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Cannabinoid Content in Cannabis Flowers and Homemade Cannabis-Based Products Used for Therapeutic Purposes in Argentina

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Abstract

Introduction: A recent law (DCTO-2020-883-APN-PTE—Law No. 27,350. Regulation) passed in Argentina put an end to the ban imposed for the last 60 years on cannabis cultivation within the country. The law permits restricted access to cannabis derivatives for medicinal, therapeutic, and palliative use by individuals and communities, allowing self- and community-based cannabis production. This is cause for concern in view of the lack of quality controls for cannabis derivatives. The several varieties of cannabis grown in Argentina have different chemical profiles and are processed in a variety of ways—mostly by alcohol extraction or maceration at different temperatures and for different amounts of times—making the cannabinoid content of these preparations highly variable. Determining the characteristics of home- and community-grown cannabis products will facilitate the implementation of public policies conducive to their safety and improvement.

Objective: The aim of this study was to determine the cannabinoid chemotypes used for therapeutic purposes in Argentina and evaluate whether the cannabinoids present in homemade derivatives are comparable to those in commercially available products.

Materials and Methods: High performance liquid chromatography with ultraviolet and diode array detector (HPLC/UV-DAD) analysis of 436 samples (oils, resins, and inflorescences) was carried out to determine the identity and concentration of five cannabinoids: tetrahydrocannabinolic acid (THCA), tetrahydrocannabinol (THC), cannabidiolic acid (CBDA), cannabidiol (CBD), and cannabinol (CBN). From three different sources, the samples represent the type of medical cannabis preparations to which patients have access.

Results: The results indicate that the medium-to-low cannabinoid concentration in a significant number of homemade oil samples is similar to that found in commercial products. Most of the samples have a THC/CBD ratio >1 or only contain THC. Acidic cannabinoids were detected in homemade preparations, but were not reported in package inserts of commercial products.

Conclusions: Our results indicate that despite their considerable variability, homemade preparations as a whole show cannabinoid levels and profiles equivalent to the commercially available products commonly used for medicinal, therapeutic, and palliative purposes in Argentina.

Keywords: cannabis oil; inflorescences; resins; cannabinoids; homemade herbal products

Introduction

Most of the effects of cannabis on the human organism are exerted through phytocannabinoids, lipophilic molecules whose therapeutic action is owed to the fact that they interact with the endocannabinoid system (ECS) receptors (CB1 and CB2), triggering the same effects as endocannabinoids, such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG).[1] Phytocannabinoids therefore act as modulators of many physiological processes that involve the intervention of the

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ECS, whose main role is to regulate the body homeostasis. New actions of ECS are being discovered, enabling us to learn more about the beneficial effects of phytocannabinoids in the treatment of the symptomatology associated with several diseases.[2,3]

Around 554 compounds have been identified in *Cannabis sativa* sp. plants, among which 113 were phytocannabinoids[3,4] and over 120 terpenes.[5]

One of the most well-known and studied phytocannabinoids is Δ9-tetrahydrocannabinol (THC), initially linked to the psychoactive effects of cannabis, but now also shown to be highly effective for treating chronic pain in adults and nausea and vomiting in chemotherapy treatment, and relieving the symptoms of spasticity in multiple sclerosis.[6] Other common phytocannabinoids that have been studied are cannabidiol (CBD), a nonpsychoactive substance claimed to have anti-inflammatory, analgesic, antianxiety, and antipsychotic properties; and cannabinol (CBN), a cannabinoid with considerably less psychoactivity than THC and known for its sedative effects, and anticonvulsant and antibacterial properties. Furthermore, since CBN derives from the oxidation of THC, it has been linked to the aging or overheating of cannabis-based preparations.[7–9] These neutral cannabinoids are present in low quantities in fresh plants and are biosynthesized as prenylated aromatic carboxylic acids such as tetrahydrocannabinolic acid (THCA) or cannabidiolic acid (CBDA), convertible to their neutral counterparts by decarboxylation when exposed to light or heat.[10,11] Unlike THC, the acidic form THCA is not psychoactive since it is unable to pass through the blood–brain barrier and has low affinity for the CB1 receptor.[12,13] Acidic cannabinoids also have pharmacological properties such anti-inflammatory effects since they are COX2-selective inhibitors.[12,14] When acidic and neutral cannabinoids are administered together, they interact with each other and with other substances contained in phytopreparations (terpenes, flavonoids, sterols, etc.), generating different effects from when they are administered separately; this phenomenon is known as "entourage effect."[14,15]

In recent years, the use of medicinal cannabis has become more widespread in Latin America, providing a therapeutic alternative being increasingly taken into account by patients and doctors. As in many countries, there are no legally available medicinal cannabis preparations on the market in Argentina for patients to include in their treatment. Thus, self-cultivation and home processing of phytopreparations based on cannabis are currently their main options. In many of these cases, physicians are aware that their patients use cannabis-based derivatives therapeutically and take this into account when prescribing other medications, monitoring for any improvement in symptoms as well as possible side effects or drug-drug interactions. In the absence of legal products for sale in pharmacies, patients are forced to access cannabis-based oils on the illegal market. As described above, this situation means that the composition of these products is not checked by quality control and can therefore vary widely. Self-cultivation and solidarity cultivation have expanded in recent years in Argentina, generating cannabis-based herbal preparations with levels and chemoptypes of cannabinoids compatible with therapeutic requirements of the user. This socially propelled process was fundamental in helping to bring about the recent changes in the law, permitting this informal cultivation to provide access to medicinal cannabis. Due to the recent law (DCTO-2020-883-APN-PTE—Law No. 27,350. Regulation) passed in, Argentina permits the access to cannabis-based derivatives for medicinal purposes through self- and community-based cannabis production; it is necessary to know the characteristics of these kinds of products. Determining the characteristics of home- and community-grown cannabis products will provide information and facilitate the implementation of public policies conducive to their safety and improvement. Therefore, the aim of this study was to analyze the cannabinoid composition of a variety of cannabis-based inflorescences, resins, and oils used for therapeutic purposes in Argentina.

Materials and Methods

Samples analyzed

The study was carried out using inflorescence ($n = 34$), resin ($n = 40$), and oil ($n = 362$) samples received between 2018 and 2019 within the framework of the Cannabis and Health project conducted at the Environmental Research Center (CIM, Institute dependent of National Council for Scientific and Technical Research of Argentina [CONICET] and National University of La Plata [UNLP], La Plata, Argentina). The samples came from different sources, together representing a cross-section of the type of medical cannabis preparations to which patients have access. Given the variability of oil sources and therapeutic objectives for which they have been used and to carry out a differential analysis of total cannabinoid content, we established three subgroups of oils based on their origin: GENERAL

($n = 220$), oil samples corresponding to patients or growers with no concrete affiliation with any nongovernmental organization or health institution; ROFFO ($n = 125$), oil samples used by patients of the Palliative Care Area of the Oncologic Institute Ángel H. Roffo (IOAR, Buenos Aires, Argentina) who participated in an observational study supervised by Dr. Saurí (Palliative Care Area Director); and ACUFALP ($n = 17$), homemade oil samples from different home-grown strains used by the civil association "Cultivo en Familia La Plata," La Plata, Argentina.

Determination of cannabinoids in studied samples

Sample conditioning and processing. The samples were conditioned and processed according to the following cannabinoid extraction protocols:

The flowers were received in the laboratory dried. However, to ensure this condition before sample processing, the inflorescences were dried in an oven (SAN JOR—SL 17C) for 1 h at low temperature (30°C) to avoid decarboxylation. Once dry, the flowers were homogenized with sterile scissors and in each case, 1 g was used for cannabinoid extraction using 20 mL of ethanol 96° (Purocol) and sonication for 10 min (Omniruptor). Filtration was then performed using sterile cotton gauzes followed by filtration with Whatman filter paper.

For resins and oils, the extraction was performed with 96° ethanol (Purocol) using 100 mL/g resin or 20 mL/g oil and shaking in vortex (10 min) to favor efficient contact and extraction. The obtained solution was then centrifuged for 10 min at 5000 rpm (Rolco Centrifuge) to separate the alcohol extract from insoluble residues. Matrix cleanup was carried out on alcohol extracts obtained from inflorescences, resins, or oils using a mixture of activated carbon, C_{18}, and Mg_2SO_4 and sonication for 10 min. The supernatant was separated by centrifugation and the salts were washed with ethanol and sonication for 10 min to recover any cannabinoids adsorbed on the salt surfaces. Centrifugation (10 min at 5000 rpm) was subsequently performed and the supernatant was incorporated into the initially treated alcoholic phase. In previous studies, we determined cannabinoid content in oil, resin, and alcoholic extract samples with and without carrying out the matrix cleanup procedure. An 8% decrease in cannabinoid content for CBD and CBD-A, 9% for THC and THC-A, and 7% for CBN were determined after the cleanup process. Based on these previous studies, we included a corresponding correction factor in the cannabinoid quantification. Finally, the alcoholic phase was filtered using Osmonics 45 μm filters, to further analyze by high performance liquid chromatography with ultraviolet and diode array detector (HPLC/UV-DAD).

Analytical determination of cannabinoids. Cannabinoid profiles were studied by HPLC/UV-DAD (Shimadzu LC-20A), employing a Thermo Hypersil BDS C18 column (150 × 4.6 mm, 5 μm) according to the analytical technique described by De Backer et al.,[16] with slight modifications. The mobile phase consisted in A: methanol and B: 25 mM ammonium acetate solution. The gradient was: 75% A: 1 min, 75% to 95% A in 15 min, 95% A: 2 min, 95% to 75% A in 2 min, and 75% A: 5 min. Total run time was 25 min, flow: 1 mL/min, and detection at 205 nm. Cannabinoid analytical standards were purchased from Cerilliant Corporation. This technique allowed us to differentiate the acidic (THCA, CBDA, etc.) from the neutral cannabinoids (THC, CBD, etc.). Based on the cannabinoid concentration obtained, ratios and derived variables were established according to the following equations:

$$\begin{aligned}\text{Total cannabinoids} &= [\text{CBDA}] + [\text{THC A}] + [\text{CBD}] \\ &\quad + [\text{CBN}] + [\text{THC}] \\ \text{THC/CBD ratio} &= [(0.877 * [\text{THC A}] + [\text{THC}])] / \\ &\quad [(0.877 * [\text{CBD A}]) + [\text{CBD}]] \\ \text{Acidic/neutral ratio} &= ([\text{CBD A}] + [\text{THC A}]) / \\ &\quad ([\text{CBD}] + [\text{THC}])\end{aligned}$$

A conversion factor of 0.877 was employed in the Total THC (sum of THC and THCA) and Total CBD (sum of CBD and CBDA) calculations to adjust for the loss of mass of THCA and CBDA after decarboxylation.[17]

Data analysis. All the results were subjected to a one-way analysis of variance (ANOVA) with the help of Systat (version 12.0 for Windows) from SPSS Science (Chicago, IL) and represent the mean ± standard error (n is indicated in each case). The differences in the mean of the groups were evaluated by a two-tailed Student's t-test, with a statistical significance level of $p < 0.05$.

Results

We analyzed a total of 436 samples (362 oils, 40 resins, and 34 inflorescences) and determined the identity and concentration of five cannabinoids: THCA, THC, CBDA,

Table 1. Parameters Used in the Characterization of the Samples

Samples	Total cannabinoids	THC/CBD	Acidic/neutral	CBN
Inflorescences	62.5 ± 6.0 A (34)	43.4 ± 14.4 A (28)	8.6 ± 1.9 A (34)	0.8 ± 0.7 A (34)
Resins	358.8 ± 40.9 B (40)	38.2 ± 22.7 A (34)	1.5 ± 0.3 B (33)	2.7 ± 0.8 A (40)
Oils	8.4 ± 1.1 C (362)	26.8 ± 3.1 A (297)	1.0 ± 0.1 B (276)	0.25 ± 0.04 B (362)

Total cannabinoids and CBN are expressed in mg/g for resins and inflorescences, and in mg/mL for oils.
The significant differences among results [mean ± SE, (*n*)] are indicated with different letters ($p > 0.05$).
CBD, cannabidiol; CBN, cannabinol; SE, standard error; THC, tetrahydrocannabinol.

CBD, and CBN. Based on these findings, the samples were analyzed according to total cannabinoid content, THC/CBD ratio, acidic/neutral ratio, and CBN content, these parameters being directly related to the quality and therapeutic potential of cannabis and its derivatives.

Total cannabinoids in resin, inflorescence, and oil samples

Of all the samples analyzed, resins—basically obtained by alcohol extraction followed by alcohol evaporation—showed the highest average concentration of total cannabinoids (358.8 ± 40.9 mg/g), followed by inflorescences, with a 5-fold lower total cannabinoid level (62.5 ± 6.0 mg/g). The total mean cannabinoid concentration in oils was 8.4 ± 1.1 mg/mL (Table 1). These groups differed significantly from one another in total cannabinoid content, with significant variability within each group.

In view of this wide variability and to analyze cannabinoid distribution, relative frequencies (%) were calculated for oil, inflorescence, and resin samples based on five total cannabinoid concentration (TCC) ranges (0–0.1, 0.1–1, 1–10, 10–100, and over 100 mg/mL [oils] or mg/g [resins and inflorescences]) (Fig. 1).

Most resin samples (82.5%) were within the highest TCC range (over 100 mg/g) with a relative frequency of 7.5% for each one in the 10–100 and 1–10 mg/g ranges. The rest of the resin samples (2.5%) presented TCCs in

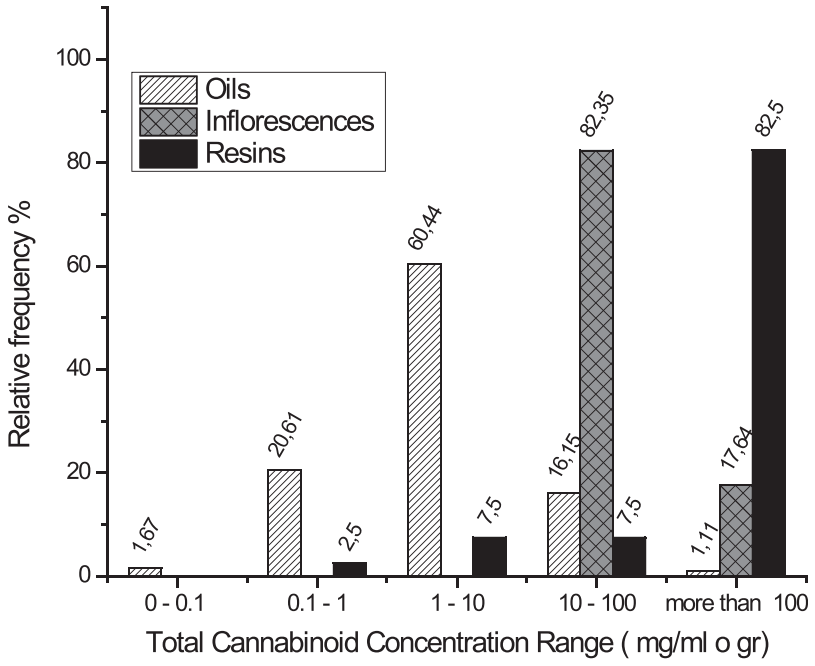

FIG. 1. Relative frequency (%) of samples of oils (total) (striped bar), inflorescences (squared gray bar), and resins (black bar) based on TCC ranges. TCC, total cannabinoid concentration.

Table 2. Parameters Used in the Characterization of the Oil Samples by Origin (GENERAL, ROFFO, and ACUFALP)

Oils	Total cannabinoids	THC/CBD	Acidic/neutral	CBN
GENERAL	11.1 ± 1.8 A (220)	21.7 ± 2.1 A (186)	1.1 ± 0.1 A (164)	0.3 ± 0.1 A (220)
ROFFO	3.2 ± 0.4 B (125)	22.2 ± 4.2 A (95)	1.1 ± 0.2 A (100)	0.1 ± 0.1 B (125)
ACUFALP	42.7 ± 23.9 C (17)	14.1 ± 4.5 A (16)	0.4 ± 0.2 A (12)	0.9 ± 0.6 C (17)

Total cannabinoids and CBN are expressed in mg/g for resins and inflorescences; and in mg/mL for oils. The significant differences among results [mean ± SE, (*n*)] are indicated with different letters ($p > 0.05$).

one of the lowest TCC range (0.1–1 mg/g), which is rare for a usually concentrated material such as resin (Fig. 1). In the case of inflorescences, 82.4% of the samples were within the 10–100 mg/g TCC range, while a smaller percentage of the samples (17.6%) were in the highest range (over 100 mg/g), some samples reaching 220 mg/g (Fig. 1).

Oils had the highest number of samples distributed in relatively low TCC ranges, with relative frequencies of 60.4% and 20.6% in 1–10 and 0.1–1 mg/mL ranges, respectively (Fig. 1). Moreover, 1.7% of oil samples were within the lowest range established (0–0.1 mg/mL) and 16.2% and 1.1% corresponded to the two highest ranges, 10–100 mg/mL and over 100 mg/mL, respectively.

Analysis of oil subgroups according to their origin

Total cannabinoid levels detected in oil subgroups differed significantly from one another. The subgroup presenting the highest TCC was ACUFALP (42.7 ± 23.9 mg/mL) followed by the GENERAL (11.1 ± 1.8 mg/mL) and ROFFO (3.2 ± 0.4 mg/mL) subgroups (Table 2).

Table 3 shows the TCC reported on the labels of the some of the more dominant brands of oils available on the international market, often alluded to in Argentina by the community, but not available in the country. They cover a varied range of cannabinoid concentrations in their different presentations. Compared to these products, ROFFO oils have the lowest TCC and those of the GENERAL subgroup were ranked fourth, presenting concentrations close to 10 mg/mL. Oils of the ACUFALP subgroup ranked in the middle of Table 3, with an average of 42.7 mg/mL, seven products having higher concentrations.

Most oil samples of the ACUFALP subgroup were within the three highest TCC ranges: over 100, 100–10, and 1–10 mg/mL, with relative frequencies of 14.3%, 42.9%, and 35.7% respectively; 7.1% of the samples were in the 0.1–1 mg/mL range and there were no samples in the lowest established TCC range.

Even though the ROFFO and GENERAL subgroups showed the highest proportion of samples in the 1–

Table 3. Total Cannabinoids and THC/CBD Ratio in Different Cannabis-Based Products Available in the Market, Produced by International Pharmaceutical Companies and Firms, as Well as the Subgroups of Oils Studied Here (GENERAL, ROFFO, and ACUFALP)

Product	Origin/pharmaceutical company	Pharmaceutical formulation	Total cannabinoids	THC/CBD ratio
ROFFO	Self-cultivation/solidarity cultivation and undetermined	Oral solution: oil	3.2 mg/mL	22.2:1
Charlotte's Web CBD Oil	Stanley Brothers	Oral solution: oil	7 mg/mL	Only CBD informed
RSHO: Green, Blue and Gold Label	Hemp Meds	Oral solution: oil	8.5 mg/mL	Only reports CBD
GENERAL	Self-cultivation/solidarity cultivation and undetermined	Oral solution: oil	11.1 mg/mL	21.7:1
Charlotte's Web CBD Oil	Stanley Brothers	Oral solution: oil	17 mg/mL	Only reports CBD
THC 20:1—Oil (Formerly Champlain)	Aphria	Oral solution: oil	21.3 mg/mL	20:1
THC:CBD 10:13—Oil (Formerly Capilano)	Aphria	Oral solution: oil	21.5 mg/mL	10:13
CBD 25:1—Oil (Formerly Ridean)	Aphria	Oral solution: oil	25.2 mg/mL	1:25
ACUFALP	Self-cultivation/solidarity cultivation	Oral solution: oil	42.7 mg/mL	14.1:1
Charlotte's Web CBD Oil	Stanley Brothers	Oral solution: oil	50 mg/mL	Only reports CBD
Sativex	GW Pharmaceutical	Solution for oral spray	52 mg/mL	1:1
Charlotte's Web CBD Oil	Stanley Brothers	Oral solution: oil	60 mg/mL	Only reports CBD
Epidiolex	GW Pharmaceutical	Oral solution	100 mg/mL	Only reports CBD
RSHO Green Label 3G Pure CBD Oil	Hemp Meds	Hemp oil	120 mg/g	Only reports CBD
RSHO Blue Label 3G Pure CBD Oil	Hemp Meds	Decarboxylated hemp oil	170 mg/g	Only reports CBD
RSHO Gold Label 3G Pure CBD Oil	Hemp Meds	Decarboxylated and filtered hemp oil	240 mg/g	Only reports CBD

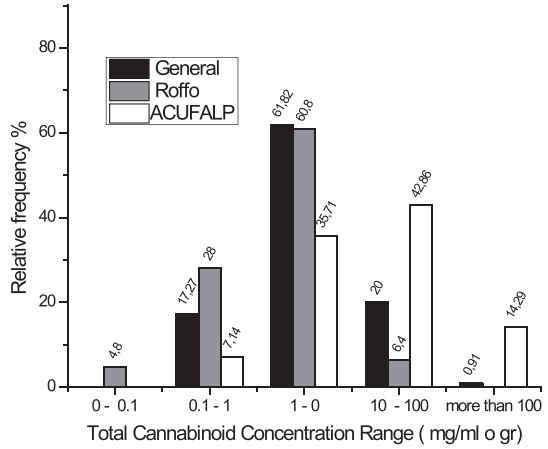

FIG. 2. Relative frequency (%) of samples from oil subgroups: GENERAL (black bars), ROFFO (gray bars), and ACUFALP (white bars) based on the TCC range.

10 mg/mL TCC range (60.8% and 61.8%, respectively), there were differences in the distribution of the relative frequencies in the other ranges (Fig. 2). Oils of the GENERAL subgroup showed the following relative frequencies: 1% in the over 100 mg/mL range, 20% in 10–100 mg/mL range, and 17.2% in the 0.1–1 mg/mL range. There were no samples with concentrations within the lowest range. The ROFFO subgroup showed relative frequencies of 28% in the 0.1–1 mg/mL range and 4.8% in the 0–0.1 mg/mL range; 8.4% of this subgroup's samples were found in the 10–100 mg/mL range, with no sample in the maximum established range (Fig. 2).

THC/CBD ratio

There were no significant differences in the THC/CBD ratio among inflorescence, resin, and oil samples, nor among the oil subgroups (Tables 1 and 2). Only 0.5% of the oil samples showed no THC content, whereas CBD was not detected in 24.3% of inflorescences, 15% of resins, and 19.1% of oils (Fig. 3A). Inflorescences and oils presented their highest relative frequency in the 10–100 THC/CBD range (37.8% and 40.4%, respectively) and resins in the 0.1–1 range (37.5%). Our results indicate that resins presented a higher proportion of samples with balanced THC and CBD content or enriched in CBD, with 42.5% of samples with THC/CBD < 1. Inflorescences and oils showed 16.2% and 14.4% of samples with THC/CBD < 1, respectively.

We detected THC and CBD in different proportions in samples of the ACUFALP oil subgroup. THC was not detected in 0.9% of the GENERAL subgroup samples, and CBD was not detected in 16.8% and 25.4% of samples of GENERAL and ROFFO subgroups, respectively.

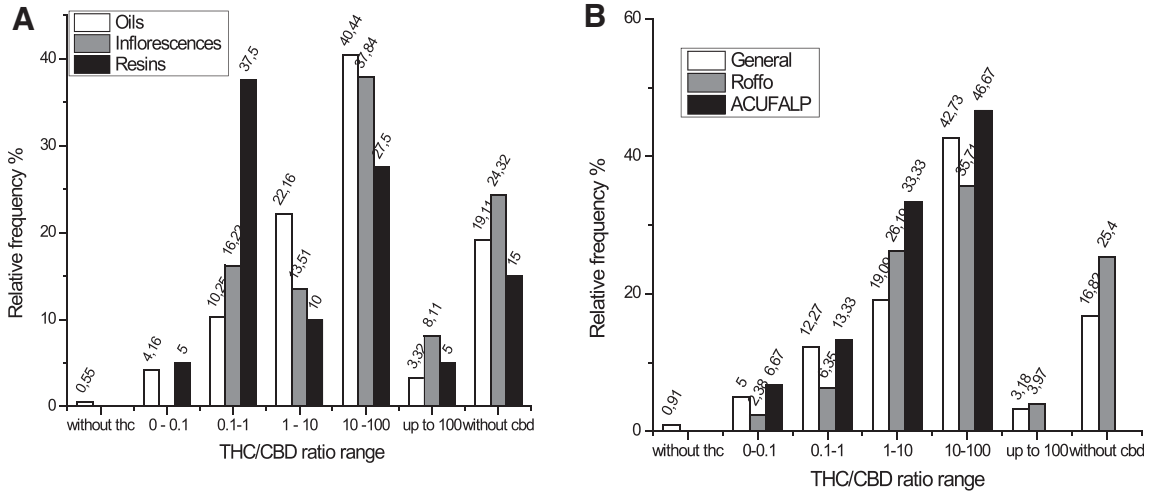

FIG. 3. Relative frequency (%) of samples based on THC/CBD ratio range. **(A)** Inflorescences (gray bars), resins (black bars), and oils (white bars). **(B)** Oil subgroups: GENERAL (white bars), ROFFO (gray bars), and ACUFALP (black bars). CBD, cannabidiol; THC, tetrahydrocannabinol.

The highest proportion of samples from the ACUFALP subgroup was found in the 10–100 (46.7%) and 1–10 (33.3%) ranges and frequencies of 6.7% and 13.3% were determined in the 0–0.1 and 0.1–1 ranges, respectively. The distribution of relative frequencies of the THC/CBD ratio for the GENERAL subgroup was as follows: 5% for 0–0.1, 12.3% for 0.1–1, 19.1% for 1–10, 42.7% for 10–100, and 3.2% for over 100 (Fig. 3B). For the ROFFO subgroup, the following relative frequency distributions were determined: 2.4% for 0–0.1, 6.4% for 0.1–1, 26.2% for 1–10, 35.7% for 10–100, and 4% for over 100.

Acidic/neutral ratio

Our results indicate that the highest proportion of samples rich in acid cannabinoids is among inflorescences, with 91.2% of the samples presenting acid/neutral >1. Resins and oils presented higher proportions of samples enriched in neutral cannabinoids, most of them presenting acid/neutral <1 or no acidic cannabinoids at all (57.5% resins and 78.4% oils) (Fig. 4A).

The GENERAL and ROFFO oil subgroups showed acidic/neutral ratios close to 1 (1.1 ± 0.1 and 1.1 ± 0.2, respectively), whereas the ACUFALP subgroup showed a slightly lower ratio (0.4 ± 0.2) (Table 2).

The relative frequency distribution was quite homogeneous among lower acid/neutral ratio ranges, with the highest relative frequency for each subgroup in the 0.1–1 range (GENERAL: 32.7%, ROFFO: 36.2%, and ACUFALP: 35.3%). However, the GENERAL subgroup presented 25% of the samples in the 1–10 range—indicating samples enriched in acid cannabinoids—followed by the ROFFO (16.5%) and ACUFALP (5.9%) subgroups. All three subgroups presented samples in which acid cannabinoids were not detected, with relative frequencies of 25%, 17.1%, and 29.4% for GENERAL, ROFFO, and ACUFALP, respectively (Fig. 4B). The ACUFALP subgroup therefore presented the highest proportion of samples enriched in neutral cannabinoids and 94.1% presenting acid/neutral <1 or no acidic cannabinoids at all, followed by the ROFFO (82.9%) and GENERAL (74.1%) subgroups.

CBN content

As expected, inflorescences and resins showed significantly higher levels of CBN (0.8 ± 0.7 mg/mL and 2.7 ± 0.8 mg/mL) than oils (0.3 ± 0.1 mg/mL), which are usually more diluted (Table 1). However, given the differences observed in relation to total cannabinoid content, these CBN levels represent 0.5% and 1% of the total cannabinoids for inflorescences and resins, respectively; in the case of oils, they constitute 6% of total cannabinoids. Moreover, most of the samples of each group (91.2% for inflorescences, 65% for resins, and 94.5% for oils) showed low CBN concentrations of below 1 mg/mL.

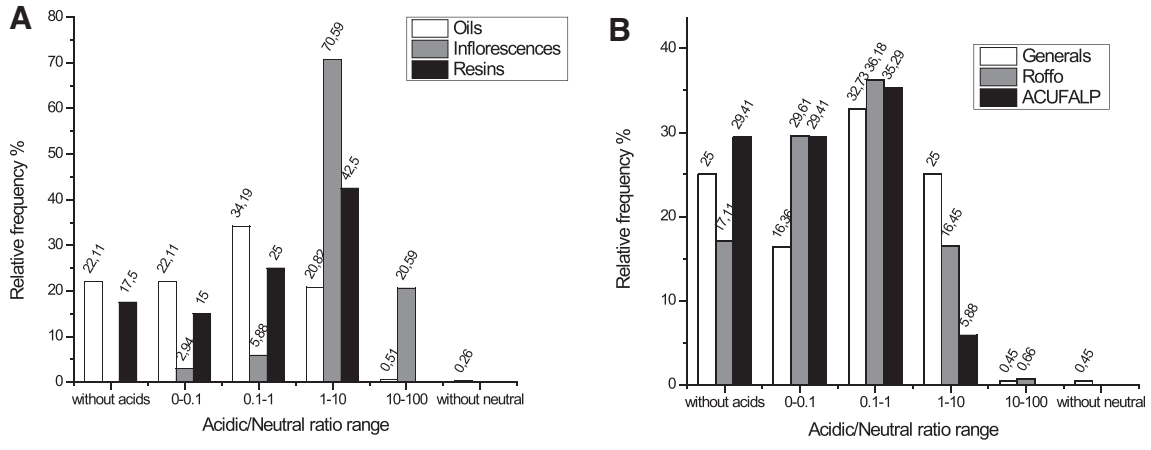

FIG. 4. Relative frequency (%) of samples based on the acidic/neutral ratio range. **(A)** Inflorescences (gray bars), resins (black bars), and oils (white bars). **(B)** Oil subgroups: GENERAL (white bars), ROFFO (gray bars), and ACUFALP (black bars).

CBN levels in the oil subgroups showed significant differences: GENERAL: 0.3 ± 0.1 mg/mL, ROFFO: 0.1 ± 0.1 mg/mL, and ACUFALP: 0.9 ± 0.6 mg/mL (Table 2), representing 6% of total cannabinoids for GENERAL; 0.1% for ROFFO; and 1% for ACUFALP subgroups. It is worth mentioning that 93.6%, 97.6%, and 83.4% of the samples from the GENERAL, ROFFO, and ACUFALP subgroups, respectively, contained CBN levels below 1 mg/mL.

Discussion

This work constitutes the first reported analysis of cannabis derivatives such as inflorescences ($n = 34$), resins ($n = 40$), and oils ($n = 362$) used therapeutically in Argentina. The analysis was based on parameters traditionally used in scientific studies to characterize cannabis strains or derivatives linked particularly to therapeutic functions, such as total cannabinoid content and THC/CBD ratio.[18–20] CBN content was also analyzed and the relationship between acid and neutral cannabinoids studied.

Our results indicate that total cannabinoid levels in resins were on average 5- and 43-fold higher than those found in inflorescences and oils, respectively. This is in line with the concentration and dilution processes associated with the elaboration of resins and oils from inflorescences. However, the variability in each group was relatively high for all analyzed parameters. This could be due to the use of different Cannabis sp. strains and growing conditions (indoor and outdoor); different processes carried out to obtain resins and oils, such as alcohol extractions or macerated in oil; short or prolonged contact times; different temperatures; and the use of the whole plant or inflorescences only. Other aspects to be taken into account are the different ways of preserving inflorescences and their derivatives such as different temperature conditions (room temperature, refrigerator, and freezer), pressure (atmospheric and vacuum), and light or darkness, during different periods of time.[14]

Our findings show no significant difference among resins, inflorescences, and oils in the THC/CBD ratio traditionally used to characterize cannabis oils and derivatives. It should be highlighted that virtually all the studied resin, inflorescence, and oil samples showed the presence of THC. Most of the samples in each group were rich in THC, the THC/CBD ratio being greater than 1 in 59.4%, 42.5%, and 65.9% of inflorescences, resins, and oil samples, respectively. In addition, 24.3% (inflorescences), 15% (resins), and 19.1% (oils) of samples contained no measurable amount of CBD.

However, the acidic/neutral cannabinoid ratio for inflorescences showed significant differences with respect to resin and oil values, evidencing the changes occurring in acidic and neutral cannabinoid content during the processes to obtain resins and oils. Thus, 91.2% (inflorescences), 42.5% (resins), and 21.3% (oils) of samples showed an acidic/neutral ratio >1; note that acidic cannabinoids were not detected in 17.5% of the resins and 22.1% of the oils employed for therapeutic proposes.

CBN could occur in cannabis in low concentrations as a degradation product of THC. High levels of CBN might indicate poorly stored or aged cannabis with prolonged exposure to elevated temperatures, light, and/or oxygen.[21] The low CBN levels observed indicate that samples were fresh and in accordance with good practices of the homemade oil production, employing usually from 5 to 20 g of inflorescences.

Our results indicate that oils form ACUFALP subgroup presented the highest concentrations of total cannabinoids, being average 4 and 13 higher than those observed in GENERAL and ROFFO subgroups, respectively. In this regard, it should be noted that only in ACUFALP subgroup, although in different proportions, as opposed to GENERAL and ROFFO subgroups, where CBD was not detectable in 16.8% and 25.4% of samples, respectively. Even though most oils presented higher THC content than CBD, it is noteworthy that the ACUFALP subgroup presented the highest proportion of CBD-rich oils, with 20% of samples showing THC/CBD <1, followed by the GENERAL (17.3%) and ROFFO (8.8%) subgroups.

Our findings show that the oils studied in this work presented medium-to-low concentrations of cannabinoids compared to a random selection of cannabis products available in other countries. Oils of the ROFFO subgroup showed the lowest TCC, at approximately half the concentration of the most diluted oil (Table 3). Oils of the ROFFO subgroup presented an average THC/CBD ratio of 22:1, whereas most of the products listed in Table 3 only show the presence of CBD.

When applying cannabis-based oil treatment concomitantly with traditional oncological medications and treatments, the medical team at the Palliative Care Service (IOAR, Oncologic Institute Ángel H. Roffo) observed that ROFFO subgroup oils employed by patients in their observational study were associated with pain reduction and a decrease in OMED (Oral Morphine Equivalent Dose)[22,23]

The presence of THC and CBD in oils of the ROFFO, GENERAL, and ACUFALP subgroups contrasts with the

exclusive CBD content of many products on the international market, including Epidiolex (approved by the U.S. Food and Drug Administration [FDA] for Lennox-Gastaut and Dravet syndrome treatment). In most cases, the proportion of acidic and neutral cannabinoids (THCA, THC, CBDA, and CBD) in studied homemade oils was different.

Conclusions

In Argentina, as in a growing number of countries around the world, the use of cannabis for therapeutic purposes is becoming increasingly widespread for treating symptoms in a number of diseases. However, access to cannabis-based products is hindered by legal/economic issues, making homemade preparations the norm. In this context, it is essential to gain more detailed information on the total cannabinoid content, the THC/CBD ratio, and the acidic cannabinoid content of these preparations, all of which contribute to the oils' various properties.[23,24] Furthermore, oils containing acidic cannabinoids constitute an alternative cannabis-based oil since most of the currently available commercial formulations contain neutral cannabinoids.

Approval in November 2020 of the new regulation of medicinal cannabis law 27,350 underscores the need to expand our knowledge of and protocolize all related processes, from strain cultivation to material processing and storage, to enhance the quality, safety, and reproducibility of homemade products.

Our findings indicate that despite their wide variability, homemade preparations in Argentina show average levels of cannabinoids and profiles, compatible with effective therapeutic action.

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Abbreviations Used

2-AG = 2-arachidonoylglycerol
AEA = anandamide
ANOVA = analysis of variance
CBD = cannabidiol
CBDA = cannabidiolic acid
CBN = cannabinol
CONICET = National Council for Scientific and Technical Research of Argentina
ECS = endocannabinoid system
FDA = U.S. Food and Drug Administration
HPLC/UV-DAD = high performance liquid chromatography/ultraviolet diode array detector
IOAR = Oncologic Institute Ángel H. Roffo
NGO = nongovernmental organization
SE = standard error
TCC = total cannabinoid concentration
THC = tetrahydrocannabinol
THCA = tetrahydrocannabinolic acid
UNLP = National University of La Plata