Universidade de Lisboa

Faculdade de Farmácia

Cannabis:
The new trendy ingredient in skincare

Ana Lúcia Pires Gomes

Monografia orientada pela Professora Doutora Joana Marques Marto, Professora Auxiliar da Faculdade de Farmácia da Universidade de Lisboa, e coorientada pela Professora Doutora Ana Margarida Martins, Investigadora da Faculdade de Farmácia da Universidade de Lisboa.

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Trabalho Final de Mestrado Integrado em Ciências Farmacêuticas apresentado à Universidade de Lisboa através da Faculdade de Farmácia

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Resumo

Cannabis sativa L. é uma planta utilizada há milhares de anos com múltiplos e variados objetivos, desde o fornecimento de fibras, alimento, óleo, mas também para fins medicinais, fins recreativos e/ou religiosos. Nos últimos anos tem-se verificado um aumento no interesse pelas aplicações terapêuticas e cosméticas da cannabis e dos seus constituintes, o que se pode justificar tanto pelas constantes descobertas do seu valor terapêutico no tratamento de várias doenças, como pelas suas inúmeras aplicações como precursor ou agente de soluções ecológicas para o desenvolvimento sustentável em várias indústrias, uma preocupação que é muito atual.

A utilização de *C sativa* L. em dermatologia tem sido cada vez mais estudada e documentada, revelando um imenso potencial no tratamento de uma grande variedade de distúrbios da pele, e identificando vários mecanismos através dos quais esta planta, e especialmente os seus canabinóides, têm a capacidade de apurar a função protetora da pele, e de controlar as respostas inflamatórias e neuroendócrinas na pele danificada.

Tendo em consideração estas mais recentes tendências, esta monografia irá incluir uma visão geral da *Cannabis sativa* L, e posteriormente focar-se-á nas suas aplicações em dermatologia, descrevendo os mecanismos de ação do sistema endocanabinóde na pele, associando-os à patologia das doenças inflamatórias da pele mais prevalentes.

Por último, irá ser realizada uma análise das terapêuticas atualmente disponíveis e perspetivas futuras da utilização da *Cannabis sativa L.* no tratamento de doenças dermatológicas, bem como uma pesquisa sobre as suas possíveis e potenciais aplicações cosméticas, de forma a proporcionar uma visão atualizada da cannabis e do seu mercado em constante evolução.

Palavras-chave: Cannabis, Canabinóides, Dermatologia, Doenças dermatológicas inflamatórias, Cosmética.

Abstract

Cannabis sativa L. has been used for millennia due to multiple and diverse purposes, such as providing for fibres, food, oil, medicine, but also for recreational and/or religious practices. Recent years have registered an increasing interest in therapeutic and cosmetic applications for cannabis and its constituents, which can be justified by the continuous research of its therapeutic activities value in several diseases, and also because it can be used as an environmentally friendly component and sustainable development in numerous industries, which is a very current concern.

Recently, the usefulness of *Cannabis sativa* L. in dermatology has been explored and documented, revealing an enormous therapeutic potential for the treatment of numerous skin conditions. Several studies have reported various ways this plant, and especially its cannabinoid components, can improve the skin's function as a protective barrier, and modulate inflammatory and neuroendocrine responses in damaged skin.

This monograph comprises an overview on *Cannabis sativa* L, then it discusses its applications in dermatology and describes the mechanisms of the endocannabinoid system in the skin, and how these relate with the most prevalent inflammatory skin diseases.

Last, a review on the current available therapies and future prospects using *Cannabis sativa L.* for skin disorders, and on existing and developing cosmetic applications will be presented, to provide an update on the ever-evolving cannabis market.

Keywords: Cannabis, Cannabinoids, Dermatology, Inflammatory skin diseases, Cosmetic.

Abbreviations

ACD - Allergic contact dermatitis

AD – Atopic dermatitis

AE - Asteatotic Eczema

AEA - Anandamide

2-AG - 2-Arachidoynyl-glycerol

CBD – Cannabidiol

CBDA – Cannabidiolic acid

CBGA – Cannabigerolic acid

CBR – Cannabinoid receptor

CB1R – Cannabinoid type-1 receptor

CB2R - Cannabinoid type-2 receptor

DNFB - 2,4-Dinitrofluorbenzene

ECB – Endocannabinoid

ECS – Endocannabinoid system

EMA – European Medicines Agency

EU – European Union

FAAH - Fatty acid amide hydrolase

FDA - US Food and Drug Administration

FD&C Act - US Federal Food, Drug, and Cosmetic Act

GMP – Good Manufacturing Practice

MAGL - Monoacylglycerol lipase

pCB – Phytocannabinoid

PEA - N-Palmitoylethanolamine

PPARs - Peroxisome proliferator-activated receptors

SGs - Sebaceous glands

THC – Δ9-Tetrahydrocannabinol

THCA - Δ9-Tetrahydrocannabinolic acid

mTOR - Mechanistic target of rapamycin receptor

TRPV - Transient receptor potential cation channel subfamily V member 1 (vanilloid receptor)

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1. *Cannabis sativa* L.: An Overview

Cannabis sativa L. is an annual, pollinated, usually dioecious[a] flowering plant from the *Cannabaceae* family. Many different varieties of this plant have developed throughout the centuries as a result of breeding and selection. However, due to the lack of a universally acknowledged taxonomic rank on the various groups of plants belonging to the genus *Cannabis,* it is commonly accepted to refer to all types as *Cannabis sativa* L. (*C. sativa* L., cannabis). (1,2)

Cannabis sativa L. shares its origins with the first agricultural societies in Asia, and over the course of history has been used for several purposes, such as a provider of fibres, food, oil, medicine, textiles, and also for recreational and/or religious practices. (3)

The first medical uses of this plant date back to the time when the emperor Chen Nung, "father" of Chinese agriculture, drafted the first Chinese pharmacopoeia, which recommended cannabis for fatigue, rheumatism and malaria, and its seeds, due to their richness in γ-linoleic acid, for eczema, psoriasis, and inflammatory diseases. However, and despite all the knowledge on numerous applications and beneficial therapeutic effects of cannabis that were collected, documented and/or shared between countries and cultures throughout history, especially in the Age of Discovery, C. *sativa* L. was banned in the twentieth century on account of its psychoactive effects. (3–5)

By the end of the twentieth century, however, there was a surge on the interest in hemp, which is currently one of the most rapidly growing products in agricultural markets. The expressions "hemp" or "industrial hemp" and "marijuana" or "medicinal cannabis" are broad classifications that were adopted into western culture, to differentiate between two types of the plant, with different purposes determined by different compositions. "Hemp" or "industrial hemp" are terms used to classify varieties of *C. sativa* L. that contain 0.3% or less tetrahydrocannabinol (THC), the main psychoactive compound in the plant, while "marijuana" or "medicinal cannabis" can contain up to 30% of THC and is considered a controlled substance. (6) These low levels of psychoactive components are what make pharmaceutical industries bet largely on hemp to obtain the non-psychoactive cannabinoid

[a] Meaning that male and female flowers are found on separate plants.

cannabidiol (CBD) that has been demonstrating a high therapeutic value in numerous diseases.

As a consequence of the blooming interest in *C. sativa L* and its potential for therapeutic use, this plant has been increasingly studied, on a wide array of scientific areas, and it is now well established that it contains hundreds of chemically active compounds, such as cannabinoids, terpenoids, flavonoids, alkaloids, and others, all relevant in the medical field. In 1974, Mechoulam and Gaoni opened the way for the study of cannabinoids, through the isolation and synthesis of Δ9-THC (Δ9-tetrahydrocannabinol) (7), and in the 1980s the pharmaceutical company Pfizer developed synthetic ligands for cannabinoid receptors (CBRs). These studies led to the discovery of the cannabinoid type-1 receptor (CB1R) in 1990, the cannabinoid type-2 receptor (CB2R) in 1993 and, subsequently other CBRs and the endocannabinoid system (ECS), among others. (3,7,8)

Most therapeutic effects of *C. sativa L* are due to the presence of cannabinoids, a very heterogeneous group of pharmacologically active compounds that are structurally and biochemically similar to the primary psychoactive compound derived from *C. sativa* L., THC.

Cannabinoids can be divided into three main classes:

- Endocannabinoids (ECBs) are endogenous compounds that occur naturally, being produced by humans and other animals, in the brain or peripheral tissues. They are typically referred to as neuromodulator agents, and have particular characteristics that distinguish them from typical neurotransmitters: they are synthesized at will in their place of action, by receptor-stimulated cleavage of precursors of the lipid membrane and are not preserved in synaptic vesicles. (9,10)

- Phytocannabinoids (pCBs) are cannabinoids produced exclusively in *C. sativa* plants. Over 100 different pCBs have been identified, the vast majority of which have little to no psychoactive activity, and mostly with acceptable side-effect profiles, which makes them particularly interesting candidates for the treatment of several diseases. (11) Phytocannabinoids are a group of agents analogous to terpenophenolic compounds, with lipophilic properties, mostly present in the resin secreted from trichomes of female plants. The major pCB is the previously mentioned Δ9-THC, the natural psychoactive ingredient of *C. sativa L.*, but there are other subclasses of pCBs, such as cannabidiol (CBD), (-)-Δ8-trans-tetrahydrocannabinol (Δ8-THC), cannabinodiol (CBND), cannabigerol (CBG), Δ-9-

tetrahydrocannabivarin (Δ9-THCV), cannabichromene (CBC), cannabicyclol (CBL), cannabinol (CBN), etc. (summarized in Table1). (3,9)

- Synthetic cannabinoids are cannabinoids developed in laboratories, that have structural similarities to both ECBs and pCBs, and act by similar biological mechanisms.

Table 1 - Examples of cannabinoids, divided by class. Proposed by Eagelston *et al*. (9)

Endocannabinoids	Phytocannabinoids	Synthetic cannabinoids
• 2-Arachidonoylglycerol (2-AG) • Anandamide (AEA) or N-arachidonoylethanolamine • Homo linoleoyl ethanolamide (HEA) • Docosa tetranyl ethanolamide (DEA) • Palmitoylethanolamide (PEA) • Oleoylethanolamide (OEA) • Virodhamine • Noladin ether	• Δ9-tetrahydrocannabinol (THC) • Cannabidiol (CBD) • Cannabichromene (CBC) • Cannabicyclol (CBL) • Cannabigerol (CBG) • Cannabinol (CBN) • Cannabinodiol (CBND) • Cannabielsoin (CBE) • Cannabitriol (CBT) • Δ-9-Tetrahydrocannabivarin (Δ9-THCV) • (-)-Δ8-Trans-tet-rahydrocannabinol (Δ8-THC);	• WIN-55,212-2 • JWH-133 • (R)-Methanandamide (MET) • CP 55,940

Cannabinoids can also be distinguished between neutral cannabinoids and cannabinoid acids, based on the presence or absence of a carboxyl group. In plants, concentrations of neutral cannabinoids are much lower than those of cannabinoid acids, thus THC and CBD are formed via non-enzymatic decarboxylation, due to stressful events, like light exposure, heating, or ageing of their acidic precursors Δ9- tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), originated from cannabigerolic acid (CBGA), as explained in Figure 1. The amounts of each component formed in the plant depend on genetic characteristics, and also environmental conditions, such as temperature, humidity, soil nutrition, and others. THCA synthase and CBDA synthase were the first cannabinoid synthases to be studied, and are potential targets for several biotechnological applications, given that they produce the direct precursors of pharmacologically active cannabinoids (12,13).

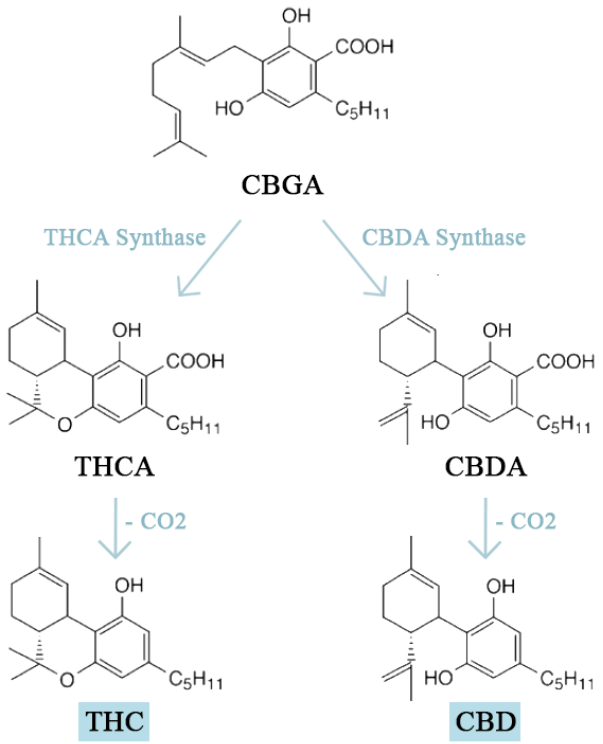

Figure 1 - Biogenesis of THC and CBD. THCA synthase and CBDA synthase catalyze oxidative cyclization of CBGA, forming THCA and CBDA respectively. These compounds then suffer non-enzymatic decarboxylation originating in THC and CBD, respectively. Adapted from Taura *et al.* (12)

As previously mentioned, ECBs, related mediators and pCBs have shown increasing potential as a therapy for numerous diseases. THC, for example, has a variety of therapeutic effects that are primarily mediated through agonistic actions at CBRs. This cannabinoid has been associated with the capacity to relieve nausea and anorexia caused by radio- and chemotherapy, and with the symptomatic mitigation of multiple sclerosis. The brain's dopamine pathway includes both CB1R and CB2R (cannabinoid receptors type 1 and 2, respectively), and studies reported that their response to the presence of THC is to increase dopamine release, which explains the possible euphoric effects of cannabis. Even though THC produces a smaller release of dopamine than other drugs, like cocaine or methamphetamines, it can, however, produce a faster release because cannabis is typically smoked.

Cannabidiol (CBD) is a pCB with a slightly different structure than THC, but without its psychoactive capabilities. CBD has also been shown to have therapeutic abilities and, because it is not psychoactive, it has garnered a great deal of attention in the medical field, in the last

few years. For example, this compound has been reported as anti-inflammatory, anxiolytic, antiepileptic, and antischizophrenic in animals and humans. CBD acts as an antagonist at the central CB1R and can inhibit several CB1R-mediated THC effects, thus having the potential to lessen the worst effects of THC. (12,14–17) Additionally, CBD seems to exert some of its pharmacological effects by binding to other receptors, such as TRPV1 (Transient receptor potential cation channel subfamily V member 1 or vanilloid-1 receptor), GPR55 (G protein-coupled receptor 55), or 5-HT1A (5-hydroxytryptamine receptor subtype 1A). (18) Nowadays CBD is a widespread ingredient in skincare products and can be found in body oils, moisturizers, lotions, lip balms, and others. However, scientific studies on safety and efficacy are still needed to better understand its actions and benefits, as it will be further discussed in this paper. (18)

The actions of cannabis are generally related to cannabinoids, but other *C. sativa* L. compounds can also have medicinal properties. Terpenoids, which have been identified in the flower, leaves and trichomes of *C. sativa* L., are also becoming increasingly relevant, and seem to mostly be responsible for the fragrance, and some protective functions of the plant. There are over 200 terpenoids identified in *Cannabis sativa* L. plants, the most common being limonene, myrcene, and α-pinene, which are highly volatile compounds. (3) These molecules are easily extracted from the plant material by steam distillation, resulting in a substance called the essential oil or volatile oil of the plant, or through vaporization. (15) Terpenes, closely related to terpenoids, have been associated with several medicinal properties including antimicrobial, antioxidant, anticancer, antiarrhythmic, anti-aggregating, anaesthetic, anti-inflammatory, antihistaminic, etc. (19) Some recent studies also reported synergistic contributions of terpenoids to cannabis-mediated effects, which can enhance cannabinoid activity, thus making this a matter worthy of further investigation. (15) Thus, terpenes may have therapeutic potential, alone or in combination with cannabinoids, however there is still little information on this matter, so this paper will not focus on these.

To be used in medicine and cosmetics, CBD, THC, and other cannabinoids must first go through processes to separate them from other cannabis components, before being infused into the product. In brief, cannabis first undergoes an initial solvent-based extraction, usually with a hydrocarbon or ethanol solvent, followed by other steps, such as winterization, filtration and decarboxylation, before going through distillation. After extraction, and depending on several factors, the crude oil obtained will typically have a THC/CBD

concentration between 60-80%, while the other fraction contains a variety of other cannabis components, like terpenes, vitamins, antioxidants, and others. To be further purified, it is then subjected to the distillation process, isolating specific molecules, such as THC and CBD, thereby producing refined cannabis oil, a "distillate", with over 90% purity of the molecule in question, and any remaining cannabis constituents will, at this point, be vestigial. (20,21)

C. sativa L. is not only relevant for medicines and cosmetics, but it can also be used as an environmentally friendly raw material in the textile, paper, fishing nets, bioplastic, biofuel, hempcrete (building material that permanently binds CO_2 for more sustainable housing), and also food industries. (6)

The hype around all that is organic and sustainable, plus the trend of a healthier lifestyle seen in the last decade, have allowed for hemp and its products to find their footing in today's market and even be at its centre sometimes. The multifunctional purpose of *C. sativa* L., the low level of initial efforts needed for growing it, the positive effect it has on the soils and its carbon fixing potential, can be a huge help in achieving the ambitious goals set by the European Commission in the Green Deal, in which it is stated that Europe will strive to be the first carbon-neutral continent by 2050. This is to be achieved with the investment in cutting-edge research, innovation and technology, like the capturing and storing of CO_2 in the ground, and the seizing of CO_2 using plant biomass. Henceforth, the farming of industrial hemp, or *C. sativa* L. in general, can be instrumental, since forests take many years to grow, while hemp only takes weeks, additionally opening doors for a whole range of other new green opportunities. (6)

Presently, *C. sativa L.* is widely distributed throughout the world, even though its large-scale production only occurs in very few places. A clear distinction can be made based upon the purpose of *Cannabis sativa* L. production: cultivars as a source of fibre and seeds, or as a source of cannabis for recreational and medical outcomes. Whether a *C. sativa* L. plant predominantly produces fibre (hemp) or narcotics is determined by both genetic and climatic factors. The different plants can only be distinguished with a molecular-level analysis, with classification dependent on the concentration of the psychoactive compound THC and the non-narcotic CBD, since the plants are not morphologically distinguishable. *Cannabis* plants are split into each phenotype, "chemo-type" or "fibre-type", based on the ratio of [THC] +

[CBN[b]] / [CBD], first reported in a study by Fetterman *et al.* (22) If the ratio is <1.0, then the plant is classified as fibre-type cannabis (hemp), while a ratio >1.0 classifies it as a biologically active type of marijuana, a "chemo-type". (1,16,22)

Cultivation of medicinal cannabis is prohibited in most countries, except for research purposes or pharmaceutical use, and the cultivation and production of industrial hemp are restricted and highly controlled due to its narcotic potential. Hemp breeders need to pay close attention to the growing variables such as soil make-up, pH levels, moisture content, etc., to create high CBD yields while maintaining THC levels under the allowable limits, with the ultimate goal of a THC content below 0.3%. (1)

In summary, *C. sativa* L. and its constituents have been showing tremendous potential in multiple industries, slowly moulding the market, and altering perceptions on what the cannabis plant can be used for. Developing the cannabis industry is a complex and demanding process, with several sensitive regulatory and cultivation requirements that need to be met, so that the plant can be used by the general public, in various forms. However, given all the potential benefits that *C. sativa* L. has been showing in the medical, beauty, fashion, construction, agriculture, sustainable development, and other fields, it is clear that investing in its research and development is very important, and will most likely bring many advantages. Most relevant for this paper is the potential of cannabis in medicine, particularly with new and better treatments in dermatology, and to shape the near future of the beauty industry.

[b] Cannabinol [CBN] is the degradation product of (-)-Δ9-trans-tetrahydrocannabinol [THC].

2. Therapeutic Potential of *C. sativa* L. in Dermatology

The skin is the human body's largest organ, and the first barrier between the external environment and the inside of the body, protecting it against pathogens and chemical, biological and/or radiation damage. Besides its protective function, the skin also plays a major role in the immune, neurologic, and endocrine responses, being composed of an intricate multicellular communication network, in which the skin and its pilosebaceous units function as neuroimmunoendocrine organs, responding to external stimuli, neuropeptides and mediators released by neighbouring cells. It is a complex and delicate process that is essential for maintaining homeostasis. (23)

Numerous studies have described how *C. sativa* L. can be used for chronic pain, spasticity, anorexia, nausea, and a plethora of other conditions and symptoms, including dermatological disorders, such as pruritus, inflammatory skin diseases, and others. The discovery of a skin ECS lead to the investigation of its role in the functions of this organ, and how disturbances in its regular actions can contribute to the development of pathological skin disorders (24). Research and evidence on this matter have suggested that modulation of the ECS´s activity, through the use of CB1R and CB2R selective agonists and antagonists, holds great potential as a possible treatment for numerous skin disturbances. (8) For example, some research has suggested that CB1R, unlike CB2R, are expressed in a hair-cycle-dependent manner in human hair follicles and as such, that a CB1R-mediated ECS might act as an autocrine–paracrine negative regulator of human hair growth. This concept suggests that CB1R agonists may have hair growth-inhibitory effects, and can be used for the management of unwanted hair growth, as seen in hirsutism. On the contrary, CB1R-antagonist-based treatment options could possibly help manage the opposite condition, where hair growth is depleted, like in alopecia areata and effluvium. (25) This notion can be applied to the therapy of several skin diseases, particularly inflammatory ones, which will be further described and discussed in the following chapters.

2.1. Cannabis Actions in the Skin: The Endocannabinoid System

The ECS is an extensive network of bioactive lipid mediators, and respective receptors that regulate a multitude of physiological processes, such as appetite, pain, mood, memory, etc.,

both in the peripheral and central nervous systems, and various peripheral organs. As such, several studies have been suggesting that modulating ECS activity may hold enormous therapeutic potential for numerous diseases affecting humans, including obesity/metabolic syndrome, diabetes and diabetic complications, neurodegenerative, inflammatory, cardiovascular, liver, gastrointestinal, skin diseases, pain, and many others. (23,26)

The ECS consists of several endogenously produced ECBs, such as 2-arachidoynyl-glycerol (2-AG), anandamide (AEA) and N-palmitoylethanolamine (PEA), endocannabinoid responsive receptors (CBRs), mainly CB1R and CB2R, and also all of the different metabolic enzymes and transporters involved in the synthesis, cellular uptake and release, and degradation of ECBs, meaning that they are responsible for all the endogenous modulation and biological activity of these compounds. (23,27,28)

CB1R is mostly concentrated in the brain and central nervous system acting mostly as a neuromodulator, even though it can also be involved in the modulation of inflammation in peripheral tissues (i.e., heart, lungs, gastrointestinal tract, liver, bladder, placenta, ovaries, testicles, adipose tissue, etc.). The expression of CB1R in the central nervous system relates to the levels of gamma-aminobutyric acid and glutamate-gated ion channels. In fact, it has been demonstrated that the CB1R is primarily pre-synaptic in the brain, on GABA-ergic and glutamatergic interneurons, which could justify its role in neuromodulation and signal transmission. This also corroborates the possible role of these receptors in the management of memory, appetite, mood, sleep and pain via the release of neurotransmitters. (9,10,23,27)

CB2R is mainly expressed in immune cells on peripheral tissues, like the spleen, tonsils and thymus, and cells of hematopoietic descent (i.e., lymphocytes B and T, NK cells, monocytes) and, for this reason, this receptor has been called the immunocannabinoid system receptor, being able to act, for example, as a key regulator of ECB-dependent suppression of various inflammatory responses. (9,10,23,27)

The balance between CB1R and CB2R signalling is imperative for physiological homeostasis, and its disruption can lead to serious pathological conditions. For example, the overactivation of CB1R has been associated with heightened psychoactivity, increases in inflammation and risk for metabolic syndrome, whereas CB2R overactivation has been linked to a decreased immune response, impeding wound healing. (23,27)

Both receptors belong to the superfamily of G-protein coupled receptors with a primary structure of seven hydrophobic α-transmembrane domains (20-25 amino acids each), connected with alternating intra- and extracellular loops. This superfamily has a few particularities that can also be observed in the CBRs, such as the formation of disulfide bridges between cysteine residues of the second and third domains, thus stabilizing the tertiary structure of these receptors, and also the existence of a highly conservative fragment, free of the amino acid proline, in the fifth hydrophobic domain. (10,29)

Cannabinoid receptors have been identified in numerous tissues and organs, including the skin, and as such, the ECS has been implicated in the homeostasis of several cutaneous processes, like proliferation, differentiation and survival, immune competence and/or tolerance, hair growth, sebaceous lipid production, melanogenesis, fibroblast activity, and others. The dysregulation of these processes can lead to skin pathologies, such as asteatotic eczema, atopic dermatitis (AD), irritant contact dermatitis, allergic contact dermatitis (ACD), chronic pruritus, skin cancer, hair growth disorders, acne vulgaris and others, revealing how important the equilibrium of all cutaneous functions is for the health and functionality of the skin, and explaining why the use of various forms of *C. sativa* L. to treat dermatological conditions has become so relevant in recent years. (11,24)

CB1R and CB2R are considered the main receptors mediating the effects of cannabinoids, but recent studies have interestingly shown the existence of CBR-independent mechanisms. For example, it has been shown that receptors belonging to the peroxisome proliferator-activated receptor (PPAR) and TRPV-1 families could also be activated by cannabinoids. Besides, experiments on mice reported by Breivogel and colleagues (30) suggested the existence of a CB3R receptor. Also, some results have shown that ECBs can simultaneously activate multiple receptors in the same cell. (10)

The aforementioned ECBs AEA and 2-AG, like CB1R and CB2R, along with the major metabolizing enzymes (e.g., FAAH - fatty acid amide hydrolase or MAGL - monoacylglycerol lipase) have been found in various cutaneous cells, such as dermal nerve cells, keratinocytes, hair follicular epithelial cells and sebocytes. (23) AEA is primarily degraded by FAAH and N-acylethanolamine-hydrolyzing acid amidase (NAAA), while 2-AG is primarily degraded by MAGL, FAAH, a/b hydrolases (ABHD) 6 and 12, although other catabolic enzymes have been implicated in the degradation process of both ECBs. Endocannabinoids can also be metabolized by lipoxygenases, cyclooxygenases and

cytochrome P450, resulting in bioactive metabolites that possibly might activate CBR--independent mechanisms. (summarized in Figure 2) (23,28)

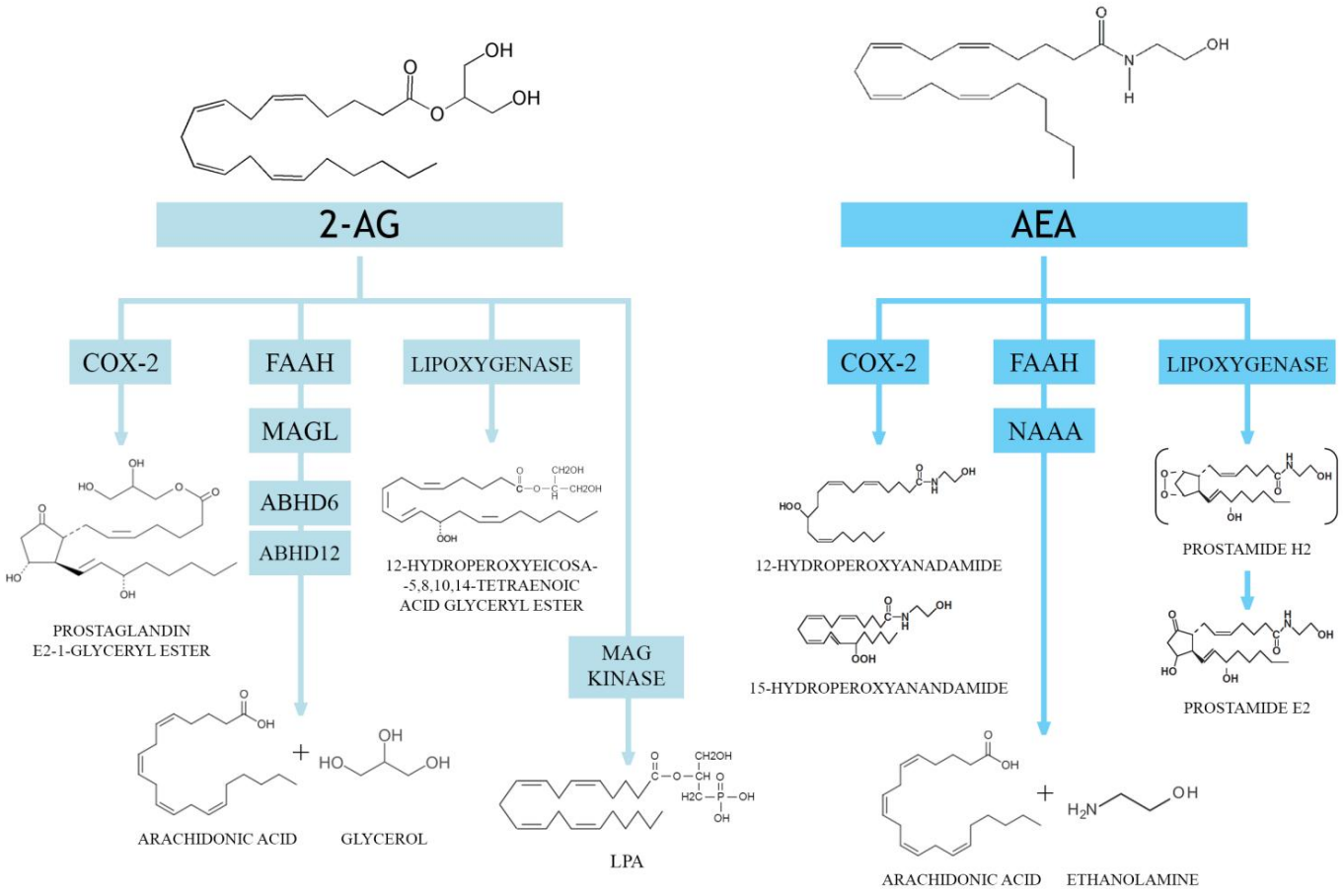

Figure 2 – Degradation pathways of Anandamide (AEA) and 2-Arachidoynyl-glycerol (2-AG). Adapted from Ueda *et al.* (2010) and Ueda *et al.* (2011) (80,81)

The different signalling pathways activated by the ECBs depend on the specific receptor engaged. For instance, AEA and 2-AG have been shown to bind both types of CBRs, type 1 in the brain, liver, lungs, and type 2 in the immune system, where they mostly exert their activity, respectively. Additionally, these ECBs bind GPR55, which is involved in the modulation of cardiovascular and inflammatory events, and PPARs α and γ, therefore regulating adipocyte differentiation, lipid and glucose metabolism and inflammatory responses. Also, AEA also seems to bind to TRPV1, triggering pro-apoptotic signalling pathways and exercising some control of brain functions. (28)

Other ECBs, like PEA, have a lower affinity for CB1R and CB2R, but can act as enhancers of AEA and 2-AG actions, by binding TRPV-1 receptors present in immune cells, keratinocytes, and neurons that supply sensation to the skin, by what is called an ‘‘entourage’’ effect. Also, Since TRPV-1 is responsible for the regulation of cutaneous pain sensation to chemical and temperature provoked injuries, PEA and other ECBs that bind to this receptor can have an important role in pain perception and inflammation responses to epidermal aggression. (23)

The ECS signalling mechanism of action can be summarized as follows: after stimuli, neurotransmitters from the presynaptic neuron bind to their respective receptors on the postsynaptic neurons and, following the intracellular rise in free Ca^{2+}, these neurons synthesize ECB precursors that are later cleaved to form the biologically active ECBs (e.g., AEA and 2-AG). Thereafter, the formed ECBs are released in the synaptic cleft, and act in a retrograde manner, binding to CB1R in presynaptic neurons, inhibiting the influx of Ca^{2+}, consequently blocking future neurotransmitter release. Afterwards, excess ECBs are degraded, with AEA primarily catabolyzed by FAAH, 2-AG by MAGL and PEA by NAAA and FAAH-2. Consequently, it is possible for the ECS to be modulated by inhibiting catabolic enzymes, resulting in longer-lasting or more robust effects on the ECB receptors. (23)

In the cutaneous ECS, endogenous ECBs, like AEA and 2-AG, are locally produced in the epidermis and pilosebaceous unit, binding to CB1R, CB2R and/or TRPV receptors available on keratinocytes, sebocytes, neurons, and hair follicular cells, so as to sustain proper function and homeostasis within the skin, controlling cutaneous functions such as sensation, growth, survival, immune competence and/or tolerance. (23,24)

Endocannabinoid signalling through CB1R and CB2R also seems to be essential in the control and maintenance of cutaneous inflammatory responses. For example, Karsak *et al*. (31) used an animal model with a cutaneous hypersensitivity prone to the development of contact dermatitis, and showed that in mice lacking both CBRs, the allergic inflammatory response was exacerbated, whereas in FAAH-deficient mice, with consequently increased levels of AEA, the allergic response was decreased, meaning that CBR antagonists exacerbated the allergic inflammation, while agonists attenuated said inflammation (31). Also, a study made by Chiurchiù *et al.* (27) showed that in AEA-treated keratinocytes CB1R suppressed the secretion of proinflammatory cytokines (IL-12 and IL-23) through the inhibition of mTOR (mechanistic target of rapamycin), suggesting that this receptor is also

involved in T-cell dependent inflammatory diseases (27). Furthermore, Oláh *et al.* (32) showed that the expression and activity of FAAH can be regulated by Toll-like receptors (TLR-2), indicating its relevance as a regulator of cutaneous inflammatory processes.

In summary, there is evidence that the modulation of the cutaneous ECS may be relevant and valuable in the management of numerous human skin diseases. For example, suppression of the skin ECS function using CBR antagonists, or other agents that lower the local production of ECBs, may be useful in the therapy of certain hair growth conditions (e.g., forms of alopecia, effluvium), as previously mentioned, and sebaceous gland disorders (e.g., acne, seborrhea). On the contrary, the increase in the cutaneous ECS function, using CBR agonists, or agents capable of stimulating the local production of ECBs, may help in the treatment of multiple benign and malignant skin tumours, hyperproliferative skin diseases (e.g., psoriasis), excessive hair growth (e.g., hirsutism), different forms of dermatitis, dry skin conditions and other sensory phenomena (e.g., pain, itch), as summarized in Table 2. (24)

Table 2 - ECS targeting approaches in the skin. Proposed by Biró T. *et al.* (24)

Disease	Target cell population	Target receptor	Possible approach	Expected effects
Skin tumours	Transformed skin cells	CB1R CB2R	○ CBR agonists ○ Agents that increase ECS tone	○ Suppression of growth, angiogenesis and metastasis ○ Induction of apoptosis
Psoriasis	Keratinocytes, immune cells	CB1R CB2R	○ CBR agonists ○ Agents that increase ECS tone	○ Suppression of keratinocytes proliferation and inflammation
Unwanted hair growth (hirsutism)	Hair follicle epithelium	CB1R	○ CB1R agonists ○ Agents that increase ECS tone	○ Suppression of hair growth ○ Stimulation of intrafollicular apoptosis and catagen regression
Alopecia areata, effluvium	Hair follicle epithelium	CB1R	○ CB1R antagonists ○ Agents that decrease ECS tone	○ Stimulation of hair shaft elongation ○ Suppression of intrafollicular apoptosis and catagen regression
Acne, seborrhea	Sebaceous gland epithelium	CB2R	○ CB2R antagonists ○ Agents that decrease ECS tone	○ Inhibition of sebum/lipid production in the sebaceous gland

Dry skin	Sebaceous gland epithelium	CB2R	○ CB2R agonists ○ Agents that increase ECS tone	○ Stimulation of sebum/lipid production in the sebaceous gland
Dermatitis	Infiltrating immune cells, keratinocytes, sebocytes	CB1R CB2R	○ CBR agonists or agents that increase ECS tone	○ Suppression of immune/inflammatory processes
Pain	Sensory neurons, keratinocytes, other skin cells	CB1R CB2R	○ CBR agonists ○ Agents that increase ECS tone	○ Suppression of release a algogenic substances ○ Inhibition of transmission of signals in the nervous system
Itch	Sensory neurons, keratinocytes, sebocytes, other skin cells	CB1R CB2R	○ CBR agonists ○ Agents that increase ECS tone	○ Suppression of release a pruritogenic substances ○ Inhibition of transmission of signals in the nervous system

All available data supports that treatments focused on targeting ECS manipulation might have high therapeutic value, and a broad spectrum of potentially useful applications that aim to regularize all skin functions, from cell growth to sebum production, hair growth, skin inflammation, and many others. However, for this to become a reality, and for the true therapeutic value of these promising new targets to be revealed, there is still much to learn and numerous questions that have to be addressed, like what are the potential side effects of targeting this system, and the need to better understand the pathological role of the ECS in various diseases and the ECB pharmacology, among others.

2.2. Inflammatory Skin Diseases

Since the discovery of CBRs, particularly CB2R, in immune cells, studies with CBR agonists, antagonists, or other agents that regulate the ECBs levels during inflammatory processes (e.g., suppression of production of various cytokines, chemokines, arachidonic acid-derived proinflammatory metabolites, etc.**)** have provided extensive evidence suggesting that the ECS has numerous important immunomodulatory effects. One example is the previously mentioned study by Karsak *et al.* (31) Also, the study by Oka *et al.* (33) using different animal models for acute and chronic contact dermatitis, reported elevated 2-AG levels and the involvement of CB2R in the inflammation process, with CB2R antagonists markedly attenuating the symptoms of skin inflammation. However, the role of CB2R in cutaneous inflammation is still controverse, as evidenced by contradictory results. For example, the

study by Zheng *et al.* (34) suggested that CBRs are involved in the promotion of *in vivo* skin carcinogenesis, and UVB-induced cutaneous inflammatory processes. Nonetheless, it is generally acknowledged that ECS wields protective functions in a large number of acute and chronic inflammatory diseases, including those affecting the skin, some of which will now be explored in greater detail. (10,24)

2.2.1. Atopic dermatitis

Atopic dermatitis (AD) is a chronic relapsing/remitting inflammatory skin disease characterized by weepy red plaques in the acute stage, and lichenified thick plaques in the chronic stage, that cause itching and discomfort. Even though its pathogenesis is not well understood yet, it is believed to be a result of various factors, such as immune dysregulation, epidermal and sebaceous barrier disruption, altered sensation to itch stimuli, impaired microbial defence, and others. (23)

Treatment for AD mainly focuses on anti-inflammatories, such as topical steroids and calcineurin-inhibitors, barrier repair using moisturizers, and the reduction of microbial colonization. Nonetheless, multiple clinical studies have already suggested that topical preparations containing ECB receptor agonists or degradation inhibitors may have a high therapeutic value in AD. (23) For example, Yuan *et al.* (35) used PEA-containing cream in combination with a class IV topical steroid cream (0.1% clocortolone pivalate) in a randomized controlled study involving children and adults with AD, and found that the combination led to longer times between flare-ups, when compared to the cohort that only used the topical steroid cream. Furthermore, Eberlein *et al.* (36) conducted an observational, non-controlled, prospective cohort study, in which patients aged 2 to 70 were treated with a PEA-based cream with a unique lamellar matrix, and found that substantial relief of objective and subjective symptoms of AD were reported after regular skincare with the studied formulation. The recorded decline of pruritus and loss of sleep indicated a gain in the quality of life in these patients, and the reduced need for topical corticosteroids, thus reducing the potential side effects of these drugs, which are always of concern when used in treating AD.

2.2.2. Allergic contact dermatitis

Allergic contact dermatitis is one of the leading causes of occupational diseases. When the skin is first exposed to the allergen, dendritic cells present said molecule to naive T-cells,

leading to an inflammatory response upon repeat exposure to the same allergenic agent, which is referred to as a delayed hypersensitivity reaction. (23)

Nowadays, the preferred treatment for ACD is, besides avoiding triggers of the disease, the use of topical corticosteroids and calcineurin inhibitors, and systemic immunosuppressive agents for severe cases. Concerning the therapeutic value of the use of cannabinoids in the treatment of ACD, studies with humans are still lacking, but mice studies have shown high potential. Examples are the previously mentioned studies by Karsak *et al.* (31) and Oka *et al.* (33). Another study, reported by Petrosino *et al.* (37) showed an increase in PEA levels after DNFB (2,4-dinitrofluorbenzene) exposure, and also an inhibition of a DNFB-induced ear ACD after injection of PEA into the peritoneum of mice, suggesting that endogenous production and exogenous administration of PEA may prevent ACD development.

2.2.3. Asteatotic eczema

Asteatotic Eczema (AE) , also refered to as xerotic eczema or eczema craquelé, is a skin disorder characterized by dry, scaly, and itchy skin that often is exacerbated by dry and cold weather, being associated with skin exposure to environmental irritants. As such, and because prevention is key in avoiding or controlling itch and irritation, patients are advised on several lifestyle alterations, such as avoiding harsh cleansing agents, or opting for lukewarm water showers, to prevent exacerbation of this uncomfortable disease. The usual treatment for AE generally involves emollients, containing ureia, lactic acid, or a lactate salt, however, severe cases can require topical corticosteroid treatment. (23,35)

Symptoms seen in AE and other forms of eczema and dermatitis are partially due to impaired skin barrier repair. Low levels of ECBs within the *stratum granulosum* of the skin have been linked to xerosis, and ECS response leads to an increase of lipid synthesis in that skin layer. (23,35) The topical application of ECBs has been shown to improve the symptoms of eczema. For example, Yuan *et al.* (35) reported that patients who used a 0.3% PEA / 0.21% AEA cream showed significant improvement in itching and skin hydration, as well as a decrease in erythema, scaling, and dryness, typical of eczema and other skin diseases.

2.2.4. Psoriasis

Psoriasis is a common inflammatory hyperproliferative skin condition, notable for the manifestation of unsightly lesions (‘scales’) that develop within the epidermis, originated by

an extremely fast turnover of epidermal keratinocyte proliferation, accompanied by the infiltration and increased expression of proinflammatory mediators into the skin. (24) It affects between 2% and 3% of the world population, presenting significant morbidity, and often causing anxiety and depression in patients. (38) Cannabinoids have shown promise in helping to treat psoriasis, with several action mechanisms being proposed:

- Inhibition of keratinocyte proliferation, as suggested by Wilkinson *et al*. (39) in an *in vitro* study testing several cannabinoids (THC, CBD, CBN and CBG) which showed proliferation inhibition in a concentration-dependent manner, independent of CBR activation. A different inhibitory mechanism was reported by Ramot *et al.* (40), this one involving the activation of CB1R by a CB1R-specific agonist, *in* vitro, which then lead to the downregulation of keratin K6 and K16 expression. (40)

  That being said, a study by Casares L. *et al.* (40) warrants some caution in using CBD as a treatment for psoriasis. This study showed CBD to be a BACH1 inhibitor. BACH1 is a potent HMOX1 inducer, which is an important cytoprotective enzyme with anti-inflammatory, antioxidant and anti-apoptotic properties, and as such BACH1 inhibitors, like CBD, should be very useful as a treatment for inflammatory disorders or oxidative stress-associated skin conditions, protecting the skin from external insults (eg. UV radiation). Much like the previously described studies by Wilkinson *et al*. (38) and Ramot *et al.* (39), the *in vitro* analysis made by Casares L. *et al* (40) recorded an antiproliferative and pro-differentiation profile for CBD, however, in the *in vivo* studies the opposite was observed, with CBD inducing keratinocyte proliferation, and increments in both skin thickness and the levels of the proliferative keratins K16 and K17 being registered. As such, because psoriasis is defined by chronic inflammation and keratinocyte hyperproliferation, until further research CBD should be used with prudence in this disorder, despite its anti-inflammatory properties.

- Inhibition of the effects of antigen processing and prevention of the release of inflammatory cytokines, such as IL-2, TNFα, and interferon-gamma, which are key mediators in the pathogenesis of psoriasis. (41) It has been shown that cannabinoids inhibit antigen processing in macrophages, macrophage/T-cell interaction, and release of pro-inflammatory cytokines and nitric oxide from immune cells. These inflammatory processes stimulate keratinocyte proliferation and expression of

adhesion molecules, which are at the core of psoriasis pathogenesis. This suggests that cannabinoids may act as inhibitors of the inflammatory mechanisms, and may be used as antipsoriatic agents. (42)

- Interaction between the immune and nervous systems occurring through a cholinergic anti-inflammatory pathway and the ECS, resulting in anti-proliferative effects on human keratinocytes and vagal nerve stimulation, leading to the enhancement of acetylcholine release and consequent immunomodulation through inhibition of TNF production by cytokine-producing macrophages. (9,43)

These findings do support a potential role for cannabinoids in the treatment of psoriasis, which is highly relevant, since antipsoriatic medications are often associated with adverse side effects, and high costs in some cases (e.g., biologics), thus a continuous search for safer agents that can be used alone or in combination with current antipsoriatic drugs, is imperative. (42)

2.2.5. Acne and seborrhea

Acne and seborrhea are the most frequent dermatological disorders, and are both characterized by highly elevated sebum (lipid) production of the sebaceous glands (SGs).

It has been reported that cultured human SZ95 sebocytes express CB2R, but not CB1R and that ECBs, such AEA and 2-AG, are present in these sebocytes in order to up-regulate lipid synthesis and induce apoptosis-driven cell death via selective CB2R-coupled signalling. (44) Thus, CB2R activation in the SG by ECBs leads to an increase of lipid synthesis, as previously mentioned, which is why agents that suppress the local output of these ECBs in the ailing SG (e.g., DAGL inhibitors), and/or that inhibit CB2R on the sebocytes (CB2R antagonists), can have some therapeutic potential in these skin disorders.

In 2014, Olah *et al*. (45) reported that CBD can inhibit the lipogenic action of numerous compounds (e.g., arachidonic acid) and suppress sebocyte proliferation via activation of TRPV4 ion channels, affecting the prolipogenic ERK1/2 MAPK pathway and nuclear receptor interacting protein-1 (NRIP-1) production, thus inhibiting sebocyte lipogenesis and affect glucose and lipid metabolism, respectively. The same study suggested that the anti-inflammatory effects of cannabinoids may occur via up-regulation of tribbles homolog 3 (TRIB3) and inhibition of nuclear factor kappa B (NF-κB) signalling, via A2a adenosine receptor. Olah *et al.* (45) therefore proposed that CBD is a promising therapeutic agent of acne, given its capacity to regulate

lipogenesis (lipostatic effect) and decrease proliferation (antiproliferative effect), without compromising cell viability or inducing apoptosis or necrosis of sebocytes, and also by preventing the actions of TLR-activation or "pro-acne" agents to elevate pro-inflammatory cytokine levels (anti-inflammatory effect). These anti-inflammatory properties were later on further explored by Olah *et al.* (32) with a study on the effects caused by inhibition of FAAH, a TLR2-dependent regulator of cutaneous inflammatory processes.

It should be noted that the transdermal penetration of cannabinoids has been reported and confirmed, which elicits the possibility for these agents to be efficiently applied to the skin in topical pharmaceutical preparations, like creams (24), facilitating the treatment of acne, and the previously mentioned dermatological conditions. For example, Ali *et al*. (46) reported in a single-blinded comparative study spanning over 12 weeks, that sebum levels and erythema were significantly decreased with the use of a 3% cannabis extract cream on the right cheek twice per day, when compared to a control cream that was similarly applied on the left cheek.

2.2.6. Pruritus

The ECS also has an essential part in the central and peripheral processing, and skin-derived sensory manifestations, such as pain and pruritus, more commonly known as itch. Cannabinoid agonists and/or ECBs have shown powerful analgesic and anti-pruritic effects, in humans and animals alike, through the activation of CB1R and/or CB2R, and possibly other receptors (e.g., TRPV1), in the sensory nerve terminals and/or inflammatory cells. (24)

Pruritus is an unpleasant localized or generalized common symptom in inflammatory skin diseases, contributing significantly to the impaired quality of life of affected patients, since it is considered one of the most bothersome symptoms. (10,24)

Despite the existence of multiple possible antipruritic regimens, they often show low efficacy rates, and thus new treatment options that may improve the lives of those affected are always welcomed. The published data generally suggests that cannabinoids can exercise anti-pruritic effects, and consequently may be a potential therapeutic option for patients suffering from pruritus, who failed to improve with other treatment modalities. As an example, the study by Eberlein *et al.* (36), previously described, explains how the use of a cream containing PEA significantly decreased objective and subjective symptoms of ACD, including pruritus. Also,

Schlosburg *et al.* (47) observed that the suppression of the neuronal FAAH reduces the scratching response through the inhibition of AEA degradation and activation of CB1R.

Moreover, dry skin can be a promoter for pruritus, and other skin diseases like dermatitis. The application of formulations containing cannabinoids that stimulate CB2R (CB2R agonists) in the SG, and/or agents that increase the local production of ECBs and/or inhibit their degradation (e.g., FAAH and/or MAGL inhibitors) in the SG, seems to be able to amplify fat production in the SG, a feature that also might attract the interest of the cosmetics industry, and that may be a novel therapeutic approach for extremely dry skin and other skin disorders. However, it is extremely important that these topical medications are made from ECS-acting substances that, on absorption to the blood, do not penetrate the brain and that henceforth will not cause psychoactive effects. (24)

2.2.7. Skin cancer

Although skin cancer is not considered an inflammatory skin disease, it involves inflammatory processes and cannabinoids have been proving to be extremely important in cancer treatment, particularly in dermatology, thus this was deemed as a relevant topic to this review.

Since the 1970s, when exogenous cannabinoids were already considered potential anticancer drugs, knowledge about the anti-tumour effect of these compounds has been increasing and further explored, suggesting that ECBs can induce apoptosis, inhibit tumour cell proliferation and migration, reduce expression of pro-angiogenic agents and receptors (essential for the growth of skin tumours), diminish vascular hyperplasia, and modulate signal transduction in various cell types (10). These effects have been observed in various cancers, from gliomas and lymphomas, to cancers of the prostate, breast, lungs and skin. (48)

Data shows that both human benign (e.g., papillomas) and malignant skin tumours (e.g., melanoma, squamous cell carcinoma) express considerable amounts of CB1R and CB2R, and as such ECBs may also be beneficial in their treatment. *In vitro* experiments with cultured cells have shown that the activation of CBRs by CBR agonists, decreased growth, proliferation, angiogenesis and metastasis formation, increasing apoptosis in tumorigenic epidermal cells, while normal epidermal cells remained unaffected. (10,49) However, as briefly mentioned previously in the study made by Zheng. *et al.* (34), there is also conflicting evidence that implicates cannabinoids in the early stages of malignant transformation, when

both CBRs were activated by UVA and UVB radiation, resulting in high levels of NF-κB activation and elevated levels of TNF-α.

Thus, further investigation is required to better understand physiopathology, mechanisms of action, feasible targets, and understand these opposite results, but the potential for cancer treatment is an exciting possible application for cannabinoids, one that defiantly should be further explored.

2.3. *Cannabis* in the Treatment of Inflammatory Skin Diseases: Available Therapeutics and Future Prospects

Despite the tremendous potential that the use of *C. sativa* L. and its constituents have shown in the management of several skin diseases, in several European countries, such as the Netherlands, Germany, Switzerland, and Catalonia (Spain), pain is still the only medically approved dermatologic-related occurrence for which cannabis is allowed. However, in other countries like Canada, or some states of the United States, cannabis has already been approved for the treatment of other conditions, such as psoriasis, dermatitis, pruritus, and more. (50,51) Even though some cultural and regulatory concerns, or sometimes even the lack of scientific evidence, have been slowing down the process of sanctioning C. *sativa* L.-based treatments for a wide array of disorders, the acceptance of its therapeutic value and the authorization of its use, is a trend that the rest of the world will most likely be following in the next few years.

With the developments in legal status observed in many international jurisdictions, especially in authorization for medical use, there has been a rapid increase in consumer demand for cannabis and cannabis-based products as a therapeutic option for treatment and management of a wide range of diseases. There has been a substantial change in policies regarding the use of *C. sativa* L. in the United States and Canada, consequently instigating other countries, including some in Europe, to also allow the use of cannabis and cannabinoids for medical purposes. Nonetheless, the shortage of evidence concerning the efficacy and safety of *C. sativa* L., as well as questions regarding addiction and adverse reactions, raise some concerns, and as consequence, many countries are wary of changes in their cannabis regulations. (5,51,52)

Clinical, academic and public international calls have been made for the development of rigorous clinical trials, to garner knowledge and establish an evidence base for the therapeutic

use of cannabis-based medicines. Even though the undertaking of human studies with *C. sativa* L.-derived pharmaceuticals is necessary to demonstrate their efficacy and safety in various clinical settings, those already performed highlighted some unique challenges, causing some apprehension, particularly with ethics and governance committees**,** when it comes to the endorsement of new trials using cannabis-based drugs, and consequently arising barriers that are slowing down the progress on their use in medicine. (53)

Most countries in Europe prohibit the use of herbal cannabis, whereas cannabinoid-based medicines, like THC capsules, cannabis extract mouth spray, and dried cannabis flowers for vaporising or making tea, are legal in several of them. As of now, there are only five cannabinoid-based medications available in Europe (Table 3), and none of them is aimed to the treatment of dermatological diseases.

Table 3 - Cannabis-based medicines authorized in Europe. Adapted from European Monitoring Centre for Drugs and Drug Addiction. (50)

Brand name	Description	Indications and Usage	Administration
Sativex (Nabiximols)	Extract of *Cannabis* (oil): THC and CBD	Multiple sclerosis	Sublingual spray
Marinol (Dronabinol[c])	Synthetic Δ9-THC	Cancer treatment, AIDS, multiple sclerosis	Gelatine capsule
Cesamet (Nabilone[d])	Synthetic cannabinoid similar to THC	Cancer treatment	Capsule
Bedrocan	Dried flower tips (sometimes powdered); five different strains available.	Various	Plant material
Epidiolex	Purified CBD	Lennox-Gastaut syndrome, Dravet syndrome	Oral solution

The only marketing authorization granted by the European Medicines Agency (EMA), the agency responsible for the scientific evaluation of medicines in the European Union (EU), was to Epidiolex, in September 2019. Epidiolex was authorized, through a centralized procedure, to be commercialized in all EU member states. This cannabis-based drug contains

[c] The WHO name (International Nonproprietary Name, INN) for a specific variant of Δ9 -THC that occurs naturally in the *Cannabis* plant is dronabinol, and in literature the terms are used interchangeably. Chemically synthesized dronabinol is marketed as Marinol.

[d] (Cesamet) is a synthetic cannabinoid not occurring in nature. (79)

a purified form of CBD, and is indicated for the treatment of seizures associated with Lennox-Gastaut syndrome and Dravet syndrome in patients 1 year of age and older. (54,55) Although this medicine is the only one approved by the EMA, other EU countries have authorized the use of some other cannabinoid-based treatments, also shown in Table 3, through other processes. (56,57)

C. sativa L.-based medicines are unique concerning dosing and administration. Dosing is individualized for each patient, relying on titration to reach an optimum dose of the cannabinoid content, if existent, and it is usually recommended to start with lower doses, which are then increased over time until a positive response is registered, or until the negative effects outweigh the positive. Patients and clinicians must work together to establish a personalized drug regiment, with initial dose, increments and decrements well-defined to maximize the patient's benefits and minimize the adverse effects. (53)

Besides, despite the fact that approved medications to treat skin disorders are not yet available in the market, several studies have provided preliminary evidence that cannabinoids can be very beneficial in these conditions, like described in the previous chapter. However, existing studies tend to be small and lacking rigorous design, thus there is a clear need for high-quality randomized controlled trials that, as per the requirement of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) E6, provide safety data supporting the study design elements (e.g., participant population, dosing, expected adverse effects), and an outline to a post-study safety monitoring approach, in order to completely assess the efficacy and safety of these compounds, before their use can be authorized for the treatment of dermatological diseases. (49) Nonetheless, there are a few clinical studies on the use cannabinoids to treat skin conditions, some of which have been described previously, and which are summarized in Table 4.

Table 4 - Outline of clinical trials studying cannabinoid efficiency and safety in the treatment of dermatological disorders. Proposed by Nickles *et al.* (58)

Study	Type of study	Number of participants	Skin Disease	Cannabinoid-based product	Outcome
Ali *et. al* (46)	Single blind comparative study	11	Acne	*C. sativa* seed extract cream (3%)	○ Decrease in sebum and erythema levels.
Yuan *et al.* (35)	Randomized, double-blind, comparative trial	60	Asteatotic eczema	Emollient PEA/AEA cream (0.3%/0.21%)	○ Significant improvement in erythema, integrity of the skin, scaling, dryness, and itching at day 28; ○ No difference shown in transepidermal water loss (TEWL) between PEA/AEA cream and control cream.
Callaway *et al.* (59)	Single-blind crossover	20	Atopic dermatitis	Dietary hempseed oil	○ Improvement of skin dryness and itchiness; ○ Decrease in dermal medication usage.
Del Rosso (60)	Investigator-blinded comparative study	43	Atopic dermatitis	PEA-containing nonsteroidal cream	○ Lengthened the mean time to the next flare on an average of 28 days.
Eberlein *et al.* (36)	Cohort	2456	Atopic dermatitis	Emollient cream containing PEA	○ Decreased use of topical steroids; ○ Improved sleep; ○ Decreased severity, flare-ups; ○ Increased improvement of symptoms and disease tolerance.
Dvorak *et al.* (61)	Double-blinded Comparative study	12	Chronic pruritus	Cannabinoid receptor agonist HU210 by skin patch	○ Reduced experimentally induced itch; ○ Attenuated increase in blood flow.
Ständer *et al.* (62)	Cohort	22	Chronic pruritus	Emollient with PEA	○ Reduced subjective severity of pruritic symptoms.
Visse *et al.* (63)	Single-blind comparative study	100	Chronic pruritus	Derma-membrane system (DMS)-based dermatocosmetic lotion containing PEA	○ No significant differences between DMS-based PEA lotion group and control group concerning itch, quality of life, or cosmetic acceptance.

Most of these studies focus on skin disorders, thus they mainly evaluate the use of topical formulations. However, oral formulations can also be studied. For example Callaway *et al.* (59) performed a randomized crossover study to study the effect of dietary hemp seed oil on

the symptoms of AD. In this case, however, it is noteworthy that the decrease in itchiness, dryness, and in the need for topical medications, was attributed to the high content of polyunsaturated fatty acids in the hemp seed oil, and not to its cannabinoids. (58,59)

In summary, cannabis-based medicines can be available in various forms, from whole-plant products to highly purified extracts, formulations with different concentrations of CBD, THC, other cannabinoids, and varying terpene combinations, etc. It is very important to understand that, even if available at the same dose, formulation and concentration, a cannabis product can be widely different from another, being able to vary in potency and purity, possibly even having distinct absorption rates or pharmacokinetic profiles, as a consequence of different carrier oils, extraction methods, and/or delivery systems (vaporization, nanotechnology, oral *vs* sublingual, etc.). (53)

Moreover, despite being used by humans for millenniums, the adverse effect profile for cannabis and cannabis-related products is not yet defined and fully comprehended. However it is known that the most common adverse effects associated with the oral administration of *C. sativa* L. products are (9,64):

- Short-term (single/irregular use)**:** asthenia, balance problems, confusion, dizziness, disorientation, diarrhoea, drowsiness, dry mouth, fatigue, hallucination, nausea, somnolence, vomiting, and alterations on the levels of consciousness, cognition, perception, behaviour, and later response to whatever stimuli.
- Long-term (daily use, especially for long periods)**:** dependence, cognitive impairment, mental disorders (i.e., psychoses, depression, anxiety, suicidal ideation. etc.), cardiovascular diseases, cancers, and a high probability of serious injuries that can happen while intoxicated.

Therefore, all adverse events resulting from cannabis-based treatments, as well as all changes registered in routine biochemical, haematological, and physiological monitoring, like heart rate or blood pressure, must be recorded and reported during clinical trials.

However, it is important to note, that most of the listed side effects were caused by the oral administration or inhalation of *Cannabis sativa* L and its constituents, and therefore topical application may present an opportunity to benefit from the therapeutic properties of cannabis with the advantage of fewer adverse effects or less serious ones.

In summary, it is clear the high potential cannabinoids, and in particular, CBD, have in the treatment of dermatological disorders. However, further development and clinical studies with high standards and rigour are necessary for better comprehending efficacy, optimal product formulation, indication-specific dosing and possible/probable side effects of C. *sativa* L.-based medicines, so that quality and safety can be ensured in a clinical setting.

3. *Cannabis sativa* L. in Cosmetics

3.1 Cannabis-based cosmetics: current market

C. sativa L has become a very coveted feature in beauty, used by several brands in innovative products, which contributed to distance this plant from the negative connotation associated to prohibited substance and even trading it for a more inclusive and up-scale undertone. Hereafter, the interest of the beauty industry on this plant will most likely continue to grow, and its use in this sector has already been referred to as one of the most exciting booms in its history. (65)

The current CBD, hemp and cannabis market is more directed towards the feminine wellbeing. For example, cannabis-based edibles are used to alleviate stress and menstrual cramps, while CBD can be used to target post-partum depression. Usually, generally speaking, men are less focused on self-care, and thus their approach to this emerging category of products is more workout-related. (65)

The design stamps of the cannabis industry have evolved from pot leaf imagery to a more high-end health-care and well-being related look, often with a more feminine take, but with gender-neutral branding also being a very common option. (65)

There is a wide range of practical applications of cannabis products in the cosmetic industry. Examples are:

- Use of hemp seed oil in unique beauty and personal care applications. For example, in 2018 Milk Makeup launched mascara containing this oil as a binding agent instead of the traditional beeswax, adding richness and viscosity to the formula, and claiming its use to prevent dry-out while keeping lashes conditioned. (65,66)
- Use of CBD-infused in hemp seed oil in cosmetic formulations for hair care and skincare, which renders the beneficial effects of both the phytonutrients from the CBD extracts and polyunsaturated fatty acids from the hemp seed oil, providing higher nutrition to the skin, or hair. (67)
- Use of hemp oil to help decrease the transepidermal water loss (TEWL), thus increasing the water retained in the epidermis, which is crucial for moisturization. This

attribute can be highly relevant in the treatment of skin disorders, like AD, ACD, and other diseases that relate to dry skin. (67)

- Use of CBD in numerous skincare products, like face masks, creams, serums, exfoliants, bath bombs, and lip treatments, due to its known smoothing, anti-acne and anti-inflammatory properties. (66)
- Use of CBD and other cannabinoids in products that target hair growth, due to the role played by ECS in this process. (67)
- Use of some pCBs in sun protection, since a broad absorption in the UV range has been reported. Also in wound care, with its reported antimicrobial and antifungal benefits being useful in soaps, deodorants, or to treat open wounds as they heal. (67,68)
- CBD can also be found in drinks and dietary supplements, in sublingual forms, in gel caps, tinctures, etc. (67,69)

Furthermore, while trying to capitalize on all the attention that CBD and other cannabinoids are getting, the beauty industry has also been expanding their interest in other cannabis-derived compounds, such as terpenes. These molecules are already being used in some creams and skincare products, like the rejuvenating cream from the brand Ayuna (72), but can also be used for their ability to enhance the effects of CBD, opening a pathway for synergistic formulations, that may be necessary for the desired skincare outcome. (66)

3.2 Cannabis-based cosmetics: formulation challenges

CBD distillates are the most commonly used in modern cosmetic formulations, appearing, at room temperature, as a thick, sticky mass similar to honey, which is why, for transferring and measuring, they need to be heated up to about 90 ºC. The CBD distillates can either be *full-spectrum* or *broad-spectrum*. *Full-spectrum* are extracts or distillates that include mostly CBD, with other cannabinoids, such as THC, CBC, CBG, CBDA and CBDV, existing in only 1% or less, while *broad-spectrum* refers to an oil or distillate from which THC has been removed to the lowest amount possible. (70)

To formulate products such as balms or body butter, CBD distillates can be readily solubilized in vegetable oils, like palm, olive, almond, avocado and sunflower oils, but are

also compatible with common emollients like *Butyrospermum parkii* (shea) butter or *Simmondsia chinensis* (jojoba) oil. As for emulsions, glyceryl monostearate, polyethylene glycol and polysorbates are compatible with most cannabinoids. (70)

To keep CBD and other phytonutrients stable in hemp oil for extended periods, oxidation problems have to be tackled. During the manufacturing of the oils some precautions may be taken to control oxidation processes and rancidity, such as (67):

- Use of high-quality, freshly produced raw materials;
- Storage in cool, dark and cold places, under an inert atmosphere of nitrogen;
- Use of stainless steel containers to avoid metal contamination;
- Heating the oils to the minimum practical temperature required for the process, avoiding excess heating;
- Avoidance of any air leakage that might allow for an influx of air that typically induces the development of thermal and oxidative polymers.

Being a highly lipophilic molecule, CBD can accumulate in the *stratum corneum* rather than penetrate the deeper layers of the skin. Thus, formulations may need to include an efficient method of delivery, like gels made of carbomer, encapsulation and transdermal patches, or also in combination with hyaluronic acid, argan oil, or boswellic acid, to facilitate absorption. (71)

Dosing is a critical factor in CBD products, and the necessary amounts of cannabis or cannabinoids must be used for effective results. Thus, economic considerations and pressures may lead to misbranded products that deceive consumers on the amount that is actually present. Such cases have been reported, even forcing the US Food and Drug Administration (FDA) to send warning letters to companies that were not complying with regulations, which only reiterates the importance of a well-defined regulatory framework, consumer education and a critical thinking when analysing the products on the market. (70,72)

CBD products, especially those for topical application, perfectly integrate the market for natural topical products. This cannabis constituent may be delivered in most topical formats, as previously listed, the most common being creams and lotions (43%) followed by balms (26%), with the most common package sizes being 60 mL, followed by 30 mL. Most CBD topical products contain less than 500 mg of CBD per package, and a quarter of that number

even contains less than 100 mg. Thus, when compared to similar non-CBD topical products, it is clear that CBD-containing products, with their small package sizes and small cannabidiol amounts, are generally more expensive. Therefore, for future reference, there should to be a better balance between quantity and price, to appeal to a larger audience and incite consumers that have yet to try them. (63)

3.3 Cannabis-based cosmetics: current global regulatory framework review

Even though cannabis beauty and adjacent markets have been booming in recent years, their success can be hindered by a lack of or insufficient laws, compromising the transparency of *C. sativa* L. regulatory boundaries and possibly leading to misinterpretation and mishaps. For instance, following the passing of the Agriculture Improvement Act of 2018 (the 2018 Farm Bill), cannabis containing less than 0.3% of THC was removed from the Controlled Substance Act. Simultaneously, the U.S. Congress preserved the FDA's current authority to regulate products containing cannabis or cannabis-derived compounds under the Federal Food, Drug, and Cosmetic Act (FD&C Act) and section 351 of the Public Health Service Act, allowing this agency to continue enforcing the law to protect the public from unsafe products. Also, while most states supported the Farm Bill, allowing the legal growth of hemp, others did not, illegalizing the growth or even the transport of hemp in or through those states boundaries, even if the destination is a state that supports the bill. These different laws and regimes lead to confusion and misunderstandings, slowing down the progress in the *C. sativa* L. industry, and preventing the mainstream use of CBD in the U.S. Since the Farm Bill will expire in October 2021, it is expected that the identified concerns and faults will be addressed. (73–75)

Furthermore, in the U.S., cosmetic manufacturers are not required to register with the FDA or submit their products for pre-market approval, however they do have the responsibility to ensure the safety and correct labelling. The FD&C Act prohibits the introduction of cosmetics "adulterated", meaning with filthy, putrid or decomposed substances, or manufactured/held under insanitary conditions, and of "misbranded" cosmetics, with false or misleading labelling, that may be harmful to consumers under customary conditions of use. As long as the CBD-containing products comply with these requirements, the products can be marketed in the US. Despite not having to comply with specific FDA regulations and good manufacturing practices (GMP), the FDA has issued a document comprising international consensus standards for manufacturing cosmetics in general: the "Cosmetics—Good

Manufacturing Practices (GMP) - Guidelines on Good Manufacturing Practices," ISO 22716:2007 that industries can use to minimize the risk of product contamination and adulteration. (76)

Concerning the EU, there is also no consensus on CBD laws, and the criminal and administrative responses toward the use of CBD are the responsibility of each EU member state. By law, cannabis-based cosmetics may be placed on the EU market if they present as "safe for human health when used under normal or reasonably foreseeable conditions of use", as depicted in articles 3 and 4 of Chapter II of the Cosmetics Regulation (Regulation (EU) No 1223/2009). (74,77) Besides, CBD is not directly listed in the Annex II of said Cosmetics Regulation, a list of substances prohibited in cosmetic products, including all those mentioned in the tables drafted for the Convention on Narcotic Drugs of 1961, in which cannabis is included. Henceforth, since CBD is not directly mentioned, it is allowed for it to be used in cosmetic products and preparations, as long as all other regulations are respected. (78)

Conclusions

Cannabis and cannabinoid-based products seem to have promising applications in skincare, both in the cosmetics industry and for the topical treatment of skin diseases and their symptoms, such as pruritus, inflammatory diseases, and even skin cancers.

However, to further explore such possibilities, our knowledge of the cutaneous cannabinoid system must expand. Because cannabinoids can bind to multiple receptors (not necessarily limited to CBRs), with varying affinities, or possibly even acting in a receptor-independent manner, they can lead to biological outcomes that currently cannot be reliably predicted, challenging the approval of cannabis-based therapies. Besides, the existing clinical trials are still typically of small dimensions, and greatly varying in formulation, route of administration, dosage, and frequency of use, not providing enough data concerning safety and efficacy. Therefore, the industry must be encouraged to keep investing in cannabis-related studies, so that an evidence base for the therapeutic use of cannabis-based drugs is established.

Moreover, additionally to its therapeutic potential, CBD is currently one of the most interesting and explored components of *C. sativa* L. in the cosmetic industry, due to its seemingly safe therapeutic profile and lack of psychoactivity. Due to its quick and increasing emergence in the beauty space, it is important that dermatologists and pharmacists, with important roles in skincare counselling, are well versed in CBD and all cannabis-based products that are introduced in the market. This counselling will be essential in clarifying consumer questions arising due to the negative attention that has been surrounding cannabis over the years, and also to help navigate all the new vocabulary and information associated with this emerging industry (CBD, hemp, cannabinoids, etc.).

Furthermore, the legal status of *C. sativa* L. is not yet clear and standardized worldwide and, with the widespread growth of markets selling products containing CBD (medicinal products, foods and cosmetics) and the continuous rise in potential pharmacological and cosmetic applications of other *C. sativa* L. constituents, the legal framework for the use of *C. sativa* L. must evolve until firmly established.

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