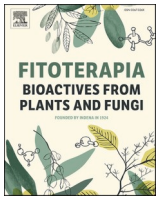
Fitoterapia 177 (2024) 106128

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote



Long-term stability and bactericidal properties of galenic formulations of *Cannabis sativa* oils

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ARTICLE INFO

Keywords:
Long-term stability
Cannabis oil
Galenic formulations
Total polyphenol content
Antioxidant capacity

ABSTRACT

The long-term stability in real and accelerated time for galenic oils based on full-spectrum cannabis has been studied, using sesame oil as a dilutant. Sesame oil is one of the most used vehicles in the cannabis pharmaceutical industry due to the costs and increased oral bioavailability of cannabinoids. The real-time assays conducted at 25 °C over twelve months demonstrated high stability and showed no significant changes in the composition of cannabinoids, total polyphenols, flavonoids, or antioxidant capacity. In these studies, it was observed that there was no development of microorganisms compromising the stability of the oils over a year. The three oil varieties exhibited a high bactericidal capacity against *E. coli, S. aureus*, and *P. larvae.*

1. Introduction

Cannabis sativa is a plant used medicinally and recreationally since ancient times. It represents a true pharmacological plethora, likely due to the abundance and heterogeneity of chemical components with extensive biological activities that act synergistically to address numerous pathologies and ailments such as epilepsy, anxiety, chronic and neuropathic pain among others [1–4]. It is also clinically used for alleviating cancer medication nausea, multiple sclerosis symptoms, cancer cell proliferation, insomnia, neurological disorders and Crohn's disease, all with different levels of evidence [5–10].

Regarding antimicrobial capacity, cannabinoid compounds such as cannabidiol (CBD) and tetrahydrocannabinol (THC) have been reported to have antimicrobial activity against numerous bacterial species [11,12]. Likewise, in *Cannabis sativa*, a multitude of non-cannabinoid compounds have been identified that also present antimicrobial activity or that can interact synergistically, improving the biological properties of cannabis formulations [13,14]. These properties are significant due to the growing need for new natural alternatives to synthetic antibiotics. This is especially important in those cases where antibiotics cannot be used either due to prohibition or the emergence of drug-resistant strains. For example, in beekeeping, the use of the antibiotic oxytetracycline hydrochloride has been prohibited for treating *Paenibacillus larvae*, the bacteria responsible for American foulbrood disease [15].

The modern medical acceptance of the palliative or medicinal properties of cannabis has led to numerous modifications in the regulatory frameworks of most countries, resulting in the global development of cannabis-based medicinal products. Nevertheless, many aspects of the medicinal use of certain pharmaceutical forms such as full-spectrum oils, obtained from inflorescences, have yet to be formalized or standardized. In a few countries, such as Italy, cannabis-based compounding preparations are standardized. The Italian Society of Preparatory Pharmacists (SIFAP) recommends carrying out prior decarboxylation to increase potency (neutral cannabinoids) and subsequent extraction in olive oil [16].

In Argentina, law 27,350, recently approved by Congress, acknowledges the need to research the *Cannabis sativa* plant, its properties, and

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https://doi.org/10.1016/j.fitote.2024.106128
Received 27 January 2024; Received in revised form 4 July 2024; Accepted 15 July 2024
Available online 24 July 2024

everything related to its potential application in human medicine [17,18].

According to the Argentine Pharmacopoeia [19], expiration dates for magistral preparations will be determined based on the physicochemical and galenic characteristics of the product, as well as established reference bibliographies when available. In the absence of a compiled monograph for a specific galenic formulation, and lacking evidence of degradation or contamination risk, maximum expiration dates will be assigned following the inherent properties of the products. For non-aqueous officinal formulations that do not require sterility, a maximum shelf life of 90 days at controlled room temperature or under refrigeration, is stipulated. Given that galenic formulations are prepared on a personalized basis, and considering the immense variety of cannabis plants with different chemical profiles, and the great stability shown by the oils over a year of monitoring, it is not necessary to determine the maximum duration of these formulas, as they are not expected to be shelf-sold medicines but rather formulations upon delivery of an individual prescription. However, since a shorter stability period of 14 days [20] and 8 months with and without antioxidant addition [21] have been reported for cannabis formulations in olive oils, we evaluated a formulation based on sesame oil aimed at standardizing a long-lasting and definitive formulation. Due to the high quantity and heterogeneity of components in the full-spectrum galenic oil, it is not appropriate to conduct stability and expiration date studies strictly according to the International Council of Harmonization (ICH) and pharmacopoeia guidelines, nevertheless, we conducted a stability study taking into account the conditions established by the ICH, as a reference [22]. ICH stability criteria involve conducting analyses of drug substances under various storage conditions, i.e., long-term tests performed during 12 months at 25 °C ± 2 °C/60% RH ± 5% RH and accelerated tests for 6 months at 40 °C ± 2 °C/75% RH ± 5% RH. The variables studied for three types of cannabis varieties, namely chemotype I, with high amounts of THC (THC: CBD $>>$ 1), II with balanced amounts of THC and CBD, (THC: CBD = 1, acceptable between 0.5 and 2) and III with high CBD content (THC: CBD $<<$1) [23] in this work were: total THC and CBD potency, neutral cannabinoids (THC and CBD), total polyphenol content (TPC), total flavonoid content (TFC), microbiological stability, antioxidant capacity expressed as Trolox Equivalent Antioxidant Capacity (TEAC), and antimicrobial capacity. This study represents the first investigation into the long-term stability of full-spectrum cannabis galenic oils, evaluated under both real-time and accelerated conditions. It addresses the critical need for information regarding the longevity, stability, and preservation methods of galenic and artisanal oils, which are extensively utilized worldwide. We used sesame oil as a diluent, one of the most commonly used vehicles in the cannabis pharmaceutical industry due to its properties, including solubility, preservation, and enhanced oral bioavailability of cannabinoids [31].

2. Experimental sections

2.1. Reagents and drugs

2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich®. Solvents were HPLC grade and other chemicals were of analytical grade. The following standard compounds, purchased from Sigma-Aldrich®, were used in this study: gallic acid (GA, purity≥97.5%), quercetin (Q, purity≥95%) and Trolox (purity of 97%). Standard solutions of cannabidiol tetrahydrocannabidiol and cannabinol (CBN) were purchased from Restek.

The inflorescences were provided by the CBG 2000 NGO from Mar del Plata City, Buenos Aires, Argentina, and characterized by the MDQ Herbarium of Vascular Plants of the Plant Diversity Laboratory of the Marine and Coastal Research Institute (IIMyC) of the National University of Mar del Plata (voucher number IMyCHer: MDQ: 00871 (chemotype I) MDQ:00630 (chemotype II) and MDQ: 00870 (chemotype III).

The vehicle used was gluten-free Nutrasem® Premium Toasted Sesame oil, purchased from NutraSem S. R.L.

2.2. Oil preparation

The oils were prepared by extraction by maceration in pharmacopoeia-grade ethanol. Twenty grams of freshly cut inflorescences, frozen overnight, were finely chopped and placed in 1 l of ethanol with magnetic stirring for 4 h. The filtrate was dried using a rotary evaporator. Subsequently, the resins were completely decarboxylated in a balloon with a refrigerant in a glycerin bath at 130 °C for half an hour, until no acidic cannabinoids were detected in TLC. The resin was diluted 1 in 50 *w*/w and stirred until a translucent solution was obtained. Samples were stored for 12 months at 25 °C and 6 months at 41 °C in a San Jor cultivation oven model SL3C from 20 to 70 °C with a humidity monitor. The three types of oils were prepared in quadruplicate for each temperature tested.

2.3. Quantification of THC and CBD

CBD and THC were quantitatively analyzed by HPLC, using a chromatograph Konik KNK-500-A with a UV–visible detector (Konik UVIS-200) with a reverse-phase Zorbax SB-Aq column 4.6 mm ID x 250 mm, 5 μm. A mixture of (85:15) methanol and ultrapure water with a flow rate of 1 mL min^{-1} was used as the mobile phase and the detection was performed at l = 220 nm (Saingam and Sakunpak 2018). The neutral CBD and THC concentration was determined using the external standards calibration curve method. All experiments were carried out at room temperature. GC–MS analysis was performed using a Shimadzu GCMS-QP2100ULTRA-AOC20i with a column of 0.25 mm ID, 30 m and 0.1 μm phase thickness Zebron ZB-5MS. Samples were injected at splitless and the injection volume was 1 ml. The interface and the ionization source were kept at 280 °C and 230 °C respectively. Helium chromatographic grade (99.9999%) was used as the carrier gas with a linear velocity of 52.1 cm seg^{-1}. The oven temperature program started at 50 °C, where it was held for 1 min and then increased to 190 °C at 40 °C min^{-1}, and to 280 at rate 10 °C min^{-1}, finishing at 300 °C at 40 °C min^{-1} where it was held for 2 min. Electron impact ionization (EI) was used at 70 eV. Full-scan and the SIM EI spectra were acquired under the following conditions: mass range 35–700 *m/z*, scan time 0.3 s, solvent delay 3.0 min. Ions used for characterization were: Δ9-THC: 299, 314 and 231. CBD: 231, 246 and 314. CBN: 295, 296, 238. Δ8-THC: 258 and 299. The total Δ9-THC, CBD and CBN content (potency) was quantified using analytical standards and the calibration curve method**.** Dilution of oils for both measurements was made 1:1000 in a mixture 3:7 ethyl acetate:methanol. Results for each measure are the mean of the values obtained for three independent processes.

2.4. Total polyphenol content

Quantification of total polyphenol was performed as reported by Torres et al. (2018), with modifications [24]. An Epoch BioteK microplate reader with a working range of 200 to 900 nm was used. 20 μL of cannabis oils (or sesame oil used as control), 100 μL of Folin-Ciocalteu reagent, and 80 μL of a Na_2CO_3 saturated solution were added and 45 min later, absorbance was read at 760 nm. Total polyphenol content was informed as μg of gallic acid equivalents (GAE) per mL of extract. The values shown are the mean of four measures.

2.5. Total flavonoid content

Total flavonoid content was measured according to Matejic et al. with some modifications [25]. 100 μL of cannabis oils (or sesame oil vehicle as control), were mixed along with 100 μL of $AlCl_3$ 2% in

methanol:acetic acid 95:5. After 45 min at room temperature, absorbance was read at 420 nm. Quercetin (Q) was used as a standard for the calibration curve and flavonoid content was expressed as μg of quercetin equivalents (QE) per mL of extract. The values shown are the mean of four measures.

2.6. Antioxidant capacity

The antioxidant capacity was determined according to Dudonné et al. [26]. Briefly, a 7 mM ABTS solution was mixed with a 140 mM $K_2S_2O_8$ solution. This mixture was allowed to react at room temperature for 16 h in the dark. a 10 μL aliquot of cannabis oil (or control) and 180 μL of ABTS were added to each well in a microplate. Absorbance was read at 734 nm after 45 min at room temperature. Trolox was used as standard. Values are shown as μg of trolox equivalent (TEAC) per mL of extract. The values shown are the mean of four measures.

2.7. Antimicrobial activity

The minimum inhibitory concentration (MIC) of cannabis oils after 12 months of storage at 25 °C was evaluated using the broth microdilution test in a 96-well microtiter [27]. To elucidate the biological properties of the extracts, we assessed their antimicrobial activity against Gram-negative (*Escherichia coli* ATCC 25922) and Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *P. larvae* ERIC I genotype). *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were obtained from the stock cultures of the Department of Chemistry and Biochemistry of the National University of Mar del Plata. *P. larvae* (ERIC I genotype) were obtained from the Research Institute in Health, Production, and Environment (IIPROSAM).

The oil samples were dissolved in 10% dimethyl sulfoxide (DMSO), and a series of two-fold dilutions were dispersed in the culture medium to achieve various final concentrations (46 to 1.44 $mgmL^{-1}$) in the wells. Mueller-Hinton broth was used as culture media for the strains *E. coli* 25922 and *S. aureus* ATCC 25923, while Mueller-Hinton broth supplemented with yeast extract, glucose, and sodium pyruvate was used for *P. larvae* (genotype ERIC I). Finally, a portion of the bacterial suspensions of the strains (10^5–10^6 $CFUmL^{-1}$) was prepared in physiological solution and added to each well. All microtiter plates (with positive and negative controls) were incubated at 35 ± 0.5 °C for 24–48 h, depending on each strain. The MIC was determined as the lowest concentration that inhibits the visible growth of each strain. The minimum bactericidal concentration (MBC) was determined by subculturing the broth dilutions from the microdilution test. The dilutions in which bacterial growth inhibition was determined were plated on Mueller–Hinton or Mueller–Hinton agar supplemented with yeast extract, glucose agar, and sodium pyruvate and incubated for 24 to 48 h, depending on each strain. The MBC was determined to be the lowest dilution of antimicrobials that prevents the growth of the microorganisms on the agar.

2.8. Evaluation of the shelf life of cannabis oils

To evaluate the shelf life of cannabis oil, samples obtained at different storage times and temperatures were analyzed. The samples were stored at different controlled temperatures and humidity of 60 ± 5%: 1) room temperature (25 ± 2 °C) and 2) growing stove (41 ± 2 °C). Microbiological counts of the samples were performed at different time intervals (0, 3, 6, 8, 10 and 12 months). Microbiological tests were conducted to ensure the microbial safety of the product over one year of storage, following the regulations established by the National Administration of Medicines, Food, and Medical Technology of Argentina (ANMAT) for non-mandatory sterile pharmaceutical products, in the form of non-aqueous preparations for oral use. Ten ml of the sample was homogenised in 90 ml of sterile phosphate buffer solution at pH 7.2. Serial dilutions were prepared by adding 1 ml to 9 ml of phosphate buffer pH 7.2. An aliquot of each dilution was plated on Casein-Soy Digest Agar (Britania, Buenos Aires) to determine the total aerobic count (35 ± 0.5 °C, 48 h). To identify the presence of *E. coli*, samples were plated on MacConkey Agar. Suspicious colonies should be subsequently inoculated on Eosin-Methylene Blue Agar (35 ± 0.5 °C, 48 h). To evaluate the total moulds and yeast count, samples were plated on Dextrose-Sabouraud Agar (Britania, Buenos Aires) and incubated at 25 °C for 5–7 days.

2.9. Statistical analysis

The concentration data were analyzed with a non-parametric Kruskal–Wallis's test using the Infostat statistical software, version 2008 [28]. The values of MIC were also evaluated with a non-parametric Kruskal-Wallis's test using SPSS 15.0 (SPSS Inc., Chicago, Ill., USA) for Windows [14] In all cases, significant levels were defined at $p < 0.05$.

3. Results and discussion

3.1. Long-term chemical characterization

The results of the chromatographic analyses by GC–MS are presented in Fig. 1. As observed, for both real and accelerated-time assays, no significant differences were observed in terms of cannabinoid potency (total CBD and THC). This fact was confirmed by HPLC, comparing values at different times as indicated by the ICH (Fig. 2). No CBN or Δ8-THC were detected at any time by the SIM method in GC–MS. This is probably due to the inhibition of the THC oxidation or isomerization in the formulation. Non-aqueous formulations have less solubilized oxygen, and this combined with the antioxidant properties of many compounds present in the full spectrum oil, diminishes or inhibits non-enzymatic transformation of cannabinoids such as THC into CBN or the Δ8-THC isomer [29–31].

Sesame oil is primarily composed of long-chain fatty acids, including saturated fatty acids (stearic and palmitic) and unsaturated fatty acids (oleic (18:1) and linoleic (18:2)) which are known to enhance oral bioavailability [32,33]. It is used as a substitute for olive oil due to its high content of unsaturated fatty acids, approximately 85%. Additionally, it contains tocopherols, which provide high oxidative stability and other antioxidant components such as sesamol, semolin, and sesamin [32,34]. This unique composition likely contributes to the greater stability of the formulation components compared to formulations using olive oil. Furthermore, the stability and preservative properties of polyphenols and flavonoids, which are widely used as additives in the food, cosmetic, and pharmaceutical industries, enhance the overall stability of the formulation [35].

Likewise, the three types of oils maintained stable determinations of total polyphenols and flavonoids, as well as their antioxidant capacity (Fig. 3).

The determination of total flavonoids was carried out in the three types of oils, under the indicated conditions. As observed, the concentration of flavonoids remained largely unchanged throughout both the six and twelve-month trials. The flavonoids present in *Cannabis sativa*, including canflavins A, B, and C, exhibit neuroinflammatory and neuroprotective activities [36], as well as antioxidant properties similar to those of epigallocatechin, quercetin, and kaempferol, which are also present in *Cannabis sativa* L [37]. Additionally, these flavonoids are known to interact with the production or release of prostaglandin E2 (PGE2), contributing to their notable anti-inflammatory, neuroprotective, and antiproliferative properties, among other bioactivities [38]. These characteristics provide value both as a preservative and a pharmacological ingredient. .

Total polyphenols in cannabis were also determined [39], showing no significative changes along the experiments at 25 °C, however, a decrease of approximately 20% was observed in the accelerated time experiment. This is following several authors that suggest polyphenol stability decreases when rising temperatures [40]. We can infer that the

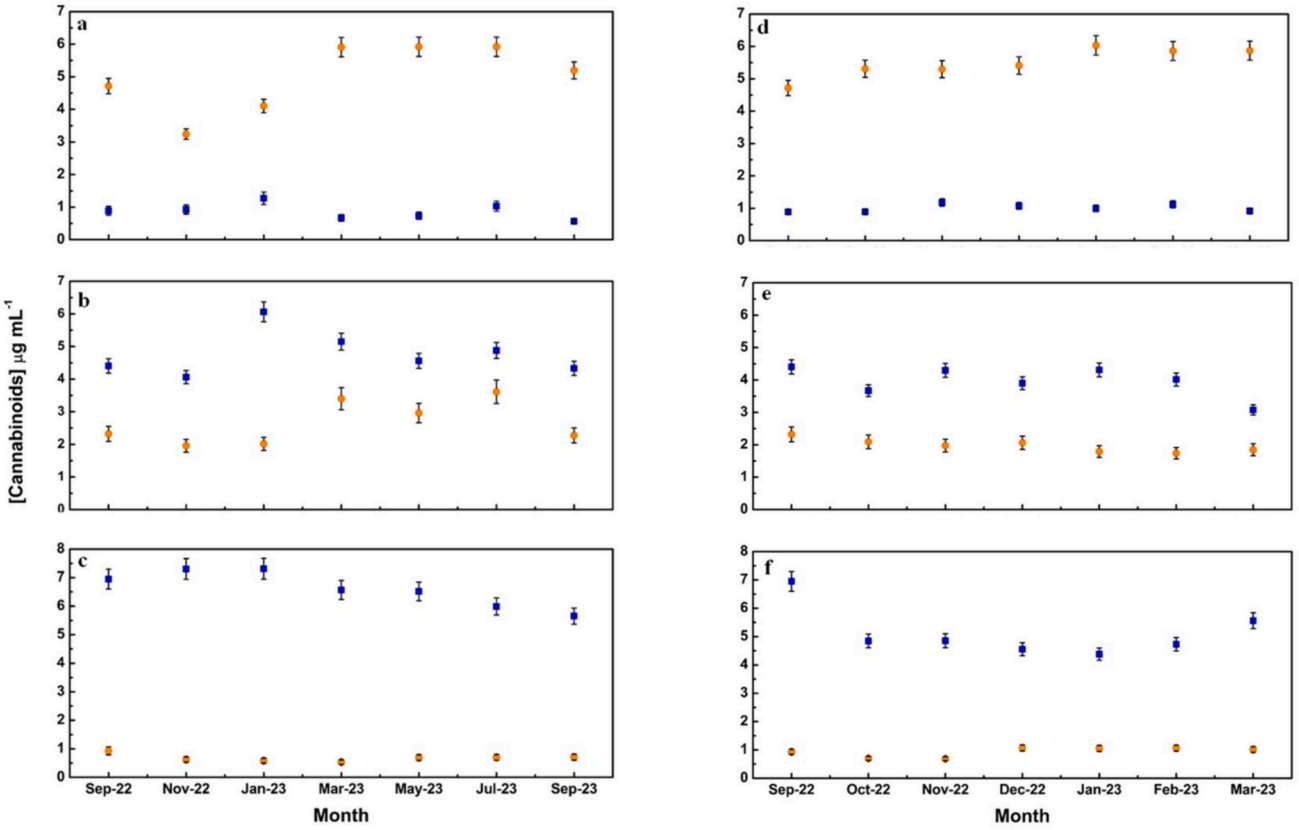

Fig. 1. Concentration profile of cannabinoids as a function of time (months) and at two temperatures. The graphs in the left column were made at 25 °C and those on the right at 41 °C. ● THC and ■ CBD. Chemotype I: a and d; Chemotype II: b and e; Chemotype III: c and f.

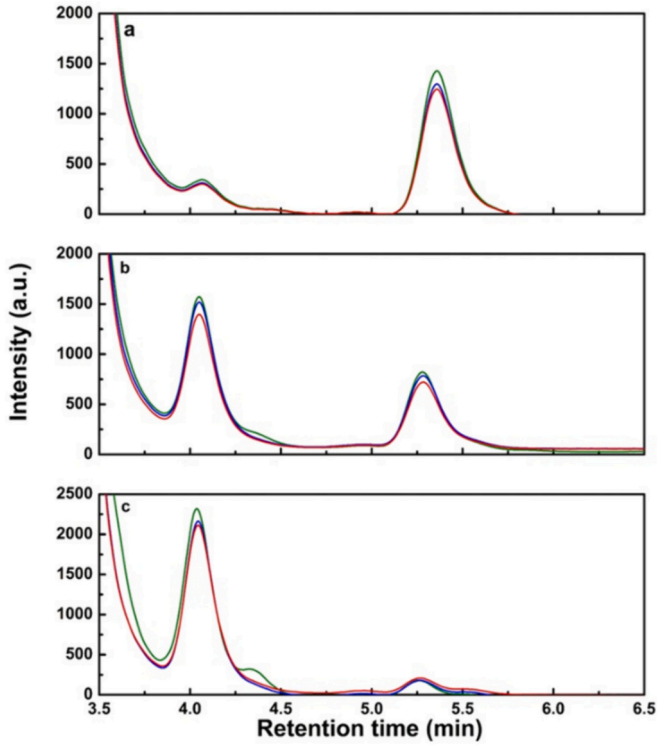

Fig. 2. HPLC chromatograms of the different cannabis oils obtained at the temperatures under study. — initial state; — 25 °C after 12 months and — 41 °C after 6 months. a) Chemotype I, b) Chemotype II and c) Chemotype III. $tR_{(CBD)}$ = 4.04 min and $tR_{(THC)}$ = 5.26 min. Detection at 220 nm.

decrease in total polyphenol content might be due to compounds other than CBD since we did not observe a decrease for this later. According to the extensive work done by Wishart et al., catechin is one of the major polyphenols present in cannabis, along with cannabidiol. It is related to a broad spectrum of biological activities such as angiogenesis, regulation of cell growth, cancer, and related disorders. It has also been shown to mediate cardiovascular protection, anti-inflammatory, and anti-proliferative effects, among others; which are likely attributed to its antioxidant capability [41]. Other polyphenol present in cannabis at elevated rates, such as 3,4-dihydroxybenzoic acid, also with high anti-oxidant activity, have been related to anti-inflammatory, anti-hyperglycemic, and antimicrobial activities [42]. Regarding antioxidant properties expressed in TEAC, Pino et al. inferred that $ABTS^{\cdot+}$ undergoes H-atom transfer (HAT) with flavonoids and phenolic compounds. Therefore, in cannabis oils, antioxidant capacity is primarily attributed to the HAT mechanism [43]. In agreement with their postulate that antioxidant capacity results synergistically from cannabinoids and all phenolic compounds present in cannabis oils, we also obtained consistent results for the three oil varieties, despite their differing chemotypes. The results of TEAC for our dilutions are in good agreement with the values reported by Pino et al. for undiluted pure resins obtained through very different methods (TEAC = 3 ± 1 mM for our oils, and 0.56–0.75 for solid–liquid extraction (SLE) and supercritical fluid extraction (SCF)). Gruschow [44] reported total polyphenol content for different oils direct extraction, namely hemp, olive and MCT oils. Our values for TPC and TEAC resulted in higher, probably because of the difference in extraction methods. Ethanol is a better solvent than oils for polyphenolic polar compounds from plant material. TEAC measured at 25 °C did not show a significant decrease. Nevertheless, following the decrease in total polyphenol content at 41 °C, the antioxidant capacity also showed a slight reduction during the six-month experiment. In this case, the antioxidant capacity was reduced by 15% to 25%. Although these values

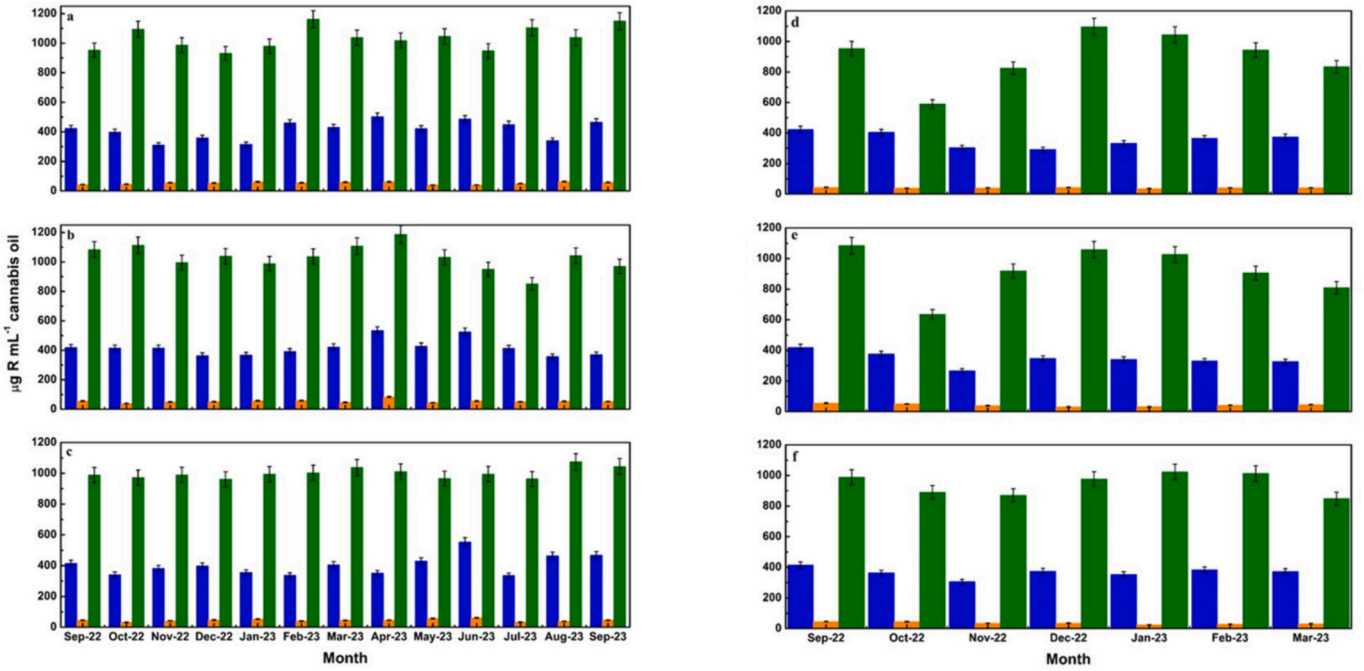

Fig. 3. Comparison of the polyphenol and flavonoid content and antioxidant capacity of cannabis oils as a function of time (months) at different temperatures. The graphs in the left column were made at 25 °C and those in the right at 41 °C. Where R represents: ● µg of gallic acid equivalents (GAE) per mL of extract; ● µg of quercetin equivalents (QE) per mL of extract; ● µg of Trolox equivalents (TEAC) per mL of extract. Chemotype I: a and d; Chemotype II: b and e; Chemotype III: c and f.

are expected at higher temperatures (60 °C) [45], we believe that exposure at 41 °C, albeit at a slower rate, could lead to similar outcomes over six months of exposure. Larrauri et al. suggested that the oxidation of polyphenols at high temperatures may also contribute to a decline in TEAC [46].

Terpenoids were not quantified in this study due to their presence in quantities approximately one hundred times smaller than cannabinoids [35], given the considerable variability among different varieties as well as changes occurring during inflorescence trimming and production methodologies [47]. We do not believe these compounds significantly alter the quality of the oils, let alone their pharmacological properties.

3.2. Evaluation of the shelf life of cannabis oils

The shelf life of cannabis oils was also evaluated. Microbiological tests were carried out to guarantee the microbial safety of the product during one year of storage. Microbiological quality analyses included total aerobic count, total combined moulds and yeast count, and investigation of *E. coli*. The results of the microbiological examination are shown in Table 1. At the beginning of storage of the cannabis oil samples with different cannabinoid content, a total aerobic count of <1 $CFUmL^{-1}$ was obtained, while *E. coli* was absent in all of the samples analyzed. In addition, values below 10^2 $CFUmL^{-1}$ were obtained for the total moulds and yeast count. In the following storage times of 3, 6, 8, 10 and 12 months, the growth of total aerobic bacteria, *E. coli* moulds and yeast were not detected in the oils incubated at 25 °C. The growth of these microorganisms was also not detected in the samples incubated at 41 °C for 3 and 6 months of storage.

The route of administration and the way of use of *C. sativa* formulations are decisive in establishing microbiological safety. In microbiological quality studies of cannabis products, bacterial indicators are generally studied as indicative of hygiene care during product production. According to the guideline for non-aqueous preparations for oral use from ANMAT up to 10^3 $CFUmL^{-1}$ total aerobic count, and 10^2 $CFUmL^{-1}$ total moulds and yeasts count, are allowed, while *E. coli* must be absent in cannabis oil samples. From the results obtained at the beginning of storage, all the samples accomplished the requirements of the legislation and were within the tolerance limits suitable for use as oral preparation. During storage for 1 year at 25 °C samples with different cannabinoid contents remained within the limits established by regulations for these products. Likewise, the different cannabis oils maintained a microbiological quality within the limits at 41 °C for six months.

Total aerobic bacteria are indicative of the manufacturer's sanitary quality and compliance with good manufacturing practices, but may not directly correlate with the presence of pathogens [48]. Likewise, the

Table 1
Number of microorganism colonies in oil cannabis samples at different temperatures and storage times.

		Total aerobic count ($CFUml^{-1}$)			Total moulds and yeast count ($CFUml^{-1}$)			*Escherichia coli* ($CFUml^{-1}$)		
Chemotype		I	II	III	I	II	III	I	I	III
25 °C	0 months	<1	<1	<1	5	10	5	n. d.	n. d.	n. d.
	3 months	<1	<1	<1	<1	<1	<1	n. d.	n. d.	n. d.
	6 months	<1	<1	<1	<1	<1	<1	n. d.	n. d.	n. d.
	8 months	<1	<1	<1	<1	<1	<1	n. d.	n. d.	n. d.
	10 months	<1	<1	<1	<1	<1	<1	n. d.	n. d.	n. d.
	12 months	<1	<1	<1	<1	<1	<1	n. d.	n. d.	n. d.
41 °C	0 months	<1	<1	<1	5	10	5	n. d.	n. d.	n. d.
	3 months	<1	<1	<1	<1	<1	<1	n. d.	n. d.	n. d.
	6 months	<1	<1	<1	<1	<1	<1	n. d.	n. d.	n. d.

Abbreviations: CFU: colony forming units; n.d.: not detected.

limits established for the count of moulds and yeast, differ according to the type and use of the product [49]. For aqueous preparations for oral use, the limit for the count of total aerobic bacteria is 10^2 CFUmL^{-1}, while for the count of moulds and yeasts, up to 10^1 CFUmL^{-1} is accepted. This limit is maintained for use via oromucosal, gingival, cutaneous, nasal, auricular, vaginal and transdermal patches, while for the rectal administration route, the limit of 10^3 CFUmL^{-1} for total aerobic bacteria and 10^2 CFUmL^{-1} for moulds and yeasts is accepted.

On the other hand, *E. coli* is a bacterium commonly found in the intestines of many animals, including humans, and it is typically not associated with disease, except for specific toxin-producing strains such as O157:H7:. While *E. coli* is susceptible to high temperatures, pathogenic strains are rare and they do not thrive under conditions of low water activity or refrigeration temperatures. Since cannabis is dried and heated during processing, it is not a likely vehicle for *E. coli* infection except in cases of fresh consumption [50]. Argentinian legislation stipulates the absence of *E. coli* in non-aqueous preparations for oral use and regulates the absence of other bacteria such as *S. aureus* and *Pseudomonas aeruginosa* in cannabis products intended for administration via oromucosal, gingival, cutaneous, nasal, auricular, and transdermal patches. Additionally, *Candida albicans* must be absent in products intended for vaginal use.

3.3. Antimicrobial activity of cannabis oils

In the present study, the antimicrobial activity of the three different oils after 12 months of storage at 25 °C was studied against strains *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *P. larvae* (ERIC I genotype) (Table 2). All cannabis oils showed antimicrobial activity against the three strains. MICs of cannabis oil to prevent the growth of *E. coli*, *S. aureus* and *P. larvae* were significantly similar for the different oils with dissimilar cannabinoid contents and between strains (P>0.05). On the other hand, for *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, MBC was 46 mgmL^{-1}, while for *P. larvae* ERIC I MBC were between 2.87 and 5.75 mgmL^{-1}.

The antimicrobial activity against the pathogenic bee bacteria, *P. larvae* ERIC I genotype presented a MIC of 2.87 mgmL^{-1} in the oil with the highest CBD content (chemotype III) and 5.75 mgmL^{-1} for the oils with the highest THC content (chemotype I) or CBD/THC in equilibrated parts (chemotype II). Fuentes et al. (2023) [14] studied the antimicrobial activity of cannabis hexane extracts against *P. larvae* strains, obtaining MIC values of 1.13 mgmL^{-1} for root extracts. On the other hand, extracts from other plants against *P. larvae* have demonstrated antimicrobial activity with MIC values of 385 mgmL^{-1} for extracts of *Citrus paradisi* [51] and 25 mgmL^{-1} for extracts of *Scutia buxifolia* [52].

Likewise, the antimicrobial activity of cannabis oil was observed against *E. coli* ATCC 25922, with a MIC of 23–46 mgmL^{-1}. Kaur et al. [53] determined a MIC of 50 mg mL^{-1} for methanol extracts of *C. sativa* against *E. coli*. and Ferrante et al [54] obtained MIC values of 7.1 mgmL^{-1} for water extracts against *E. coli* ATCC 10536. Although the low susceptibility of *E. coli* and other Gram-negative bacteria to *C. sativa* extracts has previously been reported [55], the present results are promising as a potential treatment for this group of bacteria.

Regarding the antimicrobial activity of the oils against *S. aureus* ATCC 25923, MICs of 11.5 to 46 mgmL^{-1} were determined. Zengin et al. [56] determined MIC of 8 mgmL^{-1} for essential oils from leaves, inflorescences, and thinner stems against *S. aureus*, while Fuentes et al. (2023) determined MIC values of 8.5 mgmL^{-1} of hexane extracts of *C. sativa* roots.

On the other hand, for the cannabis oil chemotype III, the MIC had a content of 2.76 mgmL^{-1} of CBD against *E. coli* ATCC 25922, 0.69 mgmL^{-1} of CBD against *S. aureus* ATCC 25923 and 0.17 mgmL^{-1} of CBD against *P. larvae* genotype ERIC I. Likewise, for the cannabis oil type I, the MIC had a content of 0.92 mgmL^{-1} of THC against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, and 0.23 mgmL^{-1} of THC against *P. larvae* genotype ERIC I. Therefore, the majority content of THC and CBD in the determined MICs presented similar values.

The greater or lesser content of CBD or THC does not seem to be critical in the antimicrobial activity of the cannabis oils against the different bacteria studied, since they present similar antimicrobial activity as individual compounds. THC presents a MIC of 1 μgmL^{-1} against *S. aureus* ATCC 25923, while previous studies report CBD MICs of 0.5-1μgmL^{-1} for *S. aureus* ATCC 25923 [10]. On the other hand, for both compounds, low activity has been reported against Gram-negative bacteria such as *E. coli*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Proteus vulgaris*, *Proteus mirabilis*, *Klebsiella pneumoniae* [11].

There is a high variability in the composition of *C. sativa* essential oils, which could explain the variability in antimicrobial activities towards the same microbial species. The antimicrobial activity of *C. sativa* oils is not only due to the presence of the cannabinoids CBD and THC but also to many other compounds. The antibacterial effect has been determined for essential oils prepared from different cultivars of *C. sativa* L with undetectable levels of Δ^9-THC and very low levels of other cannabinoids, but with other compounds such as polyphenol or terpenes (α-pinene, myrcene, trans-β-ocimene, α-terpinolene, trans-caryophyllene, and α-humulene) [57]. Similarly, the antimicrobial activity of *C. sativa* extracts that did not contain THC or CBD in their composition and that nevertheless presented antimicrobial activity against different bacterial species has been reported, supporting the idea of synergism between the compounds that determine the bactericidal capacity such as polyphenols, without attributing this action exclusively to the compounds found in a greater proportion in the extracts [14]. On the other hand, the study of the antimicrobial activity of *C. sativa* oils is significant, due to the development of resistance to traditional antibiotics which is a common reason for treatment failure. Phytocannabinoids such as CBD and cannabigerol (CBG) do not induce the appearance of strains resistant to antibacterial compounds [58].

Table 2
Antimicrobial activity (MIC and MBC) of the cannabis oils against *E. coli ATCC 25922, S. aureus ATCC 25923 and P. larvae* (ERIC I genotype).

	E. coli ATCC 25922		*S. aureus* ATCC 25923		*P. larvae* ERIC I genotype	
Chemotype	MIC (mg mL^{-1})	MBC (mg mL^{-1})	MIC (mg mL^{-1})	MBC (mg mL^{-1})	MIC (mg mL^{-1})	MBC (mg mL^{-1})
I	23	46	23	46	5.75	5.75
II	23	46	46	46	5.75	5.75
III	46	46	11.5	46	2.87	2.87

Abbreviations: MIC (minimum inhibitory concentration; MBC (minimum bactericidal concentration).

4. Conclusions

Galenic formulations based on cannabis were prepared using standardized methods. These formulations used sesame oil due to its preservative properties, solubility, and ability to enhance the bioavailability of cannabinoids through oral administration. All three varieties or chemotypes used for oil preparation demonstrated good long-term stability, remaining unchanged for 12 months at 25 °C with no observable organoleptic or physicochemical changes (such as solubility, opalescence, or turbidity). In this study, no significant changes were observed in the chemical profiles or presence of microorganisms. This stability could be attributed to the antioxidant and antimicrobial properties of various cannabinoid and polyphenolic compounds, which, alongside their pharmacological characteristics, contribute as natural preservatives. In accelerated stability studies at 41 °C, no significant changes were observed in cannabinoid profiles or the appearance of microorganisms. However, the determination of total polyphenols showed a slight decline, as did antioxidant capacity, likely due to elevated temperatures. These results suggest that full-spectrum oils

formulated in sesame oil exhibit high stability even without refrigeration potentially meeting or exceeding the standard duration specified in Argentine Pharmacopoeia for non-sterile, non-aqueous formulations.

Research involving human and animal participants

This article does not contain any studies with animals.

Funding

This work was financially supported by the University of Mar del Plata, Buenos Aires, Argentina, under projects Exa 1140/23 and EXA962/20. G.M.F, P.A.S, M.F·F and C.L.R. are members of the research staff of the National Scientific and Technical Research Council of Argentina (CONICET).

Credit authorship contribution statement

Dalila E. Orallo: Writing – review & editing, Investigation, Data curation. **Giselle M. Fuentes:** Software, Investigation, Formal analysis. **María G. Benavidez:** Investigation. **Patricia A. Suárez:** Validation. **Diego Nutter:** Investigation. **María F. Fangio:** Writing – original draft, Investigation, Funding acquisition, Formal analysis. **Cristina L. Ramirez:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

Authors declares that there is no conflict of interests.

Acknowledgements

The authors thank ONG CBG2000 staff for all the assistance and useful discussions. We also thank UNMDP and CONICET for their financial support, G.M.F. PhD scholarship and M.G.B. bachelor scholarship.

References

[1] A. Stasiłowicz-Krzemień, W. Nogalska, Z. Maszewska, M. Maleszka, M. Dobroń, A. Szary, A. Kępa, M. Żarowski, K. Hojan, M. Lukowicz, J. Cielecka-Piontek, The use of compounds derived from *Cannabis sativa* in the treatment of epilepsy, painful conditions, and neuropsychiatric and neurodegenerative disorders, Int. J. Mol. Sci. 25 (11) (2024), https://doi.org/10.3390/ijms25115749.

[2] A. Bhaskar, A. Bell, M. Boivin, W. Briques, M. Brown, H. Clarke, C. Cyr, E. Eisenberg, R. Ferreira de Oliveira Silva, E. Frohlich, P. Georgius, M. Hogg, T. I. Horsted, C.A. MacCallum, K.R. Müller-Vahl, C. O'Connell, R. Sealey, M. Seibolt, A. Sihota, B.K. Smith, D. Sulak, A. Vigano, D.E. Moulin, Consensus recommendations on dosing and administration of medical cannabis to treat chronic pain: results of a modified Delphi process, J. Cannabis Res 3 (1) (2022) 1–12, https://doi.org/10.1186/s42238-021-00073-1.

[3] M.K. Dahlgren, A.M. Lambros, R.T. Smith, K.A. Sagar, C. El-Abboud, S.A. Gruber, Clinical and cognitive improvement following full-spectrum, high-cannabidiol treatment for anxiety: open-label data from a two-stage, phase 2 clinical trial, Commun. Med. 2 (1) (2022) 139, https://doi.org/10.1038/s43856-022-00202-8.

[4] N. Chaachouaya, A. Azerouala, B. Bencharkia, A. Douirab, L. Zidaneb, *Cannabis sativa* L.: a review on traditional uses, botany, phytochemistry, and pharmacological aspects, Tradit. Integr. Med. 8 (1) (2023) 97–116, https://doi.org/10.18502/tim.v8i1.12407.

[5] P. Grimison, A. Mersiades, A. Kirby, N. Lintzeris, R. Morton, P. Haber, I. Olver, A. Walsh, I. McGregor, Y. Cheung, A. Tognela, C. Hahn, K. Briscoe, M. Aghmesheh, P. Fox, E. Abdi, S. Clarke, S. Della-Fiorentina, J. Shannon, C. Gedye, M. Stockler, Oral THC:CBD cannabis extract for refractory chemotherapy-induced nausea and vomiting: a randomised, placebo-controlled, phase II crossover trial, Ann. Oncol. 31 (11) (2020) 1553–1560, https://doi.org/10.1016/j.annonc.2020.07.020.

[6] T.S. Kafil, T.M. Nguyen, J.K. MacDonald, N. Chande, Cannabis for the treatment of Crohn's disease, Cochrane Database Syst. Rev. 11 (2018), https://doi.org/10.1002/14651858.CD012853.pub2.

[7] S. Blasco-Benito, E. Moreno, M. Seijo-Vila, I. Tundidor, C. Andradas, M.M. Caffarel, M. Caro-Villalobos, L. Urigüen, R. Diez-Alarcia, G. Moreno-Bueno, L. Hernández, L. Manso, P. Homar-Ruano, P.J. McCormick, L. Bibic, C. Bernadó-Morales, J. Arribas, M. Canals, V. Casadó, E.I. Canela, M. Guzmán, E. Pérez-Gómez, C. Sánchez, Therapeutic targeting of HER2-CB_2R heteromers in HER2-positive breast cancer, Proc. Natl. Acad. Sci. U. S. A. 116 (9) (2019) 3863–3872, https://doi.org/10.1073/pnas.1815034116.

[8] C. Sánchez García, 2.-Preparaciones de cannabis medicinal, in: Usos medicinales del cannabis: ¿qué sabemos de sus propiedades curativas?, Los Libros de la Catarata, 2019, pp. 33–44. https://produccioncientifica.ucm.es/documentos/61a718cc3ce0234e3fd34fbc.

[9] N. Kuhathasan, L. Minuzzi, J. MacKillop, B.N. Frey, The use of cannabinoids for insomnia in daily life: naturalistic study, J. Med. Internet Res. 23 (10) (2021) e25730, https://doi.org/10.2196/25730.

[10] M. de Fátima dos Santos Sampaio, Y.B. de Paiva, T.B. Sampaio, M.G. Pereira, N. C. Coimbra, Therapeutic applicability of cannabidiol and other phytocannabinoids in epilepsy, multiple sclerosis and Parkinson's disease and in comorbidity with psychiatric disorders, Basic Clin. Paharmacol. Toxicol. 134 (5) (2024) 574–601, https://doi.org/10.1111/bcpt.13997.

[11] H. Hong, L. Sloan, D. Saxena, D.A. Scott, The antimicrobial properties of cannabis and cannabis-derived compounds and relevance to CB2-targeted neurodegenerative therapeutics, Biomedicines 10 (8) (2022), https://doi.org/10.3390/biomedicines10081959.

[12] R.V. Sionov, D. Steinberg, Anti-microbial activity of phytocannabinoids and endocannabinoids in the light of their physiological and pathophysiological roles, Biomedicines 10 (3) (2022), https://doi.org/10.3390/biomedicines10030631.

[13] E.J. Addison, Review cannabis: a new strategy against methicillin-resistant *Staphylococcus aureus*, VJSTE 64 (4) (2022) 70–78, https://doi.org/10.31276/VJSTE.64(4).

[14] L. Schofs, M.D. Sparo, S.F. Sánchez Bruni, The antimicrobial effect behind *Cannabis sativa*, Pharmacol. Res. Perspect. 9 (2) (2021) e00761, https://doi.org/10.1002/prp2.761.

[15] G. Fuentes, A. Iglesias, D. Orallo, F. Fangio, F. Ramos, G. Mitton, S. Fuselli, M. Maggi, C.L. Ramirez, Antibacterial activity of cannabis (*Cannabis sativa* L.) female inflorescence and root extract against *Paenibacillus larvae*, causal agent of American foulbrood, Biocatal. Agric. Biotechnol. 47 (2023) 102575, https://doi.org/10.1016/j.bcab.2022.102575.

[16] SIFAP, Società Italiana Farmacisti Preparatori, SIFAP propone un metodo per l'Estrazione oleosa di infiorescenze femminili di Cannabis, 2016. https://www.sifap.org/newsletter/sifap-propone-un-metodo-per-l-estrazione-oleosa-di-infiorescenze-femminili-di-cannabis, 2016.

[17] PODER EJECUTIVO NACIONAL, Ley de investigación médica y científica del uso medicinal de la planta de Cannabis y sus derivados. https://www.argentina.gob.ar/normativa/nacional/decreto-738-2017-279831/texto, 2017.

[18] A. Alicia, FDA Recommends DEA Move Cannabis from Schedule I to III. https://www.medscape.com/viewarticle/fda-recommends-dea-move-cannabis-schedule-iii-2024a100010v?form=fpf, 2024.

[19] Ministerio de Salud and ANMAT, Farmacopea Argentina, 7ma edición. Segundo Suplemento. Apartado 1027. Capítulo 5. https://www.argentina.gob.ar/sites/default/files/anexo_7022520_1.pdf, 2023.

[20] R. Pacifici, E. Marchei, F. Salvatore, L. Guandalini, F.P. Busardò, S. Pichini, Evaluation of cannabinoids concentration and stability in standardized preparations of cannabis tea and cannabis oil by ultra-high-performance liquid chromatography tandem mass spectrometry, Clin. Chem. Lab. Med. 55 (10) (2017) 1555–1563, https://doi.org/10.1515/cclm-2016-1060.

[21] C. Morano, M. Dei Cas, E. Casagni, M. Pallavicini, C. Bolchi, S. Penati, A. Cuomo, M. Cascella, S. Bimonte, A. Spensiero, A. Ferrari, A. Lombardi, G. Roda, Medicinal cannabis: extended stability of cannabis extracts produced using a new ethanol-based extraction method, Planta Med. 90 (1) (2024) 73–80, https://doi.org/10.1055/a-2189-8155 (Epub 2023 Nov 14. PMID: 37963569).

[22] O. González-González, I.O. Ramirez, B.I. Ramirez, P. O'Connell, M.P. Ballesteros, J. J. Torrado, D.R. Serrano, Drug stability: ICH versus accelerated predictive stability studies, Pharmaceutics 14 (11) (2022) 2324, https://doi.org/10.3390/pharmaceutics14112324.

[23] D. Pacifico, F. Miselli, A. Carboni, A. Moschella, G. Mandolino, Time course of cannabinoid accumulation and chemotype development during the growth of *Cannabis sativa* L, Euphytica 160 (2) (2008) 231–240, https://doi.org/10.1007/s10681-007-9543-y.

[24] P. Torres, J.P. Santos, F. Chow, M.J. Pena Ferreira, D.Y.A.C. dos Santos, Comparative analysis of in vitro antioxidant capacities of mycosporine-like amino acids (MAAs), Algal Res. 34 (2018) 57–67, https://doi.org/10.1016/j.algal.2018.07.007.

[25] J.S. Matejić, A.M. Džamić, T.M. Mihajilov-Krstev, V.N. Ranđelović, Z. Krivošej, P. D. Marin, Total phenolic and flavonoid content, antioxidant and antimicrobial activity of extracts from *Tordylium maximum*, J. Appl. Pharm. Sci. 3 (1) (2013) 055–059, https://doi.org/10.7324/JAPS.2013.30110.

[26] S. Dudonné, X. Vitrac, P. Coutière, M. Woillez, J.-M. Mérillon, Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays, J. Agric. Food Chem. 57 (5) (2009) 1768–1774, https://doi.org/10.1021/jf803011r.

[27] M.F. Fangio, G.B. Herrera, S. Medici, F. Cardinali, C.L. Ramirez, Chemical characterization and antimicrobial activity of *Blumenbachia insignis* (Loasaceae) native to South America, Bull. Natl. Res. Cent. 46 (1) (2022) 269, https://doi.org/10.1186/s42269-022-00957-z.

[28] M. Balzarini, J.A. Di Rienzo, M. Tablada, L. Gonzalez, C. Bruno, M. Córdoba, W. Robledo, F. Casanoves, Estadística y biometría: ilustraciones del uso de InfoStat en problemas de agronomía, 2015.

[29] T. Moreno, P. Dyer, S. Tallon, Cannabinoid decarboxylation: a comparative kinetic study, Ind. Eng. Chem. Res. 59 (46) (2020) 20307–20315, https://doi.org/10.1021/acs.iecr.0c03791.

[30] M. Carbone, F. Castelluccio, A. Daniele, A. Sutton, A. Ligresti, V. Di Marzo, M. Gavagnin, Chemical characterisation of oxidative degradation products of Δ9-THC, Tetrahedron 66 (49) (2010) 9497–9501, https://doi.org/10.1016/j.tet.2010.10.025.
[31] P.J. Duggan, The chemistry of cannabis and cannabinoids, Aust. J. Chem. 74 (6) (2021) 369–387, https://doi.org/10.1071/CH21006.
[32] M.E. Aguirre, D.E. Orallo, P.A. Suárez, C.L. Ramirez, Galenic formulations of *Cannabis sativa*: comparison of the chemical properties of extracts obtained by simple protocols using lipidic vehicles, Nat. Prod. Res. 38 (4) (2023) 661–663, https://doi.org/10.1080/14786419.2023.2184357.
[33] P.X. Chen, M.A. Rogers, Opportunities and challenges in developing orally administered cannabis edibles, Curr. Opin. Food Sci. 28 (2019) 7–13, https://doi.org/10.1016/j.cofs.2019.02.005.
[34] M. Córdova Suárez, F. Ruiz Mora, Extracción, caracterización e índice de estabilidad del aceite de ajonjolí (*Sesamum indicum* L.) a diferentes condiciones de inhibición oxidativa, Universidad Técnica de Ambato, Ambato, Ecuador, 2022. https://repositorio.uta.edu.ec/jspui/handle/123456789/34281.
[35] I. Dini, L. Grumetto, Recent advances in natural polyphenol research, Molecules 27 (24) (2022), https://doi.org/10.3390/molecules27248777.
[36] A.K. Al-Khazaleh, X. Zhou, D.J. Bhuyan, G.W. Münch, E.A. Al-Dalabeeh, K. Jaye, D. Chang, The neurotherapeutic arsenal in *Cannabis sativa*: insights into anti-neuroinflammatory and neuroprotective activity and potential entourage effects, Molecules 29 (2) (2024), https://doi.org/10.3390/molecules29020410.
[37] N. Vetskov, N. Kaloyanov, I. Hinkov, M. Funeva-Peycheva, T. Yankovska-Stefanova, A. Garbev, Comparative study of poliphenolic composition and reducing properties of extracts of *Cannabis sativa* L., *Cannabis indica* and *Cannabis hybrid*, J. Chem. Technol. Metal. 59 (2) (2024) 237–242.
[38] J.L. Bautista, S. Yu, L. Tian, Flavonoids in *Cannabis sativa*: biosynthesis, bioactivities, and biotechnology, ACS Omega 6 (8) (2021) 5119–5123, https://doi.org/10.1021/acsomega.1c00318.
[39] D.S. Wishart, M. Hiebert-Giesbrecht, G. Inchehborouni, X. Cao, A.C. Guo, M. A. LeVatte, C. Torres-Calzada, V. Gautam, M. Johnson, J. Liigand, F. Wang, S. Zahraei, S. Bhumireddy, Y. Wang, J. Zheng, R. Mandal, J.R.B. Dyck, Chemical composition of commercial cannabis, J. Agric. Food Chem. 72 (25) (2024), https://doi.org/10.1021/acs.jafc.3c06616.
[40] L. Zeng, M. Ma, C. Li, L. Luo, Stability of tea polyphenols solution with different pH at different temperatures, Int. J. Food Prop. 20 (1) (2017) 1–18, https://doi.org/10.1080/10942912.2014.983605.
[41] R.R. Watson, V.R. Preedy, S. Zibadi, Front-matter, in: Polyphenols in Human Health and Disease, Academic Press, San Diego, 2014, pp. i–iii, https://doi.org/10.1016/B978-0-12-398456-2.00107-9.
[42] Y. Semaming, P. Pannengpetch, S.C. Chattipakorn, N. Chattipakorn, Pharmacological properties of protocatechuic acid and its potential roles as complementary medicine, Evid. Based Complement. Alternat. Med. 2015 (2015) 593902, https://doi.org/10.1155/2015/593902.
[43] S. Pino, L. Espinoza, C. Jara-Gutiérrez, J. Villena, A.F. Olea, K. Díaz, Study of cannabis oils obtained from three varieties of *C. sativa* and by two different extraction methods: phytochemical characterization and biological activities, Plants (Basel) 12 (9) (2023), https://doi.org/10.3390/plants12091772.
[44] A. Gruschow, A Comparison of Antioxidant Potential, Total Phenolic Content, and Cannabidiol (CBD) Content of Cannabis Infused Hemp, MCT, and Olive Oils, Montclair State University, E.E.U.U, New Jersey, 2020. https://digitalcommons.montclair.edu/etd/472.
[45] I. Volf, I. Ignat, M. Neamtu, V.I. Popa, Thermal stability, antioxidant activity, and photo-oxidation of natural polyphenols, Chem. Pap. 68 (1) (2014) 121–129, https://doi.org/10.2478/s11696-013-0417-6.
[46] J.A. Larrauri, C. Sánchez-Moreno, F. Saura-Calixto, Effect of temperature on the free radical scavenging capacity of extracts from red and white grape pomace peels, J. Agric. Food Chem. 46 (7) (1998) 2694–2697, https://doi.org/10.1021/jf980017p.
[47] C.N. Pegoraro, D. Nutter, M. Thevenon, C.L. Ramirez, Chemical profiles of *Cannabis sativa* medicinal oil using different extraction and concentration methods, Nat. Prod. Res. 35 (13) (2021) 2249–2252, https://doi.org/10.1080/14786419.2019.1663515.
[48] D.S. Sopovski, J. Han, M. Stevens-Riley, Q. Wang, B.D. Erickson, B. Oktem, M. Vanlandingham, C.L. Taylor, S.L. Foley, Investigation of microorganisms in cannabis after heating in a commercial vaporizer, Front. Cell. Infect. Microbiol. 13 (12) (2023), https://doi.org/10.3389/fcimb.2022.1051272.
[49] V. Vujanovic, D.R. Korber, S. Vujanovic, J. Vujanovic, S. Jabaji, Scientific prospects for cannabis-microbiome research to ensure quality and safety of products, Microorganisms 8 (2) (2020), https://doi.org/10.3390/microorganisms8020290.
[50] K. Boyar, Cannabis microbial testing - methodologies and considerations, in: R. M. Strongin, J. Meehan-Atrash, M. Vialpando (Eds.), Recent Advances in the Science of Cannabis, 2021, https://doi.org/10.1201/9780429274893-6.
[51] S.R. Fuselli, S.B.G. de la Rosa, L.B. Gende, M.J. Eguaras, R. Fritz, Antimicrobial activity of some Argentinean wild plant essential oils against *Paenibacillus larvae larvae*, causal agent of American foulbrood (AFB), J. Apic. Res. 45 (1) (2006) 2–7, https://doi.org/10.1080/00218839.2006.11101304.
[52] A.A. Boligon, T. Faccim de Brum, M. Zadra, M. Piana, C.F. dos Santos Alves, V. P. Fausto, V. dos Santos Barboza Júnior, R. de Almeida Vaucher, R.C. Vianna Santos, M. Athayde, Antimicrobial activity of *Scutia buxifolia* against the honeybee pathogen *Paenibacillus larvae*, J. Invertebr. Pathol. 112 (2) (2013) 105–107, https://doi.org/10.1016/j.jip.2012.11.009.
[53] S. Kaur, C. Sharma, S. Chaudhry, R. Aman, Antimicrobial potential of three common weeds of Kurukshetra: an in vitro study, Res. J. Microbiol. 10 (2015), https://doi.org/10.3923/jm.2015.280.287.
[54] C. Ferrante, L. Recinella, M. Ronci, L. Menghini, L. Brunetti, A. Chiavaroli, S. Leone, L. Di Iorio, S. Carradori, B. Tirillini, P. Angelini, S. Covino, R. Venanzoni, G. Orlando, Multiple pharmacognostic characterization on hemp commercial cultivars: focus on inflorescence water extract activity, Food Chem. Toxicol. 125 (2019) 452–461, https://doi.org/10.1016/j.fct.2019.01.035.
[55] T.T. Vu, H. Kim, V.K. Tran, Q. Le Dang, H.T. Nguyen, H. Kim, I.S. Kim, G.J. Choi, J. C. Kim, In vitro antibacterial activity of selected medicinal plants traditionally used in Vietnam against human pathogenic bacteria, BMC Complement. Altern. Med. 16 (2016) 32, https://doi.org/10.1186/s12906-016-1007-2.
[56] G. Zengin, L. Menghini, A. Di Sotto, R. Mancinelli, F. Sisto, S. Carradori, S. Cesa, C. Fraschetti, A. Filippi, L. Angiolella, M. Locatelli, L. Mannina, C. Ingallina, V. Puca, M. D'Antonio, R. Grande, Chromatographic analyses, in vitro biological activities, and cytotoxicity of *Cannabis sativa* L. essential oil: a multidisciplinary study, Molecules 23 (12) (2018), https://doi.org/10.3390/molecules23123266.
[57] J. Novak, K. Zitterl-Eglseer, S.G. Deans, C.M. Franz, Essential oils of different cultivars of *Cannabis sativa* L. and their antimicrobial activity, Flavour Fragr. J. 16 (4) (2001) 259–262, https://doi.org/10.1002/ffj.993.
[58] M.A. Farha, O.M. El-Halfawy, R.T. Gale, C.R. MacNair, L.A. Carfrae, X. Zhang, N. G. Jentsch, J. Magolan, E.D. Brown, Uncovering the hidden antibiotic potential of cannabis, ACS Infect. Dis. 6 (3) (2020) 338–346, https://doi.org/10.1021/acsinfecdis.9b00419.