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Extraction and chemical characterization of bioactive compounds from non-psychoactive *Cannabis sativa* L. and assessment of their antiproliferative activity against human glioblastoma cell lines

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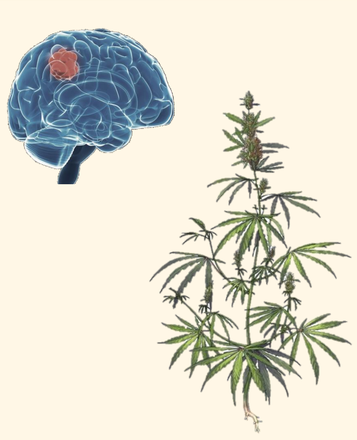
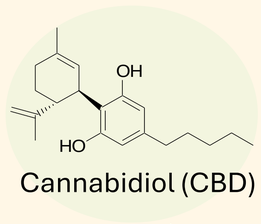
Background and Aim

Glioblastoma multiforme (GBM) is one of the most frequent malignant primary tumours. It is characterized by an average 16-month survival rate, caused by its high proliferation, invasion, migration, angiogenesis and resistance to conventional anticancer drugs. For these reasons it is crucial to find new treatments for GBM [1].

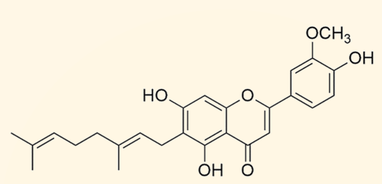
In recent years, the interest in the antiproliferative activity of the natural components of non-psychoactive *Cannabis sativa* L. (hemp) is increasing [2,3]. This plant is mainly composed of three chemical classes: cannabinoids, polyphenols, and terpenes, with cannabidiol, cannflavin A and B and β-caryophyllene, respectively, as representative components [4].

In the light of this, the **aim** of this study was to **obtain**, and fully **characterize**, three different extracts enriched in cannabinoids, polyphenols and terpenes, starting from hemp inflorescences. Then, the activity of the extracts was assessed on U87MG and T98G GBM cell lines, in order to evaluate their **antiproliferative effects** and their possible mechanism/s of action.

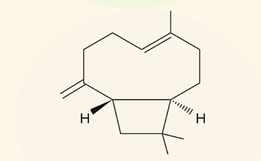




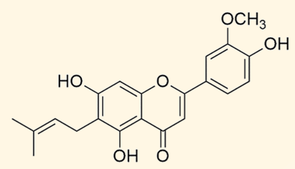
Cannabidiol (CBD)



Cannflavin A (CFL-A)



β-caryophyllene

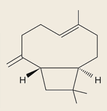


Cannflavin B (CFL-B)

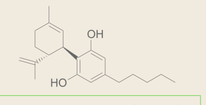
Methods

1. Extraction from hemp inflorescences

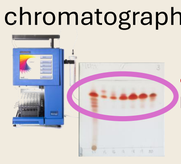
Steam distillation → **TERPENES (TEF)**

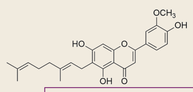


Decarboxylation → Maceration with ethanol → **CANNABINOIDS (CEF)**



Pre-maceration with *n*-hexane followed by maceration with methanol/acetone 90:10 (v/v) + 0.1% formic acid → Preparative chromatography → **POLYPHENOLS (PEF)**





2. Qualitative and Quantitative analyses

CEF and **PEF**


UHPLC-HRMS untargeted analysis


HPLC-UV quantitative analysis

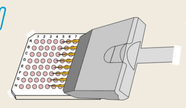
TEF


GC-MS untargeted analysis


GC-FID quantitative analysis

3. Cell viability assays

U87MG and **T98G** glioblastoma cell lines



The absorption was measured at **450 nm**, after the addition of **CCK-8**

Cells treated with **CEF**, **PEF**, **TEF** for **24** and **48** h of exposure

4. Cell migration assay

U87MG cell line treated with **20 µg/mL** of **CEF**

Cells where photographed every 7 minutes for 24 h


ImageJ

Results and Discussion

1. Qualitative and quantitative characterization of the extracts

CEF

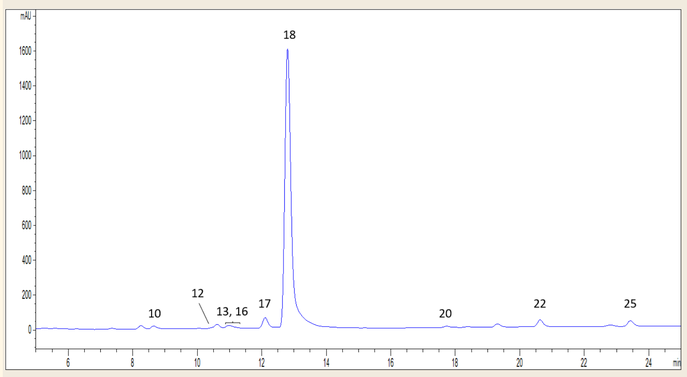


Table 1. Fragmentation pattern and quantitative results of the identified compounds in CEF after UHPLC-HRMS and HPLC-UV analysis

Peak n°	Compound	Rt (min)	Ion mode	MS	MS/MS	mg/g ± SD
10	CBDV	8.24	+	287.2007	287.2008 (100), 231.1383 (17), 165.0912 (45), 135.1169 (18)	5.4 ± 0.1
12	CBDB	9.87	+	301.18	301.2161 (100), 245.1539 (17), 179.1069 (41), 135.1170 (16)	3.6 ± 0.2
13	CBDA	10.29	-	357.207	357.2077 (100), 339.1954 (69), 313.2163 (16), 182.9926 (18)	7.5 ± 0.6
16	CBGA	10.67	-	359.2228	341.0341 (20), 222.0892 (100), 179.0341 (22), 165.0184 (11)	< LOQ
17	CBG	10.88	+	317.2477	193.1226 (100), 123.0445 (14), 135.1170 (10), 183.1021 (7)	11.2 ± 1.2
18	CBD	11.11	+	315.2321	315.2320 (100), 259.1695 (17), 193.1225 (37), 135.1170 (16)	403.1 ± 9.9
20	CBN	12.78	+	311.201	311.2003 (100), 283.1697 (17), 223.118 (17), 213.0911 (12)	3.8 ± 0.1
22	Δ⁹-THC	13.28	+	315.2325	315.2319 (100), 259.1693 (18), 193.1225 (36), 135.1170 (16)	7.2 ± 0.5
25	CBC	13.67	+	315.1962	315.2320 (100), 259.1696 (13), 193.1225 (35), 135.1170 (13)	29.5 ± 1.1
26	Δ⁹-THCA	14.11	-	357.2069	357.2066 (100), 313.2172 (25), 245.1543 (12), 191.1068 (13)	< LOQ

PEF

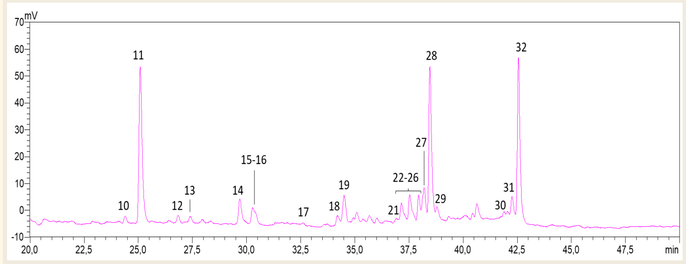


Table 2. Fragmentation pattern and quantitative results of the identified compounds in PEF after UHPLC-HRMS (ESI +) and HPLC-UV analysis

Peak n°	Compound	Rt (min)	MS	MS/MS	mg/g ± SD
11	N-trans-feruloyltyramine	24.9	314.1385	314.1385 (19), 177.0546 (100), 145.0284 (32), 121.0650 (42)	17.7 ± 2.2
27	Demethoxy CFL-B	38.7	339.1277	339.1225 (5), 283.0598 (100), 183.0287 (4), 165.0181 (11)	1.3 ± 0.6
28	CFL-B	38.9	369.1332	313.0704 (100), 298.1469 (15), 165.0182 (9)	8.1 ± 0.3
31	Demethoxy CFL-A	42.8	407.1854	283.0598 (100), 183.0287 (5), 165.0181 (9)	2.7 ± 0.3
32	CFL-A	43.0	437.1958	313.0704 (100), 298.0469 (14), 165.0182 (7)	10.7 ± 0.8

TEF

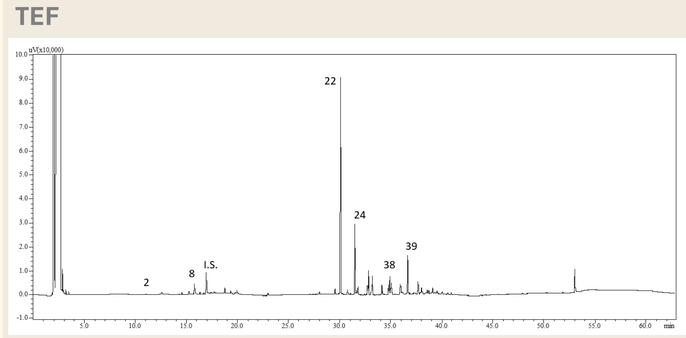


Table 3. Identified compounds in TEF after GC-MS analysis (*) and quantitative results obtained after GC-FID analysis

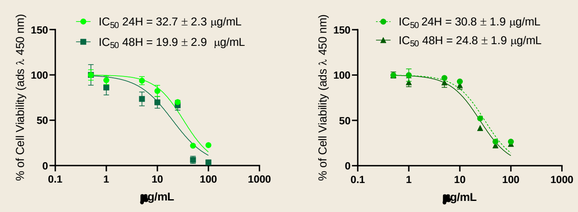
Peak n°	Compound	Rt (min)	MW	mg/mL ± SD
2	β-Myrcene	9.6	136.23	4.5 [a]
8	Linalool	14.2	154.25	24.0 [a]
22	β-Caryophyllene	28.1	204.35	279.5 ± 8.7
24	α-Caryophyllene	29.5	204.35	145.6 ± 1.9
38	trans-Nerodilol	33.9	222.37	27.7 ± 1.9
39	Caryophyllene oxide	34.5	220.35	84.2 ± 4.7

[a] SD < 0.05
(*) compounds were identified with the use of NIST library

2. Cell viability of the extracts

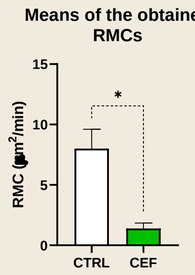
Regarding the extracts, the best results were achieved, in both cell lines, after the exposure to **CEF**
Both **PEF** and **TEF** gave IC_{50} values higher than 100 µg/mL in both cell lines after 24 and 48 h of treatment

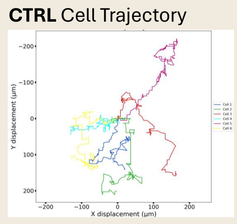
Dose-response curves obtained after the exposure of **CEF** on both cell lines:

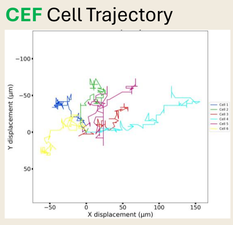


3. Cell migration of U87MG cell line

This test was carried out only on U87MG cells, because T98G do not have the high rate of cell migration between their characteristics



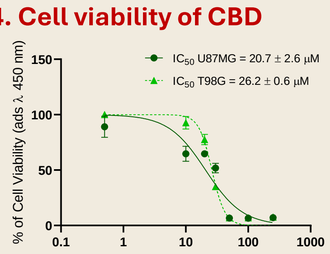




The means (n = 6) of the obtained Random Mobility Coefficients (RMCs) of U87MG cells treated with **CEF,** was significantly lower than the one of CTRL ($p < 0.05$).

In particular, **CEF** dropped the RMC value by 83% and 67%, respectively, in comparison to the RMC mean value of CTRL.

4. Cell viability of CBD



Given the previous results, the cell viability assay was also performed treating both cell lines with pure **CBD**, being it the main compound present in **CEF**.

After 48 h of treatment, **CBD** reduced the cell viability of GBM cell lines. Further studies are then necessary to understand its mechanism of action.

It was already demonstrated that CBD-enriched extracts were able to modify the mechanical properties of cells [2]. Moreover, our migration assay showed a decrease of cell mobility when cells are exposed to cannabinoids. Our data, then, support the hypothesis of the existence of a possible new CBD target involved in cell mobility and migration.

Conclusions

- In this project we were able to obtain and fully characterize three different extracts, obtained from inflorescences, enriched in cannabinoids, polyphenols and terpenes.
- These extracts were then tested for their antiproliferative activity on glioblastoma cell lines, with promising IC_{50} values obtained after the treatment of CEF.
- Even if further research is needed to characterize the mechanism/s of action of CEF and, in particular, of its main component CBD, these results support evidence describing cannabinoids as promising candidates for the study of new poly-pharmacological approaches in the treatment of GBM.

References

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