CHAPTER

53

Cannabis sativa and Hemp

Joshua A. Hartsel, Joshua Eades, Brian Hickory and Alexandros Makriyannis

INTRODUCTION

Cannabis sativa is a remarkable plant containing many valuable natural components. It has been cultivated throughout the world and history for use as a food, fuel source, nutritional supplement, body care product, source of paper, building material, medicine, and in textiles (Small and Marcus, 2002). In 1938, *Popular Mechanics* magazine noted the economic value of hemp, one member of the *C. sativa* taxon, as the new billion-dollar crop of the era. The article reported that 30,000 products could be made from only one component of the hemp plant, the fiber, and the hurd of the stalk (Mechanics, 1938). One key aspect of *C. sativa* that *Popular Mechanics* did not mention was the flower, which produces cannabinoids, terpenes, and seeds with a healthy balance of fatty acids. This chapter describes the scientific data as they relate to *C. sativa* flowers, biology, and chemistry, and how these components contribute to its recognized therapeutic value.

C. *SATIVA* TAXONOMY

Among all genera belonging to the *Cannabaceae* family, *C. sativa* has attracted special interest from the public because of its psychoactive effects. The genetic plasticity of cannabis has made it difficult to catalog, and there is still an ongoing discussion about its proper classification. In 1737, Carl Linnaeus, the father of modern taxonomy, described *C. sativa* as a genus composed of a single species, *C. sativa*. Linnaeus was unaware of drug-type cultivars that were prevalent in Asia and classified cannabis as a single species from his experience with fiber-type crops that were common in Europe (Linnaeus, 1800). It is uncertain how he would have cataloged the new Indian and Asian cannabis cultivars had he been aware of them during his era. Comparative analyses between the Indian and the European hemp varieties, based on size, shape, leaf structure, and psychoactive effects, inspired Jean-Baptiste Lamark to classify the Indian cultivars as a separate species, *Cannabis indica* (Lamarck, 1811). The Soviet botanist Janischevsky revisited the polytypic (multi-species) view when he recognized that local Russian plants did not fit the characteristics of *C. sativa* or *C. indica*, but fell within the *Cannabis* taxa. He named the short, wild Russian autoflowering plants *C. ruderalis* (Small, 1975) and divided the genus into three distinct species, *C. sativa* L., *C. indica* Lam., and *C. ruderalis* Janisch. According to the American Herbal Pharmacopeia, *C. sativa* L. was historically bred to be tall and is used mainly for fiber and seed (Figure 53.1; Upton et al., 2014). *C. sativa indica* Lam. is characterized by a short, densely branched structure and potent level of Δ^9-tetrahydrocannabinol (Δ^9-THC), which is the main psychoactive ingredient (Upton et al., 2014). To date, scholars still disagree on how to correctly catalog cannabis species. The two competing schools of cannabis taxonomy are divided between a monotypic (single-species) and a polytypic perspective. The current debate centers on one question: Are all cannabis cultivars *C. sativa*? *Sativa* and *indica* cannabis types are frequently crossbred to produce hybrid phenotypes with desired characteristics. The viability of interbred *sativa* and *indica* cannabis types supports the cataloging of all varieties as subspecies of *C. sativa* (i.e., *C. sativa sativa*, *C. sativa indica*, and *C. sativa ruderalis*) (Anderson, 1980; Schultes et al., 1974). To simplify the text, the monotypic subspecies nomenclature is used throughout this chapter.

Nutraceuticals.
DOI: http://dx.doi.org/10.1016/B978-0-12-802147-7.00053-X

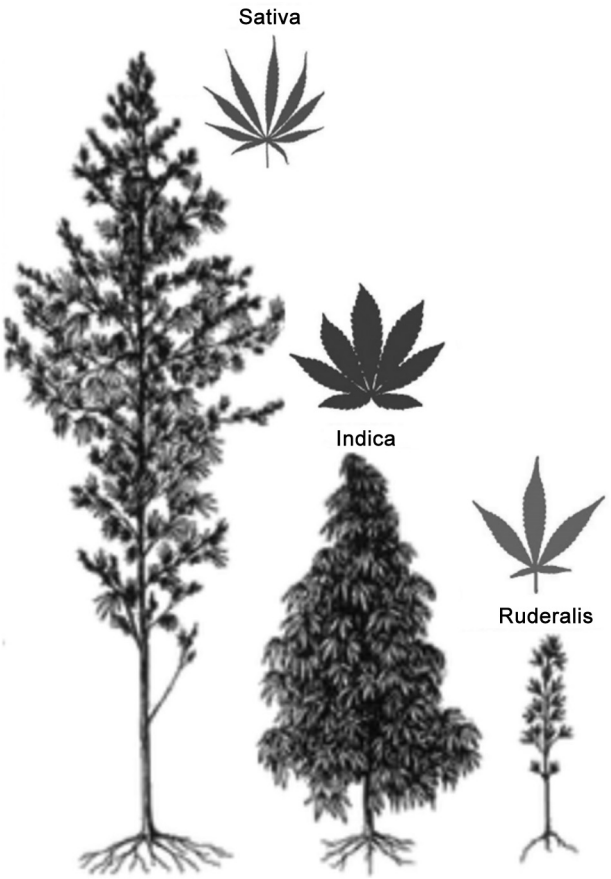

FIGURE 53.1 Subspecies of *Cannabis sativa*, include *C. sativa sativa*, *C. sativa indica*, and *C. sativa ruderalis*. *Source: wikicommons; public domain.*

EUROPEAN APPROVED HEMP CULTIVARS AND MEDICAL C. *SATIVA*

Unlike medical cannabis abundant in the psychoactive ingredient Δ^9-THC, modern hemp has been selectively bred to produce low levels of Δ^9-THC and high levels of fiber, seed, and, more recently, cannabidiol (CBD). Most of the European Union and Canada have recognized the value of hemp and have defined a legal limit of 0.3% Δ^9-THC in the dry plant material (Small and Marcus, 2003), with the exception of Italy (0.2% Δ^9-THC) (Cappelletto et al., 2001). Many hemp cultivars are rich in cellulose used for biofuel, primary fibers for pulping, fine fiber textiles, dense flowering varieties that offer abundant seed, or high CBD-to-Δ^9-THC ratios. Hemp varieties resistant to drought, high soil salinity, cold, heat, humidity, and common pests and diseases have been selected to improve yields (Watson and Clarke, 2014). To date, 51 hemp cultivars have been approved for commercial use by the European Union (Directive, 2013). These registered varieties originate from high-latitude European nations, which are not acclimated for equatorial regions of the world. Little is known about the viability of the European cultivars in the United States, and it is currently being examined by agronomists.

CHEMOTAXONOMY OF C. *SATIVA*

Chemical phenotypes (chemotypes) can be useful to classify *C. sativa* as drug- or fiber-type varieties. The United Nations Office on Drugs and Crime categorizes *C. sativa* into three chemotypes based on the proportion of THC and CBN relative to CBD (Equation 53.1: Classification of cannabis by chemotype.) (Drugs, 2009). Chemotype I (drug-type) cultivars are characterized by X values greater than 1, whereas values lower than 1 are representative of chemotype III cultivars (fiber-type).

$$X = \frac{[\text{THC}] + [\text{CBN}]}{[\text{CBD}]} \tag{53.1}$$

The evidence suggests that drug-type or chemotype I *C. sativa* cultivars with high levels of Δ^9-THC originated from below the 30°N latitude (Hillig and Mahlberg, 2004). CBD-rich cultivars containing low levels of THC are regarded as fiber-type or chemotype III cultivars. Chemotype II cultivars are characterized by equivalent levels of Δ^9-THC and CBD and, along with chemotype III cultivars, are typically found above the 30°N latitude (Hillig and Mahlberg, 2004). Hillig and Mahlberg (2004) used a statistical approach to define chemotaxonomic trends in *C. sativa* and noticed that most cultivars did not fall within the arbitrary values set by the United Nations on Drugs and Crime. Instead, most cultivars clustered into chemotype I (X>10), chemotype II (0.2< X<10), or chemotype III (X<0.2). The relative cannabinoid levels in *C. sativa* remain constant from the seedling stage throughout the plant lifecycle (Broséus et al., 2010), making it possible to determine the chemotype at early development stages prior to flowering (Barni-Comparini et al., 1984; Vogelmann et al., 1988).

Hemp- and drug-type cultivars are members of both *C. sativa sativa* and *C. sativa indica* subspecies. Chemotaxonomic analysis is useful to differentiate hemp from drug-type *C. sativa* based on acceptable levels of Δ^9-THC established by regulating bodies. Cannabinoid detection methods have not been useful to distinguish *C. sativa sativa* from *C. sativa indica*. Incremental progress has been made distinguishing the subspecies using terpene fingerprinting techniques, but has not provided the practical level of significance for routine application (Hillig, 2004). More recently, the draft genome of *C. sativa* has been published, opening new opportunities to identify genetic markers unique to *C. sativa* subspecies (van Bakel et al., 2011).

POTENCY TRENDS

Historically, *C. sativa indica* originating from Southeast Asia has been known for its high levels of Δ^9-THC. Until recently, it was unclear whether the high Δ^9-THC cultivars were present prior to human intervention. Genetic evidence suggests that the high B_T allele frequency, responsible for Δ^9-THC production, was present in *C. sativa indica* prior to human domestication (Hillig and Mahlberg, 2004). These data indicate that natural evolution played a prominent role in *C. sativa indica* cultivars with high levels of Δ^9-THC and predated selective breeding practices. Within recent decades breeders have brought the Δ^9-THC content to unprecedented levels not observed for a single component in a plant species. A potency monitoring report put forth in collaboration between The University of Mississippi and the National Institute on Drug Abuse (NIDA) identified levels up to 37% Δ^9-THC in 45,603 cannabis inflorescences confiscated internationally from 1993 to 2008 (Mehmedic et al., 2010). The High Times Cannabis Cup is a competitive event for breeders to showcase their superlative cannabis products. The organization submits competitive strains for independent testing and reports that the highest strains are approximately 25% (Hellerman, 2013). The high Δ^9-THC levels achieved over the recent decades have largely been attributed to selective breeding, indoor cultivation, and worldwide access to potent seed varieties (Brenneisen, 2007). From 1980 to 2008, the average Δ^9-THC content in confiscated cannabis increased from 1.5% to 8.8% and was the direct result of human intervention (ElSohly et al., 2000; Mehmedic et al., 2010).

Cannabinoid levels other than Δ^9-THC have remained relatively constant from 1993 to 2008. For example, average CBD levels have gone from 0.2% in 1993 to 0.4% in 2008 (Mehmedic et al., 2010). However, high-CBD medical cultivars with up to 18% CBD have recently been developed. High CBD cultivars include Harlequin, ACDC, Cannatonic, and an Israeli strain, Avidekel. Despite the high CBD levels in these cultivars, they fail to meet the 0.3% Δ^9-THC threshold to be defined as hemp. Agricultural hemp cultivars producing up to 5% CBD have been described in the literature. However, the high CBD hemp varieties also tested above the legal limit of 0.3% Δ^9-THC and could not be cultivated as agricultural hemp (Virovets, 1996). An analysis of more than 100 Italian fiber-type hemp samples indicates that CBD values range from 0.07% to 2.3%, whereas the Δ^9-THC values ranged from 0.01% to 0.44% (Rustichelli et al., 1998). These data suggest that the 5% CBD hemp cultivars were outliers and selected for high-CBD cultivar breeding projects. Alternatively, other hemp cultivars have been developed that produce little to no detectable cannabinoids (Virovets, 1996).

C. SATIVA CHEMISTRY

C. sativa produces more than 750 natural compounds of different chemical classes (Upton et al., 2014). Cannabinoids are grouped in one class composed of 86 terpenophenolic secondary metabolites identified in *C. sativa* (Radwan et al., 2008). Another important class of compounds produced by *C. sativa* includes the 140 terpenes that have been identified to date (Brenneisen, 2007). In addition to the fragrance that the terpenes contribute, there is also evidence to suggest that they are a critical component in modulating the strain-specific physiological effects (Russo, 2011). Other compound classes include 50 identified hydrocarbons, 34 sugars and related compounds, 27 nitrogenous compounds, 25 noncannabinoid phenols, 23 fatty acids, 23 flavonoids, 20 simple acids, 13 simple ketones, 13 simple esters and lactones, 12 simple aldehydes, 11 proteins, 11 steroids, 9 elements, 3 vitamins, and 2 pigments that are summarized in Table 53.1 (Brenneisen, 2007; Callaway, 2004; ElSohly and Slade, 2005; Turner et al., 1980). It is worth noting that no free flavonoids have been identified in *C. sativa*, but they are found in their corresponding glycosides (Turner et al., 1980). Covering each component of *C. sativa* is beyond the scope of this chapter.

TABLE 53.1 Chemical Constituents Identified in *C. sativa* by Chemical Class

Chemical class	Identified compounds
Terpenes	140
Cannabinoids	86
Hydrocarbons	50
Sugars and related compounds	34
Nitrogenous compounds	27
Noncannabinoid phenols	25
Fatty acids	23
Flavonoids	23
Simple acids	20
Simple ketones	13
Simple esters and lactones	13
Simple aldehydes	12
Proteins, enzymes, and glycoproteins	11
Steroids	11
Elements	9
Simple alcohols	7
Vitamins	3
Pigments	2

However, an excellent review of *C. sativa* chemistry can be found in the body of work compiled by ElSohly and coworkers (Brenneisen, 2007; Callaway, 2004; ElSohly and Slade, 2005; Turner et al., 1980).

CANNABINOIDS

Of the 86 cannabinoids identified in *C. sativa*, the majority can be categorized as analogs of Δ^9-THC, CBD, cannabichromene (CBC), cannabigerol (CBG), cannabinol (CBN), cannabicyclol (CBL), cannabielsoin (CBE), and cannabitriol (CBT). There are seven known analogs of CBD in *C. sativa*, and Figure 53.2 illustrates common structural trends that are found within cannabinoid classes (ElSohly and Slade, 2005). Although the general public is most familiar with neutral cannabinoids such as CBD and Δ^9-THC, cannabinoids exist in an acid form, meaning they contain a carboxylic acid pendant to the aromatic ring (e.g., Cannabidiolic acid, CBDA, R=H, with pendant COOH). The length of the alkyl side chain is a common site of structural variability and can range from one to five carbons. The most abundant cannabinoids in *C. sativa* contain *n*-pentyl side chains, but *n*-butyl, *n*-propyl, ethyl, and methyl side chains have also been identified in lower abundance (ElSohly and Slade, 2005; Turner et al., 1980). Cannabinoids bearing an *n*-propyl side chain are referred to as cannabivarins, indicated by the suffix -varin. Tetrahydrocannabivarin (THCV), the *n*-propyl side chain analog of THC, is often found in samples collected from the *C. sativa indica* subspecies (Hillig and Mahlberg, 2004). Although not common, the phenol portion of the cannabinoid can be protected as monomethyl ether, as is observed in analogs of CBD (R=H, Figure 53.2). In addition, there are 30 miscellaneous cannabinoids present in low concentrations, but they will be excluded from this text based on the limited understanding of their therapeutic potential.

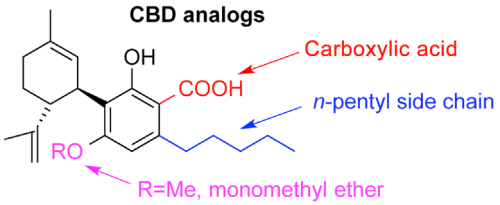

FIGURE 53.2 Typical cannabinoid analogs (e.g. CBD, R=H) differ with respect to the carboxylic acid, alkyl side chain, or methyl ether group.

It is important to note that *C. sativa* does not produce Δ^9-THC, CBD, CGG, or CBC, but rather carboxylic acid–containing precursors referred to as Δ^9-tetrahydrocannabinoic acid (Δ^9-THCA), CBDA, cannabigerolic acid (CBGA), and cannabichromenic acid (CBCA), respectively. *C. sativa* extracts are nonpsychoactive until sufficient heat is supplied to cause a chemical reaction known as decarboxylation. Decarboxylation of cannabinoic acids occurs rapidly above 105°C, which is a condition obtained during the smoking or baking process (Figure 53.3; Veress et al., 1990). Decarboxylation occurs slowly under ambient conditions, but the rate increases with temperature. High levels of decarboxylated cannabinoids in flowers can indicate that a sample has been stored improperly or is aging. Δ^9-THC can undergo oxidation to CBN and is also a chemical indicator of poor or lengthy storage conditions. Carbon dioxide is released during the decarboxylation process.

C. sativa produces four types of cannabinoic acids, CBGA-, CBDA-, Δ^9-THCA-, and CBCA-type, through biosynthetic pathways shown in Figure 53.4 (Fellermeier et al., 2001; Sirikantaramas et al., 2007; Taura et al., 2007). CBGA acts as substrate for *C. sativa*–specific oxidoreductases CBDA (Taura et al., 1996), THCA (Shoyama et al., 2005; Sirikantaramas et al., 2004), or CBCA synthase

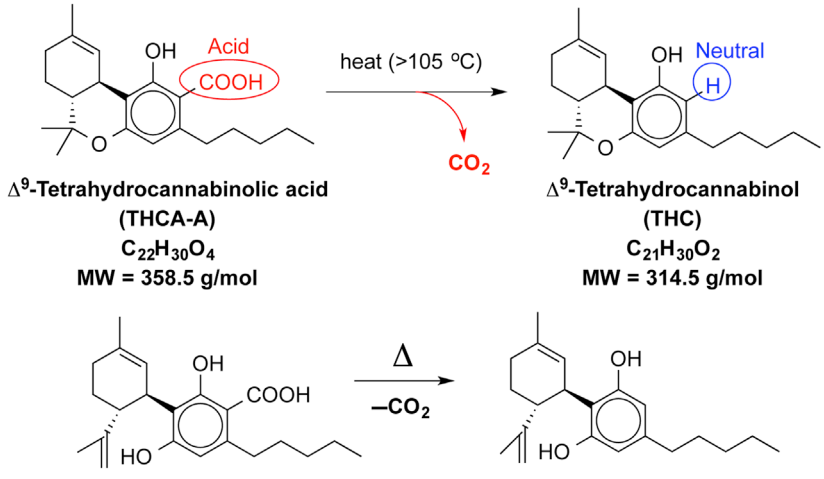

FIGURE 53.3 Conditions required to decarboxylate native cannabinoic acids to their neutral form.

(Morimoto et al., 1997) and is the convergent intermediate in cannabinoid production. Prior to the discovery of THCA synthase, Δ^9-THC and its acid form were believed to be produced through a ring closure of CBD or CBDA, respectively (Shoyama et al., 1975). It was later shown that Δ^9-THCA was produced through independent enzyme-catalyzed conversion of CBGA, and then decarboxylated to form Δ^9-THC (Shoyama et al., 2005; Sirikantaramas et al., 2004).

The CBGA carboxyl group is critical for cannabinoid synthase substrate recognition. CBG, without the pendant carboxyl group, did not undergo enzyme catalysis through the cannabinoid synthase pathways (Taura et al., 2007). Both Δ^9-THCA and CBDA are produced with greater than 95% optical purity. CBCA, however, is a 5:1 ratio of enantiomers (Morimoto et al., 1997). The evidence suggests that CBCA synthase releases the substrate before the reaction is complete, resulting in lower enantiomeric purity compared to Δ^9-THCA and CBDA (Taura et al., 2007). THCA synthase is FAD- and oxygen-dependent and generates hydrogen peroxide as a by-product. The release of hydrogen peroxide could act as a component of the plant disease defense mechanism (Taura et al., 2007). In contrast to THCA synthase, CBDA synthase does not require molecular oxygen for the conversion of CBGA to CBDA. A comparison of THCA synthase with other plant enzymes revealed that it shares high homology to the alkaloid-producing berberine-bridge enzyme from *Eschscholzia californica*, or California poppy (Dittrich and Kutchan, 1991; Taura et al., 2007).

Cannabinoids other than Δ^9-THCA, CBDA, and CBCA (primary cannabinoids) are generated through nonenzymatic degradation pathways. The primary cannabinoids either can be decarboxylated to the neutral form or can be converted to CBEs, CBNs, Δ^8-THCs, or CBLs via exposure to light, heat, and oxygen as shown in Figure 53.4 (ElSohly and Slade, 2005; Turner et al., 1980). CBDs can undergo photo-oxidation or pyrolysis to CBEs. Alternatively, THCs can be degraded to CBNs in the presence of oxygen, or to more thermodynamically

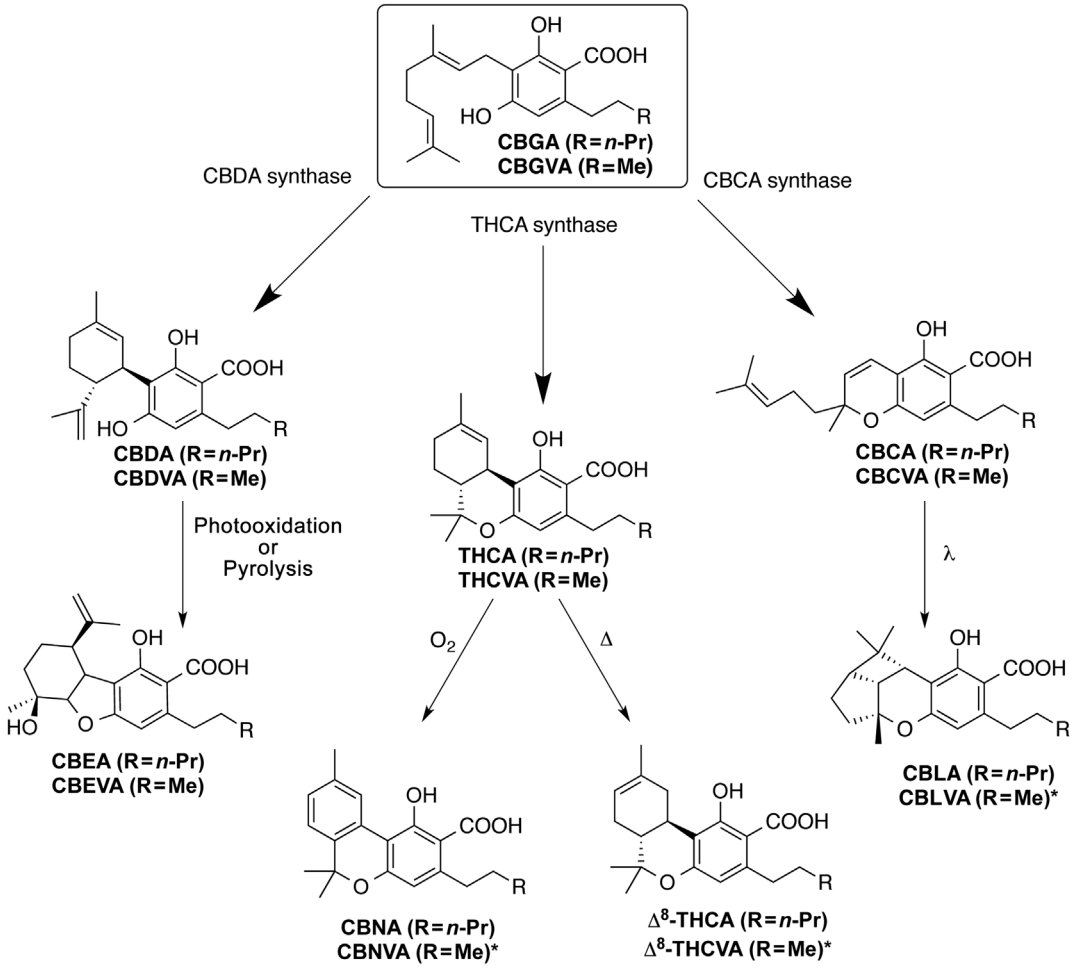

FIGURE 53.4 Biosynthesis of CBDA, THCA, and CBCA from CBGA through the cannabinoid synthase pathways. Degradation of the CBD-, THC-, and CBC-type cannabinoids leads to CBE-, CBN-, THC-, and CBL-type cannabinoids. *This compound was not identified in cannabis.

stable Δ^8-THCs when exposed to heat. CBCs are converted into CBL-type cannabinoids in the presence of light. The shorter side-chain cannabinoid analogs, such as three-carbon-containing cannabivarins, are generated through identical cannabinoid synthase pathways from cannabigerovarinic acid (CBGVA), the homologous propyl-containing CBGA precursor (Shoyama et al., 1984).

CANNABINOIDS FROM NON-C. *SATIVA* SPECIES

As a complement to *C. sativa*, South African *Helichrysum umbraculigerum* has been shown to contain CBGA, CBG, and other prenylated dibenzyls similar to cannabinoids in *C. sativa*. Cannabinoid-like molecules derived from *Helichrysum* and other *Asteracea* genera generally have a phenethyl group pendant at the *n*-pentyl region of cannabinoids and have been used in traditional medicine to treat a host of inflammation and infections (Appendino et al., 2011). *Echinacea* (Woelkart et al., 2005) and liverworts (Appendino et al., 2011) are known to produce cannabinoid-like molecules. In particular, *Echinacea* roots produce endocannabinoid-like molecules that have been shown to bind to cannabinoid receptors in rodents (Woelkart et al., 2005).

C. *SATIVA* TERPENES

The combination of more than 140 terpenes identified in *C. sativa* contributes to its unique aroma (Brenneisen, 2007). The pungent inflorescences can be described as skunky, fruity, or piney, among others, depending on the aromatic components present. Terpenes are a group of volatile acyclic, monocyclic, and polycyclic hydrocarbons that contribute to the complex aroma profile of *C. sativa* and many other terpene-containing plants (Figure 53.5; Brenneisen, 2007). The volatility of terpenes imparts aroma but these molecules evaporate upon standing. In one study, approximately 55% of the volatile terpene constituents were lost after being stored 3 months under drying conditions (Ross and ElSohly, 1996). Terpenes isolated through steam distillation from indoor drug-type *C. sativa* constituted a low percentage (0.29% w/w) of the total predried biomass. The main fraction of the distillate comprised monoterpenes (92% of total terpenes), with a small amount of sesquiterpenes (7%) and other simple ketones and esters (Ahmed et al., 2008; Ross and ElSohly, 1996). Of the monoterpenes present in the fresh flowers, myrcene (67%) was the most abundant component, followed by D-limonene (16%) and linalool (3%). Monoterpene content in *C. sativa* cultivated outdoors ranged from 48% to 92% of total terpenes present (Mediavilla and Steinemann, 1997). Sesquiterpene content was lower, ranging from 5% to 49%. Higher levels of monoterpenes α-pinene, *trans*-ocimene, and α-terpinolene were

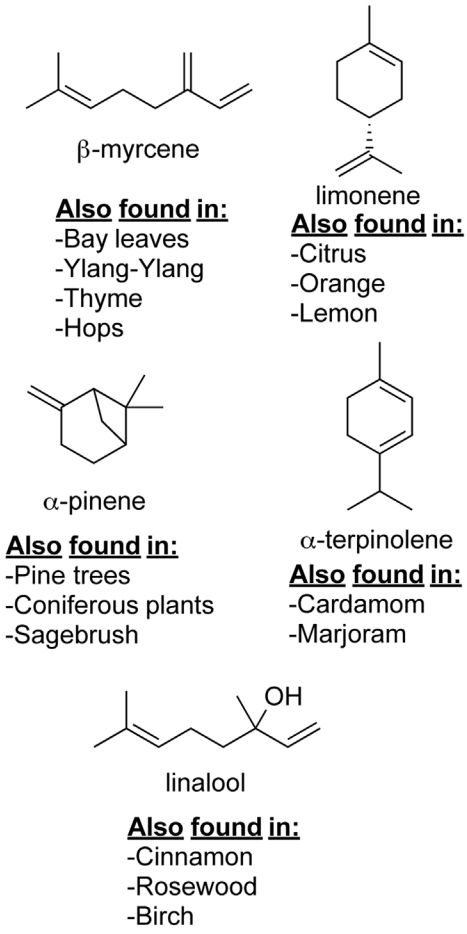

FIGURE 53.5 Common monoterpenes found in cannabis and other plants.

observed in *C. sativa* cultivated outdoors (Mediavilla and Steinemann, 1997) compared to indoor varieties (Ross and ElSohly, 1996). α-Terpinolene and α-pinene were the most abundant terpenes found in European cultivars, which contained significantly higher amounts of α-humulene (6–9% of total terpenes) compared to the varieties cultivated in the United States (Novak et al., 2001). Sabinene, α-terpinene, eucalyptol, pulegone, γ-terpinene, terpineol-4-ol, bornyl acetate, α-copaene, alloaromadendrene, viridiflorene, α-bisabolene, γ-cadinene, trans-nerolidol, and β-bisabolol were terpenes found in trace amounts (McPartland and Russo, 2001; Mediavilla and Steinemann, 1997; Ross and ElSohly, 1996). Characteristic levels of particular terpenes have been linked to geographic origin (Brenneisen and ElSohly, 1988).

The terpene composition of 10 hemp varieties was investigated over two growing seasons and ranged from 0.11% to 0.25% (w/w) of the fresh plant material. The most abundant monoterpenes present were myrcene (8–45%), terpinolene (0.12–22%), α-pinene (3–20%), *E*-ocimene (1–10%), and β-pinene (1–8%). The main sesquiterpenes present were β-caryophyllene

TABLE 53.2 Terpene Composition in Freshly Harvested Cannabis Inflorescences by Percent of Total Steam-Distillate I Drug-Type and Fiber-Type Cannabis

Compound	Drug type (%)	Average ± SD (N)	Fiber type (%)	Average ± SEM (N)
Monoterpenes				
β-Myrcene	0.4–67	21.9 ± 5.1 (17)	8.2–66	30.7 ± 2.8 (39)
α-Pinene	ND–31.0	5.2 ± 1.9 (17)	2.3–20	8.4 ± 0.7 (39)
Terpineolene	ND–15.3	3.2 ± 1.1 (17)	tr–24	10.1 ± 1.0 (39)
trans-Ocimene	0.3–5.9	3.0 ± 1.2 (5)	0.4–10.3	4.9 ± 0.5 (39)
D-Limonene	ND–16.4	2.7 ± 1.0 (17)	0–6.4	1.9 ± 0.3 (39)
β-Pinene	tr–7.8	1.7 ± 0.5 (17)	0.6–8.0	3.1 ± 0.3 (39)
cis-Ocimene	ND–3.9	0.6 ± 0.2 (17)	0–1.0	0.3 ± 0.0 (39)
Linalool	ND–2.8	0.4 ± 0.2 (12)	ND–2.7	0.5 ± 0.1 (20)
Sesquiterpenes				
β-Caryophyllene	0.5–31	5.8 ± 2.1 (17)	7.3–37.5	19.0 ± 1.0 (39)
Humulene	ND–1.2	1.0 ± 0.3 (17)	1.3–12.6	5.5 ± 0.5 (39)
Caryophyllene oxide	tr	0.4 ± 0.2 (6)	0.3–11.3	2.6 ± 0.3 (39)
β-Eudesmol	ND–0.4	0.04 ± 0.03 (12)	ND–1.6	0.2 ± 0.1 (20)
trans-Nerolidol	tr	$N = 1$	0.1–1.7	0.9 ± 0.1 (20)

ND, not detected (< LOD); tr, trace levels detected (< LOQ). Drug type: Ross and ElSohly (1996), Fischedick et al. (2010), Mediavilla and Steinemann (1997). Hemp varieties: Bertoli et al. (2010); Mediavilla and Steinemann (1997); Novak et al. (2001).

(7–28%), α-humulene (3–12%), and caryophyllene oxide (0.12–22%). The dioecious cultivars tended to have higher myrcene and terpinolene content, whereas high α-pinene and β-pinene tended to be more characteristic of the monoecious cultivars (Bertoli et al., 2010). The publicly available literature on terpene composition in *C. sativa* was compiled for drug- and fiber-type varieties, which are summarized in Table 53.2 (Bertoli et al., 2010; Fischedick et al., 2010; Mediavilla and Steinemann, 1997; Novak et al., 2001; Ross and ElSohly, 1996).

Growing techniques, environmental factors, and genetics contribute to strain-specific aromas. Although olfactory response is subjective, high sesquiterpene content was considered by test subjects to have a poor aroma compared to "pleasant" cultivars high in monoterpene content (Mediavilla and Steinemann, 1997). The unique skunk smell emitted by *C. sativa* may not be a traditional terpene. For example, skunks produce a group of nonterpene thiols that contribute the pungent aroma (Wood et al., 2002). It is possible that the skunk smell in *C. sativa* is also a thiol that does not fit within the terpene class; the constituent responsible for the skunky aroma of cannabis remains to be elucidated.

PHYTOCANNABINOID AND TERPENE BIOCHEMISTRY

Terpenes, phytocannabinoids, flavonoids, lignans, coumarins, polyketides, and lipids are produced through shared biosynthetic pathways in *C. sativa* (Figure 53.6).

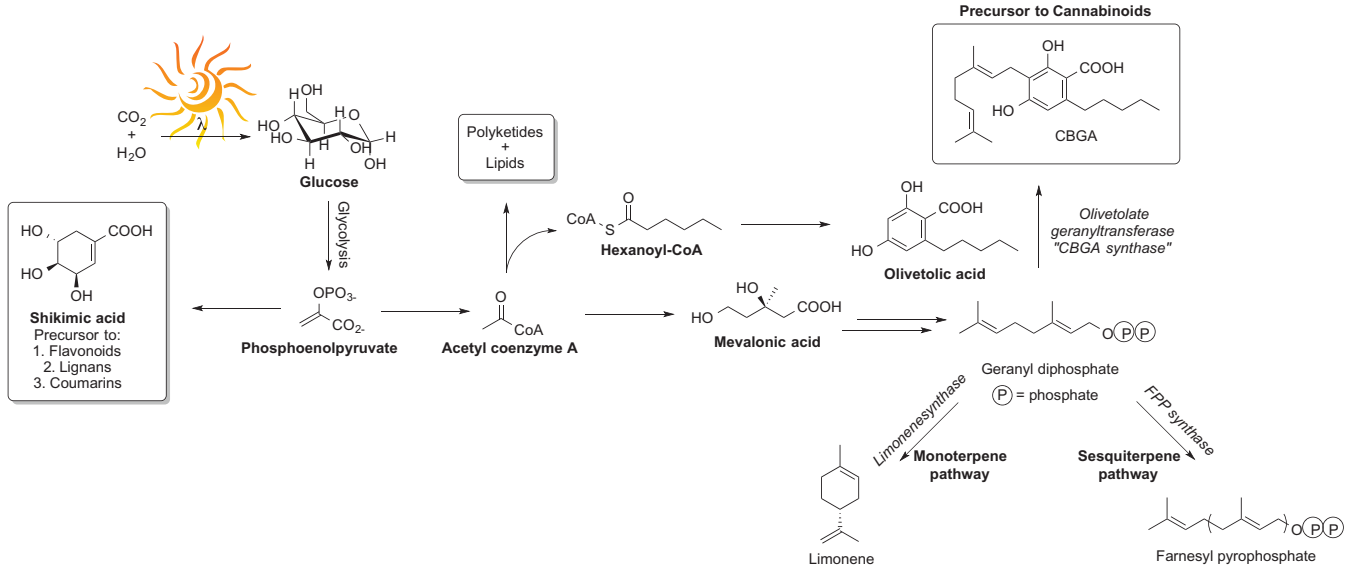

FIGURE 53.6 Plants use sunlight, carbon dioxide, and water to generate many useful botanical ingredients. Terpenes and cannabinoids are generated biosynthetically through convergent intermediate geranyl diphosphate. Geranyl diphosphate can be diverted through several competing enzymatic pathways to produce a variety of useful botanical compounds including. CBGA, monoterpenes, and sesquiterpenes.

Phosphoenolpyruvate is a key intermediate in the biosynthesis of many plant compounds and is produced through glycolysis. One pathway exploited by plants is the conversion of phosphoenolpyruvate to shikimic acid. Shikimic acid is the precursor to flavonoids, lignans, and coumarins. Alternatively, phosphoenolpyruvate can be catabolized to acetyl coenzyme A, which is a building block for botanical lipids and polyketides. Cannabis has specially adapted type III polyketide synthase and olivetolic acid cyclase enzymes to convert acetyl coenzyme A to olivetolic acid (Gagne et al., 2012). Acetyl coenzyme A is also consumed in a competing biosynthetic pathway leading to geranyl diphosphate. Geranyl diphosphate and olivetolic acid are both downstream products derived from acetyl coenzyme A. Interestingly, geranyl diphosphate and olivetolic acid can be enzymatically coupled by olivetolate geranyl transferase in CBGA biosynthesis (Marks et al., 2009). Geranyl diphosphate also acts as a substrate in monoterpene (Taura et al., 2007) and sesquiterpene (Sell, 2009) biosynthesis. The abundance of each cannabinoid and terpene is modulated by the relative expression and efficiency of the competing enzymatic pathways. Terpenes are produced in low levels compared to cannabinoids in *C. sativa*.

TRICHOMES

The biosynthesis of terpenes and cannabinoids occurs within the extracellular secretory cavity, referred to as a trichome. Trichomes are present in more than 300 plants (Dayanandan and Kaufman, 1976), and over 300 morphologically distinct trichomes have been reported (Wagner, 1991). The TrichOME project (www.planttrichome.org) is a database of EST sequences, microarray hybridizations, mass spectrometry–based metabolite profiles, and literature compiled with funding from the NSF and the Samuel Roberts Nobel Foundation. Trichomes serve several functions in plants, including keeping the frost from the surface cells, reducing transpiration, and increasing light reflectance in desert species. Specialized glandular trichomes produce and emit compounds that interact with insects deterring pests or promoting pollination (Wagner, 1991). The trichomes act as a first line of defense to protect the plant from the environment (Taura et al., 2007). Interestingly, some of the very cannabinoids (THCA and CBGA) produced within the *C. sativa* trichome have been found to be toxic to *C. sativa* cells (Sirikantaramas et al., 2005). Cannabinoids are secreted into the trichome to prevent cellular damage to the plant. THCA and CBGA have been shown to be toxic to certain insect species as well (Taura et al., 2007).

The term trichome is derived from the Greek word meaning hair, because of the hair-like appearance. Trichomes found on *C. sativa* have been divided into two types, glandular and nonglandular. Nonglandular *C. sativa* trichomes cover the shoot from early seedling stages to the end of flowering and are highly silicified unicellular hair-like appendages (Dayanandan and Kaufman, 1976). Cystolithic nonglandular trichomes are short hairs ranging from 150 to 220 µm in height and containing basal deposits of calcium carbonate. In contrast, noncystolithic and nonglandular are generally longer (340–500 µm), with a more slender structure, and do not contain a basal deposit of calcium carbonate (Upton et al., 2014). The nonglandular trichomes cover the majority of the plant's surface, including the stems, petioles, stipules, leaf blades, bracts, and both surfaces of the tepals (Dayanandan and Kaufman, 1976).

The cannabinoids are produced in two types of glandular trichomes, capitate-stalked and capitate-sessile. The capitate-stalked glandular trichome heads are composed of eight cells and reside on the bracts of the female plants and anthers of the male plants. They are easily recognized by the mushroom-like stalk and head appearance (Figure 53.7C; Dayanandan and Kaufman, 1976). Capitate-stalked trichomes are the primary source of cannabinoid and terpene resin production in flowering *C. sativa*. Dayanandan and Kaufman (1976) have shown that male *C. sativa* plants produce equal quantities of glandular trichomes compared to females and bear similar trichome types. It has also been demonstrated that some resinous cannabinoid-producing glands develop prior to flower formation. For example, capitate-sessile trichomes produce cannabinoids throughout the plant life cycle, but at lower levels compared to the capitate-stalked type. Capitate-sessile trichome heads are composed of between two and four cells. In contrast, the bulbous glandular trichomes are found throughout the plant and do not produce cannabinoids or terpenes (Figure 53.7B). The head of the trichome is composed of one, two, or four cells with stalks either one or two cells high.

The cannabinoid precursors, olivetolic acid and geranyl diphosphate, are transported through the stalk of glandular trichomes via vacuoles and plastids, respectively (Figure 53.7A). The cannabinoid precursors are released into the secretory cavity, where they are transformed biosynthetically through resident cannabinoid synthase enzymes in the extracellular space (shown in Figure 53.4). Monoterpenes are formed in the plastids via the enzymatic conversion of geranyl diphosphate. Alternatively, geranyl diphosphate can couple with isopentenyl diphosphate in the cytoplasm to form the parent compound to the sesquiterpenoids, farnesyl pyrophosphate (Russo, 2011). THCA synthase fused to green fluorescent protein helped visually demonstrate that this enzyme is accumulated in the secretory cavity (Sirikantaramas et al., 2005) located in the head of

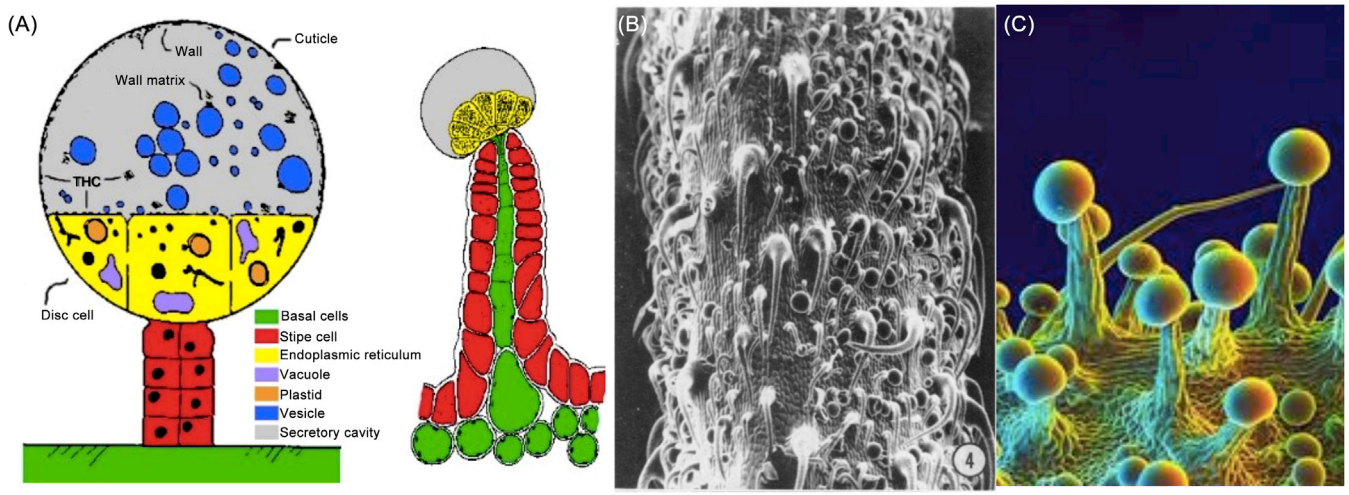

FIGURE 53.7 Structure of glandular and cystolith hair trichomes. (A) A cartoon depicts the components of the glandular capitate-stalked trichome. *Source: Photo credit:* http://medicalmarijuana.com/experts/expert/title.cfm?artID=140. (B) Scanning electron microscope image of alternative bulbous and capitate-sessile glandular, and cystolithic hair-type trichomes. (C) Scanning electron microscope image of the glandular capitate-stalked trichomes. *Source: Photo credit:* http://medicalmarijuana.com/experts/expert/title.cfm?artID=140.

glandular capitate-stalked and capitate-sessile trichomes (Figure 53.7C).

THE PHARMACOLOGY OF CANNABIS

The therapeutic properties of cannabis have been appreciated over several millennia and its pharmacological properties have been studied since the mid-1900s. This effort was enhanced in the 1960s with the isolation, characterization, and subsequent synthesis of its major psychoactive ingredient (−)-Δ^9-THC, and much attention was given to developing synthetic compounds with more potent and targeted therapeutic effects. Concurrent approaches by individual research laboratories and the pharmaceutical industry produced a plethora of structurally related new compounds, which were collectively called cannabinoids. One of these drug development projects by Eli Lilly led to the first synthetic cannabinoid, Nabilone (Cesamet), which was used to treat nausea, pain, and reduced appetite associated with cancer chemotherapy (Makriyannis, 2014; Pertwee, 2008). Notwithstanding these major efforts by the industry, the enthusiasm to develop additional medications waned principally because of lack of knowledge of their pharmacological mechanism of action. There was also accumulating evidence that the therapeutic effects of smoked cannabis in certain instances provided superior relief when compared to the synthetic compounds.

The major breakthrough in understanding the mechanism of action of cannabis, and more specifically Δ^9-THC, came with the discovery of the first biological targets for

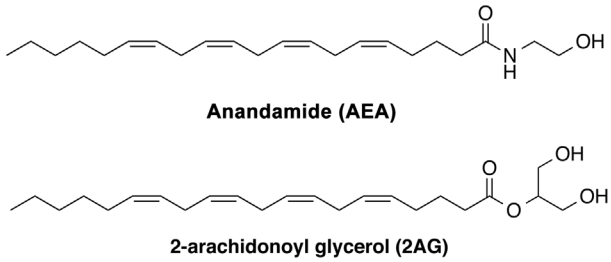

FIGURE 53.8 Naturally occurring endocannabinoids that bind to the endogenous endocannabinoid receptors in mammals.

this compound. Following a very successful research meeting in 1987 by the National Institute on Drug Abuse in Bethesda, Maryland (Makriyannis, 2014), collaborative work among the participating laboratories led to the discovery of a new G-protein-coupled receptor (GPCR) that was named CB1, to be followed 2 years later by a second THC-related GPCR named CB2. The next discovery was a group of compounds found in mammalian tissues, which were named endocannabinoids, the most representative of which were arachidonyl ethanolamide or anandamide (AEA), a long-chain fatty acid amide and 2-arachidonoyl glycerol (2AG) (Figure 53.8), the respective ester (Pertwee, 2000) These new substances are capable of activating both CB1 and CB2 receptors and, when tested in animals, produce biological effects paralleling those of Δ^9-THC.

Endocannabinoids are produced on demand by a series of enzymes that are present within the cell membrane and are activated by elevated levels of calcium

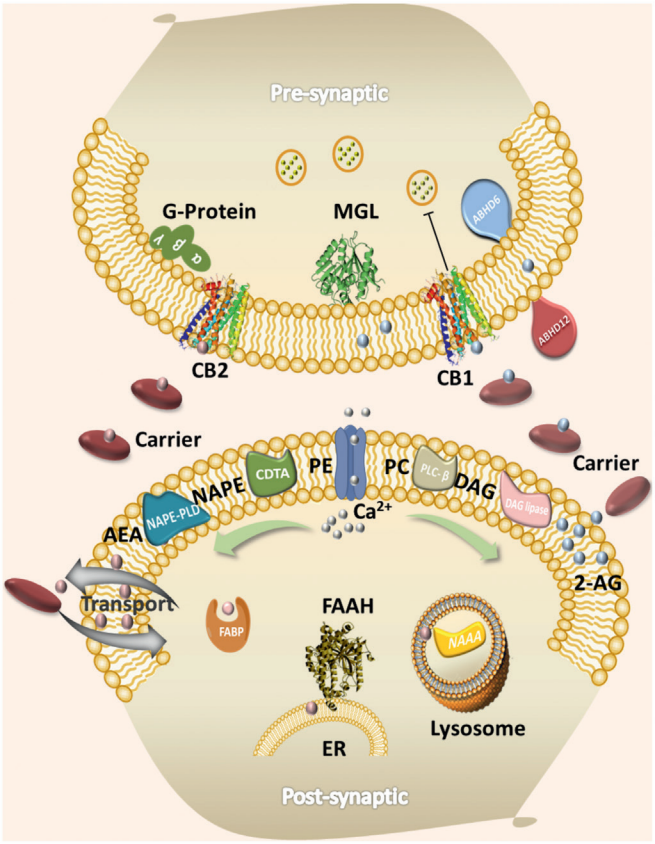

FIGURE 53.9 Schematic representation of the endocannabinoid signaling system. CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; FAAH, fatty acid amide hydrolase; MGL, monoacylglycerol lipase; ABHD6, α-β Hydrolase domain-containing protein 6; ABHD12, α-β Hydrolase domain-containing protein 12; NAPE, N-arachidonoyl phosphatidylethanolamine; PE, phosphatidylethanolamine; PC, phospholipase C; PD, phospholipase D; DGL, diacylglycerol lipase; FABP, fatty acid–binding protein; AEA, arachidonoylethanolamide; 2-AG, 2-Arachidonoylglycerol; ER, endoplasmic reticulum. *Source: Adapted from Vemuri and Makriyannis (2015).*

ions. Endocannabinoid levels also referred to as the "endocannabinoid tone" are tissue-dependent and are regulated by another set of enzymes, the most prominent of which are fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), whose role is to deactivate AEA and 2AG and their congeners, respectively. An additional feature in the regulation of endocannabinoid tone is a transport mechanism that is involved in transporting released endocannabinoids into the cell (Figure 53.9; Vemuri and Makriyannis, 2015).

These new discoveries opened the door for characterizing the endocannabinoid biochemical system (EBS) that encompasses two cannabinoid receptors, the proteins that modulate their function, and the endocannabinoid family of compounds. The EBS plays a major role in the regulation of many aspects of human physiology. It is well established by now that the CB1 cannabinoid receptor is the most abundant GPCR in the brain, but it is also present in many other organs such as the heart, blood vessels, liver, lungs, and the digestive system, as well as fat and sperm cells. However, the CB2 cannabinoid receptor is mostly found in immunity-related cells, and it is largely involved in regulating the immune system and playing a role in inflammatory conditions. Although under normal "homeostatic" conditions the CB2 receptors have a very low presence in the central nervous system (CNS), in "nonhomeostatic" situations such as inflammation, neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD), and ALS, and in cancers such as gliomas, their presence in the brain is dramatically increased in astrocytes as well as microglial and cerebromicrovascular endothelial cells. Recent published work has provided evidence that this receptor may also have a role in modulating addictive disorders (Xi et al., 2011).

It has been shown that many, although not all, of the effects of cannabis can be linked to the ubiquitous nature of the endocannabinoid system. This can explain the multitude of physiological and potentially therapeutic effects associated with smoking cannabis (Hill et al., 2012; Izzo et al., 2009; McPartland et al., 2015; Pertwee, 2008; Pertwee et al., 2007). The pharmacological profile of smoked marijuana presents many similarities to those of its key psychotropic constituent, Δ^9-THC (Hill et al., 2012; Russo, 2011). However, there is ample evidence that the two profiles do not completely overlap and that the overall experience of smoking the plant material is substantially different from that of using THC (Hill et al., 2012; Russo, 2011). For this reason there has been a major research effort to study the pharmacological properties of the herb's individual components (ElSohly and Slade, 2005).

The medicinal benefits of cannabis are derived from a variety of compounds produced naturally by the plant. These compounds can be grouped into several distinct compound classes based on structure, function, and pharmacological effect. The compound groups with the most significant body of evidence supporting therapeutic benefit are the cannabinoids and terpenes. These compounds may represent more than 20–30% of the dry cannabis flowers by weight. Another group of compounds with demonstrated therapeutic value is the flavonoids, which are generally present as a more minor fraction of cannabis (on average between 1% and 5% by weight).

Because the cannabis varieties have different phytocannabinoid and terpene compositions, as reflected in their distinct pharmacological profiles, it can be argued that overall modulation of the ratios of specific phytocannabinoids and terpenes may offer therapeutic potential dependent on the nature of the target disease.

A brief description of the pharmacological profiles of the phytocannabinoids, terpenes, and other beneficial

compounds is provided, with a particular emphasis on those that have undergone or are currently undergoing clinical trials and/or advanced preclinical evaluation with *in vivo* animal models of CNS diseases.

PHYTOCANNABINOIDS

(–)Δ^9-Tetrahydrocannabinol

Δ^9-THC is the key psychoactive phytocannabinoid and is known to have a broad spectrum of pharmacological properties. Within the EBS, Δ^9-THC is capable of activating both CB1 and CB2 receptors. THC has the highest potency at both CB1 and CB2 receptors of all the phytocannabinoids. However, it behaves as a partial agonist toward both receptors because it cannot induce their full activation to produce a maximal response. When compared to the endogenous cannabinoids, the CB1 affinities and potencies of Δ^9-THC are comparable to those of both AEA and 2AG. Δ^9-THC also resembles AEA in its functional properties (both are partial agonists) and differs from 2AG, which, as a full agonist, is capable of inducing a full response from both receptors. When tested in healthy mice or rats, Δ^9-THC is shown to suppress locomotor activity, produce a decrease in body temperature (hypothermia), induce catalepsy (immobility), and show analgesic properties, all of which are considered CB1-related effects. Additionally, Δ^9-THC has immunomodulating and anti-inflammatory properties that are produced through the activation of the CB2 receptor. Δ^9-THC is also effective in the treatment of certain disorders such as anorexia as well as nausea and emeses, and for the relief of neuropathic pain that accompanies multiple sclerosis and cancer (Hill et al., 2012; Pertwee, 2008). Currently, an orally administered medication of pure synthetic Δ^9-THC (dronabinol) is used in cancer chemotherapy-induced nausea and vomiting as well as for appetite stimulation in HIV/AIDS patients. More recently, a medication that predominantly contains plant-extracted Δ^9-THC and CBD (Sativex) is used for the relief of neuropathic pain in patients with multiple sclerosis and cancer (Hill et al., 2012; Pertwee, 2008; Russo, 2011).

(–)-Cannabidiol (CBD)

Except for Δ^9-THC, CBD is the cannabis component that has received the most attention. Unlike Δ^9-THC, CBD is not psychoactive. Furthermore, it displays low affinities for both CB1 and CB2 receptors and, for this reason, its cannabinoid receptor–related effects, until recently, had not received much attention. However, more recent studies of its functional properties revealed that it behaves as a CB1 receptor antagonist when tested in mouse brain membranes and other tissues with CB1 receptors (Hill et al., 2012; McPartland et al., 2015; Pertwee, 2008). Importantly, CBD was found to block several of the THC effects in mice, rats, rabbits, and humans and was also shown to antagonize the effects of known potent synthetic CB1 agonists. With regard to its effects on the CB2 receptor, CBD appears to behave as a low-efficacy agonist. It was also found to inhibit immune cell migration when tested in murine macrophage preparations (Hill et al., 2012; Pertwee, 2008). These *in vitro* observations may account for some of the anti-inflammatory effects observed in human studies.

Although the psychoactive properties of CBD are consistent with its effects on the two cannabinoid receptors, it has also become clear that other targets contribute to the compound's overall pharmacological profile. Among the ones to be considered are the various transient receptor potential (TRP) channels, most notably TRPV1, TRPA1, and TRPM8. The specific CBD contributions to these not yet fully characterized receptors are being studied.

Beyond the receptor-based biological properties of CBD, an important aspect of its potential therapeutic effects can be attributed to its strong antioxidant properties and its ability to interact with what are known as reactive oxygen species (ROS). These very reactive products of the oxygenation cycle interact with and damage several cellular components and contribute to inflammation. It can be argued that CBD's role in neutralizing ROS species is an important contribution to its well-known anti-inflammatory effects.

The antiepileptic effects of cannabis have been known for many centuries, having been recognized as Ayurvedic medicine for the treatment of epilepsy. A more detailed examination of cannabis constituents starting from the 1970s identified CBD's anticonvulsant properties in rats (Consroe and Wolkin, 1977) and performed initial testing in humans in small groups (Carlini and Cunha, 1981). This was followed by a larger human study using 900–1,200 mg/kg daily, which showed that CBD was effective in treating seizure states (Trembly and Sherman, 1990). Overall, CBD exhibits the most reliable anticonvulsant effects of cannabis constituents. In very recent reports describing its use in children experiencing epileptiform conditions, CBD was shown to greatly ameliorate these effects. Also, initial results from a broad clinical evaluation of CBD in a seizure-associated condition in children is very promising.

When tested in animal models, CBD was shown to have anxiolytic properties (Zuardi and Karniol, 1983), as confirmed in a number of studies involving humans. In an early study using healthy human volunteers subjected to a stressful public speaking test (SPST), a 300-mg dose reduced the volunteers' subjective anxiety to a level comparable to the standard anxiolytic diazepam (Zuardi

et al., 1993). Neuroimaging confirmed these effects in follow-up studies (Bergamaschi et al., 2011; Crippa et al., 2009). CBD has also been shown to ameliorate unwanted effects of THC and may be responsible for the improved properties of smoked cannabis compared to THC administration.

Ligresti et al. (2006) examined the antitumor effects of a variety of cannabinoids, including both neutral and acidic forms. While CBD was determined to be the most potent antitumor cannabinoid tested, CBDA was the least. The activity of CBD was due to its capability of inducing apoptosis via direct or indirect activation of cannabinoid CB2 and potential vanilloid type 1 receptors.

One potentially useful feature of the cannabinoids is the antimicrobial properties that could protect the plant and humans from disease. The prenyl-containing cannabinoids are highly effective against methicillin-resistant *Staphylococcus aureus* (MRSA) strains relevant to the clinical setting, with CBD being the most potent (Appendino et al., 2008). CBDA (Schultz and Haffner, 1958), CBC (Turner and Elsohly, 1981), and CBG (ElSohly et al., 1982) are powerful plant antibiotics and likely play a role in maintaining plant health.

(–) Δ^9-Tetrahydrocannabivarin (THCV)

THCV is the propyl analog of THC in which the 5-carbon side chain is shortened by two methylene units. Its pharmacological properties have been studied since the early 1970s, when it was recognized that it behaves as a significantly weaker agonist (approximate 5-fold) compared to THC.

Thus, as with THC, THCV produces analgesic but also cataleptic effects in animals (Hill et al., 2012; Pertwee, 2008). These effects can be antagonized by synthetic CB1 antagonists such as SR1716A or AM251. This property of being a weaker agonist than THC is consistent with extensive medicinal chemistry work showing the decreased potency of the cannabivarin family relative to the longer side chain analogs. Maximum potency is observed with side chains seven to eight carbons long (Makriyannis, 2014).

Although THCV can behave as a CB1 agonist *in vitro*, it can also act as a CB1 antagonist capable of blocking the effects of more potent synthetic CB1 agonists as well as THC and the endocannabinoids AEA and 2AG. For this reason, Δ^9-THCV can act as an *in vivo* CB1 antagonist.

At the CB2 receptor, Δ^9-THCV behaves as a partial agonist and is capable of activating this receptor, although only to a limited extent. Because CB2 receptor activation has been shown to be associated with attenuation of inflammation, the antiepileptic effects of this compound are currently being tested in humans.

Cannabigerol (CBG)

CBG is the principal precursor of phytocannabinoids in its acid form. This compound is nonpsychoactive and is a relatively weak partial agonist for both CB1 and CB2. Because of its low cannabinoid receptor potency, it can functionally antagonize the CB1 effects of THC. It has been shown to relieve intraocular pressure, which is potentially useful in the treatment of glaucoma. Additionally, its antioxidant and anti-inflammatory properties make it a potential candidate for inflammatory bowel disease. Recent evidence identifies CBG as a potential candidate for treatment of colon cancer (Ligresti et al., 2006).

Cannabichromene (CBC)

CBC is a nonpsychotropic phytocannabinoid shown to be a potent TRPA1 agonist and to have anti-inflammatory and antimicrobial properties.

Tetrahydrocannabinoic Acid (THCA) and Cannadiolic Acid (CBDA)

There is some information about Δ^9-THCA and CBDA, the two naturally nonpsychotropic precursors of Δ^9-THC and Δ^9-CBD, that shows that their therapeutic value is derived from mechanisms other than classical CB1/CB2 receptor binding (Ahmed et al., 2008). THCA *in vitro* is capable of modulating the functions of two TRP channel receptors acting as a potent TRPA1 agonist and TRPM8 antagonist and inhibits both cyclooxygenase enzymes, COX1 and COX2. When tested in rat models of nausea, THCA appears to be a better alternative for treating nausea and vomiting associated with cancer chemotherapy (Rock et al., 2014; Rock and Parker, 2013). THCA has also been shown to reduce levels of TNF-α *in vitro*, suggesting a mechanism for immune modulation (Ligresti et al., 2006). CBDA was shown to be a selective inhibitor of COX2 (Takeda et al., 2008), implying anti-inflammatory activity. Unlike its congener, CBDA effectively reduced anticipatory nausea and may be useful against acute nausea induced by chemotherapy (Rock and Parker, 2013).

Cannabinol (CBN)

CBN is a product of Δ^9-THC oxidation and was the first phytocannabinoid to be isolated in pure form. CBN can activate both CB1 and CB2 receptors with a potency approximately 10-fold lower than Δ^9-THC. It can thus be viewed as a weak Δ^9-THC relative (Izzo et al., 2009).

CANNABIS TERPENES

A comprehensive discussion of the many terpenes found in cannabis and their complex synergies with the

cannabinoids is beyond the scope of this chapter. A summary of the most abundant terpenes in *C. sativa*, as well as their effects on biological systems, is presented.

Myrcene

Myrcene, better known as the active sedating principle of hops and lemon grass, is also found in basil, mangos, and its namesake, *Myrcia sphaerocarpa*, a medicinal shrub from Brazil traditionally used to treat diabetes, diarrhea, dysentery, and hypertension (Ulbricht, 2011). In culinary and perfume use, myrcene's aroma is earthy, fruity, and clove-like; it is pungent in higher concentrations. Myrcene synergizes the activities of terpenes and other compounds in a variety of ways. One mechanism that would be of particular note in cannabis is its claimed effect on the permeability of cell membranes, particularly the blood–brain barrier (BBB), increasing transport of cannabinoids into the brain; however, perusal of claimed references in the popular literature shows a lack of hard data regarding brain transport. Myrcene has been shown to enhance transdermal absorption (Schmitt et al., 2009). It has a significant analgesic effect, which is blocked by the action of naloxone, an opioid antagonist, suggesting a mechanism of action through the opioid receptor (Rao et al., 1990). However, myrcene's lack of affinity for opioid receptors points to alpha 2-adrenoceptor–stimulated release of endogenous opiates. In contrast to morphine, no tolerance was observed after repeated dosing in rats (Lorenzetti et al., 1991). At very high doses, myrcene in mice was a sedative comparable to phenobarbital (Gurgel do Vale et al, 2002); the effect was increased by simultaneous administration of citral, a mixture of other terpenes. Al-Omari (2007) demonstrated that myrcene improved glucose tolerance in alloxan diabetic rats comparable to metformin, without an effect on glucose levels in normal rats. Myrcene also showed powerful anti-inflammatory and anticatabolic effects in a human chondrocyte model of osteoarthritis (Rufino et al., 2015). With inflammation underlying numerous diseases, myrcene is the subject of a broad array of current research.

β-Caryophyllene

β-Caryophyllene has the distinction of being the first known "dietary cannabinoid," a common component of food that has GRAS (Generally Recognized as Safe) status and is approved by the FDA for food use. β-Caryophyllene is the primary sesquiterpene contributing to the spiciness of black pepper; it is also a major constituent of cloves, hops, rosemary, copaiba, and cannabis. It was one of the first cannabis-derived compounds other than THC, CBD, and CBN shown to bind directly to endocannabinoid receptors (Gertsch, 2008). In fact, it was one of the first cannabis-derived compounds with a fundamentally different structure from the classical cannabinoids that interacts with the endocannabinoid system in humans. β-Caryophyllene is known to selectively bind to the CB2 receptor; therefore, it is sometimes also classed as an atypical cannabinoid (Gertsch et al., 2010). As described elsewhere in this chapter, CB1 is responsible for the psychoactive effects associated with certain cannabinoids such as THC. However, CB2, particularly in peripheral tissues in the body, is a therapeutic target for treatment of inflammation, pain, atherosclerosis, and osteoporosis (Gertsch, 2008; Gertsch et al., 2008). β-Caryophyllene has now been shown to be directly beneficial for colitis (Bento et al., 2011), osteoarthritis (Rufino et al., 2015), diabetes (Basha and Sankaranarayanan, 2014), cerebral ischemia (Chang et al., 2013), anxiety and depression (Bahi et al., 2014), liver fibrosis (Calleja et al., 2013; Mahmoud et al., 2014), and Alzheimer-like disease types (Cheng et al., 2014). In cancer studies, β-caryophyllene demonstrated synergy with the chemotherapy drug Paclitaxel on human tumor cell lines, and alone it stimulates apoptosis and suppresses tumor growth (Legault and Pichette, 2007). In a *Caenorhabditis elegans* model, β-caryophyllene modulated stress-related genes and extended the lifespan of the organism (Pant et al., 2014). Importantly, it has been shown to be orally bioavailable; therefore, it would provide an important medicinal benefit to oral cannabis preparations.

Limonene

Limonene is one of the most abundant terpenes in cannabis, and it may be found in concentrations as high as 16% of the essential oil fraction. Ubiquitous in citrus rind, limonene is a monoterpene commonly used in perfumes, household cleaners, food, and medicines. Limonene has numerous medicinal benefits demonstrated in human and animal studies. Limonene is among a number of plant essential oils that have been identified as having antioxidant and anticancer properties. Limonene has therefore been suggested as an excellent dietary source for cancer prevention (Aggarwal and Shishodia, 2006). Anxiolytic effects in a mouse maze model were comparable to diazepam, but not antagonized by flumazenil, implying a nonbenzodiazepine mechanism (Lima et al., 2013). This is in contrast to previous results that also demonstrated antidepressant activity via the 5-HT_{1A} receptor pathway (Komiya et al., 2006). Limonene has anti-inflammatory effects in models of osteoarthritis (Rufino et al., 2015) and asthma (Hirota et al., 2010, 2012). Multiple modes of anticancer activity were observed, including chemoprevention (Crowell and Gould, 1994). Limonene is metabolized into perillyl alcohol, which is also a subject of numerous cancer-related studies (Thomas Prates Ong et al., 2012).

Humulene

Humulene is the characteristic terpene of hops, *Humulus lupulus*, but it is also found in cannabis, sage, and ginseng. Humulene, also known as α-caryophyllene, is a ring-opened isomer of β-caryophyllene, which is notably lacking in CB2 activity. Nonetheless, it also possesses powerful anti-inflammatory activity equal to dexamethasone in an animal model (Fernandes et al., 2007). Humulene possesses both topical and systemic anti-inflammatory properties (Chaves et al., 2008) and is an effective analgesic when taken topically, orally, or by aerosol (Rogerio et al., 2009). Humulene can produce ROS, leading to an antineoplastic effect by inducing apoptosis; although at moderate levels, ROS can increase tumor growth. β-Caryophyllene was synergistic with humulene in one study (Legault and Pichette, 2007). Interestingly, humulene was shown to increase secretion of IL-8, a chemokine with various functions, including promoting angiogenesis, helpful in wound healing but not typically associated with anticancer compounds (Satsu et al., 2004).

FLAVONOIDS

Whole cannabis extracts contain approximately 20 flavonoids that contribute useful activity (McPartland and Russo, 2001; Turner et al., 1980). Volatile and lipophilic, many flavonoids retain activity in smoke (Sauer et al., 1983). Apigenin inhibits TNF-α (Gerritsen et al., 1995), a potentially disease-modifying treatment target in multiple sclerosis, rheumatoid arthritis, psoriasis, and other autoimmune diseases, and is also a potent inhibitor of CYP2C9 (Si et al., 2009), a liver enzyme that metabolizes numerous drugs, most relevantly THC. Also found in chamomile (*Matricaria recutita*), apigenin has high affinity for central benzodiazepine receptors, showing powerful anxiolytic activity comparable to diazepam but without the negative effect on memory and learning (Salgueiro et al., 1997). Cannflavin A, a flavone unique to cannabis, inhibits PGE_2 with 30 times the activity of aspirin (Barrett et al., 1986).

PHYTOSTEROLS

β-Sitosterol, a phytosterol found in cannabis, improves urinary symptoms and flow measures in benign prostatic hyperplasia (Wilt et al., 1999). It was also active in a model of skin diseases, reducing topical inflammation, and edema (Gómez et al., 1999).

C. SATIVA AS A NUTRACEUTICAL

The term "nutraceutical" was introduced to define ingredients that contain both nutritional and pharmaceutical properties; it was defined by Dr. Stephen DeFelice of the Foundation for Innovation in Medicine as, "Food, or parts of food, that provide medical or health benefits, including the prevention and treatment of disease." This broad view encompasses isolated nutrients, dietary supplements, botanicals, functional foods including prebiotics, and medicinal products thereof. However, nutraceuticals are clearly distinct from dietary supplements in that they not only supplement the diet but also aid in the prevention and/or treatment of disease (Kalra, 2003). *C. sativa* is unique in that many of its constituents can be classified as a pharmaceutical ingredient, nutrient, dietary supplement, or an herbal product.

Cannabis as Food

Cannabis seed, especially its oil, is particularly nutritious and is often consumed whole or used in food preparations. Whole hemp seed contains approximately 20–25% protein, 20–30% carbohydrates, and 10–15% insoluble fiber (Callaway, 2004; Deferne and Pate, 1996). In addition, it contains a mixture of the saturated fatty acids palmitic and stearic acid as well as oleic acid (Callaway, 2004; Leizer et al., 2000). Hemp seed oil is an extremely rich source of unsaturated fatty acids, especially the essential fatty acids linoleic acid (LA) and alpha-linolenic acid (LNA) (Callaway, 2004; Leizer et al., 2000). Essential fatty acids (EFAs) cannot be produced naturally by the human body and must be sourced from the diet—LA and LNA are omega-6 and omega-3 essential fatty acids, respectively, that are well known for their general health benefits. Hempseed oil also tends to contain high amounts of gamma-linolenic acid (GLA) and stearidonic acid (SDA), which are metabolites of LA and LNA (Callaway, 2004). Because these metabolites are produced by breakdown of dietary LA and LNA, they are not considered EFAs. However, supplementation in the diet can be extremely beneficial. Many chronic diseases of modern society, including cancer, diabetes, heart disease, arthritis, and AD, have an inflammatory component (Kapoor and Huang, 2006). Diets enriched in GLA have been shown to reduce inflammation (Tate et al., 1989); therefore, the nutritional value of GLA from hempseed oil is clear.

A common claim in popular literature is that cannabis seed oil has the proper ratio (2–3:1) of omega-6 to omega-3 acids for optimal human health. Although many benefits of supplementation have been observed, it seems a simple conclusion that consumption would lead to general health benefits. Supplementation levels are not high enough to correct an otherwise high omega-6 diet (cf. fish oil chapter). However, this claim is based on the hypothesized diet of Paleolithic humans and the reality is more complicated, with health effects depending on the individual and specific health concern

in question. For example, a ratio of 4:1 was associated with a 70% decrease in total mortality from cardiovascular disease, whereas in colorectal cancer a decrease in rectal cell proliferation was only observed when the ratio decreased to 2.5:1. A ratio of 5:1 was beneficial in asthma (Simopoulos, 2002).

Historical/Culture Uses

Throughout history, cannabis has been consumed in various ways that have provided therapeutic benefits. Cannabis preparations have been used for home remedies, as medicine, as a functional food, and as a source of nutrition.

In India, a number of oral preparations of cannabis are prevalent—these may be known as "bhang," "ghota," "thandai," or "pang" (Chopra and Chopra, 1957). The simplest bhang beverages are prepared by pounding cannabis leaves with black pepper, sugar, and water. Other added ingredients may include milk, ghee, almonds, and other herbs and spices. Although bhang is often consumed for its intoxicating properties, it has also been used for medicinal purposes. Bhang beverages have often been used for treatment of a variety of gastrointestinal and rheumatic issues, which may range from simple digestive improvement and appetite stimulation to gonorrhea and dysuria and to diarrhea and dysentery. Other medicinal uses of bhang as a household remedy include pain relief, prevention of malaria, and improvement of nervous diseases and/or epileptic disorders (Chopra and Chopra, 1957; Touw, 1981). Although many oral preparations of bhang are prevalent, such as beverages, curry, syrups, and even ice creams, other modes of application of bhang are described, for instance, as a poultice, tincture, or alcoholic extraction.

The use of cannabis in Ayurvedic medicine dates back many centuries. Early Indian texts describe cannabis as one of five sacred plants used for "freedom from distress" (Aldrich and Mathre, 1997). Cannabis has numerous known uses in Ayurvedic medicine, including sleep aid, appetite stimulant, analgesic, excitant, aphrodisiac, antiphlegmatic, and intoxicant (Aldrich and Mathre, 1997; Touw, 1981). Cannabis is described in one of the earliest Indian medical texts, the *Sushruta Samhita*, with its use characterized as an antiphlegmatic (Touw, 1981). Its medicinal benefit would thus include its value as an expectorant and diuretic; however, in this cultural context, phlegmatic tendencies also included sluggishness of energy and thought, and so the use of cannabis to "enliven" the spirit was considered another important therapeutic benefit. Although the *Sushruta Samhita* is the first documented evidence of medicinal cannabis use in India, dating back to 400–600 BC, through time its use in Indian (Ayurvedic) and Moslem (Unani) medicine has spread (Touw, 1981). Both agreed that cannabis increased digestion and appetite, and both used it as an antispasmodic and anticonvulsive. There are more cannabis-related preparations and uses in common Ayurvedic practice than are found in the Ayurvedic pharmacopeia (Dwarakanath, 1965; Touw, 1981). It has been described as the "penicillin of Ayurvedic medicine" (Touw, 1981).

Cannabis was a staple crop in ancient China, where it was valued as food, fiber, and also as a medicine (Aldrich and Mathre, 1997; Li, 1973; Touw, 1981). As a food crop, cannabis seed was one of the major grains cultivated to produce cooking oil in ancient China (Li, 1973). Along with millet, soybean, rice, and barley, hempseed was considered one of the five major grains in Chinese culture. The medicinal properties of cannabis were also well known in the early Chinese pharmacopeia. Some of the earliest pharmacopeia in the world listed more than 100 ailments treated by cannabis, including gout, rheumatism, malaria, constipation, and diarrhea. Other historical Chinese medical texts described its use as an antibiotic and anthelmintic, and for the treatment of leprosy (Li, 1973). Although less prevalent, there are also references to the medicinal value of *C. sativa* in the Persian culture.

Juicing of cannabis leaves and flowers has been used historically and is gaining in popularity as a method of preparation and consumption. The cold extraction of raw cannabis juice results in preparations with much higher proportions of naturally occurring acidic cannabinoids such as THCA and CBDA. Cold extraction precludes the decarboxylation of these acidic forms to their neutral counterparts, a reaction that typically occurs at temperatures required for combustion (smoking), vaporization, or hot extraction. Because THCA has a significantly lower affinity for the endogenous cannabinoid receptor CB1, such preparations will typically not have the same psychoactivity as preparations containing neutral THC. The ingestion of unheated cannabis preparations would therefore allow one to administer significantly higher doses of acidic cannabinoids without the concomitant psychoactive effects of THC. Despite these limitations, cold extracts of raw cannabis (i.e., cannabis juice) would have the advantage of retaining a larger percentage of terpenes and other volatile compounds, which also have important medicinal properties, as described herein, and which are typically lost when using extraction or preparation methods involving heat. It is therefore clear that different cannabis preparations may have different medicinal benefits as a nutraceutical.

GENERAL HEALTH BENEFITS

Compounds found in cannabis are known to provide a number of general health benefits that are not necessarily disease-specific. Consumption of cannabis as a

nutraceutical should therefore provide general improvement of health. For instance, certain cannabinoids and terpenes have been shown to have antioxidant and/or neuroprotective properties. They have also been shown to be effective against inflammation, which is an underlying factor in many types of disease. Such compounds may also provide beneficial effects on metabolic pathways, which may be useful for conditions related to diabetes, metabolic syndrome, and obesity.

Inflammation

Inflammation is a highly prevalent condition that contributes to the development and progression of many diseases and conditions. Compounds found in Cannabis that reduce inflammation are abundant and diverse. The most abundant phytocannabinoids in cannabis, THC and CBD, both have strong anti-inflammatory properties, whereas CBC, CBG, and THCV have also demonstrated anti-inflammatory properties. Among the terpenes, α-pinene, β-myrcene, and β-caryophyllene appear to act through prostaglandin receptors (PGE1 and/or PGE2) to have an anti-inflammatory effect.

Neuroprotection

Certain cannabinoids and terpenes have been demonstrated as neuroprotectants. The neurotransmitter glutamate is released during periods of ischemia and other traumatic brain events. In excess, glutamate itself is toxic and can lead to neuronal cell death in a process known as excitotoxic stress. Compounds with antioxidant properties are often neuroprotective, for instance, through reduction of toxic ROS produced during ischemic metabolism. Both THC and CBD have been shown to have antioxidant properties (Hampson et al., 1998). Hampson et al. (1998) also demonstrated that THC and CBD are both able to prevent glutamate-induced neurotoxicity. Interestingly, the neuroprotective effect of these compounds was found to be independent of their CB receptor binding activity. THC and CBD were both found to reduce ROS *in vitro*, with similar potency to known antioxidants such as ascorbate and butylated hydroxytoluene. CBD has been shown to protect against cerebral ischemic injury (Hayakawa et al., 2008), and also attenuates AD–related neuroinflammation in animal models (Esposito et al., 2007).

Certain terpenes also have strong antioxidant properties and may be useful neuroprotectants. In a mouse study of cerebral ischemia, Ciftci et al. (2011) demonstrated that β-myrcene protected against oxidative stress and histological damage induced by ischemia-reperfusion, and is thus an effective neuroprotectant. The compound is suggested as a good candidate for treatment of ischemic stroke.

Obesity

Certain cannabinoids have demonstrated effects that may be useful for obesity treatment and prevention. The cannabinoid THCV has been shown to produce weight loss and decreased body fat and serum leptin concentrations in obese mice (Riedel et al., 2009; Wargent et al., 2013). While seemingly paradoxical, Le Foll et al. (2013) found that the prevalence of obesity is lower in regular cannabis users compared to nonusers, even after adjusting for important variables such as age, sex, and tobacco smoking status. Additional support for this idea comes from recent work by Silvestri et al. (2015) in which THCV and CBD were both shown to reduce accumulated lipid levels in adipocytes and in a model of hepatosteatosis.

Antioxidant

Plant antioxidants are important for human health and include compounds such as ascorbic acid, tocopherols, polyphenolic compounds, and terpenes. Numerous mono- and sesquiterpenes have antioxidant properties, and the essential oils of a multitude of plant species have been tested for their antioxidant properties—this includes many plant species that are rich in terpenes, which are abundant in cannabis. Terpenes in cannabis with demonstrated antioxidant properties include β-caryophyllene (Calleja et al., 2013), limonene, and β-myrcene (Ciftci et al., 2011). Several cannabinoids also have demonstrated antioxidant properties, including THC and CBD (Hampson et al., 1998), as well as CBG (Borrelli et al., 2013).

CONCLUDING REMARKS AND FUTURE DIRECTION

C. sativa is the quintessential nutraceutical product—its seeds provide rich nutritional value and are a source of essential omega-3 and omega-6 fatty acids. Of course, dried flower products and other extracts derived from them have been consumed both medicinally and recreationally throughout history. The plant is held sacred by numerous cultures and people throughout the world, which speaks to its value to human kind, who will continue to use this nutraceutical to its full advantage.

Over recent decades, empirical evidence has provided tremendous insight into the machinery used by *C. sativa* to produce these compounds, as well as their application in disease therapy. Although there is much to learn about the complex EBS, it is clear that the components of *C. sativa* exhibit a wide range of actions. This chapter summarizes the literature available to date and provides a reference for cannabis scholars and enthusiasts working in the era of legalized cannabis.

Acknowledgments

The authors thank Osvaldo Marinotti for his insightful comments and contributions while reviewing this chapter.

References

Aggarwal, B.B., Shishodia, S., 2006. Molecular targets of dietary agents for prevention and therapy of cancer. Biochem. Pharmacol. 71 (10), 1397–1421.

Ahmed, S.A., Ross, S.A., Slade, D., et al., 2008. Cannabinoid ester constituents from high-potency *Cannabis sativa*. J. Nat. Prod. 71 (4), 536–542.

Aldrich, M., Mathre, M., 1997. History of Therapeutic Cannabis. Cannabis in Medical Practice. McFarland, Jefferson, NC, pp. 35–55.

Al-Omari, S.M., 2007. The Effect of Thujone and Myrcene on Diabetes Mellitus in Albino Rats. (MS), University of Jordan.

Anderson, L.C., 1980. Leaf variation among *Cannabis* species from a controlled garden.. Bot. Mus. Leaf. Harv. Univ. 28 (1), 61–69.http://dx.doi.org/10.2307/41762825

Appendino, G., Gibbons, S., Giana, A., et al., 2008. Antibacterial cannabinoids from *Cannabis sativa*: a structure – activity study. J. Nat. Prod. 71 (8), 1427–1430.

Appendino, G., Chianese, G., Taglialatela-Scafati, O., 2011. Cannabinoids: occurrence and medicinal chemistry. Curr. Med. Chem 18 (7), 1085–1099.

Bahi, A., Al Mansouri, S., Al Memari, E., et al., 2014. β-Caryophyllene, a CB 2 receptor agonist produces multiple behavioral changes relevant to anxiety and depression in mice. Physiol. Behav. 135, 119–124.

Barni-Comparini, I., Ferri, S., Centini, F., 1984. Cannabinoid level in the leaves as a tool for the early discrimination of Cannabis chemiovariants. Forens. Sci. Intl. 24 (1), 37–42.

Barrett, M.L., Scutt, A.M., Evans, F.J., 1986. Cannflavin A and B, prenylated flavones from *Cannabis sativa* L. Experientia 42 (4), 452–453.

Basha, R.H., Sankaranarayanan, C., 2014. β-Caryophyllene, a natural sesquiterpene, modulates carbohydrate metabolism in streptozotocin-induced diabetic rats. Acta Histochem. 116 (8), 1469–1479.

Bento, A.F., Marcon, R., Dutra, R.C., et al., 2011. β-Caryophyllene inhibits dextran sulfate sodium-induced colitis in mice through CB2 receptor activation and PPARγ pathway. Am. J. Pathol. 178 (3), 1153–1166.

Bergamaschi, M.M., Queiroz, R.H.C., Chagas, M.H.N., et al., 2011. Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naive social phobia patients. Neuropsychopharmacology 36 (6), 1219–1226.

Bertoli, A., Tozzi, S., Pistelli, L., et al., 2010. Fibre hemp inflorescences: from crop-residues to essential oil production. Ind. Crops Prod. 32 (3), 329–337.

Borrelli, F., Fasolino, I., Romano, B., et al., 2013. Beneficial effect of the non-psychotropic plant cannabinoid cannabigerol on experimental inflammatory bowel disease. Biochem. Pharmacol. 85 (9), 1306–1316.

Brenneisen, R., 2007. Chemistry and analysis of phytocannabinoids and other Cannabis constituents Marijuana and the Cannabinoids. Springer., pp. 17–49.

Brenneisen, R., ElSohly, M.A., 1988. Chromatographic and spectroscopic profiles of Cannabis of different origins: part I. J. Forens. Sci. 33 (6), 1385–1404.

Broséus, J., Anglada, F., Esseiva, P., 2010. The differentiation of fibre- and drug type Cannabis seedlings by gas chromatography/mass spectrometry and chemometric tools. Forens. Sci. Intl. 200 (1–3), 87–92.

Callaway, J.C., 2004. Hempseed as a nutritional resource: an overview. Euphytica 140 (1–2), 65–72.

Calleja, M.A., Vieites, J.M., Montero-Meterdez, T., et al., 2013. The antioxidant effect of β-caryophyllene protects rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation. Brit. J. Nutr. 109 (03), 394–401.

Cappelletto, P., Brizzi, M., Mongardini, F., et al., 2001. Italy-grown hemp: yield, composition and cannabinoid content. Ind. Crops Prod. 13 (2), 101–113.

Carlini, E.A., Cunha, J.M., 1981. Hypnotic and antiepileptic effects of cannabidiol. J. Clin. Pharmacol. 21 (S1), 417S–427S.

Chang, H.-J., Kim, J.-M., Lee, J.-C., et al., 2013. Protective effect of β-caryophyllene, a natural bicyclic sesquiterpene, against cerebral ischemic injury. J. Med. Food 16 (6), 471–480.

Chaves, J.S., Leal, P.C., Pianowisky, L., et al., 2008. Pharmacokinetics and tissue distribution of the sesquiterpene alpha-humulene in mice. Planta Med. 74 (14), 1678–1683.

Cheng, Y., Dong, Z., Liu, S., 2014. β-Caryophyllene ameliorates the Alzheimer-like phenotype in APP/PS1 mice through CB2 receptor activation and the PPARγ pathway. Pharmacology 94 (1–2), 1–12.

Chopra, I.C., Chopra, R.N., 1957. The use of the cannabis drugs in India. Bull. Narcot. 9 (1), 4–29.

Ciftci, O., Ozdemir, I., Tanyildizi, S., et al., 2011. Antioxidative effects of curcumin, β-myrcene and 1, 8-cineole against 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin-induced oxidative stress in rats liver. Toxicol. Ind. Health 27 (5), 447–453.

Consroe, P., Wolkin, A., 1977. Cannabidiol – antiepileptic drug comparisons and interactions in experimentally induced seizures in rats. J. Pharmacol. Exp. Therap. 201 (1), 26–32.

Crippa, J.A., Zuardi, A.W., Martín-Santos, R., et al., 2009. Cannabis and anxiety: a critical review of the evidence. Hum. Psychopharmacol. Clin. Exp. 24 (7), 515–523.

Crowell, P.L., Gould, M.N., 1994. Chemoprevention and therapy of cancer by d-Limonene. CRC Crit. Rev. Oncol. 5 (1), 1–22.

Dayanandan, P., Kaufman, P.B., 1976. Trichomes of *Cannabis sativa L.* (Cannabaceae). Am. J. Bot. 63 (5), 578–591.

Deferne, J.-L., Pate, D.W., 1996. Hemp seed oil: a source of valuable essential fatty acids. J. Int. Hemp. Assoc. 3 (1), 4–7.

Directive, C., 2013. Common catalogue of varieties of agricultural plant species. Off. J. Eur. Union 379 (1).

Dittrich, H., Kutchan, T.M., 1991. Molecular cloning, expression, and induction of berberine bridge enzyme, an enzyme essential to the formation of benzophenanthridine alkaloids in the response of plants to pathogenic attack. Proc. Nat. Acad. Sci. U.S.A. 88 (22), 9969–9973.

Drugs, U.N.O.O., 2009. Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products. United Nations Publications.

Dwarakanath, C., 1965. Use of opium and cannabis in the traditional systems of medicine in India. Bull. Narcot. 17 (1), 15–19.

ElSohly, H.N., Turner, C.E., Clark, A.M., et al., 1982. Synthesis and antimicrobial activities of certain cannabichromene and cannabigerol related compounds. J. Pharmaceut. Sci. 71 (12), 1319–1323.

ElSohly, M.A., Ross, S.A., Mehmedic, Z., et al., 2000. Potency trends of delta9-THC and other cannabinoids in confiscated marijuana from 1980–1997. J. Forens. Sci. 45 (1), 24–30.

ElSohly, M.A., Slade, D., 2005. Chemical constituents of marijuana: the complex mixture of natural cannabinoids. Life Sci. 78 (5), 539–548.

Esposito, G., Scuderi, C., Savani, C., et al., 2007. Cannabidiol *in vivo* blunts β-amyloid induced neuroinflammation by suppressing IL-1β and iNOS expression. Brit. J. Pharmacol. 151 (8), 1272–1279.

Fellermeier, M., Eisenreich, W., Bacher, A., et al., 2001. Biosynthesis of cannabinoids. Eur. J. Biochem. 268 (6), 1596–1604.

Fernandes, E.S., Passos, G.F., Medeiros, R., et al., 2007. Anti-inflammatory effects of compounds alpha-humulene and (–)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. Eur. J. Pharmacol. 569 (3), 228–236.

Fischedick, J.T., Hazekamp, A., Erkelens, T., et al., 2010. Metabolic fingerprinting of *Cannabis sativa* L., cannabinoids and terpenoids for chemotaxonomic and drug standardization purposes. Phytochemistry 71 (17–18), 2058–2073.

Gagne, S.J., Stout, J.M., Liu, E., et al., 2012. Identification of olivetolic acid cyclase from *Cannabis sativa* reveals a unique catalytic route to plant polyketides. Proc. Nat. Acad. Sci. U.S.A. 109 (31), 12811–12816.

Gerritsen, M.E., Carley, W.W., Ranges, G.E., et al., 1995. Flavonoids inhibit cytokine-induced endothelial cell adhesion protein gene expression. Am. J. Pathol. 147 (2), 278–292.

Gertsch, J., 2008. Anti-inflammatory cannabinoids in diet – towards a better understanding of CB2 receptor action? Towards a better understanding of CB2 receptor action? Commun. Integr. Biol. 1 (1), 26–28.

Gertsch, J., Leonti, M., Raduner, S., et al., 2008. Beta-caryophyllene is a dietary cannabinoid. Proc. Natl. Acad. Sci. U.S.A. 105 (26), 9099–9104.

Gertsch, J., Pertwee, R.G., Di Marzo, V., 2010. Phytocannabinoids beyond the Cannabis plant–do they exist? Brit. J. Pharmacol. 160 (3), 523–529.

Gómez, M.T.S., García, M.D., Fernández, M.A., 1999. Study of the topical anti-inflammatory activity of *Achillea ageratum* on chronic and acute inflammation models. Zeitschrift für Naturforschung C 54c (11), 937–941.

Gurgel do Vale, T., Couto Furtado, E., Santos Jr, J.G., et al., 2002. Central effects of citral, myrcene and limonene, constituents of essential oil chemotypes from *Lippia alba* (Mill.) N.E. Brown. Phytomedicine 9 (8), 709–714.

Hampson, A., Grimaldi, M., Axelrod, J., et al., 1998. Cannabidiol and (–)Δ9-tetrahydrocannabinol are neuroprotective antioxidants. Proc. Natl. Acad. Sci. U.S.A. 95 (14), 8268–8273.

Hayakawa, K., Mishima, K., Irie, K., et al., 2008. Cannabidiol prevents a post-ischemic injury progressively induced by cerebral ischemia via a high-mobility group box1-inhibiting mechanism. Neuropharmacology 55 (8), 1280–1286.

Hellerman, C., 2013. Is Super Weed, Super Bad? CNN News. Available from: http://www.cnn.com/2013/08/09/health/weed-potency-levels/.

Hill, A.J., Williams, C.M., Whalley, B.J., et al., 2012. Phytocannabinoids as novel therapeutic agents in CNS disorders. Pharmacol. Therap. 133 (1), 79–97.

Hillig, K.W., 2004. A chemotaxonomic analysis of terpenoid variation in Cannabis. Biochem. Systemat. Ecol. 32 (10), 875–891.

Hillig, K.W., Mahlberg, P.G., 2004. A chemotaxonomic analysis of cannabinoid variation in *Cannabis* (Cannabaceae). Am. J. Bot. 91 (6), 966–975.

Hirota, R., Roger, N.N., Nakamura, H., et al., 2010. Anti-inflammatory effects of limonene from Yuzu (citrus junos tanaka) essential oil on eosinophils. J. Food Sci. 75 (3), H87–H92.

Hirota, R., Nakamura, H., Bhatti, S.A., et al., 2012. Limonene inhalation reduces allergic airway inflammation in Dermatophagoides farinae-treated mice. Inhal. Toxicol. 24 (6), 373–381.

Izzo, A.A., Borrelli, F., Capasso, R., et al., 2009. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. Trends Pharmacol. Sci. 30 (10), 515–527.

Kalra, E., 2003. Nutraceutical-definition and introduction. AAPS Pharm. Sci. 5 (3), 27–28.

Kapoor, R., Huang, Y.-S., 2006. Gamma linolenic acid: an anti-inflammatory omega-6 fatty acid. Curr. Pharmaceut. Biotechnol. 7 (6), 531–534.

Komiya, M., Takeuchi, T., Harada, E., 2006. Lemon oil vapor causes an anti-stress effect via modulating the 5-HT and DA activities in mice. Behav. Brain Res. 172 (2), 240–249.

Lamarck, J.-B., 1811. Encyclopédie méthodique, botanique: Suppl. 2: Panckoucke.

Le Foll, B., Trigo, J.M., Sharkey, K.A., et al., 2013. Cannabis and Δ9-tetrahydrocannabinol (THC) for weight loss? Med. Hypoth. 80 (5), 564–567.

Legault, J., Pichette, A., 2007. Potentiating effect of β-caryophyllene on anticancer activity of α-humulene, isocaryophyllene and paclitaxel. J. Pharm. Pharmacol. 59 (12), 1643–1647.

Leizer, C., Ribnicky, D., Poulev, 2000. The composition of hemp seed oil and its potential as an important source of nutrition. J. Nutraceut. Funct. Med. Foods 2 (4), 35–54.

Li, H.-L., 1973. An archaeological and historical account of cannabis in China. Econ. Bot. 28 (4), 437–448.

Ligresti, A., Moriello, A.S., Starowicz, K., et al., 2006. Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. J. Pharmacol. Exper. Therap. 318 (3), 1375–1387.

Lima, N.G.P.B., De Sousa, D.P., Pimenta, F.C.F., et al., 2013. Anxiolytic-like activity and GC–MS analysis of (R)-(+)-limonene fragrance, a natural compound found in foods and plants. Pharmacol. Biochem. Behav. 103 (3), 450–454.

Linnaeus, C., 1800. Species Plantarum, vol. 4. Impensis GC Nauk.

Lorenzetti, B.B., Souza, G.E.P., Sarti, S.J., et al., 1991. Myrcene mimics the peripheral analgesic activity of lemongrass tea. J. Ethnopharmacol. 34 (1), 43–48.

Mahmoud, M.F., El Swefy, S., Hasan, R.A., et al., 2014. Role of cannabinoid receptors in hepatic fibrosis and apoptosis associated with bile duct ligation in rats. Eur. J. Pharmacol. 742, 118–124.

Makriyannis, A., 2014. 2012 Division of medicinal chemistry award address. Tracking the cannabinoid road: a personal perspective. J. Med. Chem. 57 (10), 3891–3911.

Marks, M.D., Tian, L., Wenger, J.P., et al., 2009. Identification of candidate genes affecting Δ9-tetrahydrocannabinol biosynthesis in *Cannabis sativa*. J. Exper. Bot., erp210.

McPartland, J.M., Russo, E.B., 2001. Cannabis and cannabis extracts: greater than the sum of their parts? J. Cannabis Therap. 1 (3–4), 103–132.

McPartland, J.M., Duncan, M., Di Marzo, V., et al., 2015. Are cannabidiol and Δ9-tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. Brit. J. Pharmacol. 172 (3), 737–753.

Mechanics, P., 1938. New billion-dollar crop. Popular Mechanics pp. 238–238.

Mediavilla, V., Steinemann, S., 1997. Essential oil of *Cannabis sativa* L. strains. J. Int. Hemp. Assoc. 4, 80–82.

Mehmedic, Z., Chandra, S., Slade, D., et al., 2010. Potency trends of Δ9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. J. Forens. Sci. 55 (5), 1209–1217.

Morimoto, S., Komatsu, K., Taura, F., et al., 1997. Enzymological evidence for cannabichromenic acid biosynthesis. J. Nat. Prod. 60 (8), 854–857.

Novak, J., Zitterl-Eglseer, K., Deans, S.G., et al., 2001. Essential oils of different cultivars of *Cannabis sativa* L. and their antimicrobial activity. Flav. Fragr. J. 16 (4), 259–262.

Pant, A., Saikia, S.K., Shukla, V., et al., 2014. Beta-caryophyllene modulates expression of stress response genes and mediates longevity in Caenorhabditis elegans. Exp. Gerontol. 57, 81–95.

Pertwee, R.G., 2000. Cannabinoid receptor ligands: clinical and neuropharmacological considerations, relevant to future drug discovery and development. Exp. Opin. Investig. Drugs 9 (7), 1553–1571.

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

Pertwee, R.G., Thomas, A., Stevenson, L.A., et al., 2007. The psychoactive plant cannabinoid, Δ9-tetrahydrocannabinol, is antagonized by Δ8- and Δ9-tetrahydrocannabivarin in mice *in vivo*. Brit. J. Pharmacol. 150 (5), 586–594.

Radwan, M.M., ElSohly, M.A., Slade, D., et al., 2008. Non-cannabinoid constituents from a high potency *Cannabis sativa* variety. Phytochemistry 69 (14), 2627–2633.

Rao, V.S.N., Menezes, A.M.S., Viana, G.S.B., 1990. Effect of myrcene on nociception in mice. J. Pharm. Pharmacol. 42 (12), 877–878.
Riedel, G., Fadda, P., McKillop-Smith, S., et al., 2009. Synthetic and plant-derived cannabinoid receptor antagonists show hypophagic properties in fasted and non-fasted mice. Brit. J. Pharmacol. 156 (7), 1154–1166.
Rock, E.M., Parker, L.A., 2013. Effect of low doses of cannabidiolic acid and ondansetron on LiCl-induced conditioned gaping (a model of nausea-induced behaviour) in rats. Brit. J. Pharmacol. 169 (3), 685–692.
Rock, E.M., Limebeer, C.L., Navaratnam, R., et al., 2014. A comparison of cannabidiolic acid with other treatments for anticipatory nausea using a rat model of contextually elicited conditioned gaping. Psychopharmacology 231 (16), 3207–3215.
Rogerio, A.P., Andrade, E.L., Leite, D.F., et al., 2009. Preventive and therapeutic anti-inflammatory properties of the sesquiterpene α-humulene in experimental airways allergic inflammation. Brit. J. Pharmacol. 158 (4), 1074–1087.
Ross, S.A., ElSohly, M.A., 1996. The volatile oil composition of fresh and air-dried buds of *Cannabis sativa*. J. Nat. Prod. 59 (1), 49–51.
Rufino, A.T., Ribeiro, M., Sousa, C., et al., 2015. Evaluation of the anti-inflammatory, anti-catabolic and pro-anabolic effects of E-caryophyllene, myrcene and limonene in a cell model of osteoarthritis. Eur. J. Pharmacol. 750 (0), 141–150.
Russo, E.B., 2011. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. Brit. J. Pharmacol. 163 (7), 1344–1364.
Rustichelli, C., Ferioli, V., Baraldi, M., et al., 1998. Analysis of cannabinoids in fiber hemp plant varieties (*Cannabis sativa* L.) by high-performance liquid chromatography. Chromatographia 48 (3-4), 215–222.
Salgueiro, J.B., Ardenghi, P., Dias, M., et al., 1997. Anxiolytic natural and synthetic flavonoid ligands of the central benzodiazepine receptor have no effect on memory tasks in rats. Pharmacol. Biochem. Behav. 58 (4), 887–891.
Satsu, H., Matsuda, T., Toshimitsu, T., et al., 2004. Regulation of interleukin-8 secretion in human intestinal epithelial Caco-2 cells by α-humulene. Biofactors 21 (1–4), 137–139.
Sauer, M.A., Rifka, S.M., Hawks, R.L., et al., 1983. Marijuana: interaction with the estrogen receptor. J. Pharmacol. Exper. Therap. 224 (2), 404–407.
Schmitt, S., Schaefer, U.F., Doebler, L., et al., 2009. Cooperative interaction of monoterpenes and phenylpropanoids on the *in vitro* human skin permeation of complex composed essential oils. Planta Med. 75 (13), 1381–1385.
Schultes, R.E., Klein, W.M., Plowman, T., et al., 1974. Cannabis: An Example of Taxonomic Neglect. Botanical Museum Leaflets. *Harvard University*., pp. 337–367.
Schultz, O.E., Haffner, G.A., 1958. A sedative and antibacterial principle in German hemp (*Cannabis sativa*). Naturforsch 14b, 98–100.
Sell, C., 2009. Handbook of Essential Oils: Science, Technology, and Applications. CRC Press, New York, NY.
Shoyama, Y., Hirano, H., Nishioka, I., 1984. Biosynthesis of propyl cannabinoid acid and its biosynthetic relationship with pentyl and methyl cannabinoid acids. Phytochemistry 23 (9), 1909–1912.
Shoyama, Y., Takeuchi, A., Taura, F., et al., 2005. Crystallization of 1-tetrahydrocannabinolic acid (THCA) synthase from *Cannabis sativa*. Acta Crystallographica Sect. F: Struct. Biol. Crystall. Commun. 61 (8), 799–801.
Shoyama, Y., Yagi, M., Nishioka, I., et al., 1975. Biosynthesis of cannabinoid acids. Phytochemistry 14 (10), 2189–2192.
Si, D., Wang, Y., Zhou, Y.-H., Guo, Y., et al., 2009. Mechanism of CYP2C9 inhibition by flavones and flavonols. Drug Metab. Dispos. 37 (3), 629–634.
Silvestri, C., Paris, D., Martella, A., et al., 2015. Two non-psychoactive cannabinoids reduce intra-cellular lipid levels and inhibit hepatosteatosis. J. Hepatol..
Simopoulos, A.P., 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed. Pharmacother. 56 (8), 365–379.
Sirikantaramas, S., Morimoto, S., Shoyama, Y., et al., 2004. The gene controlling marijuana psychoactivity. Molecular cloning and heterologous expression of Δ1-tetrahydrocannabinolic acid synthase from *Cannabis sativa* L. J. Biol. Chem. 279 (38), 39767–39774.
Sirikantaramas, S., Taura, F., Morimoto, S., et al., 2007. Recent advances in *Cannabis sativa* research: biosynthetic studies and its potential in biotechnology. Curr. Pharmaceut. Biotechnol. 8 (4), 237–243.
Sirikantaramas, S., Taura, F., Tanaka, Y., et al., 2005. Tetrahydrocannabinolic acid synthase, the enzyme controlling marijuana psychoactivity, is secreted into the storage cavity of the glandular trichomes. Plant Cell Physiol. 46 (9), 1578–1582.
Small, E., 1975. American law and the species problem in *Cannabis*: science and semantics. Bull. Narcot. 27 (3), 1–20.
Small, E., Marcus, D., 2002. Hemp: a new crop with new uses for North America. Trends New Crops New Uses, 284–326.
Small, E., Marcus, D., 2003. Tetrahydrocannabinol levels in hemp (*Cannabis sativa*) germplasm resources. Econ. Bot. 57 (4), 545–558.
Takeda, S., Misawa, K., Yamamoto, I., et al., 2008. Cannabidiolic acid as a selective cyclooxygenase-2 inhibitory component in cannabis. Drug Metab. Dispos. 36 (9), 1917–1921.
Tate, G., Mandell, B., Laposata, M., et al., 1989. Suppression of acute and chronic inflammation by dietary gamma linolenic acid. J. Rheumatol. 16 (6), 729–734.
Taura, F., Morimoto, S., Shoyama, Y., 1996. Purification and characterization of cannabidiolic-acid synthase from *Cannabis sativa* L. Biochemical analysis of a novel enzyme that catalyzes the oxidocyclization of cannabigerolic acid to cannabidiolic acid. J. Biol. Chem. 271 (29), 17411–17416.
Taura, F., Sirikantaramas, S., Shoyama, Y., et al., 2007. Phytocannabinoids in *Cannabis sativa*: recent studies on biosynthetic enzymes. Chem. Biodiver 4 (8), 1649–1663.
Thomas Prates Ong, M.T.C., de Conti, A., Moreno, F.S., 2012. Chemoprevention of hepatocarcinogenesis with dietary isoprenic derivatives: cellular and molecular aspects. Curr. Cancer Drug Targets 12 (9), 1173–1190.
Touw, M., 1981. The religious and medicinal uses of Cannabis in China, India and Tibet. J. Psychoact. Drugs 13 (1), 23–34.
Trembly, B., Sherman, M., 1990. Double-blind clinical study of cannabidiol as a secondary anticonvulsant. Paper presented at the Marijuana'90 International Conference on Cannabis and Cannabinoids. Kolympari, Crete, July 8.
Turner, C.E., Elsohly, M.A., 1981. Biological activity of cannabichromene, its homologs and isomers. J. Clin. Pharmacol. 21 (S1), 283S–291S.
Turner, C.E., Elsohly, M.A., Boeren, E.G., 1980. Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. J. Nat. Prod. 43 (2), 169–234.
Ulbricht, C., 2011. Focus: diabetes. J. Dietary Suppl. 8 (3), 239–256.
Upton, R., Craker, L., ElSohly, M., et al., 2014. Cannabis Inflorescence: Standards of Identity, Analysis, and Quality Control. Scott's Valley, CA.
van Bakel, H., Stout, J.M., Cote, A.G., et al., 2011. The draft genome and transcriptome of *Cannabis sativa*. Genome Biol. 12 (10), R102.
Vemuri, V.K., Makriyannis, A., 2015. The medicinal chemistry of cannabinoids. Clin. Pharmacol. Therap., 97 (6), 553–558.
Veress, T., Szanto, J., Leisztner, L., 1990. Determination of cannabinoid acids by high-performance liquid chromatography of their neutral derivatives formed by thermal decarboxylation: I. Study of the decarboxylation process in open reactors. J. Chromatogr. A 520, 339–347.
Virovets, V., 1996. Selection for non-psychoactive hemp varieties (*Cannabis sativa* L.) in the CIS (former USSR). J. Intl. Hemp. Assoc. 3, 13–15.

Vogelmann, A.F., Turner, J.C., Mahlberg, P.G., 1988. Cannabinoid composition in seedlings compared to adult plants of *Cannabis sativa*. J. Nat. Prod. 51 (6), 1075–1079.

Wagner, G.J., 1991. Secreting glandular trichomes: more than just hairs. Plant Physiol. 96 (3), 675–679.

Wargent, E., Zaibi, M., Silvestri, C., et al., 2013. The cannabinoid Δ9-tetrahydrocannabivarin (THCV) ameliorates insulin sensitivity in two mouse models of obesity. Nutr. Diabetes 3 (5), e68.

Watson, D.P., Clarke, R.C., 2014. The genetic future of hemp. Int. Hemp. Assoc. Available from: http://www.hempworld.com/hempcyberfarm_com/htms/research_orgzs/iha/ihagenetic.html.

Wilt, T.J., Macdonald, R., Ishani, A., 1999. β-sitosterol for the treatment of benign prostatic hyperplasia. A systamic review. BJU Int. 83 (9), 976–983.

Woelkart, K., Xu, W., Pei, Y., et al., 2005. The endocannabinoid system as a target for alkamides from *Echinacea angustifolia* roots. Planta Med. 71 (08), 701–705.

Wood, W.F., Sollers, B.G., Dragoo, G.A., et al., 2002. Volatile components in defensive spray of the hooded skunk, *Mephitis macroura*. J. Chem. Ecol. 28 (9), 1865–1870.

Xi, Z.-X., Peng, X.-Q., Li, X., et al., 2011. Brain cannabinoid CB2 receptors modulate cocaine's actions in mice. Nat. Neurosci. 14 (9), 1160–1166.

Zuardi, A., Karniol, I., 1983. Effects on variable-interval performance in rats of delta 9-tetrahydrocannabinol and cannabidiol, separately and in combination. Brazil. J. Med. Biol. Res. 16 (2), 141–146.

Zuardi, A., Guimaraes, F., Moreira, A., 1993. Effect of cannabidiol on plasma prolactin, growth hormone and cortisol in human volunteers. Brazil J. Med. Biol. Res. 26 (2), 213–217.