Journal of Biosciences and Medicines, 2023, 11, 192-207
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Exploring Chemovar-Specific Cannabis Extracts Quantification and Evaluation of Cytotoxic Compounds for Targeting Glioblastoma Multiforme

Ashraf Duzan[1,2,3*], Mufeed Basti[2], Travis Cesarone[4], Waldemar Debinski[5], Daniel Todd[6]

[1]Wingate University School of Pharmacy, Wingate University, Wingate, NC, USA
[2]Applied Science and Technology Department, North Carolina State University of Agriculture and Technology, Greensboro, NC, USA
[3]Department of Pharmaceutical Sciences, College of Pharmacy, Health Professions Division, Nova Southeastern University, Fort Lauderdale, USA
[4]Uprooted Concepts, British Columbia, Victoria, Canada
[5]Brain Tumor Center of Excellence, Wake Forest School of Medicine, Medical Center Blvd, Winston Salem, NC, USA
[6]Department of Chemistry and Biochemistry, The University of North Carolina at Greensboro, Greensboro, NC, USA
Email: *asduzan@aggies.ncat.edu

How to cite this paper: Duzan, A., Basti, M., Cesarone, T., Debinski, W. and Todd, D. (2023) Exploring Chemovar-Specific Cannabis Extracts Quantification and Evaluation of Cytotoxic Compounds for Targeting Glioblastoma Multiforme. *Journal of Biosciences and Medicines*, **11**, 192-207.
https://doi.org/10.4236/jbm.2023.118015

Received: July 24, 2023
Accepted: August 20, 2023
Published: August 23, 2023

Abstract

Glioblastoma Multiforme (GBM) represents one of the most aggressive and metastatic brain tumors, with a dismal success rate of less than three percent after five years, particularly in tumors with active immune checkpoints. This necessitates the development of targeted endogenous agents for precise GBM treatment. Previous experiments utilizing Chemovar Specific Cannabis Extractions (CSCEs), fractionated with polar solvents and quantified using Liquid and Gas Column Chromatography combined with Mass Spectrometry (LC/GCMS), have shown reduced viability and motility in human GBM cell lines. However, the complexity of the botanical substance has hindered the personalization of standard cannabis medicines for GBM due to unknown synergistic effects of multiple compounds. To address this limitation, our study focuses on exposing AM251 cells to chemovar fractions extracted using a non-polar solvent, thereby isolating a broader spectrum of constituents. By employing LC/GCMS in conjunction with Nuclear Magnetic Resonance (NMR), we have identified and quantified nine* compounds present in the non-polar CSCE that exhibit significant efficacy (0.1 μM) in inducing cytotoxicity* in GBM tumor cells. Conversely, the polar fraction in our experiment did not demonstrate efficacy against UM251 cells. The quantification of individual compounds within a cannabis extraction that selectively induces

cell death in brain tumors holds promise for guiding future research and facilitating the development of a standardized CSCE for GBM therapy.

Keywords

Cannabinoids, Liquid Chromatography, Mass Spectrometry (LC-MS), Glioblastoma Multiforme (GBM), Total Correlation Spectroscopy (TOCSY), Gas Chromatography-Mass Spectrometry (GC-MS)

1. Introduction

Gliomas, a type of brain tumor, present a significant challenge in the field of oncology. Among these tumors, glioblastoma (GBM) stands out as a particularly aggressive and lethal form, classified as a Stage IV glioma according to the World Health Organization (WHO). Recent advancements in understanding the molecular aspects of Central Nervous System (CNS) tumors have led to the inclusion of molecular parameters in their classification, with significant revisions made by the WHO in 2021 [1] [2]. One prevailing theory gaining support in the scientific community is the Cancer Stem Cell (CSC) theory, proposing that glioblastomas arise from glial cells undergoing aberrant differentiation, resulting in the formation of cancer stem cells. These CSCs are believed to drive tumor initiation and progression [3] [4]. However, uncovering the precise origins of individual GBM tumors remains an ongoing research endeavor, urging the need for further investigation.

In the clinical realm, standardized treatments have shown promising results in improving survival rates for GBM patients. The current Standard of Care encompasses surgical resection, radiation therapy, and chemotherapy, notably employing daily temozolomide (TMZ) [5] [6]. This combined approach has demonstrated median survival rates of up to 20 months. However, the effectiveness of treatment is highly dependent on the genetic composition of each tumor, as specific mutations can confer resistance to therapy [7]. Notably, the presence of O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation is a crucial determinant of TMZ efficacy, with GBM lacking this methylation exhibiting resistance to the drug [7] [8] [9].

In light of these challenges, novel therapeutic strategies are being explored, and cannabis metabolites have emerged as a promising avenue. These metabolites have demonstrated the ability to target several druggable mechanisms observed in genotype-specific GBM. Despite these promising findings, additional research is still needed to establish a replicable and effective therapeutic approach utilizing cannabis metabolites [10].

Cannabaceae is a Plantae family that includes *Cannabis sativa L* and comprises a unique system of non-polar metabolites. [11] [12] CBGa is a precursor for naturally occurring phytocannabinoids with a five-carbon chain, and tetrahy-

drocannabinolic acid (THCa) and cannabidiolic acid (CBDa) are its two primary metabolites. [13] Cannabis's non-polar constituents include 180 known phytocannabinoids, 111 terpenes comprising either ten or fifteen carbons, and 121 terpenoids. [14] [15] [16] Fractions containing Δ^9-THCA, CBG, and CBC derived from a crude CSCE produced with polar solvents induced ~90% cell death in the A172 GBM cell line after 48 hours of exposure. [10]. Through compliant extraction techniques and LC/GCMS quantification, personalized CSCEs can be standardized [10] [16] [17] and selected to any of the following GBM-related mechanisms: promoting 2-AG, ERK via CBrs, or PPARs [18] [19] [20] promoting or down-regulating AKT and cAMP-dependent on allosteric modulation [21] [22] [23], desensitizing a number of Transient Receptor Potential Channels (TRPs) [24], inhibiting GPR_{55} [25] [26] or reducing Prostaglandin E2 (PGE_2) via COX-2. [27] [28] [29]

Δ^9–tetrahydrocannabinol, a decarboxylated cannabinoid that activates the G protein-coupled receptors—CB_1r and CB_2r. [30] [31] It causes notable intoxication but further promotes angiogenesis, maintains ceramide homeostasis, [32] and partially inhibits autotoxin. [33] Cannabidiol (CBD), in part and in contrast to Δ^9–THC, reduces calcium current by antagonizing GPR_{55}. [25] CBD negatively binds to the allosteric pocket in CB_1r and CB_2r with low efficacy. [34] [35] Notably, CBD co-administered with an equal ratio of THC did not affect either ERK or P13K pathways in the ventral hippocampi, in contrast to each cannabinoid alone. [22] CBD induced autophagy in a neuroblastoma cell line dependent on ERK1/2 and PI3K/AKT activation. [36] Drug development must, therefore, consider the biased conformational changes downstream of CB_1r during the treatment of GBM with CSCEs. [10] [18] [22]

CBG weakly binds to CB_2r, [37] whereas cannabichromene (CBC) and beta-caryophyllene (BCP) are selective CB_2r agonists. [37] [38] Glial cells in the cerebellum express CB_2r following oxidative stress. [39] [40] [41] [42]. Cannabinoids downregulate calcium and sodium current via TRP desensitization or inactivation. [24] CBD induces cell death in glioblastoma by dephosphorylating TRPV1 and inhibiting GPR_{55}. [26] TPRV1 strongly cross-talks with PGE_2, [43] which is metabolized by COX-2. CBDa, THCa, and CBG inhibit COX-2 with significant efficacy, which likely mediates GBM proliferation by targeting arachidonic acid metabolism to PGE_2. [27] [29] The development of clinical COX-2 inhibitors to treat glioblastoma previously failed due to widespread adverse reactions.

Cannabinoid formulations affect enzymes responsible for the metabolism and catabolism of endocannabinoids. CBD inhibits FAAH, which metabolizes N-arachidonoylethanolamine (AEA/anandamide) in the post-synaptic cleft of CB_1r. [24] [44] However, glioblastoma does not migrate away from the brain where 2-AG levels are ~170-fold greater than anandamide. [45] Whole-plant extracts containing THCa, CBGa, and CBG, but not cannabinoid isolates, inhibit the serine hydrolase known as MAGl, which metabolizes 2-AG in the pre- synaptic cleft. [24]

THC purified from cannabis extracts did not enhance the cytotoxicity of CBG against glioblastoma, whereas CBG induces cell death in Cancer Stem Cells, targeting treatment-resistant tumors. [26] [46] An earlier clinical trial with cannabis containing THC, using TMZ as a control, had cytotoxic effects. [47] To improve on earlier trials, a wide array of bioactive constituents in a whole-plant extract standardized with non-polar extraction quantified with LC/GCMS coupled with NMR can replicate positive results against GBM. [10] Consistent co-administration of antagonist/agonist functions, alongside appropriate allosteric control of CB1 receptor signaling, has potential viability as a treatment for Stage Four 4 Gliomas and GBM with whole-plant CSCEs.

2. Material and Method

2.1. Chemicals and Reagents

Mass spectrometry grade formic acid, methanol, hexane and acetonitrile (methyl cyanide) were procured from Fisher Scientific (Waltham, MA). Optima grade water was used for GC-MS analysis. Acquity ultra-high-performance liquid chromatography (UPLC) BEH C18 analytical column and Vanguard pre-column for chromatography were obtained from Waters Corp., Waltham, MA. Cannabis dry flower samples were obtained from local hemp stores in Wingate, NC, USA. These products were stored at −20˚C until further analysis. Deuterated chloroform ($CDCl_3$) with 1 v/v% TSP was obtained from Acros Organics, NJ, USA.

2.2. Sample Preparation

1) Extraction: Dried cannabis flower was milled into a powder using a high-speed multifunction grinder (HC-1500Y). In an Eppendorf, 0.5 ml miscible of 0.33 ml methanol and 0.17 ml water were combined with 0.5 ml of chloroform and added to 0.04 g to 0.06 g of milled cannabis. The Eppendorf was then placed into a vortex shaker for five minutes before placement into a Micro 2 L centrifuge at a speed of 14.8×10^3 RPM at ambient temperature for 10 min. The resulting solute was separated into a: Top (polar), bottom (non-polar), and middle (solid) layer**.** The top layer is polar and contains the Methanol/water solvent. The bottom layer is non-polar and contains the chloroform solvent. The middle layer (solid) dissolves exclusively in DMSO. The organic phase was transferred into a separate Eppendorf with a micro-pipette and placed into a Savant Speed-Vac SPD1030 integrated Vacuum concentrator at ambient temperature and a pressure of 6 torr for 4 - 6 hrs.

2) GCMS: The non-polar sample was dissolved in hexane and injected into a DB-1HT MS capillary column with 2 µl of helium gas as a carrier. For MS detection, ionization energy (IE) of 70 eV was used at the source temperature, and analytes were scanned using a Shimadzu GCMS-QP2010S.

3) UPLC-MS/MS: The resulting extract was shaken in the Vortex for 1 min and filtered through a 0.22 µm filter unit. The solute was diluted to a ratio of 1:100 and 100 µl of sample was transferred to LCMS vials and centrifuged for 5

min. The Linear Gradient consisted of eluant (I) at 50% and (II) at 50% for 1 min, II for 8 min at 100%, II for 3 min at 50%, and equilibrated for 2 min. The program ran for 13 min at a flow rate of 5 ml/min. 0.5 µL of the sample was injected, and UPLC was then quantified using an ACQUITY-Target Lynx.

4) NMR: The non-polar phase was dissolved in deuterated chloroform ($CDCl_3$) with 1% TSP as an internal reference at 0 PPM. The Bruker Ascend 400 MHz spectrometer recorded NMR spectra at 25˚C. Mixing times used in the analysis varied between 0.03, 0.08, and 0.12 seconds, with the number of scans set to 256. Signals from protons in the sample were obtained by selectively exciting a spin-coupled proton whose resonance corresponds to a narrow PPM region of the 1D spectrum in each spectrum. A standard non-phase sensitive sequence (2D) homo-nuclear shift correlation generated two-dimensional NMR experiments, with data gathered by 2kX256 data points matrix.

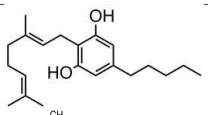
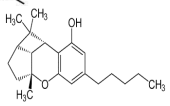
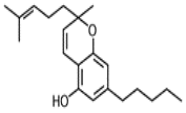
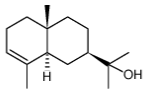
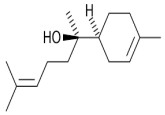
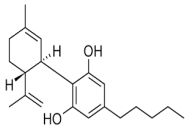
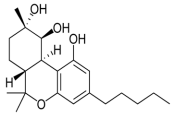
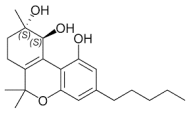
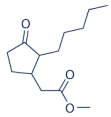
2.3. Instrumental

1) GC-MS: A Shimadzu GC-MS-QP 2010S generated the GC-MS data. Physical specifications for the DB-1HT MS capillary column used for GC were as follows: 30.0 m length, 0.25 mm i.d., 0.10 µm film thickness. The temperature program was set up from 100˚C and programmed to increase to 200˚C for 9 min and remained for 4 min and then to 300˚C for 7 min and remained for 21 min for a total program time of 45.67 min. Both the injector and detector temperatures were 260˚C. The mass spectroscopy detector used Ionization Energy (IE), with a source temperature of 260˚C and a scan range set to 40 - 600 AMUs.

2) Liquid chromatographic (LC) conditions: Analytes were separated on an Aquity U-PLC BEH C18 analytical column (2.1 × 100 mm, 1.7 µm particle size, 130 Å̊ pore size) preceded by an Acquity U-PLC BEH C18 VanGuard pre-column (2.1 × 5 mm, 130 Å̊). The flow rate was kept at 0.5 mL/min. The autosampler was maintained at 10˚C throughout the analysis, and the analytical column was maintained at 45˚C. The mobile phases consisted of 0.1% formic acid in water (I) and 0.1% formic acid in acetonitrile (II), and an ACQUITY-Target Lynx was used for to quantify the UPLC samples.

Mass spectrometry conditions: Quadrupole Time-of-flight tandem mass spectrometer system (Waters SYNAPT G2-Si Q-ToF parameters were optimized using tandem MS (MS/MS) ions for each standard solution of c. Sativa metabolites in the positive and negative modes. The method was validated and followed FDA guidelines. Electrospray ionization (ESI) in positive and negative modes was used to quantify the analytes' tandem MS/MS transitions. A total ion chromatogram (TIC) was used to quantify the analytes (Table 1). Mass spectrometry parameters included capillary voltage 1.50 kV, collision gas flow 0.15 mL·min^{-1}, extractor voltage 3 V, desolation temperature 500˚C, source temperature 150˚C, and desolation gas flow 1000 L/h, and the MS scan was 50 - 1200 m/z. MSMS mode For MS^E experiments, one acquisition function with different collision energy ramps was used for additional MS/MS experiments with electrospray ionization (ESI).

Table 1. Summarizes the LC-MS, GCMS, and NMR results for the identified compounds in the non-polar extract of cannabis sativa.

Compound	Chemical Structure	Chemical Formula	RT (min)	$[M + H]^{-/+}$ (m/z)	LCMS	GCMS	NMR
Cannabigerol (CBG)		$C_{21}H_{32}O_2$	4.6	317.2410 > 180.1375	Yes	Yes	Yes
Cannabicyclol (CIC)		$C_{21}H_{30}O_2$	5	315.8131 > 245.1577	Yes	Yes	Yes
Cannabichromene (CBC)		$C_{21}H_{30}O_2$	6	313.1870 > 214.1724	Yes	Yes	Yes
α-Eudesmol		$C_{15}H_{26}O$	10	221.8950 > 163.1525	Yes	Yes	Yes
α- Bisabolol		$C_{15}H_{26}O$	9	221.1510 > 99.1425	Yes	Yes	Yes
Cannabidiol(CBD)		$C_{21}H_{30}O_2$	4.5	315.4691 > 181.1275	Yes	Yes	Yes
Cannabiripsol (CBR)		$C_{21}H_{32}O_4$	4.5	349.2361 > 279.1576	Yes	Yes	Yes
Cannabitriol(CBT)		$C_{21}H_{30}O_4$	6.2	347.4692 > 277.1475	Yes	Yes	Yes
Hedione		$C_{13}H_{22}O_3$	8.1	227.3121 > 154.1575	Yes	Yes	Yes

3) NMR: The NMR spectra were recorded on a Bruker Ascend 400 MHz spectrometer. Standard 1D NOESY pulse sequence was used to acquire the ^1H spectrum. 1D selective TOCSY data were collected using homonuclear Hartman-Hahn (HOHAHA) transfer pulse sequence where MLEV17 sequence was used for mixing and the selective excitation was obtained using a shaped pulse and Z-filter[25]. The data were processed with LB of 0.1 - 1.0 Hz. Data were collected with 2kX256 data points matrix. The data was processed with window functions that included: F2 dimension sine bell, with line-broadening of 02 Hz. F1 dimension (second dimension) sine bell, with line-broadening of 0.1 Hz. The Gaussian maximum position was 0.05 (F2) and 0.1 (F1), and the data were zero-filled to 2KX1K data points. Phase-sensitive homonuclear Hartman-Hahn (HOHAHA) transfer acquired the Total TOCSY spectrum with an MLEV17 sequence for mixing. Single quantum correlation (SQC) data of ^1H- ^{13}C was ac-

quired using the phase sensitive, 2D H-1/X correlation via double inept transfer with sensitivity improvement pulse sequence. Data was acquired as 2KX256 data points and zero-filled to 2KX1K data points.

2.4. Biological Activity of Cannabis Extracts on Glioblastoma Cell Line Culture

Each compound was dissolved in respective amounts of DMSO and transferred to a tube with known mass. The tubes were spun at 3000 RPM for 5 min (with DMSO and pellet) and superannuant was reserved and tested on cells. Each supernatant sample was placed into a different microfuge tube with a known mass. The open microfuge tubes containing the pellets were placed on a 37˚C heat block so that any leftover DMSO would evaporate. Eventually, the mass was obtained for compounds 1 and 2. We used the maximum amount of DMSO containing compound the cells would tolerate (1%) and used 1:10 further dilutions. Compound #2 or the non-polar layer was further tested with a 1:2 serial dilution of DMSO concentration starting at 1% DMSO.

3. Result and Discussion

The results presented in the scientific paper describe the instrumental methods and conditions used for the analysis of cannabis extracts and their biological activity on glioblastoma cell lines. The paper employed three different instrumental techniques: gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR).

Gas chromatography-mass spectrometry (GC-MS) has emerged as a powerful analytical technique for identifying chemical compositions in various samples. By coupling gas chromatography with mass spectrometry, GC-MS allows for the separation and detection of complex mixtures of compounds present in a sample. The gas chromatograph separates the components based on their volatility, while the mass spectrometer identifies and quantifies individual compounds by measuring their mass-to-charge ratios. GC-MS analysis generates large datasets consisting of mass spectra, retention times, and peak intensities, which can be further processed and analyzed using advanced data analysis techniques. These datasets provide valuable information about the chemical composition of samples, enabling researchers to identify specific compounds, detect impurities, assess sample purity, and explore the metabolic profile of biological samples. The utilization of GC-MS in conjunction with comprehensive data analysis techniques facilitates the understanding of complex chemical systems, leading to advancements in various fields, including environmental analysis, forensic science, pharmaceutical research, and metabolomics.

In the GC-MS analysis, a Shimadzu GC-MS-QP 2010S instrument was used with a DB-1HT MS capillary column. The temperature program involved a gradual increase from 100˚C to 200˚C, followed by further increases to 300˚C. The

mass spectroscopy detector used ionization energy (IE) with a source temperature of 260˚C. The scan range was set to 40 - 600 atomic mass units (AMUs). The chromatogram obtained from the GC-MS analysis is shown in Figure 1, demonstrating the compounds and metabolites present in the organic extract of cannabis flower (Table 1). GC-MS analysis was performed to obtain a chromatogram of compounds and metabolites in the organic extract of cannabis flower. The obtained chromatogram provides a visual representation of the compounds present in the extract. This analysis complements the NMR data and further confirms the presence of Cannabidiol (CBD), Cannabicyclol (CBL), Cannabichromene (CBC), Cannabiripsol (CBR), Cannabitriol (CBT), and α-Bisabolol are the most abundant in the extract.

Liquid chromatography-mass spectrometry (LC-MS) has become an indispensable tool in modern analytical chemistry for identifying chemical compositions. LC-MS combines the separation power of liquid chromatography with the sensitive and selective detection capabilities of mass spectrometry. By analyzing the parent ions, daughter fragmentations, and retention times of compounds, LC-MS allows for the confirmation of the identity of each compound. The parent ions provide information about the intact molecular species, while the daughter fragmentations offer insights into the structural characteristics of the compounds. Additionally, the retention times obtained from the chromatographic separation aid in distinguishing between different compounds with similar mass spectra. Together, these features enable researchers to confidently identify and characterize a wide range of chemical compounds using LC-MS.

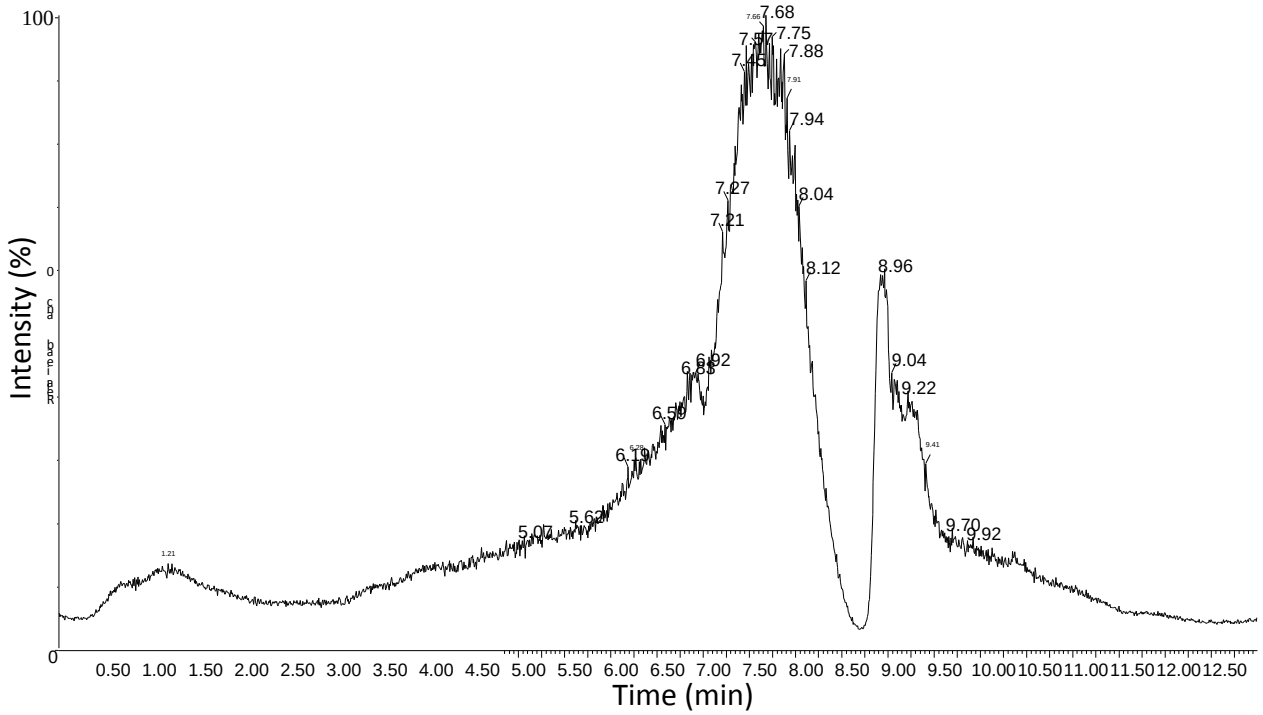


Figure 1. The chromatogram of the compounds and metabolites in the organic (Chloroform) extract of cannabis flower in GCMS.

LC analysis, an Aquity U-PLC BEH C18 analytical column was used, preceded by an Acquity U-PLC BEH C18 VanGuard pre-column. The mobile phases consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The LC analysis was coupled with a mass spectrometer (LC-MS) using a Waters SYNAPT G2-Si Q-ToF tandem mass spectrometer system. The LC-MS conditions were optimized using tandem MS/MS ions for each standard solution of cannabis metabolites.

The LC-MS chromatogram is shown in Figure 2, which represents the chloroform extract. Table 1 summarizes the LC-MS results, including the retention time, m/z values for parent and daughters' fragmentations. The UPLC-MSMS analysis using chloroform/methanol/water extraction also provided a chromatogram in Figure 2, that represents the compounds present in the extract. This technique allowed for the identification of Cannabigerol (CBG), Cannabicyclol (CBL), Cannabichromene (CBC), α-Eudesmol, α-Bisabolol, Cannabidiol (CBD), Cannabiripsol (CBR), Cannabidiol (CBT), and Hedione in the extract. The results from UPLC-MSMS corroborate the findings from NMR and GC-MS analyses, further confirming the presence of these compounds in the cannabis extract.

One approach to gaining insights into the chemical composition and structural elucidation of organic extracts is through the utilization of proton nuclear magnetic resonance spectroscopy (1H-NMR) and Selective Total Correlation Spectroscopy (TOCSY). 1H-NMR allows for the identification and quantification of various organic compounds based on their unique proton chemical shifts, providing valuable information about the functional groups present in the extract. Selective TOCSY, on the other hand, aids in the determination of connectivity between protons, enabling the assignment of complex proton spin systems and facilitating the elucidation of molecular structures. Together, these techniques offer a powerful toolset for characterizing and understanding the complex mixture of organic compounds present in an extract, paving the way for further investigations in fields such as natural product discovery and environmental analysis.

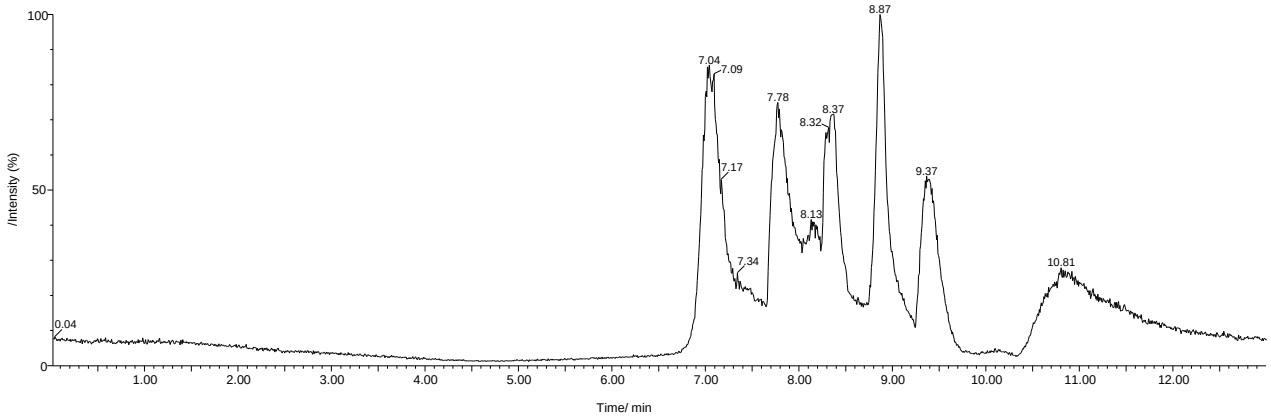
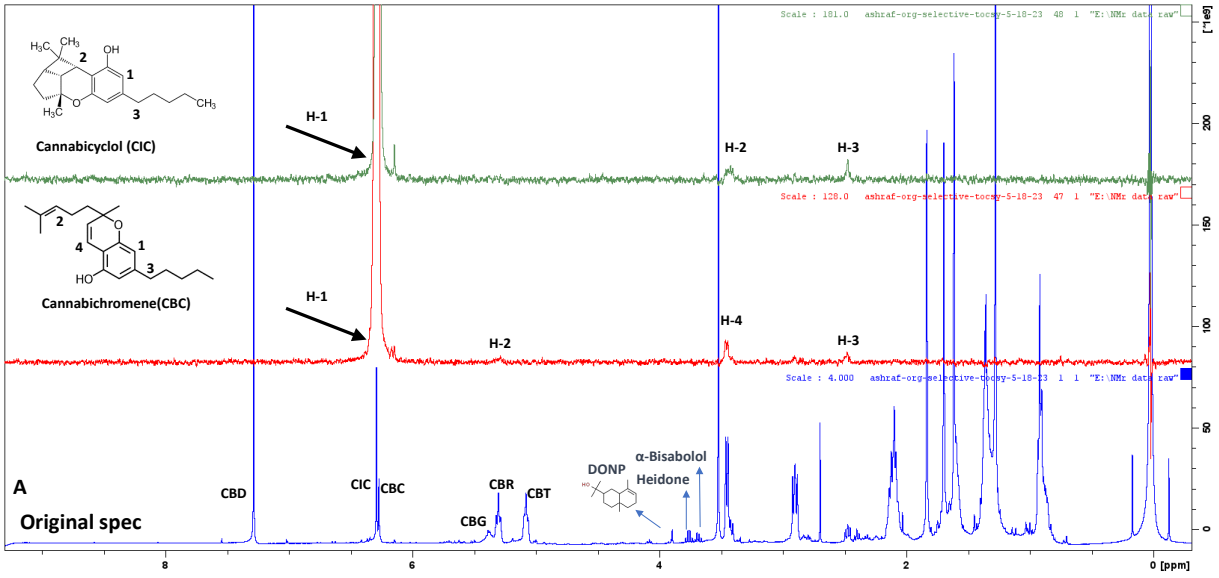
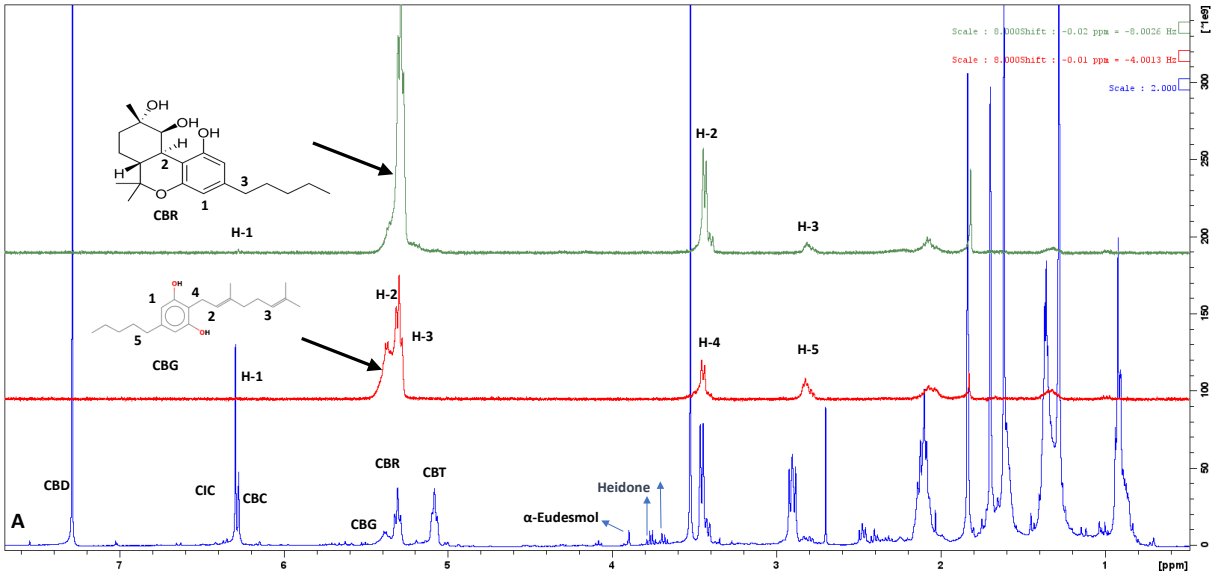


Figure 2. The chromatogram of the compounds in the organic extract of cannabis flowers in UPLC-MSMS using chloroform/methanol/water extraction, and the chromatogram represented LCMS of chloroform extract.

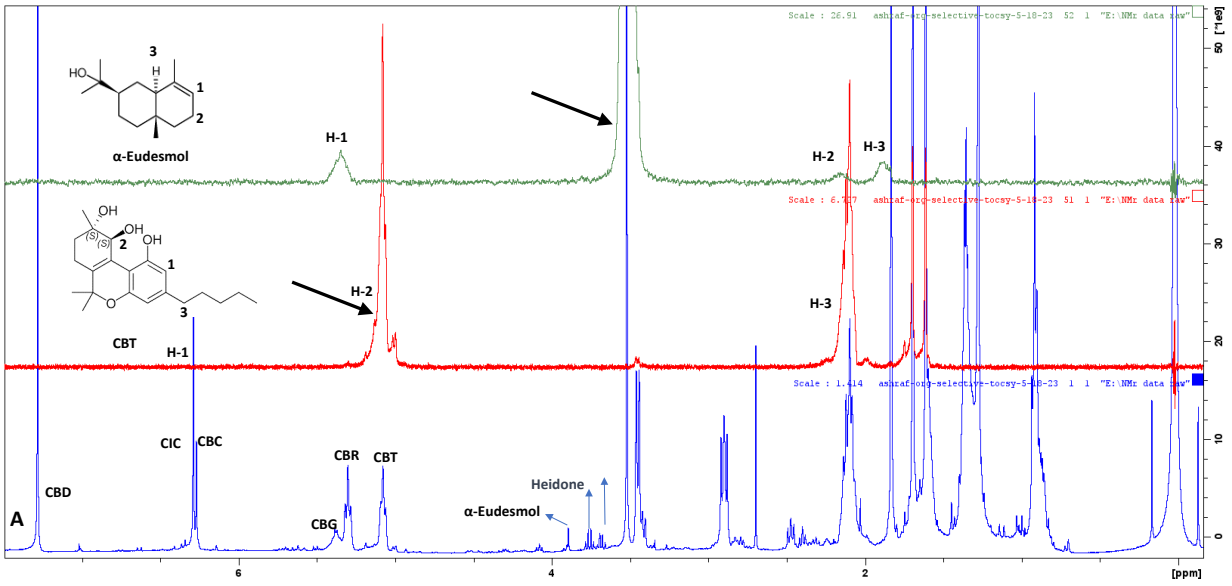
^{1}H-NMR spectrum was acquired using a standard pulse sequence, while 2D experiments, such as TOCSY, were performed to acquire additional spectral information. The NMR data provided structural information and confirmation of the identified compounds in Table 1. The NMR analysis allowed for the identification of several compounds based on their respective chemical shifts. The chemical shifts observed at 7.26 ppm and 3.49 ppm corresponded to Cannabidiol (CBD), indicating the presence of this compound in the sample. Other compounds identified by NMR included Cannabicyclol (CIC), *α*-Eudesmol, Cannabigerol (CBG), Cannabichromene (CBC), Cannabiripsol (CBR), Cannabidiol (CBT), Hedione, and Alpha-Bisabolol. The NMR data provides valuable information about the chemical shifts and allows for the identification of specific compounds in the extract (Figure 3).



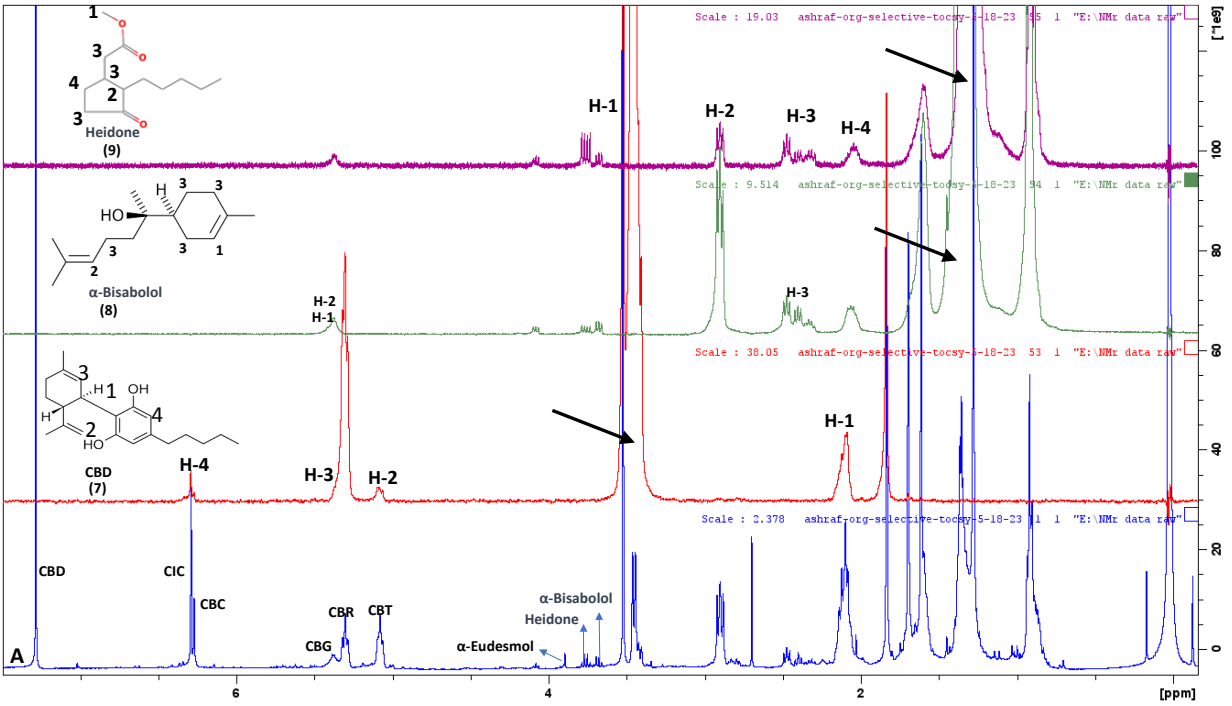
(a)



(b)



(c)



(d)

Figure 3. [1]H-NMR and Selective TOCSY of organic extract spectrum. Selective TOCSY of the compounds (1-9) showing specific protons and chemical shifts. (a) compounds CBC, CIC; (b) CBG, CBR; (c) CBT, *α*-Eudesmol; (d) CBD, *α*-Bisabolol, Heidone.

The biological activity of cannabis extracts on glioblastoma cell lines was evaluated. The extracts were dissolved in dimethyl sulfoxide (DMSO) and tested on cells. The non-polar extract

Non-polar extract showed a potent cytotoxic effect on the U250 glioblastoma cell line, while no toxic effects were observed with the polar and middle-layer

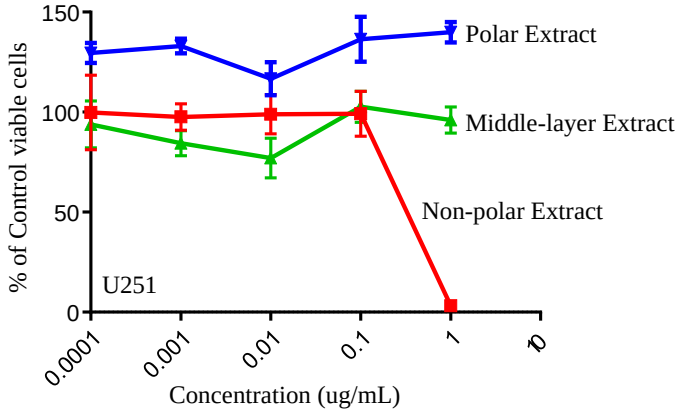


Figure 4. A: Pharmacological activity of cannabis sativa extracts in polar and non-polar solvents and the middle-layer extracts. Those extracted were tested and incubated with two different glioblastoma cell line cultures U250 l. The figure shows that non-polar extract has potent cytotoxic effect on U250 cell line culture, however, we did not see any toxic effect on cancer cell line culture using polar and middle-layer extracts.

extracts. This research explores the potential anti-cancer properties of various compounds found in cannabis extracts, including Cannabidiol (CBD), Cannabicyclol (CIC), Cannabiripsol (CBR), and Alpha-Bisabolol, Cannabigerol (CBG), Cannabichromene (CBC), and Cannabitriol (CBT). CBD, extensively studied, has shown promise in preclinical models, with studies suggesting its anti-cancer effects on glioblastoma cell lines by inducing apoptosis and inhibiting cell growth and migration. Similarly, CBG, CBC, and CBT have demonstrated potential anti-cancer properties, particularly concerning glioblastoma cells, in preclinical studies. However, it is crucial to note that evidence is still preliminary, and further research, including clinical trials, is required to establish the safety and efficacy of these compounds for glioblastoma treatment. These results are depicted in **Figure 4**, illustrating the pharmacological activity of cannabis extracts on glioblastoma cell cultures.

4. Conclusion

The presented results provide valuable insights into the chemical composition of cannabis extracts and their potential pharmacological activities. The instrumental analysis techniques, namely Nuclear Magnetic Resonance (NMR), Gas Chromatography-Mass Spectrometry (GC-MS), and Ultra-Performance Liquid Chromatography-Mass Spectrometry (UPLC-MSMS), were employed to identify and characterize various compounds present in the extracts. The instrumental analysis techniques of GC-MS, LC-MS, and NMR have provided valuable insights into the chemical composition and structure of cannabis extracts. GC-MS analysis revealed the presence of various compounds and metabolites in the organic extract of cannabis flowers. LC-MS analysis allowed for the identification and quantification of specific compounds, including amino acids and cannabi-

noids. NMR analysis further confirmed the presence of these identified compounds. Biological activity testing on glioblastoma cell lines demonstrated that the non-polar extract exhibited a potent cytotoxic effect on the U250 cell line, indicating its potential as an anticancer agent. However, the polar and middle-layer extracts did not exhibit any toxic effects on the tested cell lines. These findings significantly contribute to our understanding of the chemical composition and biological activity of cannabis extracts, particularly in relation to glioblastoma cell lines. Further research and investigations are necessary to explore the mechanisms of action and potential therapeutic applications of these extracts. The pharmacological activity of cannabis sativa extracts on glioblastoma cell lines was discussed, revealing that the non-polar extract displayed a potent cytotoxic effect on the U250 glioblastoma cell line. This suggests that the non-polar extract may possess anti-cancer properties, specifically targeting the U250 glioblastoma cell line. In summary, the instrumental analysis techniques of NMR, GC-MS, and UPLC-MSMS provided comprehensive information on the chemical composition of cannabis extracts. The identified compounds, such as Cannabidiol (CBD), Cannabicyclol, Cannabichromene, Cannabiripsol (CBR), Cannabidiol (CBT), and Alpha-Bisabolol, hold potential therapeutic and pharmacological implications. Further investigations are warranted to explore the specific mechanisms of action and potential applications of these compounds in various medical and pharmaceutical contexts.

Author Statement

Ashraf Duzan: Conceptualization, methodology, software, data curation, writing original draft preparation, Daniel A. Todd: GCMS data, Waldemar Debinski: has done the biological activity, Ashraf Duzan, Mufeed M. Basti and Travis A. Cesarone: Writing- Reviewing and Editing.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Louis, D.N., Perry, A., Reifenberger, G., *et al.* (2016) The 2016 World Health Organization Classification of Tumors of the Central Nervous System: A Summary. *Acta Neuropathologica*, **131**, 803-820. https://doi.org/10.1007/s00401-016-1545-1

[2] Louis, D.N., Perry, A., Wesseling, P., *et al.* (2021) The 2021 WHO Classification of Tumors of the Central Nervous System: A Summary. *Neuro-Oncology*, **23**, 1231-1251. https://doi.org/10.1093/neuonc/noab106

[3] Chesler, D.A., Berger, M.S. and Quinones-Hinojosa, A. (2012) The Potential Origin of Glioblastoma Initiating Cells. *Frontiers in Bioscience-Scholar*, **4**, 190-205. https://doi.org/10.2741/s261

[4] Nelander, S. (2018) Increasing the Accuracy of Glioblastoma Subtypes: Factoring in the Tumor's Cell of Origin. *Molecular & Cellular Oncology*, **6**, Article ID: 1302907. https://doi.org/10.1080/23723556.2017.1302907

[5] Fernandes, C., Costa, A., Osório, L., *et al.* (2017) Current Standards of Care in Glioblastoma Therapy. In: De Vleeschouwer, S., Ed., Glioblastoma, Codon Publications, Brisbane, 197-241. http://dx.doi.org/10.15586/codon.glioblastoma.2017.ch11

[6] Dressler, E.V., Liu, M., Garcia, C.R., *et al.* (2019) Patterns and Disparities of Care in Glioblastoma. *Neuro-Oncology Practice*, **6**, 37-46. https://doi.org/10.1093/nop/npy014

[7] Butler, M., Pongor, L., Su, Y.T., *et al.* (2020) MGMT Status as a Clinical Biomarker in Glioblastoma. *Trends in Cancer*, **6**, 380-391. https://doi.org/10.1016/j.trecan.2020.02.010

[8] Liu, D., Yang, T., Ma, W. and Wang, Y. (2022) Clinical Strategies to Manage Adult Glioblastoma Patients without MGMT Hypermethylation. *Journal of Cancer*, **13**, 354-363. https://doi.org/10.7150/jca.63595

[9] Georgescu, M.M. (2021) Multi-Platform Classification of IDH-Wild-Type Glioblastoma Based on ERK/MAPK Pathway: Diagnostic, Prognostic and Therapeutic Implications. *Cancers*, **13**, Article 4532. https://doi.org/10.3390/cancers13184532

[10] Peeri, H., Shalev, N., Vinayaka, A.C., *et al.* (2021) Specific Compositions of *Cannabis sativa* Compounds Have Cytotoxic Activity and Inhibit Motility and Colony Formation of Human Glioblastoma Cells *in vitro*. *Cancers*, **13**, Article 1720. https://doi.org/10.3390/cancers13071720

[11] McPartland, J.M. and Small, E. (2020) A Classification of Endangered High-THC Cannabis (*Cannabis sativa* subsp. *indica*) Domesticates and Their Wild Relatives. *PhytoKeys*, **144**, 81-112. https://doi.org/10.3897/phytokeys.144.46700

[12] Livingston, S.J., Bae, E.J., Unda, F., *et al.* (2021) Cannabis Glandular Trichome Cell Walls Undergo Remodeling to Store Specialized Metabolites. *Plant and Cell Physiology*, **62**, 1944-1962. https://doi.org/10.1093/pcp/pcab127

[13] Livingston, S.J., Rensing, K.H., Page, J.E. and Samuels, A.L. (2022) A Polarized Supercell Produces Specialized Metabolites in Cannabis Trichomes. *Current Biology*, **32**, 4040-4047.E4. https://doi.org/10.1016/j.cub.2022.07.014

[14] Hanuš, L.O. and Hod, Y. (2020) Terpenes/Terpenoids in *Cannabis*: Are They Important? *Medical Cannabis and Cannabinoids*, **3**, 25-60. https://doi.org/10.1159/000509733

[15] Linciano, P., Citti, C., Russo, F., *et al.* (2020) Identification of a New Cannabidiol n-Hexyl Homolog in a Medicinal Cannabis Variety with an Antinociceptive Activity in Mice: Cannabidihexol. *Scientific Reports*, **10**, Article No. 22019. https://doi.org/10.1038/s41598-020-79042-2

[16] Amirav, A., Neumark, B., Margolin Eren, K.J., Fialkov, A.B. and Tal, N. (2021) Cannabis and Its Cannabinoids Analysis by Gas Chromatography-Mass Spectrometry with Cold EI. *Journal of Mass Spectrometry*, **56**, e4726. https://doi.org/10.1002/jms.4726

[17] Mazuz, M., Tiroler, A., Moyal, L., *et al.* (2020) Synergistic Cytotoxic Activity of Cannabinoids from *Cannabis sativa* against Cutaneous T-cell Lymphoma (CTCL) *in-vitro* and *ex-vivo*. *Oncotarget*, **11**, 1141-1156. https://doi.org/10.18632/oncotarget.27528

[18] Ibsen, M.S., Connor, M. and Glass, M. (2017) Cannabinoid CB_1 and CB_2 Receptor Signaling and Bias. *Cannabis and Cannabinoid Research*, **2**, 48-60. https://doi.org/10.1089/can.2016.0037

[19] Bow, E.W. and Rimoldi, J.M. (2016) The Structure-Function Relationships of Classical Cannabinoids: CB1/CB2 Modulation. *Perspectives in Medicinal Chemistry*, **8**, 17-39. https://doi.org/10.4137/PMC.S32171

[20] Chester, L.A., Englund, A., Chesney, E., *et al.* (2022) Effects of Cannabidiol and Delta-9-Tetrahydrocannabinol on Plasma Endocannabinoid Levels in Healthy Volunteers: A Randomized Double-Blind Four-Arm Crossover Stud. *Cannabis and Cannabinoid Research*. https://doi.org/10.1089/can.2022.0174

[21] Glass, M. and Felder, C.C. (1997) Concurrent Stimulation of Cannabinoid CB1 and Dopamine D2 Receptors Augments cAMP Accumulation in Striatal Neurons: Evidence for a G_s Linkage to the CB1 Receptor. *Journal of Neuroscience*, **17**, 5327-5333. https://doi.org/10.1523/JNEUROSCI.17-14-05327.1997

[22] Hudson, R., Renard, J., Norris, C., Rushlow, W.J. and Laviolette, S.R. (2019) Cannabidiol Counteracts the Psychotropic Side-Effects of Δ-9-Tetrahydrocannabinol in the Ventral Hippocampus through Bidirectional Control of ERK1-2 Phosphorylation. *Journal of Neuroscience*, **39**, 8762-8777. https://doi.org/10.1523/JNEUROSCI.0708-19.2019

[23] Wartchow, K.M., Schmid, B., Tripal, P., *et al.* (2021) Treatment with Cyclic AMP Activators Reduces Glioblastoma Growth and Invasion as Assessed by Two-Photon Microscopy. *Cells*, **10**, Article 556. https://doi.org/10.3390/cells10030556

[24] De Petrocellis, L., Ligresti, A., Moriello, A.S., *et al.* (2011) Effects of Cannabinoids and Cannabinoid-Enriched Cannabis Extracts on TRP Channels and Endocannabinoid Metabolic Enzymes. *British Journal of Pharmacology*, **163**, 1479-1494. https://doi.org/10.1111/j.1476-5381.2010.01166.x

[25] Lauckner, J.E., Jensen, J.B., Chen, H.Y., Lu, H.C., Hille, B. and Mackie, K. (2008) GPR55 Is a Cannabinoid Receptor That Increases Intracellular Calcium and Inhibits M Current. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 2699-2704. https://doi.org/10.1073/pnas.0711278105

[26] Lah, T.T., Majc, B., Novak, M., *et al.* (2022) The Cytotoxic Effects of Cannabidiol and Cannabigerol on Glioblastoma Stem Cells May Mostly Involve GPR55 and TRPV1 Signalling. *Cancers,* **14**, Article 5918. https://doi.org/10.3390/cancers14235918

[27] Takeda, S., Misawa, K., Yamamoto, I. and Watanabe, K. (2008) Cannabidiolic Acid as a Selective Cyclooxygenase-2 Inhibitory Component in Cannabis. *Drug Metabolism & Disposition*, **36**, 1917-1921. https://doi.org/10.1124/dmd.108.020909

[28] Ruhaak, L.R., Felth, J., Karlsson, P.C., Rafter, J.J., Verpoorte, R. and Bohlin, L. (2011) Evaluation of the Cyclooxygenase Inhibiting Effects of Six Major Cannabinoids Isolated from *Cannabis sativa*. *Biological and Pharmaceutical Bulletin*, **34**, 774-778. https://doi.org/10.1248/bpb.34.774

[29] Jiang, J., Qiu, J., Li, Q. and Shi, Z. (2017) Prostaglandin E2 Signaling: Alternative Target for Glioblastoma? *Trends in Cancer*, **3**, 75-78. https://doi.org/10.1016/j.trecan.2016.12.002

[30] Devane, W.A., Dysarz III, F.A., Johnson, M.R., Melvin, L.S. and Howlett, A.C. (1988) Determination and Characterization of a Cannabinoid Receptor in Rat Brain. *Molecular Pharmacology*, **34**, 605-613.

[31] Munro, S., Thomas, K.L. and Abu-Shaar, M. (1993) Molecular Characterization of a Peripheral Receptor for Cannabinoids. *Nature*, **365**, 61-65. https://doi.org/10.1038/365061a0

[32] Casasampere, M., Ordoñez, Y.F., Pou, A. and Casas, J. (2016) Inhibitors of Dihydroceramide Desaturase 1: Therapeutic Agents and Pharmacological Tools to Decipher the Role of Dihydroceramides in Cell Biology. *Chemistry and Physics of Lipids*, **197**, 33-44. https://doi.org/10.1016/j.chemphyslip.2015.07.025

[33] Eymery, M.C., McCarthy, A.A. and Hausmann, J. (2023) Linking Medicinal Canna-

bis to Autotaxin—Lysophosphatidic Acid Signaling. *Life Science Alliance*, **6**, e202201595. https://doi.org/10.26508/lsa.202201595

[34] Navarro, G., Reyes-Resina, I., Rivas-Santisteban, R., *et al.* (2018) Cannabidiol Skews Biased Agonism at Cannabinoid CB_1 and CB_2 Receptors with Smaller Effect in CB_1-CB_2 Heteroreceptor Complexes. *Biochemical Pharmacology*, **157**, 148-158. https://doi.org/10.1016/j.bcp.2018.08.046

[35] Vrechi, T.A.M., Leão, A.H.F.F., Morais, I.B.M., *et al.* (2021) Cannabidiol Induces Autophagy via ERK1/2 Activation in Neural Cells. *Scientific Reports*, **11**, Article No. 5434. https://doi.org/10.1038/s41598-021-84879-2

[36] Nachnani, R., Raup-Konsavage, W.M. and Vrana, K.E. (2021) The Pharmacological Case for Cannabigerol. *Journal of Pharmacology and Experimental Therapeutics*, **376**, 204-212. https://doi.org/10.1124/jpet.120.000340

[37] Gertsch, J., Leonti, M., Raduner, S., *et al.* (2008) *β*-Caryophyllene Is a Dietary cAnnabinoid. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 9099-9104. https://doi.org/10.1073/pnas.0803601105

[38] Udoh, M., Santiago, M., Devenish, S., McGregor, I.S. and Connor, M. (2019) Cannabichromene Is a Cannabinoid CB_2 Receptor Agonist. *British Journal of Pharmacology*, **176**, 4537-4547. https://doi.org/10.1111/bph.14815

[39] Núñez, E., Benito, C., Pazos, M.R., *et al.* (2004) Cannabinoid CB_2 Receptors Are Expressed by Perivascular Microglial Cells in the Human Brain: An Immunohistochemical Study. *Synapse*, **53**, 208-213. https://doi.org/10.1002/syn.20050

[40] Maresz, K., Carrier, E.J., Ponomarev, E.D., Hillard, C.J. and Dittel, B.N. (2005) Modulation of the Cannabinoid CB_2 Receptor in Microglial Cells in Response to Inflammatory Stimuli. *Journal of Neurochemistry*, **95**, 437-445. https://doi.org/10.1111/j.1471-4159.2005.03380.x

[41] Stella, N. (2013) Chronic THC Intake Modifies Fundamental Cerebellar Functions. *Journal of Clinical Investigation*, **123**, 3208-3210. https://doi.org/10.1172/JCI70226

[42] Morris, G., Sominsky, L., Walder, K.R., *et al.* (2022) Inflammation and Nitro-Oxidative Stress as Drivers of Endocannabinoid System Aberrations in Mood Disorders and Schizophrenia. *Molecular Neurobiology*, **59**, 3485-3503. https://doi.org/10.1007/s12035-022-02800-y

[43] Jang, Y.W., Kim, M. and Hwang, S.W. (2020) Molecular Mechanisms Underlying the Actions of Arachidonic Acid-Derived Prostaglandins on Peripheral Nociception. *Journal of Neuroinflammation*, **17**, Article No. 30. https://doi.org/10.1186/s12974-020-1703-1

[44] Raïch, I., Rivas-Santisteban, R., Lillo, A., *et al.* (2021) Similarities and Differences upon Binding of Naturally Occurring Δ^9-Tetrahydrocannabinol-Derivatives to Cannabinoid CB_1 and CB_2 Receptors. *Pharmacological Research*, **174**, Article ID: 105970. https://doi.org/10.1016/j.phrs.2021.105970

[45] Stella, N., Schweitzer, P. and Piomelli, D. (1997) A Second Endogenous Cannabinoid That Modulates Long-Term Potentiation. *Nature*, **388**, 773-778. https://doi.org/10.1038/42015

[46] Lah, T.T., Novak, M., Pena Almidon, M.A., *et al.* (2021) Cannabigerol Is a Potential Therapeutic Agent in a Novel Combined Therapy for Glioblastoma. *Cells*, **10**, Article 340. https://doi.org/10.3390/cells10020340

[47] Guzmán, M., Duarte, M.J., Blázquez, C., *et al.* (2006) A Pilot Clinical Study of Δ^9-Tetrahydrocannabinol in Patients with Recurrent Glioblastoma Multiforme. *British Journal of Cancer*, **95**, 197-203. https://doi.org/10.1038/sj.bjc.6603236