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Research Article

Design of *Cannabis sativa* (L.) Emulsion. Characterization and Quantification of Cannabinoids and Terpenes

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Abstract

The aim of this study was to design and develop emulsions with cannabidiol-rich ethanolic extract (EE) and essential oil (EO) of *Cannabis sativa* L. as active and coactive herbal ingredients respectively. At the same time, an analytical methodology was developed to quantify the main cannabinoids and characterize the terpenes of the essential oil. Four O/W emulsions were prepared with 1% of EE (1g EE/100 g emulsion) F1, 0.5 % of EE (0.5g EE/100 g emulsion) F2, 0.25 % of EE (0.25g EE/100 g emulsion) F3 and 0.125 % of EE (0.125 g EE/100 g emulsion) F4. The co-active ingredient (EO) was added following the same proportions as the EE. All emulsions, F1, F2, F3, y F4 were found to have acceptable physicochemical characteristics such as pH, viscosity, extensibility, stability. Respect to the organoleptic properties only F1 was no acceptable.

Keywords: Cannabis Sativa; Essential Oil; Emulsion; Cannabinoids; Terpenoids

Introduction

C. sativa L. (Cannabaceae) is an herbaceous plant native to Central Asia, historically used as a source of fiber, food, oil, and medicine [1]. Its therapeutic potential lies primarily in the presence of three main groups of biologically active compounds such as cannabinoids (CB), flavonoids, and terpenoids [2], which can act either individually or synergistically [3]. Depending on the relative composition of these molecules in the plant, various modulatory and potentiating pharmacological effects have been observed [4,5]. A recent classification identified 545 cannabis components organized into chemical classes based on structural similarities [6], of which more than 100 belong to the phytocannabinoid (pCB) class, primarily extracted from female plants. Among the most prominent are Δ9-tetrahydrocannabinol (Δ9-THC), cannabidiol (CBD), and cannabigerol (CBG) [7,8].

There is substantial scientific evidence supporting the anti-inflammatory, anxiolytic, antibacterial, antioxidant, and wound-healing properties of CBD. Furthermore, it has been observed that CBD administered as a full-plant extract provides superior therapeutic outcomes compared to purified CBD, likely due to the pharmacological synergy between the various bioactive compounds present in the plant [9].

The pCB are characterized by high lipophilicity, low aqueous solubility, rapid metabolism, susceptibility to oxidation, and low oral bioavailability. For CBD, oral bioavailability ranges from 13% to 19% [10], due to incomplete absorption and significant hepatic clearance [11]. These limitations have driven the exploration of alternative routes of administration, such as topical and transdermal, which have shown significant advantages: higher bioavailability, prolonged plasma concentrations, and reduced psychoactive effects, particularly in preparations with high THC content, due to passive diffusion through the stratum corneum, the skin's primary barrier [12].

Dermal formulations are categorized into transdermal, which have systemic effects, and topical, which act locally on the skin. Emulsions, as dispersions of immiscible liquids, are particularly relevant in the pharmaceutical industry as they protect components susceptible to oxidation or hydrolysis.

The CB are divided into three groups: endocannabinoids (ECB), pCB, and synthetic cannabinoids. The presence of ECB receptors in the skin has been confirmed in both humans and rodents, and they have been shown to regulate cell growth, differentiation, survival, immune and inflammatory responses, as well as sensory phenomena [13]. Preclinical studies suggest that topical CBD administration is effective in treating skin conditions such as eczema, psoriasis, pruritus, and inflammation [14].

The objective of this study was to design o/w (oil-in-water) emulsions using EE and EO from *C. sativa* inflorescences at different concentrations. Additionally, a simple and robust methodology for the quantification of pCB in herbal ingredients using high-performance liquid chromatography (HPLC) was developed.

Material and Methods

Equipment

Viscosity was measured using a Brookfield brand viscometer model DV-E VIS 01 at 2 rpm. The centrifugation test was performed using a Thermo Scientific-Sorvall ST8 centrifuge with a range between 1000 and 6000 RPM. The pH measurements were performed using pH meter Orion Star A211.

The characterization of the EO was carried out using a Thermo Trace 1300 gas chromatograph coupled to an ITQ900 ion trap mass spectrometer (GC-MS-ITD). The analysis was performed using a DB-5 fused silica capillary column Ohio Valley (5% phenyl, 95% dimethylpolysiloxane, 30 m × 0.25 i.d., film thickness 0.25 µm).

Quantitative pCB analysis was performed on a Waters 1525 HPLC system equipped with a degasser, binary pump, temperature-controlled column compartment, and UV/DAD detector. Empower software was used for instrument control, data acquisition, and processing. All samples were analyzed using a Restek ARC-18 column (150 x 4.6 mm ID; 2.7 µm; 90 Å) equipped with a guard column.

Reagents

The excipients of the emulsions investigated are described in table 1. THC (CAS No.: 3387-41-5, Δ-9-THC: Δ-8-Tetrahidrocannabinol), THCA (CAS No.: 23978-85-0, Tetrahydrocannabinolic acid), CBD (CAS No.: 13956-29-1, Cannabidiol), CBDA (CAS No.: 1244-58-2, Cannabidiolic acid), CBG (CAS No.: 25654-31-3, Cannabigerol), CBGA (CAS No.: 25555-57-1, Cannabigerolic acid) and CBN (CAS No.: 521-35-7, Cannabinol) standards at a concentration of 1000 µg/mL in methanol, in a 1 mL ampoule of the Restek brand were obtained from Jenck S.A., Argentina. The 19 standard cannabis terpenes are composed of (-)-α-Bisabolol (CAS No: 23089-26-1), Camphene (CAS No: 79-92-5), δ-3-Carene (CAS No: 13466-78-9), β-Caryophyllene (CAS No: 87-44-5), Geraniol (CAS No: 106 -1), (-)-Guaiol (CAS No.: 489-86-1), α-Humulene (CAS No.: 6753-98-6), p-Isopropyltoluene (p-cymene) (CAS No.: 99-87-6), (-)-Isopulegol (CAS No.: 89-79-2), d-Limonene (CAS No: 138-86-3), Linalool (CAS No: 106-106-106), Spanish: No: 78-70-6), β-Myrcene (CAS No: 123-35-3), Nerolidol (CAS No: 7212-44-4), Ocimene (CAS No: 13877-91-3), α-Pinene (CAS No: 80-56-8), (-)-β-Pinene CAS °: 127-91-3), α-Terpinene (CAS No: 99-86-5), γ-Terpinene (CAS No: 99-85-4) and Terpinolene (CAS No: 586-62-9), with a concentration of 2500 µg/mL in Isopropanol, in 1 mL ampoule, RESTEK brand (PN 34095) were purchased from Jenck S.A., Argentina. Chromatographic grade solvents methanol, ethyl acetate, absolute ethanol and acetonitrile were purchased from Sigma Aldrich, Argentina. Ammonium formate HPLC grade was purchased from Chemical Center S.R.L., Argentina. Ethanol 96% was obtained from Porta S.A., Argentina.

Components	INCI name	Properties
CERAL EF	Cetearyl alcohol + sodium lauryl sulfate + ceteareth-20	No ionic self-emulsifying wax. Suitable for the production of o/w type liquid emulsions with high water content, obtaining great whiteness. Ideal for the preparation of highly stable emulsions.
VASELINE	Petrolatum	Emolient
LANOLINE	Lanolin	Co-emulsifier
VITAMIN E	Tocopherol	Antioxidant
GLYCERIN	Glycerin	Moisturizing
RM 2051	Sodium polyacrylate, dimethicone, cyclopentasiloxane, trideceth-6 (and) peg/ppg-18/18 dimethicone.	Thickening agent
DERMOCIDE L	Peg-8 + methylparaben + propyl-paraben	Preservative
DIMETHICONE	Dimethicone	Emolient
PURIFIED WATER	AQUA	Vehicle
C. sativa EE	--	Active herbal ingredient
C. sativa EO	--	Co-active herbal ingredient

Table 1: International Nomenclature of Cosmetic Ingredients (INCI) and properties.

Plant material

Fresh female inflorescences of *C. sativa* L. were supplied by the company Madre Kaya S.A., in Nogoli, province of San Luis, within the framework of the collaboration agreement No. 1516 signed with the National University of San Luis (UNSL). For this study, inflorescences from a variety Bobby Buva of *C. sativa* were used, all sourced from the same cultivation greenhouse. These inflorescences were previously analyzed and classified as belonging to chemotype III, characterized by its predominance of CBD over THC.

Obtaining and characterization of EO

Freshly cut female inflorescences were stripped and stored in vacuum sealed bags at -18 °C in a freezer. They were then cut into small pieces (approximately 1 cm in length) yielding 370 g (fresh weight). Steam distillation was performed at 96 °C using a Figmay essential oil extraction apparatus (Figure 1). The process continued until no further EO production was observed. The recovered EO was dried over anhydrous Na_2SO_4 and stored in a sealed glass vial. The sample was stored at -18 °C and kept in the dark until analysis by GC-MS.

Figure 1: Laboratory-scale steam distillation apparatus.

For GC-MS analysis, mass transfer line and injector temperature were set at 240 °C and 220 °C, respectively. The oven temperature was programmed from 60 to 246 °C at a rate of 3 °C min^{-1} and finally raised to 300 °C at 15 °C min^{-1} carrier gas was He (10 psi). A 0.1 µL sample of EO was injected in split mode (1:50). Spectra were acquired in full-scan positive mode. The EO components were identified by comparison of their Linear Retention Index in relation to the homologous series of n-alkanes (C_9-C_{26}) according to Adams [15]. MS fragmentation patterns were compared with those stored in the NIST 2.0 spectra library [16]. A match factor ≥800 was considered when comparing MS spectra with those from NIST according to [17]. Relative amounts of each individual component were expressed as percentages of each peak area relative to the total chromatogram peak area.

Obtaining of EE

The female inflorescences were harvested, and the largest leaves were removed. The inflorescences were then dried under controlled conditions in a cool, dark environment for seven days. Afterward, the samples were ground, and 250 g of this material were used for solvent extraction, utilizing Figmay equipment (Figure 2). Three consecutive extractions were carried out with ethyl alcohol 96%, completely covering the plant material, with a total volume of 4 L of solvent. The EE was filtered with a cellulose filter (Cas-10 permeability 101-200 mDarcys,) collected in a round flask and concentrated in a rotary evaporator at reduced pressure until complete removal of the solvent. Finally, the EE obtained was subjected to decarboxylation by controlled heating in an oven at 120 ºC for 30 minutes. The choice of ethyl alcohol as an extraction solvent is based not only on its extractive efficiency [18,19] but also on its low cost, availability in satisfactory purity grades and mainly its low toxicity. According to the guidelines established by the International Council for Harmonization (ICH), ethanol is classified as a class III solvent, characterized by its low toxic potential for humans and its inclusion in pharmaceutical products with permitted limits of up to 5000 ppm (United States Pharmacopeial Convention, 2019).

Figure 2: Laboratory scale steam distillation equipment Figmay brand (left) and rotary evaporator (right).

Preparation of emulsions

The proposed formulations were obtained by mixing the emulsion base (O/W) with different concentrations of EE of *C. sativa* and EO. All components of the oil phase were heated to 70°C as well as those of the aqueous phase and both phases were mixed with constant stirring up to a temperature of 50°C. The mixture was kept under constant stirring until its complete homogenization at room temperature (Table 2).

Components	Emulsion (O/W) (%)	F1 (%)	F2 (%)	F3 (%)	F4 (%)
C. sativa EE*	---	1	0.5	0.25	0.125
C. sativa EO**	---	1	0.5	0.25	0.125
Ceral F®	5	5	5	5	5
Vaseline	2	2	2	2	2
Lanoline	2	2	2	2	2
Vitamin E	0.5	0.5	0.5	0.5	0.5
Glycerin	3	3	3	3	3
RM 2051®	0.2	0.2	0.2	0.2	0.2
Dermocide L®	0.5	0.5	0.5	0.5	0.5
Dimethicone	2	2	2	2	2
Purified water	84.8	82.8	83.8	84.3	84.55

Table 2: Qualitative and quantitative composition of emulsions.

*1% of EE = 1g EE/100g of emulsion. 0.5 % of EE = 0.5g EE/100g of emulsion. 0.25 % of EE = 0.25g EE/100 g of emulsion. 0.125 % of EE = 0.125g EE/100g of emulsion.

** 1% of EO = 1ml EO/100g of emulsion.

Preparation of standards

From the standard solutions of the 7 cannabinoids (1000 µg/mL), dilutions in methanol were prepared to concentrations of 10, 20, 40, 60, 80 and 100 µg/mL in 2 mL amber glass vials.

Quantification of pCB in EE and emulsions

For the analysis of the EE, 1 g was weighed in a 100 mL volumetric flask, to which 50 mL of methanol was added. The sample was sonicated for 10 minutes at 20°C until complete dissolution, and then filled to the calibration mark. A fraction of the solution was filtered through 0.22 µm PDVF filters and diluted 1:100 with methanol. The sample was stored in refrigeration until analysis.

For the quantification of emulsions, 500 mg of each emulsion (F1, F2, F3 and F4) was weighed into 10 mL volumetric flasks, to which 5 mL of methanol was added. The samples were sonicated for 10 minutes at 20 °C until completely dissolved, and then brought up to volume. A fraction from each flask was filtered through 0.22 µm PDVF filters and diluted 1:10 with methanol. The samples were stored in the refrigerator until analysis.

In both cases, the chromatographic separation was carried out under isocratic conditions (25% A:75% B), with solvent A being water containing 5 mM ammonium formate and 0.1% formic acid, and solvent B being acetonitrile with 0.1% formic acid. The flow rate was set to 1.50 mL/min, and the column temperature was maintained at 30°C. The injection volume was 10 µL, with six washes between samples. Detection was performed at 228 nm. Cannabinoid identification was carried out by co-injection of commercial standards, and each calibration curve point for each cannabinoid was injected in triplicate.

Characterization of emulsions

Sensory analysis

The changes in color, odor, texture, visual appearance, consistency and feel to the touch of each formulation during each stability period were taken into account. (Table 4).

Stability tests

The stability study was carried out to establish the compatibility between the formulation components (intrinsic factors) and the packaging conditions (extrinsic factors). The samples were packaged in neutral, transparent glass bottles with a tight-fitting lid. The emulsions were subjected to stress conditions in order to accelerate the appearance of possible signs of instability (colour change, phase separation and liquefaction). The cycles adopted for the accelerated stability tests were 24 hours at 40 ± 2 ºC and 24 hours at 4 ± 2 ºC, for four weeks (Table 5).

pH analysis

The pH for each of the emulsions was measured at stability time T0, T1, T2 and T3 at room temperature. The pH specification of the formulation was developed according to the literature, where the pH of the skin has been reported to be in an acidic but wide range from 4.0 to 7.0 [20,21].

Extensibility

The extensibility study was carried out on each of the prepared formulations. For the test, a 25 mg sample was used, which was placed in the center of a slide, placed on a millimeter sheet, and covered with another slide previously weighed. The sample was then allowed to stand for one minute and the diameter formed was

measured using the weight of the upper slide. A 2 g weight was then added. The process was repeated and finally a 5 g weight was added. The weight change was performed every minute and the diameter of the sample was measured using a caliper [20,21].

Viscosity

The viscosity of the emulsions (diluted to 5%) was evaluated using a viscometer for approximately 5 minutes.

Statistical analysis

All results were expressed as mean values ± standard deviation (SD) R software (R version 4.1.0 and RStudio version 1.4.1717) was used to determine the level of significance ($p \leq 0.05$) by analysis of variance (ANOVA).

Results and Discussion

The steam distillation of fresh inflorescences of *C. sativa* variety Bobby Buva yielded 6 g of EO per kg of fresh plant material. Qualitative analysis using gas chromatography coupled with mass spectrometry (GC-MS) identified 41 compounds, accounting for 97.81% of the total EO area. Monoterpene hydrocarbons were the predominant group (74.05%), followed by sesquiterpene hydrocarbons (12.27%) and oxygenated terpenes (9.2%) (Figure 3). The most abundant compounds were myrcene (52.9%), α-pinene (10.95%), β-pinene (5.24%), and limonene (4.13%), with trace amounts of cannabidiol (CBD) at 0.34%.

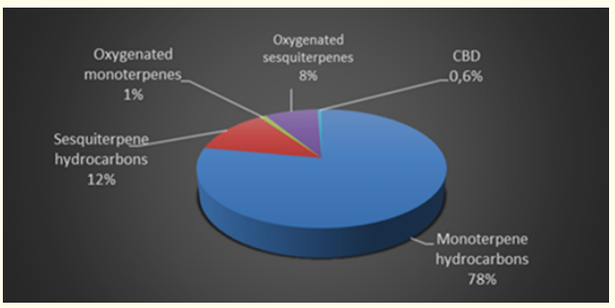

Figure 3: Essential oil (EO) composition.

Ethanolic extraction from 250 g of inflorescences yielded 40.2 g of a dark green, highly viscous resin, resulting in a yield of 160 g/kg of plant material. HPLC-UV/DAD analysis produced linear calibration curves ($R^2 > 0.99$) for all evaluated standards within the range of 10 to 100 µg/mL. The quantitative analysis of cannabinoids in the resin revealed a total content of 59.55% w/w of CBD, 2.99% w/w of THC, and 2.72% w/w of CBG, with a marked predominance of cannabinoids in their neutral forms over their acidic forms. The detailed content of each cannabinoid is presented in table 3.

The stability assessment of the developed emulsions was carried out in order to establish the conditions and times in which these pharmaceutical forms retain their physicochemical properties. During the tests carried out, the pH measurement remained within the specifications corresponding to products for topical application. The sensory analysis was carried out in parallel to the

CB	EE	
	mg/g	% P/P
THCA	18.5	1.85
Δ9-THC	13.7	1.37
Δ8-THC	ND	ND
CBDA	94.4	9.44
CBD	512.7	51.27
CBGA	5.2	0.52
CBG	22.6	2.26
CBN	ND	ND
Potency of THC	29.9	2.99
Potency of CBD	595.5	59.55
Potency of CBG	27.2	2.72

Table 3: Quantitative composition of cannabinoids in EE.

Quantification limit (QL) THC y CBD: 0.02 mg/g

ND: No detected

Potency expresses the sum of each cannabinoids in decarboxylated form.

stability, keeping the characteristic odor constant throughout the study (Table 4).

	F1			F2			F3			F4		
Times	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
Organoleptic characteristics	NA	NA	NA	A	A	A	A	A	A	A	A	A
Stability tests	A	A	A	A	A	A	A	A	A	A	A	A
pH	5,6	5.5	5.4	5.7	5.7	5.8	6.0	6.1	6.1	6.3	6.2	6.2
Extensibility (mm^2)	745	850.3	987.2	760.6	1256	1625.1	841.9	1287.5	2001.9	987.2	1865.1	2680.1
Viscosity (P)	998	998	997	990	989	991	910	912	910	890	889	889

Table 4: Characterized of emulsions.

A: Accepted

NA: No Accepted

T0: initial time. T1: at 1 minute. T2: at 2 minutes. T3: at 3 minutes.

Regarding the color, an intense green color (F1), intermediate green (F2), light green (F3) and lighter yellowish green (F4) were observed (Figure 4). The viscosity had small variations in accordance with the small modifications of the formulas. The concentration of pCB in the final emulsions (Table 5) showed a relationship with the amount of ethanolic extract (EE) used in the formulation.

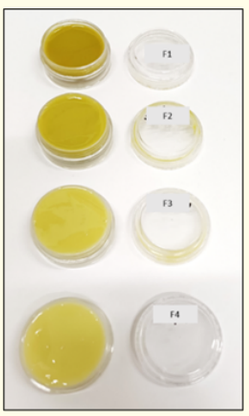

Figure 4: Emulsions.

Conclusion

This study shows the development of stable emulsions containing a cannabidiol-rich ethanolic extract and essential oil of *C. sativa*, which preserved the integrity of the active components. Although formulation F1 presented some limitations in terms of its organoleptic properties due to the high concentration of extract

CB	F1			F2			F3			F4		
	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
THCA	0.21	0.22	0.2	0.09	0.09	0.08	0.06	0.07	0.07	0.04	0.05	0.05
Δ^9–THC	0.18	0.16	0.17	0.08	0.06	0.04	0.05	0.06	0.03	0.02	0.03	0.02
Δ^8–THC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CBDA	1.09	1.08	1.09	0.56	0.57	0.56	0.29	0.3	0.3	0.14	0.16	0.15
CBD	5.43	5.45	5.41	2.74	2.73	2.75	1.41	1.43	1.38	0.76	0.73	0.74
CBGA	< QL	< QL	< QL	< QL	< QL	< QL	< QL	< QL	< QL	< QL	< QL	< QL
CBG	0.31	0.32	0.29	0.15	0.15	0.14	0.07	0.05	0.06	0.04	0.05	0.03
CBN	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Potency THC	0.36	0.35	0.35	0.16	0.14	0.11	0.1	0.12	0.09	0.06	0.07	0.06
Potency CBD	6.39	6.4	6.37	3.23	3.23	3.24	1.66	1.69	1.64	0.88	0.87	0.87
Potency CBG	0.31	0.32	0.29	0.15	0.15	0.14	0.07	0.05	0.06	0.04	0.05	0.03

Table 5: Quantitative composition of cannabinoids in emulsions.

Quantification limit (QL) THC y CBD: 0.02 mg/mL

ND: No detected

Potency expresses the sum of each cannabinoids in decarboxylated form

T0: initial time. T1: 3 months. T2: 6 months. Results expressed in mg/g.

and essential oil, the other formulations exhibited suitable physicochemical characteristics.

It is important to highlight the value of using whole plant extracts, which are not only rich in CBD but also contain other bioactive compounds that may act synergistically, enhancing both the therapeutic efficacy and safety of the formulations. This comprehensive approach to utilizing the natural components of *C. sativa* broadens the potential for developing more effective products for the treatment of skin pathologies.

Furthermore, the development of efficient extraction methods is crucial to achieve higher concentrations of cannabinoids and other active compounds, thereby optimizing the yield and quality of the formulations. In addition, the implementation of precise analytical methodologies is essential to ensure that these products meet regulatory standards, guaranteeing safety and efficacy in pharmaceutical use.

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Conflicts of Interest

The authors declare no conflict of interest.

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