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Phytochemical Aspects and Therapeutic Perspective of Cannabinoids in Cancer Treatment

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Additional information is available at the end of the chapter

Abstract

Cannabinoids comprise the plant-derived compounds and their synthetic derivatives as well as endogenously produced lipophilic mediators. Phytocannabinoids are terpenophenolic secondary metabolites predominantly produced in *Cannabis sativa* L. The principal active constituent is delta-9-tetrahydrocannabinol (THC), which binds to endocannabinoid receptors to exert its pharmacological activity, including psychoactive effect. The other important molecule of current interest is non-psychotropic cannabidiol (CBD). Since 1970s, phytocannabinoids have been known for their palliative effects on some cancer-associated symptoms such as nausea and vomiting reduction, appetite stimulation and pain relief. More recently, these molecules have gained special attention for their role in cancer cell proliferation and death. A large body of evidence suggests that cannabinoids affect multiple signalling pathways involved in the development of cancer, displaying an anti-proliferative, proapoptotic, anti-angiogenic and anti-metastatic activity on a wide range of cell lines and animal models of cancer. These findings have led to the development of clinical studies to investigate potential anti-cancer activity in humans, but reliable clinical evidence for this therapeutic option is still missing.

Keywords: cannabinoids, phytochemistry, THC, CBD, cancer

1. Introduction

Cannabis sativa L. (Cannabaceae) is one of the first plants cultivated by man and one of the oldest plant sources of fibre, food and remedies. It has a long history of medical use in the Middle East and Asia, dating back to the sixth century BC. During a period of colonial expansion in the early nineteenth century, cannabis found a way to Western Europe as a medicine to alleviate a variety of conditions, such as pain, spasms, dysentery, depression, sleep disturbance and loss of

appetite. In the beginning of the twentieth century, due to the availability of substitute drugs, absence of quality control and the risk of abuse and intoxication, cannabis medication fell into disuse. Moreover, following the UN Single Convention on Narcotic Drugs in 1961, cannabis and its products were classified as narcotics. Phytochemical analysis of cannabis in the 1940s and 1960s led to the discovery of a unique group of terpenophenolic secondary metabolites, known as cannabinoids, of which trans-(−)-delta-9-tetrahydrocannabinol (THC) was shown to be the primary active constituent which is responsible for the plant's psychoactive effect [1–3]. Many natural products besides cannabinoids have been isolated from cannabis, including terpenes, flavonoids, steroids and nitrogenous compounds. Up to date, 750 constituents have been identified from cannabis, out of which over 100 are classified as cannabinoids [4, 5]. Research of the cannabis medical properties has gained worldwide interest after the discovery of two types of cannabinoid receptors, which are G-protein coupled receptors specifically responding to endocannabinoids and phytocannabinoids, and related synthetic cannabimimetic compounds. Therefore, the term cannabinoids now includes not only the plant-derived compounds (phytocannabinoids), but also in laboratory synthesised derivatives (synthetic cannabinoids) and a family of endogenously produced compounds (endocannabinoids) [6]. The therapeutic properties of cannabis have been much debated from scientific and regulatory points of view over the years. The medical use of cannabis is still controversial and strongly limited by unavoidable psychotropic effects. However, solid scientific data indicated the potential of therapeutic value of cannabis in controlling some forms of pain, relieving chemotherapy-induced nausea and vomiting, treating cachexia and anorexia in AIDS patients and combating muscle spasms in multiple sclerosis with no evidence that giving cannabis to the patients would increase illicit drug use in the general population [7]. Nowadays, many countries legalised cannabis for medical purposes. To avoid abuse, numerous centres for cannabis therapy are founded worldwide and usually organised as clinics where cannabis can be prescribed in various forms, including dried plant material and cannabis extract. So far, only three cannabis-based medicines have been registered for certain indications. In the context of cancer, dronabinol (synthetically generated THC) and nabilone (a synthetic THC analogue) can be prescribed to prevent chemotherapy-induced nausea and vomiting. Nabiximols, plant extract enriched in THC and cannabidiol (CBD) at an approximate 1:1 ratio, are approved for the treatment of cancer-associated pain [8]. Apart from these palliative effects, recent preclinical studies suggest that various cannabinoids exert antitumour effects in different experimental cancer models [1]. In this chapter, we will focus on phytochemistry and pharmacology of cannabinoids as well as their current and potential roles in symptom management and cancer therapy.

2. The cannabis plant

The concept of Cannabis as a monotypic genus containing just a single highly polymorphic species is widely accepted, although there has been a long-standing debate among taxonomists regarding classification of the existing varieties. Other previously described species, including C. indica Lam. and C. ruderalis Janisch., are now recognised as varieties of C. sativa L. based on morphological, anatomical, phytochemical and genetic studies [9, 10]. C. sativa L. is an annual, herbaceous, taprooted and predominantly dioecious plant. Its height (0.2–6 m) and degree of branching depend on both genetic and environmental factors. Staminate (male) plants are usually

taller but less robust than pistillate (female) plants. The leaves are petiolate, palmately compound, with an odd number (3–13) of coarsely serrate, lanceolate leaflets. The male inflorescence is a lax panicle or compound cyme composed of many individual, yellowish green, pedicellate flowers containing five pendulous anthers. The pistillate flowers are green, sometimes purple to red, sessile, grouped in apical leaf axils or terminals of branches. They form short, congested pseudo-spikes among leaf-like bracts and bracteoles. Each flower has a small green bract enclosing the ovary with two long, slender pistils projecting well above the bract. The male plants commence flowering slightly before the females. When mature, the sepals on the male flowers are open to enable passing air currents to transfer the released pollens to the pistillate flowers. Soon after pollination, the male plants wither and die in order to secure more space, nutrients and water to the females so that they could produce a healthy crop of viable seeds. Following fertilisation, the ovary develops into an achene, a fruit containing a single seed with a hard shell [11–13]. The surface of aerial plant parts is covered in trichomes. These are either covering (non-glandular) trichomes or glandular trichomes containing a resin (**Figure 1**). Non-glandular trichomes are numerous, unicellular, rigid and curved hairs, with a slender pointed apex. Cystolithic trichomes found on the upper surface of the cannabis leaves are swollen at the base and have calcium carbonate crystals (cystoliths), while slender non-cystolithic trichomes occur mainly on the lower side of the leaves, bracts and bracteoles. Three morphologically distinct types of glandular trichomes have been identified: (1) a long multi-cellular stalk and a multi-cellular head with approximately eight radiating club-shaped cells (capitate-stalked); (2) sessile with a multi-cellular head (capitate-sessile); (3) a short unicellular stalk and a bi-cellular, rarely four-cell, head (bulbous). These are mainly associated with the female inflorescences, but they can also be found on the underside of the leaves and occasionally on the stems of young plants. Bulbous and capitate-sessile trichomes occur on all parts of vegetative and flowering shoots. In contrast, capitate-stalked trichomes are restricted to flowering regions. The glandular trichomes are secretory structures, where the cannabinoid-laden resin is produced and stored. Besides cannabinoids, these trichomes produce terpenes, which are responsible for the typical plant aroma. The extreme variations in cannabinoid contents of the different tissues are due to markedly different distributions of glandular trichomes on the surface of the plant [14, 15]. The unfertilised flower heads and flower bracts of the female plant are the primary source of cannabinoids (**Figure 1**).
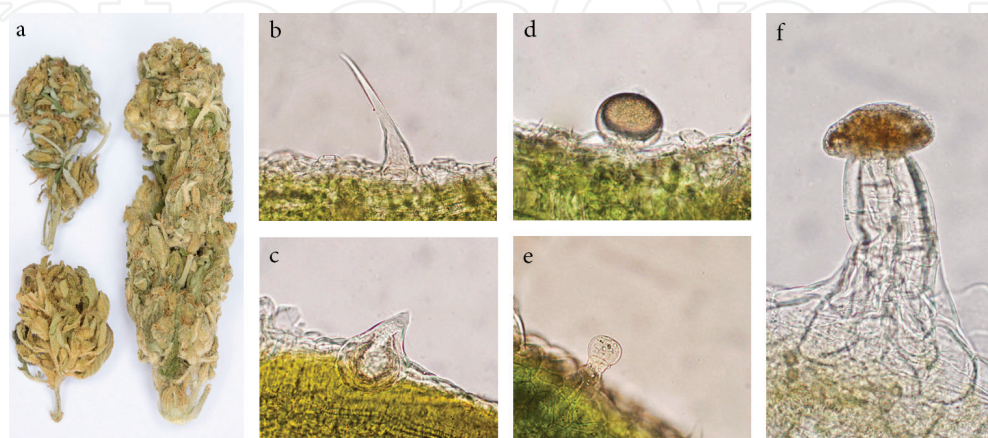

Figure 1. *Cannabis sativa* L. – dried pistillate inflorescences and trichomes on their surface. (a) dried pistillate inflorescences (50% of the size); (b) non-cystolithic trichome; (c) cystolithic trichome; (d) capitate-sessile trichome; (e) simple bulbous trichome; (f) capitate-stalked trichome (400×).

3. Biosynthesis and structure of phytocannabinoids

Phytocannabinoids represent a group of terpenophenolic compounds predominantly produced in the cannabis plant. These secondary metabolites are biosynthesised as prenylated aromatic carboxylic acids, and while almost no neutral forms can be found in fresh plants. However, cannabinoid acids may convert to their neutral homologues by spontaneous decarboxylation under the influence of light, heat or prolonged storage. The precursors of phytocannabinoids originate from two distinct biosynthetic pathways: the polyketide pathway, giving rise to olivetolic acid (OA) or divarinic acid (DA), and methylerythritol phosphate pathway, leading to the synthesis of geranyl pyrophosphate (GPP). The biogenesis of phytocannabinoids containing n-pentyl side chain starts with the condensation of OA and GPP into cannabigerolic acid (CBGA), catalysed by geranyl pyrophosphate—olivetolate geranyl transferase (GOT). The isoprenylation step is next followed by activity of three corresponding oxidative cyclases that generate tetrahydro-cannabinolic acid (THCA), cannabidiolic acid (CBDA) and cannabichromenic acid (CBCA) from CBGA as the key intermediate. The phytocannabinoid acids are non-enzymatically decarboxyl-ated into cannabigerol (CBG), delta-9-tetrahydrocannabinol (delta-9-THC), cannabidiol (CBD) and cannabichromene (CBC) [16, 17]. **Figure 2** shows the cannabinoid biosynthetic pathway and the structures of the major constituents. The biosynthesis of phytocannabinoids with C3 side-chain (propyl cannabinoids) from DA probably follows a similar pathway yielding can-nabigerovarinic acid [18].

Over 100 various phytocannabinoids have been found so far, but many of them are pro-duced in trace quantities or represent auto-oxidation artefacts [16, 19]. The structural diver-sity of naturally occurring cannabinoids is the result of differences in the nature of their isoprenyl residue, resorcinyl core and side chain. Based on the structural variation, Hanuš and coworkers [4] have classified phytocannabinoids as follows: cannabigerol, cannabi-chromene, cannabidiol, tetrahydrocannabinol, cannabinol, thymyl, cannabielsoin, canna-bicyclol and 8,9-secomenthyl types. The *Cannabigerol type* compounds are one of the most structurally diversified classes of phytocannabinoids. A linear isoprenyl residue is their main feature, as exemplified by CBG, which was the first structurally elucidated and also the first natural cannabinoid to be synthesised. The isoprenyl residue of CBG is non-oxy-genated, indicating its early biogenetic stage within phytocannabinoids. Other components of this type are propyl side-chain analogues (cannabigerovarin) and monomethyl ether derivative. The isoprenyl residue is oxidatively fused to the resorcinyl ring in the *canna-bichromene type*. Cannabichromene (CBC) is the simplest natural cannabinoid to obtain by synthesis and the only major phytocannabinoid that shows a bluish fluorescence under UV light. CBD, as the main representative of the *cannabidiol type* compounds, was isolated in 1940, but the correct structure elucidation was reported more than two decades later. CBD and its corresponding acid are the most abundant cannabinoids in the fibre-type of can-nabis (non-psychotropic). Ten CBD type phytocannabinoids with C1–C5 side-chains have been described. The *tetrahydrocannabinol type* compounds contain several bis-reduced forms of cannabinol (CBN), differing in location of the remaining double bond, the configuration of the chiral centres, or both isomeric options. The most prominent constituent of this sub-class is delta-9-THC, the main psychoactive ingredient of cannabis plant, isolated in 1942, but structurally elucidated only in 1964. Other representative of this type is delta-8-THC,
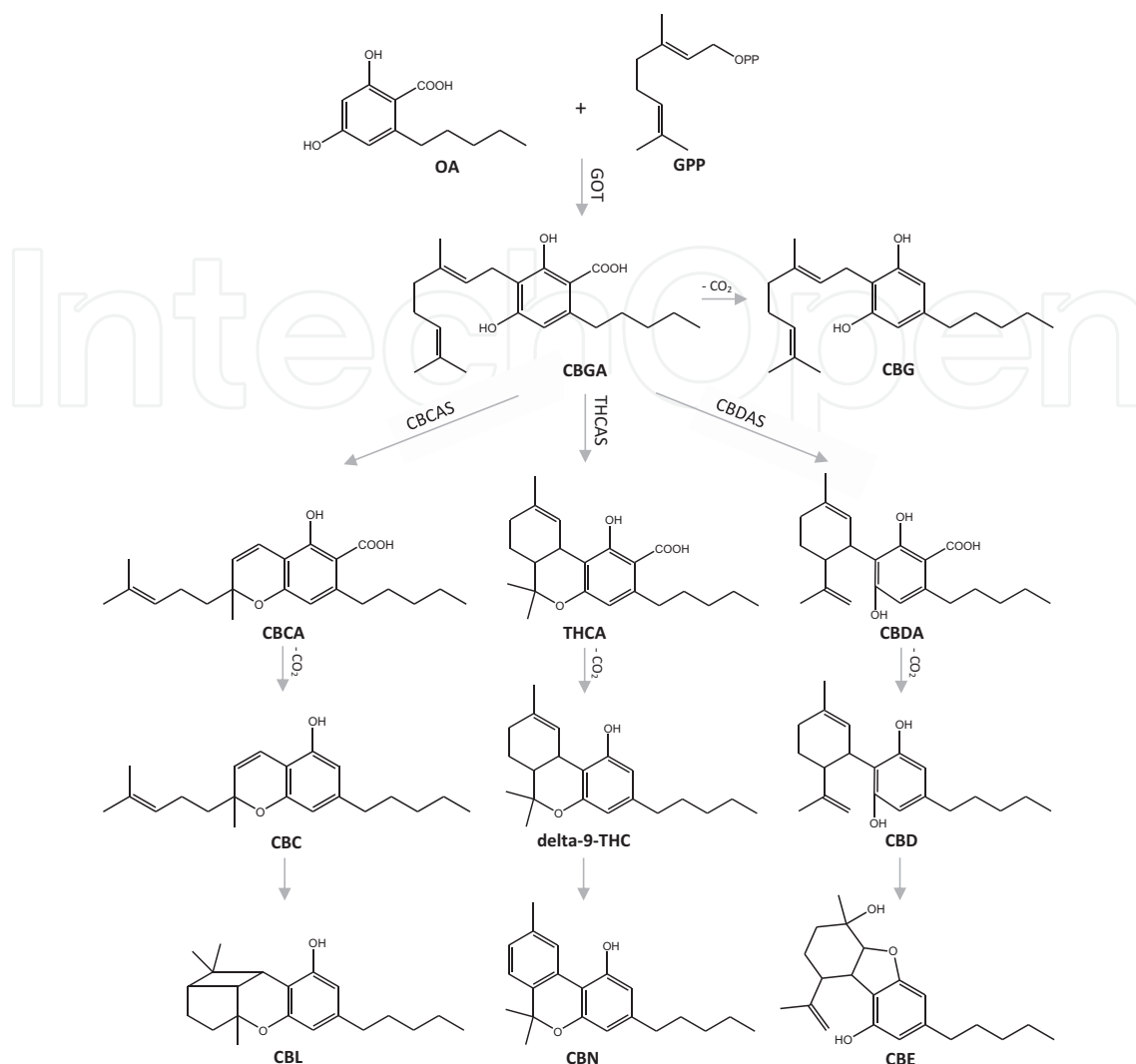
Figure 2. Biosynthesis and degradation of the major phytocannabinoids. OA—olivetolic acid; GPP—geranyl pyrophosphate; GOT—geranyl pyrophosphate—olivetolate geranyl transferase; CBGA—cannabigerolic acid; CBG—cannabigerol; CBCAS—cannabichromenic acid synthase; THCAS—tetrahydrocannabinolic acid synthase; CBDAS—cannabidiolic acid synthase; CBCA—cannabichromenic acid; THCA—tetrahydrocannabinolic acid; CBDA—cannabidiolic acid; CBC—cannabicromene; delta-9-THC—delta-9-tetrahydrocannabinol; CBD—cannabidiol; CBL—cannabicyclol; CBN—cannabinol; CBE—cannabielsoin.

most likely to be generated from delta-9-THC or CBD. It is easier to synthesise and more thermodynamically stable than delta-9-THC. CBN and its derivatives and analogues (*cannabinol type*) are considered artefacts derived from oxidative aromatisation of the corresponding THC type compounds. Their concentration in cannabis products depends on age and storage condition. CBN is highly stable towards oxidative degradation and so has been used as a marker for the identification of narcotic cannabis in archaeological findings. The structural hallmark of *thymyl type* represented by cannabinodiol and cannabifuran is the presence of thymyl group obtained by aromatisation of the menthyl moiety of CBD. The *Cannabielsoin type* compounds are the result of the intra-molecular opening of cannabidiol-type epoxides and could be isolated artefacts. Cannabielsoin (CBE) is the major pyrolytic product of CBD and therefore expected to be present in cannabis smoke. Other artefacts formed during storage of the plant material in the presence of light are *cannabicyclol* (CBL)

and its derivatives, characterised by a five-atom ring and C1 bridge instead of a typical six-membered ring in the cannabinoid structure. *8,9-Secomenthyl cannabidiols* are formed by splitting of the endocyclic double bonds of delta-9-THC (cannabicoumaronone) and CBD (cannabimovone) [4, 19, 20].

4. Phytochemical characterisation of cannabinoids

Various scientific attempts have been made to classify *Cannabis* taxa based on their cannabinoid composition, which is under strong environmental influences and also depends on plant sex and maturity. The most important classification of cannabis types in forensics and legislation is that into drug type (marijuana) and fibre type (hemp). A high amount of psychoactive THC characterises the drug type, while particularly low content defines the fibre type [21, 22]. Nowadays, cannabis is divided mainly into three chemotypes (i.e. chemical phenotypes) on the basis of the content ratio of the two major cannabinoids, THC and CBD, in dried inflorescence: (1) THC > 0.3% and CBD < 0.5% (THC predominant); (2) THC ≥ 0.3% and CBD > 0.5% (intermediate); (3) THC < 0.3% and CBD > 0.5% (CBD predominant). Two rare chemotypes with prevalence of CBG and cannabinoid-free, respectively, have also been found [23, 24]. Apart from these chemotypes, de Meijer [25] has additionally described CBC, delta-9-tetrahydrocannabivarin (THCV) and other propyl cannabinoid-rich chemotypes. A large variation of cannabis strains have been developed during a long period of breeding and selection. Over 700 different cultivars of cannabis have been catalogued and many more varieties are thought to exist [26]. With the increasing use of cannabis for medical purposes, the need for a clear chemotaxonomic distinction between varieties has become even more important. Phytocannabinoids were chosen as chemotype markers as they are considered to be the main pharmacologically active constituents in cannabis [27].

Because of the complex chemistry of cannabis, advanced separation techniques, such as gas chromatography (GC) or high performance liquid chromatography (HPLC), often coupled with mass spectrometry detection (MS), are necessary for the determination of the typical phytochemical profiles of cannabis constituents [28, 29]. Thin layer chromatography (TLC) is suitable only for identification of cannabis plant material, detection of its principal cannabinoids and distinguishing between main chemotypes. The separation of phytocannabinoids is mainly achieved by using silica gel as stationary phase, reversed phase for the non-polar system and normal phase for the polar system. Two different reagents for the visualisation of cannabinoids, fast blue and vanillin-sulphuric acid, can be used [11, 30, 31]. **Figure 3** shows high performance thin layer chromatography (HPTLC) chromatogram of cannabis ethanolic extracts, representing THC and CBD predominant types, respectively.

Gas chromatography, commonly coupled to flame ionisation detection (FID) or MS, provides data only on neutral cannabinoids. Due to the high temperature of the injection port, the rapid decarboxylation of the acidic cannabinoids to the neutral forms occurs, thus the real cannabinoid profile of the plant material does not correspond to the results obtained. Derivatisation of phytocannabinoid acids to their trimethylsilyl esters before injection is one approach that can allow the separation and detection of the acidic and neutral forms. Identification of the phytocannabinoids is most readily performed by GC-MS, method of choice for creating cannabis

profiles and metabolic fingerprints [12, 28, 32]. GC-FID is suitable for routine identification and quantification of the major phytocannabinoids as illustrated in **Figure 4**, representing THC and CBD predominant types, respectively.
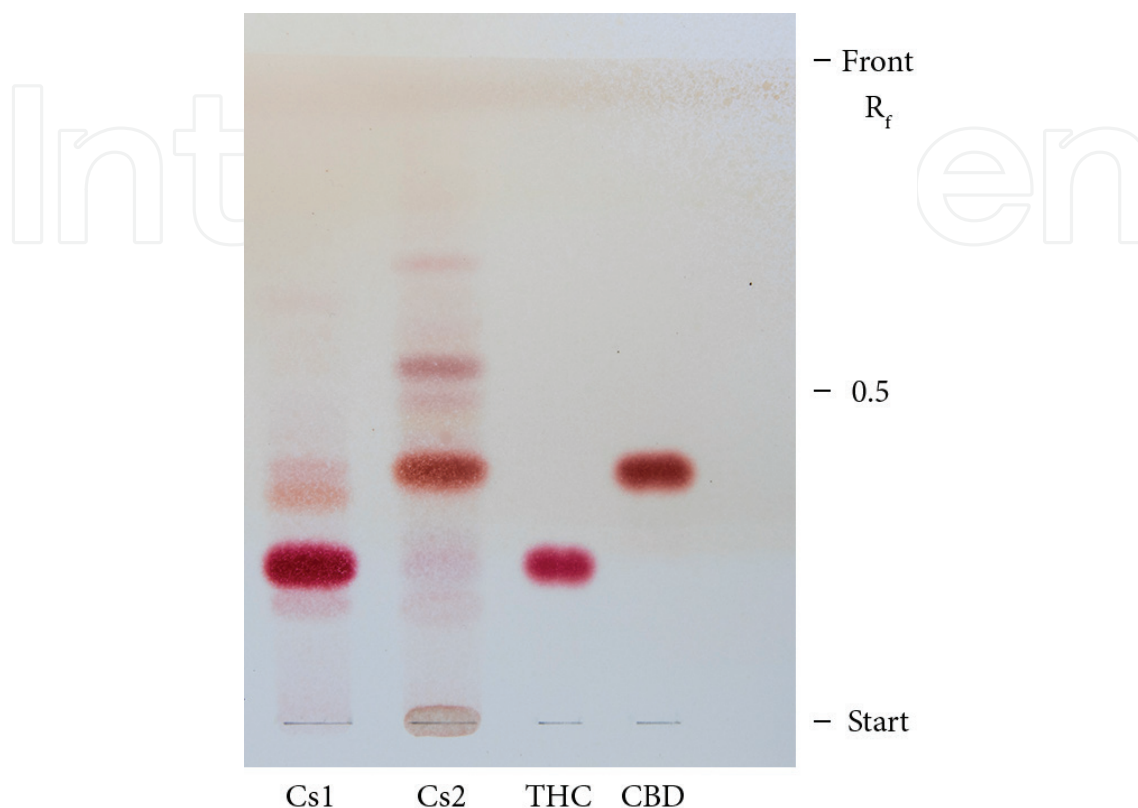

Figure 3. HPTLC chromatogram of phytocannabinoids in the concentrated ethanolic extracts of cannabis inflorescence. Cs1—THC predominant type of *Cannabis sativa* extract; Cs2—CBD predominant type of *Cannabis sativa* extract; stationary phase: HPTLC silica gel C_{18} F_{254}; mobile phase: methanol-water with 0.1% glacial acetic acid 75:25 (V/V); detection: Fast blue reagent; R_f (THC) = 0.25; R_f (CBD) = 0.38.
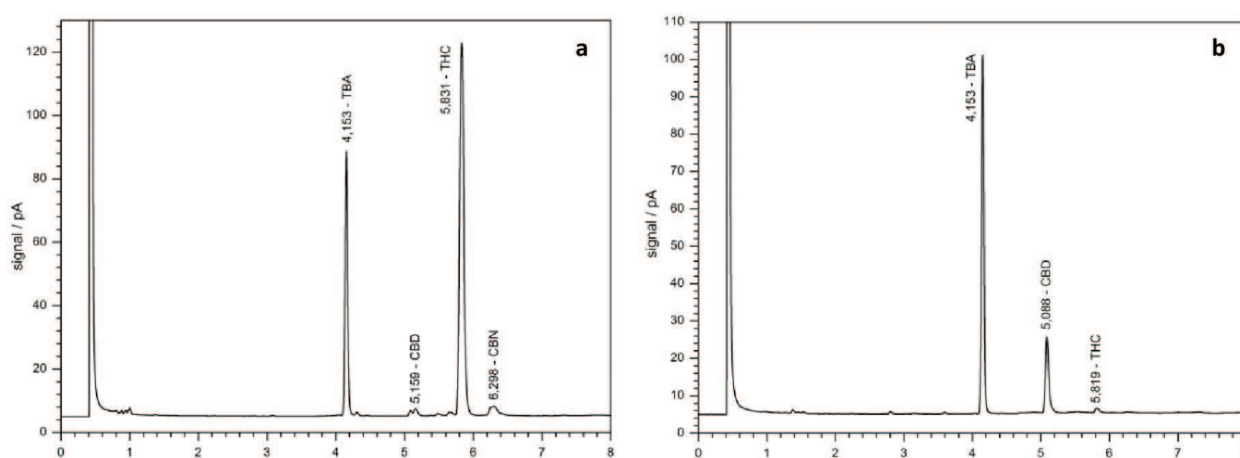

Figure 4. GC-FID chromatograms of two concentrated ethanolic extracts of cannabis inflorescence. (a) THC predominant type of cannabis extract (THC/CBD = 87;2). (b) CBD predominant type of cannabis extract (THC/CBD = 0.08). Agilent 7890A gas chromatograph equipped with FID; HP-5MS column (15 m x 0.25 mm i.d., 0.25 μm film thickness); carrier gas: helium at a constant flow rate of 2.0 mL/minute; temperature program: initial temperature 200°C for 2 minutes, increased by 10°C/minute to final temperature 240°C and held for further 2 minutes; detector temperature 300°C; injector temperature 280°C with split ratio of 20:1; injection volume 1.5 μL; i.s. – tribenzylamine (TBA).

Both acidic and neutral forms of phytocannabinoids can be directly analysed by means of HPLC without any derivatisation step. In contrast to GC, no decomposition occurs during HPLC analysis, which is the main advantage for obtaining the complete cannabinoid profiles. Analytical methods based on reversed-phase chromatography with gradient elution are commonly used. Detection of phytocannabinoids is usually performed by UV and diode array detectors (DAD), but high sensitivity can best be achieved through the use of thermospray MS. Apart from several HPLC methods, ultra performance liquid chromatography (UPLC) method has also been validated for the analysis of a wide range of phytocannabinoids in plant material [13, 29]. Moreover, a novel method of ultra-high performance supercritical fluid chromatography (UHPSFC) coupled with DAD/MS for the separation and discrimination of cannabinoids in complex matrices has been developed and validated [33]. Giese et al. [5] highlighted that typical concentration ranges for the cannabinoids vary from 0.1 to 40% of inflorescence dry weight. These data show how extreme the variations of phytocannabinoids between plant specimens can get, indicating that the cannabis for medical use should always be thoroughly profiled. Therefore, the previously mentioned analyses are of interest given the probability that both the therapeutic and adverse effects of cannabis may be dictated by the concentrations and interactions of certain phytocannabinoids.

5. The endocannabinoid system

The endocannabinoid system (ECS) consists of endogenous cannabinoids, their receptors and the enzymes responsible for their biosynthesis, transport and degradation. The endocannabinoids are lipophilic mediators, which include amides, esters and ethers of long-chain polyunsaturated fatty acids, mostly arachidonic acid. The first two identified and most studied endocannabinoids are N-arachidonylethanolamide called anandamide (AEA) and 2-arachidonoylglycerol (2-AG) (**Figure 5**). AEA and 2-AG are not pre-synthesised and stored in vesicles like classical neurotransmitters, but rather released from the cells immediately after biosynthesis. They are synthesised via enzymatic pathways from phospholipid precursors in the plasma membrane of post-synaptic cells on demand upon relevant physiological or pathological stimuli. After release, acting as retrograde messengers, AEA and 2-AG travel backwards to stimulate receptors on the pre-synaptic membrane. The main intermediate in the synthesis of AEA is *N*-acyl-phosphatidylethanolamine (NArPE), transformed into anandamide by several possible pathways among which the most investigated is the direct conversion catalysed by an enzyme of phospholipase D family. 2-AG is produced primarily by the hydrolysis of diacylglycerols (DAGs) via DAG lipases α and β. The endocannabinoids act on their receptors only locally, possibly because of their high lipophilicity, and are immediately inactivated under physiological conditions. The suggested mechanisms of endocannabinoid transport across the plasma membrane (facilitated transport, passive diffusion and/or endocytosis) are still not fully elucidated. After their cellular re-uptake, AEA is rapidly degraded by the enzyme fatty acid amide hydrolase (FAAH) while 2-AG is hydrolysed by monoacylglycerol lipase (MAGL) forming arachidonate and ethanolamine or glycerol, respectively [34, 35].
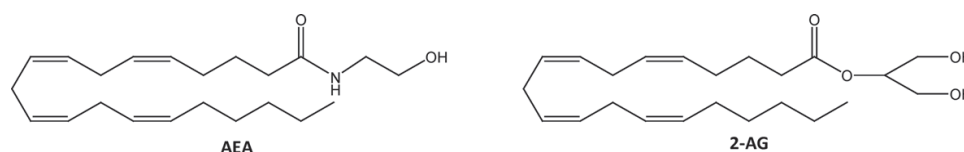
Figure 5. The structures of main endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG).

Apart from hydrolytic degradation, endocannabinoids may also be oxidised by cyclooxygenase-2, lipoxygenases and cytochrome P450 [36].

The cannabinoids exert their effects by binding to specific receptors, among which the most important are cannabinoid receptors CB1 and CB2 encoded by different genes and exhibiting 44% homology in their primary structure. They belong to the large rhodopsin family of G-protein-coupled receptors (GPCRs) with seven transmembrane domains connected by three extracellular and three intra-cellular loops, an extracellular N-terminal tail and an intra-cellular C-terminal tail. There is increasing evidence supporting the existence of additional targets for cannabinoids like transient receptor potential (TRP) ligand-gated cation channels (vanilloid type 1, TRPV1, melastatin type 8, TRPM8 and ankyrin type 1, TRPA1), certain orphan GPCRs (GPR55, GPR119 and GPR18), 5-hydroxytryptamine receptor subtype 1A ($5-HT_{1A}$) and peroxisome proliferator-activated receptors (PPARs). The functions of cannabinoid receptors can be modulated by endo-, phyto- or synthetic-cannabinoids which target the orthosteric or allosteric binding sites on the receptors. The cannabinoid receptors modulate adenylyl cyclase (AC) activity depending on its isoform expressed in the cells and, consequently, alter the cellular production of second messenger cyclic adenosine monophosphate (cAMP). The activation of CB1 and CB2 receptors mainly causes inhibition of AC and the subsequent reduction of intra-cellular cAMP levels leads to the inactivation of the protein kinase A (PKA) phosphorylation pathway. Studies have shown that cannabinoid receptors can also be coupled to other types of intra-cellular signals, such as the protein kinase B, phosphoinositide 3-kinase and phospholipase C pathway. Also, activation of CB1 and CB2 receptors leads to the downstream activation of mitogen-activated protein kinase (MAPK), p44/42, p38 and c-JUN amino terminal kinase as signalling pathways to regulate nuclear transcription factors. Unlike the activation of CB2 receptor, which generally has no effect on ion channels, CB1 receptors inhibit calcium channels and activate potassium channels. The cannabinoid receptors are widely distributed in the human body. CB1 receptors are localised predominantly in the CNS and mainly expressed in areas that are involved in the control of motor coordination and movement, memory, learning, emotions, sensory perception and autonomic and endocrine functions. In addition, CB1 receptors are present to a lesser extent in some organs and peripheral tissues, including endocrine glands, leukocytes, adipocytes, spleen, liver, heart and part of the reproductive, urinary and gastrointestinal systems. By contrast, the CB2 receptor was initially described as present in the immune system, but more recently it has also been shown to be expressed in additional cell types [37–40]. Since elevated expression of CB1 and CB2 receptors and higher levels of endocannabinoids have been found in many types of cancer, compared to normal tissues, the ECS has been recognised as attractive potential target for cancer therapy. The growing evidence over the past decade suggests that cannabinoids affect multiple signalling pathways involved in the development

of cancer, displaying an anti-proliferative, proapoptotic, anti-angiogenic and anti-metastatic activity on a wide range of cell lines and animal models of cancer [41].

6. Preclinical evidence on cannabinoids as anti-cancer agents

Despite remarkable advances in understanding and treating cancer, finding new, more effective pharmacotherapeutics still remains a key challenge for scientists worldwide. The first study suggesting that plant-derived cannabinoids might be potential anti-cancer agents, demonstrating their ability to inhibit tumour growth *in vitro* and *in vivo* and to increase the survival of lung cancer-bearing animals, was published more than 40 years ago [42]. Later discoveries of the ECS in the human body, combined with the development of numerous preclinical testing models, have paved the way for a renaissance in the study of anti-cancer properties of cannabinoids in the last two decades. A large body of *in vitro* data has been accumulated demonstrating that cannabinoids affect a wide spectrum of tumour cells, including gliomas, neuroblastomas, lymphomas, hepatocarcinoma as well as thyroid, skin, prostate, pancreatic, breast, cervical, colon, gastric, lung and some other cancers [6, 41, 43]. Several plant-derived (THC and CBD), synthetic (e.g. JWH-133, WIN-55,212-2 and KM-233) and endogenous cannabinoids (AEA and 2-AG) were found to be potent inhibitors of both cancer growth and spreading due to their ability of modulating various cell-signalling pathways [6, 37, 43, 44]. Their anti-neoplastic action mainly relies on the activation of cannabinoid CB1 and/or CB2 receptors, although some other non-CB1/CB2 receptors, like TRPV1 and PPARs, as well as mechanisms unrelated to receptor stimulation may also be involved [43, 45, 46]. Cannabinoids might stop the uncontrolled growth of cancer cells by several different mechanisms, including inhibition of cell-cycle progression, inhibition of cell proliferation as well as induction of autophagy and apoptosis [41, 43, 44]. Due to their modulatory actions on various cell cycle regulatory molecules, like cyclin A and cyclin dependent kinase (CDK) 2, cannabinoids have been shown to cause arrest of cell cycle progression in different phases (e.g. G0/G1, G2/M), leading to growth inhibition and/or apoptotic death of cancer cells [43]. The anti-proliferative activity is based on their ability to inhibit proliferative and oncogenic pathways in cancer cells, such as adenylyl cyclase and cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) pathway leading to the activation of mitogen-activated protein kinase (MAPK) pathway as well as cell cycle blockade with induction of the CDK inhibitor (CDKI) p27Kip1 and p21waf, decrease in epidermal growth factor (EGF) receptor (EGFR) expression and/or attenuation of EGFR tyrosine kinase activity, decrease in the activity and/or expression of nerve growth factor (NGF), prolactin, or vascular endothelial growth factor (VEGF) tyrosine kinase receptors. The MAPK signalling cascades, consisting of the extracellular signal-regulated kinase (ERK1/2), c-Jun N-terminal kinase (JNK) and p38 MAPK, as well as phosphatidylinositol 3 kinase (PI3K)-Akt pathways seems to have a prominent role in the control of tumour cell fate by cannabinoids [43, 45]. Cancer cell death-inducing activity of cannabinoids relies greatly on the apoptosis and, among several molecular mechanisms, the stimulation of endoplasmic reticulum (ER) stress and subsequent autophagy has been recently suggested as the most common one. Cannabinoids can induce accumulation of *de novo*–synthesised ceramide and thereby activate an ER stress-related response through up-regulation of the

stress-regulated protein p8 and several of its downstream targets, like activating transcription factor 4 (ATF4), C/EBP homologous protein (CHOP) and pseudokinase tribbles-homologue 3 (TRIB3), leading to the inhibition of the AKT–mammalian target of rapamycin complex 1 (mTORC1) signalling, and autophagy-mediated apoptosis. Cannabinoid-evoked and ER stress-dependent activation of calcium/calmodulin-dependent protein kinase β (CaCMKKβ) and AMP-activated protein kinase (AMPK) lead, together with the p8/TRIB3 pathway, to autophagy and apoptosis [1, 46]. Tumour angiogenesis represents additional important target for cancer therapy affected by cannabinoids. They can directly inhibit vascular endothelial cell migration and survival or act indirectly by modulating the expression of pro-angiogenic factors, like VEGF, matrix metalloproteinase-2 (MMP-2) or anti-angiogenic factors like tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) as well as their receptors in tumours [41, 44]. Besides influencing the growth of different cancer cells, cannabinoids may exert their anti-cancer effects by inhibiting all the steps of tumour progression. The inhibitory effect on migration, adhesion and invasion through CB receptors is related to the blocking of key pathways such as EGF-EGFR, RhoA-RhoA kinase (ROCK), focal adhesion kinase (FAK)-Src and of MMPs and TIMP-1, which are fundamental for the invasiveness and spread of tumours [41, 43, 44]. Non-CB receptors mediated anti-metastasic effects may rely on the down-regulation of the helix-loop-helix (bHLH) transcription factor inhibitor of DNA binding 1 (ID1) [46]. **Tables 1** and **2** summarise preclinical evidence collected during the last decade about the role of two most-investigated phytocannabinoids, THC and CBD, in different type of cancers and their associated cell signalling pathways.

Recent *in vivo* studies demonstrated that cannabinoids of plant, synthetic and endogenous origin are able to decrease tumour growth and metastasis of different experimental cancers [47]. Preclinical assessments have mainly been conducted using human tumour engraft models, where human cancer cells were subcutaneously injected (ectopic model) or transplanted into the same origin site of the tumour (orthotropic model) in immunodeficient mice. The syngeneic (allograft) models, established by transplantation of mice cancer cells in immunocompetence animals, as well as carcinogen-induced spontaneous tumour models and genetically engineered mouse models (GEMM) have also been used, but rarely [47, 48]. An overview of last decades' discoveries revealed the effectiveness of THC against experimental glioma, liver, pancreatic, breast and lung cancers (**Table 1**) while CBD was found to be effective against glioma and neuroblastoma, melanoma, colon, breast, prostate and lung cancers (**Table 2**). Among other phytocannabinoids, CBG could be considered as a candidate for colon cancer prevention and treatment [49]. Beside these findings, the potential clinical interest of cannabinoids is additionally strongly suggested by their selectivity for tumour cells (and even ability to protect the non-transformed cells) as well as by their good tolerance in animal studies and the absence of normal tissue toxicities that are still the major limitations of most conventional chemotherapeutics [45]. However, several studies reported that THC and some other cannabinoids can inhibit apoptosis in the transformed-cell lines, exhibit proangiogenic effect and stimulate cancer cell proliferation or show a biphasic effect in cancer cells by increasing their proliferation at lower concentrations and decreasing at higher concentrations [37, 41]. The ability to promote the tumours growth was found in two experimental animal model cancers and attributed to their suppression of anti-tumour immune response [37]. Despite the few mentioned conflicting data, the majority of recent preclinical studies provide

the supporting evidence on cannabinoids as promising anti-cancer agents, thus encouraging further clinical investigations.

Considering the possibilities for therapeutic use of cannabinoids in cancer, their combination with traditional chemotherapy or radiotherapy seems to be an interesting option. The possible advantages of combination therapy may be a synergistic effect evident as improved efficiency, lowered doses and consequently attenuated toxic side effect or reduced drug resistance. Accordingly, γ-irradiation was found to enhance CBD-induced apoptotic death in cultured leukaemia cells [50]. Synergism of plant-derived cannabinoids and radiation was confirmed *in vivo*, where the simultaneous treatment with THC and CBD enhanced the cancer-killing effects of the radiation in murine glioma model [51]. Preclinical evidence also supports the combination of phytocannabinoids and chemotherapy drug temozolomide (TMZ), commonly used in patients with glioblastoma. Torres et al. [52] proved that co-administration of TMZ with THC reduces the growth of glioma xenograft to a much higher extent than the treatment with the individual agents, observing effect in the TMZ-resistant tumours also. Interestingly, combined treatment with TMZ and submaximal doses of THC and CBD (approximate 1:1 ratio) produced similar anti-tumoural effect in both TMZ-sensitive and TMZ-resistant tumours. Usage of main cannabis constituents together may be therapeutically very attractive, since CBD has the ability to potentiate anti-cancer properties of THC and, as a non-psychotropic cannabinoid, can mitigate adverse psychoactive effects of THC that limit its clinical use [46, 52]. Recent data also revealed that CBD-enriched cannabis extract can significantly enhance the efficacy of bicalutamide or docetaxel, two standard drugs used in the treatment of prostate cancer, and taken together even prolong the survival of treated animals [53]. Overall, recent findings provide promising evidence on the benefits of cannabinoid-based combinational therapy in cancer, and suggest novel therapeutic opportunities that need to be clinically proven in future.

Cancer type	Experimental model	Findings [reference]
Brain (Glioma)	*in vitro* U251MG, U87MG	Inhibited cell cycle progression (G0/1 arrest) by down-regulation of E2F transcription factor 1 and cyclin A [54]
	C6.9, U87MG	Inhibition of migration by inhibition of TIMP-1 expression via ceramide and stress protein p8 [55]
	C6.9, U87MG	Inhibition of invasion by down-regulating MMP-2 via ceramide and p8 [56]
	U87MG	Induced autophagy-mediated cell death through ER stress–evoked stimulation of ceramide synthesis *de novo*, eIF2α phosphorylation and up-regulation of p8/TRIB3 pathway leading to inhibition of Akt/mTORC1 pathway; autophagy leads to apoptosis [57]
	in vivo C6.9 xenograft	Decreased tumour growth and tumoural TIMP-1 expression [55]
	U87MG xenograft	Decreased tumour grow and tumoural MMP-2 expression [56]
		Decreased tumour growth and activated autophagic mediated cell death pathway (↑TRIB3, ↑LC3-II, ↑caspase 3, ↓rpS6) [57]

Cancer type	Experimental model	Findings [reference]
Lung	*in vitro* A549, SW-1573 *in vivo* A549 xenograft	Inhibited proliferation, migration and invasion of tumour cells by inhibition of EGFR-mediated activation of MAPKs (ERK1/2, JNK1/2) [58]
	LL2 allograft	Reduced tumour growth and metastasis through inhibition of proliferation (↓Ki67), vascularisation (↓CD31) and decreased phosphorylation of FAK, ERK1/2 and Akt [58]
		No significant effect on tumour growth [59]
Liver	*in vitro* HepG2, HuH-7 HepG2	Induced cancer cell death through autophagy stimulation via CB2 receptors by (i) inhibition of the Akt/mTORC1 axis via ER stress with TRIB3 up-regulation and (ii) stimulation of AMPK via CaMKKβ; autophagy leads to apoptosis [60]
	in vivo HepG2 xenograft HuH-7 xenograft	Anti-proliferative action modulated by up-regulation of PPARγ-dependent pathways through TRIB3 [61]
	HepG2 orthotopic	Reduced tumour growth relies on decreased mTORC1 activation, enhanced AMPK phosphorylation and increased autophagy and apoptosis [60]
	HepG2 xenograft	Decreased hepatomegaly and ascites, ↓α-fetoprotein, in tumour ↑pAMPK, ↓pAkt, ↓pS6, ↓procaspase-3 [60]
		Reduced tumour growth via PPARγ activation [61]
Pancreas	*in vitro* MiaPaCa2, Panc1 *in vivo* MiaPaCa2 xenograft	Induced cancer cell death by apoptosis via activation of the p8-ATF-4-TRIB3 pathway (↑caspase-3, ↑ceramide) [62]
		Reduced tumour growth [62]
Breast	*in vitro* EVSA-T, HMEC *in vivo* MMTV-neu	Reduced cancer cell proliferation through apoptosis and cell cycle blockade (G2-M arrest) by CDK1 down-regulation [63]
	N202.1 xenograft	Reduced tumour growth, tumour number and metastases by cell proliferation inhibition (↓Ki67), apoptosis (↑caspase 3), decreased angiogenesis and ↓MMP2 [64]
		Decreased tumour growth via Akt inhibition [64]
Skin	*in vitro* CHL-1, A375, SK-MEL-28 *in vivo* CHL-1 xenograft	Induced cancer cell death by activating non-canonical autophagy-mediated apoptosis dependent on Atg7 but not Beclin-1 or Ambra1 [65]
		Inhibited tumour growth via autophagy and apoptosis (↓Ki67, ↑TUNEL, ↑LC3) [65]
	HCmel12 xenograft	Reduced tumour growth in CB receptor-dependent manner and decreased inflammatory immune cell infiltrates in the tumour microenvironment [66]

Table 1. Effects of THC on different types of cancer.

Abbreviations are listed in **Table 2**.

Cancer type	Experimental model	Findings [reference]
Brain	*In vitro* U87 *in vivo* U87 xenograft U251 orthotopic xenograft 3832, 387 orthotopic xenograft SK-N-SH xenograft	Induced apoptosis of cancer cells through caspase activation ($\uparrow$caspase-8, -9 and -3) and oxidative stress ($\uparrow$ROS, $\downarrow$GSH, $\uparrow$GPx, $\uparrow$GRed) [67]
		Reduced tumour growth through inhibition of 5-LOX ($\downarrow$LTB4) and ECS—activation of FAAH ($\downarrow$AEA) [68]
		Reduced tumour progression and cancer cell invasion through down-regulation of Id-1 expression [69]
		Initial inhibition of tumour growth ($\downarrow$Ki67, $\downarrow$pAkt, ($\uparrow$caspase-3) followed by tumour resistance [70]
		Suppressed neuroblastoma tumour growth via apoptosis induction ($\uparrow$caspase-3) [71]
Lung	*In vitro* A549, H460 *in vivo* A549 xenograft	Anti-invasive and anti-metastatic action via up-regulation of ICAM-1 which leads to enhanced cancer cell adhesion to LAK cells and subsequent enhance of LAK cell-mediated cancer cell lysis [72]
		Decreased tumour growth and inhibited tumour cell invasion via down-regulation of PAI-1 [73]
		Decreased tumour metastasis [74]
		Inhibited cancer cell invasion and metastasis by stimulation of TIMP-1 via up-regulation of ICAM-1 [75]
		Decreased tumour growth via apoptosis caused by up-regulation of COX-2 and PPAR-γ [76]
Colon	*In vivo* Azoxymethane-induced cancer	Reduced preneoplastic lesions, number of polyps and tumours through apoptosis by inhibition of the PI3K-Akt pathway ($\downarrow$pAkt, $\uparrow$caspase 3) [77]
Prostate	*In vitro* LNCaP, DU-145 LNCaP *in vivo* LNCaP xenograft	Induced cell death through apoptotic pathways ($\uparrow$caspase 3, $\uparrow$PUMA, $\uparrow$CHOP, $\uparrow$intra-cellular Ca^{2+}, down-regulation of AR, p53 activation, $\uparrow$ROS) [53]
		Induced phosphatase-dependent apoptosis in cancer cells via CB1/CB2 [78]
		Decreased tumour growth [53]

Cancer type	Experimental model	Findings [reference]
Breast	*In vitro* MCF-7, KiMol, C6, MDA-MB-231	Inhibited cancer cell proliferation through proapoptotic effect and by cell cycle blockade at G1/S phase, acting directly via CB2 and TRPV1 receptors and indirectly via elevation of intra-cellular Ca^{2+} and ROS [79]
	MDA-MB-231, 4T1	Anti-proliferative and anti-invasive effect by up-regulation of ERK and ROS pathways leading to down-regulation of Id-1 protein expression [80]
	MDA-MB-231 SUM159, 4T1.2	Induced cancer cell death by both apoptosis (↑PARP) and autophagy (↑LC3-II) through induction of ER stress and inhibition of Akt/mTOR/4EBP1 signalling independently of receptor activation; important role of ROS and Beclin-1 [81] Inhibited tumour cell proliferation, migration and invasion through EGF/EGFR pathway inhibiting EGF-induced activation of EGFR, ERK, Akt and NF-kB signalling and actin stress fibre formation and focal adhesion formation; Anti-metastatic effect also by decreasing secretion of MMP-2 and MMP-9 as well as chemokines CCL3, GM-CSF, MIP-2 [82]
	In vivo MDA-MB xenograft 4T1 orthotopic 4T1 allograft 4T1.2 orthotopic MVT-1 orthotopic	Decreased tumour growth and lung metastasis [79]
		Reduced tumour growth and metastasis. Anti-metastatic effect by down-regulation of tumoural Id1 expression [80, 83]
		Inhibited tumour growth and lung metastasis due to anti-proliferative (↓Ki67) and angiogenic (↓CD31) effects and inhibition of EGFR, Akt and ERK activation [82]

AEA—anandamide; Akt—serine/threonine protein kinase; AMPK—adenosine monophosphate-activated protein kinase; AR—androgen receptor; ATF-4—activating transcription factor 4; Atg7—autophagy-related protein 7; CaMKKβ—calcium/calmodulin-dependent protein kinase β; CCL3—chemokine (C-C motif) ligand 3; CD31—cluster of differentiation 31, syn. platelet endothelial cell adhesion molecule (PECAM-1); CDK1—Cyclin-dependent kinase 1; CHOP—transcription factor CAAT/enhancer binding homologous protein; COX-2—cyclooxygenase-2; 4EBP1—eukaryotic translation initiation factor 4E binding protein 1; ECS—endocannabinoid system; EGF—epidermal growth factor; EGFR—epidermal growth factor receptor; eIF2α—α subunit of eukaryotic initiation factor 2; ER—endoplasmic reticulum; ERK—extracellular signal-regulated kinase; FAAH—fatty acid amide hydrolase; FAK—focal adhesion kinase; GM-CSF—granulocyte-macrophage colony-stimulating factor; GPx—glutathione peroxidase; GRed—glutathione reductase; GSH—glutathione; ICAM-1—intercellular adhesion molecule 1; Id-1—helix-loop-helix protein inhibitor of DNA binding-1; JNK1/2—c-Jun N-terminal protein kinases 1 and 2; Ki67—biomarker of cancer cells proliferation LAK cells - lymphokine-activated killer cells; LC3—microtubule-associated protein 1 light chain 3; 5-LOX—arachidonate 5-lipoxygenase; LTB4—leukotriene B4; MAPK—mitogen-activated protein kinase; MIP-2—macrophage inflammatory protein 2; MMP—matrix metalloproteinase; mTOR—mechanistic target of rapamycin; mTORC1—mammalian target of rapamycin complex 1; NF-kB—nuclear factor-kappa B; p53—tumour protein 53; p8—stress-regulated protein; PAI-1—plasminogen activator inhibitor-1; pAkt—phosphorylated Akt; pAMPK—phosphorylated adenosine monophosphate-activated protein kinase; PARP—poly (ADP-ribose) polymerase; PI3K—phosphoinositide 3-kinase; PPARγ—peroxisome proliferator-activated receptor γ; pS6—phosphorylated-ribosomal protein S6; PUMA—p53 up-regulated modulator or apoptosis; ROS—reactive oxygen species; rpS6—ribosomal protein S6; TIMP-1—tissue inhibitor of matrix metalloproteinase 1; TRIB3-—tribbles pseudokinase 3; TUNEL—terminal deoxynucleotidyl transferase dUTP nick end labelling.

Table 2. Effects of CBD on different types of cancer.

7. Clinical studies of cannabinoids in cancer care

7.1. Clinical anti-cancer studies

The promising preclinical data have encouraged the development of clinical studies aimed at investigating the potential therapeutic value of cannabinoids as anti-cancer agents. The only clinical study published up to date was a pilot phase I trial in which nine patients with recurrent glioblastoma multiforme (GBM) that have previously failed standard therapy underwent intracranial THC administration. The study showed that THC delivery was safe without evident psychoactive effects and that THC neither facilitates tumour growth nor decreases patients' survival. Additionally, THC inhibited tumour-cell proliferation and induced apoptosis in samples obtained from two patients before and after treatment. However, evaluation of patients' survival requires a larger study with a different design and preferably oral or oromucosal application [46, 84]. According to the register of clinical trials [85], there are several on-going clinical trials evaluating anti-cancer activity of cannabinoids. Two phase I/II clinical studies in recurrent GBM patients are being conducted to assess the safety and effectiveness of the administration of an oromucosal spray containing cannabis extract (2.7 mg THC and 2.5 mg CBD in 100 µL) in combination with dose-intense TMZ (NCT01812603 and NCT01812616). These studies have passed their completion date, but the status has not yet been verified. Evaluation of pure CBD as a single-agent for solid tumour (NCT02255292) started in 2014 as a phase II clinical trial and still did not reveal any results. Dexanabinol, a synthetic cannabinoid, is currently undergoing phase I trial for the treatment of advanced solid tumours (NCT01489826). This non-psychotropic cannabinoid was applied in different doses with the intention to determine the maximum safe dose, to understand interactions between the body and the drug and to measure any reduction in size of patients' tumour. Data on tumour response and the number of adverse events have not yet been reported.

7.2. Studies on chemotherapy-induced nausea and vomiting

In contrast to rare clinical anti-cancer studies, clinical trials evaluating efficacy of cannabinoids in cancer symptom management have a long history. The 1970s and 1980s mark a period of intensive clinical trials dealing with chemotherapy-induced nausea and vomiting (CINV), but the interest in these investigations is not decreasing due to the influence of CINV on patients' life quality and compliance with future treatment [86, 87]. Modern anti-emetic treatment includes corticosteroids, serotonin receptor antagonists ($5\text{-}HT_3$) and neurokinin (NK_1) receptor antagonists, while cannabinoids (dronabinol and nabilone) are prescribed to the patients who have failed to respond to conventional anti-emetic therapy [88, 89]. Majority of clinical studies have compared efficacy of cannabinoids to dopamine receptor antagonist and neuroleptics [87], yet some recent studies have been focusing on newer generation agents such as $5\text{-}HT_3$ and NK_1 receptor antagonists. Meiri and coworkers [90] have design randomised, double-blind, placebo-controlled, parallel group, five-day study for evaluating dronabinol alone and in combination with ondansetron, a $5\text{-}HT_3$ receptor antagonist. They recruited 61 patients with delayed CINV, which is defined as nausea and vomiting occurring more than 24 hours after chemotherapy and lasting up to one week. Obtained results indicated that dronabinol or ondansetron was similarly effective and well

tolerated, but combination of these two drugs was not more effective than either drug alone. Duran and coworkers [91] conducted a pilot, double-blind, parallel, placebo-controlled phase II clinical trial with standardised oromucosal cannabis extract containing a mixture of THC and CBD (2.7 mg THC and 2.5 mg CBD per spray) in patients with CINV. To be recruited in the study, patients had to have moderately emetogenic cancer therapy caused CINV lasting more than 24 hours despite standard anti-emetic therapy. During five days patients were allowed to add up to eight sprays per day along with their standard therapy. Combination of cannabis extract with standard anti-emetic therapy was well tolerated and provided better protection against delayed CINV. The benefits of cannabinoids in CINV are undoubtedly confirmed in numerous clinical studies, but there is lack of studies dealing with cannabis plant [92]. First scientific article about use of smoked cannabis reported it as a rescue drug in case of vomiting episodes [93]. In 2001, Musty and Rossi [94] published the review about effects of smoked cannabis and oral THC based on previously unpublished USA clinical trials with cannabis and/or THC. The investigation included 748 patients who smoked cannabis prior to and/or after cancer chemotherapy and 345 patients who used the oral THC capsules. Patients who smoked cannabis experienced 70–100% relief from nausea and vomiting, while those on THC capsules reported 76–88% relief. Although it is clear that cannabinoids can serve as anti-emetic agents in cancer therapy, clinical studies on their effectiveness on nausea and vomiting in advanced cancer and metastasis are needed since there are case-reports in which cannabinoids showed potential therapeutic use for these indications [95].

7.3. Studies on cancer-related pain

In the last decades, available clinical data on benefits of cannabinoids in chronic pain were scarce; however, currently there are many clinical studies, which include various cannabinoid preparations and test different chronic pain conditions [96]. Animal studies in a variety of nociceptive assays have confirmed that activation of CB1 receptors by exogenously applied agonists can reduce pain sensitivity, while activation of CB2 receptors may promote analgesia without psychoactive side effects usual for CB1 agonist [97]. Patients who are suffering from chronic cancer-related pain usually are put on high doses of opiates, which alter their state of consciousness. It has been reported that cancer patients down-sized opioid dose after adding cannabis in their pain regimen and when selecting cannabis extract, THC-rich cannabis extract was the first choice, though many patients experienced pain relief after using CBD-rich type [92]. In multi-centre, double-blind, randomised, placebo-controlled, parallel-group, two-week study, THC:CBD extract and THC extract were evaluated in patients with intractable cancer-related pain. Study included 177 patients with inadequate analgesia despite opioid dosing. During first week patients self-titrated dose up to maximum of 48 actuations (each 100 µL containing 2.7 mg THC and 2.5 mg CBD or just 2.7 mg THC) per day and remained on that dose till the end of the study. The mean number of THC/CBD sprays was 9.26 and of THC 8.47 per day. Analysis of change from baseline in Numeral Rating Scale score was significantly in favour of THC/CBD extract, while THC extract showed non-significant change. There was no change in dose of opioid background medication as well [98]. A long-term, open-label, follow-up study investigated the long-term tolerability of THC/CBD and THC oromucosal spray in 43 patients with terminal cancer-related pain refractory to opioid who had participated in previously

mentioned trial. Patients self-administered the medication to their optimal dose, again with limitation to a maximum of 48 sprays per day. The duration of treatment with THC/CBD spray (39 patients) was from minimum of 2 and maximum of 579 days (median 25 days) while treatment with THC spray lasted from 4 up to 657 days (median 151.5 days). THC/CBD spray was found to be well tolerated in long-term use, and patients did not ask for higher dose of spray or other pain-relieving medication. Long-term use of cannabinoids did not result with loss of relieving effect on cancer pain [99]. Another randomised, placebo-controlled, graded-dose trial evaluating THC/CBD extract was conducted among opioid-treated patients with poorly-controlled chronic pain who received placebo, low (1–4 sprays/day), medium (6–10 sprays/day) or high dose (11–16 sprays/day) of 2.8 mg THC/2.5 mg CBD extract. During period of five weeks average pain, worst pain and sleep disruption were measured among 360 patients, of which 263 completed the study. Low and medium dose group of patients showed greater analgesia than placebo group and could be assumed as effective and safe, while in high-dose group dose medication was not well-tolerated and had no analgesic effect [100]. Another type of pain that usually occurs in cancer patients is chemotherapy-induced peripheral neuropathy caused by neurotoxicity of drugs such as platinum compounds, vinca alkaloids, taxols and suramin. Although chemotherapy is limited to a short period of use and to a specific tissue, there is no adequate medications for prophylaxis of this type of neuropathy and therapy is restricted to symptomatic treatment of paraesthesia and pain. Ion channel blockers and tricyclic anti-depressants are first choice for treating neuropathy symptoms [101]. Being resistant to conventional treatments, neuropathy lowers life quality in affected patients and limits dosing and duration of chemotherapy, which is crucial for extending their life. Preclinical studies implied that cannabinoid agonists can suppress neuropathy caused by chemotherapeutics, namely vincristine, paclitaxel and cisplatin; moreover, they had better efficacy than conventional treatment. For effectiveness estimation of cannabinoid extract for treating neuropathy, a randomised, placebo-controlled, cross-over pilot study with 18 patients was conducted. Patients were experiencing neuropathic pain, which persisted for three months after chemotherapy with paclitaxel, vincristine or cisplatin, and were treated with maximum of 12 oromucosal sprays (each containing 2.7 mg THC and 2.5 mg CBD) per day. First study period lasted for four weeks with the result of five patients having a decrease of 2.6 on an 11-point numeric rating scale for pain intensity, but in whole group, there was no significant difference between treated and placebo group. Ten patients have entered the extension phase for the next six months (five have completed the study), and confirmed pain reduction by average dose of 4.5 sprays per day. Despite inconsistent results, these findings support studying cannabinoids for chemotherapy-induced neuropathic pain in larger randomised controlled trials [102].

7.4. Cannabis and cancer associated anorexia/cachexia

Many cancer patients experience cachexia, anorexia as well as progressive loss of adipose tissue and skeletal muscle mass. Poor chemotherapy response and decreased survival are often connected with cachexia, a syndrome characterised by systemic inflammation, negative protein and energy balance, and an involuntary loss of body mass [103]. Majority of clinical studies dealing with cachexia and anorexia are focused on AIDS patients and as a result dronabinol

was approved for treatment of anorexia associated with weight loss in patients with AIDS. Still there are some clinical evidences that show that cannabinoids could be beneficial for patients with cancer-associated anorexia/cachexia. One of earliest trials with cancer patients, in 1976, showed that oral THC in doses up to 15 mg per day stimulated appetite and produced significant weight gain [104]. Eighteen cancer patients with anorexia and life expectancy more than 4 weeks underwent a phase II study of THC under regime 2.5 mg tree times per day, one hour after meal. Thirteen patients responded positively to the appetite stimulating effects of THC, but rather surprising was the fact that nausea was common side-effect [105]. In contrast, study conducted in 2006 did not confirm these results. Multi-centre, phase II, randomised, double blind, placebo controlled clinical trial included 164 patients with advanced incurable cancer and involuntary weight loss more than 5%. Patients were divided in placebo, cannabis extract (2.5 mg THC and 1 mg CBD in a capsule) or THC (2.5 mg in a capsule) group, and they were assigned to take capsules twice per day, one hour before meal for six weeks. There were no significant differences between groups considering appetite, quality of life, cannabinoid related toxicity, mood and nausea [106]. It is rather unusual that in this large trial there were no side effects, which suggest that administrated dose of cannabinoids is suboptimal. Moreover, in case of the use of cannabinoids for anorexia and cachexia, European Palliative Care Research Collaborative noticed that dose-regimen of THC used in clinical trials may be the reason for its lack of efficacy. They concluded that for future trials individual dose titration could be more efficient [107, 108]. These theses were confirmed in another randomised, double-blind, placebo-controlled pilot trial in which influence of THC on taste improvement, smell perception, appetite, caloric intake and quality of life was explored. Twenty-one advanced cancer patients, with poor appetite and chemosensory alterations, received THC (2.5 mg, twice per day) and had the option to increase their drug dose to a maximum of 20 mg/day. Though study population was not specifically cachexic, THC-treated patients had improvement in taste, appetite, protein consumption and sleep quality [109].

To summarise, cannabinoids show positive results in various clinical trials considering treatment of nausea, vomiting, pain and anorexia/cachexia while clinical anti-cancer studies are yet to be reported. The perspective of cannabis-based therapy also depends on a paradigm shift from illicit drug to clinically proved medicine. Due to their acceptable safety profile, with side effects that are generally tolerable and reversible [92], clinical trials testing them as single drugs or in combination therapies in various types of cancer are needed, particularly with respect to their effects on tumour growth and patient survival.

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