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Is turbo-extraction an efficient method for obtaining cannabinoids?

A turbo-extração é um método eficiente para obtenção de canabinoides?

¿Es la turboextracción un método eficiente para la obtención de cannabinoides?

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Abstract
Cannabis-based products are still not produced in many countries and the great need makes them imported, and often expensive. As an objective, a low-cost extraction method was proposed to facilitate access to the medicinal effects of cannabidiol. The samples of *Cannabis sativa* used were obtained from *in vitro* cultivation or from commercially purchased products. Two extraction techniques (ultrasonic extraction and turbo-extraction) and two solvent systems (methanol:chloroform and ethanol) were evaluated. The levels of cannabinoids, total phenolics and total flavonoids were evaluated. The best results were obtained with the turbo-extraction. Ethanol showed a higher yield of cannabinoids, in addition to being a solvent accepted in international conventions in reducing the use of toxic solvents to the environment. Turbo-extraction with ethanol proved to be effective, economical and in line with environmental concerns in the use of ecologically sustainable solvents. As future possibilities, it is proposed to use the information obtained in this study as part of the process of obtaining and subsequent analysis of *Cannabis* extracts for medicinal purposes.
Keywords: Extraction; *Cannabis*; ultrasonic extraction; Hemp; High performance liquid chromatography.

Resumo
Os produtos à base de *Cannabis* ainda não são produzidos em muitos países e a grande necessidade os torna importados e, muitas vezes, caros. Como objetivo, foi proposto um método de extração de baixo custo para facilitar o acesso aos efeitos medicinais do canabidiol. As amostras de *Cannabis sativa* utilizadas foram obtidas de cultivo *in vitro* ou de produtos adquiridos comercialmente. Foram avaliadas duas técnicas de extração (extração ultrassônica e turboextração) e dois sistemas de solventes (metanol:clorofórmio e etanol). Foram avaliados os níveis de canabinoides, fenólicos totais e flavonoides totais. Os melhores resultados foram obtidos com a turbo-extração. O etanol apresentou maior rendimento de canabinoides, além de ser um solvente aceito em convenções internacionais na redução do uso de solventes tóxicos ao meio ambiente. A turbo-extração com etanol mostrou-se eficaz, econômica e em linha com as preocupações ambientais no uso de solventes ecologicamente sustentáveis. Como possibilidades futuras, propõe-se utilizar as informações obtidas neste estudo como parte do processo de obtenção e posterior análise de extratos de *Cannabis* para fins medicinais.
Palavras-chave: Extração; *Cannabis*; Extração por ultrassom; Hemp; Cromatografia líquida de alta eficiência.

Resumen
Los productos a base de C*annabis* todavía no se producen en muchos países y la gran necesidad hace que se importen y, a menudo, sean caros. Como objetivo se planteó un método de extracción de bajo costo para facilitar el acceso a los efectos medicinales del cannabidiol. Las muestras de *Cannabis sativa* utilizadas se obtuvieron de cultivo *in vitro* o de productos adquiridos comercialmente. Se evaluaron dos técnicas de extracción (extracción ultrasónica y turbo-extracción) y dos sistemas de solventes (metanol:cloroformo y etanol). Se evaluaron los niveles de cannabinoides, fenoles totales y flavonoides totales. Los mejores resultados se obtuvieron con la turboextracción. El etanol mostró un mayor rendimiento de cannabinoides, además de ser un solvente aceptado en convenciones internacionales en la reducción del uso de solventes tóxicos para el medio ambiente. La turbo-extracción con etanol demostró ser eficaz,

económica y acorde con las preocupaciones medioambientales en el uso de disolventes ecológicamente sostenibles. Como posibilidades futuras, se propone utilizar la información obtenida en este estudio como parte del proceso de obtención y posterior análisis de extractos de Cannabis con fines medicinales.
Palabras clave: Extracción; *Cannabis*; Extracción ultrasónica; Cáñamo; Cromatografia líquida de alta resolución.

1. Introduction

Cannabidiol (CBD)-based drugs range between 160 and 500 dollars and, therefore, have led families to resort to clandestine means, which do not offer security. In this context, the possibility of producing CBD within the regulatory requirements becomes of extreme relevance social and economic importance for several countries (Chandra et al., 2012).

Cannabis extracts are obtained from dried or fresh plant materials. As the main objective of an extractive process is to remove compounds of interest from a complex matrix, the selectivity required in this process is based on two main factors: the solvent characteristics and the applied extractive process (Allen Junior et al., 2013; Där, 1981; Prista, 2011; Voigt & Bornschein, 1982). The factors that most influence the extractives processes are: temperature, agitation, granulometry of the plant material and pH. Furthermore, the extraction efficiency is entirely dependent on the solvent system chosen. In this aspect, it can be said that the selectivity of the extraction can reach a high degree, working with the relative polarity of the solvents and constituents of interest (Allen Junior et al., 2013; Simões et al., 2003).

To date, there are no studies that use turbo-extraction to obtain cannabinoids. In this sense, this study aimed to develop an efficient and low-cost method by means of turbo-extraction.

2. Methodology

2.1 Samples

C. sativa were obtained from *in vitro* cultivation (Duarte-Almeida et al., 2020; Stein et al., 2019). These samples were dehydrated in a forced air oven for 24 h at 35 ºC. Subsequently, these samples were pulverized and stored at 25 ºC. RSHO™ (HempMeds®, USA) were purchased commercially.

2.2 Extraction methods

Ultrasound assisted extraction (UAE) was performed according to Mano-Sousa et al. (2021) and Aguiar et al. (2018), at 25 ºC and 40 ºC. In the turbo-extraction method were performed using a homogenizer Ultra-Turrax®. The extractions were carried out under high-speed agitation (4,000 rpm) for 2 min. The extracts were obtained in two stages, using half the volume of the respective solvent-system, with the same rotation and extraction time. The solvent systems used were: MeOH:$CHCl_3$ (9:1 v:v) and EtOH. The extracts were prepared in the proportion of 5 g of plant material to 200 mL of solvent. This method was patented by INPI (BR1020210053992) (Duarte-Almeida et al., 2021). The extracts were lyophilized.

2.3 Chromatography in flash mode

The chromatograph used was the Pure C-850 FlashPrep (Büchi, Switzerland), controlled by Pure Navigator Software. Separation of compounds was performed using a Flash Pure FP ID HP Si 12g cartridge, flow of 20 mL/min and running time of 40.5 min. Column stabilization was accomplished in 4.8 minutes. The mobile phases, in gradient, were: chloroform (A) and hexane (B). The time and concentration of solvents are described in Table S1 (supplementary data). Compounds were determined by UV/VIS spectrum (200-800 nm), sensitivity was adjusted to the UV threshold at 0.05 AU. In addition, they were evaluated by the Evaporative Light Scatter Detector (ELSD).

2.4 Chromatography Analysis

Chromatography analysis were performed using a UFLC Proeminence liquid chromatograph system (Shimadzu, Japan), equipped with an autosampler (SIL-20AHT), binary pump system (LC-20AD) and DAD (SPD-M20A) operated by the software LC Workstation (Shimadzu, Japan).

The chromatographic separation was performed with a Phenomenex Kinetex C_{18} column (100 mm × 2,1 mm, 5 µm), stored at 14 ± 2 ºC. The mobile phases used with a flow of 0.5 mL/min as a gradient were: A – 0.1 % formic acid solution, and B – MeOH. The gradient program used was 0 to 1 min, 60 % B; 1 to 3 min, 80 % B; 3 to 5 min, 85 % B; 5 to 6.5 min, 90 % B, 6.5 to 7.0 min, 60 % B. The injection volume used for each run was 5 µL. The determination was performed at 220 nm. The samples were diluted in mobile phase, homogenized in an ultrasonic bath and filtered through a 0.45 µm syringe filter before being injected. This method was patented by INPI (BR1020210053992) (Duarte-Almeida et al., 2021).

2.5 Quantitative analysis

The determination of Total phenolic content (TPC) was performed according Mano-Sousa et al. (2022). The absorbance was determined by spectrophotometry at 750 nm (QU798U, Brazil). Gallic acid (0.2 mg/mL) was used as a reference compound, and the TPC were expressed as mg of gallic acid equivalents per g of extract (mg EAG.g^{-1}).

The determination of Total flavonoids content (TFC) was performed according Bahia et al. (2018). The absorbance was read at 425 nm (QU798U, Brazil). Quercetin was used as a reference compound to produce an analytical curve, and TFC were expressed as mg of quercetin equivalents per g of extract (mg EQ g^{-1}).

2.6 Statical analysis

The data obtained in this study were expressed as mean ± standard deviation (SD). Analysis of variance tests were performed by ANOVA, followed by Tukey's or Fisher's test ($p < 0.05$). Statistical analyzes were performed using Statistica 10.0, GraphPad Prism 8.0.2, and Excel 19 (Microsoft Office 2019) software.

3. Results and Discussion

The yields obtained for the evaluated extraction processes are shown in Table 1. Turbo-extraction using MeOH:$CHCl_3$ was significantly different for all methods, except when EtOH was used as the solvent system (Figure S1, S2 and S3 – supplementary data).

Secondary analyzes were performed in relation to the extraction technique (Table S2), and another only in relation to the solvent-system (Table S3). Turbo-extraction showed the highest significant yield ($p = 0.004$) and the highest CBD content (%) in the extract ($p = 0.009$), while there was no significant difference in terms of CBD content (%) in the raw material. There was no significant difference between the solvent-systems used.

Clearly, turbo-extraction was the most efficient technique. This fact can be explained by the decrease in the granulometry of the material and increase in the contact surface of the solvent, causing a greater potential for compound extraction. Turbo-extraction using MeOH:$CHCl_3$ as the solvent system had the highest significant yield ($p = 0.0044$) compared to UAE at 25 ºC, in both the solvent system and UAE at 40 °C ($p = 0.036$). In addition, it has advantages in relation to the extraction time, 4 min, while the UAE is 30 min.

Table 1 - Cannabidiol (CBD) yield and content obtained by ultrasound assisted extraction (UAE) and turboextraction in different system-solvents.

Extraction Type	Solvent	Yield[2] (mean ± SD)[1]	CBD content[3] (mean ± SD)[1]	CBD content[4] (mean ± SD)[1]
UAE	MeOH:$CHCl_3$	5.83 ± 3.14[b]	3.42 ± 0.38[a.b]	58.52 ± 9.38[a]
(25 ºC)	EtOH	7.21 ± 3.93[b]	3.19 ± 1.72[a.b]	42.35 ± 34.01[a]
UAE	MeOH:$CHCl_3$	7.41 ± 0.21[b]	2.92 ± 1.53[a.b]	39.10 ± 19.68[a]
(40 ºC)	EtOH	5.62 ± 0.52[b]	2.21 ± 0.20[b]	39.63 ± 6.53[a]
Turbo-extraction	MeOH:$CHCl_3$	24.87 ± 15.43[a]	5.07 ± 2.01[a]	37.55 ± 17.9[a]
	EtOH	9.77 ± 8.69[a.b]	4.84 ± 2.09[a.b]	126.07 ± 160.31[a]

CBD = cannabidiol; SD = standard deviation. MeOH:$CHCl_3$ = methanol:chloroform 9:1 (v/v); EtOH = ethanol.
[1]Values followed by the same letter in the column do not differ at the 5% probability level by Fisher's test.
[2]Extraction yield expressed as a percentage, that is, gram of extract per 100 grams of plant material.
[3]CBD content expressed in milligrams of CBD per 1 gram of plant.
[4]CBD content expressed in milligrams of CBD per 1 gram of dry extract.
Source: Authors.

Regarding the solvent-system, EtOH is the ideal solvent to extract cannabinoids, as EtOH is considered a green solvent and has less toxicity compared to other solvents generally used (Hahn et al., 2014; Kilburn, 2014). The results obtained in our study showed that there is no need to use solvents such as $CHCl_3$ and MeOH. MeOH has some toxic characteristics, such as irritation to the mucous membranes and skin, in addition to causing irritation to the optic nerves and eyes, which can lead to blindness. In cases of chronic exposure, chloroform can cause liver damage, such as hepatomegaly, while acute exposures include headache, nausea, and vomiting (Bizzo et al., 2010).

In this way, turbo-extraction presents itself as an ecologically sustainable and energy efficient alternative in the sense of increasing the mass transfer process within the plant tissue, favoring cellular rupture (shear), in relation to the currently available methods. The UAE is considered ecological and sustainable (Corrales et al., 2009; Toepfl et al., 2006), which has contributed to the increase in its use in order to increase the phenomenon of mass transfer and, consequently, reduce the time of extraction of bioactive compounds. The ultrasonic bath promotes an increase in mass transfer rates during solid-liquid extraction through acoustic cavitation forces, which cause tissue rupture and migration of substances from the intracellular medium to the extracting solvent, in addition to promoting homogenization and favor the solubility of solutes (Corrales et al., 2009; Knorr et al., 2002).

The MeOH:$CHCl_3$ associated with the turbo-extraction showed higher yields compared to the UAE, which can be attributed to its greater polarity and, consequently, greater extraction capacity of polar analytes. In addition, what may also have resulted in higher yields when MeOH is used in some techniques is its lower viscosity, which gives it greater permeation capacity in the sample, favoring the diffusion of the analyte and its migration to the organic phase. It is also worth mentioning that the extractive efficiency of UAE is enhanced with the reduction of solvent viscosity. Thus, the UAE employing MeOH as an extraction solvent should allow higher yields in relation to the use of ethanol (Chemat et al., 2017; Tiwari, 2015). In all extracts it was possible to identify the presence of CBD, CBN, Δ^9-THC, Δ^8-THC e THCA (Figure S4-S9).

A purification process was carried out to obtain enriched extracts. Twenty-seven extract fractions were obtained from a cannabis sample. It was possible to identify CBD, Δ^9- THC, Δ^8- THC e CBN in fractions 6 (Figure S10) and 7 (Figure S11). The preliminary purification method proposed was not efficient in the total separation of the main cannabinoids of interest, requiring the optimization of the method.

Table 2 - Total phenolic content (TPC) and total flavonoids content (TFC) from extracts obtained by turbo-extraction (methanol:chloroform 9:1 v:v) of Cannabis grown in vitro.

Sample	TPC[1]	TFC[2]
1	3.69 ± 0.41	–
2	6.52 ± 0.04	–
3	6.99 ± 0.34	–
4	9.43 ± 0.32	–
5	8.99 ± 0.79	–
6	0.82 ± 0.21	–
7	–	–
8	7.68 ± 0.60	–
9	5.46 ± 0.62	–
10	8.92 ± 0.36	–
11	4.60 ± 0.33	–
12	11.02 ± 1.99	–
13	8.26 ± 1.67	–

[1]Total Phenolic Content (TPC) of expressed in milligrams of Phenolic Equivalents to gallic acid in grams of dry extract (mg $EAG.g^{-1}$).
[2]Total Flavonoids Content (TFC) expressed in milligrams of total flavonoids equivalent to quercetin in grams of dry extract (mg $EQ.g^{-1}$).
–There was no significant content.
Source: Authors.

The determination of the TFC (Figure S13) of 13 samples from the *in vitro* cultivation was carried out, in which there was no significant content of flavonoids in any of the samples evaluated (Table 2). The highest TPC (Figure S14) was obtained in sample 12 (11.02 mg $EAG.g^{-1}$), while the lowest content was found in sample 6 (0.82 mg $EAG.g^{-1}$), according to Table 2.

4. Final Considerations

The extraction methods developed did not show significant differences in the extraction of CBD content. However, the turbo-extraction showed a higher total mass yield and a significant difference in relation to the other methods. The method that used turbo-extraction with EtOH proved to be effective, economical and in line with the environmental concerns of the use of ecologically sustainable solvents. As future possibilities, it is proposed to use the information obtained in this study as part of the process of obtaining and purifying cannabis extract for medical purposes.

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