

Characterization and Anticonvulsant Effects of a Colombian Cannabis sativa Extract: A Preclinical Study in Murine Models of Epilepsy

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All animal experimental procedures were reviewed and approved by the Institutional Ethics Committee for Animal Experimentation at the Universidad Nacional de Colombia, in accordance with the guidelines established by the Colombian Ministry of Health (Resolution 008430/1993). The study followed the principles of the 3Rs (Replacement, Reduction, Refinement) for the ethical use of animals in research.

Characterization and Anticonvulsant Effects of a Colombian *Cannabis sativa* Extract: A Preclinical Study in Murine Models of Epilepsy

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ABSTRACT

Background: Epilepsy represents one of the most prevalent chronic neurological disorders worldwide, affecting approximately 50 million people globally. Despite the availability of numerous antiseizure medications (ASMs), approximately 30% of patients develop drug-resistant epilepsy, necessitating the exploration of novel therapeutic alternatives. *Cannabis sativa* has emerged as a promising source of anticonvulsant compounds, particularly cannabidiol (CBD) and Delta-9-tetrahydrocannabinol (THC), which have demonstrated antiepileptic properties in preclinical models.

Methods: This study characterized a chloroform extract of *Cannabis sativa* cultivated in Colombia and evaluated its anticonvulsant potential in three established murine seizure models: the 6 Hz psychomotor seizure model, the pentylenetetrazol (PTZ)-induced seizure model, and the maximal electroshock seizure (MES) test. Phytochemical characterization was performed using thin-layer chromatography (TLC), colorimetric assays (Duquenois-Levine and Fast Blue B Salt), and gas chromatography coupled with mass spectrometry (GC-MS/MS) and flame ionization detection (GC-FID).

Results: Chemical analysis revealed a nearly 1:1 ratio of CBD to THC (51% CBD, 49% THC) in the extract, representing an atypical chemotype. In the 6 Hz model, the extract at 100 mg/kg demonstrated complete protection against seizures, comparable to levetiracetam (40 mg/kg). In the MES test, both 50 and 100 mg/kg doses significantly reduced seizure-related mortality. However, in the PTZ model, the extract showed limited efficacy and appeared to exacerbate seizure severity at higher doses, suggesting potential proconvulsant interactions.

Conclusions: This study provides evidence for the anticonvulsant potential of a Colombian *Cannabis sativa* extract in specific seizure models, particularly those involving generalized tonic-clonic and psychomotor seizures. The unique 1:1 CBD:THC ratio suggests possible synergistic interactions between these major cannabinoids. These findings support further investigation of cannabis-based therapies for drug-resistant epilepsy, while highlighting the importance of careful dose optimization and model-specific efficacy considerations.

Keywords: Cannabis sativa; cannabidiol; delta-9-tetrahydrocannabinol; epilepsy; anticonvulsant; drug-resistant epilepsy; maximal electroshock; pentylenetetrazol; 6 Hz seizure model

1. Introduction

Epilepsy constitutes one of the most common chronic neurological disorders globally, characterized by recurrent spontaneous seizures resulting from abnormal, excessive, and synchronous neuronal activity in the brain^[1]. According to the World Health Organization (WHO), approximately 50 million people worldwide live with epilepsy, with nearly 80% residing in low- and middle-income countries^[2]. The disease burden extends beyond the seizures themselves, encompassing significant psychosocial comorbidities, cognitive impairment, and reduced quality of life^[3].

In Colombia, epidemiological studies have reported that approximately 1.3% of the population suffers from epilepsy, representing roughly 400,000 individuals with active epilepsy^[4,5]. Notably, epilepsy accounts for 0.8% of mortality causes in the country, underscoring the urgent need for improved therapeutic strategies^[5].

1.1. Classification and Pathophysiology of Epilepsy

Seizures are broadly classified into two main categories: focal (partial) onset seizures and generalized onset seizures^[6]. Focal seizures originate in a localized cortical region and account for approximately 60% of all epileptic seizures, while generalized seizures involve both cerebral hemispheres from onset and represent the remaining 40%^[7]. Epilepsy can also be categorized etiologically as genetic, structural, metabolic, immune, infectious, or unknown^[8].

The pathophysiology of epilepsy involves complex alterations in neuronal excitability, synaptic transmission, and network synchronization^[9]. Key mechanisms include imbalance between excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmission, dysfunction of voltage-gated ion channels, and neuroinflammatory processes^[10]. These molecular alterations create a hyperexcitable neuronal network susceptible to generating spontaneous, recurrent seizures.

1.2. Drug-Resistant Epilepsy and the Need for Novel Therapeutics

Despite the availability of over 25 antiseizure medications (ASMs), approximately 30% of patients continue to experience seizures despite optimal treatment with conventional therapies^[11]. This condition, termed drug-resistant epilepsy (DRE) or refractory epilepsy, significantly impacts patient quality of life and increases the risk of sudden unexpected death in epilepsy (SUDEP)^[12].

Patients with DRE often require

invasive interventions such as surgical resection, neurostimulation devices, or ketogenic diet therapy^[13].

The limitations of current pharmacological options have prompted intensive research into novel therapeutic targets and alternative treatment modalities^[14]. Among these, plant-derived compounds have garnered significant attention, with *Cannabis sativa* emerging as a particularly promising source of anticonvulsant phytochemicals^[15].

1.3. *Cannabis sativa* and Cannabinoids: Historical and Contemporary Perspectives

Cannabis sativa L. has been utilized for medicinal purposes for millennia across diverse cultures^[16]. Historical records document its use in traditional Chinese medicine dating back to approximately 2700 BCE for conditions including menstrual disorders, rheumatism, and absent-mindedness^[17]. In Western medicine,

cannabis was widely employed throughout the 19th century as an analgesic and anticonvulsant agent before

the advent of modern pharmaceutical regulations^[18].

The cannabis plant contains over 460 chemical compounds, of which approximately 100 are classified as cannabinoids - terpenophenolic compounds unique to Cannabis species^[19,20]. The two most extensively studied phytocannabinoids are Delta-9-tetrahydrocannabinol (THC), the primary psychoactive constituent, and cannabidiol (CBD), a non-psychoactive compound with demonstrated therapeutic potential^[21].

1.4. Mechanisms of Cannabinoid Action in Epilepsy

The anticonvulsant mechanisms of cannabinoids involve multiple molecular targets beyond the canonical cannabinoid receptors (CB1 and CB2)^[22]. While THC acts as a partial agonist at CB1 receptors, predominantly expressed presynaptically on glutamatergic and GABAergic neurons in the central nervous system (CNS), CBD exhibits minimal affinity for these receptors^[23].

Current evidence supports several distinct mechanisms through which CBD exerts antiseizure effects^[24]:

(1) **GPR55 receptor antagonism:** CBD acts as an antagonist at the orphan G protein-coupled receptor GPR55, which modulates synaptic transmission and neuronal excitability^[25]. Recent studies have demonstrated that lysophosphatidylinositol (LPI)-mediated activation of GPR55 promotes glutamate release and weakens GABAergic inhibition, creating a proconvulsant environment that CBD can disrupt^[26].

(2) **TRPV channel modulation:** CBD activates and desensitizes transient receptor potential vanilloid type 1 (TRPV1) and type 2 (TRPV2) channels, which are involved in calcium homeostasis and neuronal excitability^[27,28].

(3) **5-HT1A receptor activation:** CBD demonstrates agonistic properties at serotonin 5-HT1A receptors, which modulate neuronal excitability and neurotransmitter release^[29,30].

(4) **Adenosine signaling enhancement:** CBD inhibits the equilibrative nucleoside transporter 1 (ENT- 1), thereby increasing extracellular adenosine levels, which possess endogenous anticonvulsant properties^[31].

(5) **Glycine receptor potentiation:** CBD positively modulates alpha-1 and alpha-3 glycine receptors, enhancing inhibitory neurotransmission^[32,33].

(6) **Sodium and potassium channel modulation:** Recent evidence indicates that CBD inhibits voltage- gated sodium channels (Nav1.6) and may modulate potassium channels (Kv7.2/7.3 and BK channels), directly affecting neuronal excitability^[34,35].

1.5. The Entourage Effect and Whole-Plant Therapeutics

An emerging concept in cannabinoid pharmacology is the "entourage effect," which posits that the therapeutic efficacy of cannabis-derived compounds may be enhanced when administered as whole-plant extracts compared to isolated individual components^[36]. This synergistic interaction between cannabinoids, terpenes, and other phytochemicals may result in improved efficacy and reduced adverse effects^[37].

Clinical and preclinical studies have provided evidence supporting this hypothesis. CBD-rich extracts have demonstrated superior anticonvulsant effects compared to purified CBD alone in some studies^[38]. Furthermore, the combination of CBD and THC at various ratios has shown enhanced therapeutic outcomes in multiple sclerosis (nabiximols, 1:1 ratio) and certain forms of epilepsy (20:1 or 50:1 ratios)^[39,40].

1.6. Study Objectives

The present study was designed to characterize the phytochemical profile of a *Cannabis sativa* extract cultivated in Colombia and evaluate its anticonvulsant potential in established murine seizure models. The specific objectives were:

(1) To characterize the cannabinoid content and chemical profile of a Colombian *Cannabis sativa* extract using chromatographic and spectroscopic techniques;

(2) To evaluate the anticonvulsant efficacy of the extract in three distinct seizure models: the 6 Hz psychomotor seizure model (focal impaired awareness seizures), the pentylenetetrazol (PTZ) model (generalized absence/myoclonic seizures), and the maximal electroshock seizure (MES) test (generalized tonic-clonic seizures);

(3) To compare the extract's efficacy against established antiseizure medications and investigate dose- response relationships.

This research contributes to the growing body of evidence supporting the therapeutic potential of cannabis-based medicines for epilepsy, particularly for drug-resistant forms, while providing novel data on Colombian cannabis varieties with unique cannabinoid profiles.

2. Materials and Methods

2.1. Plant Material and Extract Preparation

Approximately 500 grams of leaves and flowering tops of *Cannabis sativa* were cultivated and harvested at the Faculty of Agricultural Sciences, Universidad Nacional de Colombia, Bogota campus (Figure 1). Prior to delivery, the plant material underwent steam distillation to remove essential oils and monoterpenes. A voucher specimen was deposited and classified at the Colombian National Herbarium (code: COL597049) on November 12, 2016.



Figure 1: *Cannabis sativa* plants cultivated at the Universidad Nacional de Colombia (UNAL).

The plant material was dried at 40 degrees C for 72 hours, after which 348.6 g were subjected to solid- liquid extraction by percolation using chloroform as the solvent until exhaustion (Figure

2)^[41]. The resulting extract was concentrated to dryness using a rotary evaporator at 35 degrees C under reduced pressure, yielding 63.2 g of chloroform extract (yield: 18.1% w/w). The extract was stored at -20 degrees C until further analysis.



Figure 2: Percolation setup showing the interaction between the botanical matrix and the extraction solvent.

2.2. Phytochemical Characterization

2.2.1. Thin-Layer Chromatography (TLC)

TLC analysis was performed on silica gel GF254 plates (Merck, Germany) using two distinct elution systems^[42,43].

System A: Chloroform (100%) as the mobile phase.

System B: Hexane:diethyl ether (80:20 v/v) as the mobile phase.

Extract samples were dissolved in chloroform and applied to the plates using glass capillaries. After development, plates were visualized using three spray reagents: (1) ferric chloride (FeCl_3) solution, (2) vanillin/sulfuric acid (vanillin/ H_2SO_4), and (3) Fast Blue B Salt (FBBS)^[44]. Reference standards of cannabis plant material were run in parallel for comparison.

2.2.2. Colorimetric Assays

Approximately 20 mg of the dry extract was dissolved in 40 mL of chloroform, and 8 mL aliquots were distributed into four test tubes. The following colorimetric tests were performed^[43]:

Duquenois-Levine Test: To the extract solution, 5 mL of acetaldehyde, 500 mg of vanillin, and 20 mL of ethanol were added, followed by 2 mL of concentrated hydrochloric acid. The color change was observed at 10 minutes.

Fast Blue B Salt Test: FBBS reagent was added to the extract solution, and color development was observed.

Reference tests were performed using 100 mg of *Cannabis sativa* plant material extracted with chloroform in parallel.

2.2.3. Gas Chromatography-Mass Spectrometry (GC-MS/EI)

For quantitative and qualitative analysis, 50 mg of extract was weighed into an Eppendorf vial. To this, 0.05 mL of internal standard solution (tetracosane) and 2 mL of absolute ethanol were added. The mixture was vortexed for 1 minute, sonicated for 10 minutes, and centrifuged at 90 RPM for 5 minutes. The supernatant was transferred to glass vials for analysis^[45].

GC-MS/EI analysis was performed using a Shimadzu GC-2010 Plus gas chromatograph coupled to a mass spectrometer with electron ionization. Separation was achieved on an RTX-5MS column (30 m x 0.25 mm ID, 0.25 micrometer film thickness). The temperature program was as follows: initial temperature 200 degrees C (held 2 min), ramp to 260 degrees C at 10 degrees C/min (held 10 min), total run time 18 minutes. Injector temperature: 290 degrees C; detector temperature: 300 degrees C; injection volume: 1 microliter; split ratio: 20:1.

2.2.4. Gas Chromatography-Flame Ionization Detection (GC-FID)

GC-FID analysis was performed using identical chromatographic conditions as GC-MS/EI for quantification of major cannabinoids. Calibration curves were prepared using certified reference standards of THC, CBD, and CBN (Lipomed, Switzerland; National Measurement Institute, Australia).

2.3. Animals

Female ICR mice (10-12 weeks old, weight 28-38 g) were obtained from the Bioterium of the Department of Pharmacy, Universidad Nacional de Colombia. Animals were housed under controlled environmental conditions (temperature 22 plus or minus 1 degrees C, 12-hour light/dark cycle) with free access to food and water, except during experimental procedures when food was withdrawn 6 hours prior to testing. All experimental procedures were conducted in accordance with the guidelines established by the Colombian Ministry of Health (Resolution 008430/1993) and followed the principles of the 3Rs (Replacement, Reduction, Refinement)^[46].

2.4. Drug Preparation and Administration

2.4.1. Extract Formulation

Initial attempts to formulate the extract in aqueous vehicles (40% ethanol, and mixtures of ethanol:propylene glycol:glycerin at 20:10:10%) resulted in precipitation upon dilution with water. Therefore, the extract was formulated in extra virgin olive oil (Castell de Pierro), which provided adequate

solubilization for oral administration^[47]. Two doses were evaluated: 50 mg/kg and 100 mg/kg of dry extract, administered orally via gastric gavage in a volume of 0.01 mL/g body weight.

2.4.2. Reference Antiseizure Medications

The following reference drugs were prepared fresh on the day of experimentation:

Levetiracetam (Keppra, Grunenthal): Two 500 mg tablets were crushed to a fine powder and dissolved in 250 mL of physiological saline (0.9% NaCl) to achieve a final concentration of 4 mg/mL. Dose: 40 mg/kg (intraperitoneal).

Clonazepam (Coquan, Siegfried): A 1 mL aliquot of oral solution (2.5 mg/mL) was diluted to 250 mL with distilled water. Dose: 0.3 mg/kg (oral).

Phenytoin (Epamin, Parke Davis): A 1 mL aliquot of injectable solution (50 mg/mL) was diluted to 25 mL with distilled water. Dose: 20 mg/kg (intraperitoneal).

Pentylentetrazol (PTZ, Sigma): 1.5 g was dissolved in 250 mL of physiological saline. Dose: 60 mg/kg (subcutaneous).

2.5. Experimental Design

Animals were randomly assigned to four treatment groups (n=7 per group for 6 Hz and PTZ

tests; n=6 per group for MES test):

- **Group 1 (Control):** Olive oil vehicle only
- **Group 2 (Extract 50):** Cannabis extract 50 mg/kg
- **Group 3 (Extract 100):** Cannabis extract 100 mg/kg
- **Group 4 (Reference):** Established antiseizure medication (levetiracetam for 6 Hz, clonazepam for PTZ, phenytoin for MES)

Treatments were administered orally 2 hours prior to seizure induction. Each seizure model was tested on separate days with a one-week washout period between experiments (Figure 3).



Figure 3. (a) Oral administration via intragastric gavage, (b) subcutaneous administration in the interauricular region, and (c) corneal electroshock administration.

2.6. Seizure Models

2.6.1. 6 Hz Psychomotor Seizure Model

The 6 Hz seizure model induces limbic seizures resembling focal impaired awareness seizures in humans^[48]. Mice were manually restrained, and high-conductivity gel was applied to the

corneas. Electrical stimulation was delivered via corneal electrodes using a Coulbourn Instruments E13-51 programmable

stimulator. Stimulation parameters: rectangular positive pulses, 0.2 ms duration, 166 ms inter-pulse interval, 3-second total stimulation duration at 6 Hz frequency. Following stimulation, animals were immediately released into an open field arena and observed for the presence or absence of seizure activity (staring, automatisms, convulsions).

2.6.2. Pentylentetrazol (PTZ)-Induced Seizure Model

PTZ is a GABA-A receptor antagonist that induces generalized myoclonic and absence-like seizures^[49]. PTZ (60 mg/kg) was administered subcutaneously in the interauricular region. Animals were immediately placed in an open field and observed for 30 minutes for the occurrence of seizures (myoclonic jerks, clonic convulsions, tonic-clonic seizures, mortality).

2.6.3. Maximal Electroshock Seizure (MES) Test

The MES test is a validated model for generalized tonic-clonic seizures^[50]. Electrical stimulation was delivered via corneal electrodes using the following parameters: 60 Hz frequency, 0.033 ms pulse duration, 8.3 ms rectangular pulses, alternating current, 50 mA intensity. Animals were manually restrained during stimulation and immediately released afterward. The absence of tonic hindlimb extension was considered indicative of anticonvulsant activity. Survival was monitored for 24 hours post-stimulation.

2.7. Data Analysis

Results are expressed as the number of animals exhibiting seizures and mortality. Protection rates were calculated as the percentage of animals not displaying seizure activity or surviving the seizure induction. Data were analyzed using Fisher's exact test for comparisons between treatment groups, with statistical significance set at p less than 0.05.

3. Results

3.1. Phytochemical Characterization

3.1.1. Thin-Layer Chromatography

TLC analysis revealed the presence of multiple spots with retention factors (Rf) between 0.48 and 0.65 across both elution systems. Visualization with FeCl₃ and vanillin/H₂SO₄ spray reagents indicated the presence of phenolic compounds, appearing as colored spots in the violet-blue spectrum. When using the hexane:diethyl ether mobile phase with Fast Blue B Salt visualization, spots corresponding to known cannabinoid Rf values were observed: Rf approximately 0.63 (consistent with CBD), Rf approximately 0.58 (consistent with THC), and Rf approximately 0.50 (consistent with CBN)^[51]. These results confirm the presence of terpenophenolic cannabinoids in the extract (Figure 4).

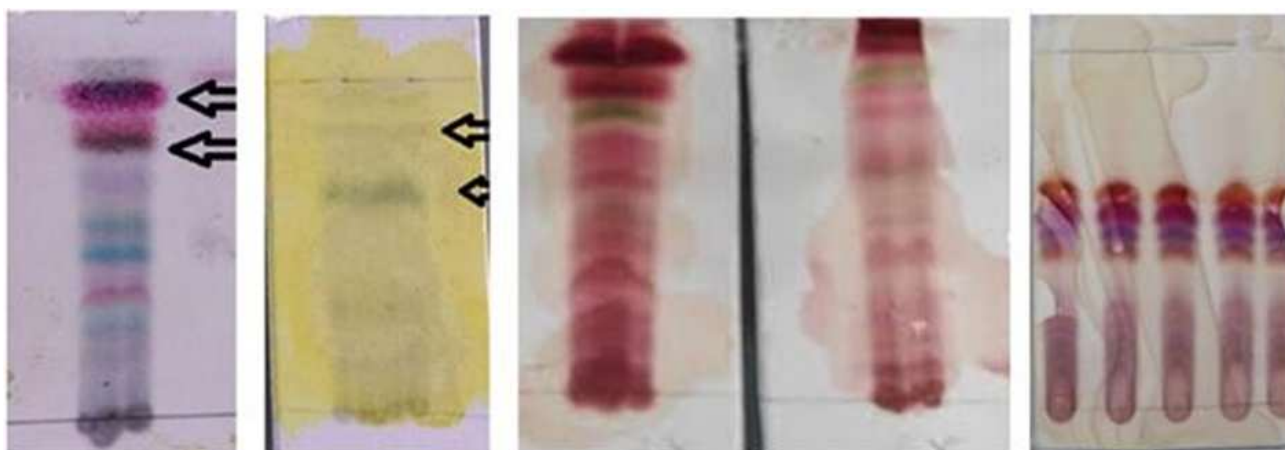


Figure 4. TLC of *C. sativa* extract. Panels A–D utilized chloroform as the mobile phase. Spraying reagents used were (A) vanillin/H₂SO₄, (B) FeCl₃, and (C, D) FBBS. In (E), the mobile phase was modified to a hexane-diethyl ether mixture (80/20 % v/v) and developed with FBBS.

3.1.2. Colorimetric Assays

The Duquenois-Levine test produced a characteristic blue-violet to red color change after 10 minutes, consistent with the presence of cannabinoids. The Fast Blue B Salt test yielded positive results with color development specific to cannabinoid phenolic structures. Both tests showed comparable results between the extract and reference cannabis plant material, confirming cannabinoid presence (Figure 5).



Figure 5. Comparative in-tube colorimetric assays of *C. sativa* extract versus crude plant drug.

3.1.3. GC-MS/EI and GC-FID Analysis

Gas chromatographic analysis identified two major cannabinoid peaks in nearly equal proportions. Mass spectrometry confirmed the identity of these compounds as cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC). The mass spectra showed characteristic fragmentation patterns for these isomeric compounds, with minor differences in base peaks and fragment ions allowing differentiation. Retention time analysis further confirmed compound identity (Figure 6-9).

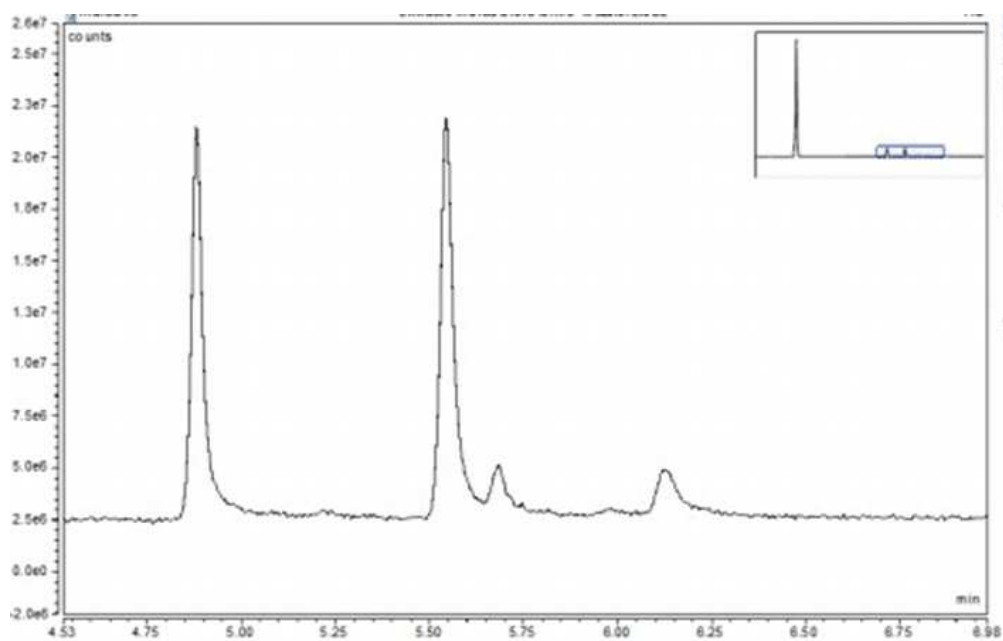


Figure 6: GC-MS/EI chromatogram of the *C. sativa* extract.

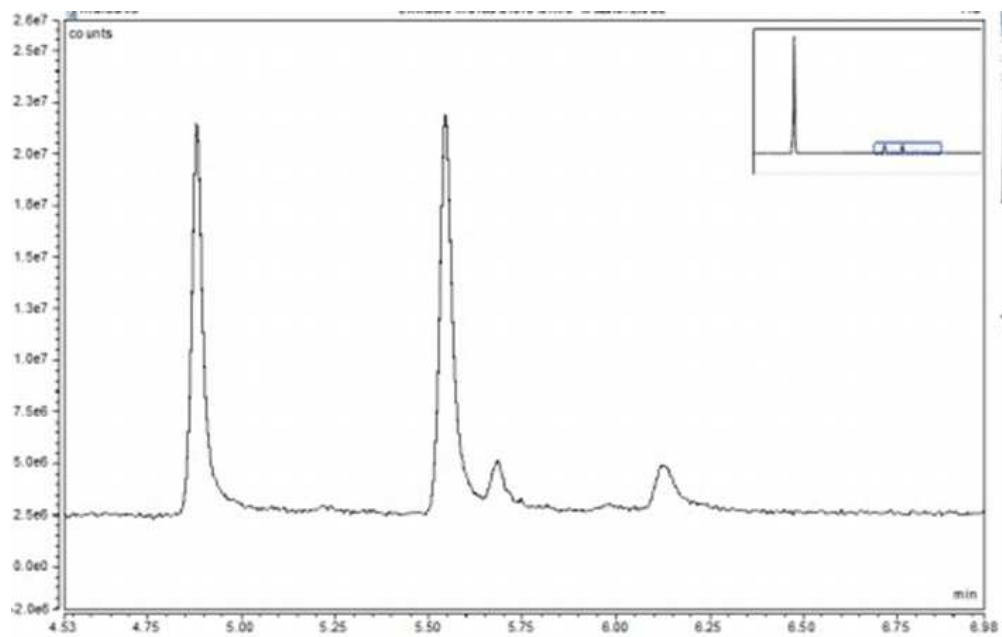


Figure 7: Mass spectrum of CBD.

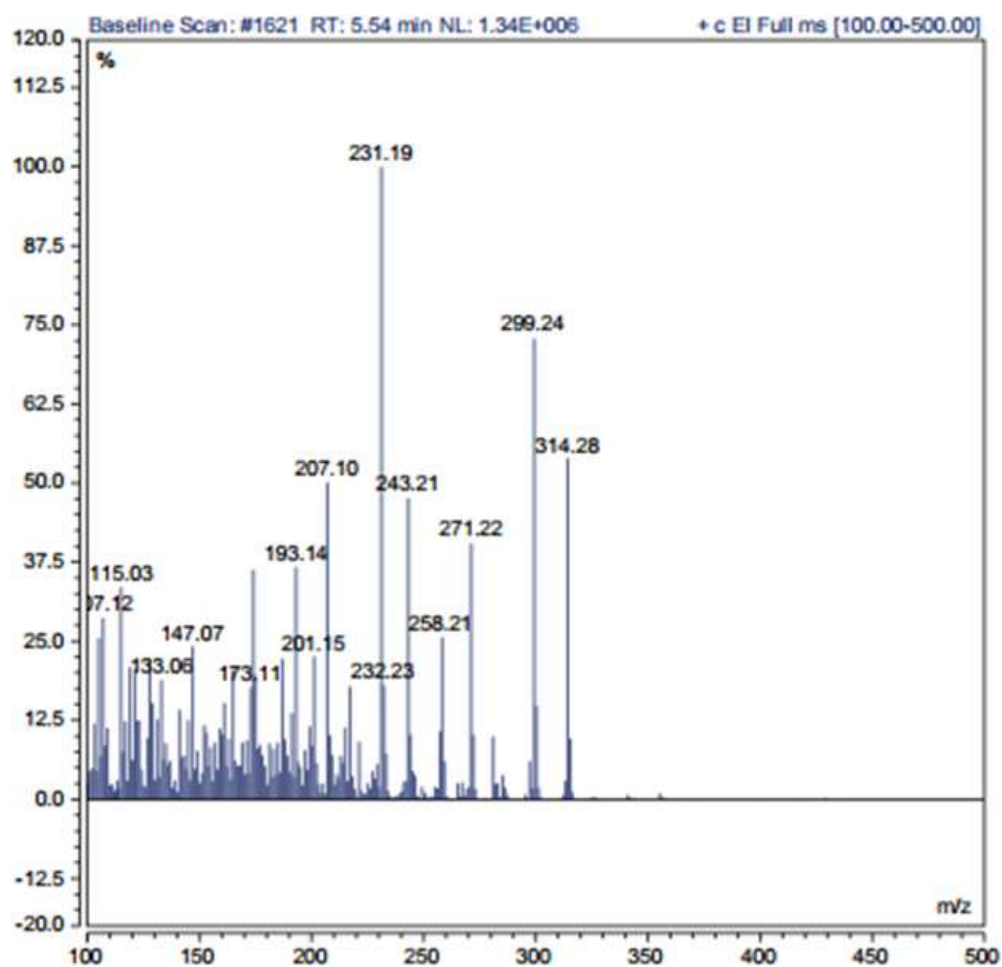


Figure 8: Mass spectrum of THC.

(Figure 8).

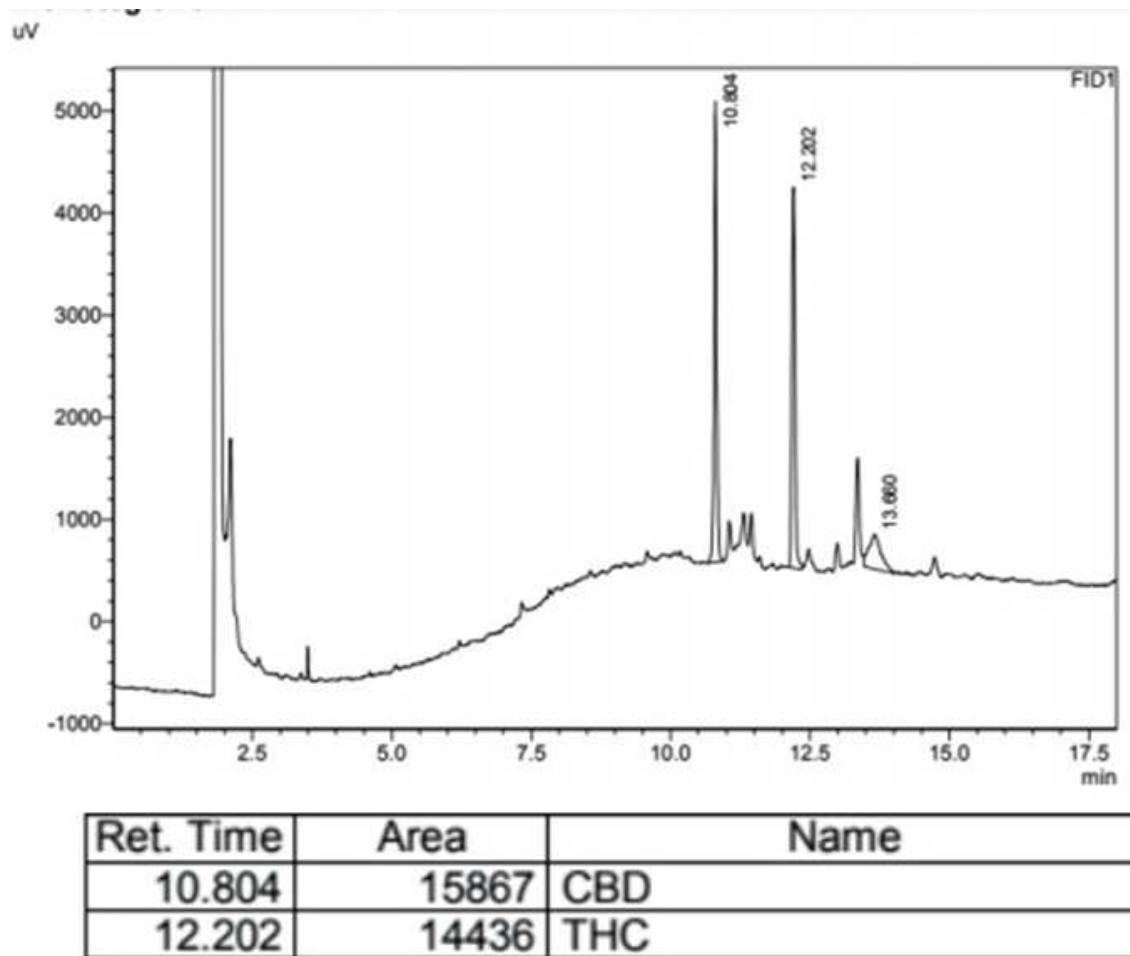


Figure 9. GC-FID chromatogram

Quantitative analysis by GC-FID revealed the following cannabinoid content per milligram of extract:

Table 1. Cannabinoid content of the *Cannabis sativa* extract determined by GC-FID.

Cannabinoid	Concentration (microgram/mg extract)	Relative Proportion (%)
Cannabidiol (CBD)	130 plus or minus 5	51
Delta-9-Tetrahydrocannabinol (THC)	125 plus or minus 5	49
Cannabinol (CBN)	Less than 5	Less than 2

This 1:1 CBD:THC ratio represents an atypical chemotype, as most commercial cannabis varieties have been selectively bred to contain either high THC (for recreational use) or high CBD (for medicinal use) content^[52].

3.2. Anticonvulsant Activity

3.2.1. 6 Hz Psychomotor Seizure Model

In the 6 Hz model, all control animals (7/7) exhibited seizure activity following electrical stimulation. Treatment with the cannabis extract demonstrated dose-dependent protection: at 50 mg/kg, 4 out of 7 animals (57%) convulsed, while at 100 mg/kg, complete protection was observed (0/7 convulsions). The reference drug levetiracetam (40 mg/kg) also provided complete protection (0/7 convulsions). These results indicate that the extract at the higher dose (100 mg/kg) exhibits anticonvulsant efficacy comparable to levetiracetam in this model of focal-onset seizures.

Table 2. Anticonvulsant effects of Cannabis sativa extract in the 6 Hz psychomotor seizure model.

Treatment	Dose (mg/kg)	Animals Tested (n)	Animals with Seizures (n)	Protection Rate (%)
Control (olive oil)	--	7	7	0
Cannabis extract	50	7	4	43
Cannabis extract	100	7	0	100
Levetiracetam	40	7	0	100

3.2.2. PTZ-Induced Seizure Model

In the PTZ model, all control animals (7/7) developed seizures, with 3 animals (43%) succumbing to status epilepticus. The extract at 50 mg/kg showed partial protection, with 3 out of 7 animals (43%) convulsing and 2 deaths (29%). However, at the higher dose of 100 mg/kg, seizure occurrence actually increased to 6/7 animals (86%), with 2 deaths (29%), suggesting a potential proconvulsant effect or exacerbation of PTZ-induced hyperexcitability at this dose. The reference drug clonazepam (0.3 mg/kg) provided complete protection with no seizures or mortality.

Table 3. Effects of Cannabis sativa extract on PTZ-induced seizures.

Treatment	Dose (mg/kg)	Animals Tested (n)	Animals with Seizures (n)	Mortality (n)
Control (olive oil)	--	7	7	3
Cannabis extract	50	7	3	2
Cannabis extract	100	7	6	2

Clonazepam	0.3	7	0	0
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3.2.3. Maximal Electroshock Seizure (MES) Test

The MES test demonstrated significant protective effects of the cannabis extract against generalized tonic-clonic seizures. In the control group, 4 out of 6 animals (67%) developed tonic-clonic seizures and died immediately following stimulation. Treatment with the extract at both 50 mg/kg and 100 mg/kg significantly reduced mortality to 1 out of 6 animals (17%) in each group. The reference drug phenytoin (20 mg/kg) provided complete protection with no seizures or mortality.

An interesting observation was made regarding potential prophylactic effects. Due to mortality in preceding PTZ experiments, some animals that had previously received extract treatment were reassigned to the control group for MES testing. These animals, which had received extract one week prior, survived the MES challenge, suggesting a possible long-lasting protective effect or preconditioning phenomenon.

Table 4. Anticonvulsant effects of *Cannabis sativa* extract in the MES test.

Treatment	Dose (mg/kg)	Animals Tested (n)	Animals with Seizures/Mortality (n)	Protection Rate (%)
Control (olive oil)	--	6	4	33
Cannabis extract	50	6	1	83
Cannabis extract	100	6	1	83
Phenytoin	20	6	0	100

4. Discussion

This study provides the first comprehensive characterization of the anticonvulsant properties of a Colombian *Cannabis sativa* extract with a unique 1:1 CBD:THC ratio. The findings demonstrate model-specific efficacy, with significant protection observed in the 6 Hz psychomotor and MES seizure models, but limited efficacy and potential exacerbation in the PTZ model.

4.1. Phytochemical Profile and Cannabinoid Content

The chemical characterization of the extract revealed a nearly equimolar ratio of CBD and THC (51:49), which is uncommon among cannabis varieties. Historically, cannabis cultivation has been directed toward either high-THC strains (for recreational psychoactive effects) or high-CBD strains (for medicinal applications)^[52]. This 1:1 ratio presents a unique chemotype that may offer distinct pharmacological properties.

The TLC and colorimetric analyses confirmed the presence of cannabinoids through characteristic R_f values and color reactions. The Fast Blue B Salt reaction specifically detects the phenolic moiety of cannabinoids, forming colored azo compounds through electrophilic aromatic substitution at the activated arene position^[53]. Similarly, the Duquenois-Levine test produces characteristic color changes through the condensation of acetaldehyde and vanillin with the cannabinoid phenolic structure in acidic conditions^[54].

The GC-MS and GC-FID analyses provided quantitative confirmation of cannabinoid identity and content. The calculated doses of individual cannabinoids administered to animals were approximately 6.5-13 mg/kg THC and 6.25-12.5 mg/kg CBD for the 50 and 100 mg/kg extract doses, respectively. These doses are notably lower than those typically reported in preclinical studies using purified cannabinoids, which often employ doses exceeding 100 mg/kg^[55,56].

4.2. Anticonvulsant Efficacy in the 6 Hz Model

The complete protection observed with 100 mg/kg extract in the 6 Hz model is particularly significant, as this model represents focal-onset seizures with impaired awareness, a common and often drug-resistant seizure type in clinical practice^[57]. The 6 Hz model is considered more refractory to conventional ASMs compared to the MES test, with some drugs effective in MES showing limited efficacy in 6 Hz^[58].

The comparable efficacy between the extract (100 mg/kg) and levetiracetam (40 mg/kg) suggests that the cannabis extract may possess broad-spectrum activity against focal seizures. This finding aligns with recent preclinical studies demonstrating CBD's efficacy in the 6 Hz model at 32 and 44 mA stimulation intensities^[59]. The NINDS Epilepsy Therapy Screening Program has identified CBD as having a unique and effective anticonvulsant profile across multiple seizure models, including 6 Hz^[60].

4.3. Efficacy in the MES Model

The significant reduction in seizure-related mortality observed in the MES test indicates protective effects against generalized tonic-clonic seizures, the most common and potentially life-threatening seizure type. The 83% protection rate at both extract doses suggests a ceiling effect, where higher doses do not provide additional benefit in this particular model.

The observation that animals previously treated with extract survived MES challenge one week later raises intriguing questions about potential prophylactic or preconditioning effects. While this finding was incidental and requires controlled investigation, it suggests the possibility of long-lasting neuroadaptive changes induced by cannabinoid exposure. Similar persistent effects have been reported in other preclinical studies, where subchronic CBD administration altered seizure thresholds for extended periods^[61].

4.4. Lack of Efficacy and Potential Exacerbation in the PTZ Model

The limited efficacy and apparent exacerbation of seizures in the PTZ model represents a complex finding that warrants careful interpretation. Several factors may contribute to this observation:

Dose considerations: The doses of CBD and THC achieved in this study (6.25-12.5 mg/kg CBD, 6.5- 13 mg/kg THC) are substantially lower than those reported effective in the PTZ model by other investigators. Jones et al. (2010) demonstrated CBD protection at 100 mg/kg (i.p.) in the PTZ model^[62]. The lower doses used in the present study may have been insufficient to overcome PTZ-induced hyperexcitability.

THC-mediated effects: THC has been reported to exhibit proconvulsant properties in certain seizure models, particularly when combined with analeptic agents^[63]. THC's effects on glutamatergic and GABAergic synapses may create a proconvulsant environment when combined with PTZ, a GABA-A

receptor antagonist. The interaction between THC's anxiogenic properties and PTZ's stimulant effects may produce synergistic proconvulsant activity^[64].

Model-specific mechanisms: The PTZ model primarily assesses drugs effective against absence seizures and myoclonic jerks, typically mediated by T-type calcium channel blockade or GABAergic enhancement^[65]. The mechanisms through which cannabinoids exert anticonvulsant effects may not optimally target the neurophysiological pathways underlying PTZ-induced

seizures.

4.5. Potential Synergistic Interactions: The Entourage Effect

A particularly noteworthy aspect of this study is the observation of anticonvulsant effects at relatively low individual cannabinoid doses compared to studies using purified compounds. This finding supports the concept of the "entourage effect," where the combined administration of multiple cannabis constituents produces enhanced therapeutic outcomes beyond what would be expected from individual components alone^[66].

Several mechanisms may underlie this potential synergy:

Complementary molecular targets: While CBD acts through GPR55 antagonism, TRPV modulation, and sodium channel blockade, THC primarily activates CB1 receptors and may modulate other ion channels. The simultaneous engagement of multiple targets may produce more robust anticonvulsant effects^[67].

Pharmacokinetic interactions: THC and CBD may influence each other's metabolism and bioavailability. CBD inhibits cytochrome P450 enzymes, potentially increasing THC plasma concentrations^[68].

Terpenoid contributions: Although the steam distillation process removed volatile monoterpenes, residual terpenoids in the extract may contribute to anticonvulsant effects through GABA-A receptor modulation or other mechanisms^[69].

Clinical evidence supporting the entourage effect includes studies showing that CBD-rich extracts may be more effective than purified CBD in reducing seizure frequency^[70]. However, purified CBD offers advantages in terms of standardized dosing, regulatory approval, and absence of psychoactive effects.

4.6. Comparison with Clinical Evidence

The findings of this study align with the clinical evidence supporting cannabinoid therapy for epilepsy. In 2018, the U.S. Food and Drug Administration (FDA) approved Epidiolex (purified CBD) for the treatment of seizures associated with Dravet syndrome and Lennox-Gastaut

syndrome in patients aged 2 years and older^[71]. Subsequent approval for tuberous sclerosis complex (TSC) in 2020 further expanded the clinical indications^[72].

Clinical trials have demonstrated that CBD significantly reduces seizure frequency in treatment-resistant epilepsies. In the GWPCARE trials, CBD treatment resulted in median seizure reductions of 43-44% compared to placebo in Dravet syndrome and Lennox-Gastaut syndrome^[73,74]. Open-label studies with

CBD-enriched cannabis extracts (CBD:THC ratios of 20:1 or 50:1) have reported similar or greater efficacy

in children with refractory epilepsy^[75,76].

The 1:1 CBD:THC ratio evaluated in this study differs from the high-CBD ratios used in most clinical studies. While high-CBD formulations minimize psychoactive effects, the equimolar ratio may offer distinct pharmacological advantages through synergistic interactions, as discussed above. However, the psychoactive properties of THC at this ratio may limit clinical applicability, particularly in pediatric populations.

4.7. Limitations and Future Directions

Several limitations of this study should be acknowledged. First, the sample sizes were relatively small, limiting statistical power and generalizability. Second, only acute administration was evaluated, and the effects of chronic treatment, tolerance development, and withdrawal phenomena were not assessed. Third, the study did not include pharmacokinetic analysis to confirm brain concentrations of cannabinoids achieved at the doses tested.

Future studies should address these limitations by incorporating larger sample sizes, chronic dosing paradigms, pharmacokinetic assessments, and additional seizure models (e.g., kindling, pilocarpine-induced status epilepticus). The potential prophylactic effects observed incidentally warrant systematic investigation. Additionally, studies comparing different CBD:THC ratios would help optimize the therapeutic window while minimizing adverse effects.

4.8. Implications for Drug-Resistant Epilepsy

The findings of this study contribute to the expanding evidence base supporting cannabinoid-based therapies for drug-resistant epilepsy. The model-specific efficacy pattern observed - effective in 6 Hz and MES but not PTZ - suggests that cannabis-based treatments may be most beneficial for specific seizure types, particularly focal-onset and generalized tonic-clonic seizures.

For the approximately 30% of epilepsy patients with drug-resistant seizures, cannabis-derived compounds represent a promising therapeutic avenue^[77]. The unique pharmacological profile of cannabinoids, targeting multiple mechanisms distinct from conventional ASMs, offers the potential for efficacy in patients who have failed existing treatments.

However, the potential for THC-mediated psychoactive effects and the exacerbation observed in the PTZ model underscore the importance of careful patient selection, dose optimization, and monitoring. The development of standardized, pharmaceutical-grade cannabis preparations with defined cannabinoid ratios will be essential for integrating these therapies into clinical practice.

5. Conclusions

This study demonstrates that a Colombian *Cannabis sativa* extract with a unique 1:1 CBD:THC ratio possesses significant anticonvulsant activity in murine models of focal-onset (6 Hz) and generalized tonic-clonic (MES) seizures. The extract provided complete protection at 100 mg/kg in the 6 Hz model and substantially reduced mortality in the MES test. However, limited efficacy and potential exacerbation were observed in the PTZ model, highlighting the model-specific nature of cannabinoid anticonvulsant effects.

The nearly equimolar ratio of CBD to THC in this Colombian cannabis variety represents an atypical chemotype that may offer distinct therapeutic advantages through potential synergistic interactions. The anticonvulsant effects observed at relatively low individual cannabinoid doses support the concept of the entourage effect and suggest that whole-plant extracts may provide therapeutic benefits beyond isolated compounds.

These findings contribute to the growing body of evidence supporting the therapeutic potential of cannabis-based medicines for drug-resistant epilepsy. Future research should focus on elucidating the mechanisms underlying model-specific efficacy, optimizing CBD:THC ratios for different seizure types, and conducting well-controlled clinical trials to translate these preclinical findings into improved patient care.

Ethics Statement: All animal experiments were conducted in accordance with the Colombian Ministry of Health Resolution 008430/1993 and followed the principles of the 3Rs (Replacement, Reduction, Refinement). Animals were maintained under appropriate bioterium conditions with humane handling throughout the study.

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