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Review Article

Pure Cannabidiol versus Cannabidiol-Containing Extracts: Distinctly Different Multi-Target Modulators

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Abstract

Cannabis saliva L. strains can be divided into a number of groups according to their content of the psychotropic phytocannabinoid delta-9-Tetrahydrocannabinol (THC) and of the non-psychotropic Cannabinoid Cannabidiol (CBD). Although the main focus has been on THC in the past, there is growing interest on strains rich in CBD. Strains with a ratio of CBD to THC above one and a content of THC of less than 1%, often legally limited to 0.3%, are commonly designed as hemp (industrial hemp or fiber-type *Cannabis*) in contrast to THC-rich strains (drug-type *Cannabis* and marijuana), and are grown as outdoor cultures in many countries. Such strains contain CBD as the main cannabinoid in addition to numerous other phytosubstances that are in general not further characterized but known to have beneficial effects on health. They are used for the preparation of extracts and other products e.g., essential oils, teas or edibles and promoted as nutraceuticals in hemp shops and on the internet. These products are increasingly popular, and a number of countries allow the cultivation of strains poor in THC. THC and CBD but also many other phytosubstances in hemp in particular terpenes and flavonoids target the so called endocannabinoid system that regulates the homeostasis of vital processes. However, the chemical profile of hemp and derivatives is subject to a wide variability due to a number of factors such as the nature of cultivars, agroclimatic conditions and methods of preparation. Hemp strains and concentrates differ not only in their chemical composition but also in their physiological effects. This heterogeneity has led to conflicting results in clinical studies with *Cannabis* formulations in the past. The physiological effects of purified cannabinoids differ from those observed with extracts. Most products from outdoor cultures cannot be sufficiently standardized, and so are currently unsuitable as medications. They may however play an important role in complementary and integrative medicine. For future clinical studies it is important that only well characterized products are used.

Keywords: Cannabidiol; *Cannabis*; Entourage effect; Flavonoids; Hemp; Terpens

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Introduction

Cannabis sativa L. is a plant with a remarkable large number of varieties. It is estimated that more than 1000 strains may exist that differ in their content of the two main cannabinoids, the psychoactive delta-9-Tetrahydrocannabinol (THC) and the non-psychoactive Cannabidiol (CBD). The genomic analysis of 340 varieties demonstrated the existence of at least three major groups [1]. Another study that included 460 *Cannabis* samples which were chemically profiled for 44 different major cannabinoids and terpenes confirmed the clear differentiation into hemp (fiber-type) and drug type *Cannabis* (*sativa* and *indica*) [2].

Industrial hemp, hemp or fiber-type *Cannabis* is the term used for those varieties of *Cannabis sativa* L. in which the THC content in dried herbal material is below 1% (in most countries below a legal limit of 0.3%) and where CBD predominates (ratio CBD:THC > 1, usually between ~5:1 to ~20:1) in contrast to drug-type *Cannabis* (marijuana, medical *Cannabis*, sometimes distinguishing "*sativa*" versus "*indica*"). In addition, "mixed type" *Cannabis* varieties exist. A number of countries permit cultivation of hemp. In the European Union, for example, varieties with a THC-concentration not exceeding 0.2% ("industrial hemp") are allowed to be grown; some countries also prescribe the maximal THC content permitted in products such as in teas or extracts or allow strains high in THC for medical prescription. Natural products are commonly believed to be effective, free from side effects and chronic toxicity; this is particularly true for *Cannabis* which has a history as folk medicine since at least 5000 years.

Although CBD is a lead substance, hemp contains more than 100 cannabinoids and numerous other phytochemicals known to be pharmacologically active such as flavonoids, terpens and carbohydrates [3]. The total number of phytochemicals is likely in the order of 550 to 600; many components remain as yet unidentified [4]. Contrary to what is widely believed, the plant does not produce cannabinoids such as CBD or THC directly but biosynthesizes their precursors which are the respective acids, i.e., Cannabidiolic Acid (CBDA) and delta-9-Tetrahydrocannabinolic Acid (the term THCA will be used to represent both isomers, THCA-A, THCA-B). Both, CBDA and THCA have Cannabigerolic Acid (CBGA), the next prominent cannabinoid, as precursor. Unsurprisingly, genetic varieties exist that produce CBG but almost no CBD or THC [5]. In nature, acids by far

outweigh the decarboxylated cannabinoids whereby decarboxylation occurs slowly through aging, not enzymatically. Commercially produced preparations are usually decarboxylated by heating. Cannabinoid acids exhibit their own pharmacologic profile, distinct from the decarboxylated form. Apart from cannabinoids, the composition and nature of terpenoids is also specific for each *Cannabis* variety, both qualitatively and quantitatively, and can be used for characterization of biotypes [6,7]. The sum of these phytochemicals makes each strain unique and therefore also the respective derivatives such as extracts and other products.

In some countries *Cannabis* is classified as a schedule I drug ("drug with a high potential of abuse, no currently accepted medical use in treatment and lack of accepted safety", Controlled Substances Act, 1970, US). Such an all encompassing classification may not be relevant to well characterized products with extremely high purity with respect to CBD content. Unfortunately, it has had - and still has - a tremendous and negative impact on scientific research. Perhaps due to such general classifications, CBD has long been a neglected and under researched substance. Early research in humans dates back to 1972 [8]. At that time, the CBD used in clinical studies was very likely not of the same quality as today when it can be produced with a purity exceeding 99% and even 99.5% with virtually no THC as byproduct (botanical drugs, e.g., CBD of BSPG, Sandwich, UK; CBD of GW Pharmaceuticals (Epidiolex™), London, UK or synthetic CBD). The interest in the potential medical utility of CBD increased rapidly few years ago, after several CNN-TV reports in 2013 and 2014 presented the case of a little girl, Charlotte Figi, suffering from treatment-resistant Dravet syndrome. It was reported that her epileptic seizures were reduced from about 40 seizures per day down to two to four per month by administering a *Cannabis* (hemp) extract containing ~ 17% CBD and 0.3 to 0.5% THC. In addition to the highly purified CBD used today in clinical studies and available Over-The-Counter (OTC) from pharmacies and health food stores, numerous hemp (*Cannabis*) extracts containing between about 4% to 20% or more of CBD are commercialized as nutraceuticals and in complementary medicines. For economic reasons, they are generally derived from outdoor cultures. Apart from these extracts which vary widely in their composition, a standardized prescription medicine exists that combines two refined extracts for medical treatment (Nabidiolex™ combined with Tetranabinex™, Sativex™).

In the following review, the physiological targets of pure CBD are summarized as well as mechanisms of other phytochemicals that may play a role as modifiers in a putative "entourage effect". Primacy is given to most recent articles and to overviews on specific subjects, rather than to original papers.

CBD, the Main Phytocompound in Hemp, is a Modulator of a Number of Endogenous Physiologic Mechanisms

CBD is the primary cannabinoid in hemp; targets and physiological effects are interconnected like a network (Figure 1), although mechanisms are very complex and still incompletely understood. A number of excellent recent reviews show that it is a multi-target modulator [9-12]. CBD does not act directly on the cannabinoid receptors CB1 and CB2. In fact, CBD is a negative allosteric modulator and many of the effects on the endocannabinoid system seem to be indirect, through a wide range of different mechanisms that are mediated in part by Endocannabinoids such as Anandamide (AEA) and 2-Arachidonoylglycerol (2-AG) and targets such as Fatty Acid Amid Hydrolase (FAAH), Monoacylglycerollipase (MAGL) or Peroxisome Proliferators Activated Receptor gamma (PPARg) some of which are shared with other phytochemicals [13]. A simplified overview is given below in figure 1.

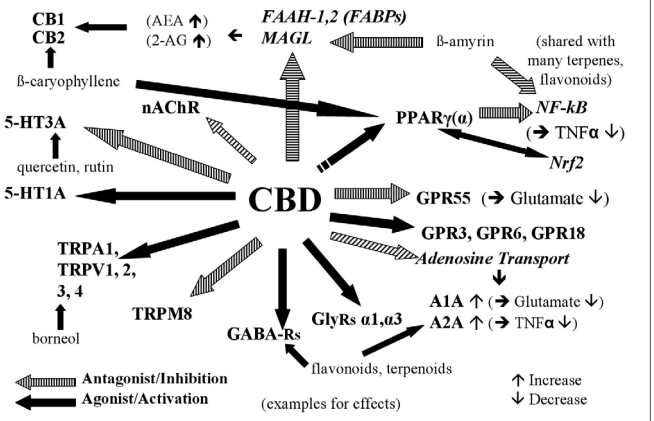

Figure 1: Examples of the interaction of CBD and non-cannabinoids in hemp with the endocannabinoid system (see tables for more details).

A1A, A2A - Adenosine receptor 1A, 2A; AEA - Anandamide; 2-AG - 2-Arachidonoylglycerol; CB1- Cannabinoid receptor 1; CB2- Cannabinoid receptor 2; FAAH - Fatty Acid Amid Hydrolase; FABP - Fatty Acid Binding Protein; GABA Rs - Gamma Aminobutyric Acid Receptors; GlyRs - Glycine Receptors; GPR3, 6, 18 - G-protein-coupled receptor 3, 6, 18; GPR55 -G-Protein-coupled Receptor 55 (orphan receptor); 5-HT - 5-Hydroxytryptamin receptor; MAGL - Monoacylglycerol-Lipase; nAChR - nicotinic Acetylcholine Receptor; NF-kB - Nuclear Factor kappa B; Nrf2 - Nuclear factor erythroid derived 2; PPAR- Peroxisome Proliferator-Activated Receptor (g-gamma, a-alpha); TNFa - Tumor Necrosis Factor alpha; TRP - Transient Receptor Potential [V - Vanilloid; A - Ankyrin repeats; M - Melastatin-type]; effects of the most prominent cannabinoids in hemp extracts are summarized below (Table 1).

A number of other phytochemicals in hemp are able to modulate not only the targets affected by CBD, but demonstrate various other physiological effects. This contributes to the previously mentioned "entourage effect" [35-37]. The two main groups that have been investigated in more details for their pharmacological activities are terpenoids and flavonoids. Of about 20,000 terpenoids known in the plant kingdom, 58 monoterpenes and 38 sesquiterpenes have been identified in hemp, but over 200 have been reported to occur in various *Cannabis* strains [7,37]. Terpenes account for about 0.01% to 3.5% of the dry weight; their evolution generally parallels the evolution of CBDA [6,38]. The nature and amounts of terpenes vary considerably between *Cannabis* strains: of 19 strains tested, the lowest versus the highest amount of myrcene was found in Fedora 19 (29.4%) and Uniko-B (65.8%), of ß-caryophyllene in B3985TE (3.8%) and Fedora 19 (37.5%), and of limonene in Fedora 19 (0.2%) and B3985TE (6.9%) respectively [7,39].

In the majority of *Cannabis* strains, ß-myrcene is the dominant terpene; antinociceptive effects were observed in animal studies after 10 - 20mg i.p./kg [40]. The next prominent is ß-caryophyllene and caryophyllene oxide, which is the substance detected by Hashish security detection dogs.

Cannabinoid	Targets	Effects (Examples)	Ref.
CBD	Agonist of 5-HT1A, TRPA1, TRPV1,2,3,4; PPARg, GPR3,6,18; antagonist of TRPM8; 5-HT3A, GPR55, adenosine transport protein; positive allosteric modulator of GABAA, GlyRs; inhibits n-AChR, NaV channels, LOX-5,-15; moderate inhibitor of FAAH	Anti-inflammatory, analgesic, anxiolytic, antidepressant; attenuates nausea, vomiting, motor and cognitive impairment; inhibits cancer cell growth	[14-24]
CBDA	Agonist of 5HT1A, TRPA1, TRPV1, TRPV4; antagonist of TRPM8; inhibitor of COX-2, NAAA	Anti-inflammatory, anxiolytic, antidepressant; attenuates nausea, vomiting, motor and cognitive impairment; antineoplastic	[16,25,26]
THC	Agonist of CB1, CB2, TRPA1, TRPV2, TRPV3, TRPV4; GPR18, PPARg; potentiates Glycine receptors (GlyRs); antagonist of TRPM8, 5-HT3A	Anti-inflammatory, anxiolytic, pro-apoptotic effects; analgesic (additive with kappa-Opioid-receptor agonists)	[16,27,28]
THCA	Weak binding to CB1, CB2; agonist of PPARg, TRPA1, TRPV2; antagonist of TRPM8; weak inhibitor of FAAH, MAGL, COX-1,-2	Anti-inflammatory, neuroprotective, pro-apoptotic effects	[29-31]
CBG	Agonist of TRPA1, TRPV1, TRPV2, TRPV4, PPARg; alpha2-adrenoceptor, Antagonist of 5-HT1A, TRPM8, CB1; inhibits NaV channels, COX-2	Antiemetic (may oppose effects of CBD), anti-inflammatory, antineoplastic, antidepressant; stimulates appetite, neuroprotective	[19,32-34]

Table 1: Main targets and effects of CBD, CBDA, THC, THCA.

CBD - Cannabidiol; CBDA - Cannabidiolic Acid; THC - delta-9-Tetrahydrocannabinol; THCA - Tetrahydrocannabinolic Acid; COX - Cyclooxygenase 1 or 2; LOX - Lipoxygenase; NAAA - N-Acylethanolamine Acid Amidase; NaV - Voltage gated Na+ (channels)

Particularly ß-caryophyllene and ß-caryophyllene oxide are used as dietary supplements and "Generally Recognized As Safe" (GRAS) by the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA). Terpenes as well as flavonoids, and not cannabinoids give *Cannabis* strains their unique scent/"perfume". As the terpenoid-profiles are strongly inherited, they may be used for taxonomic classification, although they are not clear markers [1,2,39,41,42]. The nature of terpenes differs between flowers and leaves. Buds and flowers contain more of the volatile monoterpenes such as limonene and alpha-pinene to repel herbivorous insects (*Cannabis* is wind-pollinated) whereas leaves are richer in the malodorous, bitter sesquiterpenes that protect the plant from grazing animals [39]. Interestingly, a terpenoid blend (QRD-460) that contains α-terpinene, p-cymene and d-limonene as the active substances, has been approved in the European Community as an insecticide in the cultivation of tomato, melon, cucumber and pepper.

Terpenes occur widely in the human diet and are used in a number of dietary supplements and flavor ingredients as well as in aromatherapy. In addition, (+)- and (-)-alpha-pinene and (+)-3-carene are not only potent inhibitors of Acetylcholine Esterase (AChE) but have also gastroprotective effects; AChE inhibitors are commonly used to slow down the progression of Alzheimer's disease [43,44].

Hemp (*Cannabis*) Contains Also Flavonoids

Flavonoids, a subgroup of polyphenols, are subject to considerable variation between cultivars. Both, terpenoids and flavonoids are common in the human diet and are found throughout the plant kingdom. Out of about 6000 flavonoids known, 26 have been identified in various *Cannabis* strains with apigenin, kaempferol, luteolin, orientin, quercetin and vitexin being the most common. Flavonoids are powerful antioxidants and rank among the largest group of phytonutrients. In plants, they are essential pigments and are found in flowers, leaves and stems giving them the typical color but add also to the smell and flavor of a particular *Cannabis* strain. Flowers of hemp rank among the plants with the highest flavonoid content and antioxidant power; the total content in the *Cannabis* leaves and flowers can reach 2.5% of its dry weight (green tea, for comparison: 0.5% - 1.5%); [45,46]. In an epidemiologic study, the daily intake of 25.9 mg flavonoids (quercetin, kaempferol, myricetin, apigenin and luteolin) was related to a significant decrease of cancer symptoms [47]. A number of similar more recent epidemiologic but also animal studies support these results [48,49]. In addition to potential anticancer activity, flavonoids are reported to have antibacterial, antiviral, anti-inflammatory and hepatoprotective properties and might slow the aging process including of the skin [50-52].

Similar to cannabinoids, cann (a) flavin A and cann (a) flavin B are unique to the genus *Cannabis*. Most flavonoids are soluble in water and readily absorbed [53]. Some are also volatile and found in Essential Oils (EO). Analysis of nine flavonoids of 53 individual *Cannabis* plants from nine countries demonstrated a high variation from plant to plant with no distinct taxa among them. None of the plants tested exhibited all nine compounds. Fiber cultivars contained less flavonoid material than drug type *Cannabis* [54,55].

Selected phytochemicals of hemp, their targets and effects are summarized below (Tables 2 and 3).

As can be seen, many of the terpenoids prevent the activation of the nuclear transcription factor NF-kB, thus suppressing the formation of pro-inflammatory cytokines such as TNF-α, IL-1β or IL-6. Inflammation is a characteristic of many chronic conditions including cancer and Alzheimer's disease. A number of terpenoids inhibit also the formation of pro-inflammatory metabolites notably of leukotrienes and prostaglandins by the inhibition of MAGL or COX-2 which contributes to the overall anti-inflammatory and anti-nociceptive effects. The overlapping properties of many terpenoids may be explained in parts by their chemical relationship.

As shown above, many flavonoids modulate the activation of the nuclear transcription factor Nrf-2 which is a key factor for the regulation of intracellular oxidative processes. Depending on the level, Reactive Oxygen Species (ROS) can have pro- (low intracellular levels) as well as anti-cancer effects (high levels, inducing cell death). The maintenance of the proper balance thus decreases the risk of oxidative DNA damage, genotoxicity and cancer development whereby epigenetic mechanisms play also a role.

Terpenoid	Targets	Effects (Examples)	Ref.
α,β-Amyrin	Activates CB1 (more potent than d9-THC); inhibits hydrolysis of MAGL, ABHD6, -12 and 2-AG; prevents NF-kB activation	Antinociceptive, anti-hyperglycemic, hypolipidemic; anti-inflammatory	[56-59]
Borneol	Activates TRPV3; inhibits NF-kB; positive allosteric modulator of GABAA receptors	Neuroprotective; antibacterial; occurs in hemp in low concentrations	[60,61]
ß-Caryo-phyllene	Selective CB2-agonist; PPARg,-a-agonist; nAChR antagonist	Anti-inflammatory (comparable to dexamethasone), analgesic; antibiotic, antineoplastic; reduces intracellular triglyceride accumulation	[62-67]
α-Humulene (α-caryo-phyllene)	Prevents NF-kB and activator protein 1 (AP-1) activation	Anti-inflammatory (comparable to dexamethasone), anti-nociceptive; antineoplastic; antibacterial, appetite suppressant, insecticidal	[67-69]
D-Limonene +	Prevents activation of NF-kB	Anti-inflammatory; antineoplastic; anxiolytic, insect repellent	[7,66,70-72]
D-Linalool Linalool oxide	Agonist to PPARa	Anticonvulsive, antinociceptive, sedating, local anesthetic effects; reduces plasma triglycerides	[37,65,73,74]
ß-Myrcene +	Prevents activation of NF-kB	Anti-inflammatory, analgesic, sedative, muscle relaxant, blocks hepatic carcinogenesis by aflatoxin	[37,40,66,70,75]
Nerolidol	Prevents activation of NF-kB; modulates GABAA receptors	Antinociceptive; anti-inflammatory, anxiolytic; enhances skin penetration, antimalarial	[76]
α-Pinene +	(+)-α-pinene prevents activation of NF-kB; more potent than (−)-α-Pinene	Anti-inflammatory; chondro-protective; acetylcholinesterase-inhibitor, bronchodilator, antifungal, insect repellent; antibacterial (against MRSA)	[7,37,39,43,66,71, 75,77]
α-Terpineol	Inhibition of COX-2 (superior to aspirin)	Anti-inflammatory, promotes wound healing	[70,78]
Terpinolene (delta-terpinene)[0]	Inhibits AKT-formation in leukemia cells	Antiproliferative, sedative, promotes sleep; antibacterial, antifungal, insect repellent	[79,80]

Table 2: Main targets and effects of selected terpenoids.

+ Present in hemp flower tee (Futura strain); ABHD - Alpha, Beta-Hydrolase; GABA - Gamma Aminobutyric Acid; MRSA - Methicillin-Resistant *Staphylococcus Aureus*; AKT - protein Kinase

Flavonoid	Targets	Effects (Examples)	Ref.
Apigenin +	Agonist of PPARg, Nrf-2; downregulates NF-kB; inhibits COX-1,-2; activation of GABAA receptors	Anxiolytic, anti-inflammatory, lowers formation of amyloid ß (Ab1-40, Ab1-42); nephroprotective; inhibits xanthin oxidase/anti-uricaemic effect, antibacterial, antiviral; genoprotective	[51] [81-87]
Cannflavin A,B	Inhibitor of prostaglandin PGE2	Anti-inflammatory (more effective than aspirin but less than dexamethasone); anti-protozoal-, anti-leishmanial activity	[88-90]
Genistein	Upregulation/agonist of PPARg, Nrf2; downregulates NF-kB; modest inhibitor of FAAH	Reduces hepatic fibrosis, downregulates lipogenesis; nephroprotective, anti-uricaemic effect; lowers amyloid-ß; reactivates methylation-silenced genes in cancer cells; phytoestrogen	[83,84, 86,91-93]
Kaempferol +	Inhibits COX-1, COX-2, LOX; agonist of PPARg, Nrf2; downregulates NF-kB; modest inhibitor of FAAH	Antineoplastic; anti-cholinesterase activity, lowers amyloid-ß formation, plasmatic triglycerides; weight reducing; antidepressant; antibacterial, antiviral, antifungal, antiprotozoal	[83-85,93-96]
Luteolin +	Upregulates PPARg, Nrf-2; downregulates NF-kB	Anti-inflammatory; antineoplastic, increases DNA-repair/rejoining of strand breaks; anti-uricaemic; stimulates mineralization of osteoblasts	[51,81,83-87,91,97]
Myricetin	Downregulates NF-kB	Antineoplastic; potentiates sperm function; antidiabetic	[98-100]
Naringenin (a glycone of naringin)	Agonist of PPARg, PPARa, Nrf2; inhibits NF-kB, COX-2	Inhibits osteoclast formation, decreases fibrosis, hepato- and neuro-protective; crosses the BB barrier; antigenotoxic, decreases cholesterol and metabolic syndrome; inhibits *S. aureus*	[51,65,81,83,84,86,91,100-103]
Orientin +	NF-kB inhibition;	Anti-inflammatory, antineoplastic; antibiotic, enhances repair of radiation damages	[104-106]
Quercetin +	Induces PPARg, Nrf-2, downregulates NF-kB; inhibits 5-LOX and COX-1, COX-2	Pro-apoptotic, antihistaminic; hepato-protective; anti-inflammatory; inhibits amyloid ß; anti-cholinesterase activity; antiviral, antibacterial; reduces blood pressure in hypertensive patients	[51,61,81,84-87,91,96,97,100,107-111]
Rutin	(peripheral) CB1 agonist; downregulates NF-kB; inhibits 5-HT3A, GABAc receptors, COX-2	Antifibrotic; decreases oxidative DNA damages; may reduce seizures and epilepsy-associated cognitive/behavioural symptoms;	[59,61,86,91,100]
Vitexin + (apigenin-8-C-glucoside)	Downregulates NF-kB	Anti-inflammatory, antihyperalgesic, antihypertensive, anticonvulsant; antineoplastic, protects pancreatic ß-cells, cardio- and neuro-protective, enhances memory	[112-115]

Table 3: Targets and effects of selected flavonoids.

+ Present in hemp flower tee (Futura strain); BB barrier - Blood-Brain barrier; miRNA - micro RNA; MAPK - Mitogen-Activated Protein Kinase; iNOS - inducible Nitric Oxide Synthase; *S. aureus* - *Staphylococcus aureus*.

The Composition of Phytocomponents in Outdoor Cultures is Highly Variable

The main difference between pure CBD and CBD-based concentrates (extracts named as "CBD-oil", "hemp-oil" or "*Cannabis* oil") is the relatively small and highly variable percentage of CBD in extracts in relation to the large number of other phytosubstances that are generally neither identified nor further characterized from batch to batch. In fact, it has been repeatedly observed that the declared content of CBD and/or THC in commercial products is often incorrect [116-118].

Extracts and other concentrates such as Essential Oils (EOs) are virtually cocktails of phytochemicals. These "oils" are not true oils like olive oil or hemp seed oil. Particularly their content of polyphenols and terpenoids, both known to be pharmacologically active, contribute to the postulated "entourage effect". The exact chemical composition as well as their interactions remain however essentially unknown. The composition depends not only on the cultivar but also on a number of pre- and post-harvest factors. As some components such as volatile monoterpens and flavonoids may be lost during processing, the chemotypic fingerprints of extracts, EOs and other hemp products differ significantly from that of virgin *Cannabis* flowers [119]. Examples of agro-climatic and growth conditions influencing the content of CBDA (CBD) and THCA (THC) before harvest are given below (Table 4).

Flowers have the highest content of cannabinoids, followed by the upper leaves; i.e., decreasing gradually from the top to the bottom of the plant. In flowers of hemp the content of Cannabinoids particularly of CBD (CBDA) increases during the whole growing period and accumulates in leaves and flowers at the end of the vegetative phase (peak about 10-11 weeks after cultivation). In contrast, the content of THC (THCA) in flowers of drug-type *Cannabis* (marijuana) tends to decrease at the end of the flowering period [4]. High nitrogen soil levels tend to increase CBD and to reduce the THC content of leaves, although the influence of fertilizers and other soil elements is complex. Soil nutrient affect also the production and diversity of volatile terpenoids. Further on, the quality of outdoor-grown *Cannabis* and of the products derived is a factor of considerable variability, often raising concerns as to the nature of the preparations offered for sale. *Cannabis* plants extract heavy metals from the soil and accumulate them, among others, in leaves and buds. In addition, *Cannabis* products can be contaminated with pesticides, moulds or bacteria [123].

Higher contents of THC and CBD are generally found in warmer agroclimatic conditions and particularly in high relative humidities with non-significant differences between male and female flowers [124,125]. Temperature has in general a positive influence on yield, whereas rainfalls have a negative influence on the content of cannabinoids [122]. In a six-year field experiment with eight industrial hemp varieties, a considerable variability of CBD and THC was observed, depending on the changes of agro-climatic conditions from one year to another. For a specific hemp strain, e.g., Futura 77 (Fedora 19), the variability for THC was roughly 7 times higher than for CBD; THC varied between 0.045% and 1.00% (0.0225% - 0.670%) i.e., a factor of 22 to 30, and CBD between 1.01% and 3.26% (0.568% - 2.228%) i.e., a factor of 3 to 4 [122]. Such high variability of THC - even in the same *Cannabis* strain - confirms previous observations [42].

No systematic studies on agroclimatic influences on the content of terpenes and flavonoids in hemp could be found although such influences probably exist. Factors like rainfall, mean temperature, duration of sunshine and soil composition including pH are known to affect growth. Stress factors generally increase the content of flavonoids. Studies performed on other plants reported considerable variations on the composition of essential oils in response to the stage of development and light, with an increase during flowering and a decrease in the fruiting stage. In addition, diurnal variations of the content of ß-caryophyllene (4.0% to 3.1%), a-humulene (4.0% to 2.6%), and of nerolidol (0.4% to 0.7%) have been observed [126,127]; these terpenes also occur in hemp.

Most manufacturers use buds or flowers for extraction but in addition whole plant extracts exist that capture a wider spectrum of phytocompounds. Post-harvest processes such as (sun-) drying, storage, heat treatment, soaking, distillation and extraction with more or less polar or lipophilic solvents alters the composition further. Drying at temperatures below 50°C yielded the highest amount of total phenolics; higher temperatures decrease not only the content of phenolics and volatile terpenes but also of cannabinoid acids such as CBDA and THCA that decarboxylate with an increasing speed above 100°C [53]. During drying a loss of 5% to 10% of monoterpenes may occur; a further, although much slower loss is observed thereafter over time during ambient storage [128]. Most of the commercialized hemp extracts are currently crude concentrates based on Carbondioxide (CO_2) techniques. Although the absolute concentration of cannabinoids and terpenes in concentrates is higher, the relative composition of many components remains more or less similar [42]. When specifically the cannabinoid and terpenoid contents of flowers were compared to supercritical CO_2 concentrates, the relative potencies were significantly different. Cannabinoid potency increased by factors of 3.2 for THC and 4.0 for CBD in concentrates compared to flowers. Monoterpenes were lost in the extraction process whereas monoterpene alcohols and sesquiterpenes increased by a factor between about 5 and 9 [119]. This underlines that the product after extraction has a different chemotypic fingerprint than native *Cannabis* flowers and highlights the need for more complete characterization of phytocompounds, beyond cannabinoid content.

All these observations demonstrate that there is a high variability between *Cannabis* strains as well as extracts and that it is difficult to maintain a standardized composition of phytochemicals in outdoor grown *Cannabis*, even with the same variety of the same provider. For in-door grown cultures, variability factors also apply. Under controlled climatic growth conditions a variation of average THC levels between 15.7% and 19.3% have been observed, although this depended on the genotype [129]. The chemical variability between extracts is mirrored in the physiological effects by the case of a girl with acute lymphoblastic leukemia who received five different extracts that differed in their effects on blast cells and on the profile of side effects [130]. Of interest is also a retrospective observational study on patients with spasticity from multiple sclerosis previously not improving with nabiximols (Sativex™) who had been treated with a non-activated oral formulation of Bedrocan™ (*Cannabis* flos

with 22% THC and < 1.0% CBD) [131]; 11 of 13 patients responded. Although a more detailed composition is not given, the Bedrocan extract was not only rich in THC but most likely contained a much larger spectrum of phytochemicals than Sativex where the cannabinoids THC and CBD are enriched to approximately 70%.

Composition clearly matters; there is mounting evidence that therapeutic effects differ not only between strains/extracts but also with respect to pure cannabinoids, although more systematic studies are necessary. It may be assumed that:

i. Different strains may have different therapeutic effects on the body and/or mind; CBD-rich (hemp) preparations (extracts) are likely to differ from THC-rich (drug-type) preparations (extracts)
ii. Due to the postulated entourage effect the therapeutic effects of hemp preparations (extracts) are likely to differ from pure CBD, and
iii. Drug-type preparations (extracts) are likely to differ from pure THC

As to (i), differences observed depend on the condition investigated. In a study on 77 patients it was concluded that "while *Cannabis indica* strains increased energy and appetite, it is useful to note that in treating nausea in HIV/AIDS and orthopedic diagnosis groups, *Cannabis sativa* and *C. indica* strains proved equivalent" [132]. A recent review confirms differences between THC- and CBD-rich smoked products on cognitive functions [133]. Furthermore, it seems that patients prefer specific strains for treatment of specific conditions [2].

As to the second and third assumption (ii, iii), we have found no study that compares a genuine hemp extract (THC < 0.2%) with pure CBD. A recent publication however, describing two cases of children with treatment-resistant epilepsy is of interest. Children first received CBD-enriched extracts that contained around 90% CBD in addition to 3-4% THC and standard antiepileptic therapy. After 3 to 4 months of treatment, both children presented signs of intoxication by THC (inappropriate laughter/mild euphoria, ataxia, reduced attention, irritability and eye redness). As soon as the CBD-enriched extract (which remained the same during the initial treatment) was replaced by 200-300mg/day of pure herbal CBD (purity >99.6%, BSPG, UK) a prompt and complete improvement of all intoxication signs has been observed [134].

An overview of pre-clinical studies comparing extracts to pure cannabinoids is given below (Table 5).

In the large majority of these studies CBD- or THC-enriched extracts were used with a much higher content of the main cannabinoids than usually found in extracts marketed by *Cannabis* shops. Unfortunately, in no study was reference made to the composition of phytochemicals beyond cannabinoids. Overall, these various experiments demonstrate differences but do not favour either extracts or pure Cannabinoids (CBD, THC); results seem to depend very much upon the model used. The question, as to whether a higher content of terpenoids and flavonoids or different ratios of CBD to THC would improve effects for specific purposes, remains unanswered.

Agro-climatic factor	CBD	THC	Influence on the content of CBD and THC
Soil temperature	↑	≃	Soil temperature at 5 cm has a positive influence on the content of CBD
Air humidity	≃	↑↑	Air humidity has a positive influence which is more pronounced for the content of THC than CBD
Average temperature in the entire growing period	↑↑	↑	The positive influence on CBD is about twice as high as for THC
Growing Season precipitation	↓↓	↓	The negative influence of precipitations is more pronounced for the CBD content
Fertilization (K, N, P)	↑ (NK)	↓ (PK)	Max amount of CBD observed at NK-, lowest at NPK fertilization Max amount of THC observed at PK-, the lowest at NP fertilization
Nitrogen fertilizer	↓ (NPK)	↓ (PK)	Lowest amount of CBD observed at NPK fertilization; Lowest amount of THC observed at NP fertilization
Age of leaves	↓	↓	Older leaves contained less cannabinoids than younger ones
Stage of plant development	↑	↑	The content of cannabinoids and terpenoids increases during growth in fiber-type but tend to decrease in the last stages of vegetation in drug-type *Cannabis*

Table 4: Influence of agro-climatic factors on cannabinoids.

Compiled from [120-123]

Extract	Comparator	Effects	Reference
65.6% CBD, (THC not given)	CBD	*In vitro*; effect on human bladder contractility; extract more effective than CBD	[135]
64.5% CBD, 4% THC, (CBD 10mg/kg + THC 0.62mg/kg)	CBD (10 mg p.o./kg); (THC had no effect)	Rat model; extract completely relieved thermal hyperalgesia and partially attenuated mechanical allodynia; chronic CBD had only a partial effect	[136]
17.9% CBD, 1.1% THC, 1.1% CBC (~5 to 6 times lower amount of CBD/mg extract)	CBD, dose range paw swelling: 1-50mg/kg; dose range pain: 10-150mg/kg	Mouse model; max. effect on paw swelling and pain after 5mg CBD i.p./kg compared to 50mg E i.p./kg; Orally, max. effect on paw swelling and pain after 25mg CBD/kg compared to 50mg E/kg on swelling and 150mg E/kg on pain; E was more effective on swelling after oral, CBD was more effective after i.p. administration (based on the CBD content); CBD showed a bell shaped dose-response curve	[137]
Extract with ≈. 70% CBD (Nabidiolex)	CBD	*In vitro*, eight different cancer cell liness; E mostly equipotent to CBD; CBD was the most potent (CBDA the least) out of 6 pure cannabinoids	[138]
Extract (≈. 70% CBD) 6.5mg E i.p./kg/dose	5mg CBD i.p./kg /dose	*In vivo* (mice), human breast cancer xenograft; CBD was slightly more potent than the extract	[138]
64.6% CBD, 2.5% THC (Nabidiolex)	CBD	*In vitro*, Ca++ response in neurons and glia cells; pure CBD induced a larger response than E in neurons; in glia no such difference was observed	[139]

72.6% THC, 2.5% CBD, (Tetrabinex)	THC	*In vitro*, Ca++ response in neurons and glia cells; pure THC induced a larger response than E in neurons; in glia no such difference was observed	[139]
65.9% CBD, 2.4% THC,	CBD	*In vitro*, proliferation in colorectal cancer cells; both, CBD and E reduced cells (no significant differences)	[36]
63.5% CBD, 3.6% THC,	CBD	*In vitro*, glioma cell lines; CBD was 1.1 to 2.5x more active than E	[140]
65.4% THC, 0.4% CBD	THC	*In vitro*, glioma cell lines; extract is 1.2 to 1.3x more active than pure THC	[140]
20% THC, (minor content of CBD, CBN)	THC (CBD had no effect)	*In vivo*, mouse MS-model; more rapid relief from spasticity with the extract than after pure THC but size of antispastic effect is similar;	[141]
20% THC, (minor content of CBD, CBN)	THC (CBD had no effect)	*In vitro*, rat brain slice model of epilepsy; more rapid onset of anti-convulsant activity with the extract than with pure THC	[141]

Table 5: Effects of extracts compared to effects with their primary, pure component.

E - Extract; vs - versus.

In summary, there are a number of critical aspects relating to the use of hemp products, particularly with respect to extracts:

- Choice of the strain, its composition of phytocompounds, in addition to cannabinoids
- Agroclimatic/growth conditions: precipitation, sunshine, soil, use of fertilizers, pesticides
- Harvest: time, parts of the plant harvested, transport, drying and storage conditions
- Extraction: methods, solvents, temperature

All these factors add to the considerable heterogeneity of *Cannabis* products. Thus, extrapolation of effects observed with a specific strain or product to other products is problematic, even within batches from the same provider. In the interest of future research and for the benefit of those consuming *Cannabis* for self-medication it would make sense to expand the information of products marketed beyond the declaration of the content of CBD and THC. This should include at least the name of the strain, basic information on the extraction method, temperature to which the product has been exposed during manufacturing.

Future clinical studies therefore should be conducted using well characterized, reproducible formulated products. Due to their rich content of terpenoids, flavonoids and other bioactive phytocompounds, genuine extracts can play a role as neutraceuticals and in complementary medicine. With more information on the phytocomponents it should be possible to profile *Cannabis* products for specific purposes.

Conflict of Interest

Both authors declare no conflict of interest.

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