La Granja:
Revista de
Ciencias de la Vida
pISSN:1390-3799; eISSN:1390-8596
http://doi.org/10.17163/lgr.n37.2023.02

Scientific paper/ Artículo científico

Natural Products

In-vivo anti-inflammatory activity of a topical formulation with active principles on essential oils of *Cannabis sativa* L. (cannabis) and *Baccharis latifolia* (Ruiz & Pav) per. (chilca)

Actividad antiinflamatoria *in-vivo* de una formulación tópica con principios activos de aceites esenciales de *Cannabis sativa* L. (cáñamo) y *Baccharis latifolia* (Ruiz & Pav) per. (chilca)

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Article received on July 05th, 2022. Accepted, after review, on September 19th, 2022. Early access on December 1st, 2022. Published on March 1st, 2023.

Abstract

Essential oils of *Cannabis sativa* (cannabis) and *Baccharis latifolia* (chilca), were used as anti-inflammatory ingredients in an ointment for topical application. Different formulations designed based on these two essential oils were evaluated in-vivo to measure efficacy, using the feet swelling induction method in rats, and an over-the-counter formulation with 1% diclofenac active ingredient was used as a positive control. The chemical evaluation of the two oils yielded the following main components, for chilca oil: liguloxide 14.02%, andro encecalinol 9.84%, kesane 7.53%, limonene 5.6% and Z-cadin-4-en-7-ol with 5.03%; while for cannabis essential oil: E-caryophillene 27.91%, myrcene 21.19%, α-pinene 8.05%, α-humulene 8.03%, limonene 7.18%, terpinolene 7.12% and β-pinene 4.68%. The results of the research indicate that those formulas that combined two essential oils in the formulation at 1%, are the ones with the highest anti-inflammatory activity. Statistically, the significance is high in relation to the positive control in those whose oil composition is essential oil of cannabis 75% and chilca oil 25%; and essential oil of cannabis 50% and chilca oil 50%. The other formulas have activity, but this is similar to the commercial formula used as control. Based on the results, it is possible to propose both natural products as anti-inflammatories, and to foresee the design and commercialization of topical pharmaceutical drugs using these two essential oils.

Keywords: *Cannabis sativa*, *Baccharis latifolia*, essential oils, anti-inflammatory activity, anti-inflammatory synergy.

Resumen

Dos productos naturales, aceites esenciales de *Cannabis sativa* (cannabis) y *Baccharis latifolia* (chilca), fueron empleados como ingredientes antinflamatorios en un ungüento de aplicación tópica. Para medir la eficacia, las diversas fórmulas diseñadas a base de estos dos aceites esenciales fueron evaluadas in vivo, mediante el método de inducción del edema subplantar en ratas, como control positivo se empleó una formulación de venta libre con ingrediente activo diclofenaco al 1%. La evaluación química de los dos aceites presentó para el aceite de chilca los siguientes componentes principales: ligulóxido 14.02%, andro encecalinol 9.84%, kesano 7.53%, limoneno 5.6% y Z-cadin-4-en-7-ol con el 5.03%; mientras que en el aceite esencial de cannabis las moléculas más abundantes fueron: E-cariofileno 27.91%, mirceno 21,19%, α-pineno 8.05%, α-humuleno 8.03%, limoneno 7.18%, terpinoleno 7.12% y β-pineno 4.68%. Los resultados de la investigación señalan que aquellas fórmulas con mezclas de los dos aceites esenciales en la formulación al 1%, son las que poseen la mayor actividad antiinflamatoria, desde el punto de vista estadístico la significancia es alta en relación al control positivo en aquellas cuya composición de aceites es la siguiente: aceite esencial de cannabis 75% y aceite de chilca 25%, y aceite esencial de cannabis 50% y aceite de chilca 50%. El resto de las formulaciones presentan actividad, pero esta es similar a la de la fórmula comercial usada como control. De los resultados encontrados se puede proponer a ambos productos naturales como antinflamatorios, y prever el diseño y comercialización de medicamentos farmacéuticos tópicos usando a estos dos aceites esenciales.

Palabras clave: *Cannabis sativa*, *Baccharis latifolia*, aceites esenciales, actividad antiinflamatoria, sinergia antiinflamatoria.

Suggested citation: Noriega, P., Idrobo, T., Pintag, M., Vinueza, D. and Larenas, C. (2023). *In-vivo* anti-inflammatory activity of a topical formulation with active principles on essential oils of *Cannabis sativa* L. (cannabis) and *Baccharis latifolia* (Ruiz & Pav) per. (chilca). La Granja: Revista de Ciencias de la Vida. Vol. 37(1):23-33. http://doi.org/10.17163/lgr.n37.2023.02.

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1 Introduction

Inflammatory processes are related to pathologies such as: autoimmune diseases (Urakov and Urakova, 2021; Lochhead et al., 2021; Murata, 2018), various infections (Shah, 2019; Cervilla et al., 2002); cardiovascular diseases (Golia et al., 2014; Van Eeden et al., 2012) and traumas (Brown et al., 2021; Mortaz et al., 2018). According to the technical bulletin (INEC, 2020), polytrauma is the second cause of hospital morbidity, only below gastrointestinal infections, causing a high consumption of nonsteroidal anti-inflammatory drugs (NSAIDs). The healthy inflammatory response is temporarily beneficial, but has a precarious balance that can be altered causing unintentional tissue damage and generating abnormal or chronic inflammation. This imbalance generates an uncontrolled "pro-inflammatory" state capable of causing disease as a result of oxidative stress, which is the result of damage caused by reactive oxygen species (ROS), its basis being inflammation (Schewe, 1995).

Many medicinal plants are used for their anti-inflammatory properties as part of the ancestral knowledge of peoples around the world; additionally, there are *in-vitro* and *in-vivo* assays in several of these species that prove the anti-inflammatory potential of the active principles they contain (Oguntibeju, 2018; Nunes et al., 2020; Tasneem et al., 2019; Yatoo et al., 2018). Essential oils are among the extracts used for their anti-inflammatory activity, many of these biological matrices have shown secondary metabolites with high activity (Miguel, 2010; Pérez et al., 2011; Grassmann et al., 2000). Among the promising species, either for their traditional use or for their scientific evidence, there are essential oil of *C. sativa* (cannabis) (Orlando et al., 2021; Di Sotto et al., 2022) and *B. latifolia* (chilca) (Abad and Bermejo, 2007; Sequeda-Castañeda et al., 2015).

The essential oils from the two medicinal plants were analyzed in this research and were used as active ingredients in a topical formulation, whose effectiveness was studied in vivo in an animal model and compared with an over-the-counter commercial drug widely used in the environment. In this way, an alternative formulation is proposed, which uses natural active ingredients with a high efficacy to mitigate inflammations resulting from trauma or rheumatoid pathologies.

2 Materials and Methods

2.1 Extraction of essential oils

The essential oil of cannabis was bought at Eden Garden Essentials, in San Clemente, USA. The product comes with its quality data sheet. *B. latifolia* leaves (HUPS-as-011 voucher herbarium of the Laboratories of Life Sciences-UPS), were collected in the city of Riobamba, in the province of Chimborazo at the following coordinates: 1°40′26″ S, longitude: 78°38′37″ W, altitude: 2752 m.a.s.l. The fresh material was processed in a stainless steel distiller with a capacity of 64 liters that operates with the system known as water and steam in the Life Sciences laboratories of the Universidad Politécnica Salesiana, Quito.

2.2 Chemical composition of essential oils

The technical data sheet of the essential oil of cannabis details its chemical composition. The identification of compounds of the essential oil of B. latifolia was carried out through gas chromatography coupled to mass spectrometry, using a Trace 1310 gas chromatograph coupled to a Thermo Fisher Scientific ISQ 7000 mass spectrometer with a Termo Scientific TR-5MS chromatographic column, 30 m long, 0.25 mm thick and with a film thickness of 0.25 μm. The sample was prepared by diluting 10 μL of *B. latifolia* essential oil in 990 μL of dichloromethane; the injection volume was 1 μL. The carrier gas was 99.9999 % pure helium at a flow rate of 1 1 $mL\ min^{-1}$, and a split-ratio of 1:40. The injector temperature was 250°C.

The initial temperature in the column was 60°C for 5 minutes, until reaching 100°C at a rate of 2°C/min; then it increased to 150°C, at a rate of 3°C/min, reaching 200°C at 5 °C/min, and finally it reached 230°C maintaining this temperature for 5 minutes, with a total analysis time of 60 minutes.

The mass spectrometer conditions were: ionization energy: 70 eV; emission current: 10 μAmp; scanning range: 1 scan/s; mass range: 40-350 Da; trap temperature: 230°C; transfer line temperature: 200°C.

Identification of the molecules was performed using the NIST 2001 mass spectral database. In addition, the arithmetic retention index (RI), of each compound was calculated comparing with a series of C8-C30 alkanes and finally contrasting the theoretical retention indices from Adams (2012) database.

2.3 Preparation of anti-inflammatory ointments

The type of formulation was O/W (oil in water), the formula is described in Table 1. Five formulations were prepared with different proportions of the essential oils and a control formulation without oils, where the percentage of water was 64%, the percentages of each formulation are described in Table 2.

Table 1. Formula of the anti-inflammatory ointments.

Phases	Compound	Quantity in percentage
Oily phase	Mineral oil	12
	Stearic acid	10
	Cocoa butter	5
	Cetyl alcohol	5
	Beeswax	3
	Essential oil/s	1
Aqueous phase	Water	63
	Tea	1

2.4 *In-vivo* anti-inflammatory activity

The subplantar edema method induced by carrageenan injection proposed by Winter et al. (1962), was used, using rats weighing between 180 and 220 grams, aged 4 to 8 weeks, under controlled feeding conditions. The laboratory animals were housed for 2 weeks in groups of 5 for each treatment: 5 concentrations, a control formulation and a positive control. A commercial formulation containing 1% diclofenac was used as a positive control. Each animal was injected with a 0.3% carrageenan solution in propylene glycol, with a waiting time of 30 minutes to generate edema. A total of 5 values were taken for each individual: T before carrageenan application, To at 30 minutes, T1 at 1 hour, T2 at 3 hours and T3 at 5 hours after application. The values were determined in volume units using a plethysmometer, Figure 1 shows the experiment graphically.

Table 2. Proportions of essential oils in each formulation.

Formulation with base cream	Proportion of essential oils
Formulation A	Cannabis oil 25% *B. latifolia* oil 75%
Formulation B	Cannabis oil 50% *B. latifolia* oil 50%
Formulation C	Cannabis oil 75% *B. latifolia* oil 25%
Formulation D	Cannabis oil 100%
Formulation E	*B. latifolia* oil 100%
Control formula	Essential oils 0%

2.5 Statistics

Since there were seven groups of data at different times, including both positive and control groups, the statistical study was carried out in 4 stages. Using the R program version 2021, the data were analyzed, considering as the main parameter the proportion of inflammation caused at the beginning and the level of deflation in the three times in which the measurements were taken; these parameters were expressed in net value of inflammation, having as value the volume of the inflamed paw minus the volume of the normal paw. First, it was determined if the values presented normality by means of the Kolmogorov-Smisnov test with the Lilliefors adjustment, taking into account the contrast by treatment applied and by time elapsed after the application of the treatment. Homoscedasticity analysis was then performed using Levene's test in all the inflammation measurement times to determine that the variances are homogeneous, i.e., that there is not a very large variability between the study groups and that a Tukey test can be applied later.

The main analysis was the Anova test. Previously, a covariance analysis was performed to rule out the possibility of an interference of results with a variable that is not considered, in this case a possible interaction with the volume of the rat's paw in the initial state; the analysis was conduc-

Figure 1. Graphical scheme of the carrageenan-induced subplantar edema method and measurement in a manual plethysmometer.

ted using the Ancovac test. The Anova analysis was performed in a one-way model to determine a significant difference between the means. The differences between each of the groups compared to others were observed in the three measurement times after the treatment was applied to determine which treatment is the most effective against inflammation, considering the control and positive control groups (formulation with diclofenac at 1%). Subsequently, an Anova study was performed in a linear model to see the behavior of each of the treatments as a factor within a linear regression equation, contrasting each one against the control.

3 Results and Discussion

3.1 Chemical composition of essential oils

The cannabis oil purchased at Eden Garden Essentials has a certificate of chemical composition, which is detailed in Table 3.

Table 3. Chemical composition of *C. sativa* essential oil, provided by Eden Garden Essentials.

No.	Compound	% RDA	Theorical ratio[a]
1	hexanol	0.05	863
2	Not identified	0.08	-
3	α-tujene	0.07	924
4	α-pinene	8.05	932
5	camphene	0.08	946
6	sabinene	0.95	969

No.	Compound	% RDA	Theorical ratio[a]
7	β-pinene	4.68	974
8	Not identified	0.04	-
9	3-p-menthene	0.06	984
10	myrcene	21.19	988
11	mesythilene	0.03	994
12	Not identified	0.24	-
13	α-phellandrene	0.63	1002
14	δ-3-carene	0.69	1008
15	α-terpinene	0.11	1014
16	p-cymene	0.22	1020
17	limonene	7.18	1024
18	β-phellandrene	0.32	1025
19	1,8-cineole	0.29	1026
20	Z-β-ocimene	0.24	1032
21	E-β-ocimene	2.65	1044
22	γ-terpinene	0.1	1054
23	1-octanol	0.04	1063
24	E-sabinene hydrate	0.05	1098
25	terpinolene	7.12	1086
26	fenchone	0.15	1083
27	linalool	2.25	1095
28	α-fenchol	0.7	1114
29	Not identified	0.06	-
30	borneol	0.13	1165
31	p-cimen-8-ol	0.08	1179
32	α-terpineol	0.33	1186
33	citronelol	0.16	1223
34	α-copaene	0.05	1374
35	Hexyl hexanoate	0.04	1382
36	Z-caryophyllene	0.14	1408
37	Not identified	0.04	-
38	Z-α-bergamotene	0.05	1411
39	E-caryophyllene	27.91	1417
40	α-elemene	0.04	1434
41	E-bergamotene	0.42	1432
42	Not identified	0.72	-
43	α-humulene	8.03	1452
44	Not identified	0.25	-
45	Not identified	0.1	-
46	β-selinene	0.29	1489
47	γ-amorphene	0.04	1495
48	α-selinene	0.21	1498
49	(E,E)-α-farnesene	0.08	1505
50	β-bisabolene	0.03	1505
51	Not identified	0.05	-
52	Not identified	0.05	-
53	Not identified	0.11	-
54	Not identified	0.14	-
55	selina-3,7(11)-dieno	0.18	1545
56	germacrene B	0.1	1559

No.	Compound	% RDA	Theorical ratio[a]
57	Cariophyllene oxide	0.9	1582
58	Humulene hepoxide II	0.18	1608
59	Not identified	0.62	-
60	Not identified	0.22	-
Total unidentified		2.71	
Total identified		97.29	

[a]Theoretical retention rates retrieved from Adams (2012) database.

The most important compounds are: E-caryophyllene 27.91%, myrcene 21.19%, α-pinene 8.05%, α-humulene 8.03%, limonene 7.18%, terpinolene 7.12% and β-pinene 4.68%. The chemical composition of *B. latifolia* essential oil is shown in Table 4. The main compounds are: ligulooxide 14.02%, andro encecalinol 9.84%, kesane 7.53%, limonene 5.6% and Z-cadin-4-en-7-ol with 5.03%.

Table 4. Chemical composition of the essential oil of *B. latifolia*.

No.	Name	% RDA	Theorical RI[a]	Exp. RI[b]
1	α-tujene	4.77	924	925
2	α-pinene	4.27	932	933
3	camphene	0.68	946	950
4	tuuja-2,4(10)-dieno	2.39	953	974
5	verbenone	2.49	961	980
6	β-pinene	1.1	974	992
7	3-carene	3.4	1008	1011
8	limonene	5.6	1024	1032
9	β-ocimene	0.85	1032	1048
10	terpinolen	0.66	1086	1088
11	gurjunene	0.68	1409	1406
12	caryophyllene	1.3	1417	1420
13	humulene	1.27	1454	1457
14	γ-curcumene	2.91	1475	1479
15	α-curcumene	1.97	1483	1484
16	himachalene	0.26	1481	1486
17	E-muurola-4(14),5-dieno	3.41	1493	1498
18	β-curcumene	2.19	1514	1512
19	α-7-epi-selinene	1.98	1520	1523
20	zoranene	1.01	1529	1529
21	kesane	7.53	1530	1535
22	γ-vetivevene	0.53	1531	1537
23	liguloxide	14.02	1534	1544
24	spatulenol	2.39	1578	1585
25	caryophyllene oxide	0.25	1582	1589
26	-10 epi-eudesmol	1.23	1622	1614
27	muurola-4,10(14)-dien-1-ol	0.99	1630	1633
28	epi-cadinol	1.06	1638	1638
29	Z-cadin-4-en-7-ol	5.03	1635	1647
30	α-cadinol	1.46	1652	1655
31	valerianol	0.78	1658	1668
32	andro encecalinol	9.84	1677	1679
33	α-bisabolol	1.71	1685	1698
34	ciperotundone	2.1	1695	1706

No.	Name	% RDA	Theorical RI[a]	Exp. RI[b]
35	Not identified	1.65		1751
36	Not identified	2.82		1764
37	Not identified	1.84		1769
38	Not identified	1.57		1792
Total identified		92.11		
Total unidentified		7.89		

[a]Theoretical retention rates retrieved from Adams (2012) database.

[b]xperimental retention rates compared to a homologous series of C8-C30 hydrocarbons.

3.2 *In vivo* anti-inflammatory evaluation

After homogenizing the treatments by applying the normality and homogeneity tests, the statistical analysis Anova test was performed relating the anti-inflammatory efficacy in volume for each of the treatments including the control formula (T) without active ingredients, positive control (PC) with diclofenac at 1% and the 5 formulations with different proportions of essential oils at 1% which are: A (100% E.O. cannabis), B (75% E.O. B. latifolia and 25% E.O. cannabis), C (50% E.O. B. latifolia and 50% E.O. cannabis), D (25% E.O. *B. latifolia* and 75% E.O. cannabis), E (100% E.O. *B. latifolia*). Measurements were taken 3 times as described in the trial, i.e. 1, 3 and 5 hours after product application. Figure 2 shows the results of the 3 trials.

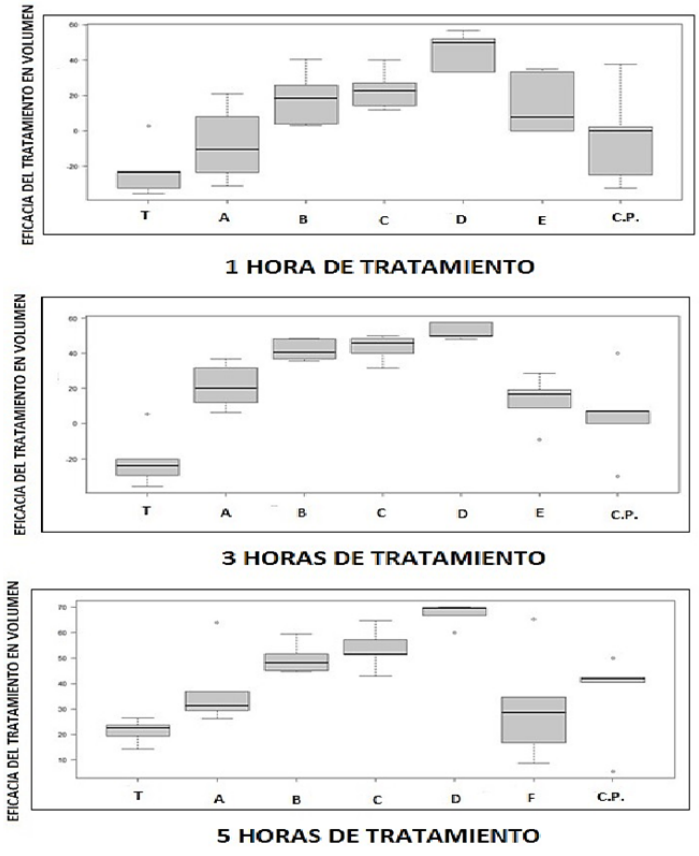

Figure 2. Treatments after 1 hour, 3 hours, 5 hours: T (control sample), A (100% E.O cannabis), B (75% E.O. *B. latifolia* and 25% E.O cannabis), C (50% E.O *B. latifolia* and 50% E.O cannabis), D (25% E.O *B. latifolia* and 75% E.O cannabis), E (100% E.O *B. latifolia*), P.C. (positive control).

Figure 3 shows a comparative analysis between the formulations with the essential oils and the positive control with diclofenac.

4 Conclusions

The chemical composition of cannabis essential oil provided in the certificate of the company Eden Garden Essentials contains as majority molecules caryophyllene, myrcene, humulene, limonene and pinenes, similar to what is stated in the literature (Malingre et al., 1975; Novak et al., 2001). For both caryophyllene(Dahham et al., 2015; Bakır et al., 2008) and myrcene (Surendran et al., 2021), there are studies confirming its anti-inflammatory activity in various assays.

Unlike the essential oil of cannabis, *B. latifolia* has few studies, which do not allow to have enough literature to compare. The study conducted by Valarezo et al. (2013), shows similarities and differences in the chemical composition of the oil, which could be due to the ecological variables of the places where the plant is harvested. Regarding the most abundant components in our research: liguloxide, andro encecalinol and kesane, there are no bioactivity studies, leaving open the possibility of isolating these molecules and verifying their medicinal properties, including anti-inflammatory properties.

Basically, all the formulations containing individual essential oils or in mixtures at 1% in the formulation show activity if considering their comparison with the positive control (commercial formulation with diclofenac at 1%). However, those formulas of essential oils whose positive activity from the point of view of statistical significance present a better anti-inflammatory bioactivity with respect to the positive control and pure essential oils, are noteworthy. The two most effective formulations are those with 25% *B. latifolia* oil (250 mg in 100 grams of ointment) and 75% cannabis oil (750 mg in 100 grams of ointment) and the one containing 50% B. latifolia oil (500 mg in 100 grams of ointment) and 50% cannabis oil (500 mg in 100 grams of ointment), practically in all tests (1, 3 and 5 hours). The statistics reveal their significance compared to the positive control, which would enable to propose them as possible formulas in the market of natural products.

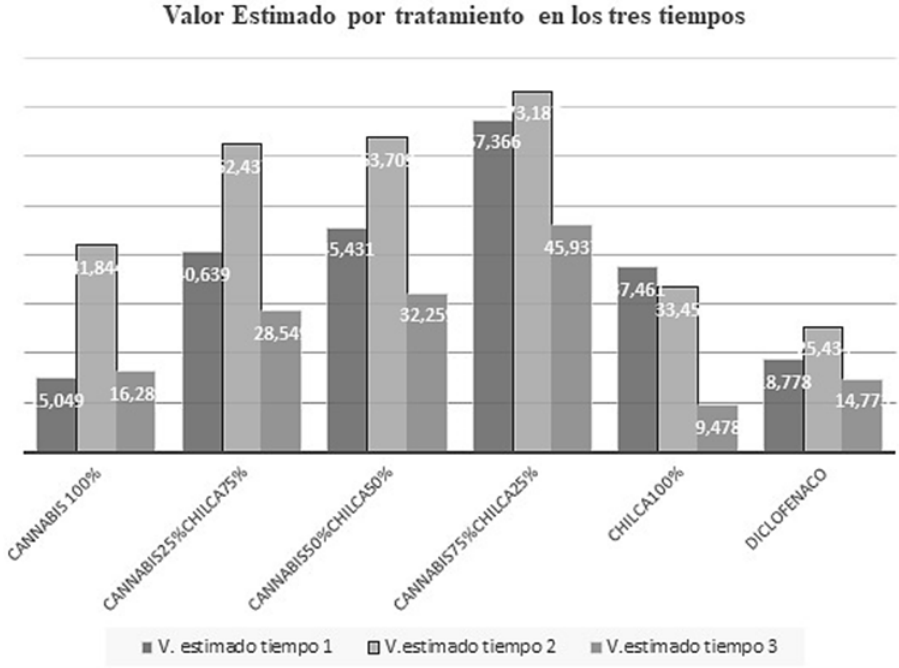

Figure 3. Comparative evaluation of the treatments and the positive control (diclofenac 1% formulation), in the 3 study times, time 1 (1 hour), time 2 (3 hours) and time 3 (5 hours).

The amounts of essential oils used are small, which would result in a commercially competitive product. Additionally, *B. latifolia* is abundant in the Andes of Ecuador and the country is beginning to produce medical cannabis, where essential oils could be one of the metabolites beyond cannabinols.

Acknowledgments

The authors thank Universidad Politécnica Salesiana by the funding awarded to Grupo de Investigación y Desarrollo en Ciencias Aplicadas a los Recursos Biológicos (GIDCARB), by the project entitled: Optimización de procesos extractivos de metabolitos secundarios de Cannabis sativa (cáñamo) fase II (Optimization of extractive processes of secondary metabolites of Cannabis sativa (cannabis) phase II).

References

Abad, M. and Bermejo, P. (2007). Baccharis (compositae): a review update. *Arkivoc*, 7(7):76–96. Online: https://bit.ly/3RHZFjo.

Adams, R. (2012). *Identification of essential oils by ion trap mass spectroscopy*. Academic press.

Bakır, B., Him, A., Özbek, H., Düz, E., and Tütüncü, M. (2008). Investigation of the anti-inflammatory and analgesic activities of β-caryophyllene. *International Journal of Essential Oil Therapeutics*, 2(1):41–44. Online: https://bit.ly/3e9KYYP.

Brown, M., Worrell, C., and Pariante, C. (2021). Inflammation and early life stress: An updated review of childhood trauma and inflammatory markers in adulthood. *Pharmacology Biochemistry and Behavior*, 211:173291. Online: https://bit.ly/3fPVwfS.

Cervilla, A., Purriños, F., and Moya, J. (2002). Faringitis crónica y macrólidos. *Acta Otorrinolaringológica Española*, 53(6):441–443. Online: https://bit.ly/3VnVKeO.

Dahham, S., Tabana, Y., Ahamed, M., and Majid, A. (2015). In vivo anti-inflammatory activity of β-caryophyllene, evaluated by molecular imaging. *Molecules & Medicinal Chemistry*, 1. Online: https://bit.ly/3TayGhu.

Di Sotto, A., Gullì, M., Acquaviva, A., Tacchini, M., Di Simone, S. C., Chiavaroli, A., Recinella, L., Leone, S., Brunetti, L., and Orlando, G. (2022). Phytochemical and pharmacological profiles of the essential oil from the inflorescences of the cannabis sativa l. *Industrial Crops and Products*, 183:114980. Online: https://bit.ly/3Mmx2qS.

Golia, E., Limongelli, G., Natale, F., Fimiani, F., Maddaloni, V., Pariggiano, I., Bianchi, R., Crisci, M., D'Acierno, L., and Giordano, R. (2014). Inflammation and cardiovascular disease: from pathogenesis to therapeutic target. *Current atherosclerosis reports*, 16(9):1–7. Online: https://bit.ly/3Mll6FX.

Grassmann, J., Hippeli, S., Dornisch, K., Rohnert, U., Beuscher, N., and Elstner, E. (2000). Antioxidant properties of essential oils. possible explanations for their anti-inflammatory effects. *Arzneimittel-forschung*, 50(2):135–139. Online: https://bit.ly/3rMJrLp.

INEC (2020). Registro estadístico de camas y egresos hospitalarios 2019. Technical Report 01-2020-ECEH, INEC.

Lochhead, R., Strle, K., Arvikar, S., Weis, J., and Steere, A. (2021). Lyme arthritis: linking infection, inflammation and autoimmunity. *Nature Reviews Rheumatology*, 17(8):449–461. Online: https://go.nature.com/3CzJcIG.

Malingre, T., Hendriks, H., Batterman, S., Bos, R., and Visser, J. (1975). The essential oil of cannabis sativa. *Planta medica*, 28(05):56–61. Online: https://bit.ly/3RWzUvZ.

Miguel, M. (2010). Antioxidant and anti-inflammatory activities of essential oils: a short review. *Molecules*, 15(12):9252–9287. Online: https://bit.ly/3fWDXLj.

Mortaz, E., Alipoor, S., Adcock, I., Mumby, S., and Koenderman, L. (2018). Update on neutrophil function in severe inflammation. *Frontiers in Immunology*, 9:2171. Online: https://bit.ly/3CMESpB.

Murata, M. (2018). Inflammation and cancer. *Environmental health and preventive medicine*, 23(1):1–8. Online: https://bit.ly/3EGJW1j.

Novak, J., Zitterl-Eglseer, K., Deans, S., and Franz, C. (2001). Essential oils of different cultivars of

cannabis sativa l. and their antimicrobial activity. *Flavour and fragrance journal*, 16(4):259–262. Online: https://bit.ly/3TjgGkX.

Nunes, C., Barreto Arantes, M., Menezes de Faria Pereira, S., Leandro da Cruz, L., de Souza Passos, M., Pereira de Moraes, L., Vieira, I., and Barros de Oliveira, D. (2020). Plants as sources of anti-inflammatory agents. *Molecules*, 25(16):3726. Online: https://bit.ly/3RWTYye.

Oguntibeju, O. (2018). Medicinal plants with anti-inflammatory activities from selected countries and regions of africa. *Journal of inflammation research*, 11:307. Online: https://bit.ly/3yC81Cl.

Orlando, G., Adorisio, S., Delfino, D., Chiavaroli, A., Brunetti, L., Recinella, L., Leone, S., D'Antonio, M., Zengin, G., and Acquaviva, A. (2021). Comparative investigation of composition, antifungal, and anti-inflammatory effects of the essential oil from three industrial hemp varieties from italian cultivation. *Antibiotics*, 10(3):334. Online: https://bit.ly/3Vkj2SH.

Pérez, S., Zavala, M., Arias, L., and Ramos, M. (2011). Anti-inflammatory activity of some essential oils. *Journal of Essential Oil Research*, 23(5):38–44. Online: https://bit.ly/3TjrGPh.

Schewe, T. (1995). Molecular actions of ebselen-an antiinflammatory antioxidant. *General Pharmacology: The Vascular System*, 26(6):1153–1169. Online: https://bit.ly/3eoaOIu.

Sequeda-Castañeda, L., Célis, C., and Luengas-Caicedo, P. (2015). Phytochemical and therapeutic use of baccharis latifolia (ruiz & pav.) pers.(asteraceae). *Pharmacology*, 2:14–17. Online: https://bit.ly/3fRoxI8.

Shah, P. (2019). Inflammation, infection and atherosclerosis. *Trends in cardiovascular medicine*, 29(8):468–472. Online: https://bit.ly/3EEcuIM.

Surendran, S., Qassadi, F., Surendran, G., Lilley, D., and Heinrich, M. (2021). Myrcene—what are the potential health benefits of this flavouring and aroma agent? *Frontiers in Nutrition*, page 400. Online: https://bit.ly/3T71p6W.

Tasneem, S., Liu, B., Li, B., Choudhary, M., and Wang, W. (2019). Molecular pharmacology of inflammation: Medicinal plants as anti-inflammatory agents. *Pharmacological research*, 139:126–140. Online: https://bit.ly/3g6WS6o.

Urakov, A. and Urakova, N. (2021). Recent insights into the management of inflammation in asthma. *Journal of Inflammation Research*, 14:4603. Online: https://bit.ly/3yzt4p8.

Valarezo, E., Rosillo, M., Cartuche, L., Malagón, O., Meneses, M., and Morocho, V. (2013). Chemical composition, antifungal and antibacterial activity of the essential oil from baccharis latifolia (ruiz & pav.) pers.(asteraceae) from loja, ecuador. *Journal of essential oil research*, 25(3):233–238. Online: https://bit.ly/3SSH8SO.

Van Eeden, S., Leipsic, J., Paul Man, S., and Sin, D. (2012). The relationship between lung inflammation and cardiovascular disease. *American journal of respiratory and critical care medicine*, 186(1):11–16. Online: https://bit.ly/3RUnuo3.

Winter, C., Risley, E., and Nuss, G. (1962). Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proceedings of the society for experimental biology and medicine*, 111(3):544–547. Online: https://bit.ly/3T0jkfS.

Yatoo, M., Gopalakrishnan, A., Saxena, A., Parray, O., Tufani, N., Chakraborty, S., Tiwari, R., Dhama, K., and Iqbal, H. (2018). Anti-inflammatory drugs and herbs with special emphasis on herbal medicines for countering inflammatory diseases and disorders-a review. *Recent patents on inflammation & allergy drug discovery*, 12(1):39–58. Online: https://bit.ly/3ECcJ6Q.