Cannabis sativa L. can reduce inflammation in human keratinocytes thanks to the inhibitory effect of cannabidiol on the NF-κB pathway

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BACKGROUND

Cannabis sativa L. contains high concentrations of cannabinoids including cannabidiol (CBD), the second major cannabinoid and devoid of psychotropic activity, and Δ-9-tetrahydrocannabinol (THC). In our previous study, a *Cannabis sativa* L. ethanolic extract (**CSE**), characterized by medium chain triglycerides (MCT) as a carrier and standardized in 5% CBD and low concentration of THC, exerted anti-inflammatory effects in keratinocytes, including the downregulation of genes involved in inflammation*. CSE and CBD inhibited the nuclear transcription factor κB (NF-κB), but only CSE showed reduction in interleukin-8 (IL-8) secretion.

***Cannabis sativa L. extract and cannabidiol inhibit in vitro mediators of skin inflammation and wound injury.**
Sangiovanni, Enrico; Fumagalli, Marco; Pacchetti, Barbara; Piazza, Stefano; Magnavacca, Andrea; Khalilpour, Saba; Melzi, Gloria; Martinelli, Giulia; Dell'Agli, Mario
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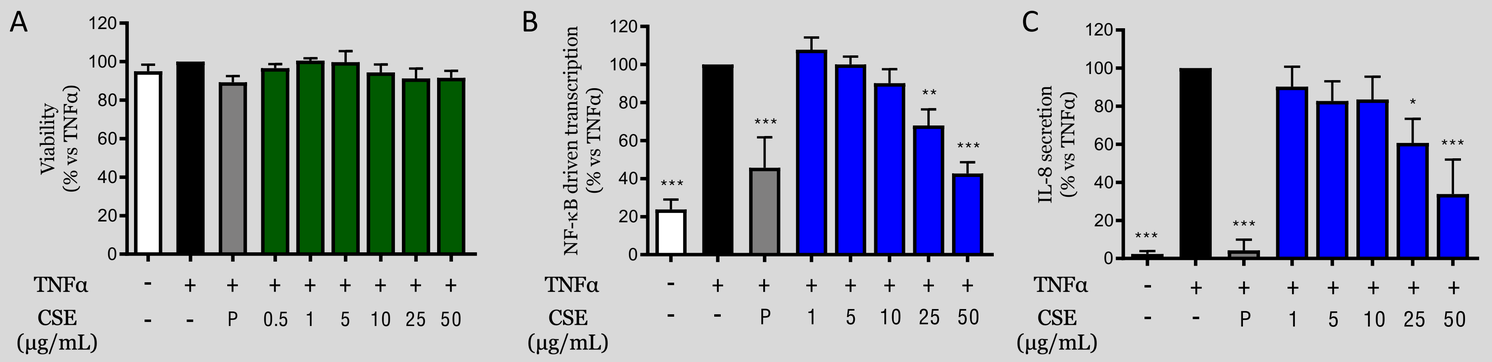
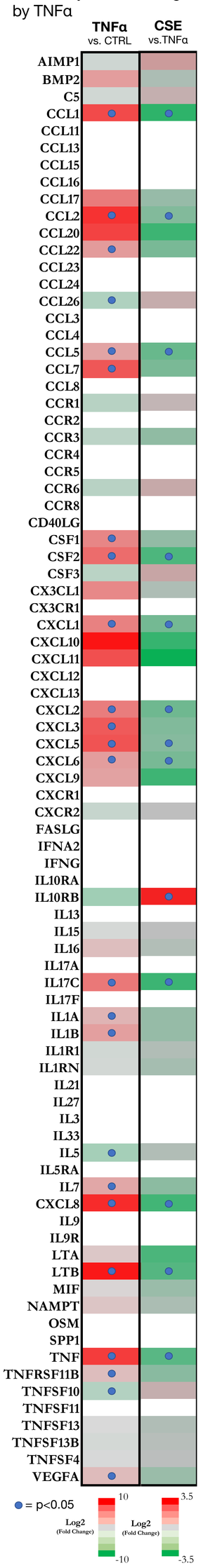
Fig. 1 Effects of **CSE** on cytotoxicity (A), NF-κB driven transcription (B), and IL-8 secretion (C) in human keratinocytes stimulated by TNFα for 6h.



Tab. 1 Composition of CSE subfractions obtained by subsequent extractions (1-4) from same plant material by solvents with increasing polarity.

Polarity increase	Percentage (%)	Cannabinoids											Terpens	Cannflavins	
	Compounds	CBD	CBDA	CBDV	CBDVA	CBDC4	THCV	CBG	CBGA	CBC	THC	THCA	Total	Cannflavin A	Cannflavin B
low	Fraction 1	28.0	0	0.3	0.1	0	0	0.7	0.1	1.0	1.0	0.1	1.8	0.1	0
	Fraction 2	13.0	0	0.3	0.2	0	0	1.0	0.1	0.6	0	0	0.2	0.4	0.3
	Fraction 3	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0
high	Fraction 4	0	0	0	0	0	0	0	0	0	0	0	0.2	0	0

Fig. 2 Effect CSE on **mRNA expression** in keratinocytes challenged by TNFα



RESULTS

CSE was not toxic to keratinocytes till the maximum concentration tested of 50 μg/mL (**Fig. 1A**) and inhibited NF-κB driven transcription (**Fig. 1B**) and IL-8 secretion (**Fig. 1C**) induced by TNFα in a concentration dependent manner. CSE significantly downregulated 12 genes induced by TNFα treatment in HaCaT cells (**Fig. 2**).

Plant material was fractionated into 4 sub-fractions and analyzed for the content of cannabinoids, terpenes, and cannflavins (**Tab. 1**). Fraction 2 resulted cytotoxic at 25 μg/mL (**Fig. 3A**), but obtained the highest inhibition of NF-κB, considering also the lower concentration tested (**Fig. 3B**). None of the fractions significantly inhibited IL-8 secretion (**Fig. 3C**), but a higher inhibition trend was observed for fraction 1.

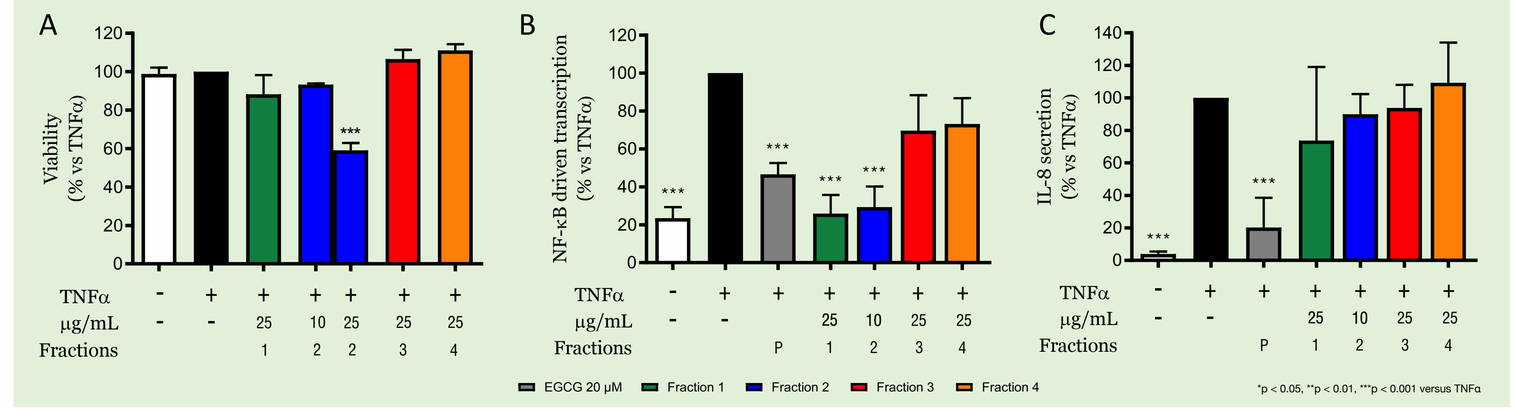
CBD was the most abundant compound of CSE (**Tab. 2**). None of purified cannabinoids were toxic to HaCaT cells till 5 μM (**Fig. 4A**) and, considering the inhibitory activity of CBD (**Fig. 4B**), this compound seems the main responsible for CSE inhibition of NF-κB.

The evaluation of a reconstituted mixture of purified cannabinoids and terpenes (compounds proportional to 25 μg/mL of CSE, except for THC **Fig. 5A**), when combined inhibited NF-κB comparably to CSE (**Fig. 5B**). The mix of terpenes did not show significant inhibition alone or in addition to CBD (4 μM), not influencing the effect of the single cannabinoid (**Fig. 5C**).

AIM

The aim of the present study was to investigate the contribution of the main constituents of CSE to the biological activities in human keratinocytes, focusing on the NF-κB pathway.

Fig. 3 Effects of **CSE fractions** on cytotoxicity (A), NF-κB driven transcription (B), and IL-8 secretion (C) in HaCaT cells stimulated by TNFα for 6h.



Tab. 2 Percentage of cannabinoids and terpenes in CSE. CBD is the most abundant compound in the extract.

Cannabinoids (%)	CBD	CBDA	CBDV	CBN	CBDC4	CBG	CBC	THC
CSE	4.836	0.020	0.019	0.001	0.020	0.138	0.235	0.222
Terpens (%)	**γ-terpineol**	**β-caryophyllene**	**α-humulene**	**Farnesene isomer 1**	**Farnesene isomer 2**	**Farnesene isomer 3**	**Caryophillene oxide**	**α-bisabolol**
CSE	0.031	0.050	0.012	0.010	0.007	0.015	0.015	0.011

CONCLUSIONS

These results suggest that CBD plays a central role in the inhibition of pro-inflammatory mediators in human keratinocytes, acting on the NF-κB pathway. However, other cannabinoids can potentially participate in the inhibition of the IL-8 release, albeit quantitatively lower than CBD, , in particular THC, cannabigerol, and cannabichromene.

Fig. 4 Effects of **purified cannabinoids, canniprene, and cannaflavin-A** on cytotoxicity (A) and NF-κB driven transcription (B) in HaCaT cells stimulated by TNFα for 6h. .

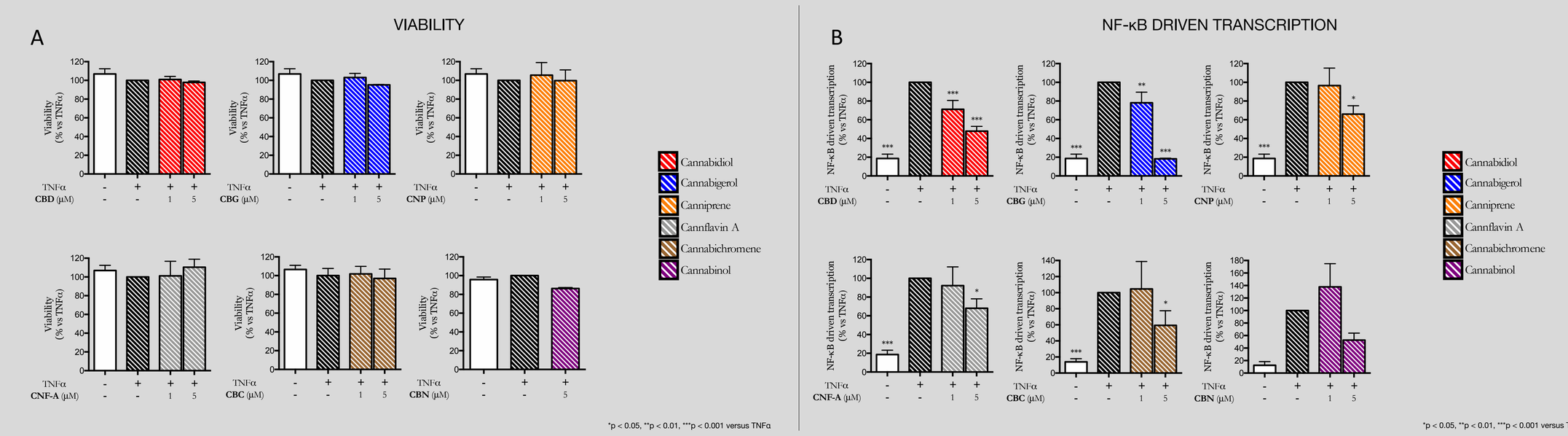
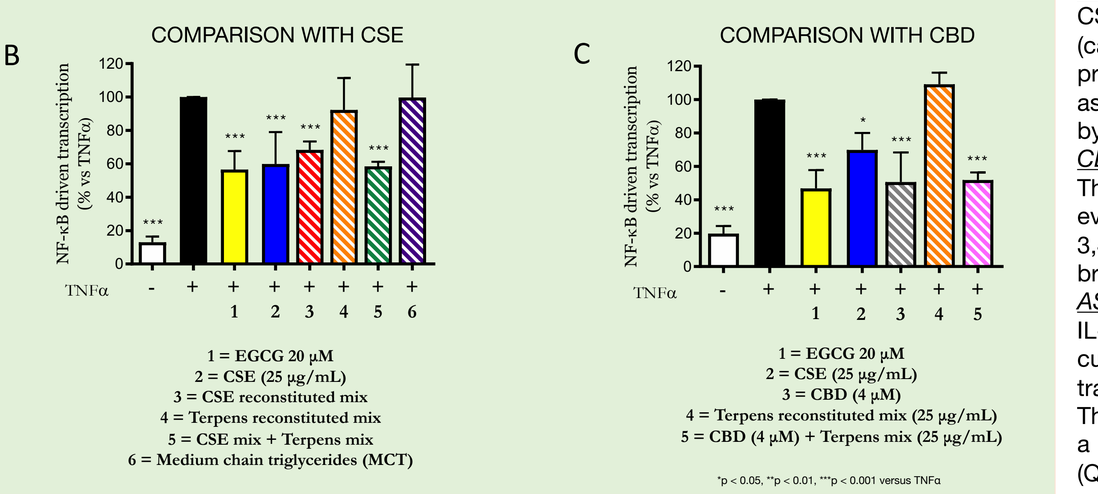


Fig. 5 Composition of the two **reconstituted mixtures**, considering compounds in 25 μg/mL of CSE (A). Effects of the reconstituted mixtures of compounds (resembling CSE) on NF-κB driven transcription in comparison with CSE (B) and CBD (C). HaCaT cells were stimulated by TNFα for 6h.

A

CSE reconstituted mix
CBD = 3.85 μM
CBG = 0.11 μM
CNP = 0.013 μM
CBC = 0.19 μM
CNF-A = 0.13 μM
CNB = 0.0008 μM

Terpens reconstituted mix
β-caryophyllene = 0.061 μM
α-humulene = 0.015 μM
Farnesene isomer 1 = 0.012 μM
Farnesene isomer 2 = 0.086 μM
Farnesene isomer 3 = 0.018 μM
Caryophillene oxide = 0.017 μM α-bisabolol = 0.012 μM



METHODS

EXTRACT AND COMPOUNDS:
CSE, MCT and other purified constituents (cannabinoids, terpenes, and cannflavins) were provided by LINNEA SA (Riazzino, Switzerland) and assayed in human keratinocytes (HaCaT) challenged by TNFα (10 ng/mL) for 6 hours.

CELL VIABILITY:
The cytotoxicity of CSE and compounds was evaluated, in HaCaT cells, by the 3,4,5-dimethylthiazol-2yl-2-5-diphenyltetrazolium bromide method (MTT)

ASSAYS FOR ANTI-INFLAMMATORY ACTIVITY:
IL-8 release was measured by an ELISA assay on cell culture media, while the NF-κB-driven transcription by transient transfection assay.

The analysis of gene expression was performed using a 384-well polymerase chain reaction (PCR) array (QIAGEN Sciences, USA).