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Cannabis sativa: Extraction Methods for Phytocannabinoids -An Update

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Abstract

This review paper highlights and updates the recent robust extraction methods for phytocannabinoids, hydrodynamic extraction technology and use of vegetable oil as the solvent system. Hydrodynamic cannabis extraction is a recent development within the cannabis industry that can be used to produce full-spectrum cannabis extracts with high bioavailability. According to the patented hydrodynamic extraction technology by **Clean Green Biosystems,** Chennai, Tamilnadu, India, the system is the first of its kind to be able to use whole, freshly harvested cannabis materials. Clean Green Biosystems, Chennai, Tamilnadu, India reports that Hydyne is innovative new hydrodynamic extraction system that uses the entire fresh plant materials to preserve all the unique phytochemicals and phytonutrients compounds in a full spectrum/broad spectrum extract. Hydrodynamic system converts the cannabis plant material into a cannabis **nano-emulsion** by means of hydrodynamic force and ultrasonification by breaking the cell walls of the plant material and releases them into the aqueous phase. **PhytoX™ (**USA) is another new hydrodynamic extraction system developed by **IASO Inc** (Incline Village, Nevada, USA) that can process whole, fresh, un-dried cannabis plants, which maximizes plant utilization, reduces processing costs, and increases yields. Many traditional extraction methods can not guarantee the integrity of unstable compounds. Hydrodynamic extraction is designed to use fresh and whole plants, ensuring these volatile molecules are kept intact. Additionally, the distillation prevents the phytocannabinoids from thermal degradation, further protecting molecule integrity. The aroma of the resultant cannabis products is stronger than traditional extracts. Because the plant material is frozen in the preparation stage of the system, it allows the aromatic compounds to remain intact.

Keywords: Clean Green Biosystems; Cannabis sativa; Extraction methods; Indian Himalayan Region; Hydrodynamic extraction technology; Phyto-cannabinoids

1. Introduction

Cannabis sativa L., belongs to *Cannabaceae* family is one of the oldest medicinal plant was found as wild noxious weed particularly in Indian Himalayan Region, Asian countries, China, Pakistan, Nepal, Bhutan, Afghanistan, Morocco, Iran and African countries[1-35]. Cannabis has been used for thousands of years for recreational, medicinal, or religious purposes. Cannabis is also a wild noxious weed with notorious psychoactive principle (THC) found growing in all the parts of India. *Cannabis sativa L*. with a diverse mixture of chemical constituents, creating a complex matrix[1-35]. Terpenes, phenols, fatty acids, amino acids, flavonoids, sugars, phenolic compounds, hydrocarbons, and phycannabinoids are only a few of the estimated 500 distinct ingredients[1-35]. *Cannabis sativa* is known for the accumulation of secondary metabolites, the phytocannabinoids as a part of its own defensive mechanism [1-35]. *Cannabis sativa* is a psychoactive plant that contains more than 500 different chemical compounds, of which

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phytocannabinoids are the main constituents [1-35-100]. These molecules are produced through the secondary metabolism, and their concentration varies between the different subspecies, age, harvesting time, and growing conditions[1-35]. *Cannabis sativa* has gained a lot of popularity in the last few decades for not only being **an illicit drug** but for its medicinal values from ancient times and a potential source for modern drugs to treat several targets for human wellness [1-35]. The pharmacologic and therapeutic properties of preparations of *Cannabis sativa* and Δ-9-THC (**Δ**9-tetrahydrocannabinol) its most psychoactive compound, have been extensively reviewed [1-39]. There is still a huge prejudice in society in relation to Medical *Cannabis sativa* L.(drug type or marijuana) due to its recreational use[1-39].

Cannabis spp. are native to the Indian sub-continent and required warm temperatures and high light intensity and some times low light intensity to achieve good yields [1-35]. Humans have a long history with *Cannabis sativa*, with evidence of cultivation dating back as far as 10,000 years[1-35]. The World Health Organization (WHO) reported that *Cannabis sativa* is the most widely cultivated, trafficked and abused illicit drug, and it constitutes over half of worldwide drug seizures [1-35]. Medical research on *Cannabis sativa* has primarily focused on isolated THC (Δ9-Tetrahydrocannabinol), and CBD (Cannabidiol) but there are hundreds of other chemical constituents in cannabis, including cannabinoids and terpenes [1-35]. *Cannabis sativa* existed prior to the development of agriculture, which began about 13, 000 years ago[1-35]. Therefore, it is assumed to have been one of the most critical crops for the development of civilization [160]. Humans across different eras have utilized it for a variety of applications, such as nutrition, recreation, generation of seed oil and fibre for industrial purposes, religious and spiritual practices and medicine [160]. Therefore, *Cannabis sativa* is one of the oldest medicinal plant on earth [160].

Cannabis sativa L. is a wind-pollinated, dioecious medicinal plant (i.e., the male and female reproductive structures are on separate plants), although monoecious plants (male and female flowers on same plant) can occur in some population [1-35]. Male plants die shortly after flowering[1-35]. The female plants live 3 to 5 weeks until seed is fully riped [1-35]. Therefore, the plants are obligatory out-crossers[1-35]. In commercial production, Medical *Cannabis sativa* (drug or marijuana type) plants are all genetically female and male plants are destroyed as seed formation reduces flower quality [1-35].

Monoecious individuals are found with hermaphrodite flower or bisexual inflorescence [1-35, 167]. The hermaphrodite inflorescences consist of few to many male flowers and female flowers within the leaf axils. *Cannabis sativa* is a diploid species with 2 n = 20 and genome size estimated at 818 Mb and 843 Mb for female and male plants, respectively[1-35, 167]. According to the karyotype of the species, female plants are homogametic (XX), and males are heterogametic (XY) [1-35, 167]. The sex determination of monoecious plants is controlled by homogametic females (XX), but the ratio of male to female flowers is controlled by autosome (XX+A) [1-35, 167]. Monoecious plants have several agricultural advantages over dioecious ones, such as seed yield, higher crop homogeneity and synchronized maturity[1-35, 167]. Monoecious plants can undergo self-pollination and minimize genetic variation[1-35, 167]. It has been observed that the sexual phenotype of monoecious plants is unstable. The multiplication of monoecious seeds in the next generation produces dioecious male and female progenies[1-35, 167].

Female *Cannabis sativa* flowers have densely packed glandular structures called trichomes that store the phytocannabinoids, tetrahydrocannabinolic acid (THCA) and Cannabidiolic acid (CBDA) which must be decarboxylated by heat to produce Δ9-tetrahydrocannabinol (THC: intoxicating) and Cannabidiol (CBD: non-intoxicating) [1-35]. The two cannabinoids the most well known for their therapeutic properties are, Δ9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) [1-35]. THC and CBD are the neutral homologs of tetrahydrocannabinolic acid (THCA) and Cannabidiol acid (CBDA) respectively [1-35]. A conventional classification model of Cannabinoids is due to their chemical contents dividing them to eleven subclasses including Cannabigerol (CBG), Δ9-tetrahydrocannabinol (Δ9-THC), Cannabidiol (CBD), Cannabichromene (CBC), **Cannabinol** (CBN), (−)-Δ8-transtetrahydrocannabinol (Δ8-THC), Cannabicyclol (CBL), Cannabinodiol (CBND), Cannabielsoin (CBE), Cannabitriol (CBT) and miscellaneous [1-35].

At present, *Cannabis sativa* is domesticated and cultivated in many countries, like USA, Canada, Australia, and many European countries, including South Africa and used as a herbal drug particularly THC and CBD for controlling many human diseases[1-35]. There are more than 750 hybrid varieties of *Cannabis sativa* are available in market. Many cannabis cultivars are obtained by crossing plants from commonly considered subspecies *Cannabis sativa*, and *Cannabis indica*, native of Indian origin are used to produce new varieties suitable for different uses, such as fiber, oil, medical drug, and recreational applications[1-35]. There are more than 750 strains of recreational cannabis, the most with colorful names. Some are strains of *Cannabis sativa* and *Cannabis ruderalis* subspecies are crossbred hybrids. The hybrid strains can be named in a variety of ways. Smell or lineage are common ways of naming. Afghan Kush, Hindu Kush, Green Kush, and Purple Kush are all pure *Cannabis indica* strains[1-35].

Additionally, the species *Cannabis sativa* L. and *Cannabis indica* are a potential source of fiber, food, oil, and protein. However, cannabis research work remains years behind than other crops because of the long legacy of prohibition and stigmatization[1-35]. *Cannabis* is the most commonly used illicit drug worldwide and the active constituents of the product were described several decades ago [1-35]. The legal status of *cannabis* is changing, fuelling an increasing diversity of *Cannabis* derived products [1-35]. New laws leading to decriminalization and legalization have given rise to a global, multibillion dollar industry that is projected to continue to grow[1-35]. As of today, there are more than 40 countries including South Africa have legalized medical *Cannabis sativa* (drug or marijuana type [1-35]. The legalization of cannabis is an important source of economic growth as it contributes to the growing revenue tax, and the creation of new workplaces[1-35]. The maturity of the cannabis market varies across the globe mainly due to differing legal environments and public attitudes towards cannabis[1-35]. Some countries such as Canada and the United States have adopted fully regulated frameworks that allow the cultivation, consumption, and retail distribution of various *cannabis* products[1-35]. **Uruguay** became the first country in the world to legalize the production, distribution and consumption of *cannabis* in 2013[1-35]. The *cannabis* market in Canada and the USA is experiencing rapid growth due to the country's legalization of recreational use. Regulations can also differ regarding how cannabis is used[1-35]. However, the use, cultivation, and marketing of Medical *Cannabis sativa* (drug or Marijuana type) is **banned** and **prohibited** in **India** due to the presence of high levels of psychoactive principle THC (Narcotic drug) [1-35].

2. *Cannabis sativa*: History in India

Cannabis sativa has a long history in India, recorded in legends and religion [1-35]. According to *Ayuverda* in India, the medicinal value of the *Cannabis sativa* plants was well documented as Vijaya and often known as Desi Vijaya [1-35]. The meaning of Vijaya is nothing but a victory[1-35]. This was the first Indian written evidence to support the medicinal value of cannabis plants which was well documented in *Ayuverda* in India [1-35]. The earliest written reference to cannabis in India may occur in the *Atharvaveda*, dating to about 2500 BCE [1-35]. Initial uses of *cannabis* date back to almost 5000 years in India which was well documented in A*yurveda* now cultivated for both medicinal and recreational applications [1-35]. The history of *cannabis* use is rooted in the Asian subcontinent particularly in India, Bhutan, Nepal, China, Pakistan, Afghanistan, and other countries like Morocco, Persians Iran ([1-35]. It is found in various habitats ranging from sea level to the temperate and alpine foothills of the **Indian Himalaya Region** from where it was probably spread over the last 10,000 years [1-35]. Therefore, medicinal uses of *Cannabis* might have started from Indian Himalayan civilization and moved to another civilization through consecutive millennia [1-35]. Many of the historians believed that Indian Himalayan Region was the centre of origin of *Cannabis sativa* and *Cannabis indica* [1-35].

Cannabis is also known as the Pot gold of **Indian Himalayan Region** (1-20). Many 19th-century practitioners described medicinal properties to *Cannabis sativa* after the drug found its way to Europe during a period of colonial expansion into Africa and Asia [1-35]. For example, William B. O'Shaughnessy, an Irish physician working at the Medical College and Hospital in Calcutta, West Bengal, India first introduced *Cannabis sativa* (Indian hemp) to Western medicine as a treatment for tetanus and other convulsive diseases[1-35]. Tribal people in the Himalayan region used cannabis as a home made herbal medicine for many diseases. During, Covid-19, the infusion of *cannabis* flower with a morning cup of tea has saved the life of many people [1-23]. Cannabis oil was used as dengue mosquito repellent for controlling dengue viral fever, bacterial infections and fungal diseases[1-35].

Introduced into Western medicine by **William O'Shaughnessy** in **1838** to treat a variety of conditions, including rheumatic pain and epilepsy, the use of cannabinoids (CBs) in clinical practice entered a period of latency and oblivion due to political barriers and problems in establishing quality control [1-35]. *Cannabis* was (re) introduced into British medical practice in the early 1840's by Irish physician Dr. William O'Shaughnessy, an army surgeon serving in Calcutta, India [1- 35]. In the Victorian period, cannabis was widely used for a variety of ailments, including muscle spasms, menstrual cramps, rheumatism, the convulsions of tetanus, rabies, and epilepsy, and as a sedative [1-35]. Cannabis extracts were typically administered orally in the form of an alcoholic tincture and were commonly incorporated in proprietary medicines [1-35]. With the introduction of synthetic drugs, herbal remedies were increasingly viewed as unpredictable and many of them, including cannabis extracts and tinctures, were removed from the British Pharmacopoeia of 1932 but retained in the **British Pharmaceutical Codex** of 1949[1-35].

3. Medical *Cannabis sativa* (drug type) : Current Legal status in India

Cannabis is one of the oldest crops grown, traditionally held religious attachments in various cultures for its medicinal use much before its introduction to Western medicine 1-35]. In India, the sacred scripture "*Atharva Veda*" claimed *cannabis* as an herb of happiness, as it elicited joy and pleasure, and hence used in ritualistic activities [1-35]. The *Ayurvedic* system of medicine also described the use of cannabis for treating various gastrointestinal, respiratory, and

urinary disorders [1-35]. The religious amalgam of cannabis was noticed in Tibetan practices and Buddhism together with Hinduism [1-35]. **Tantric** traditions also showed the unorthodox use of cannabis for different ceremonial proceedings, upholding the history of cannabis in India [1-35]. The increasing decriminalization of cannabis internationally has increased awareness within the Indian Government [3-50]. Presently, *cannabis* use in India is regulated in coherence with the **Narcotic Drugs and Psychotropic Substances Act (NDPS), 1985**, which prohibits the cultivation, possession, manufacturing, and sale of cannabis as Charas (resin), Hashish (liquid form), and Ganja (flowering or fruiting tops) excluding leaves or seed (Bhang) from the purview of the act [1-35]. However, provisions for cultivation exist but are strictly restricted for research purposes, excluding medical purposes owing to its limited proven use [1-35].

Therefore, the use, the cultivation and marketing of Medical *Cannabis sativa* (drug or Marijuana type) is **banned** and **prohibited** in India due to the presence of high levels of psychoactive principle THC (Narcotic drug) [1-35]. The concept of a Medical *Cannabis sativa* (drug or Marijuana type) program was already in progress when in 2016, BOHECO (Bombay Hemp Company) and the Council of Scientific and Industrial Research (CSIR) collaboratively organized ICARE (Indian Cannabis Analysis Research Education) to build awareness [1-35]. In the following year, the Government of India approved its first-ever license to Council of Scientific and Industrial Research–Indian Institute of Integrative Medicine (CSIR–IIIM) in collaboration with **BOHECO** to grow and formulate cannabis-based medicines [1-35]. Till today, as of November 2024, India has not approved medicinal cannabis product to date [3-28-50]. However, in 2018, the Central Council for Research in *Ayurvedic* Sciences (CCRAS) reported the pioneer *cannabis* clinical study where it reduced pain in cancer patients post chemotherapy and radiotherapy [1-35]. Presently, IIIM Jammu, India cultivates cannabis and assesses the medicinal components for cancer, epilepsy, and sickle cell anemia, seeking approval for future clinical trials [1-35]. Moreover, recently, India, during the 63rd United Nations (UN) Commission on Narcotic Drugs (CND) session, voted in favor of removing cannabis and its resin from the UN narcotic drug list [1-35]. The leniency in the decision from India in favour of cannabis legalization during the UN session indicated a future medicinal cannabis program in India [1-35].

In India, Medical *Cannabis sativa* L.(drug type or marijuana) is also commonly known as Bhang, Ganja, and Charas, which are **banned in India as an illicit drug** [1-35]. **The Narcotic Drugs and Psychotropic Substances Act, 1985** (NDPS, Act and the Drugs and Cosmetics Act, 1940), Government of India, New Delhi, defines the scope of medicinal cannabis, under the Drugs and Cosmetics Act [1-35]. As of December, 2024, sales and cultivation of Medical *Cannabis sativa* L.(drug type or marijuana) are illegal in India and considered as the narcotic substance[1-35]. The import and export of Medical *Cannabis sativa* L.(drug type or marijuana, Charas, Ganja, Bhang) into India was entirely prohibited by the Government of India nearly two decades ago. The consumption of *cannabis* resin (Charas) is prohibited everywhere in India [1-35].

4. Legalization of Industrial *Cannabis sativa* L. (Hemp or fiber type)

In 2021, FSSAI (FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA), Government of India legalized and recognized Industrial *Cannabis sativa* L. (Hemp or fiber type) with very low levels of Λ9-tetrahydrocannabinol (THC) (0 to 0.3% of dry weight) products as functional food [1-35]. This notification regulates and allows for sale of products derived from 'non-viable seeds of the *Cannabis sativa*/other indigenous cannabis species[1-35]. Further, the cultivation has to, as usual, comply with the NDPS and state laws[1-35]. The Uttarakhand, State of India is the first state which was licensed to grow hemp, sale and marketing of hemp seeds as the functional food [1-35]. There are many growing licensed Indian companies (Bombay Hemp Company, BOHECO, Satliva, Hemp Fabric Lab, Vedi, Happy Hemp, SUI, Its Hemp, Bhu:Sattva's, Health Horizons, Hemis, Hemp Republic, Hempsters, B.E. Hemp, India Hemp Co., Inc, India Hemp Organics, Its Hemp, The Trost, and Gin Gin) involved in promoting the Indian hemp products, marketing, R & D research, cultivation, harvesting, processing, manufacturing, trading, wholesaling, retailing, innovating, advocating and motivating customers across India and around the world [1-35]. This will help to boost the Indian economy and increase the productivity of the Indian hemp (fiber type) [1-35].

5. Difference between Hemp and Medical *Cannabis sativa*

Cannabis sativa L., is classified into two types as 1) Industrial *Cannabis sativa*, hemp or fiber type, and 2) Medical *Cannabis sativa* L.(drug type or marijuana) based on its Λ9-tetrahydrocannabinol (THC) content [1-35]. Medical *Cannabis sativa* (drug type or marijuana) contains very high levels of THC (above 0.3 to 38% of dry weight) and grown inside the greenhouse controlled conditions for the production of unfertilized female flowers containing very higher levels of THC [1-35]. As of December, 2024, sales and cultivation of Medical *Cannabis sativa* L.(drug type or marijuana) are **illegal** in India and considered as the narcotic substance. The import and export of Medical *Cannabis sativa* L.(drug

type or marijuana, Charas, Ganja, Bhang) into India was entirely **prohibited** by the Government of India nearly two decades ago. The consumption of *cannabis* resin (Charas) is **prohibited** and **banned** everywhere in India [1-35].

On the other hand Industrial *Cannabis sativa* L. (Hemp or fiber type) contains very low levels of Λ9-tetrahydrocannabinol (THC) (0 to 0.3% of dry weight) grown outside in a large agriculture farms for the production of fibre, seeds, oil and used as functional food [1-35]. Therefore, Industrial *Cannabis sativa* L. (Hemp or fiber type differs from marijuana in the level of Λ9-tetrahydrocannabinol (THC) present in the plant [1-21]. On 15th November 2021, FSSAI (FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA), Government of India has legalized and recognized Industrial *Cannabis sativa* L. (Hemp or fiber type) with very low levels of Λ9-tetrahydrocannabinol (THC) (0 to 0.3% of dry weight) products as functional food.

Industrial *Cannabis sativa* L. (Hemp or fiber type) and Medical *Cannabis sativa* (drug type or marijuana) share the same species, *Cannabis sativa* L, but represent different varieties [1-35]. As such, there are genetic differences that lead to different chemical characteristics, which, in turn, lead to different uses [1-21]. Industrial hemp or fibre *Cannabis sativa* is grown outside in a large agriculture land for the stalk and seeds, and maximizing yields results. in tall plants with few leaves [1-35]. On the other hand, Medical *Cannabis sativa* (Marijuana or drug type), is grown under controlled conditions in a green house conditions for its leaves and female unfertilized flower known for the parts of the plant with the largest concentrations of THC [1-35]. As a result, Medical *Cannabis sativa* (drug type or marijuana) grown for its psychoactive properties (THC) is generally managed to control height and increase bushiness: that is, encouraging many leaves and branches, thus leading to more flowers and buds [1-35]. *Cannabis* varieties also vary by planting density: Medical *Cannabis sativa* (drug type or marijuana) plants are spaced to allow bushiness while Industrial *Cannabis sativa* L. (Hemp or fiber type) plants are planted much closer together to discourage branching and flowering [1-35]. Harvest timing and strategies also vary by variety, again allowing for detection of intended use [1-35]. Thus, even in United states where Medical *Cannabis sativa* (Marijuana or drug type), is legal(in some parts of USA) , Industrial *Cannabis sativa* L. (Hemp or fiber type) may be distinguishable from *Cannabis* grown for marijuana, based on visual appearance [1-35].

Λ9-tetrahydrocannabinol (THC) is the narcotic chemical most responsible for the psychoactive properties in Medical *Cannabis sativa* L.(drug type or marijuana). Industrial hemp has traditionally been defined as having less than 0.3 percent THC, although in some U.S. states it is now defined as having no more than 0.5 % percent THC [1-35]. They have also established a maximum standard of 10 parts per million (ppm) for THC residue in hemp products, including grain, flour, and oil (Agriculture and Agri-Food Canada 2013) [1-35]. However, due to the presence of psychoactive molecules, Λ9-tetrahydrocannabinol (Λ9-THC) and Λ8-tetrahydrocannabinol (Λ8-THC), cannabis cultivation and its use is restricted/regulated in many countries [1-35]. The official discovery of Δ9-tetrahydrocannabinol (THC) is commonly attributed to **Dr. Raphael Mechoulam** affectionately referred to as the Godfather of Cannabis Science [1-21]. Δ9-tetrahydrocannabinol (THC) was discovered in 1964 by Dr. Raphael Mechoulam and his colleagues at Israel's Weizmann Institute of Science [1-35]. The credit of the discovery of Cannabidiol (CBD) in 1963 and Δ9-tetrahydrocannabinol (THC) in 1964 isolated from *Cannabis sativa* attributed to Dr. Raphael Mechoulam and his team [1-35].

6. Medical applications of *Cannabis sativa*

Today *Cannabis sativa* continues to be the most used drug in the world[1-35]. Research showed that *cannabis* use is associated with a wide range of adverse health consequences that may involve almost every physiological and biochemical system including respiratory/pulmonary complications such as chronic cough and emphysema, impairment of immune function, and increased risk of acquiring or transmitting viral infections such as HIV, HCV, and others [1-35]. Both Medical *Cannabis sativa* (Marijuana or drug type) and Industrial *Cannabis sativa* (hemp or fiber type) are used for controlling numerous diseases, such as chronic pain, asthma, rheumatoid arthritis (RA), wound healing, constipation, multiple sclerosis (MS), cancer, inflammation, glaucome, neurodegenerative disorders (Epilepsy-seizure disorder, Alzheimer's disease, Parkinson's disease, dengue viral disease, Huntington's disease, Tourette's syndrome, Dystonia, Lennox-Gastaut Syndrome (LGS) and Dravet Syndrome (DS), Obesity, weight loss, anorexia, and emesis, osteoporosis, schizophrenia, cardiovascular disorders, sleep disorders, Traumitic brain injury (TBI), Post traumetic stress injury, drug addiction (Marijuana), AIDS Wasting syndrome, Amyotrophic lateral sclerosis (ALS), depression and anxiety, diabetes, migraine (headache disorder), Covid-19 (SARS-CoV-2), Leishmaniasis (Kala-Azar), dengue fever, monkeypox, Nipah virus, Lumpy skin vital disease of cattle, and metabolic syndrome related disorders, are being treated or have the potential to be treated by cannabinoid agonists/ antagonists/cannabinoid-related compounds [1-35].

However, cannabis use is associated with a wide range of adverse economic, social, psychosocial and health consequences [1-35]. The psychosocial consequences of marijuana use—such as dropping out of school, poor school performance, and antisocial and other behaviors among youth—have been the subjects of many reviews/publications [1-35]. In the following section, this review paper highlights, the influence of external factors on extraction methods,

traditional extraction methods, and modern extraction methods, hydrodynamic extraction for phytocannabinoids has been discussed and updated.

7. Influence of Factors on Storage and Extraction of Phytocannabinoids

According to the literature survey by Lazarjani et al., (2021) [38], external factors such as light duration, oxygen, and harvest time (floral maturity) have been shown to influence the secondary metabolite production in cannabis [37-43]. Three conditions were used to store *cannabis* resin (hashish slabs) and extract (by the solvent): room temperature and 4°C both with visible light exposure and darkness, and − 20°C in darkness [37, 38-43]. One of the study identified that in cannabis resin, light exposure can affect the decarboxylation of THCA and the degradation of THC [37, 38-43]. This is evident as the half-life increased by 40% in darkness [37, 38]. It was observed that both the neutral and acidic forms of THC in the cannabis extract degraded significantly more through light exposure [37, 38-43]. However, it was observed that light was only partially influential [37, 38]. The resin samples that were placed at room temperature, either in light or dark settings, only exhibited little differences in the degradation of neutral THC [37, 38-43].

According to the literature survey by Martinez et al., [160], a crucial factor that can affect the overall quality, medical efficacy and value of *cannabis* products is the humidity level upon storage of the harvested and cured flower [160]. According to the American Society of Testing and Materials (ASTM) International, the optimal humidity level for cannabis storage is in the range of 55–65%. Exceeding the recommended values exposes the harvested flower to possible growth of mold and fungus, while failing to reach the minimum value can lead to extensive dryness. Hence, substantial loss of critical components, such as cannabinoids and volatile constituents, such as terpenes has been reported [160].

According to the literature survey by **Martinez et al**., [160], the sample processing prior to analysis or extraction is another important consideration that will largely determine the sample homogeneity and associated reliability of the analytical outcome[160]. Despite the advantageous effects of sample grinding, i.e., increased homogeneity, surface area and decreased particle size, all of which benefit extraction efficiency [160]. Further, at the same time, grinding also impacts the sample in an undesirable manner; increased contamination risk, introduction of humidity, possible losses of volatile compounds and alteration of labile ones [160]. Cryogenic grinding is an alternative that can largely circumvent the unwanted outcomes above mentioned [160]. Studies have demonstrated that Λ9-THC, both in herbal and resin cannabis preparations (marijuana and hashish), progressively decomposes over time to cannabinol (CBN), which is believed to be a mere chemical degradation product rather than a naturally/ biochemically occurring component[160]. However, decomposition of Λ9-THC (Λ9-tetrahydrocannabinol) when exposed to light and air, to cannabinol (CBN) proceeds through an oxidation reaction[160]. Although this gradual conversion of THC to CBN is an inevitable process, storage conditions can significantly delay or accelerate the conversion process [160]. Martinez et al., [160] are of the opinion that there might be either a considerable time lapse between sample reception and analysis or the need for re-evaluation of an old sample or even both, it is crucial to be able to accurately determine the effect of time and storage conditions on the sample composition [160].

It has been known since 1969 that the Λ9-THC content of marijuana stored at room temperature is decreasing at a rate of 3–5% per month[160]. Three independent 4 year long stability studies, demonstrated that light and temperature have a dramatic effect on the decomposition of Λ9-THC to CBN, while they mediate different aspects of the process; light impacts the stoichiometry of the conversion of Λ9-THC to cannabinol (CBN), whereas temperature accelerates the conversion [160]. In any case, samples stored at room temperature in direct contact with the atmospheric air, either in light or darkness, suffered the most pronounced losses in Λ9-THC ranging from 65% to almost 100%, depending on the sample origin and initial composition [160]. Since many of the substances in cannabinoids can undergo oxidation, and sealing the samples in plastic bags can reduce the losses to 25–42% [160]. It should be mentioned that the increase in the total amount of cannabinol (CBN) does not correspond with the total decrease of Λ9-THC, an observation which implies that Λ9-THC degrades to other products apart from CBN [160]. Negligible impact on the initial concentrations of cannabinoids is observed when samples are stored in the freezer at −20 °C. In contrast, storage conditions have no significant impact on the CBD content [160].

Short-term stability studies (15–30 days) of cannabinoids (THC, CBN, CBD) extracted in organic solvents (methanol, chloroform, light petroleum, methanol : chloroform 9 : 1 indicated that the form of cannabinoids and storage temperature, rather than the nature of the extractant, are crucial parameters affecting their stability in solution [160]. Specifically, neutral cannabinoids are stable for up to 15 (at 20 °C) to 30 days (at −18 °C and 4 °C) stored in the dark, while exposure to daylight leads to their dramatic decomposition[160]. In case of acidic cannabinoids, decomposition occurs both in dark and light with the rate increasing with temperature [160]. In addition to the discussed parameters,

Martinez et al., [160] are of the opinion that material of the containers in which phytocannabinoid extracts are stored can also have a significantly affect on the cannabinoid content determined in solution [160].

8. Harvesting and Pre-treatment

According to the literature survey by López-Olmos et al., (2022) [54], harvesting biomass is the first step of the global process, usually followed by treatment of the harvested biomass to make it suitable for extraction [54]. *Cannabis* plant matter contains active chemicals such as cannabinoids, terpenes, and other phenolic compounds that are produced and stored in the trichomes, which are glands on the surface of the female plants buds, mainly in the flowers [1-35, 54]. Trichomes function as a protection from winds and fungal growth and as a defence mechanism against predators due to their bitter taste and strong smell [54-56]. Glandular trichomes have a crystalline appearance; when handled, they release a resin rich in cannabinoids that can be extracted [54-56]. However, minimal amounts of the resin are formed in non-glandular tissue, such as leaves, roots, or stems, which cannot be extracted by directly soaking intact plant material in a solvent [54-59]. The accessibility of the trichomes can be increased by pre-treating the biomass, and the cannabinoids contained in non-glandular material can be extracted after pre-treatment consisting of air drying and powdering [5459]. According to Gülck nd Møller (2020) [53], *Cannabis sativa* accumulates phytocannabinoids and terpenes in glandular trichomes located all over the aerial parts of the plant and in highest density on the female flowers [1-35, 53]. No glandular trichomes are found on the root surfaces, and the root tissue. and therefore, does not accumulate phytocannabinoids [53]. Glandular trichomes may be classified as sessile trichomes or stalked trichomes [53]. Stalked glandular trichomes were recently shown to develop from sessile trichomes [53]. Glandular trichomes accumulate cannabinoids in a balloon-shaped cavity that is filled by secretory vesicles [53]. When a trichome ruptures, for example during high ambient temperatures or as a result of herbivory, its contents form a sticky coating on the plant surface that is orchestrated by the viscous, non-crystallizing properties of cannabinoids [53].

Obtaining plant material with the highest trichome concentration is crucial for the extraction of cannabinoids such as CBG, CBC, CBD, THC, and CBN [54, 56, 60]. Typical treatment of the harvested cannabis biomass includes physical processing to separate the cannabinoid-rich flowers and leaves from the stems, drying to a moisture content of 0–15 wt%, and other practices such as milling and sieving the plant material < 0.5 mm [54, 61-63]. Biomass particle size affects extraction kinetics, as particles that are too small can produce channeling phenomena, clogging phenomena, and overpressure in liquid extraction, which negatively affect extraction performance [54-64]. In contrast, too-large particles resulted in increased extraction times [54, 64]. When the stems and roots of the cannabis plant are used as raw material, they are cut into small pieces before being placed into the extraction vessel [54-64]. However, size reduction before extraction is not usually performed for flowers and leaves [54, 65]. In some cases, plant material is cut or chopped into pieces to facilitate drying to reduce the moisture present, which negatively affects extraction efficiency due to the higher total weight of humid material compared to dry material [54-65]. According to the literature survey by **López-Olmos** et al., (2022) [54], Patent US20180099236A1 (Shuja, 2018) [66], described a method to accelerate the drying and extraction steps of target compounds from cannabis plants by applying pulsed electric fields (PEF) [54, 66]. The application of a rapid and intense electric pulse (in the range of 0.1–12 kJ/kg at 20–30 kV) to the plant material positioned between two electrodes causes electroporation or modification of the cell membrane within a millisecond, with the creation of nanopores that increase the diffusion coefficients to facilitate the migration of molecules into the external solvent [54, 66].

According to the literature survey by Rantasa et al., (2024) [166], the purity of the extracts depends on the quality of the *cannabis* plant sample [166]. To provide a clean product, the flowers, seeds, and other plant components must be cleansed of dirt and pollutants like pesticides before further treatment [166]. For cannabinoids to be extracted from samples effectively, they must be appropriately prepared [166]. After being harvested, the *cannabis* plant can be dried to eliminate any extra moisture. Water can function as a barrier, lowering the quality of the intended analytes' extraction [166]. *Cannabis* samples must be stored in a dark area to prevent photochemical reactions, which may transform the composition of cannabis samples [54-166]. It is also recommended to store cannabis samples refrigerated as the content of cannabinoids drastically changes when exposed to room temperatures for longer periods of time [166]. Prior to executing the SFE, the cannabis flowers, leaves, buds, and stems should also be homogenized to produce evenly distributed, uniformly sized particles[38-54, 166]. Different types of homogenization processes for the pre-treatment of cannabis samples are listed [54, 166]. Homogenization methods may be divided into two groups, namely manual homogenization, and mechanical grinding[54-166]. Mortars and pestles, manual grinders and even metal spoons were used to manually reduce the particle size, while mechanical grinding implements different grinding machines and vibrational, ball, and knife mills [166].

9. Cryogenic Grinding

Cryogenic grinding is an alternative technique for homogenization that cools the sample using liquid nitrogen before grinding to prevent the loss of volatile compounds as the temperature rises during grinding [166]. *Cannabis* samples can also be treated using ultrasonic waves [166]. When the sample is subjected to ultrasonic irradiation, the process known as acoustic cavitation generates high temperature regions which disrupt cell walls leading to subsequent higher solvent penetration and reduced extraction times [166]. The drawback of ultrasonic irradiation is that it accelerates the chemical reactions of the compounds [166]. After homogenization, the sample is often sieved through several sieves with different mesh sizes to separate the larger particles from the finer product [166]. The use of different homogenization methods leads to different quality of the homogenized particles, evident by their average size [166]. A larger average diameter of particles requires longer extraction times to achieve similar extraction yields, whilst smaller particles may clump together and hinder effective extraction [166]. Smaller homogenized particles also lead to subsequent increased cannabinoid extraction [166]. Homogenization increases the surface-to-volume ratio of particles, thus improving the extraction efficiency by increasing the frequency at which the solvent comes into contact with the sample [166].

The best extraction results were obtained when using fine particles in the range from 0.25 mm to 0.50 mm [38]. Another study discovered that particle size affected the THC content in extracts [166]. The highest values of THC were present in sieved particles in the range of 0.063–0.125 mm, with fractions with a greater average diameter containing less THC[166]. This could also partly be contributed to variations in composition, as fractions greater than 0.200 mm contained more leaves and less resin-rich material compared to smaller fractions [166].

10. Decarboxylation of Phytocannabinoids

Cannabinoids are synthesized in the cannabis plant in an acidic, non-psychotropic form [166]. Decarboxylation is a chemical process in which the carboxyl functional group present in THCA, CBDA, and other acidic cannabinoid precursors is replaced by a hydrogen atom, at the same time CO_2 molecule is released [166]. Through this chemical process, cannabinoids are converted into neutral, bioactive molecules [38, 54, 166]. For example, THCA and CBDA are converted to THC and CBD, respectively [166]. Through decarboxylation, a higher yield of neutral cannabinoids can be achieved with SFE, as neutral compounds are more soluble in supercritical solvents, especially CO_2 [166]. There are two primary methods for the decarboxylation of cannabinoids. The first method is in-situ decarboxylation, which is performed directly on the raw plant material before extraction process [166]. The second, less commonly used approach is the decarboxylation of the extract itself [166]. *In-situ* decarboxylation not only converts acidic cannabinoids into their neutral forms, which are more soluble in supercritical CO_2 and thus improving cannabinoid yields, but it also removes the moisture from the material [54-166]. Decarboxylation of cannabinoids occurs at a slow rate naturally in cannabis plants, but the process can be accelerated if the plant or the extract are exposed to elevated temperatures, light, and O_2 [166]. Decarboxylation is generally faster when higher temperatures are employed [166]. A problem that may occur during the thermal treatment is the degradation of compounds and the loss of volatile compounds, since acidic cannabinoids such as THCA have a boiling point of 120°C, while their neutral forms have higher boiling points [166]. Furthermore, cannabis samples decarboxylated for 45 min at 120°C [166]. On the other hand the samples were decarboxylated at even higher temperatures (120–180 °C) for 15 min [166]. The maximum conversion rate of 70 % was observed at around 160°C [166]. Experiments were performed in the temperature range of 90–140 °C and the highest conversion to THC was obtained at 110 °C and 110 min[166]. Longer decarboxylation at lower temperatures favored CBD formation, while shorter reactions at elevated temperatures were preferable for THC [166].

11. Effect of Drying on Extraction of Phytocannabinoids

According to the literature survey by López-Olmos et al., (2022) [54], drying is a complex process, is difficult to optimize and implement at an industrial scale due to the requirement for long periods and large spaces [38-43, 54]. Moreover, the energy consumption during this practice affects the overall costs, and uniformly drying the plant material is a challenge that can affect the product quality [54, 67]. *Cannabis* plants can be naturally dried in a dark storage room with adequate temperature and humidity levels, a method known as “slow drying” [38-43, 54, 67, 68]. The plant material can be hung upside down or spread on drying screens, although the latter technique results in heterogeneous drying due to differences in bud sizes [38-43, 54, 67, 68]. However, natural drying requires an extended time (5–6 days) and generates large pieces of plant material[38-43, 54, 67, 68]. In addition, extended drying periods and uncontrolled environmental conditions can lead to mold growth because of incomplete drying [54, 67]. A common practice in the industry is to use dehumidifiers to dry cannabis plant material [38-43, 54, 67]. To meet the challenge of scaling up, the extraction processes and the drying step, oven drying, hot-air drying such as spray drying, and microwave drying have been used

to speed the drying process and avoid long periods between harvesting and extraction [38-43, 54, 67]. However, heat can alter bioactive compounds and evaporate volatile terpenes while microwaves may compromise the concentration of psychoactive cannabinoids [54, 66, 67-70]. However, intact fresh plant material has a larger size than dried material. Therefore, closed chambers or containers are needed to preserve the harvested material until extraction [38-43, 54, 66, 67-70].

According to the literature survey by Lazarjani et al., (2021) [38], for many applications, the dried version of the cannabis herb is required [38-43]. However, like many plants, cannabis contains approximately 80% water [38-43, 54]. For this reason, drying is considered as an essential step for product development [38-43, 54]. Drying the plant not only prevents the growth of microorganisms that would otherwise rot plant tissue [38]. Hang-drying or air-drying is considered the oldest way of drying cannabis plants after harvest that requires no dedicated equipment[38-54]. Slow drying includes placing whole plants or separated inflorescence in a cool dark room with a temperature between 18 and 25 °C and humidity between 45 and 55%, either hung from a string or laid out on drying screens [38-43, 54]. However, the disadvantage of this air-drying is that the water from the top part of the plant will absorb into the lower parts leading to a slower and uneven drying process [38-43, 54]. To speed up the procedure, heaters, fans, and dehumidifiers can be used [38]. However, fast-drying can lead to a harsher taste as opposed to slow-drying which produces smoother tasting products [38]. A faster direct method of drying is the oven-drying approach. Inflorescences were dried for 1, 4, 16, and 64 h at 65, 85, and 105° C [38]. After extraction with ethanol, gas chromatography showed that the yield of CBD and THC decreased as the temperature and time of drying increased [38]. It was also observed that at temperature 105° C, the thermal degradation of THC increased the CBN content [38-43]. CBN is of the cannabis plant considered a less potent psychoactive and mild analgesic [38]. Therefore, conversion of THC to CBN will decrease the therapeutic potential [38]. Additionally, using high temperatures and excessive drying can result in the loss of key components [38]. Therefore, air or hang drying and oven drying methods have many disadvantages and not used anymore [38-43]. A wide range of concentrations of Λ^9-THC and CBD and their carboxylic acid precursors, Λ^9-THCA and CBDA, are present in commercial medical cannabis products [38-43]. Drying negatively impacts the extraction yield, since higher yield was obtained from fresh inflorescences; specifically, drying promotes the loss of monoterpenes [160].

12. Freeze-Drying: The best method

According to the literature survey by **López-Olmos** et al., (2022) [54], freeze-drying may also facilitate the extraction of substances from *cannabis* plant material (Goldner, 2019) [71]. **Freeze-drying** or lyophilization is a sophisticated method that reduces the pieces of plant matter to a powder, which breaks the plant cells to release the active compounds [38-43, 54, 71]. Since this process operates under vacuum at low temperatures, the loss of volatiles and the degradation of other valuable components is considerably reduced compared to other drying techniques, resulting in dried cannabis of higher quality that contains the desired cannabinoids and terpenoids [54, 67, 69, 71]. However, the use of vacuums is expensive and requires high energy consumption. Thus, freeze-drying is more expensive than slow drying and costs 4–10 times more than hot-air drying such as spray drying [54, 67]. Consequently, its use is limited to high-value products such as cannabinoids. This method is described in patent US20190358278A1 (Goldner, 2019) [71], to improve the efficiency of cannabinoid extraction with liquid or supercritical carbon dioxide [71] . According to the invention, the biomass is placed into a freeze dryer and chilled to a freezing temperature that makes the moisture transition to ice crystals, with a subsequent expansion, thereby destroying microscopic structures of the plant matter and exposing cannabinoids that were difficult to extract [54, 71]. The temperature is preferably below the triple point of water to ensure the sublimation of the ice crystals [54, 71]. The result is a powder of cannabis plant matter of significantly smaller particle size than that produced by natural drying [54, 71]. The invention claims that the freeze-dried plant matter has relatively uniform particle size and higher surface area, which improves the access of the carbon dioxide during the extraction process (increasing the proportion of extracted cannabinoids) and the potential weight of biomass that can be used in the extraction vessel compared to the naturally dried biomass, with larger particle size [54, 71].

According to the literature survey by Lazarjani et al., (2021) [38], freeze-drying (also known as lyophilization) has become a popular option due to the increasing demand for high quality medicinal cannabis [38]. The freeze-drying method holds the cannabis plant at temperatures far below those of air or oven, while removing the water content, in the form of vapour, via sublimation in a vacuum chamber [38]. It is generally agreed that the end products of freeze-drying are considered high quality compared to other methods of drying [38-43]. A disadvantage of **freeze-drying** is the cost of operation [38]. This procedure requires an intense amount of energy to maintain such temperatures, vacuum, and long-running time[38-54].

According to the literature survey by Lazarjani et al., (2021) [38], the process of hang-drying cannabis was found to be time-consuming as it can take several days, while the main factors that increase the rate of drying were determined to be moving air and low humidity [38]. In contrast, the oven-drying method was observed to be faster, but readily volatile

compounds and neutral forms of cannabinoids decreased in extracts to almost non-detectable concentrations, affecting therapeutic potential [38-54-120]. **Freeze-drying** enables the preservation of flavor qualities in many foods, themselves often due to the presence of volatile compounds[38]. According to the literature survey by Lazarjani et al., (2021) [38], in all the drying methods mentioned above, humidity, temperature, ventilation rate, and time are the most important parameters to be optimized [38-43]. Incorrect drying conditions may cause decarboxylation of acidic cannabinoids and loss of terpenes [38-43-54]. The presence of light, oxygen, and heat may also cause degradation in cannabinoids and terpenes and can affect the taste[38-43-54].

13. Influence of Curing on Phytocannabinoid Extraction

According to the literature survey by Lazarjani et al., (2021) [38], **curing** is the final post-harvest procedure that allows for the development of the maximum flavour in the cannabis plant [38]. The best temperature and humidity for curing are at 18 °C and 60% RH for 14 days [38-54]. Therefore, keeping the trimmed flowers in a can for up to 4 weeks in a dark cupboard while opening the lid every day for about 6 h is the best method for curing [38, 41-54]. At temperatures between 15–21 °C and 45–55% humidity, enzymes and aerobic bacteria will be in the optimum condition to breakdown undesired sugars and degrade minerals [38, 41]. Curing can reduce the harsh smell and the sense of throat burning during smoking or vaping as well as increasing the shelf life by minimizing mold growth [38, 41-54].

It is also believed that curing can increase cannabis potency as the number of cannabinoids such as THC and CBN will increase by curing [38, 41-54]. Although curing is one of the most significant post-harvest stages for the cannabis plant, there are not enough academic investigations around this area [38, 41-54]. According to the literature survey by Lazarjani et al., (2021) [38], among different drying methods for post-harvest processing, freeze-drying is considered more appropriate when compared to other methods [38, 41, 54]. However, there is currently a lack of academic research and evidence to support this [38-54]. Hang-drying as a traditional technique is still the most convenient way to reduce the prevalence of mold and bacteria during storage before extraction [38, 41-43, 54].

14. Phytocannabinoid Extraction Methods

Phytocannabinoids are gaining attention in many sectors, including the pharmaceutical, nutraceutical, and cosmetic sectors [1-71]. To date, several conventional and alternative techniques have been applied for *Cannabis sativa* L extraction at the industrial scale [37, 38, 44, 47-52, 54-74-128-137-166]. *Cannabis* extraction can be used to concentrate target components for product development [37, 38, 44, 47-52, 54-74-128-137]. There are important parameters that can affect the yield of the cannabis extract such as mean particle size, size distribution, temperature, rate of agitation, and extraction time [37, 38, 44, 47-52, 54-74-128-137]. Solventless, solvent-based, convention, and alternative methods of extraction are explored concerning cannabis extraction [37, 38, 44, 47-52, 54-74-166]. Lewis-Bakker et al., (2019) [48] are of the opinion that there are conventional and domestic methods described for cannabis extraction such as ethanol extraction, maceration, butane extraction, and quick-wash alcohol extraction[48-120]. Recently reported extraction methods include ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE) [37, 38, 44, 47-52, 54-74-137]. Each method carries advantages and disadvantages depending on the compounds to be extracted, duration of extraction, temperature, and solvent [37, 38, 44, 47-52, 54-74-137]. It is typically desirable to use a solvent that solubilises and carries compounds from the plant, and the temperature for extraction should minimize the loss of thermally labile groups or unwanted chemical transformations[37, 38, 44, 47-52, 54-74-120]. López-Olmos et al., (2022) [54], are of the opinion that the conventional methods are liquid solvent extraction, including polar and non-polar solvents such as ethanol, hexane, petroleum ether, and other solvent extraction [54]. Pressurized gas extraction is another conventional method and comprises gaseous hydrocarbon extraction such as n-butane and n-propane, supercritical or subcritical carbon dioxide extraction, and extraction using a refrigerant gas such as 1,1,1,2-tetrafluoroethane (HFC 134a) [54]. Alternative extraction methods include microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), hydrodynamic cavitation, and pulsed electric fields (PEF) [37, 38, 44, 47-52, 54-74-128-137-166].

According to Ubeed et al., (2022) [128], numerous factors have influenced the extraction efficiency of phytochemicals from plant materials, including cannabis[37-128-139-166]. Plant materials play an important role in the extraction efficiency of their bioactive compounds [37-128-137]. Each plant material has a specific matrix, structure, and phytochemicals. Therefore, the extractability of the phytochemicals varies depending on the type of material[37, 38, 44, 47-52, 54-74-128-139]. With the same plant material, different parts, such as leaves, stems, roots, and flowers, have different extractability of the target compounds[37-128-137]. In addition, fresh, dried, or ground plant materials with small particle sizes have different extraction efficiencies when the extraction is performed under the same conditions [37, 38, 44, 47-52, 54-74-128-139]. Solvents are known to directly affect the extraction efficiency of

phytochemicals from plant materials [37, 38, 44, 47-52, 54-74-128-139]. With a range of solvents from polar to non-polar, various compounds with corresponding polarities can be extracted into solvents, depending on the polarities of the solvents and target compounds [37, 38, 44, 47-52, 54-74-128-137]. In some cases, the pH of the solvent has been found to influence the extraction efficiency of the target compounds, as acidic solvents are believed to disrupt the cell walls faster, thus increasing the extraction efficiency[37, 38, 44, 47-52, 54-74-128-137]. In addition, bioactive compounds are more stable under acidic conditions [37, 38, 44, 47-52, 54-74-128-139]. Therefore, comprehensive studies are warranted to identify the right solvents, solvent-to-material ratios, and pH for the maximum extraction of phytochemicals from cannabis [37, 38, 44, 47-52, 54-74-128-139]. According to Ubeed et al., (2022) [128], temperature and length of extraction have been reported to affect the extraction efficiency of phytochemicals from plant materials [37-128]. A higher temperature with a longer extraction time usually leads to a higher extraction efficiency [128-137]. However, the stability of phytochemicals can decrease when they are exposed to high temperatures for a long time because most phytochemicals are sensitive to heat [37-128-137]. Therefore, it is important to determine the most suitable temperature and length of extraction to extract a high level of phytochemicals with minimum degradation[54-128-139].

On the basis of literature survey by **Ubeed et al.,** (2022) [128], agitation and pressure have been found to influence the extraction efficiency of phytochemicals from plant materials[128]. Studies have reported that agitation significantly increases the extraction efficiency of phytochemicals compared to non-agitation [37-128-139]. Extraction with high pressure has improved the extraction efficiency of phytochemicals [128]. The number of extractions or extraction time has been found to affect the extraction efficiency[37-128-139]. The more extraction time is applied for the same quantity of the sample, the more phytochemicals can be extracted [128]. It is similar to the material-to-sample ratio, the use of more solvents for extraction leads to more phytochemicals in plant matrixes that can be released into solvents[128]. On the basis of literature survey by Ubeed et al., (2022) [128] it should be considered carefully, as more energy is required to heat up the larger solvent volume as well as to remove solvents [37-128-137]. Finally, extraction techniques have been reported to significantly affect the extraction efficiency of phytochemicals from plant materials [37- 128-137]. Extraction techniques can be categorized into two major groups, including conventional extraction techniques and advanced techniques [37- 128-139]. In some cases, advanced extraction techniques showed more efficiency than conventional techniques [37-128-137]. However, the cost of setting up on a commercial scale is a major limitation for the advanced techniques[128-137]. Conventional and advanced techniques have been applied for the extraction of phytochemicals from Cannabis[37-128-139].

According to the literature survey by **López-Olmos** et al., (2022) [54], the methodology used to extract cannabinoids from cannabis plant matter must be carefully selected according to key considerations [54-128-139]. Extraction yield (mass extracted vs. mass of plant material), extraction efficiency (extracted cannabinoids vs. cannabinoids present in the plant material), and extract quality are crucial factors, in addition to environmental, safety, and scalability considerations [54-128-137].

This review focuses on various drying and extraction methods while comparing conventional and most recent methods. According to the literature survey by Lazarjani et al., (2021) [38], it is found that different methods of extraction of cannabinoids from medical *Cannabis sativa* will yield varying degrees of extract quality and composition depending on the procedure and substances used [38-128-137]. According to López-Olmos et al., (2022) [54], the extraction method must preserve the biological and pharmaceutical properties of the cannabinoids while providing an acceptable extraction yield [54]. Overall, cannabinoid extraction techniques can be classified as conventional, such as liquid solvent extraction or pressurized gas extraction (including butane or subcritical or supercritical fluid extraction), and innovative or alternative, such as extraction with hot gas, ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), hydrodynamic cavitation, and pulsed electric field (PEF) [37-54-137].

15. Supercritical Fluid Extraction (SFE)

Compared to conventional methods, SCF-based processes offer the possibility of environmentally friendly, straightforward, and economical operation (e.g., supercritical conditions of supercritical CO_2 are achieved at low temperatures) to obtain exceptional solvent-free extracts[37-54-137]. In addition, depending on the process parameters, tunable thermodynamic and fluid dynamic properties of supercritical CO_2 allow better solvent selectivity and therefore higher purity of extracts [37-54-137-166]. Supercritical fluid extraction (SFE) with supercritical CO_2 is an effective method for extraction of cannabinoids from cannabis samples [37-54-137]. With temperature and pressure modifications, the density of supercritical CO_2 changes, which leads to selective and fast extraction of desired analytes [37-54-137]. Higher temperatures resulted in a lower density of supercritical CO_2, while the density increases with higher pressure. Due to supercritical CO_2 being a non-polar solvent, organic solvents such as ethanol may be added to the mixture to change the characteristics of the supercritical fluid (SCF) [37-54-137]. For optimal SFE, cannabis samples

must be adequately prepared (homogenized) and decarboxylated. SFE provides advantages compared to traditional extraction methods such as low operating costs, quick extraction times and no further extract processing[37-54-137].

16. Major drawbacks of Extraction Methods

Various extraction methods have been developed throughout the years for the extraction of cannabinoids from *cannabis* samples [38, 54, 166]. Traditional methods include dynamic maceration, Soxhlet extraction, ultrasound-assisted extraction, and microwave-assisted extraction [38, 54, 166]. These traditional methods have in common the use of organic solvents, such as ethanol, chloroform, butane, propane, diethyl ether, and hexane [38, 54, 166]. The drawback of their use is a time-consuming extraction, which may not result in a high extraction yield[38, 54, 166]. Additionally, organic solvents have poor selectivity, which affects the extract quality [38, 54, 166]. Solvents used in traditional extraction methods must also be removed from the extracts, which requires additional energy and time [38, 54, 166]. These solvents are most frequently removed by evaporation with either a rotary evaporator or vacuum ovens[38, 54, 166]. Additionally, solvents of poor quality are often used at industrial level, thereby introducing impurities into extracts [38, 54, 166]. Pesticides, herbicides, and other compounds are also frequently found in the extracts obtained from traditional extraction methods since *cannabis* plants are treated with them during growth[38, 54, 166]. As a result, these traditional methods are no longer used as much as in the past and have gradually been replaced by other methods [38-54-166].

17. Selection of Solvent and Affinity

According to the literature survey by López-Olmos et al., (2022) [54], the main parameters affecting the extraction process, the most important are the solvent nature and affinity with the target compounds, temperature, pressure, mixing rate, solvent-to- vegetable matter ratio, contact time, and particle size of the plant material (pre-treatment) [54-139]. These parameters must be properly selected to improve the extraction yield while enabling the scalability of the process[38, 54-139]. Successfully scaling up an extraction process is difficult since many factors are intertwined and must be taken into account, including safety, analytical, chemical, and engineering considerations; the availability of the required substances; installation and operating costs and times; and environmental and legal restrictions such as waste disposal [38, 54-139]. Special considerations are also required for the selection of the extraction solvent and its impact on process scale-up [37-54-139]. For example, an increased solvent-to-plant material ratio will enhance the driving forces for diffusion and increase the extraction rate[37-54-139]. In contrast, lower solvent ratios reduce the time and energy required for solvent removal, recycling, or disposal volume, at the expense of the extraction yield [37-54-139]. Another factor to consider in solvent selection is solvent toxicity due to residual solvents in the final pharmaceutical/ nutraceutical product [37-54-139]. According to López-Olmos et al., (2022) [54], these solvents are not eliminated during manufacturing and their maximum concentrations in the final product are regulated by International Council for Harmonization (ICH) guidelines [54, 75]. These standards list the class to which each solvent belongs as well as the maximum concentration limit allowed for each residual solvent in the final pharmaceutical products [54, 75] **Class I solvents** cannot be used due to their high toxicity[37-54-139]. **Class II solvents** have lower toxicity, and their use must be limited to avoid possible adverse effects[54, 75]. Finally, **class III solvents** are the least toxic but should only be used as appropriate [54, 75]. For instance, ethanol, which is class III, is less toxic than hexane, which is class II [54, 75]. Thus, CO_2, a pressurized gas that leaves no residue in the final product, appears to be the least toxic type of extraction if no co-solvents are used in the process [54, 75]. Solvents can be classified as polar or non-polar[54, 75]. Ethanol is the most widely used polar solvent for cannabinoid extraction, while typical non-polar solvents include petroleum ether, propane,butane, hexane, and CO_2 [54, 75-139]. López-Olmos et al., (2022) [54] are of the opinion that due to the concentration gradient between the biomass and the solvent, cannabinoids diffuse from the glandular trichome head and dissolve in the liquid solvent during extraction[54]. Diffusion is the rate-limiting step in extraction and determines the production output[54]. Therefore, the solubility and diffusivity of the cannabinoids in the solvent are crucial parameters affecting the process efficiency and selectivity [37-54]. Solubility and diffusivity are strongly related to the nature of the extraction solvent, particle size of the plant matter, solvent-to-raw material ratio, extraction temperature, and maceration time [54-139].

The cannabinoid profile of the plant material must also be considered, since non-decarboxylated raw material contains the cannabinoids mainly in their acid forms, which are more soluble in polar solvents than neutral cannabinoids, whose solubility in polar solvents is very low [37-54-139]. Therefore, selecting a solvent with appropriate polarity is essential to selectively dissolve the desired cannabinoids and avoid the co-extraction of unwanted substances, including nitrogenous compounds, amino acids, sugars, aldehydes, alcohols, ketones, flavonoids, glycosides, vitamins, pigments, and terpenes, among others [37-54-139]. For example, non-polar solvents remove lipid-soluble material such as fats and waxes from the biomass, while polar solvents dissolve a variety of polar compounds present in the cannabis plant

such as chlorophyll, terpenes, or alkaloids, which affect the quality of the final product in terms of aroma, flavour, and consistency, unless post-processing steps are performed to remove these impurities [54-72, 73]. Moreover, low extraction selectivity decreases the relative concentrations of the desired cannabinoids in the final product [54-72, 73].

However, even if all the above-mentioned extraction parameters are optimized, currently available techniques cannot dissolve all the cannabinoids present in the raw material [54]. They can only extract a small fraction of the cannabinoids, and undesired compounds are often co-extracted due to the lack of efficiency and selectivity of these methods [54, 71]. Therefore, to increase the solubility of cannabinoids in the solvent, pre-treatment steps to improve the accessibility of the cannabinoids are commonly reported in the patent literature [37-54-71-139]. However, some pre-treatments can disrupt plant cells, which facilitate the release of undesired compounds [54-71-139]. Therefore, the advantages and disadvantages of the pre-treatment processes must be carefully considered regarding their effects on extraction [38-54-71-139].

18. Applied Extraction Methods

According to **Ubeed et al**., (2022) [128], the extraction of *cannabis* phytochemicals is a vital step to separate bioactive molecules from the plant matrix and to enhance the diverse applications of these compounds in the pharmaceutical and food industries [38-128]. Extraction can be conducted using conventional and advanced techniques[38-128]. The efficiencies of conventional and advanced methods mainly depend on the critical input parameters[38-128]. Therefore, understanding the nature of the plant matrix and the chemistry of bioactive compounds is necessary [38-128]. Conventional and advanced techniques have also been studied to maximise the extraction of phytochemicals from cannabis [38-128].

- **Soxhlet extraction:** According to the literature survey by **Lazarjani** et al., (2021) [38], the conventional methods of extraction including Soxhlet and dynamic maceration have longer extraction time and large amounts of solvent are required to complete the extraction process [38-54]. These methods are outdated. The Soxhlet extraction has become widely employed for various extraction purposes, commonly used for the separation of bioactive compounds from plant matter [38, 54]. As commonly witnessed by other conventional processes, the long-running time and the large amount of solvent required are limitations that not only increase the cost of operation but also cause environmental complications[38, 54]. For cannabis extractions using the Soxhlet apparatus, one of the study compared different types of organic solvents for the procedure and found ethanol had exhibited the highest yields of cannabinoids [38, 54]. Soxhlet extraction involves continually extracting soluble phytochemicals from the plant under refluxing conditions of the solvent, typically ethanol [38, 54]. Soxhlet extraction may present few challenges such as duration of extraction, efficiency, and post-extraction processing [38, 54].
- **Dynamic maceration**: According to the literature survey by Lazarjani et al., (2021) [38], the dynamic maceration is a conventional solid-lipid extraction procedure that is based on soaking a sample in organic solvents (solvent varies depending on the polarity of the target compound) for a specific time at a specific temperature and followed by agitation [38, 54]. Ethanol is suggested as a preferred solvent for cannabinoid extraction [38, 54]. The use of ethanol for maceration extraction of cannabinoids was found to produce the highest yield when used twice compared to other methods of extractions, for instance, ultrasonic-assisted extraction (UAE) or supercritical fluid extraction (SFE) [38, 54-129]. The dynamic maceration with ethanol for 45 min at ambient temperature was the best way of extracting non-psychoactive cannabinoids especially the acidic forms compared to more elaborate methods like ultrasonic-assisted extraction [38, 54-129]. Methods to extract chlorophyll from plants generally required acetone as the preferred solvent; however, as acetone is considered carcinogenic, it is not recommended to be used in cannabinoid extraction[38, 54]. Soxhlet and dynamic maceration are being used as traditional methods which are time- and solvent-consuming but accurate enough to be compared with modern techniques [38, 54].
- **Decarboxylation**: According to study conducted by Lewis-Bakker et al., (2019) [48], decarboxylation of acidic phytocannabinoids could occur in an open or closed reactor [48]. In an open reactor, decarboxylation of CBs has been demonstrated to occur at 37^{o} C and 60^{o} C after exposure for several hours or at 100^{o} C for 60 min; in a closed reactor, the reaction could reach completion at 200^{o} C in just 3min [48-60]. A disadvantage of using an open reactor for decarboxylation is that there is no agreeable temperature under atmospheric conditions at which efficient decarboxylation of the acidic CBs can occur without simultaneous evaporation of the solvent, along with any volatile compounds[48-70]. Every phytocannabinoid carboxylate would have a different optimal condition for decarboxylation, thus various medical cannabis cultivars with various chemical compositions would require different conditions to achieve complete decarboxylation of all phytocannabinoids [48]. According to the literature survey by López-Olmos et al., (2022) [54], decarboxylation can be avoided when the compounds of interest are cannabinoids in their acid forms[54]. Decarboxylation is a function of time

and temperature, which depend on the desired cannabinoid [54]. In addition, decarboxylation conditions must be properly selected to minimize the thermal degradation of cannabinoids, particularly the degradation of Δ9-THC to cannabinol (CBN) [54]. Plant material can be decarboxylated before extraction when non-polar solvents such as hexane or supercritical CO2 are used since neutral cannabinoids showed higher solubility in non-polar solvents due to their lower polarity compared to the acidic forms[54]. However, extraction yield is affected by the polarities of the solvent and the cannabinoids. Hence, the extraction of a non-decarboxylated plant material (cannabinoids in their acidic forms) produces lower yields compared to the extraction of a decarboxylated plant material when using a non-polar solvent due to the lower polarity of neutral than acidic cannabinoids [54]. Overall, cannabis plant material is subjected to pre-treatment depending on the nature of the extraction process and the desired final cannabinoid profile[54]. The pre-treated (or not) biomass is then extracted to obtain the cannabinoids and remaining terpenes[54-70].

- The ultrasonic-assisted, microwave-assisted, supercritical fluid, and pressurized liquid extraction processes can be considered as an alternative, slightly greener, options as opposed to the conventional methods [37-54-129]. Pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE) [37-54-129]. One of the study conducted by **Correia et al.,** (2023) [115] reported an ultrasound-assisted solid-liquid extraction followed by high-performance liquid chromatog raphy with diode array detection (HPLC-DAD) methodology to identify and quantify Λ9 -THC, Λ 8 -THC, cannabidiol, cannabinol, Λ9-tetrahydrocannabinolic acid and cannabidiolic acid in cannabis products [115]. The herbal samples were extracted with ethanol:acetonitrile (50:50, v:v) by ultrasonication using only 50 mg of sample [115]. The plant oils were diluted in ethanol [115]. The optimized procedure allowed ≈100% extraction efficiency of the target cannabinoids[115]. The validation assays showed that the method is linear (R 2 > 0.997), selective, sensitive, precise and accurate, with suitable limits of detection (0.125–0.250 μg mL−1) and quantification (0.500 μg m−1) [115]. The method was successfully applied to cannabis samples, demonstrating its suitability for routine analyses [115]. This contribution follows the current demand for fast and straight forward analysis services of this plant and its derivatives, using small amounts of sample[115]. The study conducted by Correia et al., (2023) [115] compares very favorably against other works, particularly in regards to the extraction efficiency, speed of the overall procedure, method sensitivity, and ability to monitor Λ 8 -THC spiked samples using a novel solvent mixture [115].
- The ultrasonic-assisted, microwave-assisted, supercritical fluid, and pressurized liquid extraction procedures reduce the need for synthetic and organic solvents, cut down on operational time, and produce a better quality extract with a higher yield[48-55]. According to study conducted by Lewis-Bakker et al., (2019) [48], Microwave-assisted extraction (MAE) employs microwaves to assist in the extraction of compounds from cannabis at elevated temperatures and pressures [48-139]. The instantaneous energy transfer from solvent to biomass leads to a rapid increase in the temperature, and one can reach temperatures higher than the boiling point of the solvent if the pressure is contained[48]. MAE offers additional advantages such as shorter extraction and reaction times, smaller quantities of solvent, and reproducibility [48-54]. While all these methods help in extracting phytocannabinoids and other compounds from cannabis, activation of the extracts through the decarboxylation of phytocannabinoids remains a unique challenge [48-54-134].
- According to literature survey conducted by Lewis-Bakker et al., (2019) [48], there are conventional and domestic methods described for cannabis extraction such as ethanol extraction, maceration, butane extraction, and quick-wash alcohol extraction[48-54-139]. Recently reported extraction methods include ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE) [48-54]. Each method carries advantages and disadvantages depending on the compounds to be extracted, duration of extraction, temperature, and solvent, if any [48-124-139]. It is typically desirable to use a solvent that solubilises and carries compounds from the plant, and the temperature for extraction should minimize the loss of thermally labile groups or unwanted chemical transformations[48-139].
- **Solventless methods:** Solventless methods such as dry sieve and water extraction are particularly known to extract entire trichomes [38-48-54]. Hydrocarbon extraction methods can be used to avoid unwanted water and pigments such as chlorophyll [38-48-54]. Solventless extraction and hydrodynamic extraction are of interest due to their high yield, easy, and fast process but lack the scientific publication to promote their employment for large-scale production[38-48-54-160].
- Depending on the application, cannabinoids can be extracted in either acidic or neutral form[38-48-54]. The preservation of acidic cannabinoids requires extraction to be completed at room temperature [38-48-54].
- To decarboxylate acidic cannabinoids into neutral form, high temperatures are recommended for extraction, although a higher temperature may result in the loss of some terpenes and minor constituents [38-48-54-130].
- Solvent extraction method (solid-liquid extraction, liquid-liquid extraction), hydrodynamic extraction method, cryogenic extraction method were applied for the extraction of cannabinoids [38-48-54-90].
- **Solventless extraction:** According to the literature survey by **Lazarjani et al.,** (2021) [38], solventless extraction exploits the fact that cannabinoids are semi-liquid and can be extracted by suitable heating and

pressure[38-54]. The process of dry-sieving begins by beating dried cannabis against a mesh screen and forcing the trichomes to separate and fall off [38-54]. According to the literature survey by Lazarjani et al., (2021) [38], the final product can either be pressed further into hashish or mixed with dried flowers [38]. This simple procedure is time-consuming and labour-intensive, therefore, not popular for the industrial level[38]. Water extraction produces roughly the same potency of THC as the dry sieve method, although it also depends on the potency of the starting material [38]. According to the literature survey by Lazarjani et al., (2021) [38], the procedure begins by placing the cannabis plant in a mesh bag immersing it in ice water and finally stirring it to knock the trichome off [38]. Similarly, to dry sieving, this process is difficult to upscale as well as limited control of potency [38].

- **Solvent‑Based Extraction:** Ethanol is one of the most widely used solvents for the extraction of cannabinoids [38, 54]. Due to its polar nature and high solvent power, ethanol dissolves both cannabinoids and terpenoids as well as non-desired water-soluble molecules like chlorophyll, glycosides, sugars, and alkaloid salts that must be removed in a large number of post-processing steps to meet high purity specifications [38, 54-80]. **Ethanol** is a generally recognized as a safe (GRAS) solvent, safer than other solvents like butane, and its use does not require high pressures [38, 54-80]. Cold extraction; that is, extraction with ethanol at low temperatures (5°C to -80°C) is an advantageous modification that allows the extraction of cannabinoids and terpenoids while also minimizing the co-extraction of chlorophyll and other compounds such as triglycerides and waxes present in the plant [54, 63,161]. However, extraction with cold ethanol increases the energy consumption to cool the extraction device[54, 63,161]. In general, ethanol extraction of bio-compounds is convenient in cases where minor solvent residues are allowed in the final product or at low temperatures in applications in which the terpenoid profile of the plant must be preserved [54, 77, 78]. Hexane is a non-polar solvent commonly used to extract neutral and acidic cannabinoids, such as CBD, THC, CBG, CBGV, CBC, CBDA, Δ9-THCA, or CBGA, from plant material[54, 77, 78]. Despite its good solvent power, hexane must be completely eliminated from the final product since in pharmaceutical applications its use is controlled due to its inherent toxicity [54-80]. This is a considerable disadvantage compared to ethanol, which is considered to be of lower risk. According to the literature survey by López-Olmos et al., (2022) [54], petroleum ether is a petroleum fraction consisting of mainly aliphatic C5 to C11 hydrocarbons with a boiling point of 30–80°C and is one of the most volatile liquid hydrocarbon solvents[54]. While its relatively low cost compared to other organic solvents makes petroleum ether an attractive non-polar extraction solvent, its main disadvantages are its flammability and the lack of consistency among suppliers and even between lots, which negatively affects process controllability[54]. Thus, extraction with petroleum ether usually comprises high levels of impurities [54]. However, as previously mentioned, petroleum ether and other potentially toxic solvents can be used as extraction solvents if the final product complies with residual solvent restrictions[54]. According to the literature survey by López-Olmos et al., (2022) [54], hexane has good solvent power and, unlike ethanol, does not co-extract chlorophylls due to its non-polar nature, even though it does co-extract waxes and lipid material[54]. However, the use of hexane is limited in pharmaceutical applications due to its toxicity [54]. Therefore, post-extraction steps are compulsory for the complete removal of hexane from the final product. Despite its flammability and risk of fire and explosion, the hazard risks of hexane are less severe than those for other flammable solvents such as butane [54]. According to the literature survy by López-Olmos et al., (2022) [54], among the reviewed extraction techniques, maceration of the cannabis plant material in a polar or non-polar liquid organic solvent is the most widely used due to its simplicity, with ethanol the preferred non-polar solvent, and petroleum ether and hexane the typical non-polar solvents[54]. One disadvantage of liquid organic solvents is that they must be removed from the final product, usually by rotary evaporation, since very low solvent residues are allowed, especially for pharmaceutical purposes[54]. After analyzing various examples in patents that use the solvents mentioned above, it is clear that solvent polarity is an important factor to selectively dissolve the cannabinoids while avoiding the co-extraction of unwanted compounds that must be removed in downstream purification steps[54].

According to the literature survey by López-Olmos et al., (2022) [54], the major drawback associated with pressurized gases compared to liquid solvent extraction is the use of sophisticated equipment able to operate in severe extraction conditions, which implies high operation and maintenance costs[54]. Moreover, gaseous hydrocarbons such as n-butane and n-propane are toxic and flammable, requiring the use of safety equipment[54]. Nevertheless, one advantage over liquid solvent extraction is that the obtained extract contains practically no solvent residues since the pressurized gases can be removed and recovered at the end of the extraction by increasing the temperature due to their lower boiling point[54].

According to the literature survey by López-Olmos et al., (2022) [54], butane is the most common solvent for extracting neutral cannabinoids and terpenes and producing cannabinoid concentrates since the butane extracts reach cannabinoid concentrations of up to 90%[54]. While the obtained extracts are chlorophyll-free, a winterization step is required to remove co-extracted waxes[54]. Among non-polar solvents, winterization is commonly reported in the

patent literature as a post-extraction step in the case of butane or CO_2, but not in the case of hexane, which seems to indicate that at room temperature and atmospheric pressure, hexane dissolves less lipid material compared to pressurized gasses[54].

According to the literature survey by López-Olmos et al., (2022) [54], as it is non-toxic and easily removed from the extracts, supercritical CO_2 extraction is preferred for medicinal and pharmaceutical applications [54]. Supercritical CO_2 extraction is usually carried out in the presence of a polar co-solvent such as ethanol to increase the solubility of polar cannabinoids[54]. The decarboxylation of large amounts of cannabis material before extraction to transform the polar cannabinoids into their neutral forms, more soluble in CO_2 has also been reported [54]. The downsides of this technique include the high operating temperatures and pressures, the expensive equipment, and, as mentioned above, the requirement of a winterization step to remove wax and lipids from the extracts[54].

According to the literature survey by Lazarjani et al., (2021) [38] the solvent-based extraction methods such as Soxhlet, maceration both static and dynamic, ultrasonic-assisted extraction, and microwave-assisted extraction require a solvent to complete the extraction process [38]. According to the literature survey by Lazarjani et al., (2021) [38], a variety of solvents can be used to extract cannabinoids including ethanol, butane, propane, hexane, petroleum ether, methyl tertbutyl ether, diethyl ether, carbon dioxide (CO_2), and olive oil [38]. Gaseous solvents such as butane and propane can also be used for extraction purposes [38]. The extracted sample is collected, and the solvent is evaporated [38]. The process of pressurizing these flammable and potentially explosive gases poses safety hazards [38]. In addition, the gases used in cannabis extractions are often industrial grade and contain impurities that end up in the cannabis extracts. Moreover, the solvents themselves may become a residue in the final extract [38]. According to the literature survey by Lazarjani et al., (2021) [38], commonly used solvents to extract cannabis can be divided into three groups, low molecular mass organic solvents, vegetable fats (oils), and supercritical fluids, notably supercritical carbon dioxide[38]. The low molecular mass organic solvents are ethanol, butane, hexane, methanol and acetone[38]. Low molecular mass organic solvents are hydrocarbon based with limited polarity due to the presence of oxygen[38].

According to the literature survey by López-Olmos et al., (2022) [54], liquid solvent extraction consists of the maceration of plant material containing cannabinoids in polar or non-polar organic solvents [54, 74]. The plant material is immersed and stirred for several hours or days in the solvent, generally at room temperature or colder. Several (2–3) extraction stages are typically performed to completely extract the target cannabinoids, increasing the use of solvent and energy[54, 74]. After extraction, the solvent is commonly removed by evaporation at temperatures preferably < 60°C to produce a concentrated extract, since the presence of solvent residues compromises the quality of the product for its further application [54, 72, 73]. Very low solvent residues are allowed, especially for pharmaceutical purposes[54]. For example, the pharmaceutical limits for residual solvents set by the ICH Guideline for drug products are 290 ppm for hexane, considered a solvent to be limited, and <5,000 ppm for ethanol, considered as lower risk to human health (International Council for Harmonisation, 2020) [54, 75]. According to the literature survey by López-Olmos et al., (2022) [54], despite the simplicity of the liquid solvent extraction technique, the disadvantages of this method should be considered, including the relatively high solvent consumption, which must be evaporated or separated from the biomass, the presence of solvent residues in the final extract, environmental concerns, and the co-extraction of unwanted products and their subsequent removal in downstream purification steps[54].

Traditionally, the extraction of phytocannabinoids is performed using organic solvents, including hydrocarbons (e.g., hexane) and alcohols (e.g., ethanol, methanol) [54-90]. This method of extraction is cheap, easy to operate, and does not require sophisticated equipment[54-139]. However, the solvents used are flammable, toxic, and non-biodegradable, risking human health, besides having a huge environmental impact [54, 74-139]. Extraction using these solvents can be efficient but depending on the final product, can impact regulation, and require additional testing[54, 74-139]. For instance, residual solvent is strictly regulated and must be defined for medicines under good manufacturing practice[54, 74-139]. These solvents due to their toxicity, environmental risk, and flammability are less desirable for large scale extractions[38, 54, 74-139]. One of the study conducted by Kaczorová et al., (2023) [76] reported that, the tested bases were comparably effective in extracting cannabinoids from plant material[76]. The study conducted by Kaczorová et al., (2023) [76] confirmed that, olive oil and Synderman bases exhibited the highest cannabinoid extraction efficiencies (approximately 70%) and the best storage stabilities in terms of the content of monitored compounds[76]. The proposed preparation protocol is fast and easily implementable in pharmacies and medical facilities [76]. On the other hand, the cream bases were the least stable and problematic for extract preparation via the proposed protocol [76]. Regarding cannabinoid stability, Δ9-tetrahydrocannabinol was less stable compared to cannabidiol and decomposed rapidly in certain bases, especially when stored at laboratory temperature[76].

19. Vegetable oil is used as Solvent

According to the literature survey by **Lazarjani** et al., (2021) [38], the vegetable oils are routinely extracted from seeds or fruits such as rapeseed, sunflower, or olive, and even brans, making them an inexpensive option [38]. These oils are considered lipophilic due to their non-polar characteristic, which enables selective dissolving properties[38]. Olive oil is a well-known solvent in the cannabis extraction [38]. According to the literature survey by López-Olmos et al., (2022) [54], another process using a vegetable oil or lipid compositions, comprising canola oil, peanut oil, sunflower oil, avocado oil, grape seed oil, walnut oil, or fish oil, an ionic liquid such as tributyl methyl ammonium methyl sulfate, or an imidazolium salt such as 1-butyl-3-methylimidazolium chloride, as an extraction agent or carrier is described in invention US20180016203A1 (Raber and Elzinga, 2017) [54, 61]. After extraction by mixing, stirring, and heating to preferably 50°C for 5–90 min, the enriched lipid solvent is separated from the plant matter residue, and subjected to distillation to volatilize the cannabinoids that can be further decarboxylated or concentrated at reduced pressure [54, 61]. The obtained final product can be a cannabinoid-rich fraction containing 65–75% THC, which can be subjected to another distillation at 165°C, obtaining a resin containing THC with a purity larger than 80% [54, 61].

According to the literature survey by **López-Olmos et al.,** (2022) [54], another trend is the use of vegetable oils such as coconut, canola, sunflower, walnut, palm, or hemp oil for cannabinoid extraction, avoiding the use of toxic and hazardous hydrocarbon solvents in addition to the high pressures involved in the supercritical extraction with CO_2, a technique that is further explained later[54]. Thus, extraction with vegetable oils or lipids is considered to be solvent-free, safer, and more environmentally friendly compared to traditional extraction methods using butane or alcohols since it can be performed using certified organic solvents and a post-processing step for solvent removal is not needed as the cannabinoid product is diluted in oil [54, 61, 77, 78, 100]. Obtaining an oily cannabinoid product can be convenient since most commercialized CBD products are diluted in an oily medium like hemp seed oil [54, 56].

Many examples of the use of vegetable oils have been reported in the patent literature [54, 78]. For example, invention WO2019207554A1 (Splinter et al., 2019a) [78], which describes a method for extracting cannabinoids from cannabis in a continuous flow microwave-assisted extractor at 25–75°C, using a carrier fluid suitable for inclusion in a final formulation, such as a polyunsaturated fatty acid (PUFA), safflower oil, canola oil, cottonseed oil, soybean oil, olive oil, mono, di and triglycerides, lecithin, limonene, essential oils, or fish oil, among others [54, 78]. The invention provides a table comparing different solvents for the extraction of THCA from cannabis biomass[54, 78]. With a solvent-to-solid ratio of 8 L/kg at 30°C, a solvent comprising pentane and a medium chain triglyceride (MCT oil) produces an extract with 3.5% THCA (96% recovery from plant material), whereas pentane alone yields an extract with 92% THCA (89% recovery from plant material), indicating that the use of MCT oil results in the co-extraction of other compounds and, thus, a less pure extract, despite the higher THCA recovery [54, 78]. According to the literature survey by **López-Olmos** et al., (2022) [54], cannabinoid extraction using other liquid solvents, among which vegetable oils are the most popular method for producing cannabinoid formulations for pharmaceutical and nutritional products as the final product generally comprises the cannabinoid product diluted in oil (no need to remove the solvent in a post-processing step) [54]. However, the obtained cannabinoid concentration in oil is lower than those for other non-polar solvents (e.g., up to 80% of cannabinoids in hexane extracts) [54, 78].

20. Deep Eutectic Solvents (DESs)

According to the study conducted by **Tiago et al.,** (2022) [113], deep eutectic solvents (DESs) (DES 1= Betaine L(+)-Lactic Acid; Molar ratio Bet: Lac 1:2) (DES 2= Glucose L(+)-Lactic Acid Lac:Gluc; Molar ratio 1:5); (DES= 3 L-Proline L(+)-Lactic Acid ; Molar ration Pro:Lac 1:1); (DES= 4 Menthol L(+)-Lactic Acid; Molar ratio Men:Lac 2:1) (DES= 5 Menthol Lauric Acid; Molar ratio Men:Lau 2:1); (DES= 6 Menthol Myristic Acid; Molar ratio Men:MyA 4:1); (DES= 7 Menthol Stearic Acid; Molar ratio Men:StA 8:1) are a new class of green solvents and have received great attention as extraction media [113-124]. On the basis of the study conducted by Tiago et al., (2022) [113], DESs, introduced in the beginning of the 21st century, are prepared by simply mixing at least one hydrogen bond acceptor (HBA) with one hydrogen bond donor (HBD) at an appropriate molar ratio to form a eutectic mixture[113]. The strong bonding between HBA and HBD is the most important parameter in the formation of these systems [113]. This interaction results in a depression of the melting point of the system relative to its initial components [113]. This simple process of manufacture makes industrial scale production possible without the need for complex facilities and specialized handwork; this method does not need solvents and produces no waste or by-products [113-124]. DESs are, hence, attractive candidates for solvents due to their inherent properties, for example, short preparation time, easy storage, low cost, non-flammability, and high capacity of solvation [113-124]. Besides, DESs have other advantages, including high-thermal and electrochemical stabilities[113-124].

The study conducted by Tiago et al., (2022) [113] reported that the majority of DESs proposed so far are based on renewable resources, such as carboxylic and amino acids, sugars, amines, representing a new generation of green solvents [113-124]. These DESs based on natural compounds are known as natural deep eutectic solvents (NADES), resulting in low toxicity and biodegradable solutions [113-124].Depending on the formulation, some NADES can dissolve natural or synthetic chemicals with different polarities[113-123]. Additionally, because they are composed of natural metabolites, it makes them theoretically fully biocompatible, being a greener alternative candidate for concepts and applications involving some organic solvents and ionic liquids [113-124].

20.1. Deep Eutectic Solvents (DES): Sample Preparation

According to the study conducted by Tiago et al., (2022) [113], before extraction, hemp leaves and inflorescences were grinded using a commercial blender [113]. This method allowed a reduction of the sample size and a higher contact between the DES and the plant material, resulting in an increase of the extraction efficiency, consequently, increasing the final yield [113]. The inflorescences, leaves, and seeds were grinded in a lab mill at 6,000 rpm for 45 s, with a particle size range between 1 mm and 180 mm[113]. The samples were kept in an amber flask in a dark place to protect them from light, under room temperature[113].

20.2. Deep Eutectic Solvents Preparation

The study conducted by **Tiago et al**., (2022) [113] reported that deep eutectic solvents were produced by the heating-stirring method [113]. This method was selected since it is simple and allows the preparation of multiple DESs simultaneously, and it can be easily scaled up[113]. DESs were obtained by mixing the HBAs and HBDs at the desired molar ratio as shown as (DESs) (DES 1= Betaine L(+)-Lactic Acid; Molar ratio Bet: Lac 1:2) (DES 2= Glucose L(+)-Lactic Acid Lac:Gluc; Molar ratio 1:5); (DES= 3 L-Proline L(+)-Lactic Acid ; Molar ration Pro:Lac 1:1); (DES= 4 Menthol L(+)-Lactic Acid; Molar ratio Men:Lac 2:1) (DES= 5 Menthol Lauric Acid; Molar ratio Men:Lau 2:1); (DES= 6 Menthol Myristic Acid; Molar ratio Men:MyA 4:1); (DES= 7 Menthol Stearic Acid; Molar ratio Men:StA 8:1) [113]. The mixtures were stirred using a magnetic stirring apparatus at 40^{o} C until a homogenous solution was obtained. All DESs were then stored at room temperature [113].

20.3. Deep Eutectic Solvents (DES) Extraction

According to the study conducted by Tiago et al., (2022) [113], bioactive compounds from hemp samples were extracted by mixing the DES with the plant matrix in a solid-liquid ratio of 1:10 (W/W) [113]. After being briefly mixed, cannabinoids were extracted for 90 min in an ultrasonic bath (water temperature at 60 ^{o} C; ultrasonic power, 100 W) in cycles of 15 min; a sample was collected for future kinetic analyses[113]. Then centrifuged at 6,000 rpm for 15 min to separate the liquid from the solid phase[113]. On the basis of study conducted by Tiago et al., (2022) [113], each experiment was repeated three times for each DES, and the respective cannabinoids were quantified by HPLC[113].

20.4. Deep Eutectic Solvents (DES): HPLC and GC

Reversed phase HPLC (RP-HPLC) is the most widespread mode of chromatography[113]. It has a non-polar stationary phase and an aqueous, moderately polar mobile phase. In the reversed phase methods, the substances are retained in the system the more hydrophobic they are[113]. The ultraviolet (UV) or visible (VIS) is the most common in HPLC because it provides good stability, is simple to operate, and has a high sensitivity [113]. Common HPLC solvents include water, acetonitrile, methanol, acetone, ethanol and various other organic solvents [113]. The most common cannabinoid quantification techniques include gas chromatography (GC) and high performance liquid chromatography (HPLC) [113]. GC is often used in conjunction with mass spectrometry (MS) or flame ionization detection (FID) [113]. The major advantage of GC is terpenes quantification [113]. The main advantage of HPLC is the ability to quantify both acidic and neutral forms of cannabinoids without derivatisation which is often with MS or ultraviolet (UV) detectors [113].

20.5. Deep Eutectic Solvents (DES): Quantification by HPLC

According to the study conducted by Tiago et al., (2022) [113], the samples were prepared by diluting the obtained extracts in methanol (a 1:10 w/w ratio) and then stirred to until a homogenous solution was obtained[113]. The solution was then filtered using a hydrophilic PTFE syringe filter with a 0.20-mm pore size before analysis[113]. HPLC analysis of the hemp extracts was carried out using an Agilent Infinity 1100 HPLC System, and an Agilent 1100 series photodiode-array detector (DAD) for detection and recording at UV/Vis 220 nm[113]. On the basis of study conducted by Tiago et al., (2022) [113], the first extraction screening in hemp showed that menthol-based hydrophobic DESs showed to be more efficient in the extraction of cannabinoids[113]. Lac:Gluc had the highest TPC values (7.76 ± 1.1 mg/g) and TFC among all DESs [113]. When comparing the extraction results with ethanol, Men: Lau was the one who presented the highest yield of CBD and CBDA (11.07 ± 0.4 mg/g) [113].

According to the study conducted by Tiago et al., (2022) [113], bioactivity assays showed that Lac:Gluc and Pro:Lac also improved the solubility of CBD and CBDA in aqueous media[113]. Therefore, the results of this study proved that DESs are selective green solvents, with huge potential for use in industrial applications, involving the extraction of bioactive compounds, and can further enhance the bioavailability of the active components[113-124].

21. Supercritical CO_2 extraction

According to the literature survey by Lazarjani et al., (2021) [38], the supercritical CO_2 extraction is still considered as a novel method in cannabis industry[38]. In brief, the method involves using specialist pressure and temperature control equipment to turn gaseous CO_2 into a supercritical fluid [38-54]. When passed over cannabis material, the fluid can easily extract plant waxes and oils from the cannabis[38-54]. Supercritical CO_2 behaves like a non-polar solvent, capable of extracting a broad range of non-polar solutes, cannabinoids included [38-54]. Therefore, CO_2 is the solvent of choice due to low critical temperature and pressure[38-130]. It is also non-flammable, non-toxic, inert, renewable, easy to remove, abundant, and relatively low-cost [38-54].

According to the literature survey by Lazarjani et al., (2021) [38], with CO_2, that means processing under relatively low pressures and temperatures over a long period of time and minimizing the amount of post-processing that is done after extraction[38-54]. The most common criticism levelled at CO_2 extraction is that the high upfront costs that come with the scientific equipment needed can be prohibitive for start-ups or small businesses [38-54]. Unlike alcohol or butane, CO_2 is a highly tuneable solvent, meaning one can pull unique compounds from botanicals at different pressures and temperatures [38-54]. This opens the door for a ton of creativity in the extraction process, which was attractive to us. CO_2 is also safer than many hydrocarbon techniques which use flammable and toxic solvents like butane[38-54].

22. Fast Centrifugal Partition Chromatography (FCPC)

One of the study conducted by Maly et al., (2023) [129], demonstrated the applicability of fast centrifugal partition chromatography (FCPC) as a challenging format of counter-current preparative chromatography for the isolation of CBD and CBDA free of psychotropic compounds that may occur in *Cannabis sativa* L. plant extracts[129-139].

Currently, two different technical solutions are the most widely used, hydrodynamic high-speed counter current chromatography (HSCCC) and hydrostatic fast centrifugal partition chromatography (FCPC) [129-148]. On the basis of study conducted by Maly et al., (2023) [129],hemp was extracted by supercritical fluid extraction with ethanol as a modifier. Fifty grams of the extract was dissolved in 250 mL of ethanol (purity 96%), winterized at − 20 °C for 24 h and then filtered[129]. In this way, the waxes and other frozen solids were separated from the liquid phase rich in phytocannabinoids; ethanol was then evaporated using a rotary evaporator. For the analysis of phytocannabinoids in experimental samples, Maly et al., (2023) [129] used an ultra-performance liquid chromatograph (U-HPLC) UltiMate 3000 coupled with a tandem high-resolution mass spectrometer (HRMS/MS) [129].

The study conducted by Maly et al., (2023) [129] reported the main advantage of CPC is its generally easy scaling-up to an industrial level, once the method has been developed [129]. The large-scale separation significantly reduces the costs, mainly because of lower solvent consumption per 1 g of pure compound and the absence of expensive solid stationary phase[129]. As regards phytocannabinoids isolation, developed CPC method is a great alternative to other purification techniques used in industry, such as molecular distillation[129].

23. LabTech: Cannabis Market Extraction Methods

According to **LabTech** report, the cannabis market uses three main extraction techniques [162, 163]. In all processes, the plant material is subjected to a solvent to remove active compounds from the plant matter and filtered to yield a solution of the solvent with plant extracts[162, 163]. All processes rely on standard techniques that have been used for years in the botanical, chemical and petrochemical, and distilled spirits industries[162, 163].

23.1. LabTech: Super Critical Carbon Dioxide

According to LabTech report, use carbon dioxide (CO_2) pressurize the CO_2 to its subcritical or supercritical state[162, 163]. The CO_2 stream passes through a chamber containing cannabis material[162, 163]. The distillate can be isolated easily by reducing the pressure which evaporates the CO_2, leaving a cannabis extract with no solvent. Working on temperature and pressure affords CO_2 systems the ability to yield extracts with a complete terpene profile[162, 163].

23.2. Lab Tech: Liquefied Hydrocarbons

According to LabTech report, pressurize butane, propane or other low molecular weight hydrocarbons to a liquid state[162, 163]. The liquid hydrocarbon passes through a bed of cannabis material and filter, yielding an extract solution of hydrocarbon and plant extract. Like the CO_2 method, a reduction in pressure evaporates the hydrocarbon liquid, yielding a solvent-free plant extract[162, 163]. This method requires great attention to safety due to the flammability of the hydrocarbon used[162, 163].

23.3. Lab Tech: Ethanol Extraction

According to LabTech report, use of food grade or USP grade ethanol as a solvent to extract plant material[162, 163]. This method varies from vessels to reactors, filter reactors, spinning vessels to barrels. A popular process has the ethanol chilled to <-20 °C (- 4°F) either in a cold room or freezer and then pumped into a container of cannabis[162, 163]. After a soak period, the ethanol solution is either filtered or the plant material removed in a 'tea bag' fashion[162, 163]. The resultant mother liquor of ethanol and extract is then concentrated by removing the ethanol. Typical distillation apparatus used to remove the ethanol include rotary evaporators, falling film evaporators or a batch vacuum distillation system[162, 163].

23.4. Lab Tech: Extraction Process Residues and Winterization

According to LabTech report, all extraction methodologies described above yield an oil once the solvent has been removed[162, 163]. This oil contains plant lipids, possibly chlorophyll, waxes, fats, terpenes, THC and other cannabinoids[162, 163]. Additional processing to remove the plant lipids and waxes is necessary to produce a more desirable extract product[162, 163]. **Winterization** is the term used to describe the process of removing the plant lipids, fats and waxes[162, 163]. Dissolution of the extraction oil in ethanol and chilling to temperatures <-20°C causes the lipids, fats and waxes to precipitate[162, 163]. This cooling process is conducted in cold rooms, freezers or with jacketed vessels or jacketed filter reactors[162, 163]. The ethanol extraction is slowly making its way to an industry standard a to high-quality, low-risk, and relatively inexpensive results it produces [162, 163]. There is no huge investments needed for the Super Critical Carbon Dioxide and Liquefied Hydrocarbons methods[162, 163].

23.5. Lab Tech: Rotary Evaporator, Water Chiller, Vacuum Pump

According to LabTech report, the rotary evaporator enables the removal of solvent in a controlled manner under vacuum [161, 162]. Sizes range from bench top (to 3 L flasks) to pilot scale (20 L and up) [161, 162]. Reducing the pressure in the rotary evaporator by a vacuum pump lowers the boiling point of the solvent to be removed [161, 162]. In this case, ethanol has to be removed [161, 162]. Typically, the distilling flask is filled to 50% volume. The water bath is heated to 30-40°C[161, 162]. The condenser temperature, controlled by a recirculating chiller, is set to -5°C to 0°C [161, 162]. Once the water bath and condenser have reached the set points, the distillation flask is rotated from 150-200 rpm[161, 162]. This creates a thin film on the upper surface of the round glass flask, which increases the solution surface area and enhances the solvent evaporation rate [161, 162]. Applying an appropriate vacuum to the system lowers the boiling point [161, 162]. To achieve a recommended target, set the vacuum to achieve an ethanol vapor temperature of 15-20°C[161, 162]. As the ethanol evaporates, it will condense and collect into the distillate flask. The evaporation rate can exceed the condensation capacity of the recirculating chiller [161, 162]. Cannabis extracts required lower water bath temperatures to minimize thermal decomposition[161, 162]. Temperature control plays a vital role in the cannabis extraction workflow therefore it is paramount to use good and reliable instruments[161, 162].

24. Ethanol Extraction

According to the literature survey by **López-Olmos** et al., (2022) [54] published methods for cannabinoid extraction using ethanol at room temperature include purification steps such as filtration and decolourization to remove unwanted compounds [54, 138]. For instance, the US patent publication No 10189762 B1 (Oroskar et al., 2019) [138], described a method to produce high-purity CBD products (>95% purity) on a commercial scale from dried hemp and cannabis leaves [54, 138]. This method reports the extraction of the cannabinoids by soaking 150 Kg of plant material in ethanol (600 L) at room temperature and atmospheric pressure for 2 h with agitation[138]. The mixture stands overnight for 8–12 h to form the first ethanol layer [54, 138]. Further, the ethanol containing the cannabinoids and other impurities is removed by decantation and the raw material is mixed again with ethanol (400 L) for 2 h with agitation, followed by decantation, and the remaining wet material is pressed, resulting in spent plant material and a third liquid decanted extract, which is combined with the first and second decanted ethanolic extracts [54, 138]. The total solvent-to-plant material ratio used for the extraction is 6.66 L/kg[54, 138]. The combined decanted extract contains cannabinoids including CBD, THC, CBDA, and THCA, as well as small particles, chlorophylls, color bodies, sugars and carbohydrates, lipids, plant waxes, and other impurities [54, 138]. After the first filtration step, the filtered crude extract comprises 3.4–

3.7 wt % total cannabinoids in the liquid mixture[54, 138]. The crude cannabinoid extract is subjected to a sequence of purification steps including decolourization, decarboxylation, dewaxing, a continuous simulated moving bed process, polishing, and crystallization in hexane at −20°C or less for 24–72 h, followed by recrystallization in hexane at room temperature for 24–72 h, to obtain high purity THC-free CBD crystals (above 99 wt%) [54, 138].

Using **cold ethanol** (from −60°C to −40°C) for the large-scale extraction of cannabinoids such as CBD or THC is also described in patent US10493377B1 (Ferraro et al., 2019) [139] which reports a system for continuous biomass extraction and centrifugation[54, 139]. According to the authors, the system provides constant and repeatable extraction results and includes biomass and solvent feed tanks, several extraction vessels, and a centrifuge coupled inline [54, 139]. The process is arranged to operate in a continuous or quasi continuous manner as multiple extraction and centrifugation steps take place simultaneously[54, 139]. As described, a first slurry is formed in the first vessel, when the first portion of biomass is combined with ethanol in a typical ratio of 1:5 (80 kg of biomass and 400 kg of ethanol) for 12 min, during which the second portion of biomass and solvent is sent to the second vessel to form a second slurry[54, 139]. The first slurry is centrifuged for 4 min to separate the first extract and waste discharges, followed by centrifugation of the second slurry, and so on [54, 139]. After centrifugation of each batch, the extract discharges may be subjected to further filtering or purification to eliminate the solvent and obtain the cannabinoid in a substantially pure form [54, 139].

25. Hydrodistillation and Ethanol based Extractions

According to Chacon et al., (2024) [159], variety of techniques have been developed to extract hemp phytochemicals for research and consumption [159]. Some of the most common processes in the cannabis industry include supercritical CO_2 extraction, hydrodistillation, and solvent-based (ethanol) extractions[159]. Each of these processes has the potential to differentially extract various phytochemicals, which would impact their efficacy, tolerability, and safety [159]. Extracts were then evaluated for their terpene and cannabinoid content using GC-MS and LC-MS/MS [159]. Hydrodistilled extracts contained the most variety and abundance of terpenes with β-caryophyllene to be the most concentrated terpene (25–42 mg/g) [159]. Supercritical CO_2 extracts displayed a minimal variety of terpenes, but the most variety and abundance of cannabinoids with CBD ranging from 12.8–20.6 mg/g[159]. Ethanol extracts contained the most acidic cannabinoids with 3.2–4.1 mg/g of CBDA along with minor terpene levels [159]. The resulting extracts demonstrated substantially different chemical profiles and highlight how the process used to extract hemp can play a large role in product composition and potential biological effects[159].

Hydro-distillation is a commonly used method for the extraction of bioactive compounds from plants[160]. The key difference between steam distillation and hydrodistillation is that steam distillation uses steam, whereas hydrodistillation uses water, steam or the combination of both for the extraction[160]. Since no organic solvents are involved in this process, this can be considered as an environmentally friendly extraction method[160]. On the other hand, because of the relatively high temperature needed in this method, volatile and thermo-labile compounds may be lost during the process[160].

According to Chacon et al., (2024) [159], the inflorescence of each sampling that was used for extraction comparison were received 1–3 months prior to extraction and stored in vacuumed sealed bags at room temperature with limited light exposure[159]. Once extracted, distilled samples were stored at 4°C, ethanol extracts at room temperature, and super-critical CO_2 extracts were stored at room temperature, all extracts were stored in amber glass vials [159]. According to Chacon et al., (2024) [159], for solvent extractions, 1.0 ± 0.05 g of inflorescence material was ground to a fine powder using an electric spice grinder and transferred to a 50 mL conical tube, after which 20 mL of 96% aqueous ethanol was introduced to each tube[159]. Samples were vortexed for 10 second and then sonicated at room temperature (RT) for 30 min[159]. Samples were centrifuged at 4° C for 5 min at 3000 rpm, and the supernatant transferred to a clean 25 mL volumetric flask[159]. The extraction was repeated two additional times to reach final volume of 25 mL of supernatant[159]. The combined supernatant was vacuum filtered on a Buchner funnel with No. 1 Watman paper [159]. The collected supernatant was then placed under −20° C conditions for 24 h, filtered, then transferred to a scintillation vial and allowed to dry at room temperature[159]. Each field sample was extracted in triplicate[159].

According to **Chacon et al**., (2024) [159], the hydro-distillation was carried out using 30 g whole flower samples[159]. Each sample was placed inside a 1000 mL round bottom flask, after which 600 mL of Milli-Q water was charged[159]. The sample was then placed on a heating mantel and attached to a distilling apparatus [159]. Samples were brought to a boil then lowered to a medium temperature for a total of 60 min[159]. Oil from the return tube of the distillation apparatus was collected from the discharge stopcock into a 20 mL scintillation vial[159]. For complete removal of water remnants, each oil was filtered using a glass pipette containing a loosely packed layer of glass wool below 0.8 g of

anhydrous sodium sulfate[159]. The filtered oil was then captured in a tared 20 mL scintillation vial[159]. All samples were extracted in duplicate except for a single sample with restricted mass (F3) [159].

25.1. Supercritical CO_2 Extraction

According to Chacon et al., (2024) [159], dried hemp flower, 55–105 g, was ground and extracted using an SFT-SP1100 extraction system manufactured by Supercritical Fluid Technologies, Inc [159]. Briefly, the extraction was performed at 55 °C for 35 min at 413 bars, the extract was then collected and dissolved in ethanol to a final concentration of 10% by weight. Winterization was carried out for 24–48 h at −20 ℃ and the extract was filtered before ethanol evaporation[159]. The final extract was dissolved in fractionated coconut oil at 300 mg/mL and decarboxylated at 95 ◦C for 1 h[159]. All the samples were quantified by LC-MS/MS and GC-MS[159].

According to the study conducted by Chacon et al., (2024) [159] for LC-MS/MS, all samples were reconstituted at 50 µg/mL in methanol containing 1 µM of chlorpropamide as an internal standard, except for the distilled extracts which were prepared at 1 mg/mL[159]. Standards were prepared as a serial dilution from 30–0.003 µg/mL[159]. Samples were injected at a volume of 5 µL with the following binary system of solvent A, 0.1% formic acid in water, solvent B, 100% acetonitrile, at the following gradient: 50% B for 9 min, 100% B for 2 min, then 50% B for 2 min[159].

According to **Chacon** et al., (2024) [159], for GC-MS, samples were prepared at a concentration of 1 mg/mL in GC-MS grade hexane with 1 µM of chlorpropamide [159]. After being transferred to GC-MS vials, they were allowed to dry under a fume hood with open covers to prevent contamination of samples[159]. Once dried, samples were introduced to pyridine and BSTFA (1:1), capped and allowed to sit for 60 min at room temperature to allow for the addition of a trimethyl silyl group for increased compound separation[159]. Samples were then stored at −20 ℃until MS analysis [159]. Terpenoid standards were prepared in a similar manner but at a concentration range of 500 µg/mL– 0.3 µg/mL[159]. Cannabinoid standards were prepared at a concentration of 50 µg/mL for identification. Analysis of the samples and standards were carried on an Agilent 5975C series GC-MSD with an Electron Ionization (EI) source operated in the positive mode with a scan range of 50–600 m/z [159].

Overall, the study of Chacon et al., (2024) [159], provides evidence of the differences in cannabinoid and terpene composition obtained from hemp inflorescence after three predominant extraction techniques: supercritical CO_2, solvent extraction (ethanol), and hydrodistillation[159]. Challenges with solvent extractions involving ethanol will likely make isolation of neutral cannabinoids difficult and result in a more diverse phytochemical profile[159]. Likewise, hydro-distillation can be extremely effective at capturing terpenes within hemp inflorescences. However, the process has dramatically lower yields and a distinct lack of cannabinoids in the resulting extract[159].

26. Solid-Phase Microextraction (SPME)

According to the study conducted by **Wozniczka** et al. (2024) [165], an in vivo solid-phase microextraction (SPME) is a minimally invasive, non-exhaustive sample-preparation technique that facilitates the direct isolation of low molecular weight compounds from biological matrices in living systems [165]. This technique is especially useful for the analysis of phytocannabinoids (PCs) in plant material, both for forensic purposes and for monitoring the PC content in growing *Cannabis* spp. plants[165]. In contrast to traditional extraction techniques, *in vivo* SPME enables continuous tracking of the changes in the level of PCs during plant growth without the need for plant material collection[165].

27. Supercritical Fluid Extraction (SFE)

According to the review paper survey by **Rantasa et al.,** (2024) [166] extraction of cannabinoids from different parts of the plant matrix is often carried out by various traditional methods based on the use of organic solvents[166]. Supercritical fluid extraction (SFE) has emerged as one of the most intriguing approaches for the extraction of cannabinoids[166]. The liquid chromatography with tandem mass spectrometry (LC-MS/MS) is gaining increasing importance due to its superior identification of analytes which is based on both the retention times of analytes as well as specific qualifier ions[166]. Another interesting analytical method is supercritical fluid chromatography (SFC), which uses supercritical fluids (SCFs) such as CO_2 in combination with different modifiers, to successfully separate and determine cannabinoids [166]. Supercritical CO_2 is employed as a mobile phase to separate non-polar compounds[166]. SFC appears as a promising strategy for the analysis of cannabinoids as pure compounds and in formulations[166].

28. Water and Ethanol extracts

The high interest in non-psychoactive cannabidiol increases the need for efficient and straight forward cannabidiol (CBD) extraction methods [158]. The research work by **Szalata., et al.,** (2022) [158] compared simple methods of cannabinoid extraction that do not require advanced laboratory equipment[158]. This work by Szalata., et al., (2022) [158] reported the content of total CBD and Λ9-tetrahydrocannabinol (Λ9-THC) in popular solvents such as water and ethanol extracts[158]. Hemp raw material was analyzed with Gas Chromatography with a Flame Ionization Detector (GC-FID), while extracts were tested by High-Performance Liquid Chromatography (HPLC) [158]. The female inflorescences of three varieties of industrial hemp were tested: Futura 75, KC Dora, and Tygra (different sowing and N fertilization densities) [158]. Tygra (T/10/30) showed the highest content of CBD (0.064%) in water extracts[158]. However, in 80% tincture from Futura 75 (F/30/30), a higher CBD content of 1.393% was observed. The use of 96% ethanol for extraction and ultrasound enabled the highest CBD content to be obtained: 2.682% in Futura 75 (F/30/30) [158]. Cold water extraction showed no effect on Λ9-THC content, while hot water extraction increased content from 0.001% in KC Dora to 0.002% in Futura 75 (F/30/30) and Tygra, but the changes were statistically insignificant[158]. Application of 80% ethanol revealed the significantly highest content of Λ9-THC in KC Dora, from 0.026% (K/30/90) to 0.057% (K/30/30), as well as in Tygra (T/30/30) (0.036%.) and Futura 75 (F/30/30) (0.048%)[158]. The use of ethanol extraction in combination with ultrasound could be an efficient method of obtaining cannabinoids[158].

According to the research work conducted by **Szalata**., et al., (2022) [158], the plants were collected at the late flowering phase (the beginning of seed maturation), while the inflorescences were dried on the drying floor at 40 °C for approximately 24 h[158]. The dried material was stored in paper bags at room temperature. In plants, the cannabinoids generally occur in native forms, Λ9-tetrahydrocannabinol acid (Λ9-THCA) and cannabidiolic acid (CBDA), which through the decarboxylation process become the neutral forms Λ9-tetrahydrocannabinol and cannabidiol [158]. The proportions of these forms vary in plants, and the amount of neutral states increases during the storage of the material[158]. The total content of Λ9-THC, D9-THCA, CBD, and CBDA remains unchanged, allowing the distinction between narcotic and fibrous varieties[158].

The research work by Szalata., et al., (2022) [158] reported that temperature, solvent, and solvent polarity could influence the extraction of cannabinoids from plant material[158]. Additionally, shaking could enhance the penetration of the solution into a cell[158]. However, the latter effect was not observed in the experiment. Studies conducted by other groups also revealed that relative amounts of cannabinoids in the extract depend on an ethanol/water solution [158]. The study confirms the effectiveness of extraction methods for obtaining extracts containing CBD and Λ9-THC from fiber hemp[158]. Hot water extraction was performed: 5 g of dried inflorescences were transferred to a 250 mL flask and covered with 100 mL of hot distilled water[158]. Extraction (heating and boiling) was performed for 3 min [158]. The extract was filtered using a double-folded gauze, and the filtrate was collected[158]. Cold water extraction was performed as follows: 5 g of dried inflorescences were inserted into a 250 mL flask, and 100 mL of cold water was added[158]. The flask was closed with aluminum foil and transferred to a shaker (ES 20 Shaker) for 24 h[158]. The mixing process was performed at room temperature at a constant speed of 100 rpm [158]. After 24 h, the extract was filtered using a double-folded gauze, and the filtrate was collected[158]. Preparation of tinctures using 20%, 40%, and 80% ethanol was as follows: 10 g of dried inflorescences was transferred to the 250 mL flask[158]. For maceration 100 mL of different ethanol/water mixtures (20%, 40%, and 80% v/v) was used [158]. The flask was closed and covered by aluminum foil to protect against light [158]. The samples were stirred for three days at room temperature at a speed of 100 rpm, then extracts were filtered using a double-folded gauze, and the filtrates were collected [158]. Ultrasound extraction was performed as follows: 2 g of dried inflorescences were inserted into a 250 mL flask, and 10 mL of 96% ethanol was added[158]. The samples were sonicated in an ultrasound water bath and treated with 40 kHz for 30 min [158]. After sonication, the extract was filtered using a double-folded gauze, and the filtrate was collected[158]. Each extraction process was conducted in duplicate[158].

Samples obtained from different extraction procedures were analyzed using HPLC chromatography, and separation was performed on the Accucore C18 column [158]. Mobile phases consisted of solvents: 0.01% formic acid in acetonitrile (A) and 0.01% formic acid (B) [158]. After filtration, the samples were diluted in acetonitrile, placed in vials, and analyzed using liquid chromatography with automatic injection [158]. The experiment revealed that water's simplest extraction method was sufficient to obtain a small amount of CBD but did not allow homogeneous formulations[158].The investigation confirmed that the simplest extraction method using water was enough to obtain a small amount of CBD but did not allow homogeneous formulations[158]. The use of high concentrations of ethanol extracts, especially in combination with ultrasound and determination using liquid chromatography (HPLC), made it possible to obtain a cannabinoid profile corresponding to the standard cannabinoid determination using GCFID gas chromatography[158].

For analytical determination of cannabinoids in extracts or cannabis products, liquid chromatography (LC) and high-performance liquid chromatography (HPLC) are often used [25, 38, 34158, 166].

29. Industry Level Scale up: Hydrodynamic Phytocannabinods Extraction

Hydrodynamic cannabis extraction is a recent development within the cannabis industry that can be used to produce full-spectrum cannabis extracts with high bioavailability[140-157]. There have been accounts of companies, such as The Clean Green Biosystems, Chenni, Tamilnadu State, India[140-146] and IASO (Incline Village, Nevada, USA) [147-155], claiming to have developed a unique extraction system that produces products with high yield and increased potency[140-157]. This alternative method involves freezing fresh plant material and converting it into a nano-emulsion in water by ultrasonication[140-157]. Hydrodynamic force is then used to break the cell wall and release its contents[140-157]. This is followed by liquid– liquid extraction using solvents, centrifugal separation, and finally low-temperature drying[140-157]. Te initial step of freezing the plant matter helps preserve the volatile compounds as well as acidic cannabinoids during the following steps [140-157]. Hydrodynamic extraction is claimed to exceed conventional methods mainly due to the lack of high temperatures, short contact distillation, and low organic solvent consumption [140-157]. Ishida and Chapman (2012) [146] used this technique to extract carotenoids from tomatoes and found that the extractable lycopene, other carotenoids, and accessibility of carotenoids significantly improved (Ishida and Chapman 2012) [146] However, to this date, there has been no scientific publication that explores this method of extraction[146]. Therefore, to fully understand the efficacy of this method, more research is required[146].

Hydrodynamic extraction technology has recently been introduced to the cannabis industry, which utilizes a combination of temperature, pressure, and ultrasonication to create full spectrum cannabis extracts from whole fresh cannabis flower [140-157].

In the hydrodynamic extraction process, fresh cannabis flower is frozen and then broken down directly using ultrasonication and hydro-dynamic forces[140-157]. This produces a **nanoemulsion** that contains all the cannabinoids, terpenes, and other oils from the cannabis plant in an aqueous phase[140-157]. Centrifugal separation, followed by low-temperature vacuum distillation and drying steps, turn the nanoemulsion into a potent and highly bioavailable cannabis extract[140-157]. Because the entire process can be conducted at low temperatures and doesn't require the use of additional industrial solvents, the final extract preserves a great number of *cannabis*' aromatic compounds[140-157]. This gives the product a strong flavor and aroma that closely matches the original *cannabis* strain used in the extraction; which may be of particular interest to those involved in making vape cartridge oils who want to give their customers that "whole plant cannabis" experience[140-157].

Hydrodynamic extraction technology combines temperature, pressure, and ultrasound to create full-spectrum cannabis extracts from the whole fresh cannabis flower [140-157]. An attractive solution may be the use of bees fed only the full spectrum of cannabinoids. The Israeli company PhytoPharma International has produced hemp honey produced exclusively by bees. The result is natural honey infused with low concentrations of less than 0.3% active, highly bioavailable cannabinoids. More methods concerning aroma preservation of bioavailability may potentially be developed using simple and easily applicable techniques.

30. Clean Green Biosystems: Hydrodynamic Extraction System

Clean Green Biosystems has developed a patented technology of extracting highly bio available phytochemicals from fresh herbs, fresh fruits and fresh vegetables [140-146]. Hydyne is Innovative new hydrodynamic extraction system that uses the entire fresh plant materials to preserve all the unique phytochemicals and phytonutrients compounds in a full spectrum/broad spectrum extract[140-146]. The bio availability of many of the Herbal extracts, Nutraceuticals and fruit juices are the range of 10-35%. This poor bio availability results in poor pharmacological activity which requires high and repetitive dosages[140-146]. his patented hydrodynamic extraction process produces highly bioavailable phytochemicals /phytonutrients[140-146].

Hydrodynamic Extraction System: The Clean Green Biosystems [140-146]. (Dr. S. Prem Mathi Maran Ph. D, D.Sc CEO, Plot No-160, Karpagam Nagar, Thirumudivakkam, Chennai-600044, Tamilnadu state, India) have developed a novel patented technology of extracting highly bio available broad spectrum Phyto-cannabinoids extract from fresh Hemp / Cannabis plants[140-146]. According to Clean Green Biosystems, fresh Hemp / Cannabis plant cell fragmentation taking place because of the epidermis breaking, cuticle disintegration and membrane disintegration under intense hydrodynamic shear force and acoustics which releases bioactive compounds from the plant cells into an aqueous

media[140-146]. The controllable Hydrodynamic force provides faster and more complete release of full spectrum bio active compounds into an aqueous media, facilitates improved mass transfer[140-146]. Nano emulsification happens, which enhance the bioavailability. For further discussion, and updated information on hydrodynamic cannabinoids extraction system based projects, please contact following address[140-146].

Clean Green Biosystems

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31. Clean Green Biosystems : Principle Of Hydrodynamic Extraction

According to the patented hydrodynamic extraction technology by **Clean Green Biosystems,** Chennai, Tamilnadu, India [140-146], fresh plant materials (herbs, fruits and vegetables) are subjected to epidermis breaking, cuticle disintegration, membrane disintegration and cell fragmentation using intense hydrodynamic shearing and acoustics that releases bioactive compounds from the plant cells[140-146]. Cell disruption is a sensitive process, and control of the cell disruption is required, to avoid uncontrolled release of cell debris or biomolecules denaturation[140-146]. The bioactive compounds in the plant cells are released and dissolved into an aqueous phase. Hydrodynamic force is a well-controllable means for cell disintegration[140-146]. It provides faster and more complete release of bio active compounds into a aqueous media, improves the mass transfer[140-146]. Hydrodynamic shear waves disrupt cell walls and releases a full spectrum matrix of compounds[140-146]. As the fluid waves break the cell wall mechanically by the shear forces, it facilitates the transfer of bioactive compounds from the cell into the solvent[140-146]. Particle size reduction also increases the surface area in contact between the solid and the liquid. The solvent can enter into the cell, transporting essential oils and bioactive compounds from the cell interior into the surrounding solvent[140-146]. This enables to produce high-potency extracts, with the highest contents of phenols, flavonoids, non-flavonoids, carotenoids, terpenoids, phytosterols, etc[140-146].

32. Clean Green Biosystems: The USP of Hydrodynamic System

According to the patented hydrodynamic extraction technology by Clean Green Biosystems, Chennai, Tamilnadu, India[140-146] the system is the first of its kind to be able to use whole, freshly harvested plant materials. Concentrates can preserve the full phytochemicals/phytonutrients profile, unlike many other systems where they are compromised or even lost[140-146]. The Hydyne system uses a unique pre-processing step, freezing[140-146]. By freezing the plant material before processing, the phyto molecules retain their aromatic compounds and have a stronger scent[140-146]. Intrinsic heat generated by the hydrodynamic extraction normally activates the inactive phytochemicals /phytonutrients precursors[140-146]. Hydrodynamic technology converts fresh plant material into nano-emulsions[140-146]. According to the patented hydrodynamic extraction technology by Clean Green Biosystems, Chennai, Tamilnadu, India the proprietary technologies and procedures are designed to optimize the extraction of active phytochemicals/phytonutrients from different herbs, fruits and vegetables, maximizing yield and creating higher bioavailability, by converting the plant materials into nano emulsions[140-146]. Integrated modules include: biomass preparation, emulsion making, separation and isolation of phytochemicals/phytonutrients, extraction using a suitable solvent/solvent mixture, and evaporating the solvent and drying[140-146]. Uses a liquid-liquid extraction system, using

food-grade/pharmaceutical solvents. Able to distill the majority of solvents in the distillation system, using both a proprietary molecular distillation process in our commercial-grade systems, and a low temperature, short contact distillation path in our smaller scale systems[140-146]. Creates between 15-25% waste solids from plant material, which are converted into bio-compost that can be recycled into soil/substrate or farming manure, creating a closed loop system[140-146].

33. Clean Green Biosystems: Hydrodynamic Extraction System

According to the patented hydrodynamic extraction technology by **Clean Green Biosystems,** Chennai, Tamilnadu, India[140-146] harvesting the Fresh Cannabis and freeze it in dry ice for 30 minutes which fixes the biomolecules, which retains the natural, fresh aroma of the extract and enhances the bio availability of the extract[140-146]. The Hydrodynamic system converts the cannabis plant material into a cannabis nano emulsion by means of hydrodynamic force and ultrasonification by breaking the cell walls of the plant material and releases them into the aqueous phase[140-146]. This also enhances the bioavailability of the extract[140-146].

34. Clean Green Biosystems: Filtration System

According to the patented hydrodynamic extraction technology by Clean Green Biosystems, Chennai, Tamilnadu, India[140-146], the filtration system is optional as Clean Green Biosystems already have integrated filter in the extractor itself[140-146]. Clean Green Biosystems normally supply plate and frame filter press[140-146]. The numbers of filter plates vary on the basis of the volume of miscella to be filtered. The filter plates can be PP or SS. For small volume filtration, we supply zero hold up filter press[140-146].

35. Clean Green Biosystems: Distillation and Concentration System

According to the patented hydrodynamic extraction technology by **Clean Green Biosystems,** Chennai, Tamilnadu, India[140-146] the miscella (oil and solvent) after the extraction of phytocannabinoids from solid-liquid extraction methods of dry cannabis biomass or hydrodynamic methods of extraction of phytocannabinoids from fresh cannabis biomass must be distilled and concentrated (solvent recovery), to get a solvent-free, highly concentrated full-spectrum extract[140-146]. Also, the full-spectrum phytocannabinoids can be subjected to distillation to remove much of the Λ9-tetrahydrocannabinol (THC) and produce a broad-spectrum distillate[140-146]. At Clean Green Biosystems, the patented technology have designed a PLC-SCADA-based method (Programmable Logic Controller-Supervisory Control and Data Acquisition) for separating molecules from cannabis, called Low Temperature Short – Contact Distillation Path, which combines forced circulation evaporation and molecular distillation[140-146].

Once the extraction is complete (whether solid-liquid or liquid-liquid extraction in case of patented hydrodynamic technology), the miscella (containing the extracted phytochemicals and solvent) is collected in a tank and is subsequently evaporated through a forced circulation evaporator and molecular distillation system[140-146]. Depending upon the miscella volume that needs to be processed, single, double, or multistage systems may be employed[140-146]. According to the patented hydrodynamic extraction technology by **Clean Green Biosystems,** Chennai, Tamilnadu, India[140-146], the forced circulation evaporation system is composed of shell and tube heat exchangers, a flash evaporator, and primary and secondary condensers[140-146]. The ASME-compliant shell and tube heat exchangers heat the miscella so that the vapour is created[140-146]. The primary condenser distils the first-pass vapour whereas the secondary condenser further refines vapor from the primary condenser[140-146]. This integration helps in efficient recovery of vapours[140-146]. The forced circulation evaporation system removes around 75-80 % of the solvent from the miscella[140-146]. The output of this evaporation process is concentrated miscella, which can be further purified using molecular distillation[140-146].Thermally unstable compounds can be safely purified or separated by applying molecular distillation methods[140-146].

As phytocannabinoids are higher molecular weight, thermo labile compounds, the application of high vacuum distillation methods, such as molecular distillation, are used to isolate/separate/purify components without thermally degrading them[140-146]. A molecular distillation unit has a feed vessel that introduces miscella into the unit, whereupon the wipers spread an even coating as a thin film on the heated walls of the unit, thereby increasing the surface area[140-146]. Specific and proprietary wipers can produce a thin layer of extract that's 0.05 – 0.1 mm in thickness, significantly enhancing the evaporation surface area[140-146]. The speed of the wiper blades' rotation can be optimized and controlled to ensure uniform flow of the miscella for a rapid and efficient evaporation[140-146].

According to the patented hydrodynamic extraction technology by **Clean Green Biosystems,** Chennai, Tamilnadu, India[140-146], the molecular distillation uses short-path evaporators, and the distance (varies with flow and evaporation rates, but typically between 1 and 30 cm) between the evaporator and the condenser minimizes pressure drop[140-146]. The distance between the heating and cooling surfaces is carefully designed to have a faster, rapid, and efficient separation of solvents from the miscella[140-146]. The thermally labile molecules have short contact times with heat (may be as low as one millisecond), and thus, decomposition of the molecule(s) is kept to a minimum or does not occur at all[140-146]. The evaporated solvent is condensed using chilled water, and the thick/concentrated miscella is expelled by the wipers[140-146]. The solvent/solvent system is collected through an external vent condenser and can be used to extract further materials[140-146]. The highly concentrated cannabis extract is collected for further processing or for direct use[140-146]. The resultant product can be further distilled to get THC-remediated, broad-spectrum product[140-146]. Various fractions of the broad-spectrum phytocannabinoids can also be isolated using the molecular distillation system by adopting different standard operating procedures[140-146]. According to the patented hydrodynamic extraction technology by **Clean Green Biosystems,** Chennai, Tamilnadu, **India**[140-146], the molecular distillation provides a way to retain molecular integrity and to purify thermally unstable molecules and related compounds having low volatility and elevated boiling points[140-146]. The method uses low pressures (1-5 Pa, 0.01-0.05 mbar) [140-146]. All process control systems like temperature control, flow control, vacuum control, and product viscosity are controlled by PLC-SCADA software[140-146].

36. Clean Green Biosystems: Drying System

Clean Green Biosystems offer vacuum tray driers, rotacone driers, ribbon driers of various capacities[140-146]. Clean Green Biosystems supply suitable vacuum systems for the driers[140-146].

37. Clean Green Biosystems : THC Remediation System

Tetrahydrocannabinol (THC) remediation is the removal of THC to the legalised level (0.2 to 0.3%) in any of the CBD products[140-146]. Clean Green Biosystems systems are equipped and integrated with a THC remediation system which has a molecular distillation system, heat exchangers, condensers and chilling units[140-146].

38. Clean Green Biosystems: Decarboxylation System

Clean Green Biosystems equipments do the decarboxylation process considering the boiling points of converting the acidic forms of CBD to CBD[140-146]. The solvents and its traces are completely removed from the CBD highly concentrated miscella in the VTD[140-146]. After completing it, the decarboxylation process starts in the VTD itself at specific temperatures with a progressive temperature increase which is controlled by PLC-SCADA based automation[140-146].

39. Clean Green Biosystems: Fractionation / Purification System

Clean Green Biosystems offer downstream processing systems to produce various fractions of CBD[140-146]. The downstream processing equipments like column chromatography, ANFD, crystallizer, fractionation columns, washing columns, separating columns, pulverizers, mixers, blenders and rotary evaporators[140-146]. Clean Green Biosystems support clients with the operation manuals of the supplied plants, SOP's and maintenance services[140-146]. Also, Clean Green Biosystems impart training of the technical staffs of clients during and after the commissioning of the plants [140-146].

40. IASO: Hydrodynamic Cannabis Extraction

Hydrodynamic Cannabis Extraction: Hydrodynamic Cannabis Extraction is an innovative new technology that the IASO corporation has recently introduced to the cannabis industry. IASO has its name rooted in ancient healing, connected to the Greek goddess of healing, wellness, and cures[148-155]. IASO, (www.iasocorp.com), a science-based cannabis products and technology company, is changing the dynamics of the industry by introducing two significant and transformative new products to the medical marijuana community at the Terpene and Testing World Conference on April 10-11, 2018 in San Jose, California, USA. IASO was founded in 2015 by César Cordero-Krüger, a successful serial entrepreneur and accomplished real estate developer and consultant, who has assembled one of the most remarkable leadership and science teams in the cannabis industry[148-155]. Further IASO provides a wide range of scientifically-based innovative and proprietary products, technologies, and services to the cannabis industry[147-155]. This symbolism is the core of the company's mission to provide consumers with the highest quality cannabis products and

new and innovative cannabis technologies[147-155]. The company was founded in 2015 and brought new technologies to the cannabis industry in 2018, all of which are set to change the current cannabis landscape dramatically[147-155]. One of these technologies is PhytoX™, the first-ever system to use hydrodynamic cannabis extraction in the development of cannabis products[147-155].

PhytoX™is the world's first hydrodynamic extraction system that can process whole, fresh, undried cannabis plants, which maximizes plant utilization, reduces processing costs, and increases yields[147-155]. The highly innovative system can process freshly-harvested plants into superior extracts[147-155]. Unlike any other extraction methods, the PhytoX produces a highly bio-available product[147-155]. The system preserves plant characteristics, and produces oils that are unrivaled for purity, quality and consistency[147-155].

41. IASO: Hydrodynamic Cannabis Extraction Processing

Hydrodynamic cannabis extraction is one of the newest technologies to hit the budding cannabis industry[147-155]. It uses a combination of heat, pressure, and hydrocavitation to process whole plants that have been freshly harvested and extract uncompromised full-spectrum compounds, cannabinoids, and terpenes[147-155]. Unstable and volatile compounds are kept intact due to using fresh plant material and the method of processing[147-155].

42. IASO: Hydrodynamic Extraction Stages

Hydrodynamic cannabis extraction system has several stages which complete the process[147-155]. **Stage 1**: While preparing the fresh plant materials, compounds are frozen to preserve the biomolecules, increase bioavailability and retain aroma[147-155]. **Stage 2**: Plant material is converted in nanoemulsions using hydrodynamic force and ultrasonication[147-155]. During this process, the plant material's cell walls are broken thus transmuting them into the aqueous phase[147-155]. **Stage 3**: Centrifugal separation methods are used to separate the molecules[147-155]. **Stage 4**: The temperature from the hydrodynamic process is used in a cold extraction process to isolate the active cannabinoids and other molecules[147-155]. The total active particles are now in the solvent phase[147-155]. **Stage 5**: This step distills the solvents by using low temperature and a short combination path, ensuring the extracted elements are kept intact[147-155]. **Stage 6:** A low-temperature drying system is used, further ensuring the molecules remain intact. Residual solvents are removed during this stage using VTD[147-155].

43. IASO: Hydrodynamic Extraction: How it differs

The system is the first of its kind to be able to use whole, freshly harvested plants or dry cannabis plants[147-155]. Therefore, it is also the first to produce cannabis concentrate from whole, fresh cannabis[147-155]. Concentrates can preserve the full cannabinoid and terpene profile, unlike many other systems where they are compromised or even lost[147-155]. The PhytoX™ system uses a unique pre-processing step[147-155]. By freezing the plant material before processing, the molecules retain their aromatic compounds and have a stronger scent. The process does not use any other solvents[147-155]. Due to the Hydrodynamic Cannabis Extraction methods, the process only uses low temperatures. Intrinsic heat generated by the hydrodynamic extraction decarboxylates THCA into active THC[147-155].

44. IASO: Superior to Other Extraction Methods

The innovative ability to process whole, fresh plants renders the extracts to be of a superior nature due to their increased bioavailability[147-155]. Besides increased bioavailability, the hydrodynamic cannabis extraction method presents many benefits above the other more traditional methods[148-155]. The system has been designed to achieve the maximum possible efficiency from the extractions of the phytocannabinoids from the active trichomes[148-155]. This, therefore, increases the potency and enables a higher bioavailability because the plant is transformed, through this process, into nanoemulsions[148-155]. Many extraction methods can not guarantee the integrity of unstable compounds [147-155]. Hydrodynamic extraction is designed to use fresh and whole plants, ensuring these volatile molecules are kept intact[148-155]. Additionally, the proprietary distillation prevents the phytocannabinoids from thermal degradation, further protecting molecule integrity[148-155]. The aroma of the resultant cannabis products is stronger than traditional extracts. Because the plant material is frozen in the preparation stage of the system, it allows the aromatic compounds to remain intact[148-155]. Additionally, the **PhytoX™** system employs the use of a liquid extraction system which requires no other co-solvents[148-155]. This method reduces production costs as well as increases the product quality for consumers[148-155]. However, if any solvents do remain after the process, the system also uses a vacuum during the drying phase, which subsequently removes any residual solvent which may remain[148-155]. Another main advantage of the hydrodynamic extraction system is that while it maximizes plant utilization unlike

any other method, it also reduces costs related to processing while still increasing yield[147-155]. This is one advantage that businesses and cultivators in the cannabis industry will undoubtedly be interested in, because the innovative hydrodynamic cannabis extraction method, **PhytoX™**, produces a product with superior bioavailability and quality and less overall processing costs[148-155].

45. Sheen Biotech Inc (www. zesthabiotech.com)

Sheen Biotech Inc (www. zesthabiotech.com) [156] by S. U. Zafar and Shailandra Singh is another company located at B-58, Sector-63, Noida-201301 UP state, India[156]. Site-Pantgoon, Bhikiyasin, Almore, Uttarakhand-263680, India is also having a license for cultivation of medical cannabis and extraction of CBD oil and its derivatives in India[156]. According to the website, this company is also involved in the hydrodynamic extraction of phytocannabinoids [156].

46. Biosensors based Quantification method

The literature survey by Clément et al., (2024) [164] updated the latest trends in the development of portable technologies and commercial products to detect THC in biofluids [164]. Two main categories of portable biosensors were identified in this review: optical and electrochemical biosensors[164]. Optical-based biosensors include various techniques such as SERS, ultraviolet–visible-near infrared spectroscopy, colorimetry, and chemiluminescence detection with THC LODs varying from 0.01 to 10 ng/ mL[164]. The study of Clément et al., (2024) [164] described the recent cutting-edge portable technologies for the detection of THC in biofluids [164]. It also indicated promising potential for the rapid onsite determination of THC in biofluids [164].

Highlights

- External factors such as light duration, oxygen, humidity and harvest time (floral maturity) have been shown to influence the secondary metabolite production in cannabis.
- According to the American Society of Testing and Materials (ASTM) International, the optimal humidity level for cannabis storage is in the range of 55–65%.
- It was observed that both the neutral and acidic forms of THC in the cannabis extract degraded significantly more through light exposure.
- Studies have demonstrated that Λ9-THC, both in herbal and resin cannabis preparations (marijuana and hashish), progressively decomposes over time to cannabinol (CBN), which is believed to be a mere chemical degradation product rather than a naturally/ biochemically occurring component.
- However, decomposition of Λ9-THC (Λ9-tetrahydrocannabinol) when exposed to light and air, to cannabinol (CBN) proceeds through an oxidation reaction during long term storage. Hence long term storage should be avoided.
- Λ9-THC content of marijuana stored at room temperature is decreasing at a rate of 3–5% per month. The studies, demonstrated that light and temperature have a dramatic effect on the decomposition of Λ9-THC to CBN, while they mediate different aspects of the process. Light impacts the stoichiometry of the conversion of Λ9-THC to CBN, whereas temperature accelerates the conversion.
- Since many of the substances in cannabinoids can undergo oxidation, and sealing the samples in plastic bags can reduce the losses to 25–42%.
- Neutral cannabinoids are stable for up to 15 (at 20 °C) to 30 days (at −18 °C and 4 °C) stored in the dark, while exposure to daylight leads to their dramatic decomposition.
- After being harvested, the cannabis plant can be dried to eliminate any extra moisture. Water can function as a barrier, lowering the quality of the intended analytes' extraction. Cannabis samples must be stored in a dark area to prevent photochemical reactions, which may transform the composition of cannabis samples. It is also recommended to store cannabis samples refrigerated as the content of cannabinoids drastically changes when exposed to room temperatures for longer periods of time.
- It is also recommended to store cannabis samples refrigerated as the content of cannabinoids drastically changes when exposed to room temperatures for longer periods of time.
- **Freeze-drying** is the best method which may also facilitate the extraction of substances from cannabis plant material. Freeze-drying or lyophilization is a sophisticated method that reduces the pieces of plant matter to a powder, which breaks the plant cells to release the active compounds.
- **Cryogenic grinding** is an alternative technique for homogenization that cools the sample using liquid nitrogen before grinding to prevent the loss of volatile compounds as the temperature rises during grinding.
- It is generally agreed that the end products of freeze-drying are considered high quality compared to other methods of drying.

- **Curing** is the final post-harvest procedure that allows for the development of the maximum flavour in the cannabis plant. The best temperature and humidity for curing are at 18 °C and 60% RH for 14 days. Therefore, keeping the trimmed flowers in a can for up to 4 weeks in a dark cupboard while opening the lid every day for about 6 h is the best method for curing.
- Curing can reduce the harsh smell and the sense of throat burning during smoking or vaping as well as increasing the shelf life by minimizing mold growth.
- The traditional methods of solventless, solvent-based, convention, and alternative methods of extraction are explored concerning cannabis extraction. In some cases, advanced extraction techniques showed more efficiency than conventional techniques.
- Traditional methods include dynamic maceration, Soxhlet extraction, ultrasound-assisted extraction, and microwave-assisted extraction. These traditional methods have in common the use of organic solvents, such as ethanol, chloroform, butane, propane, diethyl ether, and hexane. The drawback of their use is a time-consuming extraction, which may not result in a high extraction yield. Additionally, organic solvents have poor selectivity, which affects the extract quality. Solvents used in traditional extraction methods must also be removed from the extracts, which requires additional energy and time. These solvents are most frequently removed by evaporation with either a rotary evaporator or vacuum ovens. Moreover, the solvents themselves may become a residue in the final extract
- A variety of solvents can be used to extract cannabinoids including ethanol, butane, propane, hexane, petroleum ether, methyl tertbutyl ether, diethyl ether, carbon dioxide (CO_2), and olive oil. Gaseous solvents such as butane and propane can also be used for extraction purposes. The extracted sample is collected, and the solvent is evaporated. The process of pressurizing these flammable and potentially explosive gases poses safety hazards. In addition, the gases used in cannabis extractions are often industrial grade and contain impurities that end up in the cannabis extracts.
- The solvents of poor quality are often used at industrial level, thereby introducing impurities into extracts. Pesticides, herbicides, and other compounds are also frequently found in the extracts obtained from traditional extraction methods since cannabis plants are treated with them during growth. As a result, these traditional methods are no longer used as much as in the past and have gradually been replaced by other methods.
- However, the stability of phytochemicals can decrease when they are exposed to high temperatures for a long time because most phytochemicals are sensitive to heat. Therefore, it is important to determine the most suitable temperature and length of extraction to extract a high level of phytochemicals with minimum degradation.
- An increased solvent-to-plant material ratio will enhance the driving forces for diffusion and increase the extraction rat. In contrast, lower solvent ratios reduce the time and energy required for solvent removal, recycling, or disposal volume, at the expense of the extraction yield. Another factor to consider in solvent selection is solvent toxicity due to residual solvents in the final pharmaceutical/ nutraceutical product. These solvents are not eliminated during manufacturing and their maximum concentrations in the final product are regulated by International Council for Harmonization (ICH) guidelines.
- Traditionally, the extraction of phytocannabinoids is performed using organic solvents, including hydrocarbons (e.g., hexane) and alcohols (e.g., ethanol, methanol). This method of extraction is cheap, easy to operate, and does not require sophisticated equipment. However, the solvents used are flammable, toxic, and non-biodegradable, risking human health, besides having a huge environmental impact. Extraction using these solvents can be efficient but depending on the final product, can impact regulation, and required additional testing. For instance, residual solvent is strictly regulated and must be defined for medicines under good manufacturing practice. These solvents due to their toxicity, environmental risk, and flammability are less desirable for large scale extractions.
- The **use of vegetable oils** such as coconut, canola, sunflower, walnut, palm, or hemp oil for cannabinoid extraction, avoiding the use of toxic and hazardous hydrocarbon solvents in addition to the high pressures involved in the supercritical extraction with CO_2. Thus, extraction with vegetable oils or lipids is considered to be solvent-free, safer, and more environmentally friendly compared to traditional extraction methods using butane or alcohols since it can be performed using certified organic solvents and a post-processing step for solvent removal is not needed as the cannabinoid product is diluted in oil. Obtaining an oily cannabinoid product can be convenient since most commercialized CBD products are diluted in an oily medium like hemp seed oil.
- The vegetable oils are routinely extracted from seeds or fruits such as rapeseed, sunflower, or olive, and even brans, making them an inexpensive option. These oils are considered lipophilic due to their non-polar characteristic, which enables selective dissolving properties. Olive oil is a well-known solvent in the cannabis extraction.

- **Deep eutectic solvents** (DESs) are a new class of green solvents and have received great attention as extraction media. Hence, attractive candidates for solvents due to their inherent properties, for example, short preparation time, easy storage, low cost, non-flammability, and high capacity of solvation. Besides, DESs have other advantages, including high-thermal and electrochemical stabilities.
- Hydrodynamic cannabis extraction is a recent development within the cannabis industry that can be used to produce full-spectrum cannabis extracts with high bioavailability. There have been accounts of companies, such as The Clean Green Biosystems, Chenni, Tamilnadu State, India and IASO (Incline Village, Nevada, USA) claiming to have developed a unique extraction system that produces products with high yield and increased potency.
- According to the patented hydrodynamic extraction technology by Clean Green Biosystems, Chennai, Tamilnadu, India, hydrodynamic extraction technology has recently been introduced to the cannabis industry, which utilizes a combination of temperature, pressure, and ultrasonication to create full spectrum cannabis extracts from whole fresh cannabis flower.
- According to the patented hydrodynamic extraction technology by Clean Green Biosystems, Chennai, Tamilnadu, India, the system is the first of its kind to be able to use whole, freshly harvested plant materials. Hydyne is Innovative new hydrodynamic extraction system that uses the entire fresh plant materials to preserve all the unique phytochemicals and phytonutrients compounds in a full spectrum/broad spectrum extract.
- Patented hydrodynamic extraction technology by Clean Green Biosystems, Chennai, Tamilnadu, India can process of 5-10 tons of fresh cannabis plant material at a industrial scale processing.
- For further discussion, and updated information on hydrodynamic cannabinoids extraction system based projects, please contact following address[140-146].

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- According to the patented hydrodynamic extraction technology by Clean Green Biosystems, Chennai, Tamilnadu, India, harvesting the fresh cannabis and freeze it in dry ice for 30 minutes which fixes the biomolecules, which retains the natural, fresh aroma of the extract and enhances the bio availability of the extract. The hydrodynamic system converts the cannabis plant material into a cannabis nano emulsion by means of hydrodynamic force and ultrasonification by breaking the cell walls of the plant material and releases them into the aqueous phase. This also enhances the bioavailability of the extract.
- Hydrodynamic cannabis extraction is an innovative new technology that the **IASO** corporation, USA has recently introduced to the cannabis industry. **PhytoX™** is the world's first hydrodynamic extraction system that can process whole, fresh, un dried cannabis plants, which maximizes plant utilization, reduces processing costs, and increases yields. The highly innovative system can process freshly-harvested plants into superior extracts. Unlike any other extraction methods, the PhytoX produces a highly bio-available product. The system preserves plant characteristics, and produces oils that are unrivaled for purity, quality and consistency.

- The quantification of extracted final cannabis products are done by using HPLC and GC-MS using chemical standards.
- Using **cold ethanol** (from −60°C to −40°C) for the large-scale extraction of cannabinoids such as CBD or THC is also described for continuous biomass extraction and centrifugation. This method is generally used in the food industries for the detection and quantification of THC and CBD content.

47. Conclusion

Cannabis has been used for thousands of years for recreational, medicinal, or religious purposes. *Cannabis sativa* and *Cannabis indica* are the native of Indian origin found as wild noxious weed in the Indian Himalayan Region and other parts of India, China, Nepal, Bhutan, Sri Lanka, Pakistan, Afghanistan, Persian, Iran, and Morocco. Now a days *Cannabis sativa* is a globally domesticated, cultivated and introduced species occurring in North and South America, Europe, Africa, Australia, Asia and other parts of world. These cannabis species are hybrid varieties and known for very high levels of THC (0.3 to 38%) as compared to wild noxious weed found in all the parts of India. There has been an increased interest in medical applications of cannabis over the last decades. Cannabis can be classified based on genetics, phenotypic properties, and chemical composition. All these types are rich in bioactive phytochemicals. However, the phytochemical composition varies in different types. The cannabis industry is rapidly growing, therefore, product development and extraction methods have become crucial aspects of cannabis research. The evaluation of the current extraction methods implemented in the cannabis industry and scientific literature to produce consistent, reliable, and potent medicinal cannabis extracts is prudent. Furthermore, these processes must be subjected to higher levels of scientific stringency, as cannabis has been increasingly used for various ailments, and the cannabis industry is receiving acceptance in different countries. Therefore, consumer demand has created a strong and growing interest in the extraction and isolation of cannabinoid compounds for medicinal, pharmaceutical, and nutraceutical applications.

Regarding sample storage and preparation prior to extraction, both can drastically influence the chemical profile of the starting material and the yield and composition of the collected extract, as demonstrated in the research discussed in this review. Therefore, a reconciliation in the inconsistencies regarding storage conditions (fridge, freezer, RT, dark, light) and sample preparation (particle size, moisture content) is necessary in order to accurately evaluate the obtained results and compare the experimental outcomes of different research efforts. Furthermore, the merit of the developed extraction approaches could benefit from their further application to various cannabis sources (fibres, flowers, seeds, processing residues) and different cannabis varieties.

The selected extraction method must preserve the cannabinoid biological and pharmaceutical properties while meeting the required specifications in terms of extraction yield, extraction efficiency, and extract quality, as well as environmental, safety, and scalability considerations. As the cannabis market is expected to continue growing, there remains much work to do in optimizing current extraction techniques for the industrial mass production of high-quality extracts while meeting environmental and safety standards. There are several factors such as the types of plant materials, extraction techniques/time, solvents, pH, temperature, pressure, and material-to-solvent ratio can influence the extraction efficiency of bioactive compounds from plant matrices. Therefore, further investigations to optimise these factors could be advantageous to recover bioactive compounds from both industrial hemp and medicinal marijuana. As the optimization of these factors can be highly expensive, time-consuming, and labour-intensive, the utilisation of mathematical prediction models could accelerate the optimisation process for both conventional (e.g., Soxhlet extraction, maceration, hydro-distillation, and steam distillation) and advanced extraction techniques. The most common extracted methods used in the cannabis industry are pressurised liquid extraction, subcritical CO_2 extraction, supercritical fluid CO_2 extraction, non-thermal pulsed electric field-assisted, MAE, UAE, and enzyme-assisted extraction. However, all these traditional extraction methods are time, and energy consuming and found very expensive too. Many traditional extraction methods can not guarantee the integrity of unstable compounds. The modern Hydrodynamic extraction technology is designed to use fresh and whole plants, ensuring these volatile molecules are kept intact.

Another trend is the **use of vegetable oils** such as coconut, canola, sunflower, walnut, palm, or hemp oil for phytocannabinoid extraction, avoiding the use of toxic and hazardous hydrocarbon solvents in addition to the high pressures involved in the supercritical extraction with CO_2 technique. Thus, extraction with vegetable oils or lipids is considered to be solvent-free, safer, and more environmentally friendly compared to traditional extraction methods using butane or alcohols since it can be performed using certified organic solvents and a post-processing step for solvent removal is not needed as the cannabinoid product is diluted in oil . Obtaining an oily cannabinoid product can be convenient since most commercialized CBD products are diluted in an oily medium like hemp seed oil.

The extraction of phytochemicals is a complex process and, till now, progress in extraction of target compounds has been achieved with various levels of performance/effectiveness. It has been demonstrated that by substituting the

conventional solvent extraction techniques with modern strategies such as hydrodynamic extraction technology has led to reduction of extraction time, decrease of solvent consumption, high extraction yields, increased selectivity, fractionation and stability of targeted compounds.

Clean Green Biosystems, Chennai, Tamilnadu, India, has developed a patented technology of extracting highly bio available phytochemicals from fresh herbs, fresh fruits and fresh vegetables including cannabis [140-146]. Hydyne is Innovative new hydrodynamic extraction system that uses the entire fresh plant materials to preserve all the unique phytochemicals and phytonutrients compounds in a full spectrum/broad spectrum extract[140-146]. The bio availability of many of the herbal extracts, nutraceuticals and fruit juices are in the range of 10-35%. This poor bio availability results in poor pharmacological activity which requires high and repetitive dosages. The Clean Green Biosystems, Chennai, Tamilnadu, India patented hydrodynamic extraction process produces highly bioavailable phytochemicals /phytonutrients.

Hydrodynamic cannabis extraction is an innovative new technology that the IASO Inc (USA) corporation has recently introduced to the cannabis industry. According to IASO Inc (USA) **PhytoXTM** is the world's first hydrodynamic extraction system that can process whole, fresh, undried cannabis plants, which maximizes plant utilization, reduces processing costs, and increases yields. The highly innovative system can process freshly-harvested plants into superior extracts. Unlike any other extraction methods, the PhytoX produces a highly bio-available product. The system preserves plant characteristics, and produces oils that are unrivaled for purity, quality and consistency.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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