EXTRACTION OF ACTIVE INGREDIENTS FROM MEDICINAL CANNABIS

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Abstract: Profound knowledge and comprehension of medical history of cannabis is necessary for its integration in the modern clinical practices. There is a recognized and verified usage of cannabis in the past. People consumed this plant and developed practices and various methods of growing for improvement of the pharmaceutical properties and features of the plant. The perception of the value and potential of cannabis changes all over the world today. Thanks to research efforts on the chemical features of cannabinoids and the endocannabinoid system, medical cannabis is obtaining a lot of attention from patients, pharmacists, doctors, and government regulators. Due to its bioactive compounds, medical cannabis is well known after its numerous therapeutic activities, which can be proved in preclinical and clinical studies. These new researches provided evidence for the efficacy and safety of medical cannabis in the treatment of various illnesses, by using extracts with known concentration of cannabinoids. Researches for cannabis are concentrated on the methods for development and extraction of new products and because of that, the industry for cannabis increases rapidly. The most appropriate procedure for obtaining the medical product should be chosen in accordance with the characteristics of the desired product. In this paper, we will present the two most common methods of extracting cannabinoids from medical cannabis; we will analyze their advantages and disadvantages. There are several procedures for extracting cannabinoids from medical cannabis. Two of these procedures for the extraction of the medical cannabis are distinguished. To achieve the set goal, we made a review of relevant literary sources of recent published papers, extraction protocols, we have analyzed them, and we have compared the data and made appropriate conclusions. The first one is a conventional method for extraction with organic solvent. This type of extraction involves the maceration of plant material in an organic solvent. In order to concentrate the extract the solvent is removed under reduced pressure. The main disadvantage of this type of extractions is the change in the chemical structures of the active components due to usage of high temperatures. The increasing of the yield of this type of extraction can be improved by ultrasound or microwave treatment. The second innovative extraction method is supercritical extraction. The conditions under which this type of pressure and temperature extraction occurs are responsible for the solubility and selectivity of various plants in the supercritical extraction. Carbon dioxide is used as a supercritical solver because of its safety and reasonable cost. Carbon dioxide provides non-oxidizing atmosphere and consequently prevents the destruction of the extracts. Temperature, pressure, pretreatment of the herb and the nature of the solvent are parameters, which should be taken care of and should be taken into account as the most important in the extraction process. The extraction is successful with optimal solubility of the active components in the appropriate solvent. The active components are necessary to be available and able to interact with the solvent. Cannabis extracts are obtained from the flowers. The plant material prior to the extraction process is exposed to various procedures including the moisture control procedure in the plant material, which concentrates the fragments of the plant containing the active ingredients and increases the contact surface between the solvent and the active ingredients. Both conventional organic solvent extraction and supercritical carbon dioxide extraction have their own advantages and disadvantages, but the list of advantages of supercritical carbon dioxide extraction is greater and hence this type of extraction for extracting cannabinoids from medical cannabis is of first choice recently.
Keywords: Medical cannabis, cannabinoids, supercritical extraction, carbon dioxide.

1. INTRODUCTION

Cannabis is a plant that belongs to the Cannabaceae family. The three spices Cannabis sativa, Cannabis indica, and Cannabis ruderalis are well-known. Cannabis is believed to have its origins in the northwest Himalayas more than ten thousand years ago and has since spread to other areas. Growing occurs in four stages**:** germination, juvenile, blooming, flowering**.** Low levels of phytochemical compounds are present throughout the initial stages of growth. During the flowering stage, a plant's growth, phytochemical development, and biomass start to rise. (Qamar et al.,2021). The medical use of medical cannabis has attracted a lot of attention recently. Cannabis can be categorized according to its genetic makeup, phenotypic characteristics, and chemical make-up. Bioactive phytochemicals are abundant in all of these plants. However, the composition of phytochemicals varies between species. For instance, Cannabis sativa contains more cannabidiol (CBD) than Cannabis indica and Cannabis ruderalis, whereas Cannabis

indica contains more delta-9-tetrahydrocannabinol (9-THC), a psychoactive cannabinoid, and Cannabis ruderalis contains less 9-THC. The majority of the cannabinoids in cannabis are produced by the enzyme olivotolate geranyl transferase from cannabigerolic acid (CBGA), which is subsequently transformed into the primary cannabinoids, delta 9-tetrahydrocannabinolic acid (THCA), THCA synthase, CBDA synthase, and CBCA synthase are oxide cyclase enzymes that produce cannabidiol acid (CBDA) in trichomes. Drying, heating, or burning non-enzymatic processes may also result in the production of additional active substances. Tetrahydrocannabinol (THC) can be changed into 8-THC, cannabinol (CBN), and cannabinolic acid, for instance (CBNA). Cannabichromene (CBC), cannabicyclol (CBL), and cannabigerol can all be produced from CBD and CBC (CBG).
With more than 125 different cannabinoids and 400 non-cannabinoids, including flavonoids, alkaloids, phenols, and terpenes, cannabis is a rich source of phytochemicals. These phytochemicals have a number of positive health effects. THC and CBD, for instance, lessen pain and inflammation. Antipsychotic, anxiolytic, and antiepileptic properties of CBD are well-known. Additionally, Alzheimer's illness, Parkinson's disease, schizophrenia, psychosis, and numerous cancers have all been linked to CBD, according to reports. Other substances like GBG, CBC, and CBN are also linked to a number of health advantages. Autism, Parkinson's disease, glaucoma, cardiovascular diseases, arthritis, and inflammatory ailments have all been treated with THC. CBN has an impact on insomnia and sleep disorders, while GBG and CBC have potent antibacterial and antifungal effects (Al Ubeed et al. 2022). The most active substances with therapeutic potential, which are primarily synthesized in trichomes, are cannabinoids and terpenes (Figure 1).

Figure 1. The most common cannabinoids and their molecular masses

Name of cannabinoids	Abbreviations	Molecular mass (g mol^{-1})	Molecular formula
(-)-trans-Δ^9-tetrahydrocannabinol	Δ^9- THC	314.472	$C_{21}H_{30}O_2$
(-)-trans-Δ^8-tetrahydrocannabinol	Δ^8- THC	314.472	$C_{21}H_{30}O_2$
(-)-trans-Δ^9-tetrahydrocannabinol acid	THCA	358.482	$C_{22}H_{30}O_4$
Cannabidiol	CBD	314.472	$C_{21}H_{30}O_2$
Cannabidiolic acid	CBDA	358.482	$C_{22}H_{30}O_4$
Cannabinol	CBN	310.440	$C_{21}H_{26}O_2$
Cannabinolic acid	CBNA	354.450	$C_{22}H_{26}O_2$
Cannabigerol	CBG	316.488	$C_{21}H_{32}O_2$
Cannabigerolic acid	CBGA	360.498	$C_{22}H_{32}O_4$

A crucial step in separating bioactive molecules from the plant matrix is the extraction of phytochemicals from cannabis. Both traditional and revolutionizing techniques can be used for extraction. For an extraction to be effective and successful, understanding the chemical makeup of the bioactive compounds and the nature of the plant is essential. For maximizing the extraction of phytochemicals from cannabis, both conventional and cutting-edge extraction methods are being studied more and more (Al Ubeed et al., 2022).
A growing number of efforts have been made in recent years to improve cannabinoid extraction and phytochemical isolation efficiency while lowering costs as a result of the high demand for medical cannabis. Therefore, product development and extraction techniques have taken center stage as the cannabis industry makes the transition from the illegal to the legal markets. Cannabis products are being produced industrially in an increasing number of different forms and concentrations. Currently, topical products, edibles, drinks, extracts, and products for both medical and recreational use are available and in use. The extraction and isolation of the active ingredients is a crucial step for the food and pharmaceutical industries, and it should be studied. Depending on the process and the ingredients used, various extraction techniques will result in extracts of varying quality and composition.
(Lazarjani et al., 2021) When selecting which method to employ, one must take into account the characteristics of the product. For instance, cannabinoids can be produced in any acid or neutral form depending on the application. Many medical cannabis preparations are not smoked; instead, they are mixed with oil or made from resin that has been extracted. To ensure maximum decarboxylation, the cannabinoids should be extracted from the dried cannabis before or during the extraction with heating for a specific amount of time at a specific temperature. The main psychoactive cannabinoid is THCA, followed by CBGA, a cannabigerolic acid.
Patients use a variety of methods to consume medical cannabis, including smoking, vaporizing, ingesting oils, and consuming other edible products. One of the problems with smoking or vaping is dosage; patients are unsure about it because herbal products can vary and because of how they inhale and hold the vapor. Smoking and vaping have

well-known detrimental effects on health, and they are especially harmful to young patients. This is the rationale behind the rising demand for extract production.

In order to extract cannabinoids, which are non-polar compounds with low solubility in water, a variety of organic solvents, such as alcohol and hydrocarbons, are used. Although using organic solvents in the extraction process can be very effective, they may have an impact on the control, necessitating additional testing and research depending on the final product. For instance, the residual solvent concentration in the medicines must be specified in accordance with good manufacturing practice GMP. The environment is at risk from the toxicity of these solvents, which can also be very expensive (Rochfort et al., 2020). Selecting the proper solvent also slows down and complicates the extraction process. It is possible to increase the yield of cannabinoids in extract by using low molecular weight alcohols or hydrocarbons as co-solvents, for instance. To separate the bioactive components from cannabis, a variety of extraction methods are being developed, including Soxhlet extraction, accelerated solvent extraction, microwave-assisted extraction, and ultrasound assisted extraction. However, the majority of extraction methods also generate waste that poses a risk to the environment. It is necessary to develop highly efficient and selective extraction methods.

When producing medical cannabis extracts, supercritical fluid extraction (SFE) can do away with the requirement for these organic solvents. CO_2 the primary solvent in SFE is carbon dioxide. When exposed to typical atmospheric conditions, it evaporates from the extract and is GRAS-designated (generally recognized as safe). The most interest has been shown in supercritical fluid extraction (SFE). Due to its strong fractionating, solvating, high selectivity, and environmental safety properties, CO_2 is used as supercritical material in SFE for the extraction of the majority of these substances (Qamar et al.,2021).

2. MATERIAL AND METHODS

This paper is a review of pertinent data that is concentrated on cannabinoid extraction techniques from medicinal cannabis. In search engines like PubMed, Google Scholar, and EMBASE I MEDLINE, combinations of the keywords medical cannabis, cannabinoid supercritical extraction, and carbon dioxide are used.

3. RESULTS

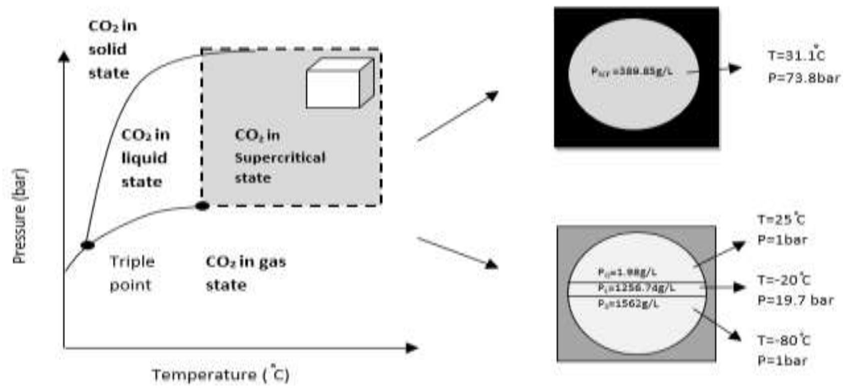
Cannabinoid extraction using conventional techniques

Terpenes, waxes, chemicals, and cannabinoids make up the complex compound known as cannabis. These phytochemicals can be extracted from dried cannabis at room temperature in amounts ranging from 98 to 99% by weight by chloroform, 80 to 90% by hexane, 50 to 60% by ethanol, and 88 to 95% by weight by petroleum ether. Cannabis contains 125 individual cannabinoids, but THCA and CBDA are the most predominant. (Marzorati et al.,2020).These two compounds undergo decarboxylation to yield Δ^9-THC and CBD. CBD (Sirikantaramas & Taura,2017). Both Soxhlet extraction and hot maceration of cannabinoids have shown ethanol to be an efficient solvent (Lewis-Bakker et al.,2019). Nevertheless, compared to advanced microwave extraction, the extraction efficiency of these two conventional techniques was significantly lower (Lewis-Bakker et al., 2019). Even though traditional extraction methods have some benefits over modern ones, such as straightforward procedures, simple operations, and availability, they also have a number of drawbacks, such as a need for more solvents and a longer extraction time, which subsequently increases operating costs overall and has a negative effect on the environment. Additionally, a high temperature for the Soxhle extraction process speeds up the conversion of THCA to THC and then to CBN, resulting in high concentrations of CBN in the extract (Wianowska et al.,2015). Cannabinoids are also extracted using a traditional method known as dynamic maceration, primarily from industrial hemp (Fathordoobady et al.,2019) The sample is soaked in organic solvents that have been chosen based on the polarity of the target compounds at a specific temperature for a specific amount of time during the dynamic maceration solid lipid extraction procedure (Fathordoobady et al.,2019). Cannabinoids are typically extracted using ethanol, acetonitrile, and hexane. Hexane, acetone, and methanol have all been found to be less effective than ethanol at extracting acidic cannabinoids using dynamic maceration; ethanol, on the other hand, is equally effective at extracting neutral cannabinoids (Fathordoobady et al.,2019). Ethanol has been shown in numerous studies to be more efficient than other organic solvents (Brighenti, et al., 2017). Furthermore, under constant agitation, the extraction of cannabinoids was successful using organic solvent mixtures like methanol and chloroform at a 9:1 (v/v) ratio. Intriguingly, discovered that olive oil was superior to ethanol at extracting cannabis oils that contained cannabinoids and terpenes. Ethanol also extracted chlorophyll, which gave the finished product a distinct green hue and unpleasant flavor. When compared to ethanol extracts, it was discovered that the rate of cannabinoid degradation in olive oil extracts was significantly slower (Romano & Hazekamp, 2013)

Supercritical fluid extraction SFE

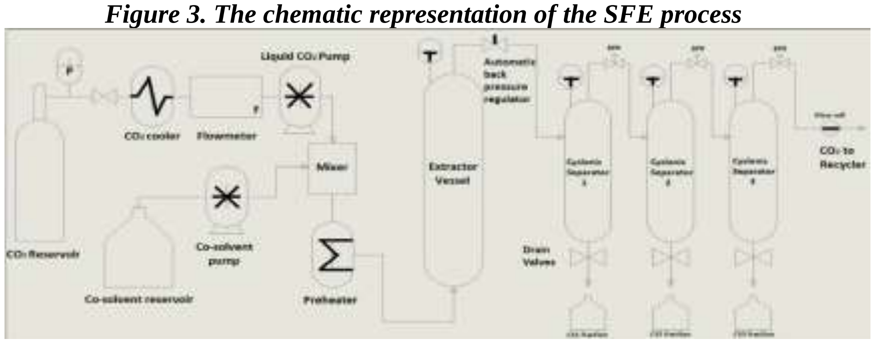
CO2 is frequently used in the SFE method as the primary solvent for extracts of bioactive components. Due to a variety of benefits, including low cost, safety, ease of recycling, and high selectivity. Due to its low surface tension, supercritical CO_2 ($scCO_2$) also has good humidity and diffusion power. It reaches the supercritical state at 73.8 bar and 31.1°C and transitions back to the gaseous state when exposed to ambient conditions. In Figure 2, the various CO_2 states are depicted. The diffusivity and viscosity of CO_2 are also altered during the extraction process by varying the minute changes in temperature and pressure. It can be introduced into the substance as a gas and used as a solvent to dissolve the desired material, increasing the yield of the bioactive substance (Qamar et al.,2021).

Figure 2. Carbon dioxide phase diagram and range of pure CO_2 densities in various states



A CO_2 , cooler is used to achieve a temperature of -5°C in the tank where the incoming CO_2 is placed. A high pressure pump can manage the flow of liquid CO_2 at its maximum mass flow. To control the polarity and density of the CO_2, the co-solvent can also be manually added to the extraction chamber or mixed with the liquid CO_2 from the co-solvent tank. The extraction vessel's conversion of liquid CO_2 to sc CO_2 to dissolve all of the sample's targeted components is controlled by a back pressure regulator. Following that, the mixture is moved to cyclone separators. The density of CO_2 gradually decreases in cyclonic separators, allowing for the separation of various components in samples. The SFE procedure allows for the collection of these fractions (Qamar et al.,2021).

Figure 3. The chematic representation of the SFE process



Supercritical fluid extraction benefits

• SFE is widely used to produce higher yields of cannabinoids because it is a high yielding drug method. The scCO2 extraction method was successful in obtaining 92% pure 9-tetrahydrocannabinol (THC) and 9-tetrahydrocannabinolic acid (THCA) from cannabis strains. Different pressure conditions (170, 240, and 340 bars) were used for this procedure and a temperature of 55℃ to assess the method's effectiveness. The best conditions for obtaining a high concentration of particular cannabinoids, however, were 340 bar and 55°C (Farag & Kayser,2015).

• As a technique for rapid cannabinoid extraction and separation.

Different detection techniques can be used to examine the effectiveness and specificity of cannabinoids after they have been extracted from plant material. Different analytical techniques and their combination have been used for quick cannabinoid screening. Techniques like LC-MS, GC-MS, and 1H NMR are frequently employed. Additionally, supercritical fluid chromatography, which was developed, can provide efficient, quick, and selective separation

(Breitenbach et al.,2016).

• As a method of selective cannabinoid extraction

Through factorial design, selective extraction of cannabinoids in the supercritical region produced an SCF extract with a high THC content. In this study, various variables were used to produce SCF extract with higher amounts of THC content (37.85%), including the amount of ethanol used as a co-solvent (0 to 5% w/w), temperature (40 to 80 °C), and pressure (150 to 330 Bars). To obtain the final fraction with 90.1% THC content, this extract underwent solid phase extraction purification once more. (Gallo-Molina et al.,2019). Another study used the SFE method to develop a selective method for the extraction of cannabidiol (CBD), 8- or 9-THC, and their carboxylic acids. In this method, high-cannabinoid-content $scCO_2$ extract was passed through a high-pressure separation vessel containing diatomaceous earth and zeolite molecular sieves to produce an extract with high concentrations of different cannabinoids, including 8-THC (1.5%), 9-THC (96.0%), and CBD (90.0%) (Mueller,2014).

• As a green cannabinoid extraction technique

SFE is regarded as a highly effective technique because of all of its benefits. Due to their combined mass transfer (as a gas) and dissolution (as a liquid) properties, SCF has the potential to behave as both a liquid and solid at the same time can use a solvent (most commonly CO_2) regarded as adaptable to extract the desired material. Considering that there is no leftover solvent after extraction, it is generally method-free. Additionally, the sample's natural state is unaffected by the low-temperature operation. It reduces processing time, process handling, energy use, and environmentally harmful emissions by avoiding the use of hazardous solvents (Perrotin-Brunel et al.,2010). CO_2 used in the extraction process can be recycled and used again. Overall, it costs very little and uses very little energy. Although the equipment costs for both subcritical and supercritical extraction are higher, the overall extraction process is thought to be safer and greener with a lower current cost of the experiment (Auerbach & Reilly, 2018). $ScCO_2$ extraction is not regarded as an expensive technique when the entire procedure is taken into account, rather than just the cost of the instruments.

Disadvantages of SFE

• While SCF solvents are adaptable, e.g. some SCFs can change their polarity at high pressure and are able to dissolve a target substance. However, the "fine-tuning" process can be difficult, because each degree Celsius and each unit of pressure change affects the diffusivity and density. Therefore, it directly affects the solvent power of SCF.

• The necessary equipment must be made of high-quality stainless steel that can withstand high pressure, which further increases the cost of the equipment.

4. DISCUSSION

In the conventional method for organic solvents to effectively extract cannabinoids, a substantial amount of solvent and additional time are needed. Additionally, other elements like sugars and pigments dissolve in the extract during solvent extraction, complicating the purification process. To enhance the solvent extraction system, a number of techniques are being developed, including microwave extraction, ultrasound extraction, and Soxhlet extraction. However, molecular degradation also takes place during these extraction methods (Rochfort et al., 2020).

Supercritical fluid extraction SFE process can be carried out at low temperatures without the use of hazardous solvents, which prevents the components from degrading as a result of exposure to high temperatures.The liquid above its critical point enters a supercritical liquid SCF state under the influence of temperature and pressure. SCF is a state where it is impossible to tell the difference between a gas and a liquid. As a result, in supercritical conditions, a solvent like CO_2 has a viscosity that is similar to a gas and a density that is similar to a liquid (Qamar et al.,2021).

5. CONCLUSION

This review paper lists the benefits and drawbacks of various extraction techniques. The best extraction techniques for industrial use are examined and recommended. A review of the literature revealed that the extraction methods used have the biggest impact on the yield and bioactivity of phytochemicals. As previously stated, a number of variables, including the kinds of plant materials, extraction methods/time, solvents, pH, temperature, pressure, and the material to solvent ratio, can influence how effectively bioactive compounds are extracted from plant matrices. The use of mathematical predictive models can expedite the optimization process for both traditional and advanced extraction techniques because optimizing these factors can be very costly, labor-intensive, and time-consuming.

SFE has the capacity to identify a variety of cannabinoids with high sensitivity and throughput. It has made it possible to find new drugs for the treatment of various disorders by fusing the SFE technique and separation. SFE is regarded as the primary analytical platform to confirm and establish a trustworthy method for cannabinoid qualitative and quantitative analysis. It becomes the primary contemporary method for the separation and detection of cannabinoids and other bioactive contents by coupling with LC (UHPSFC), GC (SFE-GC-MS), MS (SFC MS), and selecting an appropriate modifier (water, methanol, etc.). SFE's effective extraction of the entire cannabinoid profile offers a great alternative to extraction methods that use organic solvents.

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