Thieme

Cannabis sativa and Skin Health: Dissecting the Role of Phytocannabinoids

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ABSTRACT

The use of *Cannabis sativa* is currently recognized to ease certain types of chronic pain, reduce chemotherapy-induced nausea, and improve anxiety. Nevertheless, few studies highlighted the therapeutic potential of *C. sativa* extracts and related phytocannabinoids for a variety of widespread skin disorders including acne, atopic dermatitis, psoriasis, pruritus, and pain. This review summarized the current evidence on the effects of phytocannabinoids at the cutaneous level through the collection of *in vitro, in vivo,* and clinical studies published on PubMed, Scopus, Embase, and Web of Science until October 2020. Phytocannabinoids have demonstrated potential anti-inflammatory, antioxidant, anti-aging, and anti-acne properties by various mechanisms involving either $CB_{1/2}$-dependent and independent pathways. Not only classical immune cells, but also several skin-specific actors, such as keratinocytes, fibroblasts, melanocytes, and sebocytes, may represent a target for phytocannabinoids. Cannabidiol, the most investigated compound, revealed photoprotective, antioxidant, and anti-inflammatory mechanisms at the cutaneous level, while the possible impact on cell differentiation, especially in the case of psoriasis, would require further investigation. Animal models and pilot clinical studies supported the application of cannabidiol in inflammatory-based skin diseases. Also, one of the most promising applications of non-psychotropic phytocannabinoids is the treatment of seborrheic disorders, especially acne. In conclusion, the incomplete knowledge of the role of the endocannabinoid system in skin disorders emerged as an important limit for pharmacological investigations. Moreover, the limited studies conducted on *C. sativa* extracts suggested a higher potency than single phytocannabinoids, thus stimulating new research on phytocannabinoid interaction.

Introduction

Cannabis sativa L. is an herbaceous plant, which is generally found in temperate and tropical regions of the world [1]. Since ancient times, this plant has been an important source of fibers for the preparation of ropes, textiles, or paper. Among the different human applications, a special mention should be made of the therapeutic use of *C. sativa*, which finds its roots in traditional Chinese medicine about 5000 years ago as a remedy for pain and inflammation [2]. Today, the major clinical evidence shows that *C. sativa* is a valuable support to ease certain types of chronic pain, to reduce chemotherapy-induced nausea, and to improve anxiety [3].

Nevertheless, old and recent evidence suggests the extension of *C. sativa* benefits to dermatological disorders. The first Western observations on topical use were recorded at the end of the 19th

* The authors contributed equally to this work.

ABBREVIATIONS

$[C]_{max}$	maximum concentration
2-AG	sn-2-arachidonylglycerol
5HT	serotonin receptor
A	adenosine receptor
AEA	N-arachidonoylethanolamine, anandamide
Akt	protein kinase B (PKB)
ASK	apoptosis signal-regulating kinase
AUC	area under the curve
BACH1	transcription factor BTB and CNC homology 1
C. sativa	*Cannabis sativa* L.
CB	cannabinoid receptor
CBC	cannabichromene
CBCV	cannabichromevarin
CBD	cannabidiol
CBDA	cannabidiolic acid
CBDV	cannabidivarin
CBDVA	cannabidivarinic acid
CBE	cannabielsoin
CBG	cannabigerol
CBGA	cannabigerolic acid
CBGV	cannabigerovarin
CBL	cannabicyclol
CBM	cannabinoid-based medicine
CBN	cannabinol
CBND	cannabinodiol
CBT	cannabitriol
CCL	C-C motif chemokine ligand
CGRP	calcitonin gene-related peptide
CREB	cAMP-response element-binding protein
CXCL	C–X-C motif chemokine ligand
DNFB	dinitrofluorobenzene
DNMT1	DNA (cytosine-5)-methyltransferase 1
DOR	δ-opioid receptor
ECM	extracellular matrix
EGCG	epigallocatechin-3-gallate
FABP	fatty acid-binding protein
GABA	γ-aminobutyric acid
GlyR	glycine receptor
GPCR	G protein-coupled receptor
GPR	putative cannabinoid receptor
HO-1	heme oxygenase-1
HPV	human papillomavirus
ID_{50}	infective dose
IFNγ	interferon gamma
IKKα/IKB	inhibitor of nuclear factor kappa-B kinase complex – subunit alpha
IL-	interleukin-
JKN	c-Jun N-terminal kinase
K	keratin
Keap-1	Kelch ECH associating protein 1
LTB	lymphotoxin-beta
MAPK	mitogen-activated protein kinase
MCP-2	monocyte chemoattractant protein 2 (CCL8)
MMP-	matrix metallopeptidase-
MOR	μ-opioid receptor
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NK	natural killer
NLRP3	NLR family pyrin domain containing 3
NRF-2	nuclear factor erythroid 2-related factor 2
PBQ	parabenzoquinone
PI3K	phosphoinositide 3-kinase or phosphatidylinositol 3-kinases
p-IκB	phosphor-nuclear factor of kappa B inhibitor
PKA	protein kinase A
PPAR	peroxisome proliferator-activated receptor
PUFA	polyunsaturated fatty acids
Ref-1	redox effector factor 1
ROS	reactive oxygen species
TGase5	transglutaminase 5
THCV	tetrahydrocannabivarin
THCVA	tetrahydrocannabivarinic acid
TIMP	tissue inhibitor of metalloproteinases
TLR	toll-like receptor
T_{max}	the amount of time that a drug is present at the maximum concentration
TPA	tissue polypeptide antigen
TRP	transient receptor potential
TRPA	transient receptor potential channel, ankyrin subtype
TRPM	transient receptor potential channel, melastatin subtype
TRPV	transient receptor potential channel, vanilloid subtype
VEGF	vascular endothelial growth factor
α2-AR	α2-adrenergic receptor
Δ^8-THC	(−)-Δ^8-*trans*-tetrahydrocannabinol
Δ^9-THC	THC, (−)-Δ^9-*trans*-tetrahydrocannabinol

century for the reduction of itching [4]. Subsequently, several studies have evaluated the cutaneous effects of this plant, focusing mainly on the individual cannabinoids.

Cannabinoids are a group of bioactive compounds, classified as endocannabinoids (physiologically synthesized in our organism), phytocannabinoids (produced by plants), or synthetic cannabinoids (artificially synthesized in laboratories), according to their source.

The endocannabinoids act as neuro- or immunomodulatory agents in humans and interact with several targets in a specific context-dependent way, thanks to their lipophilic nature [5]. Phytocannabinoids have different affinities toward human receptors; furthermore, the biological effects are often the result of the interaction with several molecular targets [3].

The biological activities of phytocannabinoids include the psychoactive effects of *C. sativa*, and other adverse effects of recreational use, which have been extensively reviewed [6]. Neverthe-

less, phytocannabinoids can significantly influence skin biology and may also potentially exert therapeutic activities.

This review aims to collect the existing scientific evidence of the beneficial effects of phytocannabinoids from *C. sativa* at the cutaneous level, focusing on the effects on peculiar cell types in the skin, such as keratinocytes, fibroblasts, melanocytes, and sebocytes. A specific effort was conducted to document the relevance of single phytocannabinoids for the bioactivity of whole extracts.

Molecular targets of phytocannabinoids

C. sativa synthesizes an abundant number of secondary metabolites exhibiting the typical C_{21} terpenophenolic skeleton, called "cannabinoids" or more specifically "phytocannabinoids". Some biological effects of phytocannabinoids are connected to the modulation of the endocannabinoid system, through a class of GPCRs, CB_1, and CB_2 [7] differently distributed in various cell types, including human keratinocytes, melanocytes, dermal fibroblasts, and myoepithelial cells [8]. Other receptors involved in the biological activities of *C. sativa* are the TRP channels, in particular TRPV1 and the PPARs [9]. The main recognized regulatory pathways modulated by phytocannabinoids are described below.

GPCRs

Both CB_1 and CB_2 are expressed in different skin structures, such as the epidermal layer, sebaceous glands, and hair follicles, and their regulation is involved in inflammatory processes, cell proliferation, and sebum production [7]. Although these receptors are found at the cutaneous level, CB_1 is preferentially expressed in the central nervous system, in particular in presynapses of GABA-ergic and glutamatergic neurons [10]. In contrast, CB_2 expression is predominantly diffused in immune cells, such as monocytes, lymphocytes, and NK cells [11]. CB_1 and CB_2 belong to a sub-group of a class A GPCRs able to bind lipid-derived endogenous ligands, such as AEA anandamide and 2-AG [12]. High ligand lipophilicity is required for ligand binding to CB_1 or CB_2. In the skin, CB_1 is preferentially localized in the stratum spinosum and granulosum of keratinocyte layers of the epidermis, while CB_2 is found in basal keratinocytes, and sebaceous and follicle epithelial cells. Among additional class A GPCRs that have been implicated in various phytocannabinoid actions, there are adenosine (A_{1A}, A_{2A}), α2-adrenergic (α2-AR) serotonin ($5HT_{1A}$, $5HT_{2A}$, $5HT_{3A}$), μ- and δ-opioid (MOR and DOR) receptors, GPR55, and GPR18 [5].

PPARs

Some phytocannabinoids can activate the transcriptional activity of PPARs and these effects can be blocked by the use of PPARs antagonists. The general activation of PPARα and PPARγ isoforms is associated with some of the neuroprotective, antinociceptive, antiproliferative, anti-inflammatory, and metabolic properties of cannabinoids. The mechanism of action is still not clear, but the metabolic conversion to active PPARs interactors has been suggested [13]. A possible mechanism enhancing this interaction is the active transport of cannabinoids to the nucleus by FABPs [14]. Recent findings have shown that some phytocannabinoids can be transported to the interior of the cell by these proteins, and, therefore, they could be delivered for PPARs activation [15]. At the cutaneous level, the expression of PPARγ has been reported in fibroblasts, keratinocytes, melanocytes, and sebocytes, in the latter case related also to lipid biosynthesis [8]. Phytocannabinoids can bind PPARγ, enhancing the related transcriptional activity and inducing apoptotic effects [16], but little information is known about the interaction between PPARα and phytocannabinoids, while the potential involvement of the PPARβ/δ isotype remains unknown.

TRP channels

TRP is a family of ion channels that are strictly involved in different cutaneous processes such as itch, temperature and pain perception, barrier homeostasis, inflammation, and regulation of hair follicles and sebaceous glands [17]. In humans, 27 TRP channels have been identified and divided into 6 subfamilies. Phytocannabinoids have shown activities on TRP channels from 3 different subfamilies: TRPV (vanilloid), TRPA (ankyrin), and TRPM (melastatin). To date, 6 types of TRP channels of the aforementioned 3 subfamilies have been identified as targets of phytocannabinoids: TRPV1, TRPV2, TRPV3, TRPV4, TRPM8, and TRPA1 [18,19]. TRPM8 is expressed in sensory neurons and is involved in cold perception, while TRPV1, TRPV4, and especially TRPV3 are connected to mechanical and heat-evoked pain and are found in keratinocytes and epithelial cells of human hair follicles. TRPV1 and TRPV2 are diffused in sensory neurons and immune cells and participate in pain sensation and inflammation. Lastly, TRPA1 can be found in keratinocytes and melanocytes, in which it regulates pro-inflammatory processes and melanin synthesis, therefore being involved in photoprotective mechanisms [17].

GlyR

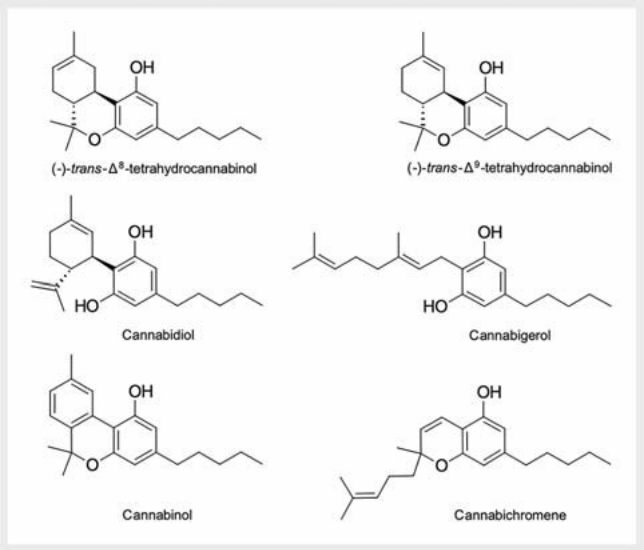
Glycine is an important neurotransmitter in the human central nervous system, and GlyRs are relevant targets for central cannabinoid action. One phytocannabinoid, CBD, has already been shown to reduce the peripherical inflammatory and neuropathic pain by potentiating GlyRs [20,21]. In mice, topical application of glycine accelerates skin barrier recovery [22]. GlyRs are also expressed in human keratinocytes and could have a role in epidermal permeability and barrier homeostasis [23].

Phytocannbinoids

Up to 120 cannabinoids have been identified so far and classified into 11 classes: Δ^9-THC, Δ^8-THC, CBG, CBN, CBD, CBND, CBC, CBE, CBL, CBT, and miscellaneous types [24]. However, the proportion among different classes is dependent on growing conditions, geographical area, plant processing methods, and plant variety/chemotype [25]. The most important classes of phytocannabinoids active at the cutaneous level are summarized below (▶ **Fig. 1**).

Tetrahydrocannabinol (Δ^9-THC and Δ^8-THC) types

The Δ^9-THC type is the most abundant, representing 17.3% of the total phytocannabinoid content, and together with Δ^8-THC type, which derives from an acidic isomerization of Δ^9-THC [26], are recognized as the psychoactive substances of *C. sativa*. Δ^9-THC is a partial agonist at both cannabinoid receptors: CB_1, a modulator of psychoactive effects, and CB_2, a modulator of immunological and anti-inflammatory pathways. Different studies have shown



▶ **Fig. 1** Chemical structures of the main phytocannabinoids investigated at cutaneous level.

that Δ^9-THC acts as an agonist of PPARγ, TRPA1, TRPV2, TRPV3, and TRPV4, and as an antagonist of TRPM8. Furthermore, Δ^9-THC can modulate the activity of μ/δ opioid- and GlyRα1/α3 receptors [27]. THCV is the homolog of THC characterized by a propyl chain instead of a pentyl chain.

CBD type

CBD is a non-psychotropic phytocannabinoid, and the CBD type class is the third most abundant in *C. sativa*, after Δ^9-THC and CBG [28]. This molecule has a low binding affinity for CB_1 and CB_2 receptors [29–31], but through the modulation of multiple targets, it reduces pain, inflammation, and anxiety and displays potential antitumor activities [32]. Despite the low affinity *in vitro*, a recent meta-analysis concluded that CBD may affect CB_1 receptor activity *in vivo* via an indirect mechanism [33–35]. Also, CBD can reduce the uptake of anandamide at the cellular level, but at concentrations far from those relevant for a physiological effect *in vivo* [18], and antagonize GPR55 [36–38] and GPR18 [39].

Outside of the endocannabinoid system, the mechanisms by which CBD possibly mediates anti-inflammatory and immunosuppressive effects include the activity at the A_{1A} adenosine receptor [40], the inhibition of the equilibrative nucleoside transporter [40, 41], and the activation of strychnine-sensitive α_1 and $\alpha_1\beta$ Glyrs [20, 21, 42]. CBD interacts differently with the serotonin receptors, as a full agonist, a weak agonist, and a noncompetitive antagonist (respectively for $5HT_{1A}$, $5HT_{2A}$, and $5HT_{3A}$) [43, 44]. *In vitro*, CBD has been reported by numerous studies to activate the TRPV1, TRPV2, and TRPA1 channels [18, 45, 46], while antagonizing TRPM8 *in vitro* [47]. *In vivo*, CBD shows possible activity at the TRPV1 channels in mice and rats and the TRPA1 channels in rats [48]. In addition, CBD acts as an agonist of PPARγ receptors and as an allosteric modulator of μ/δ opioid receptors [49] and GlyRα1/α3 receptors [20, 21]. CBDV is the homolog of CBD with the propyl chain.

CBG type

CBG has been the first compound to be isolated in a pure form from the resin of *C. sativa*. Many novel cannabinoids belonging to the CBG-type have been reported recently [50], most of them isolated from the buds of the mature female plant of a high-potency variety of *C. sativa* [51–53]. As opposed to CBD, CBG acts as a partial agonist for CB_1 and CB_2, but in common they share a weak affinity for these receptors [54] and the inhibition of anandamide uptake [55]. CBG is an agonist of TRP channel receptors (TRPA1, TRPV1, and TRPV2) and an antagonist of TRPM8, TRPV4 [19, 55], and $5HT_{1A}$ receptors [56]. CBGV is the homolog of CBG with the propyl chain.

CBC type

CBC was discovered in 1966 [57] as an agonist of CB_2 receptors [58] and is recognized as one of the strongest TRPA1 agonists among the phytocannabinoids, together with CBD and CBN [55]. It also displays agonism to TRPV3 and TRPV4 channels [19] and antagonism for TRPM8. CBC is also able to increase anandamide levels by inhibiting its uptake [55].

CBN type

Seven cannabinol derivatives, which are aromatized derivatives of THC, have been described. CBN is commonly found in the aged plant of *C. sativa* or in related products, in which the concentration increases during storage [50]. Compared to Δ^9-THC, CBN shows a higher and lower affinity for CB_2 and CB_1 receptors, respectively [59].

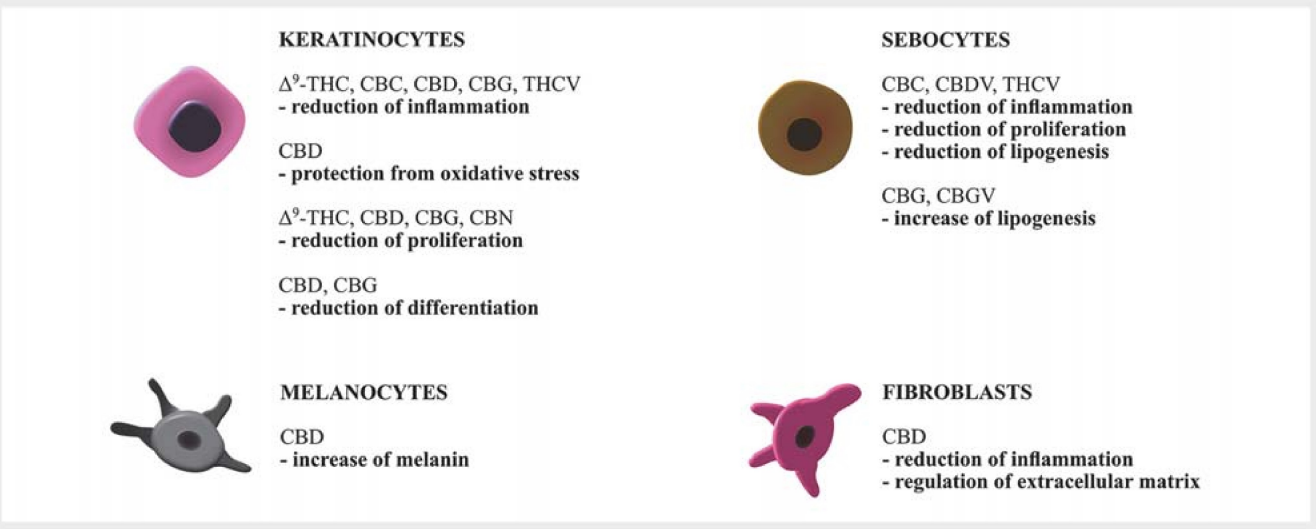
A wider and detailed comparison of the molecular mechanisms of phytocannabinoids and their specific affinities towards human receptors can be found in recently published reviews [25, 27].

Almost all the works concerning the cutaneous effects of phytocannabinoids are based on the study of pure or isolated compounds. The scientific data were collected and commented below, divided according to the evidence on cellular models, *in vivo* or human.

Search Strategy

This review aimed to collect *in vitro, in vivo*, and clinical evidence of the role of phytocannabinoids and *C. sativa* extracts in skin health, as well as their use against skin diseases.

A systemic search of electronic databases, including PubMed, Scopus, Embase, and Web of Science, was conducted in October 2020, for papers reporting *in vitro, in vivo,* or clinical evidence of the effects of phytocannabinoids at a cutaneous level, using the following search terms: ("cannabis" OR "cannabichromene" OR "cannabidiol" OR "cannabigerol" OR "cannabicyclol" OR "cannabinol" OR "cannabidivarin" OR "tetrahydrocannabinol") AND ("skin" OR "keratinocytes" OR "fibroblasts"). In a second step, duplicate articles were removed, and the references listed in the remaining ones were sifted through to identify documents that might have eluded the primary search. The search limit was the English language, whereas no limit was applied for the year of publication. This review includes only published articles and does not consider unpublished works or non-peer-reviewed articles.



▶ **Fig. 2** Summary of the main in vitro pharmacological actions of phytocannabinoids in keratinocytes, sebocytes, melanocytes, and fibroblasts.

Preclinical and clinical studies concerning the effects of *C. sativa* extracts or phytocannabinoids found in the plant on skin pathophysiology were considered eligible. Full titles and abstracts were checked for adherence to the eligibility criteria. Then, full texts were carefully read and checked for inclusion by all the authors. Papers including results obtained from the combination of extracts from plants other than *C. sativa* or phytocannabinoids together with other non-*C. sativa* molecules, except for specific pharmacological formulations, were out of the scope of the present review.

Relevant information (the type of phytocannabinoid, type of evidence, details about the model, dose/concentration, presence/absence of a positive control, schematic results, and biological context) was extrapolated from articles and summarized in a table propaedeutic to the text of the article.

In vitro Evidence

Several *in vitro* researches on cannabinoids described their biological activity (▶ **Fig. 2**) and mechanisms of action in skin cells, like keratinocytes, fibroblasts, melanocytes, and sebocytes (▶ **Table 1**). The role of $CB_1/_2$-dependent or independent signaling was considered in most of the studies, which regarded inflammation and oxidative stress, proliferation, differentiation, and migration.

The most investigated nonpsychotropic cannabinoid at the cutaneous level was CBD. The antioxidant effect of CBD was recently evaluated against UV or hydrogen peroxide models of cellular oxidation. CBD (4 µM) was able to rescue keratinocytes and melanocytes from UV-B-induced cytotoxicity [60]. The cytoprotective mechanism was deepened in keratinocytes by Skrzydlewska et al. [61,62], who ascribed it to the preservation of antioxidant defenses, the protection of plasma membrane, and the modulation of the endocannabinoid system. The authors correlated the accumulation of CBD at the membrane level with the reduction of markers of lipid peroxidation (malondialdehyde, 8-isoprostanes), the preservation of antioxidant proteins (thioredoxin, reduced glutathione, catalase) and lipophilic vitamins (A, E), and the restoration of PUFA composition [61,62]. Moreover, CBD reduced the level of endocannabinoids (anandamide, palmitoylethanolamide) in irradiated keratinocytes from healthy donors, while it increased the expression of CB receptors. Notably, the effect was controversial in irradiated cells from psoriatic patients, in which CBD tended to increase the oxidative status and exerted opposite effects on the endocannabinoid system (increased anandamide levels and decreased CB_2 expression). The authors had taken into consideration the confounding factors related to the different basal conditions of healthy and psoriatic keratinocytes and the impact of UV irradiation per se on the endocannabinoid system [61]. However, this work has opened the investigation on the use of CBD as an antioxidant for psoriasis.

The same group evaluated also the antioxidant and anti-inflammatory activity of CBD (1 µM) at the transcriptional level in keratinocytes, pointing out the role of the transcription factors NRF-2 and NF-κB, and their interplay [63]. In physiological conditions, CBD enhanced the activity of NF-κB and NRF-2, acting, respectively, on the upstream proteins IKKα/p-IKB and p62/Keap-1. The results suggested the concomitance of antioxidant and pro-inflammatory activity; on the contrary, after UV-A or UV-B irradiation, CBD revealed mostly antioxidant and anti-inflammatory properties, despite the production of TNF-α being elevated: in fact, CBD enhanced the phosphorylation of NRF-2 and the expression of the NRF-2-dependent proteins heme oxygenase 1 (HO-1) and thioredoxin reductase; moreover, the cytoprotective proteins ASK and Ref-1, elevated by UV irradiation, were normalized.

From the inflammatory point of view, CBD inhibited the inflammasome NLRP3, the levels of MAPKs, and the translocation of p52 (NF-κB). Despite the interplay between NF-κB and NRF-2 being still not clear in the scientific literature, the authors asserted the plausibility of the hypothesis that the activation of NRF-2 impaired the activity of NF-κB, according to their data and the literature. On the other hand, our group [64] excluded that CBD (1 µM) may act on NRF-2 translocation, which resulted in impairment

▶ **Table 1** Summary table of *in vitro* and *in vivo* skin studies conducted with phytocannabinoids.

Phytocannabinoids	Evidence	Details about the model	Concentration/dose	Positive control	Schematic results	Biological context	Ref.
Preclinical research							
Δ^9-THC, CBD, CBG, CBN, and olivetol	*In vivo*	CDI male mice; PBQ-induced writhing and TPA-induced ear erythema		Acetylsalicylic acid and trifluoperazine	CBD, CBN, and olivetol ↓ erythema (topical administration) and writhing (oral administration) with the strongest inhibitory potency (< 10 mg/kg); CBN was only marginally active; Δ^9-THC was fully active only at doses > 10 mg/kg	Skin inflammation and pain	[82]
CBD	*In vitro*	HaCaT keratinocytes and HDF fibroblasts cells, TNF-α induction	0.05–5 µM in HaCaT cells, 0.1–2.5 µM in HDF cells	EGCG, resveratrol, and quercetin	In keratinocytes: ↓ NF-κB; ↓ MMP-9; CBD was able to downregulate 15 TNF-α-induced genes. In fibroblasts: no inhibition of NF-κB; CBD impaired gene expression of 11 TNF-α-induced genes	Potential effect against inflammatory skin diseases	[64]
CBD	*In vitro*	Normal human dermal fibroblasts (NHDF) and normal human epidermal keratinocytes (NHEK)	CBD 0.1–0.2 µg/mL	//	↓ metalloprotease inhibitors TIMP1/2 and the expression of collagen in fibroblasts; ↓ LPS-induced IL-6 expression in fibroblasts, but not in keratinocytes	Wound healing	[66]
Δ^8-THC	*In vivo/ ex vivo*	Human skin samples from abdominoplasty surgery; male and female hairless IAF and Hartley guinea pigs	*Ex vivo*: the highest concentration of THC in any diffusion sample was 0.77 µg/mL; *In vivo*: 1 mg/kg was infused for 30 s and application of a TTS patch in guinea pigs	//	Similar permeability of Δ^8-THC in human and guinea pig	Permeability study	[71]
CBD	*In vitro/ in vivo*	NHEK, HaCaT; six-month-old female BALB/cByJRj mice	10 µM for 24 h *in vitro*; 0.1–10 % *in vivo* (topically, 1/day for 5 days)	//	↑ skin thickness, K16, and K17, but not pro-inflammatory cytokines *in vivo*; ↑ NRF-2 and HO expression trough ↓ BACH1 *in vitro*	Inflammatory skin diseases	[65]
CBD	*In vitro*	Fibroblast (CCD-25Sk) 2D and 3D culture models; UV-A 20 J/cm^2 and UV-B 200 mJ/cm^2 irradiation	4 uM	//	↓ UV-induced collagen degradation in 2D and 3D fibroblast models through PI3K/Akt pathway; ↓ lipid peroxidation; ↓ NF-κB pathway, ↑ PPARγ expression	//	[67]
CBD	*In vitro*	Human epidermal melanocytes	6 µM	Forskolin 5 µM/PMA	↑ melanin levels through tyrosinase activation, ↑ p-CREB, ↑ p-p38, p-p42/44, but not p-JNK; the mechanism was cAMP/PKA-independent and CB-1 dependent	Hypopigmented skin disorder	[68]
CBD	*In vitro*	Human keratinocytes (CDD 1102 KERTr) exposed to UV-A (30 J/cm^2) and UV-B (60 mJ/cm^2)	1 µM	//	↑ NF-κB and NRF-2 activity (physiological conditions); ↓ NF-κB, NLRP3, MAPK, ↑ NRF-2, and HO-1 expression, ↓ ASK and Ref-1 (during irradiation)	//	[63]
CBD (ethosomal formulation)	*In vivo*	CD1 nude mice, patch for 24 h; ICR mice, patch or 2–73 h and carrageenan paw edema	//	//	↓ paw edema; transdermal absorption; detection in the plasma (0.67 µg/mL at the steady state); accumulation in the hip skin (37.43 µg/cm^2), abdominal skin (110.07 µg/cm^2), and underlaying muscle (11.54 µg/cm^2)	//	[84]

cont.

▶ **Table 1** *Continued*

Phytocannabinoids	Evidence	Details about the model	Concentration/dose	Positive control	Schematic results	Biological context	Ref.
CBD	*In vitro*	SZ95 (sebocytes)	1–10 µM	//	↓ lipogenesis vs. arachidonic acid and testosterone; ↓ proliferation; ↓ TNF-α expression and NF-κB activation induced by TLR2 and TLR4 agonists; ↑ intracellular Ca^{2+} levels through TRPV4 agonism; ↑ cAMP through $A_{2\alpha}$ agonism	//	[69]
Δ^9-THC	*In vivo/ in vitro*	Wildtype vs, $CB_{1/2}$ knock-out mice, DNFB-induced dermatitis model; primary keratinocytes and macrophages from mice	30 µg (*in vivo*), 0.1–1 µM (*in vitro*)	//	↓ ear dermatitis and IFNγ *in vivo*; ↓ pro-allergenic chemokines CCL2, CCL8, CXCL10 through CB1/2-independent mechanisms in IFNγ-activated keratinocytes; ↓ macrophage migration in co-culture experiments	Atopic dermatitis	[74]
Δ^9-THC	*In vivo/ in vitro*	CB_1 and CB_2 receptor-deficient mice; melanoma cells	5–10 µM (*in vitro*), 5 mg/kg (*in vivo*)	//	The growth of murine melanomas was not affected by $CB_{1/2}$ depletion; Δ^9-THC (5–10 µM) ↓ cancer growth through anti-inflammatory activity (↓ infiltration of immune cells) and not $CB_{1/2}$-dependent mechanisms	Melanoma	[75]
Δ^8-THC	*In vivo/ ex vivo*	Rat vs. human skin models	Two formulations containing 26.5 mg/g Δ^8-THC were tested *in vivo* and in cell diffusion test	//	Δ^8-THC is 13-fold more permeable in rat than human skin and accumulates in the epidermis	Permeability study	[71]
CBC, CBDV, CBG, CBGV, THCV	*In vitro*	SZ95 (sebocytes)	0–10 µM	//	CBC ↓ basal and AA-induced lipid synthesis; CBG ↑ basal lipid synthesis and ↓ anandamide inhibitory effect	//	[69]
CBD, CBDA, CBDV, CBDVA, CBC, CBG, CBGA, CBGV, THCV, THCVA	*In vitro*	HaCaT keratinocytes stimulated with poly-(I:C)	1–20 µM	//	CBD: ↓ MCP-2, IL6-, TNF-α, IL-8, ↑ anandamide levels; CBDA, CBDV, CBDVA: no effect on MCP-2; CBC, CBG: ↓ MCP-2, IL-6, IL-8; CBGA: no effect on MCP-2 protein levels; CBGV: ↓ MCP-2 (only at 10 µM); THCV: ↓ MCP-2, IL-6. THCVA: no effect on MCP-2 protein levels.	Allergic contact dermatitis (ACD)	[79]
CBD, CBDV, CBG	*In vitro*	HaCaT and HNEK keratinocytes; TPA- and calcium-induced differentiation in HaCaT	0.1–0.5–1.0 µM	Endogenous cannabinoid AEA (anandamide)	↓ keratinocyte differentiation by increasing the general methylation state of genes	Skin diseases involving proliferation and differentiation unbalance	[77]
Δ^8-THC, CBD, CBN	*In vitro*	Human skin samples from abdominoplasty surgery; diffusion cell test	Δ^8-THC formulation: 9.09 mg/mL drug solution in propylene glycol:water:ethanol (9:1:1) or 16.67 mg/mL in propylene glycol:water:ethanol (1:1:1); CBD and CBN formulations: saturated solutions in mineral oil (7:3 propylene glycol:water or 4:5:4 propylene glycol:water:ethanol)	//	Δ^8-THC accumulated in skin tissue; CBN and CBD exhibited 10-fold higher permeability	Permeability study	[73]

cont.

▶ **Table 1** *Continued*

Phytocannabinoids	Evidence	Details about the model	Concentration/dose	Positive control	Schematic results	Biological context	Ref.
CBD, CBDV, CBCV, CBC, THCV, THC	*In vivo*	Croton oil-induced ear edema in mice	Dose range of 0.1–1 µmol/cm^2	Indomethacin 0.1–1 µmol/cm^2	↓ edema; Δ^8-THC, Δ^8-THCV, and Δ^9-THC were more effective (ID_{50} = 0.46–0.55 µmol/cm^2) than CBD, CBC, CBDV, CBCV (ID_{50} > 2 µmol/cm^2), but only slightly less potent than indomethacin	//	[83]
Δ^9-THC, CBD, CBN, CBG (the phytocannabinoids THC, CBD, CBN, and CBG were isolated from a hexane extract of *C. sativa*, grown domestically under controlled hydroponic conditions. Structures were verified by NMR spectroscopy.)	*In vitro*	The principal cells selected for use in these experiments were HPV-16 E6/E7 transformed human skin keratinocytes (ATCC; CRL-2309 KERTr). The antiproliferative effect was measured after 72 h of exposure.	//	//	↓ proliferation by CB_1- and TRPV1-independent mechanisms (2.0 < IC_{50}s < 2.9 µM)	//	[78]
Δ^9-THC	*Ex vivo*	Wildtype and CB-1$^{-/-}$ or TRPV-1$^{-/-}$ mice; rat skin explants	0.1–100 µM	Anandamide	↓ CGRP release via CB_1 (low conc.) or TRPV1 (high conc.)	Nociception	[76]
CBD	*In vitro*	Primary keratinocytes from healthy vs. psoriasis patients	4 µM	//	↓ oxidation in healthy keratinocytes, but only partially in psoriasis keratinocytes; CBD accumulated in membranes, especially after UV stimulation in psoriatic cells	Psoriasis and oxidative stress	[61]
CBD	*In vitro*	Primary keratinocytes and melanocytes	1, 4, 8 µM	//	↓ cell mortality (vs. UV-B irradiation)	UV damage	[60]
CBD	*In vitro*	CDD 1102 KERTr human keratinocytes	4 µM	//	↓ lipid peroxidation, ROS, cell mortality (vs. UV and H_20_2); the effect correlated with membrane protection through the restoration of PUFA composition	Skin protection against oxidative stress (UV, ROS)	[62]

after UV-B irradiation in human keratinocytes, while we confirmed the anti-inflammatory effect of CBD in TNF-α-challenged cells. In particular, CBD was able to interfere with NF-κB activity (IC_{50} = 2.85 µM), affecting the NF-κB-dependent protease MMP-9 in a significant manner (IC_{50} = 5 µM) but not VEGF and IL-8. In qPCR arrays, TNF-α treatment induced the upregulation of 26 genes including chemokines (e.g., CXCL8/IL-8 and CXCL10), interleukins (e.g., IL17C and IL1B), TNF family members (e.g., TNF and LTB), and other genes such as VEGFA, while the treatment with CBD was able to downregulate 15 of those genes. Different from keratinocytes, NF-κB and inflammatory mediators (MMP-9 and IL-8) were not influenced by CBD in human dermal fibroblasts. However, CBD impaired gene expression, similar to what was observed in keratinocytes. In fact, TNF-α upregulated 16 genes involved in wound healing and inflammation; in particular, the most upregulated ones were ECM enzymes (e.g., MMP-1 and MMP-9), cytokines (e.g., CXCL11, CXCL2, and IL-6), growth factors and signal transducers, while CBD treatment downregulated 11 of those genes.

In line with previous works [63, 64], Casares et al. [65] selected NRF-2 as a possible target of CBD for skin protection. This work was performed on human keratinocytes and confirmed that CBD (10 µM) may act as a weak inducer of NRF-2 (in comparison to sulforaphane, a known NRF-2 activator) and added new information on the mechanism of action. CBD strongly enhanced the expression of HO-1 and other antioxidant genes, but this effect was not sustained by strong NRF-2 activation. Moreover, the increase of HO-1 expression was observed even in Keap-1 knock-out cells and paralleled with the prevention of ROS generation, thus leading the authors to investigate ROS- and NRF-2-independent ways for HO-1 expression by CBD. Consequently, BACH1, another important transcription factor involved in oxidative stress and HO-1 expression, was pointed out as a potential target: CBD induced a strong degradation of this transcription factor. Since BACH1 represses p62 expression, its degradation by CBD paralleled with enhanced levels of p62, which, in turn, may stabilize NRF-2 competing with Keap-1. This mechanism was recognized as an indirect way for the weak induction of NRF-2 by CBD. A direct consequence of HO-1 induction is the promotion of cell survival and resilience to oxidative stress, useful in pathological conditions characterized by the occurrence of skin lesions.

This indication is also sustained by other authors, who focused on the wound healing properties of CBD [66]. The cannabinoid (in the concentration range of 0.1–0.2 µg/mL, corresponding to 0.3–0.6 µM) impaired the matrix metalloprotease inhibitors TIMP1/2 and the expression of collagen in fibroblasts, while keratinocytes were less influenced. Consequently, the observed effect was in favor of the degradation of the ECM. Similarly, CBD inhibited LPS-induced IL-6 expression in fibroblasts, but not in keratinocytes, thus exhibiting a potential anti-inflammatory activity.

Another work reported opposite effects on collagen turnover in fibroblasts [67]. CBD (4 µM) was able to prevent UV-induced collagen degradation in 2D and 3D fibroblast models. The biological mechanism was attributed to the activation of the PI3K/Akt pathway, which is also involved in cell proliferation. Of note, in line with the fact that CBD may act as stabilizer and weak inducer of NRF-2 [65], this work confirmed the protective effect of CBD against lipid peroxidation. Moreover, CBD exerted an anti-inflammatory effect acting on the NF-κB pathway, which correlated with the increase of PPARγ expression.

As previously mentioned, most of the authors focused on the role of CBD for keratinocyte and fibroblast functions. Hwang et al. [68] demonstrated that CBD may also influence melanogenesis. In particular CBD 6 µM was able to increase melanin levels in melanocytes through tyrosinase activation. The effect was independent from cAMP and the related PKA activation (forskolin was used as a positive control), although the downstream transcription factor, CREB, was equally phosphorylated. A possible explanation was attributed to the activation of MAPKs (p38, p42/44, but not JNK). Moreover, the biological activity was CB_1-dependent, since CB_1 silencing counteracted CBD-induced melanogenesis.

A specific work regarding the role of cannabinoids against acne was conducted by Olah et al. [69, 70]. The authors had previously investigated the effect of CBD (1–10 µM) in sebocytes: the compound inhibited lipogenesis, counteracting acne-inducing agents, such as arachidonic acid and testosterone; moreover, CBD suppressed the proliferation of sebocytes in the absence of cytotoxicity and impaired TNF-α expression induced by TLR2 and TLR4 agonists (LPS and lipoteichoic acid, respectively). The main mechanism of all the aforementioned effects was recognized in the increase of intracellular Ca^{2+} levels through TRPV4 agonism. In addition, the activation of A2α receptor was revealed as another fundamental anti-inflammatory mechanism, again ascribed to cAMP elevation and consequent NF-κB impairment. In a following work on sebocytes [69], the same authors compared the evidence on CBD with experiments on other cannabinoids (CBC, CBDV, CBG, CBGV, THCV), thus revealing a differential effect on lipogenesis. CBDV, THCV, and CBC impaired basal lipogenesis, resembling CBD, while CBG and CBGV slightly induced it with an opposite effect. However, following acne-like phenotype induction by arachidonic acid, all compounds were able to impair lipogenesis, with CBDV, CBC and, especially, THCV being the most effective ones. THCV (0.1 µM) was further selected for anti-inflammatory assessment, thus revealing the inhibition of LPS-induced expression of IL-8, IL-6, TNF-α, IL-1α, and IL-1β. The mechanism behind the differential functions of cannabinoids in basal lipogenesis was not verified; however, the authors suggested the involvement of the CB_2/PPARγ pathway in the activity of CBG and CBGV, which would resemble the lipogenic effect of endocannabinoids (anandamide and 2-AG), contrarily to CBD. As a consequence, several cannabinoids (CBG-like) may be of interest against dry skin conditions (xerosis, skin aging), while others (CBD-, THC-, CBC-like) may counteract seborrheic disturbs.

The second most investigated cannabinoid is THC, in the isoforms Δ^8-THC and Δ^9-THC. Despite the pharmacological equivalence among the 2 isoforms, Δ^8-THC is usually preferred for the higher stability. Three works investigated the permeability of Δ^8-THC [71–73] in skin explants from animals and humans. Valiveti et al. measured a similar permeability coefficient in human skin and hairless guinea pig skin after the application of 0.77 µg/mL (around 2.5 µM), thus sustaining the validity of the *in vivo* models for THC studies: transdermal flux was found to be 649 and 717 ng/cm²/h with a lag period of 12.4 and 13.2 h, respectively. The permeability was further confirmed *in vivo*, using a

transdermal patch system. On the contrary, Touitou et al. underlined a great difference in the diffusion of Δ^8-THC among human and rat skin: Δ^8-THC was 13-fold more permeable in the latter [71]. Moreover, the cannabinoid accumulated in the upper epidermis.

Similarly, Stinchcomb et al. compared the permeability of Δ^8-THC with CBN and CBD [73]; in line with the previous work [71], they observed the accumulation of Δ^8-THC in skin tissue, while CBN and CBD exhibited 10-fold higher permeability. THC accumulation in the epidermis suggests its preferential interaction with keratinocytes. Gaffal et al. [74] exploited this possibility in *in vitro* studies on skin allergy: Δ^9-THC (0.1–1 µM) suppressed the secretion of pro-allergenic chemokines (CCL2, CCL8, CXCL10) by IFNγ -activated wild type and *Cnr1/2*$^{-/-}$ (murine $CB_{1/2}$ receptor genes) keratinocytes cultured from the respective knock-out mice. The inhibitory effect resulted in the impairment of macrophage migration in co-culture experiments with IFNγ -activated keratinocytes. These results indicated that Δ^9-THC decreased the production of chemokines in a $CB_{1/2}$-independent manner. In line with this evidence, Glodde et al. [75] demonstrated that the growth of murine melanomas is not affected by $CB_{1/2}$ depletion; thus Δ^9-THC (5–10 µM) was not able to inhibit the tumor in a CB-dependent manner. However, as reported in the next section devoted to *in vivo* studies, THC showed anti-cancer activity through anti-inflammatory effects on infiltrating immune cells.

Δ^9-THC is also well known for its anti-nociceptive effect. Engel et al. [76] validated the effect of Δ^9-THC in skin explants from rats and mice, pointing out the role of TRPV1 and CB_1 receptors. Δ^9-THC, in comparison with anandamide, exerted moderate inhibitory activity at low (0.1 µM) and high (100 µM) concentrations on heat and capsaicin-induced calcitonin CGRP release from nociceptive nerve endings in skin. On the contrary, lower (10 nM) and intermediate (1–10 µM) concentrations of the cannabinoid were not sufficiently effective on CGRP release. Specific experiments on knock out animals revealed that the inhibitory effect at low concentrations was entirely CB_1-dependent, while, at higher concentrations, TRPV1 desensitization occurred as major mechanism.

Several authors performed a biological comparison among different cannabinoids within the same experimental setting. Due to their common anti-inflammatory properties, cannabinoids were investigated for their potential application in inflammatory-based skin diseases [77–79]. However, Petrosino et al. [79] observed different behaviors in poly(I:C)-activated keratinocytes, an immunogenic model that mimics viral infection and hypersensitivity through TLR3 and IFNγ induction. CBD, CBC, CBG, and THCV exhibited a concentration-dependent (5–20 µM) inhibitory effect on IL-6 and MCP-2, while the respective carboxylic forms (CBDA and CBGA) were inactive. The effect of CBD was further investigated, thus including IL-8 and TNF-α inhibition. The biological activity correlated with anandamide elevation and depended on CB_2 and TRPV1 agonism. Accordingly, further experiments [77] regarded the role of CBD, CBG, and anandamide in keratinocyte differentiation, a fundamental step for skin barrier constitution. CBD inhibited the expression of all the considered differentiation markers (K1, K10, involucrin, TGase5), while CBG decreased the levels of K10 and TGase5, only. Both compounds acted at the epigenetic level by increasing the methylation state of genes via selective DNMT1 up-regulation. All the biological activities were resembled by anandamide, which was found to be directly elevated by CBD in the previously described work [79]. These results indicate that CBD and CBG may counteract inflammation while impairing differentiation in keratinocytes, which is in line with the discussion from Casares et al. [65], who suggested to take into consideration the proliferative potential of CBD at skin level. In fact, although anti-inflammatory, the use of CBD may theoretically result in undesired effects when proliferation or skin barrier alteration occur, such as in plaque psoriasis or atopic dermatitis: in those patients, the differentiation markers like K10 and involucrin are downregulated [80, 81]. On the other hand, the results from Pucci et al. [77] prompted the authors to promote the use of CBD and CBG in skin cancer, which is only apparently contradictory. Indeed, Reichelt et al. clarified the role of K10 in skin papillomas, since *Krt10*$^{-/-}$ mice exhibited keratinocyte hyperproliferation, but also lower susceptibility to tumors due to the enhancement of skin turnover [22]. Consequently, the inhibition of K10 levels by CBD and CBG may result in skin tumor repression and should require further clinical investigation.

Regarding the context of psoriasis, Wilkinson et al. [78] demonstrated that phytocannabinoids (Δ^9-THC, CBD, CBG, CBN) may counteract the proliferation of human HPV 16-transformed keratinocytes. The effect was independent from TRPV1 or $CB_{1/2}$ agonism and was slightly superior for nonpsychotropic cannabinoids (IC_{50} ranging from 2.0 and 2.3 µM) than for THC (IC_{50} = 2.9 µM).

This evidence seems in conflict with the previously mentioned one on the proliferative potential of CBD: on the contrary, *in vitro* experiments from Casares et al. showed that CBD may up-regulate a cluster of genes involved in differentiation and counteract proliferation, while the *in vivo* system confirmed an opposite effect with a proliferative profile [65]. As a consequence, the effect of CBD on the proliferative/differentiative balance of epidermis may vary *in vitro* or *in vivo* and may depend on the pathological context under study. In particular, the effect of CBD and CBG on differentiation may require further validation against skin cancer, while specific studies are suggested to criticize their role in psoriasis.

In vivo Evidence

In vivo studies on cannabinoids at skin level, collected in ▶ **Table 1**, are few and mostly focused on the anti-inflammatory and antioxidant properties. As mentioned in the previous section, only a small number of authors translated *in vitro* evidence to the *in vivo* level. Casares at al. [65] characterized the antioxidant effect of CBD in human keratinocytes, demonstrating that this compound acts through NRF-2 and BACH1 degradation, thus leading to antioxidant gene expression, such as HO-1. In line with the anti-apoptotic role of HO-1, the authors observed an elevated skin thickness *in vivo*, due to keratinocyte hyperproliferation, after the topical treatment of BALB/c mice with CBD (0.1–10%). In parallel, markers of wound repair, inflammation and proliferation (K16, K17) were increased in the epidermis of mice, but the expression of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) was absent. The results suggested a possible role for CBD in skin protection but underlining a theoretical risk for the treatment of psoriasis.

Formukong et al. [82] evaluated the effect of the oral administration of Δ^9-THC, CBD, CBG, CBN and the pharmacophore olivetol on PBQ-induced writhing and the topical application of the same compounds on TPA-induced ear erythema. CBD, CBG, and olivetol showed the strongest inhibitory potency (< 10 mg/kg) in both tests, and were more potent than acetylsalicylic acid, used as a positive control in a PBQ-induced writhing test, and trifluoperazione, used as a positive control in TPA-induced ear erythema test. CBN showed only negligible effects; Δ^9-THC was effective only at doses greater than 10 mg/kg. Notably, the biological effect of individual compounds was not able to clearly explain that of petroleum or ethanolic cannabis extracts.

The anti-inflammatory effect of the topical application of Δ^9-THC (30 µg) was verified also in the mouse model of DNFB-induced ear contact dermatitis [74]. Δ^9-THC inhibited the infiltration of monocytes and granulocytes (Gr-1$^+$ staining) in the inflamed tissue and the release of IFNγ by hapten-activated T cells *ex vivo*. The mechanism of action was CB-independent, since Δ^9-THC acted also in *Cnr*$^-$/$^-$ mice and keratinocytes. Moreover, the inhibitory effect of Δ^9-THC (5 mg/kg, subcutaneous injection) on immune cell infiltration was also considered responsible for the reduction of melanomas in *Cnr*$^-$/$^-$ mice. In fact, CD45$^+$ cells, largely consisting of myeloid derived macrophages and neutrophils, were lowered in tumoral tissues, while the growth of tumors was not inhibited *in vitro*.

Similarly, Tubaro et al. [83] compared the topical effect of different cannabinoids (CBD, CBDV, CBC, CBCV, THC, THCV) against croton oil-induced ear edema in mice, in the dose range of 0.1–1 µmol/cm^2. Δ^8-THC, Δ^8-THCV, and Δ^9-THC were more effective (ID_{50} = 0.46–0.55 µmol/cm^2) than CBD, CBC, CBDV, and CBCV (ID_{50} > 2 µmol/cm^2), while only slightly less potent than indomethacin, used as reference anti-inflammatory compound.

Another *in vivo* study documented the anti-inflammatory effect of CBD, with a particular focus on its absorption after topical application [84]. The authors enhanced the bioavailability of CBD with an ethosome formulation, which was applied at the abdominal site of ICR mice: in parallel, paw inflammation was induced by carrageen injection. The transdermal absorption was measured after 12 h and 73 h and resulted in 1.37 mg and 2.60 mg, respectively, starting from the administration of 200 mg. CBD was detected in the plasma at the concentration of 0.67 µg/mL (about 2.1 µM) at the steady state (72 h). The treatment strongly reduced the paw edema. In a parallel experiment, the topical administration in CDI nude mice led to the accumulation of CBD in the hip skin (37.43 µg/cm^2), abdominal skin (110.07 µg/cm^2), and underlying muscle (11.54 µg/cm^2) after 24 h.

The bioavailability of CBD also was investigated in healthy dogs following oral or topical administration of 3 formulations (oral microencapsulated oil beads, oral CBD-infused oil, or CBD-infused transdermal cream) with doses of 75 mg or 150 mg. The highest systemic exposure and the best pharmacokinetic profile were observed with the oral CBD-infused oil formulation: the $[C]_{max}$ in the plasma was 625.3 ng/mL, the T_{max} was 1 h, and the AUC was 8 %, with comparable results for the 2 administered doses [85]. In line with previous works, the authors observed a low bioavailability after oral administration and suggested the study of formulative strategies to avoid first-pass effect by improving the transdermal passage and circumventing skin accumulation.

Valiveti et al. investigated if the topical application of Δ^8-THC may account for a different permeability on the basis of the model: they observed a similar permeability for Δ^8-THC (0.77 µg/mL, i.e., about 2.5 µM) in human and guinea pig skin explants and validated a patch system that guaranteed the transdermal passage of the cannabinoid (4.4 ng/mL at the steady state) *in vivo* [72].

On the contrary, as mentioned in the *in vitro* section, Touitou et al. [71] underlined the great differences in the diffusion of Δ^8-THC in human and rat skin and, again, its accumulation in the upper epidermis, thus remarking how important is the selection of predictable *in vivo* models for preclinical investigations.

In general, despite the clear anti-inflammatory properties of cannabinoids, there are still few *in vivo* studies concerning the biological effects in the context of common autoimmune skin disease or skin cancer. From the collection of the studies concerning topical permeability of cannabinoids, the following aspects emerged: 1) the bioavailability of cannabinoids other than THC or CBD is poorly investigated; 2) thanks to the relatively easy diffusion of cannabinoids after topical application, related to their lipophilicity, transdermal delivery is considered preferable to oral administration to reach a systemic effect, both for safety and bioavailability aspects, but innovative formulative strategies are required. However, topical administration has been only partially explored as an advantage to target the epidermis in inflammatory skin diseases, limiting diffusion to other untargeted organs.

Clinical Evidence

Clinical evidence on the therapeutic use of cannabinoids against skin diseases is rare and has been summarized in ▶ **Table 2**. Most are anecdotal or observational studies concerning CBD. Chelliah [86] et al. described 3 case reports of self-initiated topical CBD oil use against a rare genetic disease occurring in children, epidermolysis bullosa. Those patients suffer for skin frailty and the consequent lesions, but resolutive treatments are still missing. During the study, family members noted fewer blisters, shorter healing time, and less analgesic need, but these results were not verified in a randomized, double-blind, controlled study. Similarly, other authors reported 3 cases of patients with epidermolysis bullosa, who were prescribed sublingual pharmaceutical preparations of CBD and THC (20 mg/mL and 13 mg/mL, respectively), called CBM oil [87]. The CBM caused improved pain scores and reduced pruritus and the overall analgesic drug intake, but, once again, rigorous controlled trials were not conducted.

Another clinical study regarded the safety and efficacy of CBD-based preparations against skin inflammation. A patented formulation (5 % BTX 1503) for topical delivery reached phase II clinical trial against moderate to severe acne, after the first safety and efficacy assessment reported by Spleman et al. [88]. The phase I trial was an open-label and single arm study firstly performed on 20 healthy volunteers and subjects with inflammatory (n = 23) or noninflammatory (n = 20) face lesions, who were treated twice a day. At day 28, safety and efficacy were observed and recorded as preliminary evidence for further clinical trials.

▶ **Table 2** Summary table of cutaneous clinical studies conducted with phytocannabinoids.

Phytocannabinoids	Evidence	Details about the model	Concentration/dose	Positive control	Schematic results	Biological context	Ref.
CBD oil	Clinical	Observational study (3 case reports)			↓ healing time; ↓ analgesic need	Epidermolysis bullosa	[86]
CBD in a formulation called Permetrex (a pure synthetic form of CBD was manufactured for topical delivery and formulated)	Phase I clinical trial	Open-label, single-arm, 28-day evaluation of the safety of 5% BTX 1503 in moderate to severe acne (n = 23)	5% formulation of CBD applied twice daily to the entire face.	//	↓ acne lesions	Treatment of acne	[88]
CBD, THC	Clinical	3 case reports	Sublingual pharmaceutical preparation of CBD and THC (20 mg/mL and 13 mg/mL, respectively)	//	↓ pain score; ↓ pruritus; ↓ analgesic drug intake	Epidermolysis bullosa	[87]

Conclusions

The scientific evidence reported in this review underlines the complexity of the mechanisms regulating the effects of phytocannabinoids at cutaneous level. Data collected *in vitro* demonstrate that the biological effects of phytocannabinoids involve many different cell populations other than immune cells, whose role is still fundamental in the pathogenesis of numerous skin disorders, such as keratinocytes and sebocytes, implicated in the etiology of psoriasis and atopic dermatitis, and acne and dry skin, respectively.

Most of the evidence concerns isolated phytocannabinoids and, as it can be easily seen from ▶ **Table 1** and **2**, CBD is the most investigated compound, considering all the clinical and preclinical studies in several fields of application. CBD protects keratinocytes from oxidative damage induced by UV rays and stimulates the production of melanin from melanocytes, thus suggesting multiple photoprotective mechanisms that may be useful for treating skin disorders such as photo-aging or skin aging.

Despite not always being in agreement, data reported herein highlight the general anti-inflammatory effect of CBD, which in addition exploits the concomitant promotion of endogenous antioxidant factors, through the stabilization of NRF-2. The results from preliminary clinical studies and animal models support the anti-inflammatory activity of CBD for the skin; however, due to its inhibitory effects on keratinocyte differentiation, targeted studies are needed to evaluate the possible consequences in diseases such as psoriasis and atopic dermatitis, in which differentiation and proliferation are already dysregulated. It is important to point out that in the only 2 investigations in which *C. sativa* extract was compared to pure CBD, the extract was superior in terms of anti-inflammatory effects, both *in vitro* [64] and *in vivo* [82]. This observation is certainly linked to the plurality of action of different components; in fact, the oral administration of PUFA of *C. sativa* seeds also seems to have a role in the improvement of clinical symptoms of atopic dermatitis [89], but the involvement of other non-cannabinoid secondary metabolites cannot be excluded [90]. In this context, the topical applications of Δ^8-THC and Δ^9-THC also obtained good results in terms of skin inflammation reduction, probably helped by their preferential accumulation in the epidermis, but their intrinsic psychotropic activity limits the development of new dedicated therapeutic solutions.

One of the most promising applications of phytocannabinoids, supported by preclinical and clinical evidence, is the treatment of seborrheic disorders, especially acne. Although only some compounds, such as CBD, CBC, and THCV, reduce lipogenesis *in vitro*, a clinical study conducted with a mixture of phytocannabinoids (a cream based on *C. sativa* seed extract) confirmed the reduction of sebum and erythema, even if the actual composition in phytocannabinoids of the formulation was not fully described [91]. In addition, a hexane extract from *C. sativa* seeds reduced the inflammatory markers of keratinocytes challenged by *Propionibacterium acnes in vitro*, one of the main etiological agents of acne, demonstrating also a direct antimicrobial effect [92].

In conclusion, phytocannabinoids possess a great potential for the treatment of several cutaneous pathological conditions, ranging from photo-aging and inflammatory diseases to seborrheic and autoimmune disorders. However, this review suggests that the biological plausibility for the use of phytocannabinoids in human diseases still needs explanations. Only few molecular mechanisms peculiar to phytocannabinoids have been causally associated with the improvement of skin diseases. In particular, the action on PPARs, GlyRs, and TRP channels was rarely considered in comparison with CB receptor modulation. In analogy, whole extracts have been sometimes reported to exert a wider and more potent bioactivity than single phytocannabinoids, but their targets were only partially discovered. Moreover, despite the promising evidence found in the preclinical field, the small number of rigorous clinical studies, the lack of data on safety and data specifically related to *C. sativa* extracts and their intrinsic complexity limit the understanding of the real benefits for human skin.

Contributors' Statement

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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