

Comparison of *Cannabis* Flower Oil Extract from Microwave, Ultrasound, and Combined Processes: Yield, Cannabinoids Content, and Analgesic Effect in Situ

Alfonso Topete-Betancourt¹, Luis E. Sámano Muñoz¹, Héctor E. Martínez-Flores^{1,*}, Osvaldo Álvarez-Cortés², Jose J. Virgen Ortiz³, M.G. Garnica-Romo⁴, Luis F. Ortega-Varela⁵, Rosa M. García-Martínez¹

¹Facultad de Químico Farmacobiología. Universidad Michoacana de San Nicolás de Hidalgo, Tzintzuntzan 173, Col. Matamoros. C.P. 58240. Morelia, Michoacán, México,

²Programa Institucional de Doctorado en Ciencias Biológicas. Universidad Michoacana de San Nicolás de Hidalgo. Avenida Francisco J. Múgica. S/N. Ciudad Universitaria. C.P. 58030. Morelia, Mich., México.

³CONAHCYT - Centro de Investigación en Alimentación y Desarrollo, A. C. – CIDAM, Antigua Carretera a Pátzcuaro km. 8. C.P. 58341. Morelia, Michoacán, México.

⁴Facultad de Ingeniería Civil. Universidad Michoacana de San Nicolás de Hidalgo. Santiago Tapia 403. Col. Centro. C.P. 58000. Morelia, Mich., México.

⁵Facultad de Salud Pública y Enfermería. Gertrudis Bocanegra 330, Cuauhtémoc. C.P. 58000. Morelia, Mich., México.

*Corresponding author: hector.martinez.floresauthor@umich.mx

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Abstract *Cannabis Sativa L.* has been studied due to the psychoactive ingredients it contains. Demand for *Cannabis* oil has shown consistent growth since outbreak of the COVID-19 pandemic, and therefore more studies are required to improve or develop new extraction methods. The purpose of this research was to apply microwave combined with ultrasound technologies to enhance the oil extraction yield without compromising quality. Total phenolic and cannabinoid (THC, CBD) compounds content, yield, and analgesic properties were evaluated in the oil samples. The combination of processes showed higher oil yield (48.93%); however, the oil had lower values of THC content (32.14 mg/ml oil), lower analgesic quality, and low DPPH• (1.39 mg/ml) and ABTS•+ (23.37 mg/ml) scavenging activity, as well as low content of total phenol compounds (13.53 mg GAE/g oil) and high peroxide value (9.09 mEq O₂/Kg). The ultrasound process showed 40.36% yield, 1.02 mg/ml DPPH• value, and 2.30 mg/ml ABTS•+, 24.51 mg GAE/oil g total phenolic content, 7.93 meq/ peroxide value, and enhanced analgesic capacity, followed by the values obtained by microwave. The results suggest that ultrasound represents an alternative process to produce *Cannabis* oil of high quality. In addition, the *Cannabis* oil had elevated THC content, which could be related to anti-inflammatory capabilities.

Keywords: *Cannabis sativa L.*, oil, cannabinoids, ultrasound, microwave

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1. Introduction

Cannabis sativa L. was one of the first plants to be used by humans for fiber, food, medicine, and in social and religious rituals [1]. *Cannabis* is classified based on genetics, phenotypic properties, and chemical composition. The different types of *Cannabis* are rich and have a variety of bioactive phytochemicals [2,3]. Bioactive compounds from *C. Sativa* have been relevant for the pharmaceutical and food industries, with wide potential for application in diverse areas. There are over 100 different cannabinoid compounds in this herbaceous

flowering plant. The most abundant and best-known phytocannabinoids are the (–)-*trans*- Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD) [4]. *Cannabis sativa* or industrial hemp has a higher cannabidiol (CBD) level as compared to *Cannabis indica* and *Cannabis ruderalis*, whereas *Cannabis indica* has a higher level of the psychoactive THC [2]. THC is a psychoactive compound and has therapeutic activity against arthritic and inflammatory conditions, autism, Parkinson's, glaucoma, and cardiovascular disorders.

Cannabis oil has been used as a therapeutic agent due to its analgesic properties. *C. Sativa* has bioactive compounds in different concentrations, such as β -sitosterol (1905.00 $\pm$ 59.27 mg/Kg oil), campesterol

(505.69±32.04 mg/Kg oil), phytol (167.59±1.81 mg/Kg oil), cycloartenol (90.55±3.44 mg/Kg oil), and γ -tocopherol (73.38±2.86 mg/100 g oil) [5]. *C. sativa* also contains linoleic acid (55%), α -linolenic acid (16%), and oleic acid (11%). Terpenes are the primary constituents of essential oils and are responsible for the aromatic characteristics of *Cannabis* [6,7].

An extraction process is commonly employed to obtain the target bioactive compounds from complex plant matter [8]. The common method applied to *Cannabis* oil considers extraction using ethanol, methanol, butane, hexane/acetone, water, and even vegetable oil (olive oil), as well as supercritical carbon dioxide (CO₂), dynamic maceration (DM), pressurized liquid extraction (PLE), or supercritical fluid extraction. However, the increasing demand for environmentally friendly and sustainable industrial processes has provoked the development of alternative processes like ultrasound and microwave. Ultrasound assisted extraction has been widely adopted in the food and chemical industry for its ability to apply varying intensity of sound waves, generated commonly in a frequency between 20 to 100 kHz, which enables the penetration of solvents into a sample matrix to promote a major extraction of compounds during the process of cavitation. This process is described as the formation, expansion, and collapse of bubbles within the solution, which allows the solvent access into cell material, facilitating a high leach of several compounds present, with low decomposition during the extraction procedure as compared to other methods such as subcritical water or hot maceration [9]. An alternative green method is microwave-assisted extraction, which uses the electromagnetic energy provided in the form of microwaves, with frequencies between 300 MHz and 300 GHz, that is responsible for rapid heating following ionic conduction and dipole rotation [10,11]. This procedure directly exposes each molecule to a microwave field which is converted to kinetic energy that can break cell walls and release their contents into a liquid phase. The advantages of this green extraction process include improved solubility, increased surface equilibrium, efficient mass transfer, so that all these factors result in a system that uses less energy with fast processes and lesser solvent consumption. Different factors can influence the extraction efficiency of phytochemicals and cannabinoids from *Cannabis* flowers, such as the type of solvent, sample size, sample-to-solvent ratio, time, and current. The purpose of this work was to evaluate the *Cannabis* oil yield and cannabinoid compounds content using emerging technologies such as ultrasound and microwave, either singly or in combination. Another objective was measuring the analgesic property of *Cannabis* oil in Wistar rats.

2. Materials and Methods

2.1. Samples Preparation

Cannabis sativa L. flowers were cultivated under standardized conditions according to the requirements of good agricultural practice (GAP). The *Cannabis* flowers

were gathered in October 2022. After harvest, the flower sample was dried in an oven with air circulation (Model 9053A, ECOSHEL, Vela Quin, Mexico) at a constant temperature of 35°C for 24 h. The dried flowers were manicured to remove leaves and stems, then milled and sieved using a 20 μ m mesh. The sample was stored at -4°C and protected from light until analysis.

2.2. Microwave-Assisted *Cannabis* Oil Extraction

Microwave-assisted extraction (MI) was performed using a commercial microwave (Hamilton Beach, model HP-P100N30AL-T1S, VA, USA). The experiments were carried out at atmospheric pressure using a straight glass plate of 25 cm. Twenty grams of the dried sample was put on the glass plate, then irradiated at 50% power for 60 sec. Afterward the irradiated sample was poured into a glass Erlenmeyer flask containing 100 ml of ethanol at a mixing rate of 1:5, and placed in a thermo bath (Julabo, Model SW22, Seelbach, Germany) at 50°C and stirred at 40 rpm for 12 h. Past this time the fluid extracted was separated from the solid residue by using filter paper # 20. The collected solution was concentrated in vacuum rotary evaporator (HAHNVAPOR, model HS-2001NS, HAHN-SHIN Scientific, South Korea) at 40°C for 15 min. The oil obtained was stored at -4°C.

2.3. Ultrasound-Assisted *Cannabis* Oil Extraction

An ultrasound system (UT) was used for oil extraction in an Erlenmeyer flask. Twenty grams dried *Cannabis* flowers were mixed with 100 ml of ethanol at a rate of 1:5 as recommended by [12]. The mixture was treated using ultrasonic equipment (Model VC505, Sonics & Materials Inc., Newtown, CT, USA), at a frequency of 35 kHz and power of 800 W for 10 min at 20°C. Afterward, the sample was placed in a thermo bath at 50°C, and stirred at 40 rpm for 12 hr. The fluid extracted was separated from the solid residues using filter paper, and concentrated in a vacuum rotary (HAHNVAPOR, model HS-2001NS) at 40°C for 15 min. The oil was stored at -4°C.

2.4. Microwave and Ultrasound Coupled for Oil Extraction (MX)

Microwave and ultrasound were coupled (MX) for *cannabis* oil extraction. Briefly, 20 g of dried flowers were placed on a glass plate and irradiated in a microwave oven (Hamilton Beach, Model HP-P100N30AL-T1S) at 50% power for 60 sec. The irradiated sample was placed in an Erlenmeyer flask with 100 ml solvent ethanol, and the mixture was treated using ultrasonic equipment with a frequency of 35 kHz and power of 800 W for 10 min at 20°C. At the end of the extraction process the fluid extracted was separated from the solid residues using filter paper. The collected solution was concentrated in a vacuum rotary evaporator (HAHNVAPOR, model HS-2001NS) at 40°C for 15 min. The oil obtained was poured and stored at -4°C.

2.5. Efficiency of oil extraction

To determine the efficiency of oil extraction, the dried flowers and the extracted oil were weighed, and the efficiency of oil extraction was calculated [10].

2.6. Peroxide Value (PV) in *Cannabis* Oil

Five grams of *Cannabis* oil was mixed with 30 ml of a mixture of 3:2 (v/v) acetic acid: chloroform. Then 0.5 ml of saturated potassium iodide (KI) was added to the solution, and the mixture kept in darkness for 1 min. After adding 30 ml of distilled water to this solution a titration was carried out with sodium thiosulfate (0.01 N) until the solution became colorless [13]. The peroxide value was expressed as mEq of oxygen/kg.

2.7. FTIR-ATR

The Fourier Transform Infrared spectra (FTIR) with attenuated transmission (ATR) measures were recorded using a FTIR spectrophotometer (Vertex, Bruker Optics, model VERTEX 70/70 V, Germany) with a resolution of 4 cm^{-1} at 32 scans and an internal reflection accessory. Ten microliters of each oil sample were deposited on a diamond ATR crystal plate. The collection time for each sample spectrum was 2 min. Spectra were recorded for all the oil samples, scanned in the absorbance mode from 4000 to 400 cm^{-1} . The spectra were subtracted against background air spectrum. The spectra were recorded in absorption mode at each data point. The most relevant FT-MIR spectroscopic wavenumber regions were recorded to identify the THC and CBD compounds in the oils.

2.8. Total Phenolic Content

The total phenolic compounds content in the methanolic extracts was determined by the Folin–Ciocalteu method. An aliquot of 0.2 mL of the methanolic extract was placed in a volumetric flask of 10 mL, to which 0.5 mL of Folin–Ciocalteu reagent was added. After 3 min, 1 mL of saturated sodium carbonate solution also was added. The flask was filled with water up to 10 mL. After 1 h, absorbance was measured at 725 nm against a reagent blank using a UV–Vis spectrophotometer (VELAB, Model VE-5600UV, TX, USA). Total phenolic compounds were determined after preparation of a standard curve and reported as acid gallic equivalents per gram of oil (mg GAE/g oil).

2.9. Antioxidant Activity Determination

Inhibition of the DPPH• (1,1-diphenyl-2-picrylhydrazyl) radical was determined. The DPPH• radical was dissolved in methanol until an absorbance of 0.75–0.78 at 517 nm was obtained (using Spectrophotometer UV/Vis Smartec Plus, Bio-Rad, Philadelphia, PA, USA.). Then, the mixture was shaken and kept in a dark space at room temperature for 30 min, thereafter being read at 517 nm. A curve was created using gallic acid as a standard, expressing the results in terms of percentage inhibition.

The inhibition of the radical ABTS•+ (2,2'-azinobis-3-ethylbenzothiazoline 6-sulfonic acid) was determined.

This radical was formed after incubation of the ABTS (7 mM) reaction with potassium persulfate (2.45 mM) at room temperature, under dark conditions, for 12 h. Once formed, it was diluted with ethanol until an absorbance value of 0.700 ± 0.100 at 754 nm was obtained (UV/Vis Smartec Plus Spectrophotometer, Bio-Rad, Philadelphia, PA., USA.). A curve was created using gallic acid as a standard, expressing the results in terms of percentage inhibition.

2.10. Quantitative Analysis of Cannabinoids

The content of cannabinoids was determined by high performance liquid chromatography [14]. *Cannabis* oil samples were diluted with 2-propanol, filtered through $0.45\text{ }\mu\text{m}$ nylon syringe filters, and injected (10 μL) into the high-performance liquid chromatography (HPLC) system. The HPLC system was equipped with a quaternary pump unit, a diode array detector, an automatic sample injector, and a column oven. The separation of cannabinoids was performed on a Discovery® C18 column ($5\text{ }\mu\text{m}$ particle size, $L \times \text{I.D.}$: $250 \times 4.6\text{ mm}$) using 0.1% acetic acid in water and methanol (85/15, v/v) as mobile phase. The column-oven temperature was set to 45°C , and the flow rate was 0.8 mL/min. Cannabinoids were detected at 220 nm and identified by comparing their retention times with those of the corresponding standards and by spiking samples with the appropriate standard.

2.11. Formalin-induced Nociception

The formalin test is the standard method used for inflammatory pain probe. Male Wistar rats were randomly divided into five groups containing six animals each. The five groups received an intraplantar injection of a formalin solution of 1% (v/v). A volume of 20 μL of formalin solution was injected into the right hind paw of each animal, and 60 μL each of *cannabis* oil extracted using the ultrasound, microwave, and combined methods was added topically as single treatment to obtain three different groups. The fourth group was tested with formalin solution plus dexamethasone ointment, and to the fifth group received only formalin solution. Each treated rat was placed in an acrylic chamber and the rat's behavior recorded. The time which the animal spent licking, shaking, or biting its injured paw was observed and was interpreted as a sign of nociception or pain. Observation phases were undertaken every 5 min over 1 h. The nociceptive responses were divided into two phases: early phase (neurogenic pain) and late phase (inflammatory pain). The number of instances of response was plotted versus time (minute). The experiments were designed and carried out under the Mexican official normativity for the production, care, and use of laboratory animals NOM-062-ZOO-1999 [15], the general law of health of Mexico, and the American National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.12. Statistical Analysis

Results are presented as means $\pm$ SD from three replicates of each experiment. A P value <0.05 was used to denote significant differences among mean values

determined by analysis of variance using the Origin software.

3. Results and Discussion

3.1. Extraction Methods for Cannabis Oil Yield

In this work, three methods were employed to extract *Cannabis* oil, using ethanol as solvent. The microwave and ultrasound processes individually or their combination produced different *Cannabis* oil yields. Using the ultrasound process, the oil yield was $41.17 \pm 0.41\%$, a higher value as compared to that obtained by [13], who had reported oil extraction yield in a range from 23% to 31.7% using hempseed. The hempseed oil yield using microwave was $40.2 \pm 0.53\%$, a similar value as reported by [10]. The ultrasound and microwave methods use different mechanisms of action to subtract the oil in the *Cannabis* extracts. Ultrasound produces cavitation bubbles, thus allowing the solvent better penetration into the cells, which facilitates the ethanol–oil interaction [11]. Microwave method uses microwaves to penetrate the plant tissue, creating a temperature gradient in the plant matrix due to increase in the molecular water kinetic energy by rotation; this induces a high temperature until the water is released for evaporation, even severely damaging the cell walls, which facilitates the phenomena of permeability and mobility of oil [16,17,18]. When the ultrasound and microwave technologies were combined, the oil yield was $49.3 \pm 2\%$, promoting an increase in the mass transfer rate, probably because it led to cellular damage in the same sample, caused by the empty spaces following water release induced by the microwave and the resulting cavitation. Fiorini et al. [19] showed that the highest *Cannabis* oil yield applying microwave irradiation was $9.33 \pm 0.69\%$, whereas in our results the combined process presented a higher efficiency than the individual procedures, although the content of cannabinoids obtained by the combined method was lower than that achieved by applying the two extraction treatments separately. On the other hand, the characteristic aroma of *Cannabis* was high in intensity when the ultrasound process was used, in which the terpenes represent the most important constituents of *Cannabis* oil. Our results showed a similar tendency to the values reported in the literature [20], who pointed out that UT and MI, either alone or combined, can greatly improve the extraction of bioactive substances, as shown in Table 1. An extractions process combining ultrasound and microwave has the potential to improve the *Cannabis* oil yield percentage relative to conventional methods, besides being a green method with high positive environmental impact.

3.2. Determination of THC and CBD in Oil from Cannabis Flowers

UT and MI, whether individually or in combination, improve the bioactive substances' extraction. On the other hand, it is important to find the method and optimal conditions to increase the oil yield, although these conditions may not always ensure the best extraction of

the oil's chemical constituents. For example, in our results (Table 1) the combined method presented a significant increase in *Cannabis* oil yield, but the quality in terms of bioactive compounds deteriorated, possibly due to the excessive energy applied, and the phytochemical compounds could be affected. Table 1 shows the THC and CBD content in the *Cannabis* oil obtained. According to previous report [21], the yield in Δ^9 -THC rose at the expense of the native acidic compound of Δ^9 -THCA. Moreover, our study did not use harsh conditions to induce the decarboxylation reaction of THCA to THC, which is favored when the applied temperature is in a range from 100°C to 140°C [22]. In our study the highest THC yield was found when ultrasound was used, followed by the microwave method, with decarboxylation reaction from THCA and CBDA into neutral THC/CBD with CO_2 release during the extraction process [23,24]. Despite the low temperature used, of $35\text{--}40^\circ\text{C}$, this reaction probably was due to the type of energy applied. THC is accepted to be the main psychoactive agent in *Cannabis* oil, and it possesses analgesic, anti-inflammatory, appetite stimulant, and antiemetic properties [25].

3.3. Antioxidant Capacity in Cannabis Oil Extracted using Different Methods

Table 1. *Cannabis* oil yield, when extracted using microwave and ultrasound individually or in combination.

Determination	Ultrasound	Microwave	Microwave + Ultrasound
Total phenol content (mg GAE/g oil)	24.51 ± 0.87^a	20.84 ± 0.63^b	13.53 ± 0.92^c
DPPH• IC ₅₀ (mg/ml)	1.02 ± 0.04^c	1.18 ± 0.03^b	1.39 ± 0.02^a
ABTS•+ (Trolox Kg ⁻¹)	2.303 ± 0.01^c	3.12 ± 0.01^b	23.37 ± 0.04^a
THC (mg/g oil)	54.42 ± 0.54^a	44.86 ± 0.21^b	32.14 ± 0.50^c
Peroxide value (mEq O ₂ /Kg)	7.93 ± 0.53^{ab}	6.08 ± 0.74^b	9.09 ± 0.59^a
<i>Cannabis sativa</i> oil yield (%)	40.36 ± 1.13^b	38.254 ± 2.34^b	48.93 ± 0.53^a

Different superscripts letters in the same row represent statistically significant differences between experimental values ($p < 0.05$).

Table 1 shows the phenolic compounds content and the antioxidant capacity (ABTS and DPPH•) of *Cannabis* oil extracted under ultrasound, microwaves, and ultrasound combined with microwaves. Ultrasound process resulted in the highest total phenol content, 24.51 ± 0.87 mg GAE/g oil, followed by microwave with 20.83 ± 0.63 mg GAE/g oil, whereas the combined process had the lowest value, at 13.53 ± 0.92 mg GAE/g oil. In the DPPH• test, the oil extracted with ultrasound has a major inhibition capacity, with the lowest value of IC₅₀ at 1.02 ± 0.04 mg/ml, followed by microwave with 1.18 ± 0.03 mg/ml, and finally the combined process with the highest IC₅₀ of 1.39 ± 0.02 mg/ml. Somewhat similar results were achieved by [26], with 21 ± 5 mg GAE kg⁻¹ of total phenolic compounds content in oil determined with Folin-Ciocalteu method; also, using RP-HPLC the polyphenols were semi-quantified, with 23.5 ± 4 mg CAE kg⁻¹ in oil. Otherwise, in the case of the combined treatment, the result obtained was similar to that reported in the literature [27], who reported values ranging from 16.13 ± 0.003 to 6.62 ± 0.01

(mg GAE/g oil) of phenolic content in the plant after flowering. Siger et al. [28] reported that the highest total phenolic compounds content was for hemp oil, with 2.45 ± 0.05 (mg CAE/100 g) extracted using the cold-pressed process, whereas in other study [10] was mentioned that the hemp oil antioxidant activity showed a IC_{50} value of 30.82 mg/ml when extracted using a microwave-assisted process, although a IC_{50} value of 32.47 mg/ml resulted in hemp oil extracted under Soxhlet extraction. Based on our results, while the antioxidant activity increased when using ultrasound for extraction, it decreased in the case of the combined and microwave antioxidant methods. The importance of this finding is that hemp oil has high content of phenolic compounds, which probably reduces the oxidative damage in cells [29].

3.4. Vibration Spectra for FTIR-ATR

FTIR-ATR is a powerful analytical technique which has been used in the study of edible oils. The spectra from the three processes of oil extraction used in this study are shown in Figure 1. The specific vibrational bands for THC and CBD have been assigned [30]. The differences in the absorbance intensity in some bands between *Cannabis* oil samples from distinct extraction processes are slightly visible; this difference might be a consequence of the process employed to obtain the oil. The main cannabinoid molecule present in the oil samples was the THC. The most important region to identify the vibration modes assigned to THC is found in the $1700\text{--}500\text{ cm}^{-1}$ wavenumber zone [31]. Gigopulu et al. [32] monitored *in situ* the decarboxylation process of THCA to THC using THCA standard and IR spectroscopy experiment. The THC intensity in oil samples extracted from the ultrasound, microwave, and coupled processes was in agreement with the concentration determined through HPLC, with higher values being obtained using the ultrasound, followed by the microwave method, while the process of combining ultrasound with microwaves resulted in the lowest concentration. In the region from $2900\text{--}2800\text{ cm}^{-1}$ are shown the bands from CH_3 and CH_2 stretching vibrations, corresponding to the carbon chain of all edible oils. The intensity of the band at 2926 cm^{-1} was higher for the ultrasound process, followed by the microwave method and the combined process. In this wavenumber region a point determinant is not present to indicate the decarboxylate reaction from THCA to THA. The bands at 1621 cm^{-1} and 1572 cm^{-1} correspond to vibrations from the aromatic ring. On the other hand, the band at 1262 cm^{-1} is an indicator of decarboxylation from THCA to THC, and these bands gained more intensity, although the bands from the individual ultrasound and microwave processes had higher intensity at 2930 cm^{-1} , 1575 cm^{-1} , and 1184 cm^{-1} , with respect to each band belonging to specific vibration molecules of C—H on olefinic double bonds. The band at 1925 cm^{-1} represents stretching vibrations of the methylene group; slight bands at $1703\text{--}1744\text{ cm}^{-1}$ and at $1300\text{--}100\text{ cm}^{-1}$ appear in the ultrasound and microwave individual methods, which are characteristic of the ester group. In the same case a slight band appears at 1653 cm^{-1} when using ultrasound and microwave, which corresponds to C=C unsaturated acyl group. Other subtle differences in ATR intensities can be

noticed at 2358 cm^{-1} ; this band is assigned for CO_2 [33], present due to the process of decarboxylation of THCA or CBDA during the extraction procedure, as was mentioned in [22]. However, in our result this happened due to the type of energy applied rather than high temperature. The 2362 cm^{-1} band corresponds to CO_2 which was produced during the interconversion of THCA to THC. Gigopulu et al. [32] have shown that a correlation exists between the quantity of CO_2 eliminated and the conversion of THCA to THC.

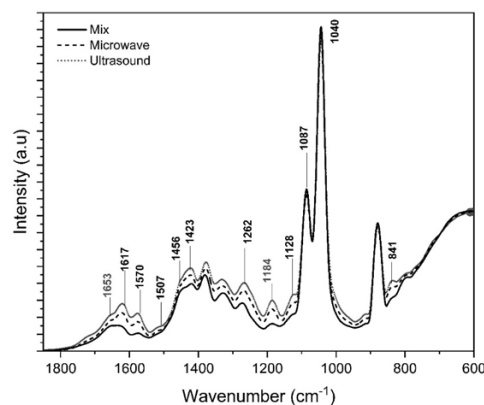


Figure 1. FTIR-ATR spectrogram for *cannabis* S. oil samples extracted with ultrasound (short, dotted line), microwaves (short, dashed line), and the process combining ultrasound with microwave (mixed with straight line).

3.5. Formalin Test in Rats

Formalin test was used to assess the analgesic activity of *Cannabis sativa* L. oil extract in rats. Nociceptive behavior in rats was observed from 0 to 15 min (phase I, early phase). The formalin presents a toxic effect on the peripheral fibers, inducing intense inflammation. When comparing the analgesic effect of cannabis oil extracted using ultrasound, microwave, and combined methods, the early phase showed that extract from the combined process helped most to reduce pain in the rats, followed by the ultrasound extract, resulting in a decreased value when compared with the control negative ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, respectively) (Figure 2). Oil extracted with ultrasound, microwave, and combined methods, as well as dexamethasone cream, showed significant decrease in licking time and frequency of licking of their formalin-injected paw by the rats. The ultrasound method, with a THC of 54.42 ± 0.54 content, showed the highest effect with respect to pain. The analgesic potential of Δ^9 -THC has been reported; among others by Wade et al. [34], who mentioned a reduction in pain with administration of $60\text{ }\mu\text{L}$ of *cannabis* oil in each rat, which reduced the pain in distinct phases compared with the control (only formalin). The latest phase at 20 to 60 min promoted chemical stimulation of nociceptors, except for the positive control [35,36]. The response of the rats in this phase showed differences; especially with regard to the combined method extract the rats' behavior during 10 to 40 min was lower, but at 30 min the pain intensity begins to increase. However, the ultrasound method extract maintained the tendency constant during

the rest of the time; this could be due to the high content not only of THC (mg/ml) but also of total phenolic compounds (24.51 mg GAE/g oil), and probably of sesquiterpenes such as β -Caryophyllene which increased aroma intensity [6]. All these compounds induced high analgesic and anti-inflammatory properties, but without antinociceptive effects systemically, when the cannabis oil samples were applied in rats topically in the formalin test [37,38].

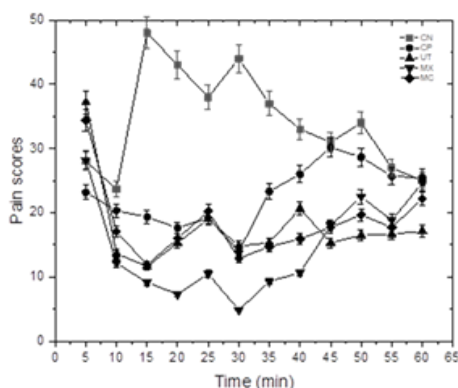


Figure 2. Formalin test to assay analgesic intensity of extracts from different processes CN: Control only formalin, CP: Control Formalin + Dexamethasone, UT: Ultrasound process, MI: Microwave process. MX: Combined processes (UT+MI).

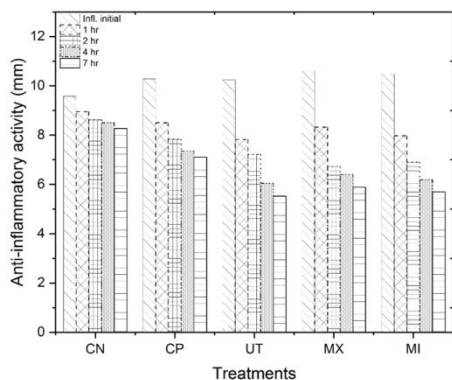


Figure 3. Anti-inflammatory effects of topical cannabis oil during 7 hours in rat's paw model, single dose treatments: formalin 20 μ L solution 1% (CN), formalin 20 μ L solution 1% plus 1 g of dexamethasone (CP), ultrasound 60 μ L cannabis oil (UT), microwave 60 μ L cannabis oil (MI), coupled 60 μ L cannabis oil (MX = UT+MI).

The results, during the mitigation of pain in rats, mainly observed in the *Cannabis* oil extracted by ultrasound, suggest that the substrate could be applied to inflamed human tissue [39,40,41]. Nevertheless, more studies are required to confirm the analgesia. For example, the correct amount of extract should be applied to avoid secondary effects, although THC and phenolic compounds may have interactions with the enzyme's pain-relieving properties [42,43]. These results show that substrate with high levels of THC and total phenolics compounds combined significantly improve the efficiency of pain relief.

For the edema test, the results illustrate that ultrasound substrate could slightly decrease paw volume from

2 to 7 h, compared to the control group; this behavior is in agreement with results reported in [44], who mentioned that THC had anti-inflammatory properties (Figure 3). Unlike dexamethasone cream, it affected paw volume after 12 h. After 3 h. This might suggest that ultrasound substrate was more effective than cream analgesic, with a progressive decrease in pain. Considering the physiology of rat paw after 4 h treatment, overall, treatment with 80 μ L of extract might show a high efficacy in decreasing paw volume from 1 to 4 h (Figure 2).

4. Conclusion

The results indicate that the combined method had a significant effect with an increased efficiency in extraction of Cannabis oil with respect to yield, over that from ultrasound and microwave methods. However, the elevated yield did not imply that this *Cannabis* oil had high cannabinoid compounds content. Our results showed that in the case of the combined method the extract had lower values with respect to DPPH, ABTS, total phenol content, peroxide value, as well as THC content compared to extracts from ultrasound method, with these having higher values, as did the extracts produced using the microwave method. The formalin test result presented the capabilities of cannabis oil as an analgesic, with the ultrasound extracted oil being more effective than the extracts from the combined and microwave methods.

Conflict of Interest

All authors declare no conflicts of interest.

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