Universidade de Lisboa

Faculdade de Farmácia

Cannabis sativa e o seu uso medicinal

Ana Sofia Ferreira dos Santos

Monografia orientada pela Professora Doutora Olga Maria Duarte Silva, Professora Associada

Mestrado Integrado em Ciências Farmacêuticas

2022

Universidade de Lisboa

Faculdade de Farmácia

Cannabis sativa e o seu uso medicinal

Ana Sofia Ferreira dos Santos

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

Monografia orientada pela Professora Doutora Olga Maria Duarte Silva, Professora Associada

2022

Resumo

Introdução: A *Cannabis sativa* L. tem sido cultivada ao longo dos séculos pela sua vasta gama de aplicações, nomeadamente, como fonte de matéria-prima, para fins recreativos e religiosos e pelas suas propriedades medicinais. O presente trabalho tem como objetivo avaliar a utilidade do uso de *Cannabis sativa* flos e seus principais metabolitos secundários no tratamento do cancro, inflamação e dor através de uma revisão da literatura científica disponível.

Métodos: Foi realizada uma pesquisa bibliográfica nas bases de dados *PubMed*, *Cochrane* e *Web of Science*, utilizando como palavra-chaves "cannabis sativa" AND "inflammation" / OR "cancer" / OR "pain", no período temporal de 1 de janeiro de 2017 e 31 de dezembro de 2021. A metodologia PRISMA foi utilizada para selecionar os artigos científicos a serem considerados.

Resultados: De um número inicial de 2664 estudos, aplicando os critérios de inclusão e exclusão definidos, foram considerados 26 estudos científicos, realizados essencialmente a nível pré-clínico, sendo 10 estudos relativos a atividade antitumoral *in vitro*; 10 visando a atividade anti-inflamatória *in vitro* e *in vivo*; e, relativamente à atividade na dor, 4 estudos *in vitro* e *in vivo*, 1 *brief report* e 1 estudo clínico piloto.

Os resultados pré-clínicos mostraram a atividade antitumoral de *C. sativa*, flor em células tumorais de cancro da próstata, cérebro, pele, mama, pulmão, fígado e colorretal, tendo também o seu principal constituinte marcador, o canabidiol (CBD), demostrado este tipo de atividade.

Na inflamação aguda, COVID-19, neuroinflamação e inflamação induzida por *Cutibacterium acnes*, preparações e constituintes de *C. sativa* flor mostraram atividade inibitória de moléculas pró-inflamatórias. No entanto, um extrato rico em CBD provocou um aumento das moléculas pró-inflamatórias, em modelo de inflamação intestinal e de COVID-19.

Um efeito sinérgico foi observado, *in vivo*, para o THC (Δ9-tetrahydrocannabinol), com a administração de gabapentina, tendo-se verificado um aumento da janela terapêutica, potência e eficácia deste constituinte na redução da dor neuropática. Diferentes tipos de administração de *C. sativa*, flor, como a intraperitoneal, oral e a absorção bucal, produziram efeitos notórios na redução da dor. A absorção bucal mostrou particular interesse, pois permitiu eliminar o efeito colateral amnésico do THC.

Conclusões: Apesar dos dados existente são ainda necessários mais estudos pré-clínicos e clínicos para confirmar a utilidade terapêutica concreta de *C. sativa* flor.

Palavras-chave: *Cannabis sativa*, canabidiol, cancro, inflamação, dor.

Abstract

Introduction: Cannabis sativa L. has been cultivated over the centuries for its wide range of applications, namely, as a source of feedstock, for recreational and religious purposes and for its medicinal properties. The present work aims to assess the usefulness of the use of *Cannabis sativa* flower and its constituent indicators in the treatment of cancer, inflammation and pain through a review of the available scientific literature.

Methods: A bibliographic search was carried out in the *PubMed*, *Cochrane* and *Web of Science* databases, using the keyword "cannabis sativa" AND "inflammation" / OR "cancer" / OR "pain" and the time period of January 1, 2017 and December 31, 2021. The PRISMA methodology was used to select the scientific articles to be considered.

Results: From an initial number of 2664 studies, applying the defined inclusion and exclusion criteria, 26 scientific studies were considered, carried out essentially at a pre-clinical level, of which 10 are related to *in vitro* antitumor activity; 10 are related to *in vitro* and *in vivo* anti-inflammatory activity; and, relating to pain activity, 4 *in vitro* and *in vivo* studies, 1 brief report and 1 pilot clinical study.

Pre-clinical studies showed the antitumor activity of *C. sativa*, flower in prostate, brain, skin, breast, lung, liver and colorectal cancer tumor cells, also having its main marker constituent, cannabidiol (CBD), demonstrated this type of activity.

In acute inflammation, COVID-19, neuroinflammation and *Cutibacterium acnes*-induced inflammation, *C. sativa* flower extracts and compounds down-regulated pro-inflammatory molecules. However, a CBD-rich extract caused an increase in pro-inflammatory molecules in a model of intestinal inflammation and COVID-19.

A synergistic effect was observed, *in vivo*, for THC (Δ9-tetrahydrocannabinol) with the administration of gabapentin, with an increase in the therapeutic window, potency and efficacy of this constituent in reducing neuropathic pain. Different types of administration of C. sativa, flor, such as intraperitoneal, oral and buccal absorption, have produced remarkable effects in reducing pain. Oral absorption showed particular interest, as it allowed to eliminate the amnesic side effect of THC.

Conclusions: Despite the existing data, more preclinical and clinical studies are still needed to confirm the concrete therapeutic utility of C. sativa flor.

Keywords: *Cannabis sativa*, cannabidiol, cancer, inflammation, pain.

Index:

Table index:

Index of figures:

Abbreviations

$5HT_{2A}R$	5-hydroxytryptamine receptor 2A
ACM	Autorização de colocação no mercado
AIDS	Acquired immunodeficiency syndrome
CBC	Cannabichromene
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBE	Cannabielsoin
CBG	Cannabigerol
CBGA	Cannabigerolic acid
CBL	Cannabicyclol
CBN	Cannabinol
CBND	Cannabinodiol
CBT	Cannabitriol
CCL	C–C Motif Chemokine Ligands
CCR	C-C chemokine receptor
CD	Cluster of differentiation
CNS	Central nervous system
COVID-19	Coronavirus disease 19
COX-2	Cyclooxygenase 2
CRCE	Cannabidiol-rich cannabis extracted
CXCL	C-X-C motif chemokine ligand
DGAV	Direção-Geral de Alimentação e Veterinária
DIO	Diet induced obesity
EFC	Extract of Fructus Cannabis
EFSA	European Food Safety Authority
EMV	Exosome and microvesicle
ER	Endoplasmatic reticulum
ERK	Extracellular-signal-regulated kinase
EU	European union
F_{CBD}	Extract fraction from C.sativa Arbel strain
FOJ	Felina 32 organic fraction from inflorescences collected in June
FOS	Felina 32 organic fraction from inflorescences collected in September
GACP	Good Agricultural and Collection Practice
GMB	Glioblastoma
GMP	Good Manufacturing Practice
HIV	Human immunodeficiency virus
HPH	Hemp protein isolate
HPP	Hemp protein products
HSHE	Hemp seed hexane extracts
IKK	IκB kinase

IL	Interleukin
IP	Intraperitoneal
JNK	c-Jun N-terminal cinase
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MDA	Malondialdehyde
MRC-1	Mannose Receptor C-Type 1
NA	Not available
ND	Not defined
NO	Nitric oxide
iNOS	Inducible nitric oxide synthase
PGE2	Prostaglandin E2
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PS1	Presenilin 1
rACC	Rostral anterior cingulated cortex
FcγRII	Type II receptor for the Fc region of IgG
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SD	Sprague-Dawley
SM	Standard mix
SOD	Superoxide dismutase
THC	Δ9-tetrahydrocannabinol
THCA	Δ9-tetrahydrocannabinolic acid
TLC	Thin-layer chromatography
TMPRSS2	Transmembrane Serine Protease 2
TNFα	Tumour Necrosis Factor alpha
US	United states

1 Introduction

1.1 General characteristics

The medicinal use of *Cannabis sativa* L. is being intensively investigated. (1) The term "medical cannabis" refers to the use of cannabis herbal medicines, preparations and substances in order to exploit their therapeutic properties. (2)

Cannabis sativa L. (Table 1), also known as Indian hemp or *cannabis*, is an annual dioecious species that is native to Central Asia and, over the centuries, has been cultivated as a source of hemp, food, oil as well as for recreational and religious purposes and for its medicinal properties. (1) (3)(4)

Figure 1 *Cannabis sativa* flower

https://commons.wikimedia.org/wiki/File:Cannabis_sativa-flowering_phase_side.jpg

Table 1 Taxonomy of *Cannabis sativa* L.

Taxonomy	
Family	Canabaceae
Genus	*Cannabis* L.
Species	*Cannabis sativa* L.
Subspecies	*sativa* *indica* *ruderalis*

Adapted from (5) (6) (7) (8) (9).

For medicinal purposes, it is used *C. sativa* flower (Cannabis flos), an official drug with a monograph in the German Pharmacopoeia, but not yet in the European and Portuguese

Pharmacopoeias in force. In the German Pharmacopoeia, Cannabis flowers consist of the dried, flowering shoot apexes, whole or crushed, of the female plants of *Cannabis sativa* L.

C. sativa flower contains no less than 90.0 and not more than 110.0 % of cannabinoid quantities indicated in the label, such as Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), as well as cannabinoid-carbon acids, such as Δ9-tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), calculated as THC and CBD, respectively, referenced to the dried drug. The scent is characteristic of cannabis flowers. Identity testing is carried out by thin-layer chromatography (TLC) (2.2.27).

C. sativa flower-based preparations should contain a minimum of 1 % and a maximum 25 % (m/m) for the THC content and do not contain a limit (or range) for CBD. Botanical identification and chemical identification by high-performance thin-layer chromatography are analyzed according to the specifications for medicinal plants of European Pharmacopoeia (Ph. Eur. 2.8.25). The assay and the tests are carried out by liquid chromatography (Ph. Eur. 2.2.29). The maximum water content (Ph. Eur. 2.5.12) is 0,5 %. It is also required a test for a limit for cannabinol (CBN) (a maximum of 2.5 %), and a test on residual solvents (Ph. Eur.5.4). (10) (11) (12)

C. sativa flower contains more than 500 secondary metabolites already identified, including approximately 125 cannabinoids, a group of compounds with a common chemical structure: a characteristic C_{21} terpenophenolic backbone. (13) This group is divided into the 11 subclasses listed in Table 2.

Table 2 Classification of phytocannabinoids.

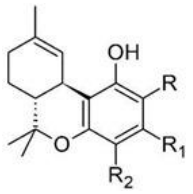
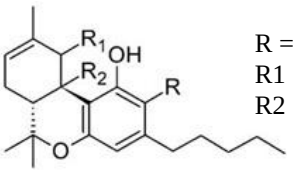
Class	First Representative Compound of the Class	Chemical Structure of the Representative Compound
Δ^9-trans-tetrahydrocannabinol	Δ^9-THC	 R = H R1 = C_5H_{11} R2 = H
Δ^8-trans-tetrahydrocannabinol	Δ^8-THC	 R = H R1 = H R2 = H

Table 3 Continuation

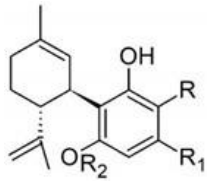
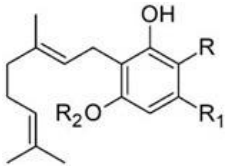
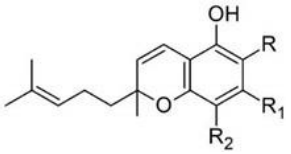
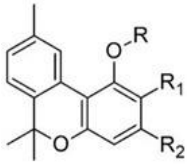
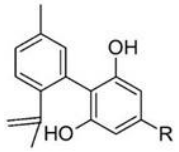
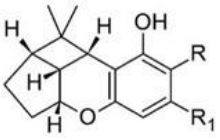
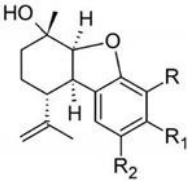
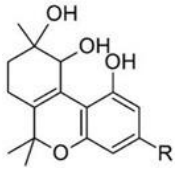
Class	First Representative Compound of the Class	Chemical Structure of the Representative Compound
Cannabidiol	CBD-C5	 R = H R1 = C_5H_{11} R2 = H
Cannabigerol	CBG	 R = H R1 = C_5H_{11} R2 = H
Cannabichromene	CBC	 R = H R1 = C_5H_{11} R2 = H
Cannabinol	CBN	 R = H R1 = H R2 = OH
Cannabinodiol	CBND-C3	 R = C_3H_7
Cannabicyclol	CBL	 R = H R1 = C_5H_{11}
Cannabielsoin	CBE-C5	 R = H R1 = C_5H_{11} R2 = H

Table 4 Continuation

Class	First Representative Compound of the Class	Chemical Structure of the Representative Compound
Cannabitriol	CBT-C5	 R = C_5H_{11}
Miscellaneous-type cannabinoids	(30 compounds)	
Δ^9-THC-Delta-9-tetrahydrocannabinol; Δ^8-THC-Delta-8-tetrahydrocannabinol; CBD-C5–cannabidiol-C5; CBG-Cannabigerol; CBC-Cannabichromene; CBN-Cannabinol; CBND-C3 – Cannabinodiol-C3; CBL-Cannabicyclol; CBE-C5–Cannabielsoin-C5; CBT-C5-Cannabitriol-C5;		

Adapted from (8) (9) (13).

C. sativa flower also contains non-cannabinoid constituents like phenol acid derivatives, flavonoids, terpenoids, and alkaloids. (13)

The discovery of cannabinoids in *C. sativa* flower led to the identification of cannabinoid receptors (CB1R and CB2R) and endogenous ligands of the endocannabinoid system, anandamide and 2-arachidonylglycerol. Cannabinoid receptors are widely expressed in different tissues and organs, such as the liver, the pancreas, the gonads and gametes, the skeletal muscle, the adipose tissue, and the skin. The highest concentration of cannabinoid receptor 1 (CB1R) can be found in the nervous system, but they can also be found in the immune cells and the gastrointestinal, reproductive, adrenal, heart, lung and bladder tissues. Although not as abundant, cannabinoid receptor 2 (CB2R) can also be found in the CNS, especially in microglia and other cells of immune origin. This wide distribution of cannabinoid receptors suggests that the endocannabinoid system is extremely complex and multifunctional, interacting with several different signaling and modulating a plethora of endogenous processes, playing an essential role in many normal physiological processes, such as memory, cognition, learning, motor control, anxiety, appetite, sleep, lipogenesis, fertility, formation of insulin and muscle fibers, vasomotricity, intestinal and bronchial motility, immune modulation, pathological-like pain, inflammation, and cancer. (8) (14)

Cannabinoids, just as endocannabinoids, can bind to and activate cannabinoid receptors, resulting, from this activation, cellular changes with an impact on several physiological processes. (15)

Among this kind of compounds, THC and CBD are the main ones, and the most studied. (15) Both are present in the plant as THCA and CBDA, respectively. The acidic forms, which are pharmacologically less active, first have to be converted into active neutral compounds through decarboxylation. (1) (13)

The THC is the major psychoactive component of cannabis as it exhibits potent in vitro anti-inflammatory, anti-cancer, analgesic, muscle relaxant, neuro-antioxidative and anti-spasmodic activities by interacting with different receptors (other than CB1R and CB2R) distributed

throughout the entire body. However, due to its mechanism (it is a partial agonist of both CB1 and CB2 receptors, but with a higher affinity for the CB1R), this constituent has also been associated with a number of side effects, including anxiety, cholinergic deficits, and immunosuppression. (14)

The CBD, in contrast with THC, is usually considered a nonintoxicating and a non psychoactive constituent of *C. sativa* flower. However, different pharmacological properties, such as analgesic, anti-inflammatory, anxiolytic, antiemetic, antipsychotic, neuroprotective, anti-arthritic, immunomodulatory, anti-fungal and anti-bacterial were already described in *in vitro* and animal studies to this compound. It also acts as an important entourage compound by reducing the side effects of THC, which may, thereby, increase the safety of *C. sativa* flower based preparations. (14) (16)

1.2 Legislation

Law n. º 33/2018, of July 18, regulated by Decree-Law n. º 8/2019, of January 15, establishes the legal framework for the use of medicines, preparations and substances based on *C. sativa* flower for medicinal purposes, namely its prescription and dispensing in a pharmacy.

The main objective of this legal framework was to regulate the prescription and dispensing in pharmacy, detention and transport, scientific research and information to professionals, as well as the regulation and supervision of activities related to the use of *C. sativa* flower for medicinal purposes, making accessible the treatment with medicines, preparations and substances based on this medicinal plant.

For marketed of *C. sativa* flower herbal medicinal, interested companies must submit an application for a marketing authorization (ACM) to Infarmed, which will then be evaluated in order to ensure that patients have access to products with quality and safety, thus avoiding unnecessary risks. (17)

The cultivation, manufacture, wholesale trade, import and export of medicines, preparations and substances based *C. sativa* flower for medicinal purposes can only be carried out after authorization from Infarmed. (18)

Entities wishing to carry out activities related to cultivation, manufacture, wholesale trade, import, export of preparations and substances based on *C. sativa* flower for medicinal, veterinary and scientific research purposes must submit the respective licensing application, mandatorily, through the Portal Licenciamento+. (19)

Medicinal products based on the cannabis plant are medicinal products for human use, so their introduction on the market is subject to a marketing authorization and payment of specific fees.

It is the physician's responsibility to assess, for each patient, the benefits of using this type of product, limiting its use to cases in which conventional treatments have not produced the expected effects, which have caused relevant adverse effects and for a limited list of situations (table 3).

This way, products based on the cannabis plant are only sold in pharmacies upon presentation of a medical prescription and their dispensation must follow the rules applicable to psychotropic and narcotic drugs, and the pharmacist must also provide the patient with all the necessary instructions for correct use of the product. (2) (17) (18)

Table 5 List of therapeutic indications considered appropriate for cannabis plant-based preparations and substances.

Therapeutic indications
Spasticity associated with multiple sclerosis or spinal cord injuries
Nausea, vomiting (from chemotherapy, radiation therapy, and combination therapy for HIV and hepatitis C medication)
Appetite stimulation in palliative care of patients undergoing cancer treatments or with AIDS
Chronic pain (associated with oncological diseases or the nervous system, such as neuropathic pain caused by nerve damage, phantom limb pain, trigeminal neuralgia or after shingles)
Gilles de la Tourette syndrome
Epilepsy and treatment of severe childhood seizure disorders such as Dravet and Lennox-Gastaut syndromes
Therapy resistant glaucoma

Adapted from (20)**.**

Each *cannabis* subspecies *(sativa, indica, ruderalis)* has a different concentration of the cannabinoids THC and CBD, as well as other compounds.

In agreement with registration of this plant in the EU's 'Common Catalogue of Varieties of Agricultural Plant Species', in the European Union, it is allowed to marked food supplements containing *C. sativa* if on this the THC content does not exceed 0.2 % (w/w) and the product cannot present health claims and therapeutic properties on their labeling/advertising. However, not all parts of the plant can be used since only the seeds, seed oil obtained by cold pressing and flour from seed milling have a history of consumption as food.

In accordance with the Regulation (EU) No 2015/2283 on Novel Foods and Food Ingredients, the flowers, leaves and extracts of any part of the *Cannabis sativa*, as well as foods to which these parts have been added and /or extracts, are novel foods and in order to be placed on the market they will have to go through the authorization procedure, as established in Regulation (EU) No 2015/2283, to which a risk analysis will be carried out by the European Food Safety Authority (EFSA). In Portugal the placing on the market procedure consists of an electronic notification to the Competent Authority (DGAV).

The use of cannabinoids (CBD and THC, CBG, CBN and others) in foods is not authorized because they don´t have significant or safe history of consumption in the EU prior to May 15, 1997. (21) (22)

1.3 *Cannabis sativa* flower producers in Portugal

According to data from the Licensing + portal, there are 18 entities approved for the cultivation of C. sativa flower in Portugal and 7 approved for the manufacture of cannabis-based preparations and substances. (23)

To obtain an authorization for the cultivation of the cannabis plant for medicinal purposes, the applicant must demonstrate that it complies with the Good Agricultural and Collection Practice

(GACP) Guidelines, an assessment that is carried out in the context of regular inspections of cultivation facilities.

To obtain an authorization for the manufacture of medicinal products for human use and/or investigational medicinal products and/or preparations and substances based on the cannabis plant for medicinal purposes, the applicant must demonstrate that it complies with Good Manufacturing Practices for medicinal products for human use. (Good Manufacturing Practice (GMP) Guidelines), and/or with the requirements of Good Manufacturing Practice for active substances (Commission Delegated Regulation (EU) No. regular inspections of manufacturing facilities. (17)

1.4 Products in the Portuguese market

There are, in Portugal, two cannabis-based medicines available on the market, Sativex and Tilray, which are presented in Table 4.

Table 6 Medicines available on the Portuguese market and their characteristics.

Drug name	Active substance Dosage	Pharmaceutical form	Therapeutic indications
Sativex	THC 27mg/ml CBD 25 mg/ml	Oral spray solution	Treatment of symptoms in adult patients with moderate to severe spasticity due to multiple sclerosis who have not responded adequately to other antispastic medication
Tilray Flor Seca THC 18	18 % THC <1% CBD	Substance of plant origin for inhalation by vaporization	(1)

(1) See Table 3. Adapted from Infomed (accessed on April 30th 2022)

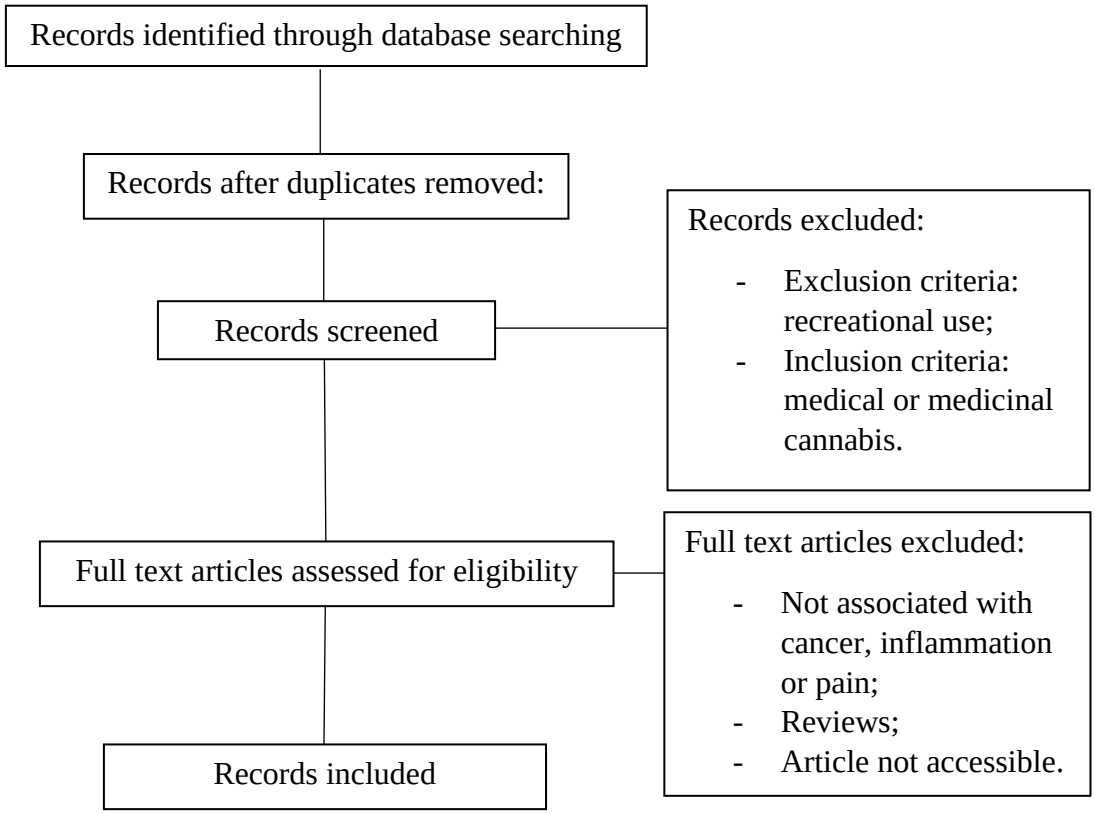
2 Objectives

The present work aims to determine whether there is clinical evidence and/or preclinical data regarding the therapeutic utility of *C. sativa* flower and its constituent indicators in the treatment of inflammation, pain and cancer, through a review of the available scientific literature.

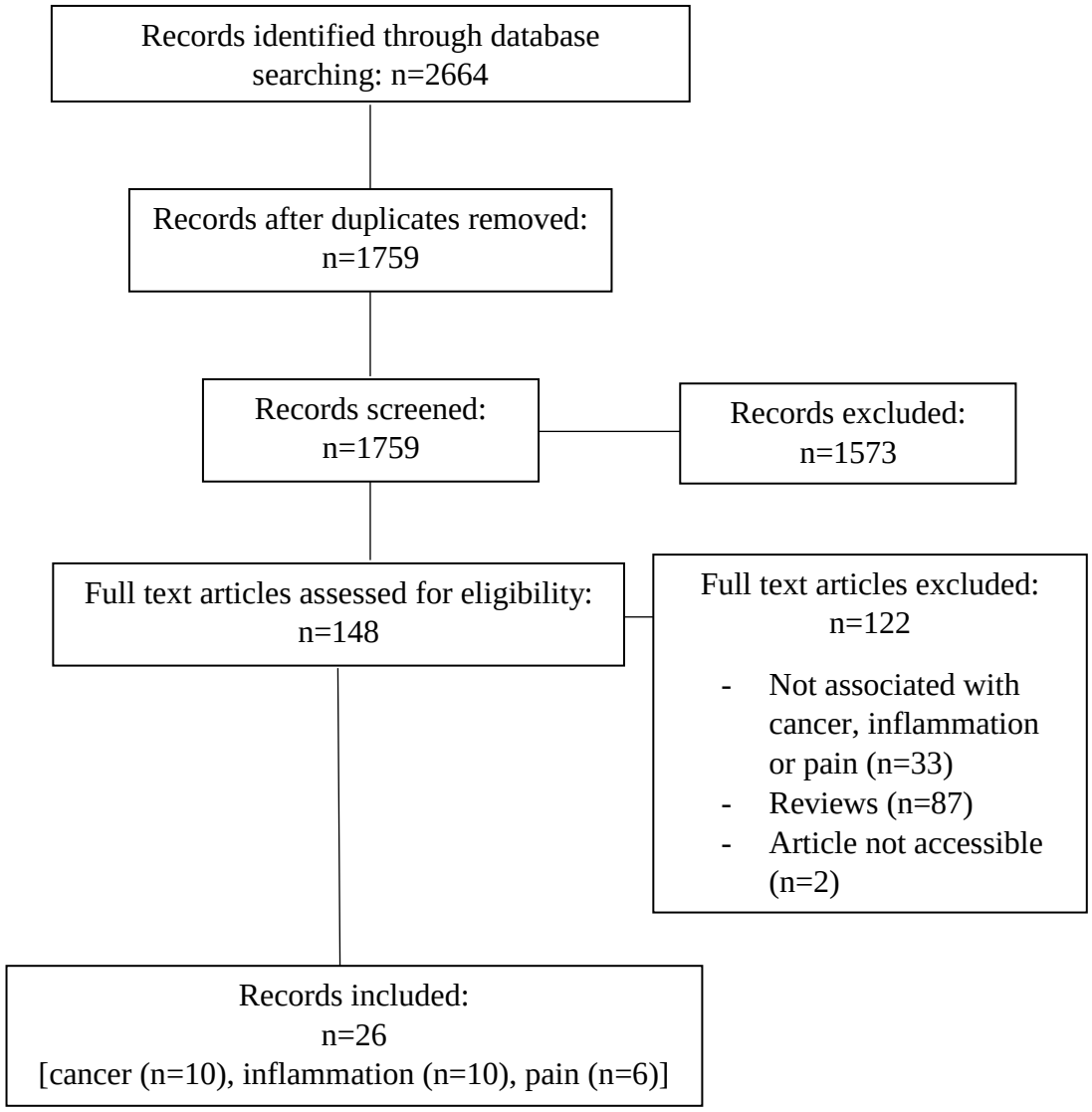
3 Materials and methods

A bibliographic search was carried out in the *PubMed*, *Cochrane* and *Web of Science* databases, using the keyword “cannabis sativa” AND “inflammation” / OR “cancer” / OR “pain” and the time period of January 1, 2017 and December 31, 2021. The PRISMA methodology was used to select the scientific articles to be considered and is portrayed in Chart 1.

Chart 1 Representation of the article selection process based on the PRISMA flow diagram.



4 Results



From an initial number of 2664 studies, applying the defined inclusion and exclusion criteria, 26 scientific studies were considered, carried out essentially at a pre-clinical level, of which 10 are related to *in vitro* antitumor activity; 10 are related to *in vitro* and *in vivo* anti-inflammatory activity; and, relating to pain activity, *4 in vitro* and *in vivo* studies, 1 brief report and 1 pilot clinical study.

The main results are presented in Tables 5-7.

Table 7 *C. sativa* flower preparations and isolated compounds *in vitro* antitumor activity studies

Cancer type	Cell lines	Results	Refs
Prostate	PC3	CBD (1 and 5 μM) showed a significant reduction of EMV release in a dose dependent manner. CBD (5 μM) showed a greater inhibitory effect on total EMV release that was greater than observed with Cl-amidine, while Cl-amidine had a significantly stronger EMV inhibitory effect than 1 μM CBD. It also reduced the expression of CD63 and modulated mitochondrial function and the expression of mitochondrial associated proteins prohibitin and STAT3.	(24)
Brain (GMB)	T98G; U87MG	CBD (10 μM) reduced cell viability in T98G cells, but not in U87MG cells. None of the CBD oils tested were more potent than pure CBD at reducing cancer cell viability.	(25)
	A172; U87MG	Fractions F4 and F5 (12.5 μg/mL) of a *C. sativa* flower extract showed a higher cytotoxic activity than that of the crude extract. Both fractions inhibited cell migration and invasion, altered cell cytoskeletons, and inhibited colony formation. U87 cells were less sensitive to the cytotoxic effect of F4 or F5 but more sensitive to the F4 effect on sphere formation, in comparison to A172 cells. Fractions F4-SM and F5-SM (10 μg/mL) were more cytotoxic than the corresponding extract fraction and led to cell apoptosis and the expression of ER-stress associated-genes.	(26)

Table 8 Continuation

Cancer type	Cell lines	Results	Refs
Melanoma	1205Lu; A375M	CBD (10 μM) reduced cell viability in 1205L cells but did not cause significant reduction of viability A375M cells. None of the CBD oils tested were more potent than pure CBD at reducing cancer cell viability.	(25)
	B16F10	Administration of a *C. sativa* flower extract(1) alone or alongside radiation substantially inhibited cell viability and proliferation in the extract dose response-dependent manner (25, 12.5, 6.25 μg/mL). The inhibition of cell viability was paralleled by an increase in necrosis but not apoptosis when cells were treated with the extract alone (6.25 ug/mL). Radiation alone did not have any antiproliferative effects and did not synergize antiproliferative effects of the extract when the extract and radiation were combined.	(27)
	B16F10	100 μg/ml of a *C. sativa* flower extract (2) showed significant reduction of the expression of the tau gene while 10 μg/ml had the most significant effect on stathmin gene expression. 100 and 10 μg/ml significantly decreased cell migration.	(28)
Breast	MCF-7	Compounds 5* (9,10-dihydro-2,3,5,6-tetramethoxyphenanthrene-1,4-dione), 9* (Cannflavin A) and 16* (4'-methoxy orientin), isolated from a *C.sativa* flower extract, induced significant inhibition of proliferation; compounds 5* and 9* promoted significantly apoptosis, while compound 16* induced weaker apoptosis.	(29)

Table 9 Continuation

Cancer type	Cell lines	Results	Refs
Breast	MDA-MB-231	CBD (1 and 5 µM) showed a dose-depended reduction in cell viability. CBD significantly reduced EMV release in a dose dependent manner (1 and 5 µM), but had a less potent total EMV inhibition than Cl-amidine. CBD (5 µM) in combination with Cl-amidine had a significantly higher inhibition when compared to CBD (5 µM) alone, while there was no significant difference compared to 1 µM CBD treatment. CBD (5 µM) reduced the expression of CD63 and modulated mitochondrial function and the expression of mitochondrial associated proteins prohibitin and STAT3.	(24)
	MDA-MB-468	*C.sativa* flower fractions, FOJ and FOS (50–250 µg/mL), showed significant reduction of cell viability, with a higher potency of FOS. CBD (10-100 µg/mL) and CBC (25-100 µg/mL) were the most effective compounds in reducing cell viability, followed by α-humulene and β-caryophyllene (25-100 µg/mL). 50 and 100 µg/mL of β-caryophyllene oxide only showed significant reduction of cell viability.	(30)
Lung	A549	Compounds 3* (Cannabispirenone-A), 5* (9,10-dihydro-2,3,5,6-tetramethoxyphenanthrene-1,4-dione), 9* (Cannflavin A), 12 (Cannflavin B) and 16* (4'-methoxy orientin), isolated from a *C.sativa* flower extract, significantly inhibited cell proliferation.	(29)
	H358	*C.sativa* flower fractions, FOJ and FOS (10–250 µg/mL), showed a slight reduction of cell viability, with a higher potency of FOS. CBD (25-100 µg/mL) and CBC (25-100 µg/mL) were the most effective compounds in reducing cell viability, followed by α-humulene (50-100 µg/mL) and β-caryophyllene (25-100 µg/mL). β-caryophyllene oxide only showed significant reduction of cell viability at 100 µg/mL	(30)

Table 10 Continuation

Cancer type	Cell lines	Results	Refs
Lung	A549	A *C.sativa* flower extract (300-900 ng/mL) significantly reduced cell viability in a dose and time-dependent manner. The treatment of cells with the extract enhanced the rate of early apoptotic cells in a dose-dependent manner (100, 300, and 500 ng/mL). The extract also induced cell cycle arrest in the subG1 phase (100 and 300 ng/mL), induction of elevation of cellular ROS levels and an increase in caspase 3 activity (300 ng/mL).	(31)
Liver	HepG2	Compounds 5* (9,10-dihydro-2,3,5,6-tetramethoxyphenanthrene-1,4-dione), 9* (Cannflavin A) and 16* (4'-methoxy orientin), isolated from a *C.sativa* flower extract, significantly inhibited cell proliferation.	(29)
	HEPG2	CBD (1 and 5 µM) showed a dose-depended reduction in cell viability. Pre-treatment with CBD (1 and 5 µM) before EMV isolation, resulted in a significant reduction of total EMV release and was more potent than for Cl-amidine. CBD (5 mM) in combination with Cl-amidine, produced a significantly higher inhibition compared to Cl-amidine alone. CBD (5 µM) reduced the expression of CD63, showing significant reduction in exosome biogenesis. CBD (5 µM) showed modulation of the expression of mitochondrial associated proteins prohibitin and STAT3.	(24)
Colorectal	HT-29	Compounds 5* (9,10-dihydro-2,3,5,6-tetramethoxyphenanthrene-1,4-dione), 9* (Cannflavin A) and 16* (4'-methoxy orientin), isolated from a *C.sativa* flower extract, significantly induced inhibition of cell proliferation.	(29)

Table 11 Continuation

Cancer type	Cell lines	Results	Refs
	SW480; HCT116	CBD (10 μM) reduced cell viability in SW480 cells but did not cause significant reduction of viability in HCT116 cells. None of the CBD oils tested were more potent than pure CBD at reducing cancer cell viability.	(25)
	Caco-2	*C. sativa* flower extracts CAN1 - CAN6, THC and CBD affected cell survival in a dose-dependent manner (0.6 – 20 ug/mL). CAN2 (10 ug/mL) exhibited the most potent anticancer properties and significantly outperformed the other extracts. Pure CBD showed potent anticancer properties while pure THC increased the cell viability at low micromolar concentration.	(32)
	HCT 116, HT-29, Caco-2	C. sativa flower extracts, C2F (1.25 mg/mL), F7 (100, 125, 250, or 400 μg/mL) and F3 (75, 107, or 176 μg/mL), showed cytotoxic activity against colon cancer cells. Synergistic interaction was found between F7 and F3. The F7 and F7 + F3 cytotoxic activity involved cell apoptosis and cell cycle arrest. Cells treated with F7 + F3, but not cells treated with F7 or F3 only, showed distinct gene expression. F7, F3, and F7 + F3 treatments were able to induce cell death of polyp cells.	(33)
	Caco2	*C.sativa* flower fractions, FOJ and FOS (10–250 μg/mL), showed a slight reduction of cell viability, with a higher potency of FOS. CBD (10-100 μg/mL) and CBC (25-100 μg/mL) were the most effective compounds in reducing cell viability, followed by α-humulene and β-caryophyllene (25-100 μg/mL). 50 and 100 μg/mL β-caryophyllene oxide showed a significant reduction of cell viability.	(30)

***- Concentration not available; GMB – Glioblastoma; NA – not available; CBD – cannabidiol; EMV – exosome and microvesicle; CAN1 - *C. sativa* flower extract obtained by maceration with MeOH; CAN2 - *C. sativa* flower extract obtained by maceration with EtOH; CAN3 - *C. sativa* flower extract obtained by Soxhlet with MeOH; CAN4 - *C. sativa* flower extract obtained by ultrasonic-assisted extraction with MeOH; CAN5 - *C. sativa* flower extract obtained by supercritical fluid extraction CO^2 at 100 bar, 40ºC; CAN6 - *C. sativa* flower extract obtained by**

supercritical fluid extraction CO^2 at 100 bar, 60ºC; F4 and F5 – C. sativa Dairy Queen fractions containing mainly CBG and THC, respectively; F4-SM and F5-SM – Standard mix fractions of *C. sativa* Dairy Queen; ER - Endoplasmatic reticulum; FOJ – Felina 32 organic fraction from inflorescences collected in June; FOS – Felina 32 organic fraction from inflorescences collected in September; CBC – Cannabichromene; ROS – reactive oxygen species; C2F – Ethanol extracts of *C. sativa* fresh inflorescences; F3 – Ethanol extracts of *C. sativa* fresh inflorescences containing mainly CBGA; F7 – Ethanol extracts of *C. sativa* fresh inflorescences containing mainly THCA.
(1) - C. sativa flower extract obtained by extraction at room temperature with CO2 and standardized based on 4% cannabidiol;
(2) – *C. sativa* flower extract obtained by the maceration method with 80% ethanol for 48 hr. It was standardized with 4% cannabidiol.

Table 12 *C. sativa* flower preparations and isolated compounds *in vitro* and *in vivo* anti-inflammatory activity studies

Disease	Study type / Cell lines	Results	Refs
Acute inflammation	In vivo Mouse strains: wild-type C57BL/6J; B6.12931-Trpv1tm1Jul/J; B6.129P2-Cnr2tm1Dgen/J; FVB.Cg-Tg(HIV-EGFP,Luc)8Tsb/J-NGL; B6(Cg) Il10tm1.1Karp/J	Administration of Δ^9-THC (5 mg/kg i.v.) caused a dramatic early upregulation of plasma IL-10 levels and reduced plasma IL-6 and CCL-2 levels, which reduced organ injury and improved clinical parameters.	(34)
COVID-19	In vitro: A549	*C. sativa* flower extract fraction, $F_{CBD,}$ substantially reduced IL-6 and -8 levels in a dose-dependent manner (5 μg/mL) Treatments with *C. sativa* flower extract fractions F_{CBD} and $F_{CBD:std}$ reduced IL-6, IL-8, CCLs 2 and 7, and ACE2 expression. Treatment with F_{CBD} induced macrophage polarization and phagocytosis, increased CD36 and FcγRII expression and IL-6 and IL-8 expression in macrophages. $F_{CBD:std}$, while maintaining anti-inflammatory activity in alveolar epithelial cells, led to reduced phagocytosis and pro-inflammatory IL secretion in macrophages in comparison to F_{CBD}.	(35)

Table 13 Continuation

Disease	Study type / Cell lines	Results	Refs
COVID-19	In vitro: EpiDermFTTM tissues; WI-38 cells	*C. sativa* flower extracts 4, 8 and 14 (ND) caused profound down-regulation of COX2, TNFα, IL-6, CCL2, and other cytokines and pathways related to inflammation and fibrosis. Extracts 6 and 13 down-regulated numerous pro-inflammatory cytokines and upregulated CXCL12. Extract 12 promoted expression of pro-inflammatory genes.	(36)
	In vitro: Tissues: AIR-100, ORL-200, SMI-100, AFT-100	Several high-CBD *C. sativa* flower extracts (NA) down-regulated ACE2 and TMPRSS2 gene expression. The effects of extracts were more pronounced than those of CBD or CBN alone.	(37)
Gut inflammation	In vivo: Male C57BL6/J mice	CRCE (with a total of 61.5, 184.5 and 615 mg/kg of CBD) increased the expression of pro-inflammatory cytokines and chemokines (Il1ß, Cxcl1, and Cxcl2) in the colon tissue.	(38)
Liver fibrosis	In vitro: LX-2 and NIH-3T3- Col1A2-luc In vivo: Male C57BL/6 mice (CCl4-induced liver fibrosis and high fat diet-induced liver fibrosis models)	Δ^9-THCA (NA) inhibited the expression of fibrotic markers Tenascin C and Col3A1 and the transcriptional activity of the Col1A2 promoter in fibroblasts. Δ^9-THCA (20 or 40 mg/kg) also significantly attenuated CCl4-induced liver fibrosis and inflammation and reduced T cell and macrophage infiltration. Δ^9-THCA (20 mg/kg) significantly reduced body weight and adiposity, improved glucose tolerance, and drastically attenuated DIO-induced liver fibrosis and immune cell infiltration.	(39)

Table 14 Continuation

Disease	Study type / Cell lines	Results	Refs
Neuroinflammation	In vivo: Sprague-Dawley (SD) male rats (SPF grade, wt 259–278 g)	EFC (400mg/kg) improved the behavioral performance of rats in the Morris water maze. EFC significantly increased the activity of SOD while lowering levels of MDA in the hippocampus. EFC inhibited the activation of astrocytes and remarkably attenuated phosphorylated tau and suppressed the expression of PS1.	(40)
	In vitro: LPS-stimulated BV2	HPH20A and HPH60A + 15AF (50 or 100 μg/mL) down-regulated TNF-α, IL-1β, and IL-6. Both HPH's (100 μg/mL) also up-regulated the gene expression of IL-10.	(41)
***Cutibacterium acnes*-induced inflammation**	In vitro: HaCaT; Hs68	HSHE 0.6% suppressed the induction of inflammatory enzymes iNOS and COX-2 and their products NO and PGE2. HSHE reduced the secretion of inflammatory cytokines IL-1β and IL-8. HSHE inhibited the phosphorylation of IKK, IκB, NF-κB, p38, JNK, and ERK, regulating NF-κB and MAPK signal pathways.	(42)
ND	In vitro: CD14 Monocytes	HPPs (50 or 100 ug/mL) decreased the pro-inflammatory mediators (TNF-α, IL-1β, and IL-6) and increased the anti-inflammatory mediators (IL-10 and IL-4). M1 polarization marker gene expression (CCR7 and iNOS) was downregulated and M2 polarization marker gene expression (CD200R and MRC1) was upregulated. The mRNA expression of chemotaxis genes (CCR2 and CCL2) was downregulated.	(43)

NA - Concentrations not available; ND – Not defined; Δ9-THC – Delta-9-tetrahydrocannabinol; IL-6 and IL-10 – Interleukin 6 and 10; CCL – C–C Motif Chemokine Ligands; ACE-2 - Angiotensin I converting enzyme 2; F_{CBD} – Extract fraction from C.sativa Arbel strain: $F_{CBD:std}$ - F_{CBD} formulation using phytocannabinoid standards; CD36 – Cluster of differentiation 36; FcγRII (type II receptor for the Fc region of IgG); COX2 – Cyclooxygenase-2; TNFα – Tumour Necrosis Factor alpha; TMPRSS2 - Transmembrane Serine Protease 2; CRCE - Cannabidiol-rich cannabis extracted with hexane as a solvent; DIO – Diet induced obesity; EFC – Extract of Fructus Cannabis; SOD – Superoxide dismutase; MDA – Malondialdehyde; PS1 - Presenilin 1; HPH20A and HPH60A + 15AF – hemp protein isolate; HSHE – Hemp seed hexane extracts; iNOS – Inducible nitric oxide synthase; MRC1 - Mannose Receptor C-Type 1; CD200R - Cell surface transmembrane glycoprotein CD200 receptor; CCR2 and 7– C-C chemokine receptor type 2 and 7;

Table 15 *C. sativa* flower preparations and isolated compounds pre-clinical and clinical anti-pain activity studies

Disease	Study type/ Cell lines	Results	Refs
Burning mouth syndrome	Pilot study	70% of patients experienced a decrease in pain sensation with Bediol.	(44)
Neuropathic pain	Original research In vitro: HCT-116, OVCAR, A549, MCF7, PC-3, HepG2, SH-SY5Y	*C. sativa* flower compounds 1 (cannabidivarin), 2 (CBD) and 2a (cannabidiol 2, 3-epoxy derivative) exhibited potent inhibitory activity against Wnt/β-catenin pathway in a dose-dependent manner (1-100 μM). Compound 2a inhibited this pathway at slightly lower concentrations than its parent molecule 2, under similar conditions.	(45)
	Original research In vivo: Male Wistar rats	Allodynia was less intense after intraperitoneal CBD (3 and 10 mg/kg). CBD (10-40 nmol/0.25 mL) injected into the rACC reduced mechanical allodynia in a dose-dependent manner.	(46)
	Original research In vivo: Adult C57BL/6 male mice	Gabapentin enhances the therapeutic window of THC, and improves its anti-allodynic potency and efficacy (total THC plus gabapentin dose range of 0.3–300 mg kg−1).	(47)

Table 16 **Continuation**

Disease	Study type/ Cell lines	Results	Refs
ND	Original research In vivo (ND)	Analgesic effects of THC (10 mg/kg, IP) were preserved after pretreatment with peptide 17, a $CB_1R{-}5HT_{2A}R$ altering agent (5, 10, and 20 mg/kg, OR). Amnesic effects of THC (3 mg/kg, IP) were abrogated by pretreatment with peptide 17 (5, 10, and 20 mg/kg, OR).	(48)
ND	Brief report	Participants using Trokie® lozenges reported a mean reduction in pain intensity of 4.9±2.0 points, from a median value of 7.4 to 2.4 on an 11-point scale (0 = no pain, 10 = worst pain imaginable).	(49)
Bediol - Oil of C. *sativa* with mean amounts of 6.5% for THC and 8% for CBD; rACC - rostral anterior cingulated cortex; CB1R - Cannabinoid Receptor Type 1; $5HT_{2A}R$ - 5-hydroxytryptamine receptor 2A; Trokie® lozenges - standardized formulation containing cannabis extracts and delivers cannabinoids via buccal absorption.			

5 Discussion

Cannabis sativa flower has several therapeutic applications that have been studied extensively in several areas of medicine, namely in the area of cancer, inflammation and pain, which are presented here.

In vitro studies showed promising activity of *C. sativa* flower extracts in the treatment of several cancers, namely, prostate, brain, skin, breast, lung, liver and colorectal, with the last one being object of the largest number of studies (5 studies). *C. sativa* flower antitumor activity is demonstrated by the reduction of cell viability, cell migration, EMV release and mitochondrial function, induction of apoptosis, necrosis and cell cycle arrest, inhibition of colony formation, expression of genes associated with ER-stress and the modification of cell cytoskeletons.

In brain, breast, lung and colorectal cancer, extracts of *C. sativa* flower and their fractions showed different activity within each cancer cell type since the variation in the extraction method can lead to different chemical composition of the final extract and fractions and thus to different biological effects. The cytotoxic activity of the extracts was similar for the four cancer types. (26) (30) (32)

CBD was the constituent object of the largest number of antitumor activity studies (4 studies). In brain, melanoma and colorectal cancer, CBD (10 μM) reduced cell viability in the T98G, 1205Lu and SW480 cell lines respectively, but not in the U87MG, A375M and HCT116 cell lines, supporting the idea that CBD acts in a cell type, and not cancer type, specific manner.

(25) In prostate, breast and liver cancer, CBD (1 and 5 µM) also reduced cell viability in a dose-dependent manner. (24)
Other molecules such as CBC, THC, caryophyllane sesquiterpenes and other less known cannabinoid and non-cannabinoid compounds also showed potential *in vitro* antitumor activity in breast, lung, liver and colorectal cancer cells. THC also showed potential *in vitro* antitumor activity in breast, lung and liver cancer cells, however, it increases the colorectal cancer cell viability at a low micromolar concentration. (29) (30) (32)

In breast, lung and colorectal cancer cells, CBD and CBC were the most effective compounds in reducing cell viability, followed by α-humulene and β-caryophyllene at similar concentrations (25-100 µg/mL). (30) In brain, melanoma and colorectal cancer cell types, pure CBD was more potent than the CBD oils tested at reducing cell viability. (25)

In melanoma cells, a *C. sativa* flower extract was able to inhibit cell viability and proliferation in a dose-dependent manner (25, 12.5, 6.25 µg/mL), but no synergistic effects were produced when the extract was combined with another cancer treatment, like radiation. In colorectal cancer, a synergistic interaction was found between two different fractions (F7 and F3). (27) (33)

In melanoma cells, concentrations of a *C. sativa* flower extract of 10 and 100 µg/ml, showed selectivity in the reduction of stathmin and tau genes expression, respectively. Both concentrations showed significant decrease in cell migration. (28)

In the *C. sativa* flower pre-clinical anti-inflammatory activity studies, **e**xtracts and the respective fractions were the main object of study in the articles selected. Compounds such as Δ^9-THC and Δ^9-THCA were also investigated for their anti-inflammatory properties. COVID-19 was the inflammatory disease with the highest number of studies, in vitro and in vivo (3 studies).

In acute inflammation, COVID-19, neuroinflammation and *Cutibacterium acnes*-induced inflammation, the major mechanism of action consists of the down-regulation of pro-inflammatory molecules such as IL-6 and 8, CCL-2 and7, ACE2, iNOS, COX2, IL-1β and TNFα. In acute and neuroinflammation, an up-regulation of anti-inflammatory molecules, such as IL-10, was also observed. (34) (35) (36) (37) (40) (41) (42)

In a COVID-19 study, different *C. sativa* flower extracts down-regulated different pro-inflammatory cytokines, showing selectivity of the extracts to the cytokines. (36)

In another COVID-19 study, *C. sativa* flower extracts up-regulated pro-inflammatory molecules, induced macrophage polarization and reduced phagocytosis. (35) In gut inflammation, a CBD-rich extract also showed an increase of the expression of pro-inflammatory molecules, such as Il1ß, Cxcl1, and Cxcl2. (38) Therefore, caution should be taken in proposing *C. sativa* flower as a treatment as it may lead to a worsening of the inflammatory state. (35)

In vivo, Δ^9-THC and Δ^9-THCA were able to reduce organ injury in acute inflammation and liver fibrosis, respectively, while both compounds improved clinical parameters. (34) (39)

The *C. sativa* flower anti-pain activity studies focused predominantly on neuropathic pain (3 studies). In an *in vitro* study, cannabidivarin, CBD and cannabidiol 2, 3-epoxy derivative reduced pain in a dose-dependent manner. Cannabidiol 2, 3-epoxy derivative showed higher potency than its parent molecule, under similar conditions. (45) In an *in vivo* study, a synergistic effect was observed with the administration of gabapentin with THC, which enhanced the latter's therapeutic window, potency and efficacy at reducing pain. (47)

Different types of administration of *C. sativa* preparations were used for general pain management. In a pre-clinical, *in vivo* study, intraperitoneal administration was able to reduce pain in a dose-dependent manner. (46) In two clinical studies, oral ingestion and buccal absorption were evaluated, both producing notorious effects on pain reduction, but with special interest in the latest, since it allowed to eliminate the amnesic side effect of THC. (44) (49)

6 Conclusion

The present work aimed to assess the usefulness of the use of *Cannabis sativa* flower and its constituent markers, in the treatment of cancer, inflammation and pain.

Pre-clinical studies showed antitumor activity of *C. sativa* flower in several types of cancer, such as prostate, brain, skin, breast, lung, liver and colorectal, with an emphasis on the latter, being object of the largest number of studies. CBD was the compound object of the largest number of anti-cancer activity studies, however, other compounds and different extracts and fractions of C. *sativa* flower also showed promising results.

In *C. sativa* flower anti-inflammatory activity studies, COVID-19 was the inflammatory disease with the highest number of pre-clinical studies.

In both COVID-19 and gut inflammation, a CBD-rich extract also showed an increase of the expression of pro-inflammatory molecules, which advises for caution when proposing *C. sativa* flower as a treatment as it may lead to a worsening of the inflammatory state.

Neuropathic pain was the main object of study in the *C. sativa* flower anti-pain activity studies. A synergistic effect was observed in vivo with the administration of gabapentin with THC, enhancing the latter's therapeutic window, potency and efficacy at reducing pain.

Different types of administration of C. *sativa* preparations were assessed for pain management. Intraperitoneal, oral ingestion and buccal absorption produced notorious effects on pain reduction. Buccal absorption showed particular interest since it allowed to eliminate the amnesic side effect of THC.

There is no clinical data to assess the *C. sativa* flower activity on cancer, inflammation and pain. Therefore, more pre-clinical and clinical studies are necessary to determine the therapeutic utility of *C. sativa* flower and to obtain reliable results, allowing for a correct and efficient use of the plant in the medicinal field.

7 References

1. Casiraghi A, Roda G, Casagni E, Cristina C, Musazzi U, Franzè S, et al. Extraction Method and Analysis of Cannabinoids in *Cannabis* Olive Oil Preparations. Planta Med. Março de 2018;84(04):242–9.

2. Diário da República, 1.ª série — N.º 137 — 18 de julho de 2018 [Internet]. [citado 1 de Maio de 2022]. Disponível em: https://files.dre.pt/1s/2018/07/13700/0324103242.pdf

3. Bonini SA, Premoli M, Tambaro S, Kumar A, Maccarinelli G, Memo M, et al. *Cannabis sativa:* A comprehensive ethnopharmacological review of a medicinal plant with a long history. J Ethnopharmacol. 5 de Dezembro de 2018;227:300–15.

4. Meissner H, Cascella M. Cannabidiol (CBD). Em: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [citado 30 de Abril de 2022]. Disponível em: http://www.ncbi.nlm.nih.gov/books/NBK556048/

5. Cascini F. Chapter e1 - Tetrahydrocannabinol Concentration and Genetic Characterization of *Cannabis*. :10.

6. McPartland JM. *Cannabis* Systematics at the Levels of Family, Genus, and Species. *Cannabis* Cannabinoid Res. Dezembro de 2018;3(1):203–12.

7. ITIS - Report: *Cannabis sativa* [Internet]. [citado 3 de Maio de 2022]. Disponível em: https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=19109#null

8. Filipiuc LE, Ababei DC, Alexa-Stratulat T, Pricope CV, Bild V, Stefanescu R, et al. Major Phytocannabinoids and Their Related Compounds: Should We Only Search for Drugs That Act on Cannabinoid Receptors? Pharmaceutics. 1 de Novembro de 2021;13(11):1823.

9. Bonini SA, Premoli M, Tambaro S, Kumar A, Maccarinelli G, Memo M, et al. *Cannabis sativa:* A comprehensive ethnopharmacological review of a medicinal plant with a long history. J Ethnopharmacol. Dezembro de 2018;227:300–15.

10. German Pharmacopoeia Draft Monograph for *Cannabis* Extracts - ECA Academy [Internet]. [citado 26 de Junho de 2022]. Disponível em: https://www.gmp-compliance.org/gmp-news/german-pharmacopoeia-draft-monograph-for-cannabis-extracts

11. German Pharmacopoeia Monograph for *Cannabis* Extracts - ECA Academy [Internet]. [citado 26 de Junho de 2022]. Disponível em: https://www.gmp-compliance.org/gmp-news/german-pharmacopoeia-monograph-for-cannabis-extracts

12. admin. Cannabis: New text of the German Pharmacopoeia | M A N O X B L O G [Internet]. 2019 [citado 30 de Junho de 2022]. Disponível em: https://manoxblog.com/2019/09/24/cannabis-new-text-of-the-german-pharmacopoeia/

13. Radwan MM, Chandra S, Gul S, ElSohly MA. Cannabinoids, Phenolics, Terpenes and Alkaloids of *Cannabis.* Molecules. Janeiro de 2021;26(9):2774.

14. Andre CM, Hausman JF, Guerriero G. *Cannabis sativa:* The Plant of the Thousand and One Molecules. Front Plant Sci [Internet]. 4 de Fevereiro de 2016 [citado 30 de Abril de 2022];7. Disponível em: http://journal.frontiersin.org/Article/10.3389/fpls.2016.00019/abstract

15. Parecer do Grupo de Trabalho da Ordem dos Farmacêuticos sobre a Utilização de Canábis com Fins Terapêuticos [Internet]. [citado 30 de Abril de 2022]. Disponível em: https://www.ordemfarmaceuticos.pt/fotos/documentos/proposta_grupo_trabalho_cannabis_20fev2018_13278071805a8c65c40f023.pdf

16. Meissner H, Cascella M. Cannabidiol (CBD). Em: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [citado 30 de Abril de 2022]. Disponível em: http://www.ncbi.nlm.nih.gov/books/NBK556048/

17. Substâncias controladas [Internet]. [citado 5 de Maio de 2022]. Disponível em: https://www.infarmed.pt/web/infarmed/substancias-controladas

18. Canábis para fins medicinais [Internet]. [citado 1 de Maio de 2022]. Disponível em: https://www.infarmed.pt/web/infarmed/canabis-medicinal

19. Licenciamento de atividades [Internet]. [citado 24 de Junho de 2022]. Disponível em: https://www.infarmed.pt/web/infarmed/licenciamento-de-atividades

20. DELIBERAÇÃO N.º 11/CD/2019 [Internet]. [citado 1 de Maio de 2022]. Disponível em: https://www.infarmed.pt/documents/15786/2893227/lista+das+indica%C3%A7%C3%B5es+terap%C3%AAuticas+aprovadas+para+as+prepara%C3%A7%C3%B5es+e+subst%C3%A2ncias+%C3%A0+base+da+planta+da+can%C3%A1bis/294b3a2d-326b-46c3-9c08-a3b57427d027

21. Uso de Cânhamo (Cannabis sativa) e/ou CBD (canabidiol) em Alimentos [Internet]. [citado 5 de Maio de 2022]. Disponível em: https://www.asae.gov.pt/newsletter2/asaenews-n-124-novembro-2021/uso.aspx

22. Suplementos Alimentares [Internet]. [citado 5 de Maio de 2022]. Disponível em: https://www.dgav.pt/wp-content/uploads/2021/01/faq_02.12.2019.pdf

23. Portal Licenciamento : Listagem de Entidades do Circuito da Distribuição e da Produção e Aquisição Direta [Internet]. [citado 5 de Maio de 2022]. Disponível em: https://extranet.infarmed.pt/LicenciamentoMais-fo/pages/public/listaECDPAD.xhtml

24. Kosgodage US, Mould R, Henley AB, Nunn AV, Guy GW, Thomas EL, et al. Cannabidiol (CBD) Is a Novel Inhibitor for Exosome and Microvesicle (EMV) Release in Cancer. Front Pharmacol. 2018;9:889.

25. Raup-Konsavage WM, Carkaci-Salli N, Greenland K, Gearhart R, Vrana KE. Cannabidiol (CBD) Oil Does Not Display an Entourage Effect in Reducing Cancer Cell Viability in vitro. Med Cannabis Cannabinoids. Dezembro de 2020;3(2):95–102.

26. Peeri H, Shalev N, Vinayaka AC, Nizar R, Kazimirsky G, Namdar D, et al. Specific Compositions of *Cannabis sativa* Compounds Have Cytotoxic Activity and Inhibit Motility and Colony Formation of Human Glioblastoma Cells In Vitro. CANCERS. 5 de Abril de 2021;13(7).

27. Naderi J, Dana N, Javanmard SH, Amooheidari A, Yahay M, Vaseghi G. Effects of standardized *Cannabis sativa* extract and ionizing radiation in melanoma cells in vitro. J Cancer Res Ther. Dezembro de 2020;16(6):1495–9.

28. Vaseghi G, Taki MJ, Javanmard SH. Standardized *Cannabis sativa* extract attenuates tau and stathmin gene expression in the melanoma cell line. Iran J Basic Med Sci. Outubro de 2017;20(10):1178–81.

29. Guo TT, Zhang JC, Zhang H, Liu QC, Zhao Y, Hou YF, et al. Bioactive spirans and other constituents from the leaves of *Cannabis sativa* f. sativa. J Asian Nat Prod Res. Agosto de 2017;19(8):793–802.

30. Di Giacomo S, Mariano A, Gulli M, Fraschetti C, Vitalone A, Filippi A, et al. Role of Caryophyllane Sesquiterpenes in the Entourage Effect of Felina 32 Hemp Inflorescence Phytocomplex in Triple Negative MDA-MB-468 Breast Cancer Cells. MOLECULES. Novembro de 2021;26(21).

31. Hosami F, Manayi A, Salimi V, Khodakhah F, Nourbakhsh M, Nakstad B, et al. The pro-apoptosis effects of Echinacea purpurea and *Cannabis sativa* extracts in human lung cancer cells through caspase-dependent pathway. BMC Complement Med Ther. 14 de Janeiro de 2021;21(1):37.

32. Rozanc J, Kotnik P, Milojevic M, Gradisnik L, Hrncic MK, Knez Z, et al. Different *Cannabis sativa* Extraction Methods Result in Different Biological Activities against a Colon Cancer Cell Line and Healthy Colon Cells. PLANTS-BASEL. Março de 2021;10(3).

33. Nallathambi R, Mazuz M, Namdar D, Shik M, Namintzer D, Vinayaka AC, et al. Identification of Synergistic Interaction Between *Cannabis*-Derived Compounds for Cytotoxic Activity in Colorectal Cancer Cell Lines and Colon Polyps That Induces Apoptosis-Related Cell Death and Distinct Gene Expression. CANNABIS CANNABINOID Res. 2018;3(1):120–35.

34. Joffre J, Yeh CC, Wong E, Thete M, Xu F, Zlatanova I, et al. Activation of CB $_1$ R Promotes Lipopolysaccharide-Induced IL-10 Secretion by Monocytic Myeloid-Derived Suppressive Cells and Reduces Acute Inflammation and Organ Injury. J Immunol. 15 de Junho de 2020;204(12):3339–50.

35. Anil SM, Shalev N, Vinayaka AC, Nadarajan S, Namdar D, Belausov E, et al. *Cannabis* compounds exhibit anti-inflammatory activity in vitro in COVID-19-related inflammation in lung epithelial cells and pro-inflammatory activity in macrophages. Sci Rep. 14 de Janeiro de 2021;11(1):1462.

36. Kovalchuk A, Wang B, Li D, Rodriguez-Juarez R, Ilnytskyy S, Kovalchuk I, et al. Fighting the storm: could novel anti-TNFα and anti-IL-6 *C. sativa* cultivars tame cytokine storm in COVID-19? Aging. 19 de Janeiro de 2021;13(2):1571–90.

37. Wang B, Kovalchuk A, Li D, Rodriguez-Juarez R, Kovalchuk I, Kovalchuk O. In search of preventive strategies: novel high-CBD *Cannabis sativa* extracts modulate ACE2 expression in COVID-19 gateway tissues. :20.

38. Skinner CM, Nookaew I, Ewing LE, Wongsurawat T, Jenjaroenpun P, Quick CM, et al. Potential Probiotic or Trigger of Gut Inflammation - The Janus-Faced Nature of Cannabidiol-Rich *Cannabis* Extract. J Diet Suppl. 2020;17(5):543–60.

39. Carmona-Hidalgo B, Gonzalez-Mariscal I, Garcia-Martin A, Prados ME, Ruiz-Pino F, Appendino G, et al. Delta 9-Tetrahydrocannabinolic Acid markedly alleviates liver fibrosis and inflammation in mice. PHYTOMEDICINE. Janeiro de 2021;81.

40. Chen NY, Liu CW, Lin W, Ding Y, Bian ZY, Huang L, et al. Extract of Fructus Cannabis Ameliorates Learning and Memory Impairment Induced by D-Galactose in an Aging Rats Model. Evid Based Complement Altern Med. 2017;2017:4757520.

41. Rodriguez-Martin NM, Toscano R, Villanueva A, Pedroche J, Millan F, Montserrat-de la Paz S, et al. Neuroprotective protein hydrolysates from hemp (Cannabis sativa L.) seeds. FOOD Funct. 16 de Outubro de 2019;10(10):6732–9.

42. Jin S, Lee MY. The ameliorative effect of hemp seed hexane extracts on the Propionibacterium acnes-induced inflammation and lipogenesis in sebocytes. PLOS ONE. 2018;13(8):e0202933.

43. Rodriguez-Martin NM, Montserrat-de la Paz S, Toscano R, Grao-Cruces E, Villanueva A, Pedroche J, et al. Hemp (*Cannabis sativa* L.) Protein Hydrolysates Promote Anti-Inflammatory Response in Primary Human Monocytes. BIOMOLECULES. 22 de Maio de 2020;10(5).

44. Alessio G, Evangelos P, Marco C, Adriana C, Roberto B, Giacomo AP. Usefulness of a *cannabis*-based medication in patients with burning mouth syndrome: preliminary results of a prospective pilot study. J Oral Pathol Med. 2019;48:20-.

45. Nalli Y, Dar MS, Bano N, Rasool JU, Sarkar AR, Banday J, et al. Analyzing the role of cannabinoids as modulators of Wnt/beta-catenin signaling pathway for their use in the management of neuropathic pain. Bioorg Med Chem Lett. 1 de Maio de 2019;29(9):1043–6.

46. Genaro K, Fabris D, Arantes ALF, Zuardi AW, Crippa JAS, Prado WA. Cannabidiol Is a Potential Therapeutic for the Affective-Motivational Dimension of Incision Pain in Rats. Front Pharmacol. 2017;8:391.

47. Atwal N, Casey SL, Mitchell VA, Vaughan CW. THC and gabapentin interactions in a mouse neuropathic pain model. NEUROPHARMACOLOGY. Janeiro de 2019;144:115–21.

48. Gallo M, Moreno E, Defaus S, Ortega-Alvaro A, Gonzalez A, Robledo P, et al. Orally Active Peptide Vector Allows Using *Cannabis* to Fight Pain While Avoiding Side Effects. J Med Chem. 27 de Maio de 2021;64(10):6937–48.

49. Crowley K, de Vries ST, Moreno-Sanz G. Self-Reported Effectiveness and Safety of Trokie (R) Lozenges: A Standardized Formulation for the Buccal Delivery of *Cannabis* Extracts. Front Neurosci. 14 de Agosto de 2018;12.