Vol. 15 (núm 1) pp. 178 - 193
Enero - Junio 2024
https://doi.org/10.19053/01217488.v15.n1.2024.15214

Antioxidant activity of non-psychoactive cannabis varieties from North Cauca, Colombia

Actividad antioxidante de variedades de cannabis del Norte del Cauca, Colombia

Paola Andrea Arango Romero[1*], Jhan Carlos Delgado[2], Sandra Marcela Muñoz[2], Luis Llanos[2], Ricardo Benítez[3]

Abstract

The present study aims to publicize the chemical composition and possible medicinal potential of varieties of non-psychoactive cannabis grown in the department of Cauca. Cannabinoids were identified and quantified by high-performance liquid chromatography coupled to an ultraviolet detector (HPLC/UV) for the analysis of the flower, and gas chromatography coupled to a mass spectrometer (GC-MS) for the analysis of the ethanolic extracts and content terpenes. Phenols were quantified by reaction with the Folin & Ciocalteau reagent; for the determination of flavonoids and anthraquinones, the extracts were treated with AlCl3. Finally, to determine the antioxidant activity, three methods were used: DPPH, ABTS and FRAP. It was possible to determine that varieties A and B contained percentages of total tetrahydrocannabinol (THC) less than 1 % and percentages of total cannabidiol (CBD) between 9-15 %. In the ethanolic extracts concentrations (w/w) of CBD were reached in varieties A and B, of 10 % and 13.7 %, respectively. Nine terpenes from sample A and seven from sample B were identified and quantified, with $\beta-$caryophyllene being the most abundant in both. Considering that there is evidence in the literature that the CBD/THC ratio influences biological activity, ethanolic extracts of varieties A and B are expected to have moderate to low antioxidant activity, which, according to some researchers, it may be associated with the neuroprotective effect, which may be favored by the presence of $\beta-$caryophyllene.

Palabras Clave: Antioxidant activity, Cannabinoid, Cannabis, Terpenoid

Recepción: 15-Noviembre-2022
Aceptación: 09-Mayo-2023

[1]PhD. in Chemical Sciences, M.Sc. in Environmental Sciences, Professor of the Department of Chemistry, University of Cauca, Popayán, Colombia. Author: parango@unicauca.edu.co
[2]Master's student, University of Cauca, Popayán, Colombia.
[3]PhD. in Enzymiology, Professor of the Department of Chemistry, University of Cauca, Popayán, Colombia.

1. Introduction

The three species of plants of the Cannabis genus - C. ruderalis janisch (exista), C. sativa L., and C. indica Lam belong to the Cannabaceae family, although their taxonomic classification has suffered from some controversy as the plant is polymorphic [1]. Through the centuries, Cannabis has been used to obtain fiber, food, oils and medicines, in addition to its use for recreational and religious purposes [2–4]. Publications on cannabis have recently been on the rise [5] given that multiple therapeutic effects have been attributed to the cannabinoids present in the plant in their acid and neutral form (See Figure 1), cannabinoids with psychoactive activity such as tetrahydrocannabinol (THC) and non-psychoactive such as cannabidiol (CBD) [6, 7]. Therapeutic effects are also attributed to the entourage effect, *i.e.* the synergistic effect between components [8, 9].

The chemical composition of Cannabis is complex since the plant generates more than 545 phytomolecu-les. These mainly comprise cannabinoids and terpenoids, but flavonoids, phenolic compounds, sterols and carotenoids are also present [10, 11]. The canna-binoids and terpenoids are concentrated in the female inflorescences [2,12] and are the most interesting pharmacologically, having been used to treat a range of pathological and physiological conditions [8,13,14]. Cannabinoids and monoterpenes have in common geranyl diphosphate (GPP), a 10-carbon isoprenoid precursor, while sesquiterpenes are produced from the 15-carbon farnesyl diphosphate (FPP) [15]. In total, more than 150 terpenes and approximately 100 different cannabinoids have been identified [15].

Terpenoids are volatile hydrocarbons (monoterpenes and sesquiterpenes), the monoterpene myrcene and the sesquiterpenes β-caryophyllene and α-humulene are found in most strains of cannabis. The monoterpenes α-pinene, limonene and linalool and the sesqui-terpenes β-bisabolol and (E)-β-farnesene can also be found (See Figure 2) [15].

Cannabis sativa has a wide range of biological properties including neuroprotective, antioxidant and anti-inflammatory activity, which is why it has been the focus of attention in the pharmaceutical industry, treating neurodegenerative disorders such as Alzheimer's disease [16], multiple sclerosis, Huntington's disease, Parkinson's disease, chronic pain management in cancer patients [17], among others [18]. Many authors affirm that the biological properties of *Cannabis sativa* L. are due to a synergistic effect between its components [19]. It has thus been found that both cannabinoids and some terpenoids (β-caryophyllene) possess important biological properties [18]. It should be noted that the biological activity depends on the profile of secondary metabolites [15], and specifically in *cannabis* extracts. The composition is affected not only by the species, variety and strain, but also by the conditions of cultivation, age of the plant, type of sampling, sample treatment, and the method and conditions of extraction [20].

The study of *cannabis* in different disciplines is booming. Legal restrictions have not until now permitted a sufficient scientific basis on which to verify the medicinal properties of the plant and the adverse effects of its products [21]. Colombia became the fourth country to bring forward legislation on the use of *cannabis* for therapeutic and palliative purposes, after Chile, Puerto Rico and Uruguay. Through Decree 2467 of December 22, 2015, the Ministry of Health and Social Protection approved the legalization of *cannabis*. Currently, modifications are expected to the current decree, 613 of 2017, according to which *cannabis* is considered non-psychoactive when the percentage of Δ9-THC is less than 1 % in dry weight and psychoactive if it is equal to or greater than 1 % [22]. To date the Ministry of Health has granted 305 licenses for Cannabis research [23], of which just 15 are from the department of Cauca, so there is little information available on the cannabinoid profile [24], Furthermore, no reports were found on the biological activity of *Cannabis sativa* cultivated in Cauca. In this research, the cannabinoids and terpenes present in two varieties of *cannabis* cultivated in northern Cauca were identified and quantified by means of high resolution liquid chromatography coupled to an ultraviolet detector (HPLC-UV) and gas chromatography coupled to mass spectrometry (GC -MS). respectively, and the antioxidant activity was evaluated by DPPH, ABTS and FRAP. Additionally, total phenols, flavonoids and anthroquinones were quantified.

2. Methods

2.1. Reagents

Methanol (instrumental grade, purity $>$ 99.99 %) and ethanol and other reagents used in analytical gas chromatography determinations were provided by Merck. The 1000 μg/mL CBD cannabinoid standard in methanol (Catalog # 34011) was purchased from Restek.

Terpenoid Standard #1 Restek: ((-)-α-Bisabolol (23089-26-1), Camphene (79-92-5), δ-3-Carene (13466-78-9), β-Caryophyllene (87-44-5), Geraniol (106-24-1), (-)- Guaiol (489-86-1), α-Humulene (6753-98-6), p-Isopropyltoluene (p-cymene) (99-87-6), (-)- Isopulegol (89-79-2), d-Limonene (5989-27-5), Linalool (78-70-6), β-Myrcene (123-35-3), Nerolidol (7212-44-4), Ocimene (13877-91-3), α-Pinene (80-56-8), (-)-β-Pinene(18172-67-3), α-Terpinene (99-86-5), γ-Terpinene (99-85-4), Terpinolene (586-62-9)).

2.2. Plant material

The cannabis samples were supplied by Natura Parma Cauca S.A.S Zomac, a company in northern Cauca in possession of a non-psychoactive cannabis license. The plant material (buds) delivered by the producer corresponded to two varieties previously denominated as varieties that differ in their composition of cannabinoids and terpenes. For this article

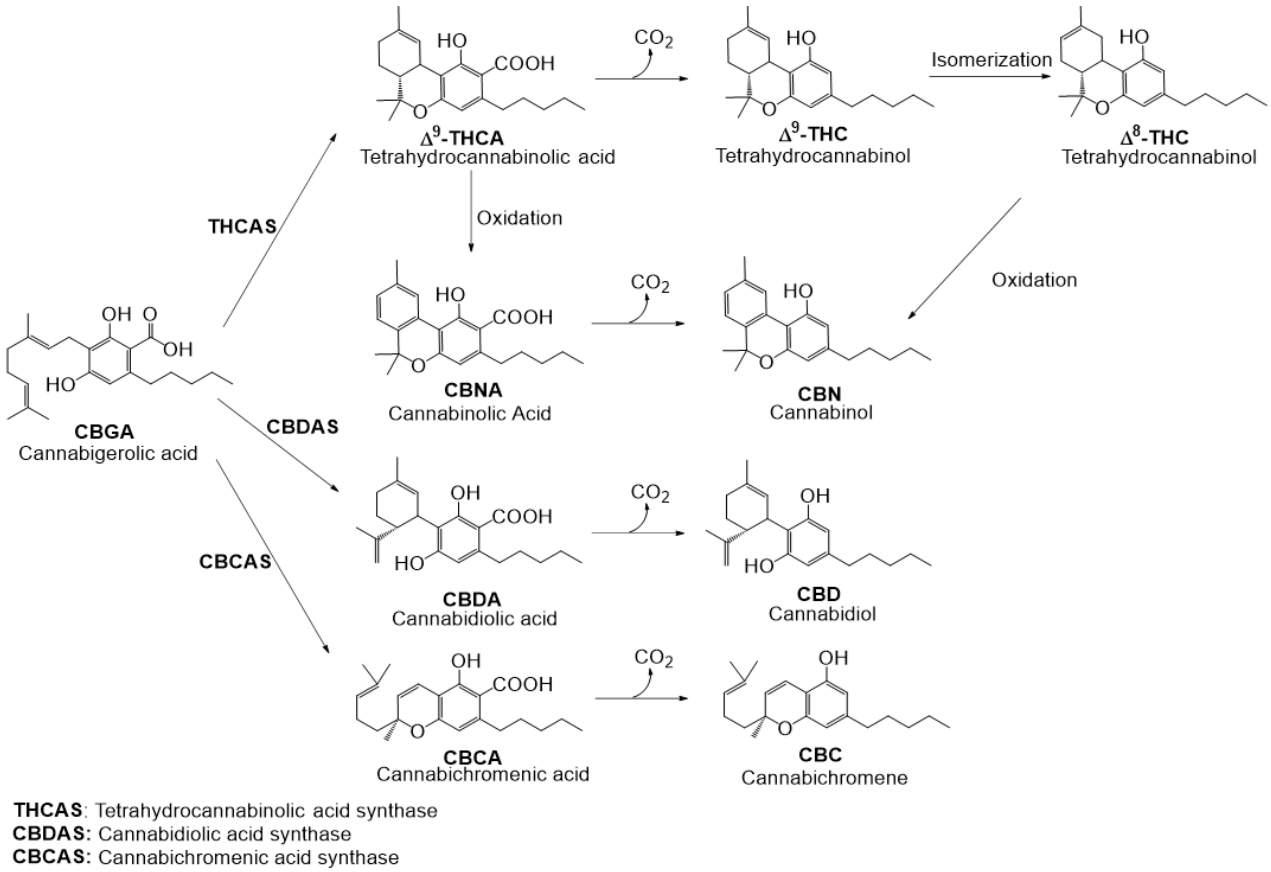


Figura 1: Biosynthesis and decarboxylation of cannabinoids.

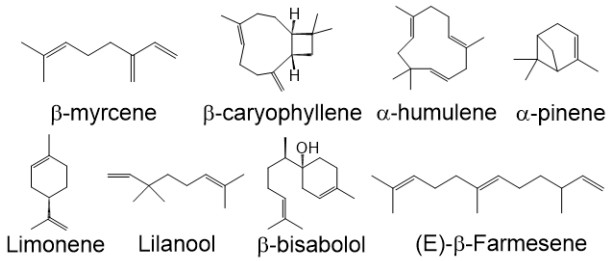


Figura 2: Biosynthesis and decarboxylation of cannabinoids.

the varieties were referred to as samples A and B. Plant material samples A and B were dried and subsequently ground to reduce particle size and improve extraction efficiency. The particle size after grinding and screening was 425 microns. These were then stored in a sealed bag at room temperature until use.

2.3. Extraction of plant material

Extraction was carried out by percolation using a 1:10 ratio, using 1,000 g of Cannabis of each variety (samples A and B), and 10 mL of ethanol.

2.4. Quantification of cannabinoids:

High-performance liquid chromatography coupled to an ultraviolet detector (HPLC-UV).

2.5. Quantification of terpenoids

Injector temperature 280 °C. Split 50 mL/min. Ramp: rises from 80 °C to 250 °C at 20 °C/min for an analysis time of 9 min. Column: TG-5MS (30 m, ID 0.25 um Film: 0.25 um). Transfer line temperature 250 and ion groove at 230 °C. Electronic Impact MS Detector mass range 40-450. 0.6 μL was injected. Quantification was carried out by external standard.

2.6. Quantification of total phenols

The test was developed following the methodology proposed by Waterman & Mole, 1994 (Waterman & Mole, 1994), through the reaction of the Folin & Ciocalteau reagent with organic compounds that have a hydroxylated aromatic ring in an alkaline medium, it is possible to determine the content of polyphenols by spectrophotometry. In the determination, 500 µL of extract (1 mg/mL) were mixed with 2.5 mL of Folin & Ciocalteau reagent (0.2 N) and incubated at room temperature for 5 min. At the end of this time, the solution was mixed with 2 mL of Na2CO3 (7.5 %). The mixture was incubated in the dark at room temperature for 2 hours and evaluated spectrophotometrically at 700 nm. Absorbance data were extrapolated onto a gallic acid calibration curve. The total polyphenol content was expressed in millimoles of gallic acid equivalents (GAE mM).

2.7. Quantification of flavonoids

The flavonoid content was determined by spectrophotometry following the methodology proposed by Arcouet-Grand et al., 1994 (Arcouet-Grand et al., 1994). The samples were treated with AlCl3 for the formation of colored chelates typical of flavonoids when treated with the Lewis acid aluminum trichloride. The analysis consisted of mixing 5 mL of extract (1 mg/mL) with 5 mL of an AlCl3 solution (2 % in ethanol) and incubating for 15 minutes at room temperature. After the time, the absorbance of the samples measured at a wavelength of 415 nm was obtained. Data were extrapolated into a quercetin calibration curve. Total flavonoids were expressed in millimole quercetin equivalents (QE mM).

2.8. Quantification of anthroquinones

The analysis was carried out following the methodology proposed by Mellado et al., 2012 (Mellado et al., 2012). As for the determination of flavonoids, anthraquinones form chelates when they react with AlCl3, but they are detected at a different wavelength. For this, 5 mL of extract (1 mg/mL) was mixed with 5 mL of an AlCl3 solution (2 % in ethanol) and incubated for 15 minutes at room temperature. After the time, the absorbance of each extract was obtained, through spectrophotometry, at a wavelength of 486 nm. The data obtained were extrapolated in a calibration curve of emodin. Total anthraquinones were expressed in millimole of emodin equivalents (mM EE).

2.9. Determination of antioxidant activity

2.9.1. Total Antioxidant Capacity – TRAP (Romay et al., 1996)

This method is based on the ability of some antioxidants to sequester an unpaired electron from the target radical. To do this, the 2,2-azinobis-(3-ethylbenzothiazolin-6-sulfonate) radical cation trapping assay is used, which presents an intense blue-green color, whose absorbance is measured by spectrophotometry (Romay et al., 1996). The presence of antioxidants in the analyzed sample is determined by the decrease in radical coloration, therefore, a decrease in absorbance. The test was developed following the methodology proposed by Romay et al., 1996 (Romay et al., 1996). The technique consisted of preparing a 10 mM solution of AAPH (6 hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid) and mixing it with a 150 μM solution of ABTS (radical cation 2,2 – azinobis – (3 -ethylbenzothiazolin-6-sulfonate)), in a 100 mM phosphate buffered saline (pH 7.4). The mixture was incubated at 45 °C for 30 minutes, resulting in the formation of the ABTS radical. bluish green. Then, 10 μL of extract (1mg/mL) were mixed with 990 μL of ABTS radical solution. and the absorbance was determined in a kinetic of 50 seconds at 734 nm. From the absorbances recorded for each extract, the percentage of radical inhibition (IR) was obtained, which was calculated through the equation:

$$\mathrm{IR}(\%) = \left(\frac{A_0 - A_{50}}{A_0}\right) \times 100 \qquad (1)$$

Where A_0 is the absorbance recorded at time 0 seconds and A_{50} represents the absorbance obtained at 50 seconds.

Once the IR percentages of each extract were obtained, the data were extrapolated into a TROLOX curve (6-hydroxy-2,5,7,8 -tetramethylchroman -2-carboxylic acid), a synthetic antioxidant mimetic of vitamin E and expressed in millimole of antioxidant capacity of TROLOX equivalents (TEAC mM). The TEAC results obtained were compared with two powerful pure antioxidants, gallic acid and BHT (butyl hydroxytoluene).

2.9.2. DPPH radical scavenging activity assay. (Brand-Williams et al., 1995)

This method is used to measure the reducing capacity of some antioxidants against the DPPH radical. (2,2'-dinitrophenyl-1-picrylhydracil), a stable radical in ethanolic solution. The assay was developed following the methodology proposed by Brand-Williams et al., 1995 (Brand-Williams et al., 1995). 100 µL of extract was mixed with 2.9 mL of DPPH solution. 50 µM in ethanol. Then, the samples were vortexed and incubated for 15 minutes at room temperature and subsequently the absorbance at 517 nm was measured. This procedure was performed for each extract at three concentrations, 1, 5 and 10 mg/mL. With the absorbance information at the three concentrations for each extract, the DPPH radical sequestration percentage was calculated. (RSA %) through the equation:

$$\mathrm{RSA}(\%) = \left(\frac{A_{\mathrm{Control}} - A_{\mathrm{sample}}}{A_{\mathrm{Control}}}\right) \times 100 \qquad (2)$$

Where, $A_{Control}$ corresponds to the absorbance of the DPPH solution. without the sample and A_sample is the absorbance of the DPPH solution. with the corresponding extract. From obtaining the RSA % and the concentration of the extracts, the IC50 was obtained, which represents the concentration at which 50 % of the radical is neutralized. Said IC50 were compared with two reference antioxidants, TROLOX and BHT.

2.9.3. Iron reducing antioxidant power – FRAP (Dudonné et al., 2009)

The FRAP assay is a colorimetric method used to determine the reducing power of the ferric ion to the ferrous ion of a given sample. The assay was developed following the methodology proposed by Dudonné et al., 2009 (Dudonné et al., 2009). For this analysis, a FRAP solution was prepared, containing 10 volumes of 300 mM acetate buffer, 1 volume of 20 mM FeCl3 and 1 volume of 10 mM TPTZ (2,4,6-tri(2-pyridyl)-S-triazine). Then, 100 µL of extract (1 mg/mL) was taken and mixed with 300 μL of the FRAP solution, shaking for 15 seconds. Afterwards, the mixture was incubated for 30 minutes at 37 °C in a thermoregulated bath. Finally, the absorbance at 593 nm was measured. Absorbance values were extrapolated onto a TROLOX calibration curve and expressed as TEAC (Mm).

3. Results

3.1. Quantification of cannabinoids

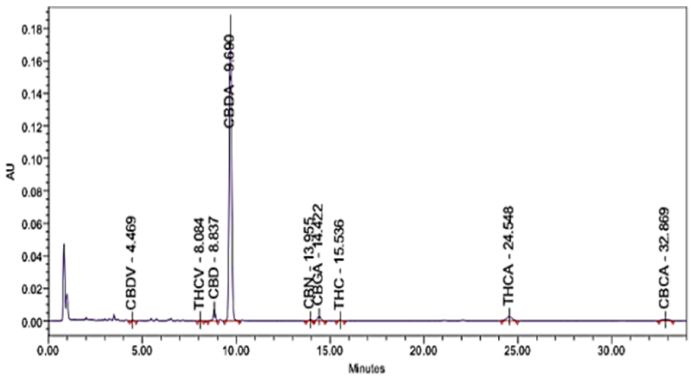
The results of the HPLC-UV analysis to quantify the composition of cannabinoids of samples A and B are shown in Table 1, and Graph 1 and 2.

The results of the GC analysis to quantify the composition of cannabinoids and terpenoids of samples A and B are shown in Tables 2 and 3.

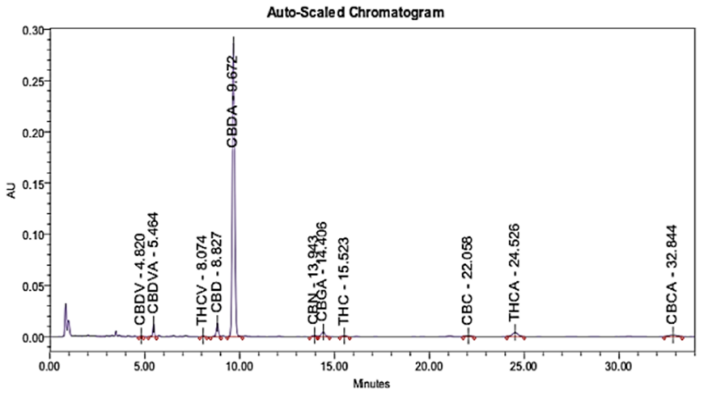
Table 1: Cannabinoid Concentrations in Different Varieties.

Cannabinoid	Variety A		Variety B	
	w/w	mg/g	w/w	mg/g
CBDV	0.11	1.1	0.12	1.2
CBDVA	—	—	0.33	3.3
THCV	0.15	1.5	0.17	1.7
CBD	0.61	6.1	1.16	11.6
CBDA	10.07	100.7	15.37	153.7
CBN	0.03	0.3	0.06	0.6
CBGA	0.13	1.3	0.31	3.1
Δ^9-THC	0.08	0.8	0.15	1.5
CBC	—	—	0.10	1.0
THCA	0.33	3.3	0.56	5.6
CBCA	0.26	2.6	0.71	7.1
Total cannabinoids	11.77	117.7	19.04	190.4

Total THC = % THC + % THCA * 0.877
Total CBD = % CBD + % CBDA * 0.877



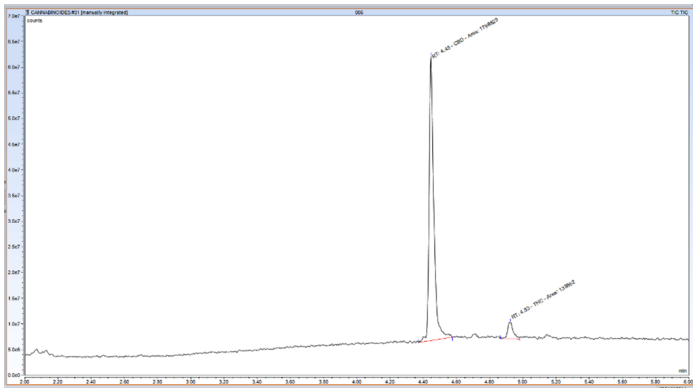
Graph 1: HPLC results for cannabinoids, variety A.



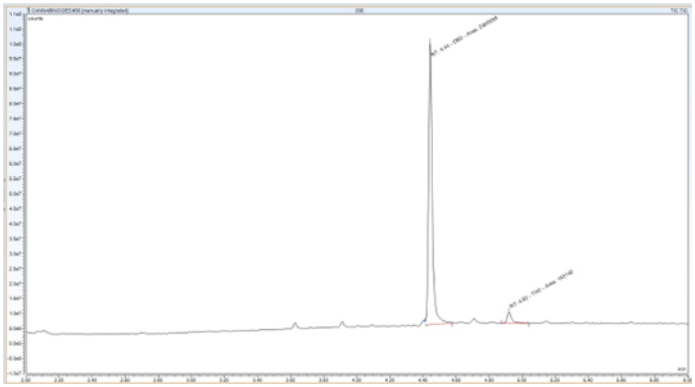
Graph 2: HPLC results for cannabinoids, variety B

Table 2: Quantification of Cannabinoid CBD

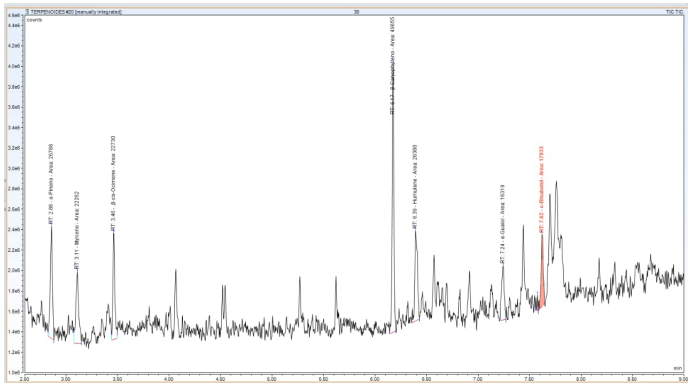
Sample ID	Concentration of CBD % (w/w)	Ratio of CBD/THC
A	10.0	13.4
B	13.7	17.2



Graph 3: Cannabinoid chromatogram, sample A



Graph 4: Cannabinoid chromatogram, sample B.



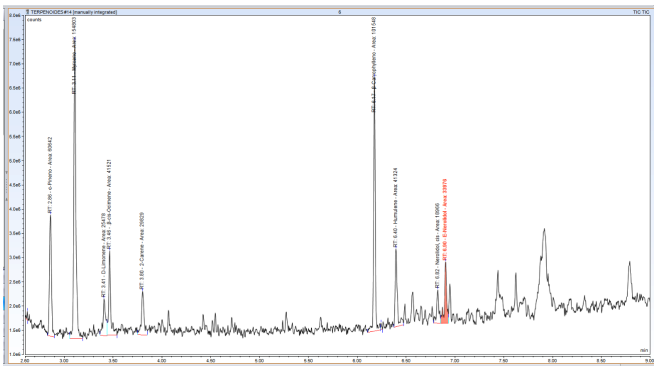
Graph 6: Terpene chromatogram, sample B.

Quantification of terpenes sample A and B

Table 3: Quantification of Terpenes sample A and B.

Terpenes	A % (w/w)	B % (w/w)
α-pinene	0.21	0.11
β-myrcene	0.28	0.07
D-limonene	0.13	/
β-ocimene	0.11	0.07
2-carene	0.08	/
β-cariophyllene	0.31	0.17
Humelene	0.15	0.11
Cis-nerolidol	0.16	/
Trans-nerolidol	0.18	/
α-guaiol	/	0.08
α-bisabolol	/	0.07
Total terpenes	**1.61**	**0.68**

Graphs 5 and 6 show the chromatograms obtained for analyzed samples A and B.



Graph 5: Terpene chromatogram, sample A.

Table 4: Content of total phenols, flavonoids and anthraquinones.

Extract	Total phenols (mg GAE/L) ± DE	Total flavonoids (QE mg/L) ± DE	Total anthraquinones (EE mg/L) ± DE
A	25.432 ± 1.327	2.347 ± 0.329	4.005 ± 0.142
B	29.375 ± 1.258	3.169 ± 0.359	4.175 ± 0.066

Table 5: Quantification of ABTS, DPPH, and FRAP in Various Extracts

Extract	ABTS (TEAC mM) ± DE	DPPH (IC50 mg/mL) ± DE	FRAP (TEAC µM) ± DE
A	0.263 ± 0.034	56.556 ± 0.282	0.43 ± 0.03
B	0.345 ± 0.066	53.509 ± 4.294	0.49 ± 0.02
Gallic acid	1.139 ± 0.014	N.A	1729 ± 0.026
BHT	1.063 ± 0.028	0.061 ± 0.000	1525 ± 0.077
TROLOX	N.A	0.107 ± 0.006	N.A

4. Discussion

4.1. Chemical composition of the Cannabis varieties cultivated in the department of Cauca

The two selected varieties grown in the department of Cauca, samples A and B, vary in their cannabinoid and terpenoid composition, depending on the influence of seed-associated factors such as species, quality, growth, geographical and climatic conditions. Harvest conditions, storage of plant material and extraction processes also ought to be taken into account.

A number of different studies over the last few years report results using a range of chromatographic methods including thin layer chromatography (TLC), gas chromatography (GC) and high performance liquid chromatography (HPLC), studies carried out by means of different extraction methods and in different plant materials and biological matrices [25–32]. This study presents the results of the analysis of the flower and the ethanolic extract of varieties A and B. The flower of each of the A and B samples was analyzed by HPLC-UV. Variety A was found to contain 0.37 % total THC and 9.44 % total CBD. Neutral and acidic cannabinoids were found in smaller quantities - cannabidivarin (CBDV), tetrahydrocannabivarin (THCV), cannabinol (CBN), cannabigerolic acid

(CBGA), and cannabichromenic acid (CBCA). Variety B contained a total THC of 0.64 % and total CBD of 14.64 %. In addition to those cannabinoids found in variety A, cannabichromene (CBC) and cannabidivarinic acid (CBDVA) were found in lower proportions, with values of less than 1 %. The results can be seen in Tables 1 and 2, corresponding to varieties A and B, respectively. The chromatograms are presented in Graphs 1 and 2.

The ethanolic extracts of varieties A and B were prepared by percolation, analyzed by mass coupled gas chromatography and the amounts of cannabinoids and terpenoids present in the samples were obtained.

Table 2 records the results obtained in varieties A and B. Respective concentrations (w/w) of CBD of 10 % and 13.7 % were obtained. The CBD/THC ratios were likewise recorded, with values of 13.4 and 17.2 for sample A and B. Graphs 3 and 4 show the peaks corresponding to THC and CBD for each of the varieties.

It was possible to identify and quantify the percentage (% w/w) of terpenes for samples A and B, which are recorded in Table 3. Sample A registered the presence of nine terpenes, among which α-pinene, β-myrcene, β-caryophyllene stood out for their higher quantities. In sample B, seven terpenes were identified with concentrations lower than those registered for sample A. It should be noted that in sample B the terpenes α-guaiol and α-bisabolol were present, terpenes that were not found in sample A, the graphs 5 and 6 show the peaks corresponding.

The ethanolic extracts of varieties A and B were prepared by percolation, analyzed by mass coupled gas chromatography and the amounts of cannabinoids and terpenoids present in the samples were obtained.

Table 2 records the results obtained in varieties A and B. Respective concentrations (w/w) of CBD of 10 % and 13.7 % were obtained. The CBD/THC ratios were likewise recorded, with values of 13.4 and 17.2 for sample A and B. Graphs 3 and 4 show the peaks corresponding to THC and CBD for each of the varieties.

It was possible to identify and quantify the percentage (% w/w) of terpenes for samples A and B, which are recorded in Table 3. Sample A registered the presence of nine terpenes, among which α-pinene, β-myrcene, β-caryophyllene stood out for their higher quantities. In sample B, seven terpenes were identified with concentrations lower than those registered for sample A. It should be noted that in sample B the terpenes α-guaiol and α-bisabolol were present, terpenes that were not found in sample A, the graphs 5 and 6 show the peaks corresponding.

4.2. Content of flavonoids, phenols and anthraquinones

Flavonoids are secondary metabolites that in Cannabis vary depending on the species, in addition, the highest content is found in the leaves compared to the inflorescences. We found a content of 2.35x10-2 mg QE /g and 3.17x10-2 mg QE/g in extracts A and B, respectively (0.002 and 0.003 % w/w). Jin D et. al (2020) report flavonoid content between 0.07–0.14 % in inflorescences and 0.34–0.44 % in Cannabis leaves [33, 34], showing, first, that inflorescences the content is lower than in leaves, and second, that in our ethanolic extracts the content was lower in both extracts compared to what was found with Jin D. et al. On the other hand, in methanolic extracts of female inflorescences of Cannabis sativa by an HPLC/MS method, Nagy et. al (2018) report flavonoid contents of 7.79 mg/g [35], higher than what was found in our work. However,it is noteworthy that the polarity of the solvent influences the extraction of these metabolites [36]. According to the literature, the flavonoids found only in the Cannabis genus are the Cannflavins [37]. Pellatti et. al (2018) quantified Cannflavin A and B in 4 samples of hemp inflorescences and found percentages that add up to between 0.01 and 0.07 %, showing that they are found in low proportions in this part of the plant [38], although not we quantify Cannflavin A and B, its determination is important in future research since they are unique in Cannabis.

Analyzing the results of total phenols, we found contents of 0.25 and 0.29 mg GAE/g for extracts A and B, respectively (0.025 and 0.029 %), lower than that reported by Izzo et. al (2020) for methanolic extracts of inflorescences of Cannabis sativa L in a range of 10.5 mg GAE/g and 52.6 mg GAE/g [39], on the other hand, Ferrante et al (2019), made extracts in water of flowers of hemp and report total phenol content between 4.7 and 8.1 mg GAE/g [40]. These differences may be due to the polarity of the extraction solvent [36]. Thus, taking into account the research of Drinić Z et. al (2018), who made ethanol/water extracts in different proportions of hemp aerial parts, found that the best extraction solvent for both flavonoids and total phenols is 50 % ethanol, with an extraction yield of 14.52 % , compared to 8.1 6 % when they used 90 % ethanol (in this work we used 99.8 % ethanol), so it may be one of the reasons why extracts A and B had a low content of phenols (See table 6).

In Cannabis, these total phenols correspond to both flavonoid and non-flavonoid type compounds [41], however, we also have cannabinoids that are metabolites of the terpenophenolic type [42], therefore, analyzing the structure of the cannabinoids found in higher concentration in extracts A and B, which are CBD and CBDA (see such table), we note that the molecular aromatic ring can be classified as phenol according to what was stated in Alexa's thesis (2020) [43], and due to Since these cannabinoids are concentrated in the female inflorescences, it would explain why much more total phenols were obtained than total flavonoids in ethanolic

Table 6: Modified from Drinić Z et. al 2018

Extract	Extraction Solvent	Total flavonoids	Total phenols	Reference
Aerial parts	8.16 Ethanol 90%	3.18	5.85	[36]
Aerial parts	8.16 Ethanol 50%	5.21	9.25	[36]

extracts A and B, in addition to the fact that ethanol allows these metabolites to be solubilized due to its adequate polarity. Added to this, according to the literature review reported by Izzo et. al (2020), we can make a comparison of the total phenols of leaves, seeds, oil, flour and aerial parts, it is from the inflorescences that more total phenols are extracted, so it is consistent with the phenol and flavonoid content that we obtained in our results, since the latter are found more in the leaves of the plant.

On the other hand, anthraquinones are secondary metabolites belonging to polyketides, and their importance derives from their antibacterial, antifungal, antiviral, insecticide, laxative, and anticancer potential, among others [44]. This group of pigments have been isolated from plants, fungi and lichens [45], in Cannabis inflorescences we did not find reports, however, if we compare with flower extracts from other plants, we find, for example, what was reported by Montenegro et. al (2020) for ethanolic extract of L. rivularis flowers, they report total anthraquinone content of 75.2023 EE mg/L [46], much higher than our findings for Cannabis extracts.

4.3. Antioxidant activity

Extracts A and B presented a higher content of CBDA followed by CBD. According to Dawidowicz et. al (2021), taking into account their structure and the antioxidant activity of these individual cannabinoids by the three methods studied, the neutral form (CBD) would provide greater activity followed by the acid or carboxylated form.

4.4. Potential biological activity of cannabinoids and terpenoids present in Cannabis samples A and B

Many recent studies on Cannabis have focused on the endocannabinoid system (ECS). This system, comprising receptors, endogenous ligands (endocannabinoids) and metabolizing enzymes, became the platform for understanding the action of phytocannabinoids and synthetic cannabinoids, seeking to determine the activity and potential of different varieties of *Cannabis* with variable cannabinoid and terpenoid profiles and to find out the effect of these in different physiological and pathological processes [32, 50–54].

The varieties A and B of non-psychoactive *Cannabis* samples grown in northern Cauca were found to have CBD/THC ratio values that ought to be considered important in the projection of phytotherapeutic products. Different studies carried out by research groups and pharmaceutical laboratories in the world project the scope of varieties of *Cannabis* with high potency in CBD and low THC.

A number of authors have evaluated the efficacy of cannabinoid-based therapies with the presence of CBD or THC to reduce pain, focusing on the management of chronic and neuropathic pain associated with cancer patients [17, 50, 55–62]. Other authors refer to the anticancer effects not only of cannabinoids, but also of terpenes and flavonoids present in Cannabis [63]. Cannabidiol – CBD - as a non-psychoactive compound has gained importance owing to its preclinically established anticancer properties and a favorable risk-benefit profile, as manifested by Hinz et al. In 2019 [64]. De Gregorio et al. in 2019 demonstrated that increasing intravenous doses of CBD induces analgesia through the activation of TRPV1 receptors, reduces anxiety through the activation of the 5-HT1A receptor, and recovers the neurotransmission of altered 5-HT in neuropathic pain conditions [65]. Kisková et al., 2019 report the use of cannabinoids in patients with breast cancer in advanced stages, but also refer to their use in early stages to slow down tumor progression [66].

A few years ago, more specific studies were carried out, such as that presented by Takeda et al. in 2012, which highlighted the biological activity of CBD, the main component in fiber-type Cannabis plants [67]. The study, based on cannabidiolic acid (CBDA), reported the inhibition of the migration of highly invasive human breast MDA-MB-231 cancer cells through a mechanism that involves the inhibition of cAMP-dependent protein kinase A, together with an activation of RhoA GTPase. Cannabinoids can be an effective adjunct in the treatment of pancreatic cancer, although more information is required regarding the dosage and mode of action [68]. Singh and Bali in 2019 presented the case of a patient with Cannabis extract treatment suffering from terminal acute lymphoblastic leukemia with a mutation of the Philadelphia chromosome [69]. They stated that Cannabis resin extract was used as an effective treatment, dependent on dosage. More recent studies focused on the effect of Cannabis in cancer patients, such as those reported by other researchers [7, 70–72]. Mazza, in 2021 concluded that medicinal Cannabis may offer a treatment option for patients with fibromyalgia syndrome but may be limited by non-serious adverse events [7].

Berman et al. in 2020 sought to understand the effect of Cannabis treatment on the endocannabinoid metabolome (ECM). The results showed that variations in the low contents of phytocannabinoids of the different extracts can produce varied effects on the concentrations of endocannabinoids and

Table 7: Bibliographic reports of antioxidant activity

Extract	DPPH	ABTS	FRAP	Ref.
Inflorescences of C. sativa by ultrasound	45.04 ± 1.23 (mg TEAC/g)	381.26 ± 9.05 (mg TEAC/g)	77.22 ± 0.92 (mg TEAC/g)	(49)
Inflorescences of C. sativa by maceration	32.43 ± 0.32 (mg TEAC/g)	502.16 ± 5.62 (mg TEAC/g)	83.14 ± 1.63 (mg TEAC/g)	(49)
C. sativa aerial part extract	IC50 0.7563 (mg/mL)			(36)
C. sativa extract by supercritical fluids	IC50 0.1419-0.6401 (mg/mL)			(19)
Inflorescences of C. sativa by percolation	A: IC50 56.556 mg TEAC/mL B: IC50 53.509 mg TEAC/mL	A: 0.263 (TEAC mM) B: 0.345 (TEAC mM)	A: 0.43 (TEAC µM) B: 0.49 (TEAC µM)	This work

the profile of metabolites in the peripheral and central system [29]. Studies are related in which doses were administered ranging between 0 and 40 mg/day of CBD, or combining varieties of THC and CBD, in which CBD ranges between 2.5 to 10 mg [54, 73–75]. These investigations make it possible to validate the application of varieties A and B in future studies aimed at the manufacture of phytotherapeutics.

Cannabis has also been used for the treatment of neurodegenerative diseases such as multiple sclerosis, Parkinson's and Alzheimer's, where cannabinoids act as neuroprotectors, possibly through antioxidant mechanisms [16, 76]; In addition to the neuroprotective effect of the terpenoid β-caryophyllene [77].

For example, Raja et al., 2020 studied the attenuation of oxidative stress induced by H2O2 and also with Aβ1-42 and Cu (II), similar to multiple sclerosis, by cannabinoids and Cannabis extracts in differentiated SY-SH5Y neuronal cells [78].

The authors reported that 98 % THC has an IC50 of 0.44 μg/mL, much better than CBD with an IC50 of 42.71 μg/mL and comparable to ascorbic acid with an IC50 of 0.25 μg/mL. In the extracts with different THC/CBD ratios. They also found that two of the extracts, with THC contents of 71.08 % and 72.88 % and non-detectable CBD, reduced ROS by more than 70 %, while the extracts with low content of THC (11.54 % and 3.9 %) and high CBD content (64.34 % and 50.34 %), with ratios of 5.58 CBD/1 THC and 12.91 CBD/1 THC respectively, reduced ROS by more than 60 %, leading the authors to affirm that other metabolites can contribute to the antioxidant effect. To verify if the CBD/THC ratio influences antioxidant activity, the authors varied this ratio and found that the mixture with a low amount of THC and high CBD (90 CBD/10 THC), was the least effective with an IC50 of 54 μg/mL. However, when the amount of THC increased, the IC50 improved. Thus, with a ratio of 75 CBD/25 THC they obtained an IC50 of 14 μg/mL, 50 CBD/50 THC (0.54 μg/mL) and the most effective was 25 CBD/75 THC with an IC50 of 0.54 μg/mL. It should be noted that, when there was a ratio of 10 CBD/90 THC, the antioxidant activity decreased and the IC50 increased again to 2.54 μg/mL. The authors concluded that the CBD/THC ratio is key to obtaining biological activity by this method in vitro. In addition, it is possible that other metabolites such as terpenoids influence the antioxidant activity of the extract. Furthermore, there is no antagonistic effect of CBD versus THC.

Antiexcytotoxic activity is key in neuroprotective processes. Khaksar & Bigdeli (2017) therefore studied the response of CBD in rats with occlusion of the right middle cerebral artery, and they concluded that 100 and 200 ng/rat of CBD significantly reduces neurological deficit, infarction, and cerebral edema. In addition, it modulates the NCX pathway (Na+/Ca2+ exchange proteins) important in the activation of the endogenous neuroprotective mechanism [79].

Libzon et al. (2018) researched CBD-enriched Cannabis oils, at different CBD/THC ratios, in movement disorders in pediatric children. They concluded that CBD/THC ratios of 6/1 and 20/1 were effective for this type of disorder; in addition, that the mood and appetite of the children improved [80]. The latter CBD/THC ratio is similar to that obtained in this study for variety B (Table 2).

It is important to note that many of the biological properties of cannabinoids may have antioxidant mechanisms. For example, dos-Santos-Pereira, et al. (2020) evaluated the anti-inflammatory and antioxidant activity of CBD in mouse microglial cells cultured for lipopolysaccharide, and found that CBD significantly inhibited the release of cytosines TNF-α and IL-β, and that of glutamate, concluding that this cannabinoid has anti-inflammatory effects on microglia through an intrinsic antioxidant mechanism, in addition to inhibiting glucose-dependent NADPH synthesis [81].

CBD also has potential for anti-anxiety treatments. For example, De Gregorio et al. (2019) state that it prevents mechanical allodynia and alleviates pain-induced anxiety-like behavior in an in vivo model in rats [65]. CBD also has potential for the treatment of eye [82]. However, several authors question its activity in glaucoma disease, where CBD did not generate a positive response [65].

There is evidence that Cannabis extracts in which there is a high content of cannabinoids along with other types of molecules, such as terpenoids, can improve activity (synergistic effect). In a meta-analysis by Pamplona, da Silva, & Coan, (2018), an analysis was made of clinical studies in the treatment of refractory epilepsy with CBD-rich products. Compared to purified CBD, it was found that patients treated with the extracts have a lower average dose [83].

Taking the above into account and comparing with samples A and B, in which the percentage of CBD predominates over THC, with a CBD/THC ratio between 13.4 and 17.2, the samples can be expected to have a moderate to low antioxidant activity and that this will depend not only on the CBD/THC ratio, but on the synergistic effect of their components, such as the content of β-caryophyllene present in both samples. In addition, as the extracts are rich in CBD, they could be expected to have great biological potential related to the content of this cannabinoid – a cannabinoid that, since it is not psychoactive, can facilitate dosing at a wide range of concentrations. Regarding the synergistic effect, variety A was found to contain α-pinene, D-limonene, and β-caryophyllene, all of which enhance the therapeutic activity of CBD.

The results reveal that in the ethanolic extracts of varieties A and B, terpenoids are less well represented (1.61 % and 0.68 % respectively) than CBD (10.0 % and 13.7 % respectively). In addition, the most abundant terpenoid in both varieties was found to be β-caryophyllene, in agreement with the literature, where it is stated that this is the sesquiterpene most readily extracted from Cannabis [84], and that along with β-myrcene, these are the most common terpenes in the Cannabis sativa plant and that play an important role in the variety of biological properties of this plant.

The many biological activities of terpenes are well known and include antifungal, antioxidant, and antimicrobial activities [16, 85]. For example, Gallily, et al. (2018) analyzed the anti-inflammatory properties of terpenoid-rich Cannabis essential oils, through in vivo (7 to 8-week-old female Sabra mice) and in vitro (RAW 264.7 murine monocyte/macrophage cell line), and they found that the anti-inflammatory and antinociceptive activities by the two methods vary according to the terpenoid profile. In addition, they found that the evaluated oils are not as effective as purified CBD [86]. Oils rich in terpenes could therefore be used to relieve acute inflammation, and not chronic as in the case of CBD. However, as already mentioned, many researchers affirm that the biological properties of Cannabis may be due to the synergistic effect of its components. Thus, Namdar et al. (2019) showed that in strains with a high content of THC or CBD, cannabinoids are produced with a certain group of terpenoids; in addition, that in the relationships inherent to the extract of 17 strains of Cannabis sativa, the cytotoxic activity against cancer cells (MDA-MB-231 and HCT-116) improves, which means that a synergistic effect between the components of the extracts is very likely [87]. According to the results obtained, and if we compare with those reported by Namdar et al., (2019), it can be seen that, for varieties with high CBD like the two studied in this article, it is possible that some terpenoids can be strongly correlated with CBD and influence a possible cytotoxic activity of the extract, such as alpha-bisabolol and alpha guaiol in variety B. This does not mean that other terpenoids are not able influence the possible cytotoxic activity, since many of those found in varieties A and B (α-pinene, β-myrcene, D-limonene, β-caryophyllene, α-humulene, α-bisabolol, would not have a specific effect.

From a medical perspective, some researchers have established that, depending on the THC/CBD ratio and the presence of some terpenoids, the biological activity of Cannabis-based products could be improved. For example, Lewis, et al., (2018) state that a variety with a high concentration of CBD, low in THC and with predominant terpenoids β-caryophyllene, limonene and α-humulene - which is similar (though not identical) to varieties A and B - could have a potential for treatment of pain, inflammation and addiction, possibly mediated by the affinity of β-caryophyllene for the CB2 receptors [88]. It should be noted that the mechanism by which the cannabinoid-terpenoid interaction occurs is not yet fully established, but Santiago, et al., (2019) state that direct interactions with endocannabinoid receptors can be ruled out, such thatresearch should focus on other pathways [89]. However, it is noteworthy that α-humulene and β-caryophyllene have an affinity for the CB2 receptor [90].

Cannabis medicines contain a wide variety of chemical compounds: cannabinoids with psychoactive (THC) and non-psychoactive (CBD) activity, terpenes, flavonoids, etc. The consumption of Cannabis and the products derived from it is associated with both pathological and behavioral toxicity. The effects will depend on the formulation and the route of administration that must be adapted to individual needs [6]. Studies of cannabis-based medicines are being approved in many European countries, while in Colombia in 2020, INVIMA, the Colombian agency in charge of regulating medicines granted the first batch of authorizations or good manufacturing practices (GMP) to Cannabis companies interested in producing generic formulations.

Typical products include CBD oil capsules and CBD or THC mouth sprays used in therapies [73]. The European Pain Federation (EFIC) convened a group of European experts from a wide range of disciplines of basic and clinical sciences. They prepared a document to inform specialists and non-specialists about the appropriate use of medicines based on Cannabis for chronic pain [91]. Busardò et al. in 2021 presented a study revealing a high interindividual variability regarding the disposition of phytocannabinoids, acid precursors and their metabolites in biological matrices of individuals treated with medicinal Cannabis by vaporization [92]. Pichini et al. in 2021 developed and validated a method using ultra-high-performance liquid chromatography in tandem with mass spectrometry (UHPLC-MS/MS) to quantify CBD and its metabolites in serum and urine samples from individuals treated with medicinal Cannabis [31].

There remains the need to conduct studies and clinical trials to demonstrate the effectiveness and safety of medicinal Cannabis and its possible means of administration [93, 94].
Toxicity

4.5. Toxicity

In recent years, medicinal Cannabis has been slowly accepted on the planet through laws that render more flexible its study and use. One of the main research focuses is the pharmacological and toxicological properties of cannabinoids and extracts in eukaryotic cells, microorganisms, animals and humans, in order to support their safety for human consumption. Thus, Ewing et al. (2019) studied the hepatotoxicity of extract rich in cannabidiol (The solvent used was hexane and flowers and leaves were used) in 8-week-old male B6C3F1 mice, varying the intake from 0 to 615 mg/kg of CBD for 10 days (subacute toxicity), and 0 to 2460 mg/kg of CBD per 24 h (acute toxicity). In the subacute study, the authors found that 75 % of the mice fed the highest concentration of CBD developed a terminal illness between days 3 and 4. In the acute study, the authors found that when the mice were fed 2,460 mg/kg there was a significant increase in the liver/body weight ratio, plasma ALT, AST and total bilirubin. Therefore, they concluded that CBD has hepatotoxicity, probably of the cholestatic type [95].

Dziwenka et al. (2020) meanwhile evaluated the toxicity of a patented CBD-rich hemp extract in Sprague-Dawley rats. They affirmed that, in the 90-day study, no significant increase in body weight was found. In addition, the feeding efficiency in the low and medium dose groups was less than 10 %, which is not toxicologically relevant. However, in male rats that received 800 mg/Kg/day it was greater than 10 %, which they considered toxicologically relevant. The authors state that the extract, whether diluted in olive oil or not, is not mutagenic and that the NOAEL value for 90 days was 800 mg/Kg/day for female rats and 400 mg/Kg/day for male rats. Therefore, the authors concluded that hemp extracts are probably safe for human consumption, but that they should be supported by additional studies [96].

In a study by Harpaz et al. (2021), the toxicity of pure cannabinoids and six ethanolic extracts of Cannabis inflorescences was studied in a panel of bio-reporter E. coli bacteria. They reported that only THC and THCA induce possible damage in DNA. On the other hand, in the ethanolic extracts of Cannabis flowers, they induced a bacterial response in a bacterial strain sensitive to DNA damage (DPD1718). It is possible, however, that this response is due to the ethanol. Furthermore, the extracts inhibited the bacterial response to the strain sensitive to genotoxicity stress (DPD2794). The authors state that these differences are due to the different mode of action of toxicity between standard cannabinoids and extracts, and it is the THC and THCA content of the extracts that determine the mode of action of toxicity in bacteria. It is noteworthy that, according to this study, only a few extracts generated cytotoxicity in the bio-reporter bacteria [97]. In accordance with the above, the entourage effect influences the overall toxicity of the extracts. There is no concrete information on the toxicity of non-psychoactive Cannabis varieties with CBD/THC concentrations between 10-20, like the varieties analyzed in the study, samples A and B.

4.6. Final observations and future perspectives.

Since the legalization of Cannabis through Decree 2467 with modification in Decree 613 April 2017 for scientific and medicinal purposes in Colombia, the Ministry of Health has granted several licenses for the use of Cannabis [23]. At the beginning of 2020, INVIMA approved the first master formulas based on medicinal cannabis, thus opening up a possibility to innovate and generate new knowledge in the country [98]. It is noteworthy that innovation in medicine has generated many research projects that have managed to solve pathologies that seemed intractable, so researching to innovate is the future of health [98]. According to Khiron Life Sciences Corp., in Colombia there are approximately six million people who suffer from epilepsy, Parkinson's, chronic pain and anxiety, for whom medicinal Cannabis can be an option to relieve their suffering [99]. Given that cannabis-based products require more research, it is important not only to investigate the profile of cannabinoids and terpenoids of Cannabis varieties from our country, but also to evaluate the biological properties of these extracts - such as antioxidant, anticancer and neuroprotective activity, in addition to evaluating the toxicity of the extracts to come to a conclusion on their safety. Studies are currently being planned in human cancer lines, a MCF-7 breast cancer line (human mammary gland adenocarcinoma), a HT-29 colon cancer line (human rectal colon adenocarcinoma) and a PC-3 prostate cancer line (human prostate adenocarcinoma), all being cancer types of great interest nationally and internationally.

5. Conclusions

The two varieties of non-psychoactive Cannabis sativa cultivated in the department of Cauca presented representative cannabinoids CBDA and THCA. In addition, other cannabinoids were found that have been less widely studied, such as CBDV, THCV, CBN, CBCA and CBGA. In the analysis of ethanolic extracts, a CBD/THC ratio of 13.4 was found for sample A and 17.2 for sample B, similar to the CBD/THC ratios used in different studies on biological activity, which demonstrates the therapeutic potential of these varieties.

The ethanolic extracts expressed a terpenoid content of 1.61 % in sample A and 0.68 % in sample B, identifying terpenoids such as α-pinene, D-limonene, β-caryophyllene which enhance the therapeutic activity of CBD. The presence of β-caryophyllene, which is the one found in the highest proportion in samples A and B (0.31 % and 0.17 % respectively), could favor this neuroprotective effect.

According to the literature, the CBD/THC ratio influences the biological potential of a Cannabis extract. According to our results, the extracts are expected to have a moderate to

low antioxidant activity, which, according to some researchers, may be associated with the neuroprotective effect that has already been reported in the literature.

In addition, the presence of percentages (w/w) of CBD of 10 % and 13.7 % for samples A and B respectively enhances the possible use of the extract in therapies to reduce pain in cancer patients. There is a need to continue carrying out studies and clinical trials that make it possible to demonstrate the effectiveness and safety of Cannabis of the varieties that are cultivated in the north of Cauca.

6. Acknowledgments

The authors would like to thank the Natural Product Chemical Research Group of the Universidad del Cauca, for the projects of the internal funding call VRI ID: 4961 of 2018 and ID:5507 of the Universidad del Cauca. We are grateful too to the Ministry of Science, Technology and Innovation for scholarships within the framework of the funding call for Doctoral Excellence Scholarships of the Bicentennial 2019-COLCIENCIAS, to the funding call 823-2018 for High Level Training for the regions – Cauca, and the project for young researchers and innovators in the department of Cauca. Finally, we are especially grateful to Colin McLachlan for suggestions relating to the English text.

7. Conflicts of interest

The authors declare that they have no conflicts of interest.

Referencias

[1] K. Hillig, "A Systematic Investigation of Cannabis," 2005. doi: 10.13140/RG.2.1.2648.3680.

[2] S. A. Bonini et al., "Cannabis sativa: A comprehensive ethnopharmacological review of a medicinal plant with a long history," J. Ethnopharmacol., vol. 227, pp. 300–315, Dec. 2018, doi: 10.1016/j.jep.2018.09.004.

[3] M. Charai, H. Sghiouri, A. Mezrhab, and M. Karkri, "Thermal insulation potential of non-industrial hemp (Moroccan cannabis sativa L.) fibers for green plaster-based building materials," J. Clean. Prod., vol. 292, p. 126064, Apr. 2021, doi: 10.1016/j.jclepro.2021.126064.

[4] F. P. Gomez, J. Hu, and M. A. Clarke, "Cannabis as a Feedstock for the Production of Chemicals, Fuels, and Materials: A Review of Relevant Studies To Date," Energy Fuels, vol. 35, no. 7, pp. 5538–5557, Apr. 2021, doi: 10.1021/acs.energyfuels.0c04121.

[5] E. M. Mudge, "Chemometrics and metabolomics of Cannabis sativa L.," University of British Columbia, 2019. doi: 10.14288/1.0376606.

[6] C. J. Lucas, P. Galettis, and J. Schneider, "The pharmacokinetics and the pharmacodynamics of cannabinoids," Br. J. Clin. Pharmacol., vol. 84, no. 11, pp. 2477–2482, Nov. 2018, doi: 10.1111/bcp.13710.

[7] M. Mazza, "Medical cannabis for the treatment of fibromyalgia syndrome: a retrospective, open-label case series," J. Cannabis Res., vol. 3, no. 1, p. 4, Feb. 2021, doi: 10.1186/s42238-021-00060-6.

[8] H. Koltai and D. Namdar, "Cannabis Phytomolecule 'Entourage': From Domestication to Medical Use," Trends Plant Sci., vol. 25, no. 10, pp. 976–984, Oct. 2020, doi: 10.1016/j.tplants.2020.04.007.

[9] J. Gonçalves et al., "Cannabis and Its Secondary Metabolites: Their Use as Therapeutic Drugs, Toxicological Aspects, and Analytical Determination," Med. Basel Switz., vol. 6, no. 1, p. 31, Feb. 2019, doi: 10.3390/medicines6010031.

[10] M. A. ElSohly, M. M. Radwan, W. Gul, S. Chandra, and A. Galal, "Phytochemistry of Cannabis sativa L," Prog. Chem. Org. Nat. Prod., vol. 103, pp. 1–36, 2017, $doi : 10{,}1007/978-3-319-45541-9_1$.

[11] H. Lowe, B. Steele, J. Bryant, N. Toyang, and W. Ngwa, "Non-Cannabinoid Metabolites of Cannabis sativa L. with Therapeutic Potential," Plants Basel Switz., vol. 10, no. 2, p. 400, Feb. 2021, doi: 10.3390/plants10020400.

[12] K. Solymosi and A. Köfalvi, "Cannabis: A Treasure Trove or Pandora's Box?," Mini Rev. Med. Chem., vol. 17, no. 13, pp. 1223–1291, 2017, doi: 10.2174/1389557516666161004162133.

[13] N. Maurya and B. K. Velmurugan, "Therapeutic applications of cannabinoids," Chem. Biol. Interact., vol. 293, pp. 77–88, Sep. 2018, doi: 10.1016/j.cbi.2018.07.018.

[14] E. C. Rosenberg, P. H. Patra, and B. J. Whalley, "Therapeutic effects of cannabinoids in animal models of seizures, epilepsy, epileptogenesis, and epilepsy-related neuroprotection," Epilepsy Behav. EB, vol. 70, no. Pt B, pp. 319–327, May 2017, doi: 10.1016/j.yebeh.2016.11.006.

[15] J. K. Booth and J. Bohlmann, "Terpenes in Cannabis sativa – From plant genome to humans," Plant Sci., vol. 284, pp. 67–72, Jul. 2019, doi: 10.1016/j.plantsci.2019.03.022.

[16] K.-Y. Yoo and S.-Y. Park, "Terpenoids as potential anti-Alzheimer's disease therapeutics," Mol. Basel Switz., vol. 17, no. 3, pp. 3524–3538, Mar. 2012, doi: 10.3390/molecules17033524.

[17] H. McTaggart-Cowan, C. Bentley, A. Raymakers, R. Metcalfe, P. Hawley, and S. Peacock, "Understanding cancer survivors' reasons to medicate with cannabis: A qualitative study based on the theory of planned beha-

vior," Cancer Med., vol. 10, no. 1, pp. 396–404, 2021, doi: 10.1002/cam4.3536.

[18] M. A. Holgado, L. Martín-Banderas, J. Álvarez-Fuentes, and M. Fernández-Arévalo, "Neuroprotective effect of cannabinoids nanoplatforms in neurodegenerative diseases," J. Drug Deliv. Sci. Technol., vol. 42, pp. 84–93, Dec. 2017, doi: 10.1016/j.jddst.2017.04.023.

[19] A. C. M. Hacke et al., "Probing the antioxidant activity of Δ9-tetrahydrocannabinol and cannabidiol in Cannabis sativa extracts," The Analyst, vol. 144, no. 16, pp. 4952–4961, Aug. 2019, doi: 10.1039/c9an00890j.

[20] D. Namdar, M. Mazuz, A. Ion, and H. Koltai, "Variation in the compositions of cannabinoid and terpenoids in Cannabis sativa derived from inflorescence position along the stem and extraction methods," Ind. Crops Prod., vol. 113, pp. 376–382, Mar. 2018, doi: 10.1016/j.indcrop.2018.01.060.

[21] Y. Chye, R. Kirkham, V. Lorenzetti, E. McTavish, N. Solowij, and M. Yücel, "Cannabis, Cannabinoids, and Brain Morphology: A Review of the Evidence," Biol. Psychiatry Cogn. Neurosci. Neuroimaging, vol. 6, no. 6, pp. 627–635, Jun. 2021, doi: 10.1016/j.bpsc.2020.07.009.

[22] "Decree 613 of 2017.pdf." Accessed: May 09, 2023. [online]. Available: https://www.minsalud.gov.co/Normatividad_Nuevo/Decreto%20613%20de%202017.pdf

[23] "Procedures and services window - Ministry of Health and Social Protection." https://tramites.minsalud.gov.co/tramitesservicios/cannabis/default.aspx (accessed May 09, 2023).

[24] J. M. Giraldo et al., "Determination and qualitative comparison of cannabinoids present in commercial natural products from the department of Cauca, Colombia," Rev. Colomb. Cienc. Quím. - Farm., vol. 48, no. 3, pp. 789–810, Dec. 2019, doi: 10.15446/rcciquifa.v48n3.84992.

[25] B. De Backer et al., "Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material," J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci., vol. 877, no. 32, pp. 4115–4124, Dec. 2009, doi: 10.1016/j.jchromb.2009.11.004.

[26] W. Peschel and M. Politi, "1H NMR and HPLC/DAD for Cannabis sativa L. chemotype distinction, extract profiling and specification," Talanta, vol. 140, pp. 150–165, Aug. 2015, doi: 10.1016/j.talanta.2015.02.040.

[27] C. Citti, D. Braghiroli, M. A. Vandelli, and G. Cannazza, "Pharmaceutical and biomedical analysis of cannabinoids: A critical review," J. Pharm. Biomed. Anal., vol. 147, pp. 565–579, Jan. 2018, doi: 10.1016/j.jpba.2017.06.003.

[28] G. Corni, V. Brighenti, F. Pellati, and G. E. Morlock, "Effect-directed analysis of bioactive compounds in Cannabis sativa L. by high-performance thin-layer chromatography," J. Chromatogr. A, vol. 1629, p. 461511, Oct. 2020, doi: 10.1016/j.chroma.2020.461511.

[29] P. Berman et al., "A new ESI-LC/MS approach for comprehensive metabolic profiling of phytocannabinoids in Cannabis," Sci. Rep., vol. 8, no. 1, Art. no. 1, Sep. 2018, doi: 10.1038/s41598-018-32651-4.

[30] A. Nemeškalová, K. Hájková, L. Mikulů, D. Sýkora, and M. Kuchař, "Combination of UV and MS/MS detection for the LC analysis of cannabidiol-rich products," Talanta, vol. 219, p. 121250, Nov. 2020, doi: 10.1016/j.talanta.2020.121250.

[31] S. Pichini et al., "UHPLC-MS/MS analysis of cannabidiol metabolites in serum and urine samples. Application to an individual treated with medical cannabis," Talanta, vol. 223, no. Pt 2, p. 121772, Feb. 2021, doi: 10.1016/j.talanta.2020.121772.

[32] H. Peeri et al., "Specific Compositions of Cannabis sativa Compounds Have Cytotoxic Activity and Inhibit Motility and Colony Formation of Human Glioblastoma Cells In Vitro," Cancers, vol. 13, no. 7, p. 1720, Apr. 2021, doi: 10.3390/cancers13071720.

[33] F. Pollastro, A. Minassi, and L. G. Fresu, "Cannabis Phenolics and their Bioactivities," Curr. Med. Chem., vol. 25, no. 10, pp. 1160–1185, 2018, doi: 10.2174/0929867324666170810164636.

[34] D. Jin, K. Dai, Z. Xie, and J. Chen, "Secondary Metabolites Profiled in Cannabis Inflorescences, Leaves, Stem Barks, and Roots for Medicinal Purposes," Sci. Rep., vol. 10, no. 1, Art. no. 1, Feb. 2020, doi: 10.1038/s41598-020-60172-6.

[35] D. U. Nagy, K. Cianfaglione, F. Maggi, S. Sut, and S. Dall'Acqua, "Chemical Characterization of Leaves, Male and Female Flowers from Spontaneous Cannabis (Cannabis sativa L.) Growing in Hungary," Chem. Biodivers., vol. 16, no. 3, p. e1800562, Mar. 2019, doi: 10.1002/cbdv.201800562.

[36] Z. Drinić, S. Vidović, J. Vladić, A. Koren, B. Kiprovski, and V. Sikora, "Effect of extraction solvent on total polyphenols content and antioxidant activity of Cannabis sativa L.," Lek. Sirovine, no. 38, pp. 17–21, 2018, doi: 10.5937/leksir1838017D.

[37] R. Pavlovic et al., "Phytochemical and Ecological Analysis of Two Varieties of Hemp (Cannabis sativa L.) Grown in a Mountain Environment of Italian Alps," Front. Plant Sci., vol. 10, 2019, Accessed: May 09, 2023.

[online]. Available: https://www.frontiersin.org/articles/10.3389/fpls.2019.01265

[38] F. Pellati, V. Brighenti, J. Sperlea, L. Marchetti, D. Bertelli, and S. Benvenuti, "New Methods for the Comprehensive Analysis of Bioactive Compounds in Cannabis sativa L. (hemp)," Mol. Basel Switz., vol. 23, no. 10, p. 2639, Oct. 2018, doi: 10.3390/molecules23102639.

[39] L. Izzo et al., "Analysis of Phenolic Compounds in Commercial Cannabis sativa L. Inflorescences Using UHPLC-Q-Orbitrap HRMS," Molecules, vol. 25, no. 3, p. 631, Jan. 2020, doi: 10.3390/molecules25030631.

[40] C. Ferrante et al., "Multiple pharmacognostic characterization on hemp commercial cultivars: Focus on inflorescence water extract activity," Food Chem. Toxicol., vol. 125, pp. 452–461, Mar. 2019, doi: 10.1016/j.fct.2019.01.035.

[41] F. A. Méndez Pérez, "Optimización de la obtención del extracto hidroalcohólico de las inflorescencias de Cannabis sativaL. 'marihuana'. Ayacucho 2018.," Univ. Nac. San Cristóbal Huamanga, 2018, Accessed: May 09, 2023. [online]. Available: http://repositorio.unsch.edu.pe/handle/UNSCH/4182

[42] M. M. Radwan, S. Chandra, S. Gul, and M. A. ElSohly, "Cannabinoids, Phenolics, Terpenes and Alkaloids of Cannabis," Mol. Basel Switz., vol. 26, no. 9, p. 2774, May 2021, doi: 10.3390/molecules26092774.

[43] A. Gruschow, "A Comparison of Antioxidant Potential, Total Phenolic Content, and Cannabidiol (CBD) Content of Cannabis Infused Hemp, MCT, and Olive Oils," Theses Diss. Culminating Proj., May 2020, [online]. Available: https://digitalcommons.montclair.edu/etd/472

[44] A. N. Yadav et al., "Chapter 20 - Metabolic Engineering to Synthetic Biology of Secondary Metabolites Production," in New and Future Developments in Microbial Biotechnology and Bioengineering, V. K. Gupta and A. Pandey, Eds., Amsterdam: Elsevier, 2019, pp. 279–320. doi: 10.1016/B978-0-444-63504-4.00020-7.

[45] G. Diaz-Muñoz, I. L. Miranda, S. K. Sartori, D. C. de Rezende, and M. A. N. Diaz, "Chapter 11 - Anthraquinones: An Overview," in Studies in Natural Products Chemistry, Atta-ur-Rahman, Ed., Elsevier, 2018, pp. 313–338. doi: 10.1016/B978-0-444-64056-7.00011-8.

[46] I. Montenegro et al., "Chemical Composition, Antioxidant and Anticancer Activities of Leptocarpha rivularis DC Flower Extracts," Mol. Basel Switz., vol. 26, no. 1, p. 67, Dec. 2020, doi: 10.3390/molecules26010067.

[47] X.-F. Shang et al., "Insecticidal and antifungal activities of Rheum palmatum L. anthraquinones and structurally related compounds," Ind. Crops Prod., vol. 137, pp. 508–520, Oct. 2019, doi: 10.1016/j.indcrop.2019.05.055.

[48] A. L. Dawidowicz, M. Olszowy-Tomczyk, and R. Typek, "CBG, CBD, Δ9-THC, CBN, CBGA, CBDA and Δ9-THCA as antioxidant agents and their intervention abilities in antioxidant action," Fitoterapia, vol. 152, p. 104915, Jul. 2021, doi: 10.1016/j.fitote.2021.104915.

[49] S. Palmieri, M. Pellegrini, A. Ricci, D. Compagnone, and C. Lo Sterzo, "Chemical Composition and Antioxidant Activity of Thyme, Hemp and Coriander Extracts: A Comparison Study of Maceration, Soxhlet, UAE and RSLDE Techniques," Foods, vol. 9, no. 9, Art. no. 9, Sep. 2020, doi: 10.3390/foods9091221.

[50] D. I. Abrams and M. Guzman, "Cannabis in cancer care," Clin. Pharmacol. Ther., vol. 97, no. 6, pp. 575–586, Jun. 2015, doi: 10.1002/cpt.108.

[51] F. Grotenhermen and K. Müller-Vahl, "The Therapeutic Potential of Cannabis and Cannabinoids," Dtsch. Ärztebl. Int., vol. 109, no. 29–30, pp. 495–501, Jul. 2012, doi: 10.3238/arztebl.2012.0495.

[52] D. Slawek, S. R. Meenrajan, M. R. Alois, P. Comstock Barker, I. M. Estores, and R. Cook, "Medical Cannabis for the Primary Care Physician," J. Prim. Care Community Health, vol. 10, p. 2150132719884838, Oct. 2019, doi: 10.1177/2150132719884838.

[53] T. Byars, E. Theisen, and D. L. Bolton, "Using Cannabis to Treat Cancer-Related Pain," Semin. Oncol. Nurs., vol. 35, no. 3, pp. 300–309, Jun. 2019, doi: 10.1016/j.soncn.2019.04.012.

[54] L. Sawtelle and L. M. Holle, "Use of Cannabis and Cannabinoids in Patients With Cancer," Ann. Pharmacother., vol. 55, no. 7, pp. 870–890, Jul. 2021, doi: 10.1177/1060028020965224.

[55] J. Fernández-Ruiz et al., "Cannabidiol for neurodegenerative disorders: important new clinical applications for this phytocannabinoid?," Br. J. Clin. Pharmacol., vol. 75, no. 2, pp. 323–333, Feb. 2013, doi: 10.1111/j.1365-2125.2012.04341.x.

[56] D. I. Abrams, "Integrating cannabis into clinical cancer care," Curr. Oncol., vol. 23, no. Suppl 2, pp. S8–S14, Mar. 2016, doi: 10.3747/co.23.3099.

[57] F. Pellati, V. Borgonetti, V. Brighenti, M. Biagi, S. Benvenuti, and L. Corsi, "Cannabis sativa L. and Nonpsychoactive Cannabinoids: Their Chemistry and Role against Oxidative Stress, Inflammation, and Cancer," BioMed Res. Int., vol. 2018, p. 1691428, 2018, doi: 10.1155/2018/1691428.

[58] B. Dariš, M. T. Verboten, Ž. Knez, and P. Ferk, "Cannabinoids in cancer treatment: Therapeutic potential and

legislation," Bosn. J. Basic Med. Sci., vol. 19, no. 1, pp. 14–23, Feb. 2019, doi: 10.17305/bjbms.2018.3532.

[59] P. B. Dall'Stella, M. F. L. Docema, M. V. C. Maldaun, O. Feher, and C. L. P. Lancellotti, "Case Report: Clinical Outcome and Image Response of Two Patients With Secondary High-Grade Glioma Treated With Chemoradiation, PCV, and Cannabidiol," Front. Oncol., vol. 8, p. 643, 2018, doi: 10.3389/fonc.2018.00643.

[60] I. Turgeman and G. Bar-Sela, "Cannabis for cancer - illusion or the tip of an iceberg: a review of the evidence for the use of Cannabis and synthetic cannabinoids in oncology," Expert Opin. Investig. Drugs, vol. 28, no. 3, pp. 285–296, Mar. 2019, doi: 10.1080/13543784.2019.1561859.

[61] J. M. J. Oultram, J. L. Pegler, T. A. Bowser, L. J. Ney, A. L. Eamens, and C. P. L. Grof, "Cannabis sativa: Interdisciplinary Strategies and Avenues for Medical and Commercial Progression Outside of CBD and THC," Biomedicines, vol. 9, no. 3, p. 234, Feb. 2021, doi: 10.3390/biomedicines9030234.

[62] B. Leiva et al., "Equol and daidzein decrease migration, invasion and matrix metalloproteinase (MMPs) gene expression in prostate cancer cell lines, DU-145 and PC-3," Bol Latinoam Caribe Plantas Med Aromát, pp. 251–262, 2015.

[63] A. M. Tomko, E. G. Whynot, L. D. Ellis, and D. J. Dupré, "Anti-Cancer Potential of Cannabinoids, Terpenes, and Flavonoids Present in Cannabis," Cancers, vol. 12, no. 7, p. 1985, Jul. 2020, doi: 10.3390/cancers12071985.

[64] B. Hinz and R. Ramer, "Anti-tumour actions of cannabinoids," Br. J. Pharmacol., vol. 176, no. 10, pp. 1384–1394, May 2019, doi: 10.1111/bph.14426.

[65] D. De Gregorio et al., "Cannabidiol modulates serotonergic transmission and reverses both allodynia and anxiety-like behavior in a model of neuropathic pain," Pain, vol. 160, no. 1, pp. 136–150, Jan. 2019, doi: 10.1097/j.pain.0000000000001386.

[66] T. Kisková, F. Mungenast, M. Suváková, W. Jäger, and T. Thalhammer, "Future Aspects for Cannabinoids in Breast Cancer Therapy," Int. J. Mol. Sci., vol. 20, no. 7, p. 1673, Apr. 2019, doi: 10.3390/ijms20071673.

[67] S. Takeda et al., "Cannabidiolic acid, a major cannabinoid in fiber-type cannabis, is an inhibitor of MDA-MB-231 breast cancer cell migration," Toxicol. Lett., vol. 214, no. 3, pp. 314–319, Nov. 2012, doi: 10.1016/j.toxlet.2012.08.029.

[68] G. Sharafi, H. He, and M. Nikfarjam, "Potential Use of Cannabinoids for the Treatment of Pancreatic Cancer," J. Pancreat. Cancer, vol. 5, no. 1, pp. 1–7, Jan. 2019, doi: 10.1089/pancan.2018.0019.

[69] Y. Singh and C. Bali, "Cannabis extract treatment for terminal acute lymphoblastic leukemia with a Philadelphia chromosome mutation," Case Rep. Oncol., vol. 6, no. 3, pp. 585–592, Sep. 2013, doi: 10.1159/000356446.

[70] E. R. Pritchard, L. Dayer, J. Belz, B. Forseth, S. E. Harrington, and J. T. Painter, "Effect of cannabis on opioid use in patients with cancer receiving palliative care," J. Am. Pharm. Assoc., vol. 60, no. 1, pp. 244–247, Jan. 2020, doi: 10.1016/j.japh.2019.10.013.

[71] S. Karim, W. Y. Cheung, J. Bu, E. Jess, and M. Kerba, "Medical Cannabis Authorization in Patients With Cancer in the Prelegalization Era: A Population-Based Study," J. Pain Symptom Manage., vol. 59, no. 6, pp. 1223–1231, Jun. 2020, doi: 10.1016/j.jpainsymman.2019.12.367.

[72] Y. Zolotov, L. Eshet, and O. Morag, "Preliminary assessment of medical cannabis consumption by cancer survivors," Complement. Ther. Med., vol. 56, p. 102592, Jan. 2021, doi: 10.1016/j.ctim.2020.102592.

[73] A. Blake et al., "A selective review of medical cannabis in cancer pain management," Ann. Palliat. Med., vol. 6, no. Suppl 2, pp. S215–S222, Dec. 2017, doi: 10.21037/apm.2017.08.05.

[74] C. A. MacCallum and E. B. Russo, "Practical considerations in medical cannabis administration and dosing," Eur. J. Intern. Med., vol. 49, pp. 12–19, Mar. 2018, doi: 10.1016/j.ejim.2018.01.004.

[75] G. Bar-Sela, D. Zalman, V. Semenysty, and E. Ballan, "The Effects of Dosage-Controlled Cannabis Capsules on Cancer-Related Cachexia and Anorexia Syndrome in Advanced Cancer Patients: Pilot Study," Integr. Cancer Ther., vol. 18, p. 1534735419881498, 2019, doi: 10.1177/1534735419881498.

[76] R. S. Borges and A. B. F. Da Silva, "Cannabidiol as an Antioxidant," in Handbook of Cannabis and Related Pathologies, Elsevier, 2017, pp. e122–e130. doi: 10.1016/B978-0-12-800756-3.00093-4.

[77] Y. Hu, Z. Zeng, B. Wang, and S. Guo, "Transcaryophyllene inhibits amyloid β (Aβ) oligomer-induced neuroinflammation in BV-2 microglial cells," Int. Immunopharmacol., vol. 51, pp. 91–98, Oct. 2017, doi: 10.1016/j.intimp.2017.07.009.

[78] A. Raja, S. Ahmadi, F. de Costa, N. Li, and K. Kerman, "Attenuation of Oxidative Stress by Cannabinoids and Cannabis Extracts in Differentiated Neuronal Cells," Pharm. Basel Switz., vol. 13, no. 11, p. 328, Oct. 2020, doi: 10.3390/ph13110328.

[79] S. Khaksar and M. R. Bigdeli, "Anti-excitotoxic effects of cannabidiol are partly mediated by enhancement of NCX2 and NCX3 expression in animal model

of cerebral ischemia," Eur. J. Pharmacol., vol. 794, pp. 270–279, Jan. 2017, doi: 10.1016/j.ejphar.2016.11.011.

[80] S. Libzon et al., "Medical Cannabis for Pediatric Moderate to Severe Complex Motor Disorders," J. Child Neurol., vol. 33, no. 9, pp. 565–571, Aug. 2018, doi: 10.1177/0883073818773028.

[81] M. dos-Santos-Pereira, F. S. Guimarães, E. Del-Bel, R. Raisman-Vozari, and P. P. Michel, "Cannabidiol prevents LPS-induced microglial inflammation by inhibiting ROS/NF-κB-dependent signaling and glucose consumption," Glia, vol. 68, no. 3, pp. 561–573, 2020, doi: 10.1002/glia.23738.

[82] D. Thapa, E. A. Cairns, A.-M. Szczesniak, J. T. Toguri, M. D. Caldwell, and M. E. M. Kelly, "The Cannabinoids Δ8THC, CBD, and HU-308 Act via Distinct Receptors to Reduce Corneal Pain and Inflammation," Cannabis Cannabinoid Res., vol. 3, no. 1, pp. 11–20, Feb. 2018, doi: 10.1089/can.2017.0041.

[83] F. A. Pamplona, L. R. da Silva, and A. C. Coan, "Potential Clinical Benefits of CBD-Rich Cannabis Extracts Over Purified CBD in Treatment-Resistant Epilepsy: Observational Data Meta-analysis," Front. Neurol., vol. 9, p. 759, 2018, doi: 10.3389/fneur.2018.00759.

[84] E. C. D. Gonçalves, G. M. Baldasso, M. A. Bicca, R. S. Paes, R. Capasso, and R. C. Dutra, "Terpenoids, Cannabimimetic Ligands, beyond the Cannabis Plant," Molecules, vol. 25, no. 7, p. 1567, Mar. 2020, doi: 10.3390/molecules25071567.

[85] L. O. Hanuš and Y. Hod, "Terpenes/Terpenoids in Cannabis: Are They Important?," Med. Cannabis Cannabinoids, vol. 3, no. 1, pp. 25–60, Aug. 2020, doi: 10.1159/000509733.

[86] R. Gallily, Z. Yekhtin, and L. O. Hanuš, "The Anti-Inflammatory Properties of Terpenoids from Cannabis," Cannabis Cannabinoid Res., vol. 3, no. 1, pp. 282–290, Dec. 2018, doi: 10.1089/can.2018.0014.

[87] D. Namdar et al., "Terpenoids and Phytocannabinoids Co-Produced in Cannabis Sativa Strains Show Specific Interaction for Cell Cytotoxic Activity," Mol. Basel Switz., vol. 24, no. 17, p. 3031, Aug. 2019, doi: 10.3390/molecules24173031.

[88] M. A. Lewis, E. B. Russo, and K. M. Smith, "Pharmacological Foundations of Cannabis Chemovars," Planta Med., vol. 84, no. 4, pp. 225–233, Mar. 2018, doi: 10.1055/s-0043-122240.

[89] M. Santiago, S. Sachdev, J. C. Arnold, I. S. McGregor, and M. Connor, "Absence of Entourage: Terpenoids Commonly Found in Cannabis sativa Do Not Modulate the Functional Activity of Δ9-THC at Human CB1 and CB2 Receptors," Cannabis Cannabinoid Res., vol. 4, no. 3, pp. 165–176, Sep. 2019, doi: 10.1089/can.2019.0016.

[90] D. B. Finlay, K. J. Sircombe, M. Nimick, C. Jones, and M. Glass, "Terpenoids From Cannabis Do Not Mediate an Entourage Effect by Acting at Cannabinoid Receptors," Front. Pharmacol., vol. 11, p. 359, Mar. 2020, doi: 10.3389/fphar.2020.00359.

[91] W. Häuser et al., "European Pain Federation (EFIC) position paper on appropriate use of cannabis-based medicines and medical cannabis for chronic pain management," Eur. J. Pain Lond. Engl., vol. 22, no. 9, pp. 1547–1564, Oct. 2018, doi: 10.1002/ejp.1297.

[92] F. P. Busardò et al., "Disposition of Phytocannabinoids, Their Acidic Precursors and Their Metabolites in Biological Matrices of Healthy Individuals Treated with Vaporized Medical Cannabis," Pharm. Basel Switz., vol. 14, no. 1, p. 59, Jan. 2021, doi: 10.3390/ph14010059.

[93] A. S. Kleckner et al., "Opportunities for cannabis in supportive care in cancer," Ther. Adv. Med. Oncol., vol. 11, p. 1758835919866362, Aug. 2019, doi: 10.1177/1758835919866362.

[94] H. Meng, T. Dai, J. G. Hanlon, J. Downar, S. M. H. Alibhai, and H. Clarke, "Cannabis and cannabinoids in cancer pain management," Curr. Opin. Support. Palliat. Care, vol. 14, no. 2, p. 87, Jun. 2020, doi: 10.1097/SPC.0000000000000493.

[95] L. E. Ewing et al., "Hepatotoxicity of a Cannabidiol-Rich Cannabis Extract in the Mouse Model," Mol. Basel Switz., vol. 24, no. 9, p. 1694, Apr. 2019, doi: 10.3390/molecules24091694.

[96] M. Dziwenka, R. Coppock, M. Alexander, E. Palumbo, C. Ramirez, and S. Lermer, "Safety Assessment of a Hemp Extract using Genotoxicity and Oral Repeat-Dose Toxicity Studies in Sprague-Dawley Rats," Toxicol. Rep., vol. 7, pp. 376–385, Jan. 2020, doi: 10.1016/j.toxrep.2020.02.014.

[97] D. Harpaz, B. Veltman, Y. Sadeh, R. S. Marks, N. Bernstein, and E. Eltzov, "The effect of cannabis toxicity on a model microbiome bacterium epitomized by a panel of bioluminescent E. coli," Chemosphere, vol. 263, p. 128241, Jan. 2021, doi: 10.1016/j.chemosphere.2020.128241.

[98] Semana, "Medical cannabis, a necessity in science," Semana.com Latest News from Colombia and the World, Apr. 10, 2020. https://www.semana.com/nacion/articulo/cannabis-medicinal.

[99] "Khiron signs agreement with Locatel to distribute medical cannabis Companies Business Briefcase." https://www.portafolio.co/negocios/empresas/ (accessed May 09, 2023).