability to activate the cannabinoid receptors. THC is a partial agonist on both CB1 and CB2. Conversely, CBD has low affinity for cannabinoid receptors and acts as a potent antagonist for CB1 and CB2 [49]. In addition, CBD interacts with other cannabinoid receptor types and different targets receptors of cannabinoids, i.e. TRPV1, orphan G-protein coupled receptor 55 (GPR55), peroxisome proliferator-activated receptors, transient receptor potential melastatin 8 (TRPM8), and transient receptor potential ankyrin 1 (TRPA1) [50,51]. Activation of both CB1 and CB2 resulting in inhibition adenylate cyclase with corresponding to inactivation of the protein kinase A phosphorylation pathway and activates several mitogen-activated protein kinases. Furthermore, cannabinoids also

inhibit certain voltage dependent calcium channels and activate inwardly rectifying potassium channels. After the cannabinoid receptor is activated, various metabolic pathways are activated, such as phosphoinositide 3-kinase pathway, cyclooxygenase-2 pathway, accumulation of ceramide, modulation of protein kinase B, and ion channels [50,52].

To date, there are still conflicting data of an important role of cannabinoid system on carcinogenesis. Alteration of cannabinoid receptor level is an important factors for estimate the effects of endocannabinoids. The overexpressions of CB1 and CB2 have been shown in various types of cancers cells, such as skin, prostate and colon cancer, hepatocellular carcinoma, endometrial sarcoma, glioblastoma multiforme, meningioma and pituitary adenoma, Hodgkin lymphoma, chemically induced hepatocellular carcinoma, mantel cell lymphoma, when compared with normal cells [53,54]. However, a low expression of CB1 was detected in metastatic colorectal cancer and colon cancer [55,56].

Furthermore, raised concentration of endocannabinoids, including anandamide and 2-arachidonoylglycerol have been reported in several types of cancer, especially in glioblastoma, meningioma, pituitary adenoma, prostate and colon carcinoma, and endometrial sarcoma [50,54]. Many studies have exhibited the relationship between expression levels of cannabinoid metabolizing enzymes and tumor aggressiveness. Endocannabinoid degrading enzymes is upregulated in aggressive human cancer cells and primary tumors. Accordingly, the tumor growth was decreased when the endocannabinoid degrading enzymes expression was knocked down. This suggests that an endocannabinoid system might play an essential role in cancer physiopathology [57-59].

Over the last few decades, numerous *in vitro* and *in vivo* studies have indicated that cannabinoids can reduce tumor growth and progression. Among several reported mechanisms of endocannabinoids, the most prevalent anticancer effects of cannabinoids are the induction of cancer cell death by apoptosis and the inhibition of cancer cell proliferation through regulation of the important signaling pathways [60]. In addition, cannabinoids can decrease tumor angiogenesis and block tumor invasion and metastasis in a many *in vivo* studies [54,60].

Anticancer Activity of THC

There are abundant studies on antitumor activity of THC focused on glioma cells. THC has been shown as an induced apoptosis in glioma cells through the pathway of ceramide synthesis *de novo* (Figure 4). Ceramides are waxy lipid molecules that consist of sphingosine and a fatty acid. Ceramides are found mainly in cell membrane of eukaryotic cells, and they play an important role in cellular signaling, such as regulating differentiation, proliferation, and programmed cell death of cells [61,62]. The sphingomyelin hydrolysis and ceramide synthesis are two major pathways that may contribute to the intracellular ceramide accumulation. It has been reported that the inhibitors of sphingomyelin hydrolysis pathway, i.e. desipramine, scyphostatin and phorbol myristate acetate could not prevent THC-induced death of glioma cells when treatments of glioma cells with THC and the selective inhibitors. In contrast, the inhibitors of ceramide synthesis pathway, i.e. L-cycloserine and fumonisin B1 could prevent THC-induced death of glioma cells. This implied that *de novo* synthesized ceramide was involved in cannabinoid-induced apoptosis of glioma cells [62]. An accretion of ceramide induces an activation of endoplasmic reticulum stress-related signaling pathway resulting in an upregulation of the expression of the stress-regulated protein p8, a transcriptional factor that overexpress in various malignancies, together with its downstream targets, i.e. transcription factor 4, C/EBP homologous protein, and tribbles homolog 3 (TRIB3), which take part in control of tumor development [63,64]. Afterwards, activation of TRIB3 causes inhibition of pro-survival protein kinase B (Akt) that in turn leads to an inhibition of the mammalian target of rapamycin complex 1 (mTORC1), taking part in activating translation of proteins, and eventually induced autophagy-mediated cell death [65,66]. In addition to glioma cell, the mechanism of the upregulation of protein p8 induced autophagy pathway in the anti-tumor action of cannabinoids has been evidently exhibited in pancreatic and hepatic cancer cells [67,68].

Another probable mechanism of anticancer action of THC has been investigated in glioma cell. The cannabinoids receptor agonist induced downregulation of Akt signaling and led to a depletion of Bcl-2-associated death promoter, a proapoptotic Bcl-2 family member, which may play an important role in downregulation of survival pathways, resulting in cancer cell apoptosis [69].

Furthermore, some additional mechanisms have been shown to provide the process of an induction of cancer cell death by THC. In hepatocellular carcinoma cells, THC reduced the viability of the human hepatocellular carcinoma cell lines by stimulation of CB2 receptors that bring on upregulation of TRIB3 that cooperate with the adenosine monophosphate-activated kinase (AMPK) stimulation (Figure 4). In addition, some evidence supports the claim that calcium/calmodulin-dependent protein kinase kinase β (CaCMKKβ) was responsible for cannabinoid-induced AMPK activation and subsequent inhibition of AKT–mTORC1 axis, resulting in the stimulation of autophagy-mediated cell death [68,70].

A study on the antiproliferative mechanism of THC in human breast cancer cells has exhibited that THC activated JunD, an activator protein-1 transcription factor that is essential for cell proliferation in some cancer cells, by upregulating gene expression and translocating the protein to the nuclear compartment (Figure 4). The cyclin-dependent kinase inhibitor p27 and p21 and tumor suppressor gene testin are the JunD targets of THC for preventing cyclin-dependent kinase 1 activation, which lead to the subsequent decreased phosphorylation of the retinoblastoma protein, and eventually cell cycle arrest and apoptosis [71-73]. Moreover, THC may act as an inhibitor of epidermal growth factor induced phosphorylation of mitogen-activated protein kinases and Akt to control the growth and metastasis of lung cancers [74].

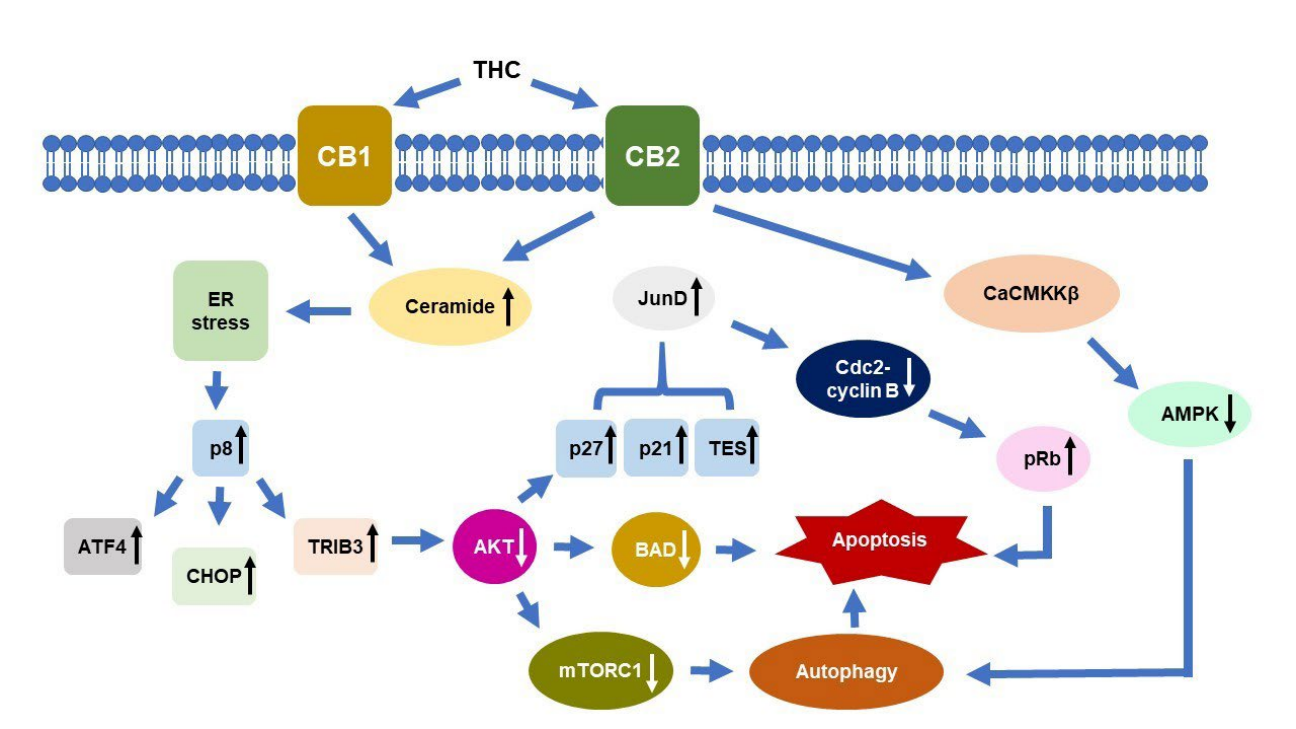

Figure 4: Some signaling pathways induced by THC in cancer cells.
THC, Δ9-tetrahydrocannabinol; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; ER, endoplasmic reticulum; p8, protein p8; CHOP, C/EBP homologous protein; ATF4, activating transcription factor 4; TRIB3, tribbles homolog 3; Akt, protein kinase B; mTORC1, mammalian target of rapamycin C1; p21, cyclin-dependent kinase inhibitor 1; p27, cyclin-dependent kinase inhibitor 1B; TES, tumor suppressor gene testin; Cdc2-cyclin B, cyclin-dependent kinase 1; pRb, retinoblastoma protein; AMPK, adenosine monophosphate-activated kinase (AMPK); CaCMKKβ, calmodulin-activated kinase kinase β.

Antitumor Activity of CBD

Recently, many studies have revealed that CBD decreased cancer cell viability in many cancer types including neuroblastoma, glioblastoma, melanoma, leukemia, colorectal, breast, lung, and prostate cancer [50]. Regarding the potential effects of CBD on cancer treatment, the mechanism of action of CBD is not related to a direct activation of the CB receptors (Figure 5). It has been reported that interactions of CBD with other types of receptors including GPR55, TRPV1 and TRPM8 may play an essential role on anticancer activity [75]. Recently, the proposed mechanism of action mainly relies on the stimulation of production of reactive oxygen species, which lead to autophagy-mediated apoptosis [75,76]. In addition, CBD induced downregulation of ERK and Akt signaling pathway that lead to inhibition of hypoxia-inducible factor 1-alpha (HIF-1α) has been reported as another mechanism of action of CBD in human glioblastoma cells [77]. HIF-1α plays a role in initiating angiogenesis and regulating cellular metabolism to overcome hypoxia that lead to promoting tumor growth and metastasis [78].

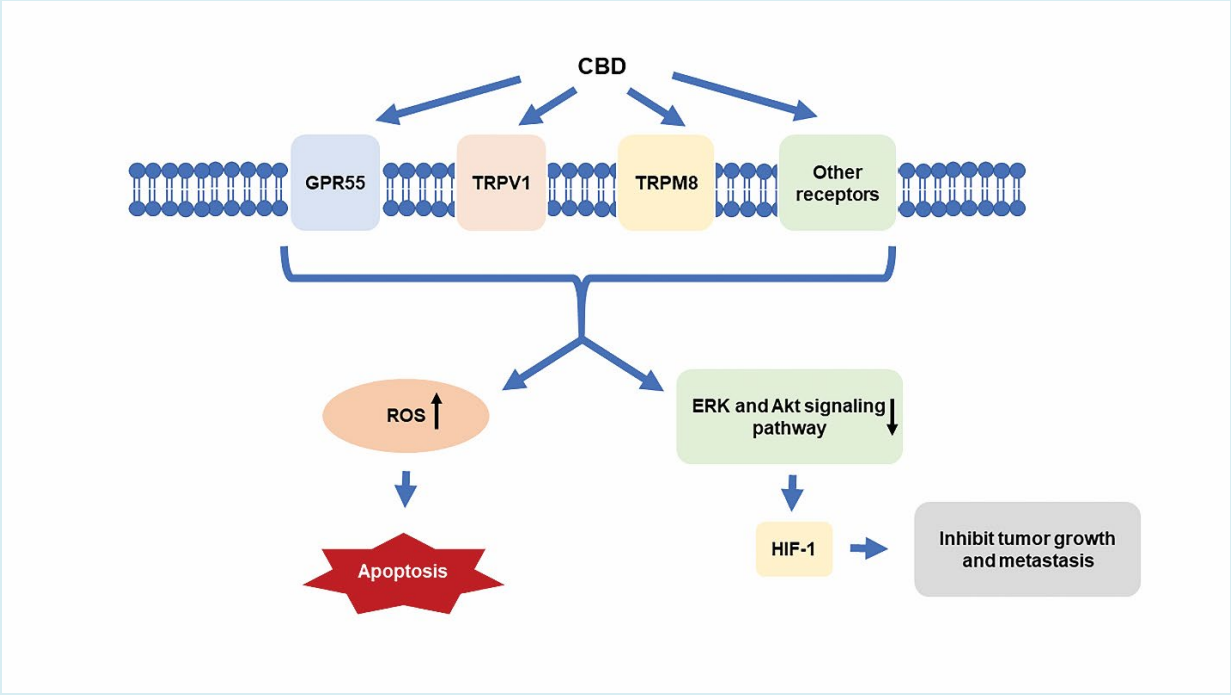

Figure 5: Some signaling pathways induced by CBD in cancer cells.
CBD: Cannabidiol; TRPV1: Receptor potential channel subfamily V member 1; GPR55: Orphan G-protein coupled receptor 55; TRPM8: Transient receptor potential melastatin 8; ROS: Reactive oxygen species; HIH-1: Hypoxia-inducible factor 1-alpha.

Inhibition of Angiogenesis and Metastasis

Cannabinoids inhibited tumor cell growth and impair tumor progression at various levels. The induction of cancer cell death by apoptosis and the inhibition of cancer cell proliferation are the most common mechanism of cannabinoids. In addition, many studies have shown that cannabinoids also impair tumor angiogenesis and block invasion and metastasis in a variety of models of cancer, i.e. gliomas, skin cancer, thyroid cancer, and breast cancer [50,79].

Based on *in vitro* and *in vivo* studies, THC inhibited tumor angiogenesis by inhibition of ceramide biosynthesis that lead to inhibition of vascular endothelial growth factor (VEGF) production and VEGF receptor (VEGFR)-2 activation. These changes in the VEGF pathway were coordinated with a decrease in size of gliomas [80]. Likewise, in skin tumors, the cannabinoid receptor agonists showed an antitumor effect through the process of impairment of tumor vascularization, as determined by disrupted vessel structure and downregulated expression of proangiogenic factors, namely VEGF, placental growth factor, and angiopoietin 2 [81]. Similarly, the cannabinoid receptor agonists also inhibited the growth of thyroid carcinoma cells in mice as well as reduced the expression of the VEGF and VEGFR. Moreover, the cannabinoid receptor agonists were able to inhibit the expression of cyclin-dependent kinase inhibitor p27, another protein advocated to play a role as a proangiogenic factor that correlated with an increased spreading of thyroid cancer cells to lymph nodes [82,83]. In addition, it has been demonstrated that the cannabinoid receptor agonists inhibited cancer cell proliferation and metastasis in other mechanisms, including modulating the cyclooxygenase-2/prostaglandin E2 signaling pathway by inhibiting the activity of the downstream molecules such as c-Fos, c-Jun, and Cdc42 [84].

In contrast, CBD has been shown to exhibit angiogenesis and metastasis of some cancer cells by acting separately from the cannabinoid receptors. In breast cancer, CBD downregulated Id-1 (helix-loop-helix transcription factor inhibitor of DNA binding 1) gene expression, an essential factor of the metastatic potential of breast cancer that involved in neovascularization [85]. In addition, CBD can lead to a decrease in lung tumor cell invasion and metastasis through a mechanism relied on the upregulation of the intercellular adhesion molecule 1 (ICAM-1), a protein that expressed on endothelial cells and cells of the immune system. An increase of ICAM-1 has been related to the upregulation of tissue

inhibitor of matrix metalloproteinases-1, a glycoprotein that may have an anti-apoptotic function [86]. A recent study has exhibited that CBD significantly reduced VEGF expression and inhibit metastasis on colon cancer, glioma, and prostate cancer [87].

Pharmacokinetics of Cannabinoids

The acronym ADME is used to describe absorption, distribution, metabolism, and excretion of a drug from human body. Generally, the pharmacokinetics of cannabinoids is varied by the routes of administration [88]. THC and CBD are highly lipophilic compounds and rapidly absorbed by the lungs resulted in an increased maximum plasma concentration (Cmax) of THC and CBD and reached faster after administration by smoking, inhalation, or intravenous route. Pulmonary administration route of THC resulted in a maximum blood concentration within minutes. In contrast, the oral bioavailability of THC and CBD is low. The absorption rate is lower in oral administration. However, their Cmax are increased after oral administration with high fat or high calorie meals [89,90]. In addition, it has been found that time to the Cmax of CBD was mostly shown between 1 and 4 hours and not a dose-dependent kinetic [91,92].

The distribution of cannabinoids in the body is depended on their lipophilicity and solubility. THC and CBD are extremely lipophilic, which imply that they are highly protein bound (94 to 99%). THC has a volume of distribution of 10 L/kg, while that of CBD is 32 L/kg [93-95].

Because of the high lipophilicity of cannabinoids, they are needed to metabolize to increase hydrophilicity before excretion. The cannabinoids are mainly metabolized *via* hepatic cytochrome oxidases enzyme. Metabolism of THC occurs principally by hepatic cytochrome P450 (CYP) 2C19 and 3A4. Besides the liver, other tissues such as heart and lung can metabolize cannabinoids, but to a lesser amount. Biotransformation of THC produces mono-, di-, and tri-hydroxy metabolites and the inactivated metabolite is THC-carboxylase [88,89,96]. Similarly, CBD is also metabolized *via* hepatic and gut by CYP2C19, CYP3A4, UGT1A7, UGT1A9, and UGT2B7 to the primary active hydroxy metabolites, and then to be an inactive metabolite by CBD-carboxylase [92,93].

THC is mostly excreted mainly as the acid metabolites in urine (20-35%) and feces (65-80%) within days and weeks. Based on lipophilic property, THC is slowly release from lipid storage compartments and enterohepatic circulation and makes its elimination half-life to be slow (25-36 hours). Unlike THC, a major part of CBD is excreted unchanged. A high percentage of CBD inactive metabolites are excreted in the feces and minor in urine. The elimination half-life of CBD is around 18-32 hours [92].

Toxicity Assessment of Cannabinoids

The toxic evidence of cannabinoids often shows when cannabis is used for recreational purposes because there is no clear demonstration of doses that achieve symptoms desired by a cannabis user. In adolescents and adults, inhaled doses of 2 to 3 mg of THC and oral administration doses of 5 to 20 mg THC can lead to impaired attention, concentration, short-term memory loss and executive functioning [97]. More serious adverse effects, including nausea, postural hypotension, delirium, panic attacks, anxiety, and myoclonic jerking, may occur when high doses of THC are administered [98]. Psychosis has also been related with a use of higher concentrated cannabis products [99].

CBD is also not risk-free. The adverse effects of CBD including developmental toxicity, embryo-fetal mortality, central nervous system inhibition and neurotoxicity, hepatocellular injuries, spermatogenesis reduction, organ weight alterations, male reproductive system alterations, and hypotension have been shown *in vivo*, at doses higher than recommendation for human pharmacotherapies. Some clinical studies have reported that CBD can induce hepatic abnormalities, diarrhea, fatigue, vomiting, and somnolence [100].

Conclusion

Cannabinoids are a large and important group of secondary metabolites found in *C. sativa* that tend to have therapeutic potential for the treatment of variety of diseases, including cancer. Cannabinoids were able to effectively inhibit tumor growth by modulation of the signaling pathways crucial in the control of cancer cell survival, in both *in vitro* and *in vivo* experiments. The two major mechanisms of cannabinoids for antitumor effects are induction of apoptosis and the inhibition of cancer cell proliferation as well as inhibition of tumor angiogenesis, invasion, and metastasis. *C. sativa* produces more than 100 different cannabinoids, including THC and CBD. THC mainly exhibit antitumor effects *via* CB1 and CB2 dependent stimulation of the *de novo* synthesis. On the other hand, CBD acts independently of the CB1 and CB2, by interactions with other types of receptors (GPR55, TRPV1, TRPM8). However, there is still a lack of high quality of safety and efficacy clinical trials. Therefore, it is very difficult to assess the potential benefits and risk of using cannabinoids in human. The uses of cannabinoids in cancer treatment still needs elucidation such as dosages, interactions with other drugs/food supplement, or assessing adverse effects. Currently, the use of cannabinoids as a treatment for cancer still has a long way to go. However, research on anticancer potential of cannabinoids is being conducted to support their evidence for the anticancer treatment.

Acknowledgement

The authors wish to thank Ms. Maria Mullet for assistance with English language editing.

References

1. WHO (2018) Cancer.

2. Arruebo M, Vilaboa N, Sáez Gutierrez B, Lambea J, Tres A, et al. (2011) Assessment of the evolution of cancer treatment therapies. Cancers 3(3): 3279-3330.

3. Falzone L, Salomone S, Libra M (2018) Evolution of cancer pharmacological treatments at the turn of the third millennium. Front Pharmacol 9: 1300.

4. Zhang QY, Wang FX, Jia KK, Kong LD (2018) Natural product interventions for chemotherapy and radiotherapy-induced side effects. Front Pharmacol 9: 1253.

5. Fu B, Wang N, Tan HY, Sha Li, Cheung F, et al. (2018) Multi-component herbal products in the prevention and treatment of chemotherapy-associated toxicity and side effects: A review on experimental and clinical evidences. Front Pharmacol 9: 1394.

6. Kinghorn AD, Chin YW, Swanson SM (2009) Discovery of natural product anticancer agents from biodiverse organisms. Curr Opin Drug Discov Devel 12(12): 189-196.

7. Ngan VK, Bellman K, Hill BT, Wilson L, Jordan MA, et al. (2001) Mechanism of mitotic block and inhibition of cell proliferation by the semisynthetic Vinca alkaloids vinorelbine and its newer derivative vinflunine. Mol Pharmacol 60(1): 225-232.

8. Thirumaran R, Prendergast GC, Gilman PB (2007) Cytotoxic chemotherapy in clinical treatment of cancer. In: Prendergast GC, et al. (Ed.), Cancer Immunotherapy, Academic Press, Cambridge, Massachusetts, United States, pp: 101-116.

9. DeLigio JT, Velkova A, Zorio DA, Monteiro A (2009) Can the status of the breast and ovarian cancer susceptibility gene 1 product (BRCA1) predict response to taxane-based cancer therapy? Anti-Cancer Anticancer Agents Med Chem 9(5): 543-549.

10. Ojima I, Lichtenthal B, Lee S, Wang C, Wang X, et al. (2016) Taxane anticancer agents: a patent perspective. Expert Opin Ther Pat 26(1): 1-20.

11. Zhang X, Rakesh K, Shantharam C, Manukumar HM, Asiri AM, et al. (2018) Podophyllotoxin derivatives as an excellent anticancer aspirant for future chemotherapy: A key current imminent needs. Bioorg Med Chem 26(2): 340-355.

12. Xiao X, Wang S, Xia S, Zou M, Li Y, et al. (2015) Retrospective study of irinotecan/cisplatin followed by etoposide/cisplatin or the reverse sequence in extensive-stage small cell lung cancer. Onco Targets Ther 8: 2209-2214.

13. Pommier Y (2006) Topoisomerase I inhibitors: camptothecins and beyond. Nat Rev Cancer 6(10): 789-802.

14. Liu LF, Desai SD, LI TK, Mao Y, Sun M, et al. (2000) Mechanism of action of camptothecin. Ann N Y Acad Sci 922: 1-10.

15. oggs DL, Peckham A, Boggs AA, Ranganathan M (2016) Delta9 tetrahydrocannabinol and cannabidiol: Separating the chemicals from the "weed," apharmacodynamic discussion. Men Health Clin 6(6): 277-284.

16. Viveros M, de Fonseca FR, Bermudez Silva F, McPartland JM (2008) Critical role of the endocannabinoid system in the regulation of food intake and energy metabolism, with phylogenetic, developmental, and pathophysiological implications. Endocr Metab Immune Disord Drug Targets 8(3): 220-230.

17. Kleckner AS, Kleckner IR, Kamen CS, Tejani MA, Janelsins MC, et al. (2019) Opportunities for cannabis in supportive care in cancer. Ther Adv Med Oncol.

18. Sarfaraz S, Adhami VM, Syed DN, Afaq F, Mukhtar H (2008) Cannabinoids for cancer treatment: progress and promise. Cancer Res 68(2): 339-342.

19. Velasco G, Sánchez C, Guzmán M (2012) Towards the use of cannabinoids as antitumour agents. Nat Rev Cancer 12(6): 436-444.

20. Dariš B, Verboten MT, Knez Ž, Ferk P (2019) Cannabinoids in cancer treatment: Therapeutic potential and legislation. Bosn J Basic Med Sci 19(1): 14-23.

21. Gyombolai P, Pap D, Turu G, Catt KJ, Bagdy G, et al. (2012) Regulation of endocannabinoid release by G proteins: a paracrine mechanism of G protein-coupled receptor action. Mol Cell Endocrinol 353(1-2): 29-36.

22. Zou S, Kumar U (2018) Cannabinoid receptors and the endocannabinoid system: signaling and function in the central nervous system. Int J Mol Sci 19(3): 833.

23. Castillo PE, Younts TJ, Chávez AE, Hashimotodani Y (2012) Endocannabinoid signaling and synaptic

function. Neuron 76(1): 70-81.

24. Fulmer ML, Thewke DP (2018) The endocannabinoid system and heart disease: the role of cannabinoid receptor type 2. Cardiovasc Hematol Disord Drug Targets 18(1): 34-51.

25. Messina F, Rosati O, Curini M, Marcotullio MC (2015) Cannabis and Bioactive Cannabinoids. Stud Nat Prod Chem 45: 17-57.

26. Thoma B, ElSohly M (2015) The Analytical Chemistry of Cannabis. 1st (Edn.), Elsevier, Amsterdam, pp: 1-135.

27. Gloss D (2015) An overview of products and bias in research. Neurotherapeutics 12(4): 731-734.

28. Hazekamp A, Fischedick J, Dıez M (2010) Chemistry of cannabis. In: Mander L, Lui H (Editors.) Comprehensive natural products II 1st (Edn.), Chemistry and biology, Elsevier, Oxford, UK, pp: 1033-1084.

29. Bonini SA, Premoli M, Tambaro S, Kumar A, Maccarinell G, et al. (2018) *Cannabis sativa*: A comprehensive ethnopharmacological review of a medicinal plant with a long history. J Ethnopharmacol 227: 300-315.

30. Pellati F, Borgonetti V, Brighenti V, Biagi M, Benvenuti S, et al. (2018) *Cannabis sativa* L. and nonpsychoactive cannabinoids: their chemistry and role against oxidative stress, inflammation, and cancer. Biomed Res Int.

31. Schafroth MA, Zuccarello G, Krautwald S, Sarlah D, Carreira EM (2014) Stereodivergent total synthesis of Δ9-tetrahydrocannabinols. Angew Chem Int Ed Engl 53(50): 13898-13901.

32. Rosenkrantz H, Thompson GR, Braude MC (1972) Oral and parenteral formulations of marijuana constituents. J Pharm Sci 61(7): 1106-1112.

33. McPartland JM, Russo EB (2001) Cannabis and cannabis extracts: greater than the sum of their parts? J Cannabis Ther 1: 103-132.

34. Brunel HP, Kroon MC, Van Roosmalen MJ, Spronsen J, Peters JC, et al. (2010) Solubility of non-psychoactive cannabinoids in supercritical carbon dioxide and comparison with psychoactive cannabinoids. J Supercrit Fluids 55(2): 603-608.

35. Lovestead TM, Bruno TJ (2017) Determination of cannabinoid vapor pressures to aid in vapor phase detection of intoxication. Forensic Chem 5: 79-85.

36. WHO (2018) Cannabidiol (CBD): Critical review report.

37. Degenhardt F, Stehle F, Kayser O (2017) The biosynthesis of cannabinoids. In: Preedy VR, (Eds.), Handbook of cannabis and related pathologies, Academic Press, Cambridge, Massachusetts, United States, pp: 13-23.

38. Atakan Z (2012) Cannabis, a complex plant: different compounds and different effects on individuals. Ther Adv Psychopharmacol 2(6): 241-254.

39. Marks MD, Tian L, Wenger JP, Omburo SN, Fuentes WS, et al. (2009) Identification of candidate genes affecting Δ9-tetrahydrocannabinol biosynthesis in *Cannabis sativa*. J Exp Bot 60(13): 3715-3726.

40. Gagne SJ, Stout JM, Liu E, Boubakir Z, Clark SM, et al. (2012) Identification of olivetolic acid cyclase from *Cannabis sativa* reveals a unique catalytic route to plant polyketides. Proc Natl Acad Sci USA 109(31): 12811-12816.

41. Fellermeier M, Eisenreich W, Bacher A, Zenk MH (2001) Biosynthesis of cannabinoids: Incorporation experiments with 13C-labeled glucoses. Eur J Biochem 268(6): 1596-604.

42. Saingam W, Sakunpak A (2018) Development and validation of reverse phase high performance liquid chromatography method for the determination of delta-9-tetrahydrocannabinol and cannabidiol in oromucosal spray from cannabis extract. Rev Bras Farmacogn 28(6): 669-672.

43. Križman M (2020) A simplified approach for isocratic HPLC analysis of cannabinoids by fine tuning chromatographic selectivity. Eur Food Res Technol 246: 315-322.

44. Mandrioli M, Tura M, Scotti S, Toschi TG (2019) Fast detection of 10 cannabinoids by RP-HPLC-UV method in *Cannabis sativa* L. Molecules 24(11): 2113.

45. Zivovinovic S, Alder R, Allenspach MD, Steuer C (2018) Determination of cannabinoids in *Cannabis sativa* L. samples for recreational, medical, and forensic purposes by reversed-phase liquid chromatography-ultraviolet detection. J Anal Sci Technol 9: 1-10.

46. Brighenti V, Pellati F, Steinbach M, Maran D, Benvenuti S (2017) Development of a new extraction technique and HPLC method for the analysis of non-psychoactive cannabinoids in fibre-type *Cannabis sativa* L.(hemp). J Pharmaceut Biomed 143: 228-236.

47. Di Marzo V, Bifulco M, De Petrocellis L (2004) The endocannabinoid system and its therapeutic exploitation. Nat Rev Drug Discov 3(9): 771-784.

48. Gong JP, Onaivi ES, Ishiguro H, Liu QR, Tagliaferro PA, et al. (2006) Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. Brain Res 1071(1): 10-23.

49. Pertwee R (2008) The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ9-tetrahydrocannabinol, cannabidiol and Δ9-tetrahydrocannabivarin. Brit J Pharmacol 153(2): 199-215.

50. Śledziński P, Zeyland J, Słomski R, Agnieszka Nowak (2018) The current state and future perspectives of cannabinoids in cancer biology. Cancer Med 7(3): 765-775.

51. Di Marzo V, Piscitelli F (2015) The endocannabinoid system and its modulation by phytocannabinoids. Neurotherapeutics 12(4): 692-698.

52. Guindon J, Hohmann AG (2011) The endocannabinoid system and cancer: therapeutic implication. Brit J Pharmacol 163(7): 1447-1463.

53. Velasco G, Tiedra SH, Dávila D, Lorente M (2016) The use of cannabinoids as anticancer agents. Prog Neuro-Psychoph 64: 259-266.

54. Hermanson DJ, Marnett LJ (2011) Cannabinoids, endocannabinoids, and cancer. Cancer Metast Rev 30(3-4): 599-612.

55. Tutino V, Caruso MG, De Nunzio V, Lorusso D, Veronese N, et al. (2019) Down-regulation of cannabinoid type 1 (CB1) receptor and its downstream signaling pathways in metastatic colorectal cancer. Cancers 11(5): 708.

56. Laezza C, Pagano C, Navarra G, Pastorino O, Proto MC, et al. (2020) The endocannabinoid system: A target for cancer treatment. Int J Mol Sci 21(3): 747.

57. Nomura DK, Long JZ, Niessen S, Hoover HS, Shu Wing Ng, et al. (2010) Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. Cell 140(1): 49-61.

58. Thors L, Bergh A, Persson E, Hammarsten P, Stattin P, et al. (2010) Fatty acid amide hydrolase in prostate cancer: association with disease severity and outcome, CB1 receptor expression and regulation by IL-4. Plos One.

59. Wang D, Wang H, Ning W, Backlund MG, Dey SK, et al. (2008) Loss of cannabinoid receptor 1 accelerates intestinal tumor growth. Cancer Res 68(15): 6468-6476.

60. Velasco G, Sánchez C, Guzmán M (2016) Anticancer mechanisms of cannabinoids. Curr Oncol 23(Suppl 2): S23-S32.

61. Mencarelli C, Martinez PM (2013) Ceramide function in the brain: when a slight tilt is enough. Cell Mol Life Sci 70(2): 181-203.

62. Pulgar TG, Velasco G, Sanchez C, Haro A, Guzmán M (2002) De novo-synthesized ceramide is involved in cannabinoid-induced apoptosis. Biochem J 363(1): 183-188.

63. Carracedo A, Lorente M, Egia A, Blázquez C, García S, et al. (2006) The stress-regulated protein p8 mediates cannabinoid-induced apoptosis of tumor cells. Cancer Cell 9(4): 301-312.

64. Encinar JA, Mallo GV, Mizyrycki C, Giono L, Gonzalez-Ros JM, et al. (2001) Human p8 is a HMG-I/Y-like protein with DNA binding activity enhanced by phosphorylation. J Biol Chem 276(4): 2742-2751.

65. Salazar M, Carracedo A, Salanueva ÍJ, Tiedra SH, Lorente M, et al. (2009) Cannabinoid action induces autophagy-mediated cell death through stimulation of ER stress in human glioma cells. J Clin Invest 119(5): 1359-1372.

66. Zou Z, Tao T, Li H, Zhu X (2020) mTOR signaling pathway and mTOR inhibitors in cancer: progress and challenges. Cell Biosci 10: 31.

67. Carracedo A, Gironella M, Lorente M, Garcia S, Guzmán M, et al. (2006) Cannabinoids induce apoptosis of pancreatic tumor cells *via* endoplasmic reticulum stress-related genes. Cancer Res 66(13): 6748-6755.

68. Vara D, Salazar M, Olea Herrero N, Guzmán M, Velasco G, et al. (2011) Anti-tumoral action of cannabinoids on hepatocellular carcinoma: role of AMPK-dependent activation of autophagy. Cell Death Differ 18(7): 1099-1111.

69. Ellert Miklaszewska A, Kaminska B, Konarska L (2005) Cannabinoids down-regulate PI3K/Akt and Erk signalling pathways and activate proapoptotic function of Bad protein. Cell Signal 17(1): 25-37.

70. Dando I, Donadelli M, Costanzo C, Pozza ED, Alessandro AD, et al. (2013) Cannabinoids inhibit energetic metabolism and induce AMPK-dependent autophagy in pancreatic cancer cells. Cell Death Dis 4(6).

71. Caffarel M, Bueno GM, Cerutti C, Palacios J, Guzman M, et al. (2008) JunD is involved in the antiproliferative effect of Δ 9-tetrahydrocannabinol on human breast cancer cells. Oncogene 27(37): 5033-5044.

72. Blázquez C, Carracedo A, Barrado L, Real PJ, Luna FJL, et al. (2006) Cannabinoid receptors as novel targets for the treatment of melanoma. FASEB J 20(14): 2633-2635.

73. Caffarel MM, Sarrió D, Palacios J, Guzmán M, Sánchez C (2006) Δ9-tetrahydrocannabinol inhibits cell cycle progression in human breast cancer cells through Cdc2 regulation. Cancer Res 66(13): 6615-6621.

74. Preet A, Ganju R, Groopman J (2008) Δ 9-Tetrahydrocannabinol inhibits epithelial growth factor-induced lung cancer cell migration *in vitro* as well as its growth and metastasis *in vivo*. Oncogene 27(3): 339-346.

75. McAllister SD, Soroceanu L, Desprez (2015) The antitumor activity of plant derived non psychoactive cannabinoids. J Neuroimmune Pharm 10(2): 255-267.

76. Shrivastava A, Kuzontkoski PM, Groopman JE, Prasad A (2011) Cannabidiol induces programmed cell death in breast cancer cells by coordinating the cross-talk between apoptosis and autophagy. Mol Cancer Ther 10(7): 1161-1172.

77. Solinas M, Massi P, Cinquina V, Valenti M, Bolognini D, et al. (2013) Cannabidiol, a non-psychoactive cannabinoid compound, inhibits proliferation and invasion in U87-MG and T98G glioma cells through a multitarget effect. Plos One.

78. Yang MH, Wu KJ (2008) TWIST activation by hypoxia inducible factor-1 (HIF-1): implications in metastasis and development. Cell Cycle 7(14): 2090-2096.

79. Caffarel MM, Andradas C, Mira E, Gómez EP, Cerutti C, et al. (2010) Cannabinoids reduce ErbB2-driven breast cancer progression through Akt inhibition. Mol Cancer 9: 196.

80. Blazquez C, González Feria L, Alvarez L, Haro A, Casanova ML, et al. (2004) Cannabinoids inhibit the vascular endothelial growth factor pathway in gliomas. Cancer Res 64(16): 5617-5623.

81. Casanova ML, Blázquez C, Palacio JM, Villanueva C, Fernández Aceñero MJ, et al. (2003) Inhibition of skin tumor growth and angiogenesis *in vivo* by activation of cannabinoid receptors. J Clin Invest 111(1): 43-50.

82. Portella G, Laezza C, Laccetti P, Petrocellis LD, Marzo VD, et al. (2003) Inhibitory effects of cannabinoid CB1 receptor stimulation on tumor growth and metastatic spreading: actions on signals involved in angiogenesis and metastasis. FASEB J 17(12): 1771-1773.

83. Khoo ML, Freeman JL, Witterick IJ, Irish JC, Rotstein LE, et al. (2002) Underexpression of p27/Kip in thyroid papillary microcarcinomas with gross metastatic disease. Arch Otolaryngol 128(3): 253-257.

84. Qamri Z, Preet A, Nasser MW, Bass CE, Leone G, et al. (2009) Synthetic cannabinoid receptor agonists inhibit tumor growth and metastasis of breast cancer. Mol Cancer Ther 8(11): 3117-3129.

85. McAllister SD, Murase R, Christian RT, Lau D, Zielinski AJ, et al. (2011) Pathways mediating the effects of cannabidiol on the reduction of breast cancer cell proliferation, invasion, and metastasis. Breast Cancer Res Treat 129(1): 37-47.

86. Ramer R, Bublitz K, Freimuth N, Merkord J, Rohde H, et al. (2012) Cannabidiol inhibits lung cancer cell invasion and metastasis *via* intercellular adhesion molecule-1. FASEB J 26(4): 1535-1548.

87. Kis B, Ifrim FC, Buda V, Avram S, Pavel IZ, et al. (2019) Cannabidiol-from plant to human body: A promising bioactive molecule with multi-target effects in cancer. Int J Mol Sci 20(23): 5905.

88. Grotenhermen F (2003) Pharmacokinetics and pharmacodynamics of cannabinoids. Clin Pharmacokinet 42(4): 327-360.

89. Sharma P, Murthy P, Bharath MS (2012) Chemistry, metabolism, and toxicology of cannabis: clinical implications. Iran J Psychiatry 7(4): 149-156.

90. Perucca E, Bialer M (2020) Critical aspects affecting cannabidiol oral bioavailability and metabolic elimination, and related clinical implications. CNS Drugs 34: 795-800.

91. Millar SA, Stone NL, Yates AS, Sullivan SE (2018) A systematic review on the pharmacokinetics of cannabidiol in humans. Front Pharmacol 9: 1365.

92. Huestis MA (2007) Human cannabinoid pharmacokinetics. Chemistry & Biodiversit 4(8): 1770-1804.

93. Taylor L, Gidal B, Blakey G, Tayo B, Morrison G (2018) A phase I, randomized, double-blind, placebo-controlled, single ascending dose, multiple dose, and food effect trial of the safety, tolerability and pharmacokinetics of highly purified cannabidiol in healthy subjects. CNS Drugs 32(11): 1053-1067.

94. Huestis MA (2007) Human cannabinoid pharmacokinetics. Chem Biodivers 4(8): 1770-1804.

95. Lucas CJ, Galettis P, Schneider J (2018) The pharmacokinetics and the pharmacodynamics of cannabinoids. Brit J Clin Pharmaco 84(11): 2477-2482.

96. Watanabe K, Yamaori S, Funahashi T, Kimura T, Yamamoto I (2007) Cytochrome P450 enzymes involved in the

metabolism of tetrahydrocannabinols and cannabinol by human hepatic microsomes. Life Sci 80(15): 1415-1419.

97. Turner AR, Spurling BC, Agrawal S (2020) Marijuana toxicity. Stat Pearls: StatPearls Publishing LLC.

98. Adams IB, Martin BR (1996) Cannabis: pharmacology and toxicology in animals and humans. Addiction 91(11): 1585-1614.

99. Pierre JM, Gandal M, Son M (2016) Cannabis-induced psychosis associated with high potency wax dabs. Schizophr Res 172(1-3): 211-212.

100. Huestis MA, Solimini R, Pichini S, Pacifici R, Carlier J, et al. (2019) Cannabidiol adverse effects and toxicity. Current Neuropharmacol 17(10): 974-989.