OPTIMIZATION OF CANNABINOID EXTRACTION CONDITIONS INCLUDING SOLVENT CHOICES USING MICROWAVE AND ULTRASONICATION METHODS

by

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APPRECIATION

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ABSTRACT

Cannabinoid compounds extracted from *Cannabis sativa* have demonstrated high therapeutic potential. Hence, efficient extraction together with reliable quantitative determination of these compounds is crucial for utilization of hemp extract. This research focuses on the optimal extraction conditions for hemp samples, which involves the comparison of ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) using ethanol as a primary solvent.

The effects of varying parameters such as extraction time, temperature, solvent-to-sample ratio, and the ratio of mixed solvents were evaluated to determine their impact on the yield and chemical profile of extracted cannabinoids. The extraction solvents evaluated for UAE and MAE are ethanol, limonene, olive oil, propylene glycol, and glycerol mixed in different volume ratios.

The comparison of cannabinoid yields for MAE and UAE under different extraction conditions including the use of solvent mixtures containing various combinations of ethanol, glycerol, propylene glycol, limonene, and olive oil. The solvent mixtures producing the highest MAE yields in cannabidiol (CBD), delta.8-tetrahydrocanabinol (delta.8-THC) and delta.9-tetrahydrocanabinol (delta.9-THC) were further evaluated for UAE yields using the optimized extraction procedures.

The comparison of the percent yields of cannabinoid compounds between cannabis flower and cannabis stem was also examined.

In conclusion, this study highlights the importance of selecting the appropriate extraction method and conditions to achieve the optimal yield and cannabinoid quality.

The profile of cannabinoid compounds extracted using different solvent mixtures that are generally regarded as safe (GRAS) is discussed.

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INTRODUCTION

1.1 Origin of *Cannabis Sativa*

In recent years, there has been a great deal of attention towards the widespread usage, trafficking, and cultivation of the *Cannabis sativa* plant, more often known as marijuana. First used by humans in 3000 B.C., it is one of the world's earliest cultivated crops.[1-2] The third century B.C. Chinese Emperor Shen Nung is credited with starting both scientific inquiry and the medical use of cannabis. The king recommended "hemp elixir" in his Flora Pharmacopoeia, an old document that is still considered the definitive guide to traditional Chinese medicine. Marijuana's initial intended usage was as an edible oil, beverage, or remedy for religious and medical purposes. In the nineteenth century, Jacques-Joseph Moreau and William Brooke O'Shaughnessy (O'Shaughnessy, 1843), who were treating patients with hashish and cannabis, found the medicinal advantages of cannabis. Patients reported improved appetite, less headaches, and a more restful night's sleep after using these analgesics and myorelaxants.[3]

The golden age of medicinal cannabis was the second half of the nineteenth century and the first part of the twentieth century. Numerous investigations have led to greater incorporation of cannabis into pharmaceuticals manufactured across the world.[4] *C. Sativa* is an annual plant that is dioecious, meaning that it possesses male and female reproductive organs on separate plants. Its leaves are sharply toothed and pinnately veined, and it may grow to a height of around 5 meters.[6] Female plants, which are distinguished by their inflorescences, produce several flower clusters, one large cluster at the plant's crown and countless smaller clusters along each branch. Their trichome glands containing resin is high in phytocannabinoids and terpenoids. Phytocannabinoids are found mostly in the glands of capitate-sessile trichomes and are also in the capitate-stalked trichomes. Swedish scholar Carl von Linné (often known as Carolus Linnaeus) was the first to classify *C. sativa* into its own taxonomic category in 1753.[6] *Cannabis sativa* has been in use for almost 200 years, yet its taxonomic status and evolutionary background are still up for debate.[5-7]

1.2 Cannabis Classification

Cannabis may be divided into numerous groups based on its chemical make-up, behavioral traits, and genetic background. However, each cannabis strain has a unique chemical composition of phytochemicals. Recent research has established that there are primarily three types of cannabis, distinguished largely by their cannabinoid concentration. *Cannabis sativa*, a plant grown for its fiber, is frequently referred to as hemp or industrial hemp. This plant produces cannabinoids, delta-9-tetrahydrocannabinol (9-THC) that has little psychotropic effects. This cannabis strain is confidently marketed as hemp vitamins, cosmetics, and tobacco replacement goods to your customers. They can also be employed in the production of foods and textiles. *Cannabis indica*, which is a broad-leaf plant that originates from the Indian subcontinent as well as Central and Southern Asia and has become a popular source for pharmaceuticals and recreational drugs. Finally, this plant's resin often contains more cannabidiol (CBD) than tetrahydrocannabinol (THC), in comparison to other *Cannabis sativa*.[8]

Cannabis sativa or industrial hemp has significantly more delta-9-tetrahydrocannabinol (9-THC) than *Cannabis indica* or *Cannabis ruderalis*, but significantly less cannabidiol (CBD). The enzyme geranylpyrophosphate:olivatolate geranyltransferase alkylates olivetolic acid with geranyl pyrophosphate to produce cannabigerolic acid (CBGA), a precursor of delta-9-tetrahydrocannabinolic acid (THCA).[7] CBGA is converted into delta-9- tetrahydrocannabinolic acid (THCA), cannabidiolic (CBDA), and cannabichromenic acid (CBCA) by oxidoreductase enzymes found in the trichomes.[9] When cannabis is roasted, dried, or burnt, a nonenzymatic process happens that creates more active chemicals. THC undergoes a series of chemical transformations throughout its metabolism, yielding compounds such delta-8-THC, CBN, and CBNA. Cannabidiol (CBD) and cannabichromene (CBC) may be broken down to produce CBL, CBLA, and cannabigerol (CBG).[11]

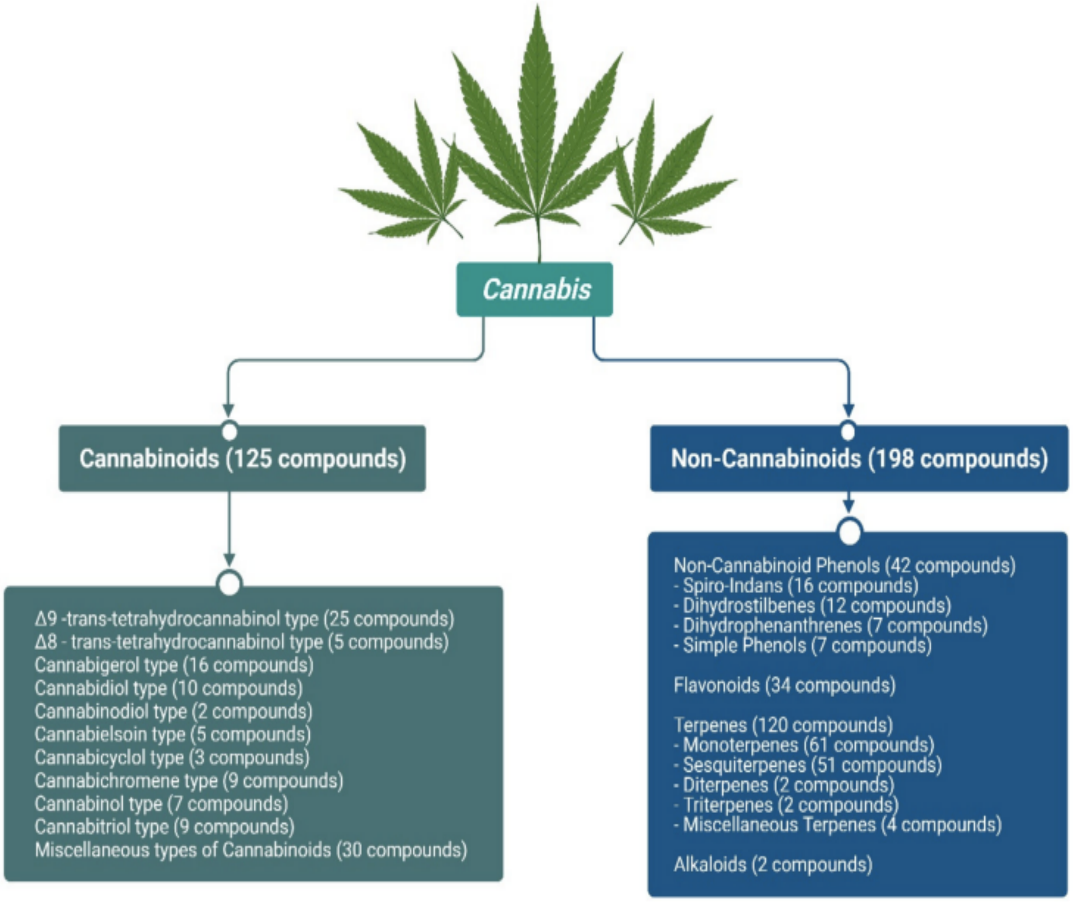


Figure 1.1. Primary Phytochemicals Isolated from Cannabis

1.3 Applications of Cannabinoids

1.3.1 Nutritional and Health Benefits of Hemp

Early in history, people have eaten hempseed in a variety of preparations, including raw, boiled, and roasted. Soluble fiber, protein, vitamins, minerals, and nutrient-rich oil are all abundant in hempseed. Hempseed oil provides various benefits and a pleasant flavor, as reported by Montserrat-De La Paz et al. (2014). Hempseed oil is a kind of vegetable oil that is also a good source of minerals including phosphorus, potassium, magnesium, sulfur, and calcium, as well as the fat-soluble vitamins A and E.

Recent studies have revealed that the polyunsaturated fatty acids (PUFAs) present in hempseed oil can significantly alter plasma fatty acid profiles and alleviate the clinical signs of atopic dermatitis. More than 80% of hempseed oil is made up of polyunsaturated fatty acids (PUFAs)[12]. Among the PUFAs are the 2:1 to 3:1 ratio of omega-6 to omega-3 essential fatty acids (EFAs) seen in these PUFAs is considered appropriate for human metabolism. Tocopherols (up to 0.1%), sterols (up to 0.7%), and phenolic compounds (up to 0.1%) are all dietary antioxidants beneficial to heart health and inflammation reduction.[13]

1.3.2 Application in Cosmetic Industry

Cannabinoids have been found to cure a wide variety of skin conditions, including severe skin diseases, skin cancer, and itching.[14] Hemp oil used directly to the scalp is rumored to nourish and protect hair, boost hair growth, and repair split ends. As a result, hemp oil has been promoted for use in the beauty industry as a hair treatment.

Several retailers on the Internet provide hemp oil shampoos and other hair care products with a negligible concentration of hemp oil, despite the absence of scientific proof.[15]

1.3.3 Applications in Medical Field

Although there are many strains of cannabis, the most well-known ones are hemp and marijuana. The hemp strains of cannabis are the most visible and rapidly growing of all the cannabis species, and contain less of the psychotropic component, THC, than other forms. Hybrids of marijuana with high levels of THC and CBD are being created for medical and recreational use.[16] There are many kinds of cannabis available, including both hybrids and the original species, such as *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis*. Chemotaxonomy classifies cannabis strains according to their relative levels of psychoactive cannabinoids (THC and CBD).[17] Sativa and indica are far more commonly used for producing medicinal and industrial cannabis, but ruderalis is less common due to its small size and low THC concentration.[18] *Cannabis indica*, which is typically used as a sedative for relaxation, is more generally referred to as a "body high" or "couch lock," whereas *Cannabis sativa*, which has moderate psychoactivity, is sometimes referred to as a "head high".[19,20]

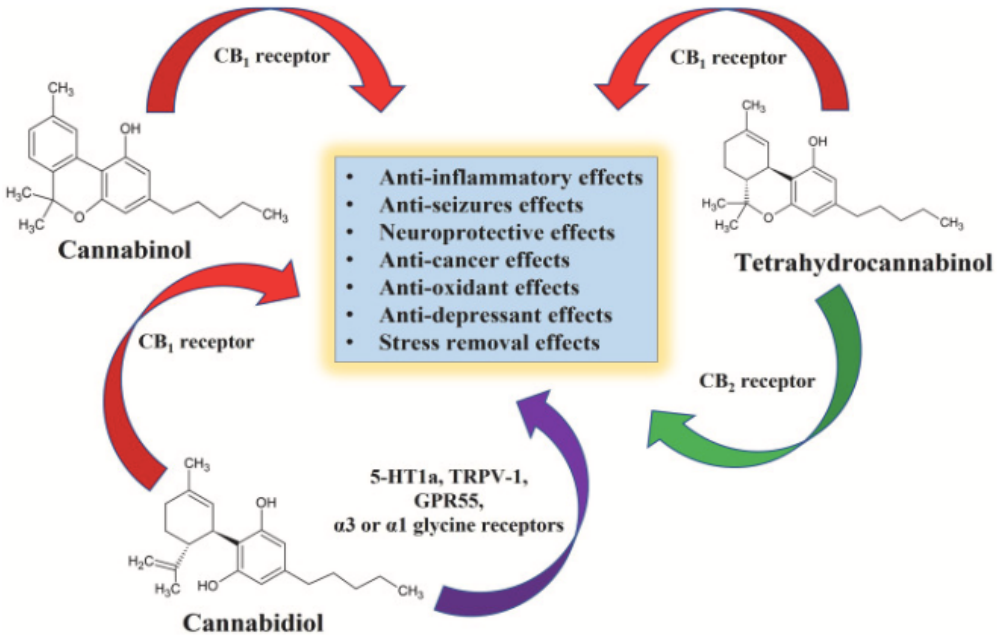


Figure 1.2. Pictorial representation of therapeutic effects of THC, CBD, and CBN interactions with metabolic system bio-receptors.

Cannabinoids, including tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN), generate therapeutic effects by interacting with several bio-receptors in the human body. Delta-3-cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC), the two most popular cannabinoids, are both formed from the CBGA. Identifying and quantifying cannabis at subnanomolar levels has become possible due to recent advances in analytical methodologies.[21]

The minimum effective dose (0.003 mg/mL) of THC, the principal psychotropic lipid, is insoluble in water.[22] To increase their bioavailability, researchers have created several solubility agents and emulsifiers, as well as improved nanoscale drug delivery systems.[23-28] Cannabis can be processed in different methods, and the pharmacological and pharmacokinetic variability decreases.[29,30] Cannabinoids such as cannabigerol (CBG), cannabidiol (CBD), cannabidiol (CBD), cannabielsoin (CBE), cannabicyclol (CBL), cannabinol (CBN), cannabibitriol (CBT), and cannabichromene (CBC) were found and identified using cannabis extracts. Several disorders, including multiple sclerosis , inflammatory bowel disease (IBD) and huntington's disease HD have reacted successfully to drugs produced from THC and CBD, and these targets the 5-HT1A and -2 adrenoreceptors.[31] Even though its major downside is its psychotropic effects, CBD may have applications in the treatment of neuropsychiatric illnesses, cancer, and chronic pain.[32-38] This is achieved by employing a wide range of biological targets and methods. By blocking the Wnt/beta-catenin signaling pathway, the cannabinoid CBE has been shown in recent clinical studies to reduce neuropathic pain.[39] Further analysis identified eleven CBN PCs and nine CBC PCs. The capacity to function as neuroprotective phytocannabinoids (PCs) was found for CBG, CBDV, CBC, CBN, 9-THCV, and 9-THCA in a recent study.[40] There are 42 distinct forms of non-cannabinoid (NC) phenols found in cannabis, including 12 different dihydrostilbenes, 7 different dihydrophenanthrenes, 16 different spiroindans, and 7 different simple phenols.[41] In addition to certain di- and triterpenoids, Radwan et al. studied the separation of 61 monoterpenes (C10) and 51 sesquiterpenes (C15) from cannabis.[41] Recent research has focused on the potential anticancer qualities and far-reaching biological implications of two types of phenolics found in cannabis: terpenes and flavonoids. Several in vitro and in vivo investigations have shown that cannaflavins have several beneficial biological effects, including antiviral, neuroprotective, antiparasitic, antioxidant, and anti-inflammatory properties.[42-44] The leaves and roots of the *C.sativa* prepared in methanol and ethanol were detected in the spermidine alkaloids, such as, cannabisativine and anhydrocannabisativine.[45,46] There is fascinating evidence that cannabis roots can be used as an additional therapy to decrease inflammation and pain, even though cannabis roots are not a very rich source of cannabinoids.[47]

1.4 Influencing Factors on the Efficiency of Phytochemical Extraction from Plant Materials

There is a wide range of approaches to phytochemical extraction from plant material. The main one is seen in Figure 2. Understanding the factors that affect extraction efficiency can help in optimizing the conditions for economically extracting the required components from the plant matrix

(a) Plants: It is crucial to isolate cannabinoids from *C.sativa* strain. Different plant materials may require distinct phytochemical extraction methods due to differences in matrix, structure, and phytochemical composition. Leaves, stems, roots, and flowers can all be picked from the same plant to yield varying concentrations of active chemicals.[48] Furthermore, the efficacy of removing bioactive components from plant material varies on whether the material is fresh, dried, or crushed. Nothing in the literature thus far has addressed the need for improved initial material selection, processing, drying conditions, or particle sizes to affect cannabis bioactive components.[49-50]

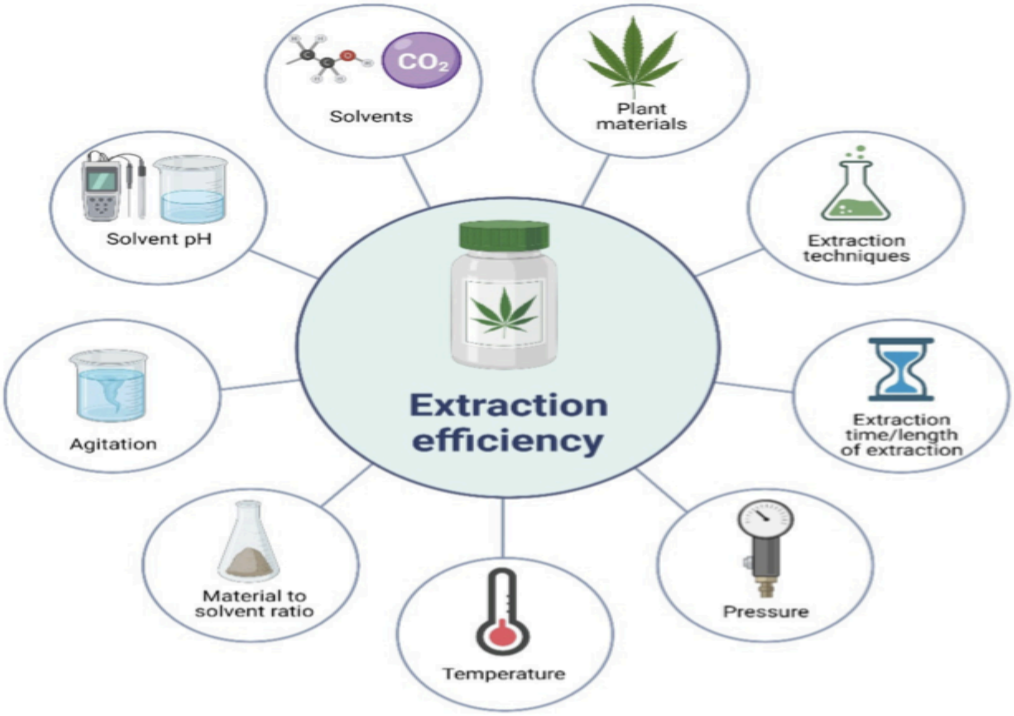


Figure 1.3. Factors Affecting the Efficiency of Phytochemical Extraction from Plant Material

(b) Material to Solvent Ratio: It is well-known that the efficacy of phytochemical extraction from plants is affected by the ratio of plant material to solvent. Both polar and nonpolar compounds can be extracted using a broad of solvents, with the choice ultimately determined by the relative polarity of the solvent and the chemicals in question. Extraction of phytochemicals is enhanced when polar and less nonpolar solvents are used together.[51] Scientists have shown that the efficiency of phytochemical extraction is dependent on the amount of plant material and the solvent combination utilized.

As the amount of solvent utilized decreases, extraction efficiency reduces due to saturation. Adjusting the material-to-solvent ratio can reduce costs even though a larger volume can be heated using the same amount of energy. Water removal during concentration or powder production consumes more energy.[52]

(c) Extracting Temperature and Length of Extraction: Phytochemical extraction efficiency has been demonstrated to vary based on both extraction temperature and extraction time. Increasing both the extraction time and temperature has been demonstrated to improve extraction results. Most phytochemicals are unstable when exposed to heat for an extended time. However, determining the optimal temperature and extraction time allows for the maximum concentration of phytochemicals to be extracted with little loss of quality.[49, 52]

(d) Solvent pH: A more rapid breakdown of cell walls using an acidic solvent increases the efficiency and yield of the extracted chemical. There is evidence that a slightly acidic environment improves the stability of bioactive substances. In order to extract phytochemicals from cannabis, the appropriate solvents, solvent-to-material ratios, and pH must be identified.

(e) Pressure and Agitation: The effectiveness of phytochemical extraction from plant materials is also affected by agitation and pressure. High-pressure extraction has improved this process. Extraction of phytochemicals is shown to be much more effective when agitation is applied as opposed to no agitation.[54, 53]

(f) Extraction Techniques: It has been demonstrated that the extraction processes used have a significant effect on the efficiency with which phytochemicals are recovered from plant sources. The phytochemical extraction of cannabinoids and other cannabinoid-derived substances for food and pharmaceutical industries would be a cost effective for consumers. Both contemporary and traditional techniques can be utilized for extraction.

Traditional and cutting-edge procedures' output efficiencies are primarily determined by their respective input parameters. Therefore, knowing the chemical properties of cannabinoids and plant matrices is essential.[55, 56]

1.5 Extraction Methods of Cannabinoid Compounds

The conventional extraction methods and the advanced extraction methods are the two primary kinds of extraction procedures. Modern extraction techniques usually perform more efficiently than traditional techniques.[57]

1.5.1 Conventional Extraction Method

Cannabinoids have been isolated from cannabis using a variety of traditional extraction methods, including Soxhlet extraction, maceration, and dynamic maceration.[58,59] It has been demonstrated via research that traditional extraction methods have limitations, such as the loss of desirable molecules and because of decomposition of heat-sensitive chemicals during the extraction process.[60]

It has been proven that ethanol is a useful solvent for extracting cannabinoids using hot maceration and Soxhlet extraction. Both methods were effective, but neither came close to the efficiency of microwave-assisted extraction used today.[58-61] There are advantages to using traditional extraction methods, such as their simplicity, low cost, and accessibility. Studies suggest that a continual high temperature during the Soxhlet extraction technique promotes the conversion of THCA to THC, and then to CBN, which explains why there is a lot of both in the extract.[62,63]

In addition, a standard procedure termed dynamic maceration is frequently used to extract cannabinoids from industrial hemp. The dynamic maceration solid-lipid extraction method involves soaking a sample in organic solvents selected according to the polarity of the target chemicals, followed by agitation for a certain amount of time at a predetermined temperature.[63,64] Most often, ethanol, acetonitrile, and hexane are used to extract cannabinoids from cannabis.[65,67] Extraction of neutral cannabinoids by ethanol was shown to be equally efficient as extraction of acidic cannabinoids by hexane, acetone, and methanol in dynamic maceration testing.[64] Interestingly, Romano and Hazekamp found that olive oil is superior to ethanol for extracting cannabis oils like terpenes and cannabinoids.[68] Olive oil extracts of cannabis are known to preserve cannabinoids for significantly longer than ethanolic extracts.[63,69]

1.5.2 Modern Extraction Methods

1.5.2.1 Pressured liquid extraction (PLE)

Pressured liquid extraction (PLE) is a fast and efficient method for extracting organic molecules. It is also known as accelerated solvent extraction (ASE).[70] Because the surface tension and viscosity of the solvent are reduced at higher temperatures, the analyte becomes more soluble, and extraction is made easier.[58] The solubility of the target chemical may be altered by changing the environmental factors such as temperature and pressure.[62] Since the insoluble matrix components are separated out during extraction, filtering is unnecessary. By including this, the process may be automated to guarantee continuous functioning.[64]

1.5.2.2 Supercritical fluid extraction (SFE)

This is a sustainable alternative to traditional extraction methods including pressing and organic solvent extractions. The use of supercritical fluids in these procedures lessens the amount of hazardous waste produced and the impact on ecosystems.[71] Plant material is initially dissolved in a supercritical solvent (often CO_2) for the extraction of essential compounds. The extracted compounds are then used in the manufacturing process. Since at room temperature supercritical fluids are gases, the extract may be easily recovered via evaporation.[72] Selectivity can be affected by changes in temperature, pressure, or both. Selective extraction is possible due to the varying solubilities of various solvents.[73] Low temperatures are preferred because they are more energy efficient and protect heat-sensitive compounds like THC. CO_2 is nonpolar, but in the supercritical state, it can become more polar. When supercritical CO_2 is not polar enough to act as a solvent on its own, other compounds can be added as co-solvents to increase the polarity of the solvent.[74] However, due to the mostly nonpolar nature of CBD and THC, supercritical CO_2 is the preferred solvent.[75]

1.5.2.3 Hydrodynamic cannabis extraction

This is a relatively recent innovation in the cannabis market, allowing to production of full-spectrum extracts with high bioavailability. Some businesses, like IASO (Incline Village, Nevada), have made headlines by claiming they've discovered a secret extraction technique that may provide higher yield and more profitable outcomes. In this alternate technique, fresh plant material is ultrasonically frozen before being used to make a nanoemulsion in water. The cell wall then ruptures, releasing the contents. The subsequent processes include centrifugal separation, liquid-liquid extraction utilizing a solvent, and low-temperature drying. Cannabinoids are highly volatile and acidic; thus, the plant material must be frozen to preserve them for subsequent processing. Hydrodynamic extraction is favored over more conventional approaches because it requires neither high temperatures nor lengthy periods of contact distillation, and because it employs fewer organic solvents. Researchers observed that both the availability and the amount of lycopene and other carotenoids extractable from tomatoes using this technique had greatly increased.[76,77]

1.5.2.4 Ultrasonic-assisted extraction (UAE)

This is a novel method that uses high-frequency sound waves (20-100 kHz) to help with the extraction process. This allows solvents to extract the necessary components from a sample matrix, often by cavitation. Cavitation is the process of bubble creation, development, and collapse in a solution that facilitates rapid solvent entry into the cellular structure and high rates of mass transfer.[58] Because of micro-mixing and quicker energy transmission, Ultrasonic-assisted extraction (UAE) has a higher mixing efficiency.[77] Several variables, such as sample moisture, particle size, milling intensity, solvent, temperature, pressure, and sonication time, must be considered and adjusted for optimal extractions.[58]

De Vita et al. (2018) looked at many commercial hemp and medical cannabis extraction processes to compare cannabinoid changes. The experiment using ethanol as a solvent showed that ultrasonication for 50 minutes at 60°C produced the most cannabis.[78] Total THC and CBD extracted were only marginally greater under ideal circumstances compared to controls prepared by refluxing ethanol for 50 minutes at 90°C. Despite the decreased yield, the ultrasonication method is safe, energy-efficient, and ecologically good because it uses less heat. The yield of the ethanol extract was also found to be three to four times greater than that of the olive oil extract, according to this investigation.[79] To get the most out of the normal extraction techniques, using UAE as a conditioning step first would be ideal. UAE was utilized to boost crude lipid production before Soxhlet extraction by around 24% without lowering extract quality.[64]

1.5.2.5 Microwave-assisted Extraction (MAE)

Microwave-assisted Extraction was developed in 1980 in response to the increased need for environmentally benign and long-lasting industrial operations.[77] Rapid heating occurs in the material as a result of ionic conduction and dipole rotation when electromagnetic radiation in the form of microwaves is applied with frequencies between 300MHz to 300GHz.[58] The contents of cells may be liquefied by exposing them to a microwave field, where the energy absorbed by the molecules is transformed into kinetic energy. In addition, greater surface equilibrium, greater mass transfer, and better solubility all contribute to the efficacy of this eco-friendly extraction method. When put together, these factors allow for the rapid, efficient, and low-energy production of a high purity extraction with little solvent utilization.[80] The effects of solvent, temperature, ramping time, and duration were studied by De Vita et al. (2018). When compared to the standard method, which was obtained by ethanol reflux at 90°C for 50 minutes, the extraction yield of CBD increased by a factor of at least four-fold, when the temperature and time were increased (De Vita et al., 2020). Appropriate extraction techniques must be applied for the extraction of neutral phytocannabinoids, such as THC 5 and cannabidiol 6, which have therapeutic benefits. Examining the degree of decarboxylation of phytocannabinoid acids can shed light on the techniques used to extract neutral cannabinoids. When comparing the effectiveness of several separation techniques, Lewis-Bakker et al. discovered that MAE produced the highest quantity of neutral cannabinoids. Over ninetynine percent of acidic cannabinoids were observed to be decarboxylated (>130ºC) during MAE, according to the research.[61]

1.6 Limitations of Conventional Methods on Cannabinoid Compounds

- Maceration with alcohol or water: Compared to MAE or UAE procedures, the maceration method is ineffective and produces a relatively low extraction yield of the desired cannabinoids.

- Soxhlet-accelerated solvent extraction: This approach is less effective than MAE or UAE procedures because the temperature varies on either side of the Soxhlet extractor.

- Supercritical fluid extraction: Apart from using ethanol (a polar solvent) as a co-solvent during the extraction process, supercritical fluid extraction only extracts nonpolar cannabinoids.

- All the methods require more solvent, have longer extraction processes, and are less environmentally friendly compared to MAE and UAE.

1.7 Objectives of Current Research

- Optimizing cannabinoid extraction conditions including solvent mixture choices for microwave and ultrasonication methods.
- Analyzing cannabis stem and bud using attenuated total reflectance (ATR)-FT-IR and GC-MS to examine variations in their infrared spectra and chromatograms, respectively.

- Comparison of percent yield of cannabinoid compounds between cannabis flower and cannabis stem.
- Studying and evaluating changes in chemical profile of cannabinoid compounds of cannabis extract under various sample preparation and analytical conditions.

CHAPTER 2

MATERIALS AND METHOD

Hemp plants grown in Tennessee and produced producing no more than 0.3% THC as regulated by the Tennessee Department of Agriculture (TDA) are purchased from a Tennessee-approved hemp grower. Cannabinoid standards including Delta-9-Tetrahydrocannabinol (9-THC), Cannabicromene (CBC), Cannabidiol (CBD), Delta-8-Tetrahydrocannabinol (8-THC), Dronabinol (THC), and Cannabinol (CBN) were purchased from Cerilliant Corporations, Round Rock, TX.

Cannabigerol (CBG) from Chiralix Catalog, Nijmegen, Netherlands, Cannabielsoin (CBE) was bought from 1st Scientific, San Diego, CA, and Cannabicylol (CBL) was bought from Sigma-Aldrich in St. Louis, Missouri. All these standards are in a methanol solvent at a concentration of 1 mg/mL. Pure olive oil (Kroger Co., Cincinnati, OH), Propylene glycol high purity (manufactured for VWR, AMRESCO LLC, Fountain Parkway Solon, OH), d-Limonene high purity food grade (Santa Monica, CA), Glycerol 99+% (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA), and ethanol 200 proof ACS/USP grade (Greenfield Global, Brookfield, CT) were utilized as the extraction solvents. Supel™ QuE 55286-U, PSA/C18/ENVI-Carb Tube (purchased from Sigma-Aldrich, St. Louis, MO) was used for eliminating chemical interferences and impurities and iso-Octane, pesticide grade was from Fisher Scientific, Fair Lawn, New Jersey.

Cannabis phytochemicals were extracted using an Antor Paar Microwave MW5000 and a Hielscher UP200St (200W, 26kHz) ultrasonic processor (Hielscher Ultrasound Technology, Teltow, Germany). Thermo Scientific iS50R ATR-FT-IR was used for determining wavenumbers of cannabidiol (CBD) being absorbed for cannabis stem and cannabis bud.

The extracted samples were analyzed using a Shimadzu QP2010S Gas Chromatography-Mass Spectrometry (GCMS) outfitted with an autosampler.

2.1 Microwave-Assisted Extractions

2.1.1 Sample Preparation of Cannabis Bud and Cannabis Stem for Extraction: Dry cannabis buds and stems were separately homogenized with a Nutri Ninja Auto iQ Blender BL482 1000w for a duration of 20 minutes. Using a Mettler Toledo weighing balance (Monobloc Weighing Technology, Columbus, OH) for the extraction experiments, 0.3000 ± 0.0062 g of dry hemp plant (*Cannabis sativa*) of a Tennessee-approved hemp grower was weighed and placed into a microwave vial containing 30.00 ± 0.31 mL ethyl alcohol. This procedure was repeated for the extraction of cannabinoid compounds with different solvent mixtures.

2.1.2 Solvent Mixtures Preparation: Cannabinoids were also extracted using a mixture of high-quality propylene glycol, glycerol (99+%), d-Limonene (also high purity and food grade), and pure olive oil. All these solvents are considered GRAS (generally regarded as safe) by the Food and Drug Administration (FDA). The solvent mixtures each totaling 3.5000 ± 0.0051 mL were measured using a Kimax kimble USA graduated cylinder, and contained either propylene glycol and ethanol, limonene and ethanol, glycerol and ethanol, or limonene and olive oil at ratio ranging from 20:80 to 80:20

respectively. Solvent mixtures including 3 different solvents (Ethanol, water, and glycerol) were also prepared before extraction. Samples prepared for microwave-assisted extraction were vortexed for 5 minutes with Vortex-Genie 2, while the samples prepared for ultrasonic-assisted extraction were sonicated so that the solvent mixture was all miscible.

The table below shows the various solvent mixtures prepared in different proportions.

Table 1: Solvent Mixtures and their Percentage compositions

Solvent Mixtures	Percentage Composition
Propylene glycol: Ethanol Glycerol: Ethanol Limonene: Ethanol	20:80
Propylene glycol:Ethanol Glycerol:Ethanol Limonene:Ethanol	40:60
Propylene glycol:Ethanol Glycerol:Ethanol Limonene:Ethanol Limonene:Olive oil	50:50
Propylene glycol:Ethanol Glycerol:Ethanol Limonene:Ethanol Limonene:Olive oil	60:40
Glycerol:Ethanol Limonene:Ethanol	70:30
Propylene glycol:Ethanol Glycerol:Ethanol Limonene:Ethanol Limonene:Olive oil	80:20
Glycerol:Ethanol	90:10

Limonene:Ethanol	
Ethanol:H_2O:Glycerol	70:10:20
Ethanol:H_2O:Glycerol	40:40:20

2.1.3 Microwave-assisted Extraction of Cannabinoids Using Ethanol as Solvent: The Antor Paar Microwave MW5000 was used to extract cannabinoids from cannabis buds and stems by heating them in microwave vials sealed with microwave-cap inserts and microwave-screwcaps to prevent the loss of organic compounds. Triplicate microwave extractions were performed, with each vial containing either cannabis bud or cannabis stem. Each vial is placed in a predetermined location on the microwave plate identical to the placement of the other microwave vials. To compensate for the solvent loss during extraction, the raw extracts volume was measured after the microwave run and if necessary, the volume is top-up with ethanol to a final volume of 3.5mL. The extraction conditions for microwave-assisted extraction of hemp using ethanol are shown in the Table 2

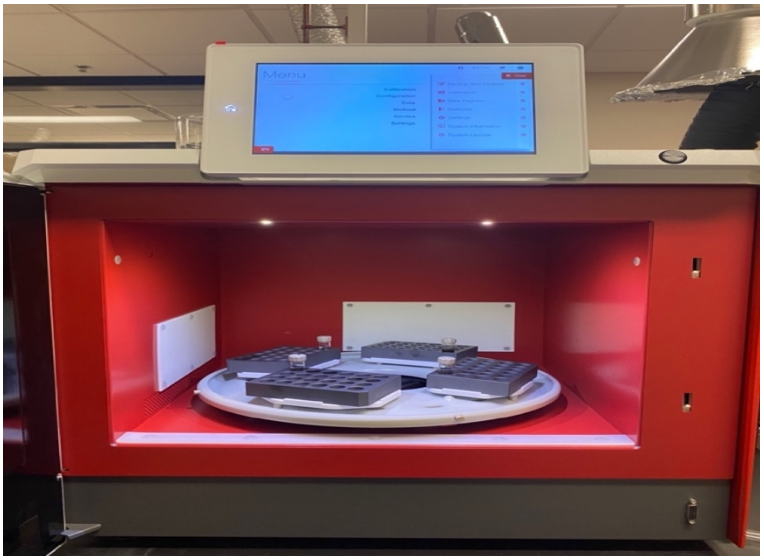


Figure 2.1. Microwave-assisted Extraction Set-Up

Table 2: Microwave Extraction Conditions for Cannabis Plant Material in Extraction Solvent

Properties	**Conditions**
Power	260 watts
Total Time	30 minutes
Cooling Temperature	55 °C
IR Temperature	70 °C
Cooling fan level	3
Stirrer	High

2.1.4 Microwave Extraction of Cannabis Bud Using Solvent Mixtures: The Antor Paar Microwave MW5000 was used to extract cannabinoids from cannabis buds prepared in different volume ratios of propylene glycol and ethanol, limonene and ethanol, glycerol and ethanol, or limonene and olive oil. Microwave vials were sealed with microwave-cap inserts and microwave-screwcaps to prevent the loss of volatile organic compounds. All microwave extraction with each vial were performed in quadruplicate in a predetermined location on the microwave plate, identical to the placement of the other microwave vials. The same extraction conditions were used for the extraction of hemp with ethanol solvent shown in Table 1.

2.2 Ultrasonication-assisted Extractions

2.2.1 Ultrasonication-assisted Extraction of Cannabinoids Using Ethanol as Solvent: Using a Mettler Toledo weighing balance (Monobloc Weighing Technology, Columbus, OH) for the extraction experiments, 2.5000 ± 0.0062 g of homogenized dry cannabis bud (*Cannabis sativa*) of a Tennessee-approved hemp grower was weighed and placed into a 100 mL flask containing 30.0000 ± 0.0051 mL ethyl alcohol.

The ethyl alcohol containing the homogenized cannabis bud sample was parafilmed to prevent the loss of volatile organic compounds and a small opening was made in the middle of the parafilm to allow the insertion of the ultrasonic probe (S26d7d probe) into the flask.

2.2.2 Ultrasonication-assisted Extraction of Cannabinoids Using Solvent Mixtures:

The cannabinoid compounds were extracted using the same procedure from ultrasonication-assisted extraction with ethanol. Different solvent mixtures including glycerol:ethanol, limonene:ethanol, and ethanol:H2O:glycerol were prepared in volume ratios of 10:90, 10:90, and 70:10:20, respectively.

The sample for cannabinoid extraction with limonene and ethyl alcohol solvent mixture was prepared with a mass of 1.0000 ±0.0062 g of homogenized dry cannabis bud weighed into a 100 mL flask holding 30.0000 ±0.0051 mL of limonene and ethyl alcohol before the extraction.

2.3 Chlorophyll Removal Procedure: The raw extracts from both the microwave and ultrasonication were treated with 0.0625g of Supel^{TM} Que 55286-U (Sigma-Aldrich) per 1.5 mL. The vials holding the samples and Supel^{TM} Que 55286-U were vortexed until the sample and the Supel^{TM} Que in the tube were equally saturated, and then centrifuged in a Fisher Scientific Micro Centrifuge (Model 235C) for 5 minutes at 3000 r.p.m. A 1mL aliquot of the extract was filtered through a polytetrafluoroethlene (PTFE) 0.45-microns syringe filter before being analyzed on GC-MS.

2.4 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Hemp Extracts

2.4.1 Analysis of Ethanol Extracts from Cannabis Bud and Cannabis Stem: Chlorophyll-free ethanol extracts of cannabis bud and cannabis stem were diluted with ethanol in a volume ratio of 1:1 prior to GC-MS analysis using a Shimadzu QP2010S Gas Chromatography-Mass Spectrometry (GCMS) with an autosampler. Triplicate extracts of both the cannabis bud and the cannabis stem were analyzed by GC-MS.

Figure 2.2. Analysis of Cannabis extract with Shimadzu QP2010S Gas Chromatography-Mass Spectrometry (GC-MS) with an autosampler

2.4.2 Analysis of Solvent Mixture Extracts from Cannabis Bud: Chlorophyll-free solvent mixture extracts of cannabis bud were diluted with ethanol in a volume ratio of 1:1 prior to GC-MS analysis using a Shimadzu QP2010S Gas Chromatography-Mass Spectrometry (GCMS) with an autosampler. The limonene and olive oil extract were diluted with iso-octane, pesticide grade (Fisher Scientific), at a 1:1 volume ratio since they were not miscible with ethanol. Shimadzu QP2010S Gas Chromatography-Mass Spectrometry (GC-MS) with an autosampler was used to analyze the solvent mixture extracts from the cannabis bud in triplicate or quadruplicate. The optimum conditions for the GC-MS analysis of both cannabis bud and cannabis stem are shown in the table below.

Table 3. GC-MS Conditions for the Analysis of Cannabis Bud and Stem Extracts

Properties	**Conditions**
Column	Phenomenex ZB-30, 30.0 m x 0.25 µm x 0.25 mm
Column Oven Temperature	55.0 °C
Injection Temperature	270.0 °C
Injection Mode	Splitless
MS Detector Voltage	Relative to the Tuning Result (0kV)
Split Ratio	40:1
Pressure	9.2 psi
Total Flow	48.2 mL/min
IonSource Temperature	260.0 °C
Linear Velocity	38.2 cm/sec
Interface Temperature	300.0 °C
Solvent Cut Time	2.5 min
MS Acquisition Mode	Scan
Mass Scan Range (m/z)	35 amu to 350 amu
Column Flow	1.10 mL/min
Oven Temperature Program	Rate. Temp. (°C) Hold Time (min)
	- 55.0 1.00
	18.00 100.0 8.00
	40.00 300.0 15.00

2.5 Infrared Spectroscopy on Cannabis Bud and Cannabis Stem: The cannabis stem and homogenized buds were analyzed independently by means of the Thermo Scientific iS50R ATR-FT-IR. Prior to analyzing the cannabis bud and stem, the ATR compartment's diamond crystal station was cleaned with methanol, both before and after sample collection. A small amount of homogenized dry cannabis sample was transferred to the (ATR)-FT-IR diamond crystal station, where it was secured with a knob to ensure that the anvil was touching the sample directly.

The conditions for the collection of infrared spectra for cannabis stem and cannabis bud is shown in Table 4.

Table 4: Infrared Parameter Conditions for Cannabis Bud and Cannabis Stem

Parameters	Settings
Number of Scans	30
Resolution	0.25 cm^{-1}
Apodization	Happ-Genzel
Wave number range	400-4000 cm^{-1}

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Data Analysis: Gas Chromatography-Mass Spectrometry (GC-MS) Results:

The need of selecting a green, sustainable solvent is important for the extraction of cannabinoid compounds, which this research was able to achieve by making different solvent mixtures containing solvents that are regarded as safe by the Food and Drug Admnistration (FDA) including propylene glycol, d-limonene, glycerol and olive oil, as compared to the previous cannabinoid extraction solvents, such as; hexane, ethanol, isopropyl alcohol and methanol.

Szalata et al (2022) reported the use of UAE and 96% ethanol, the maximum CBD level of 2.682% was achieved, as compared to the CBD yield in this research using UAE with 100% ethanol, and 90% ethanol + 10% limonene which gave a maximum yield of 3.50 and 5.57 %w/w respectively.

It is noteworthy to state the advantages ad the disadvantages of MAE and UAE methods as decribed by López-Olmos et al (2022).

MAE method is known for its speed, energy efficiency, higher yied of extracted cannabinoids and potentially good for selective extraction, while its disadvantages includes, equitment cost, scaling up, temperature control.

UAE methods has low environmental impact, effective for extracting a range of compounds, increases the surfaces area of extraction solvents through cavitation. The drawbacks of this method includes, equipment cost, potential for emulsification and scaling up.

In summary, there are benefits and drawbacks to both MAE and UAE. The objectives of the extraction, the characteristics of the compounds of interest, and the available resources are all potential determinants of the decision between the two methods.

Various researchers suggest that environmental variables including light intensity, oxygen levels, and heat affects the amount of cannabinoid compounds present in *Cannabis sativa.* Lazarjani et al (2021) and Fairbairn et al (2011) reported that Cannabinoids can be degraded by extended exposure to light or high temperatures. However, it is noteworthy that the amount of cannabinoids extracted in this research was reduced due to storage time of *Cannabis sativa* material.

3.1.1 Microwave-assisted Extraction versus Ultrasonication-assisted Extraction Using Ethanol as Solvent: The ultrasonication and microwave method both gives similar chromatograms of extracted cannabinoids from cannabis buds at retention times that are approximately comparable. The eight cannabinoid peaks identified by the NIST library are shown in the table below.

The overlay chromatograms of cannabis bud for MAE and UAE are shown in Figure 3.1

Table 5: GC Retention Characteristics of Extracted Cannabinoid Compounds

Retention Time (min)	**Retention Indices (iu)**	**Cannabinoid Compounds**
16.955	2475	Delta.8-tetrahydrocannabinol (Δ8-THC)
17.138	2486	Cannabichromene (CBC)
17.338	2256	Cannabicylol (CBL)
17.419	2605	Cannabidiol (CBD)
17.647	2475	Delta.9-tetrahydrocannabinol (Δ^9-THC)
17.771	N/A	Cannabielsoin (CBE)
17.867	2610	Cannabigerol (CBG)
17.086	2582	Cannabinol (CBN)

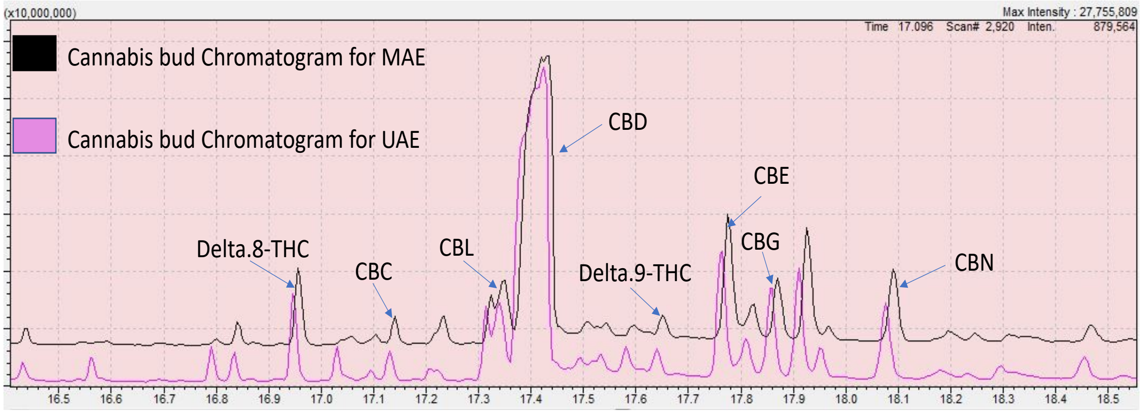


Figure: 3.1 Overlaid GC-MS Chromatograms of Cannabinoids from Cannabis bud with Ethanol Using Microwave-assisted Extraction and Ultrasonication-assisted Extraction

The chromatogram shows that the cannabinoid peaks elute at similar retention times in MAE and UAE.

The eluted peaks at retention times 16.56, 16.78 and 17.03 min are aliphatic hydrocarbon (hexadecane).

3.1.2 GC-MS Chromatogram Data Comparison for Cannabis Bud and Cannabis Stem Using Ethanol as Solvent: The ultrasonication and microwave methods both give similar chromatograms of cannabis bud and stem extract. The cannabinoid peaks of CBD identified by the NIST library at retention times are approximately comparable. The CBD concentration for cannabis bud and cannabis stem are 1,010 ppm and 328 ppm, respectively.

CBD peak was only identified Cannabis stem chromatogram. The overlay chromatograms of cannabis bud and cannabis stem are shown in Figure 3.2

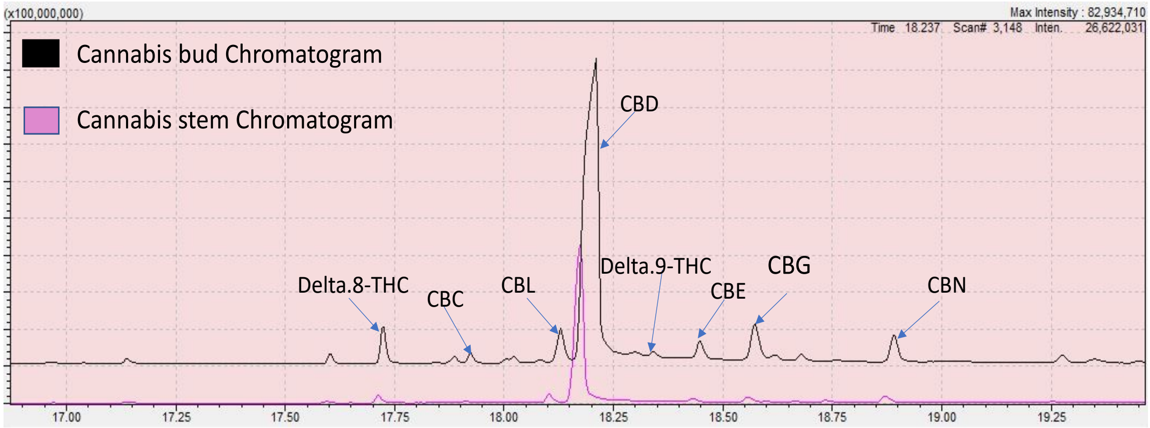


Figure: 3.2 Overlay GC-MS Chromatograms Plot of Cannabinoids from Cannabis bud and Cannabis stem in Ethanol Using Microwave-assisted Extraction and Ultrasonication-assisted Extraction

3.1.3 Data Analysis: Infrared Spectroscopy (ATR-FTIR) Results: At wavenumber 1430.04 cm^{-1}, it was observed that the peak area of CBD in cannabis bud is twice that of cannabis stem as confirmed on the GC-MS.

The comparison (ATR-FTIR) results of cannabis bud and cannabis stem are shown in Figure 3.3, while the reference IR spectra for CBD are shown in Figure 3.4

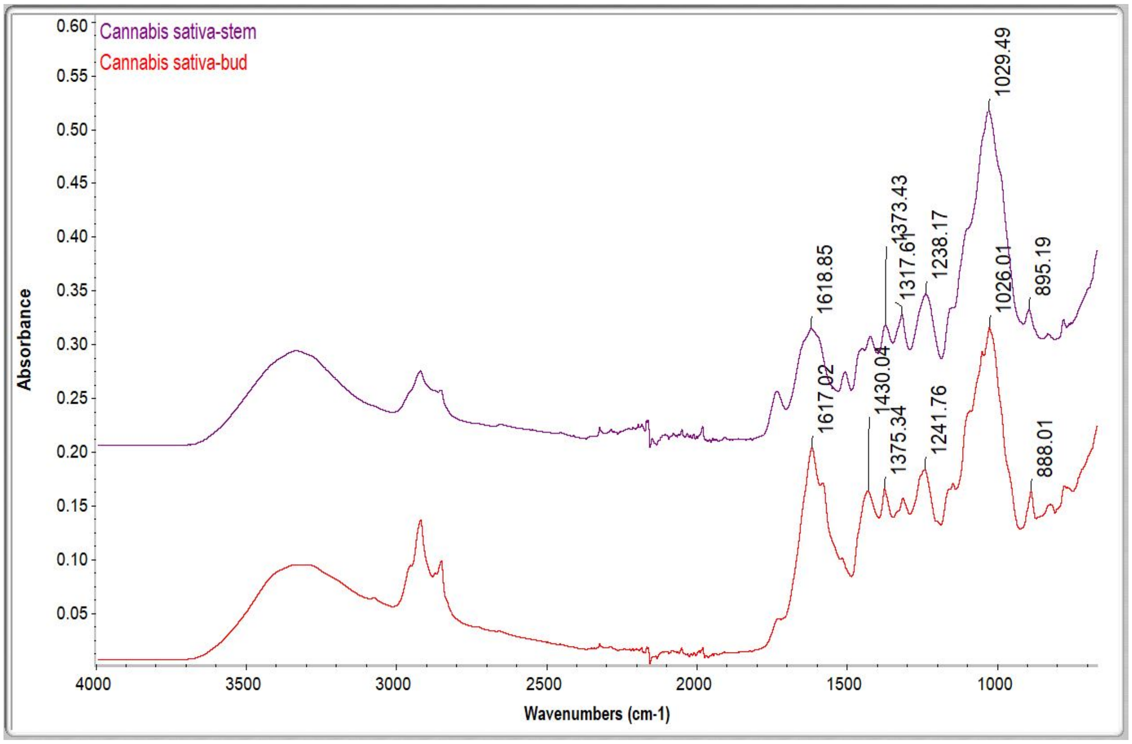


Figure 3.3 Infrared Spectra of Cannabis Bud and Cannabis Stem Analyzed in Attenuated Total Reflectance Mode

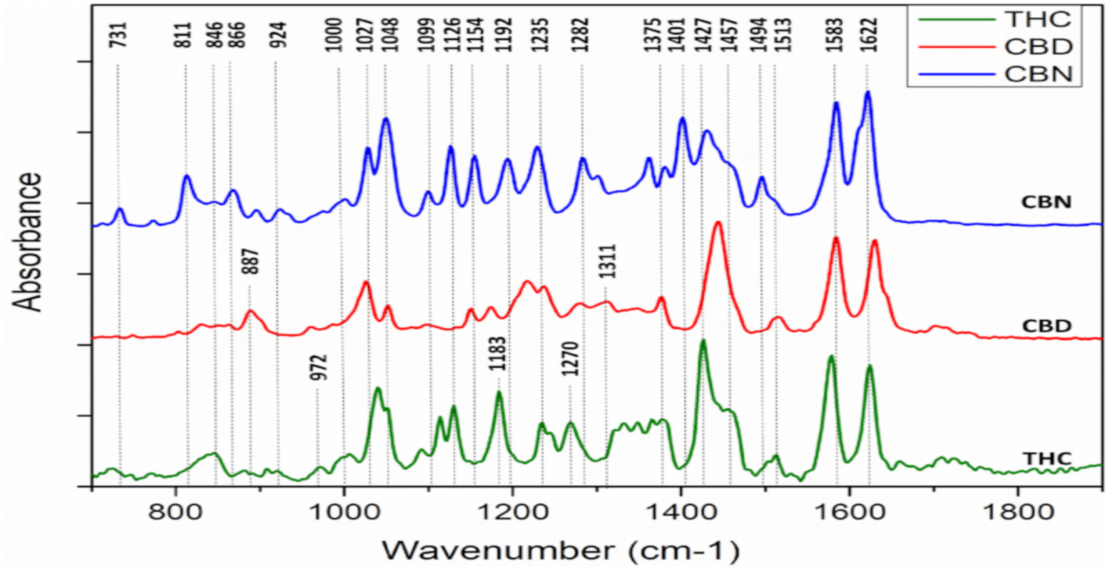


Figure 3.4 Infrared Reference Spectra of Three Cannabinoid Compounds

3.1.4 Percentage Comparison of Microwave Extracted Cannabinoids Using Glycerol and Ethanol Solvent Mixture: The optimal solvent mixture proportion for microwave-assisted extraction is 10% glycerol + 90% ethanol, extracting CBD and delta 9-THC with a percentage composition of 4.10% w/w and 0.09% w/w respectively from *Cannabis sativa* plant.

A logarithmic plot of cannabinoid concentrations (ppm) versus combinations of glycerol and ethanol at varying percentages is shown in Figure 3.5

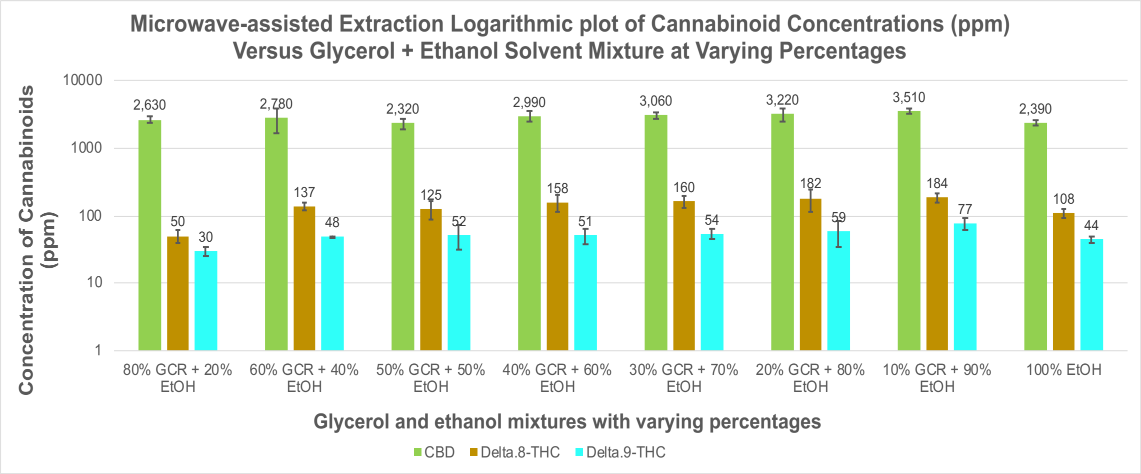


Figure 3.5 Microwave-assisted Extraction Logarithmic plot of Cannabinoid Concentrations (ppm) Versus Glycerol + Ethanol Solvent Mixture at Varying Percentages.

The concentration of cannabinoid axis (y-axis) is in a logarithmic scale. The numbers on the bar chat represent the actual concentrations (ppm) of the cannabinoid compounds.

3.1.5 Percentage Comparison of Microwave Extracted Cannabinoids Using Limonene and Ethanol Solvent Mixture: The optimal solvent mixture proportion for microwave-assisted extraction is 10% limonene + 90% ethanol, extracting CBD and delta 9-THC with a percentage composition of 3.50% w/w and 0.07% w/w respectively from *Cannabis sativa* plant.

However, as compared to the percentage composition of extracted cannabinoids using 10% limonene + 90% ethanol solvent mixture, 30% limonene + 70% ethanol solvent mixture have close percentage weight values of 3.48% and 0.09% for both CBD and delta.9-THC respectively.

A logarithmic plot of cannabinoid concentrations (ppm) versus combinations of limonene and ethanol at varying percentages is shown in Figure 3.6

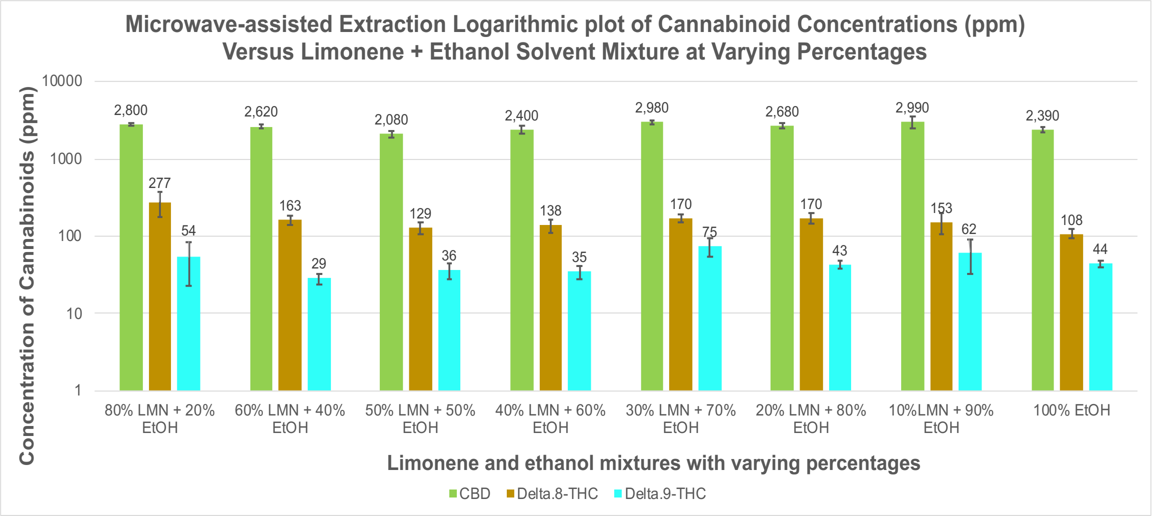


Figure 3.6 Microwave-assisted Extraction Logarithmic plot of Cannabinoid Concentrations (ppm) Versus Glycerol + Ethanol Solvent Mixture at Varying Percentages.

The concentration of cannabinoid axis (y-axis) is in a logarithmic scale. The numbers on the bar chat represent the actual concentrations (ppm) of the cannabinoid compounds.

3.1.6 Comparison of Percentage Yield of Cannabinoids in Ethanol and Glycerol + Ethanol Solvent Mixture Using Different Extraction Methods: Using the cannabinoid percentage composition (%w/w), the table below shows that the extracted cannabinoids for both MAE and UAE methods is higher in 90% ethanol + 10% glycerol solvent mixture as compared with ethanol (primary solvent). The table below also shows that UAE is more efficient for the extraction of cannabinoid compounds as compared to MAE for either ethanol or 90% ethanol + 10% glycerol.

A plot of cannabinoid concentrations (ppm) versus extracted cannabinoids in ethanol and 90% ethanol + 10% glycerol using different extraction methods is shown in the Figure 3.7

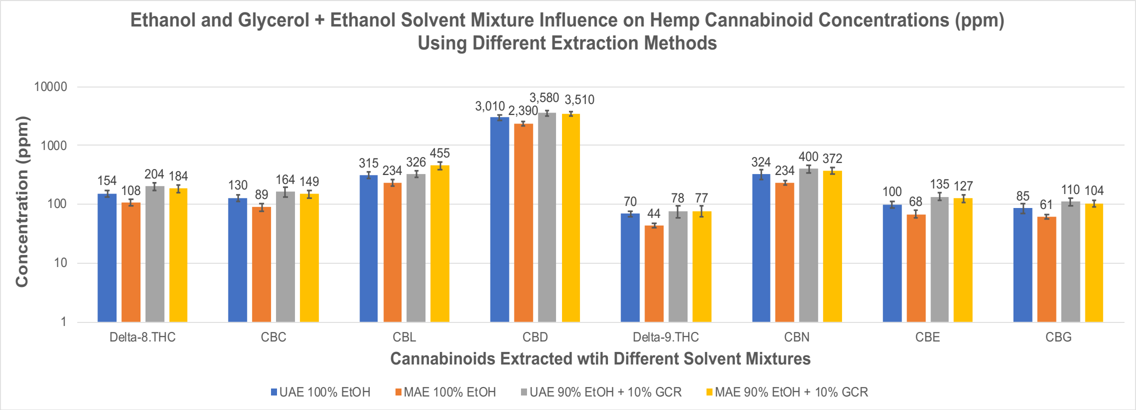


Figure 3.7 Plot of cannabinoid concentrations (ppm) versus extracted cannabinoids in ethanol and 90% ethanol + 10% glycerol using different extraction methods.

The concentration of cannabinoid axis (y-axis) is in a logarithmic scale. The numbers on the bar chat represent the actual concentrations (ppm) of the cannabinoid compounds. The standard deviation error for the CBD and delta.9-THC in solvent mixtures using different extraction methods is shown in table 10.

Table 6: Percentage Composition of Extracted Cannabinoids (%w/w) in Ethanol and Glycerol + Ethanol Solvent Mixture Using Different Extraction Methods

Solvent	**Cannabinoid Percentage Composition (%w/w)**			
	UAE		MAE	
	CBD	Delta.9-THC	CBD	Delta.9-THC
100% Ethanol	3.50 ± 0.37	0.08 ± 0.009	2.80 ± 0.24	0.05 ± 0.005
90% Ethanol + 10% Glycerol	4.18 ± 0.43	0.09 ± 0.02	4.10 ± 0.39	0.09 ± 0.02

3.1.7 Comparison of Percentage Yield of Cannabinoids in Ethanol and Limonene + Ethanol Solvent Mixture Using Different Extraction Methods

Using the cannabinoid percentage composition (%w/w), the table below shows that the extracted cannabinoids for both MAE and UAE methods is higher in 90% ethanol + 10% limonene solvent mixture as compared with ethanol (primary solvent). The table below also shows that UAE is more efficient for the extraction of cannabinoid compounds as compared to MAE for either ethanol or 90% ethanol + 10% limonene.

A plot of cannabinoid concentrations (ppm) versus extracted cannabinoids in ethanol and 90% ethanol + 10% limonene using different extraction methods is shown in Figure 3.8

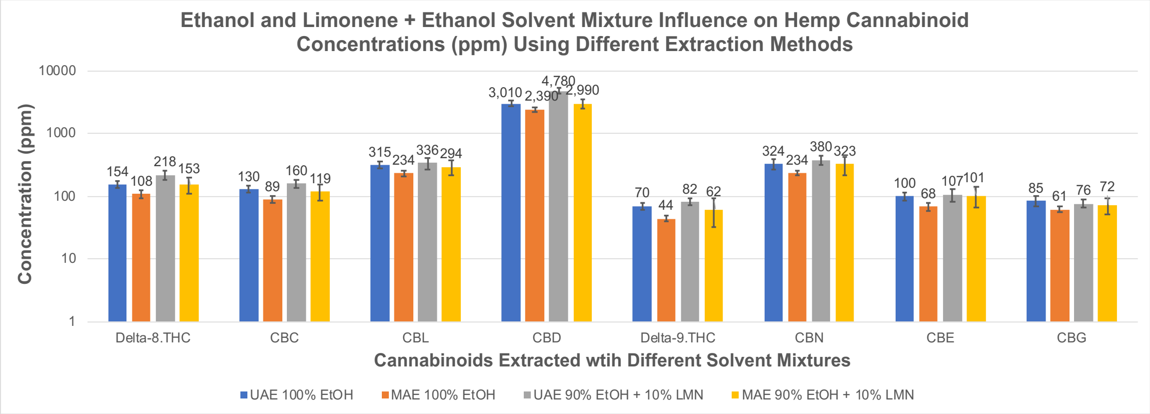


Figure 3.8 Plot of cannabinoid concentrations (ppm) versus extracted cannabinoids in ethanol and 90% ethanol + 10% limonene using different extraction methods

The concentration of cannabinoid axis (y-axis) is in a logarithmic scale. The numbers on the bar chat represent the actual concentrations (ppm) of the cannabinoid compounds. The standard deviation error for the CBD and delta.9-THC in solvent mixtures using different extraction methods is shown in table 10.

Table 7: Percentage Composition of Extracted Cannabinoids (%w/w) in Ethanol and Limonene + Ethanol Solvent Mixture Using Different Extraction Methods

Solvent	**Cannabinoid Percentage Composition (%w/w)**			
	UAE		MAE	
	CBD	Delta.9-THC	CBD	Delta.9-THC
100% Ethanol	3.50 ± 0.37	0.08 ± 0.009	2.80 ± 0.24	0.05 ± 0.005
90% Ethanol + 10% Limonene	5.57 ± 0.52	0.10 ± 0.01	3.50 ± 0.64	0.07 ± 0.03

3.1.8 Comparison of Percentage Yield of Cannabinoids in Different Solvent Mixtures Using Microwave-assisted and Ultrasonication-assisted Extraction Methods: Using the cannabinoid percentage composition below, UAE is more efficient for cannabinoid extraction in both 90% ethanol + 10% glycerol and 90% ethanol + 10% limonene.

A plot of cannabinoid concentrations (ppm) versus extracted cannabinoids in 90% ethanol +10% glycerol and 90% ethanol + 10% limonene using different extraction methods is shown in Figure 3.9

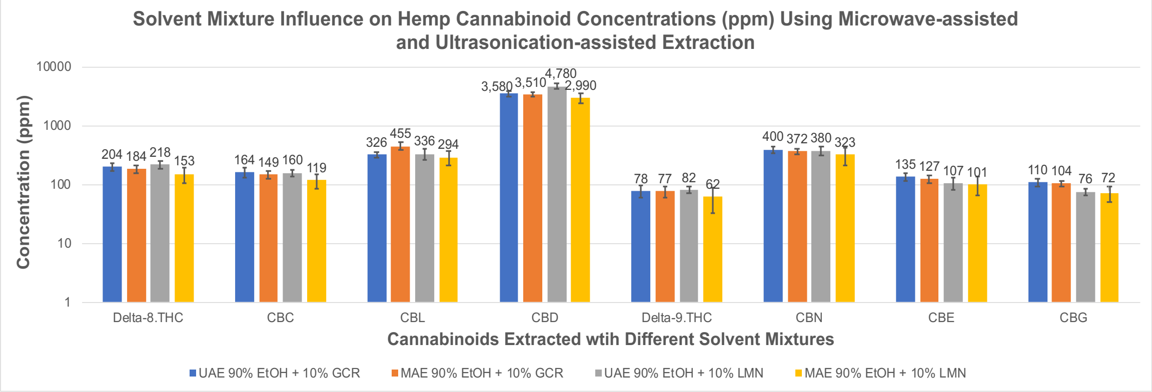


Figure 3.9 Plot of cannabinoid concentrations (ppm) versus extracted cannabinoids in different solvent mixtures using microwave-assisted and ultrasonication-assisted extraction methods.

The concentration of cannabinoid axis (y-axis) is in a logarithmic scale. The concentration of cannabinoid axis (y-axis) is in a logarithmic scale. The numbers on the bar chat represent the actual concentrations (ppm) of the cannabinoid compounds. The standard deviation error for the CBD and delta.9-THC in solvent mixtures using different extraction methods is shown in Table 10.

Table 8: Percentage Composition of Extracted Cannabinoids (%w/w) in Ethanol + Glycerol and Limonene + Ethanol Solvent Mixtures Using Different Extraction Methods

Solvent	**Cannabinoid Percentage Composition (%w/w) (Average ± 1 standard deviation)**			
	UAE		MAE	
	CBD	Delta.9-THC	CBD	Delta.9-THC
90% Ethanol + 10% Glycerol	4.18 ± 0.43	0.09 ± 0.02	4.10 ± 0.39	0.09 ± 0.02
90% Ethanol + 10% Limonene	5.57 ± 0.52	0.10 ± 0.01	3.50 ± 0.64	0.07 ± 0.03

3.1.9 Comparison of Percentage Yield of Cannabinoids in Ethanol and Ethanol +Water + Glycerol Solvent Mixture Using Different Extraction Methods: Using UAE for cannabinoid extraction, the table below shows that 70% ethanol + 10% H_2O + 20% glycerol has higher percentage composition (%w/w) in CBD as compared to 100% ethanol (primary solvent), while the solvent mixture has a lower percentage composition (%w/w) in delta.9-THC as compared to 100% ethanol (primary solvent).

Using MAE for cannabinoid extraction, the table shows 70% ethanol + 10% H_2O + 20% glycerol solvent mixture has a higher percentage composition (%w/w) in CBD and delta.9-THC as compared to 100% ethanol (primary solvent).

The table below shows that MAE is more efficient for the extraction of cannabinoid compounds as compared to UAE using 70% ethanol + 10% H_2O + 20% glycerol as the extraction solvent.

A plot of cannabinoid concentrations (ppm) versus extracted cannabinoids in ethanol and 70% ethanol + 10% H2O + 20% glycerol solvent mixture using different extraction methods is shown in the Figure 3.10

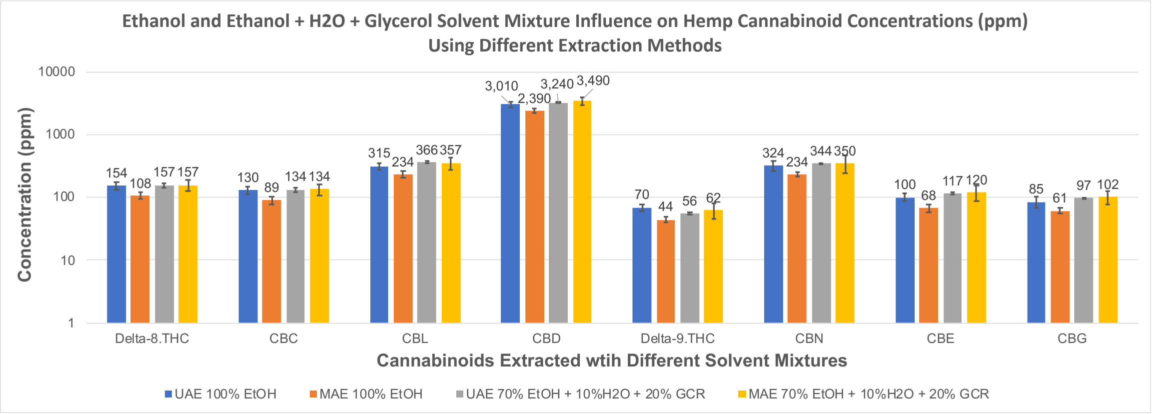


Figure 3.10 Plot of cannabinoid concentrations (ppm) versus extracted cannabinoids in ethanol and 70% Ethanol + 10% H_2O + 20% glycerol using different extraction methods

The concentration of cannabinoid axis (y-axis) is in a logarithmic scale. The concentration of cannabinoid axis (y-axis) is in a logarithmic scale. The numbers on the bar chat represent the actual concentrations (ppm) of the cannabinoid compounds. The standard deviation error for the CBD and delta.9-THC in solvent mixtures using different extraction methods is shown in table 10.

Table 9: Percentage Composition of Extracted Cannabinoids (%w/w) in Ethanol and Ethanol + Water + Glycerol Solvent Mixture Using Different Extraction Method

Solvent	**Cannabinoid Percentage Composition (%w/w)**			
	UAE		MAE	
	CBD	Delta.9-THC	CBD	Delta.9-THC
100% Ethanol	3.50 ± 0.37	0.08 ± 0.009	2.80 ± 0.24	0.05 ± 0.005
70% Ethanol + 10% H_2O + 20% Glycerol	3.78 ± 0.08	0.065 ± 0.002	4.07 ± 0.6	0.07 ± 0.02

Table 10: Concentrations and Standard Deviations of CBD and Delta.9-THC in Solvent Mixtures Using different Extraction Methods

Solvent	**Concentration (ppm) and Standard deviation error value**	
	CBD	Delta.9-THC
UAE 90% EtOH + 10% GCR	3580 ± 370	78 ± 18
MAE 90% EtOH + 10% GCR	3510 ± 331	77 ± 16
UAE 90% EtOH + 10% LMN	4780 ± 176	82 ± 4
MAE 90% EtOH + 10% LMN	2990 ± 547	62 ± 29
UAE 70% EtOH + 10%H2O + 20% GCR	3240 ± 69	56 ± 2
MAE 70% EtOH + 10%H2O + 20% GCR	3490 ± 514	62 ± 18
UAE 100% EtOH	3010 ± 316	70 ± 8
MAE 100% EtOH	2390 ± 201	44 ± 4

3.2 Mass Fragments for Extracted Cannabinoids Using ACDLabs: The mass fragments of cannabidiol (CBD) and delta.9-tetrahydrocannabinol (delta.9-THC) was analyzed using ACDLabs software.

1. Cannabidiol

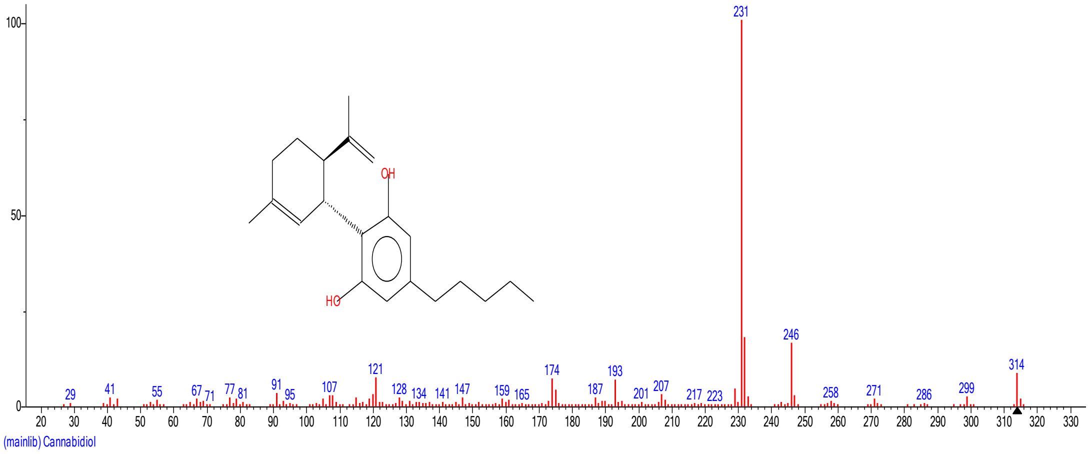
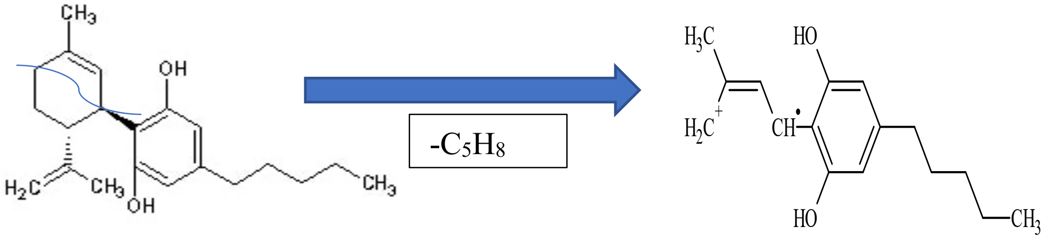


Figure 3.11 NIST Mass Spectrometry of Cannabidiol (CBD)

Molecular Structure= $C_{21}H_{30}O_2$

Molecular Structure= $C_{16}H_{22}O_2$



$M_n = 314$

$M_{mi} = 314.224580$

$M_{av} = 314.\ 469000$

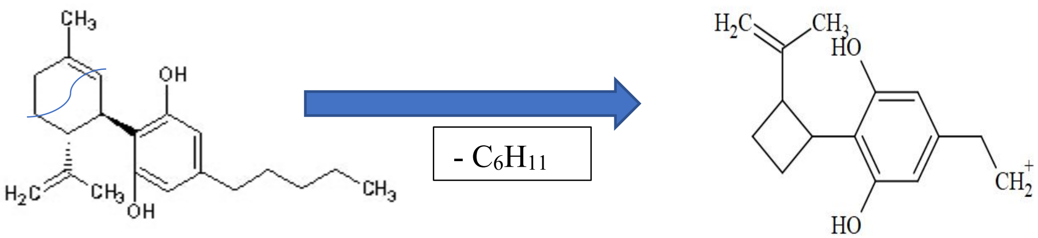
$M_n = 246$

$M_{mi} = 246.161431$

$M_{av} = 246.\ 349451$

Molecular Structure= $C_{21}H_{30}O_2$

Molecular Structure= $C_{15}H_{19}O_2$



M_n = 314

M_{mi} = 314.224580

M_{av} = 314. 469000

M_n = 231

M_{mi} = 231.137956

M_{av} = 231.314451

Figure 3.12 Mass Fragments of Cannabidiol (CBD)

2. Delta.9-Tetrahydrocannabinol

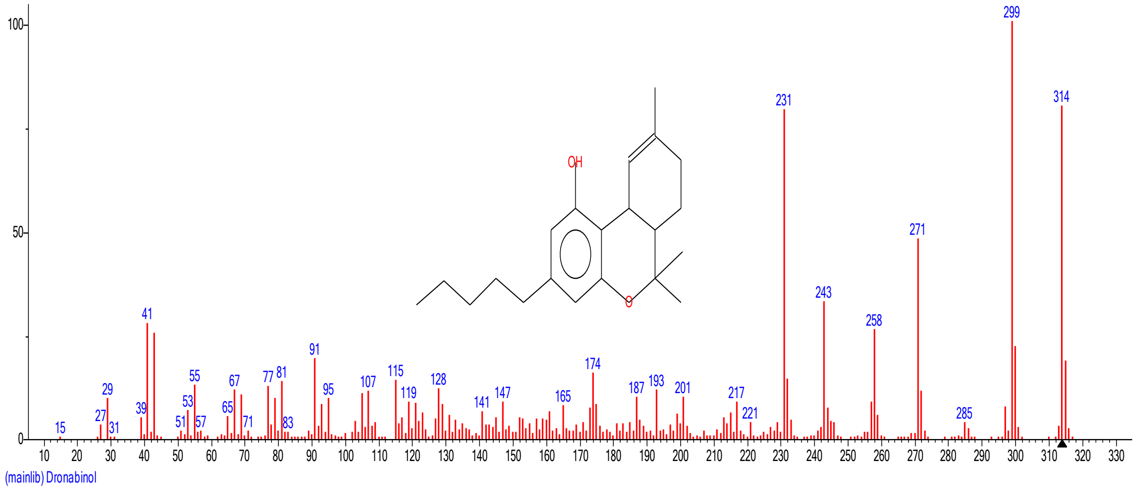
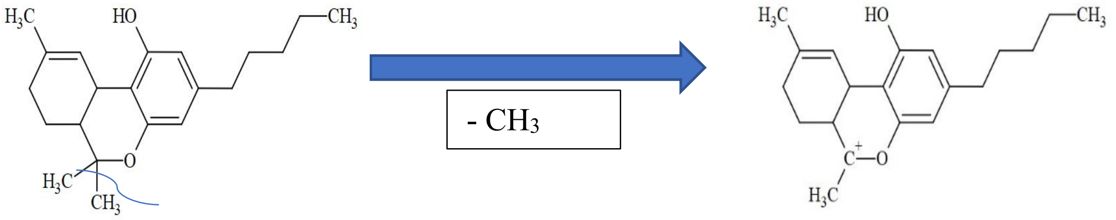


Figure 3.13 NIST Mass Spectrometry of Delta.9-tetrahydrocannabinol (delta.9-THC)

Molecular Structure= $C_{21}H_{30}O_2$ Molecular Structure= $C_{20}H_{27}O_2$



$M_n = 314$

$M_{mi} = 314.224580$

$M_{av} = 314. 469000$

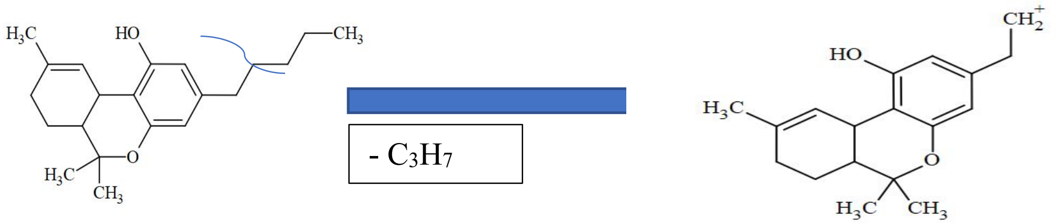
$M_n = 299$

$M_{mi} = 299.200557$

$M_{av} = 299.433451$

Molecular Structure= $C_{21}H_{30}O_2$

Molecular Structure = $C_{18}H_{23}O_2$



M_n = 314

M_{mi} = 314.224580

M_{av} = 314. 469000

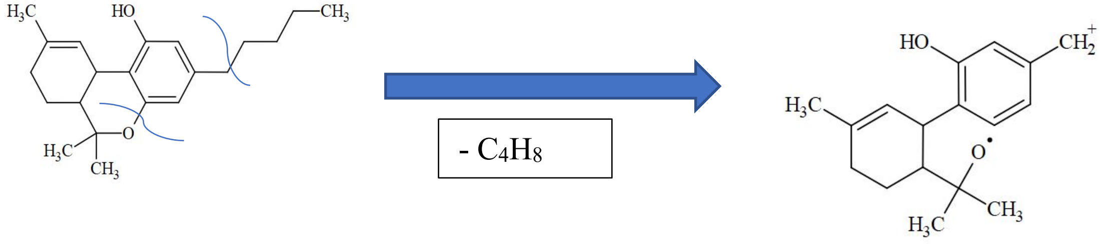
M_n = 271

M_{mi} = 271.169256

M_{av} = 271.379451

Molecular Structure= $C_{21}H_{30}O_2$

Molecular Structure = $C_{17}H_{22}O_2$



M_n = 314

M_{mi} = 314.224580

M_{av} = 314. 469000

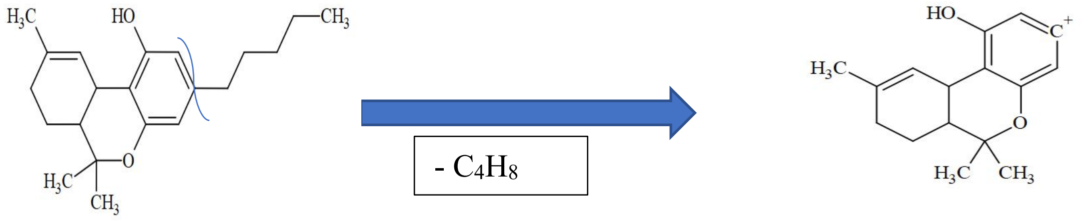
M_n = 258

M_{mi} = 258.161431

M_{av} = 258.360451

Molecular Structure= $C_{21}H_{30}O_2$

Molecular Structure = $C_{17}H_{22}O_2$



$M_n = 314$

$M_{mi} = 314.224580$

$M_{av} = 314. 469000$

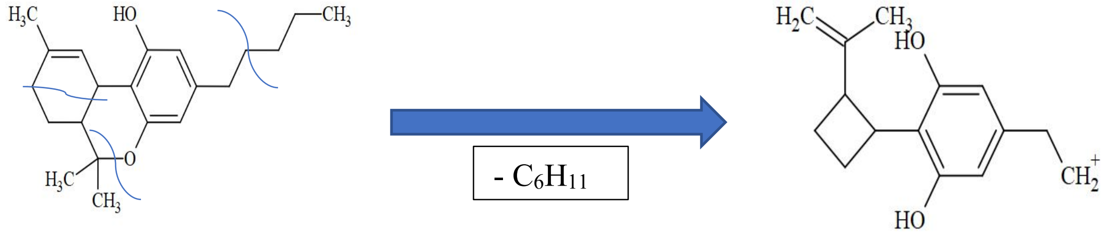
$M_n = 243$

$M_{mi} = 243.1743$

$M_{av} = 243.369451$

Molecular Structure= $C_{21}H_{30}O_2$

Molecular Structure = $C_{15}H_{19}O_2$



$M_n = 314$

$M_{mi} = 314.224580$

$M_{av} = 314. 469000$

$M_n = 231$

$M_{mi} = 231.137956$

$M_{av} = 231.314451$

Figure 3.14 Mass Fragments of Delt.9-tetrahydrocannabinol

3.3 Cannabinoid Standards Calibration plots:The peak areas of cannabinoid standards for all the eight extracted cannabinoids were plotted against its concentration (ppm).

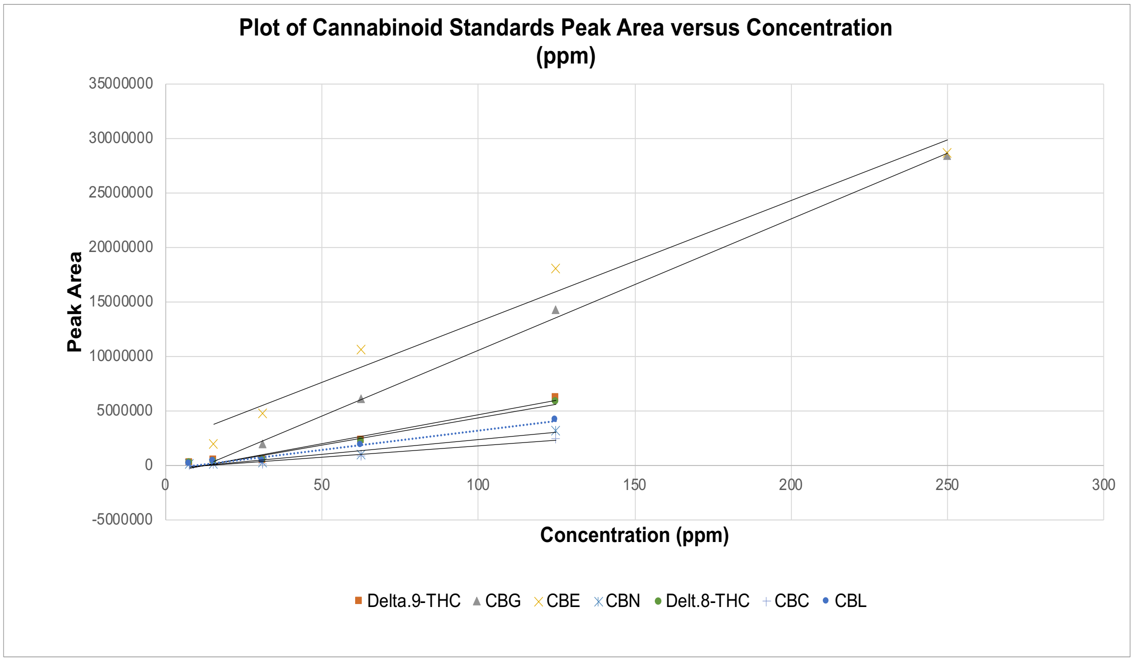


Figure 3.15 : Plot of cannabinoid standards peak area versus concentration (ppm)

The mixture of cannabinoid standards containing delta.8-THC, delta.9-THC, CBC, CBL and CBN were prepared in different concentrations, including 125ppm, 62.5ppm, 31.25ppm, 15.625ppm, 7.8125ppm. However, CBE and CBG were prepared in different conentrations, including 250ppm, 125ppm, 62.5ppm, 31.25ppm, 15.625ppm.

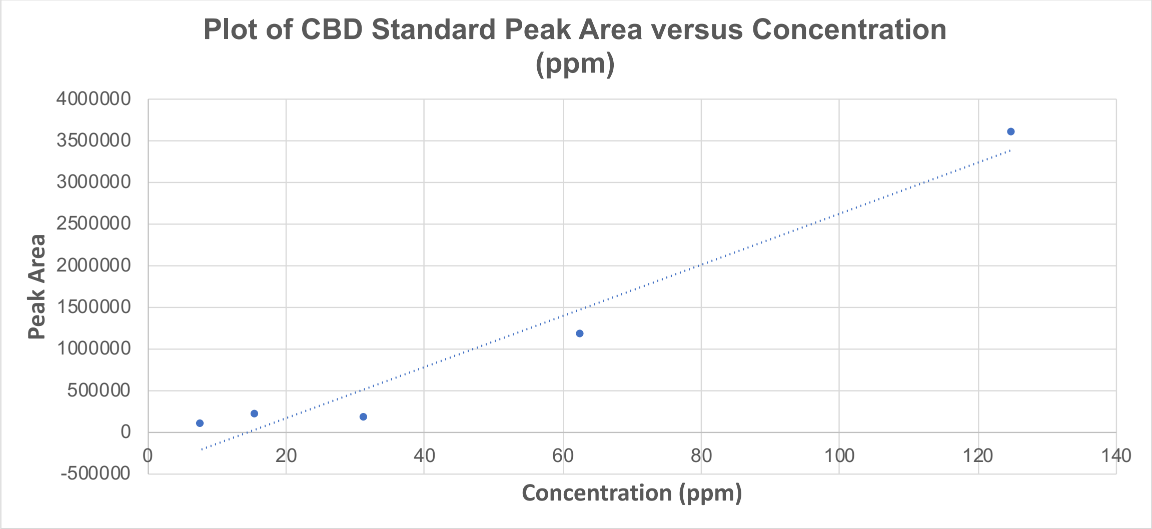


Figure 3.16 : Plot of CBD standard peak area versus concentration (ppm)

The CBD standard was prepared in different concentrations, including 125ppm, 62.5ppm, 31.25ppm, 15.625ppm, 7.8125ppm.

Table 11: Cannabinoid Standards Calibration equations and R-squared Value

Cannabinoid Standard	Calibration equation	R^2 Value
Delta.8-tetrahydrocannabinol (Δ^8-THC)	y = 49580x - 602112	0.9743
Cannabichromene (CBC)	y = 20965x - 309871	0.9665
Cannabicylol (CBL)	y = 35343x - 344829	0.9787
Cannabidiol (CBD)	y = 30594x - 429792	0.9586
Delta.9-tetrahydrocannabinol (Δ^9-THC)	y = 52562x - 624647	0.9728
Cannabielsoin (CBE)	y = 111253x + 2E+06	0.9736
Cannabigerol (CBG)	y = 120601x - 1E+06	0.9987
Cannabinol (CBN)	y = 27033x - 391649	0.9641

CONCLUSION

Statistics from post-run analysis results show that the optimum extraction conditions for microwave-assisted extraction (MAE) is at 70℃ for 20 minutes, and ultrasonication-assisted extraction (UAE) is optimal between the range of 65℃ and 75℃ for 20 minutes.

The optimal percentage solvent mixture proportion using MAE and UAE for cannabinoid extraction is 90% ethanol + 10% glycerol, 90% ethanol + 10% limonene, 90% ethanol + 10% propylene glycol, while 90% ethanol + 10% propylene glycol is an alternative solvent mixture.

Among the solvent mixtures, including propylene glycol:ethanol, glycerol:ethanol, limonene:ethanol, and olive oil:limonene, the data analysis shows that 90% ethanol + 10% glycerol and 90% ethanol + 10% limonene are more effective in extracting cannabis compounds.

Ultrasonication-assisted extraction is more efficient for cannabinoid extraction in both 90% ethanol + 10% glycerol and 90% ethanol + 10% limonene as compared to microwave-assisted extraction, while microwave-assisted extraction is more efficient for cannabinoid extraction using 70% Ethanol + 10% H_2O + 20% glycerol as compared to ultrasonication-assisted extraction.

The advantage of glycerol commonly used in CBD creams and other skincare products justifies its usage as a co-solvent for cannabinoid extraction. However, glycerol is hygroscopic, which helps to maintain skin hydration by drawing moisture from the air and retaining it on the skin's surface. This can contribute to a more effective and longer-lasting moisturizing effect.

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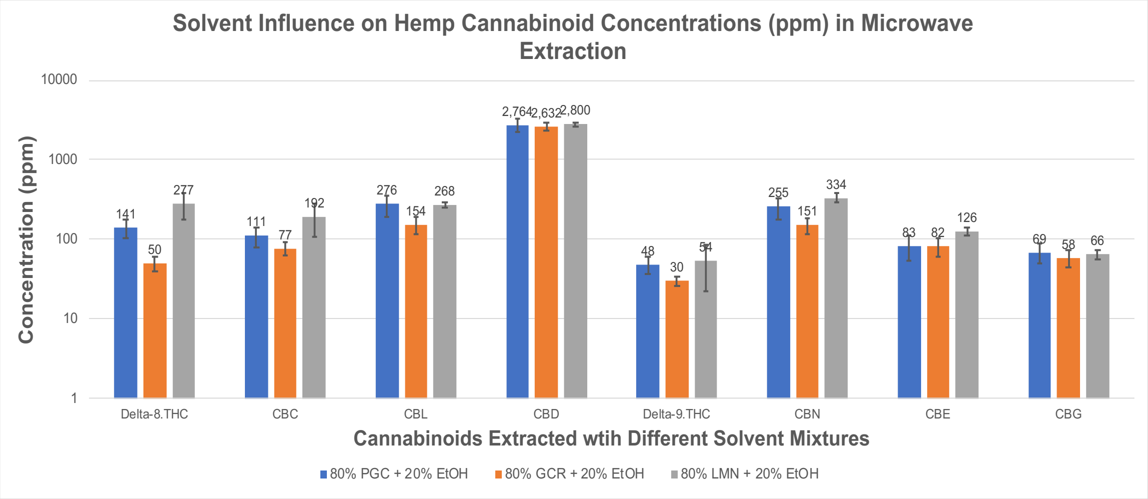
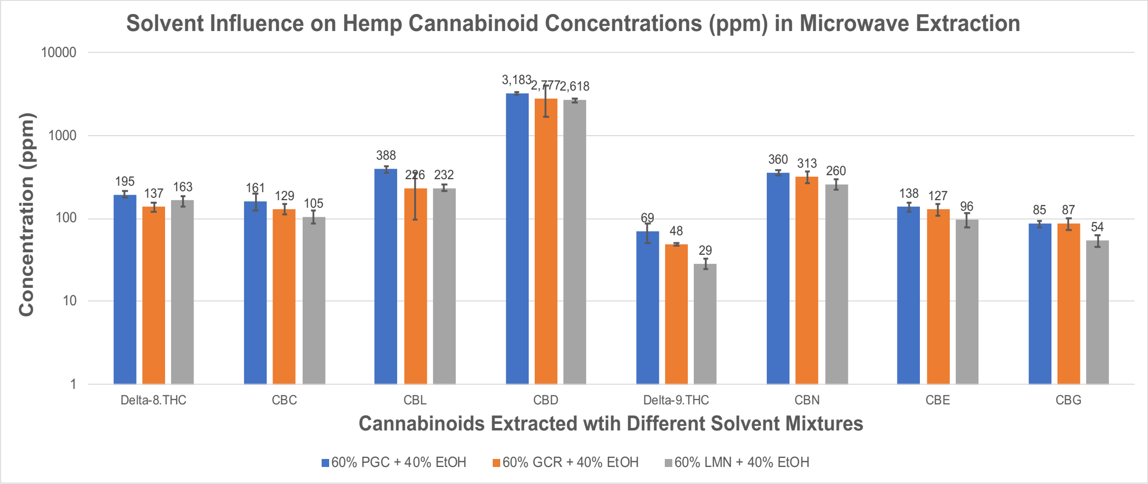
APPENDICES

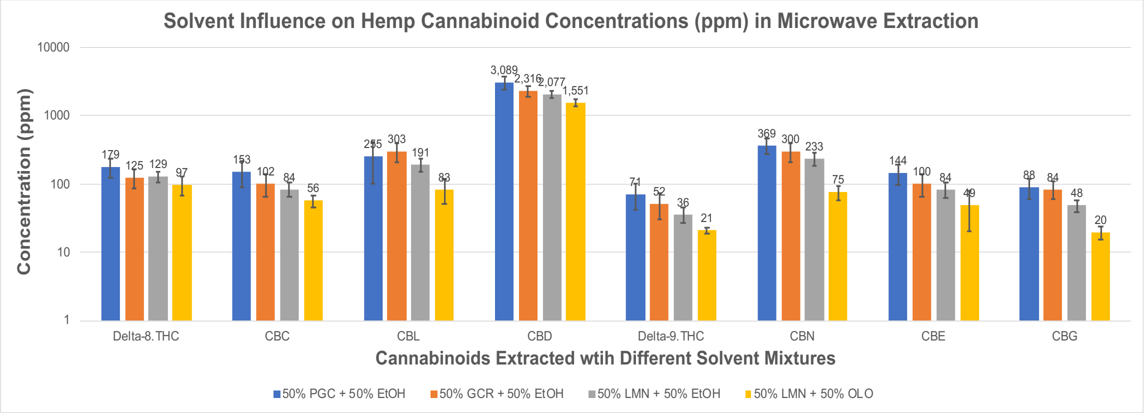
The concentration of cannabinoid axis (y-axis) of the plots below is in a logarithmic scale.



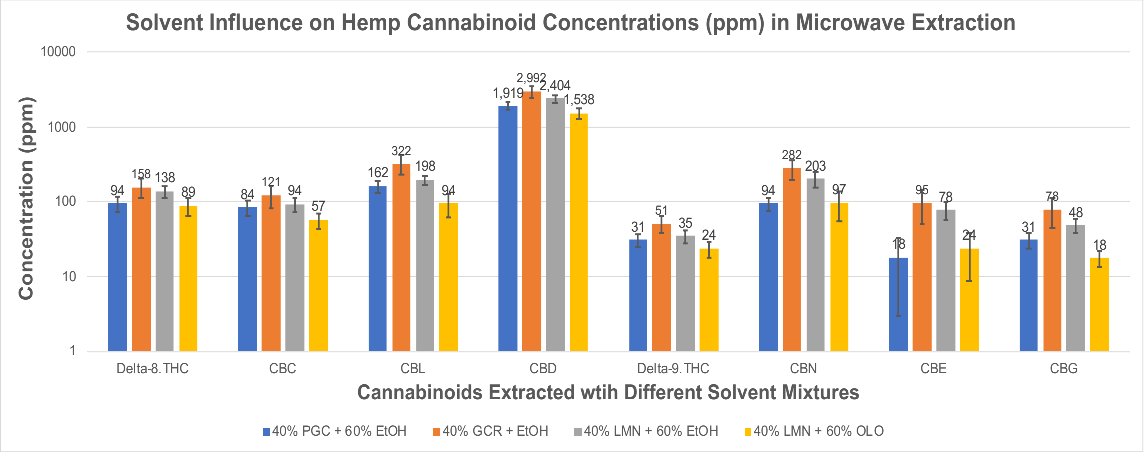
Appendix A. Logarithmic plot of cannabinoid concentrations (ppm) versus extracted cannabinoids in different solvent mixtures containing 20% ethanol using microwave extraction.



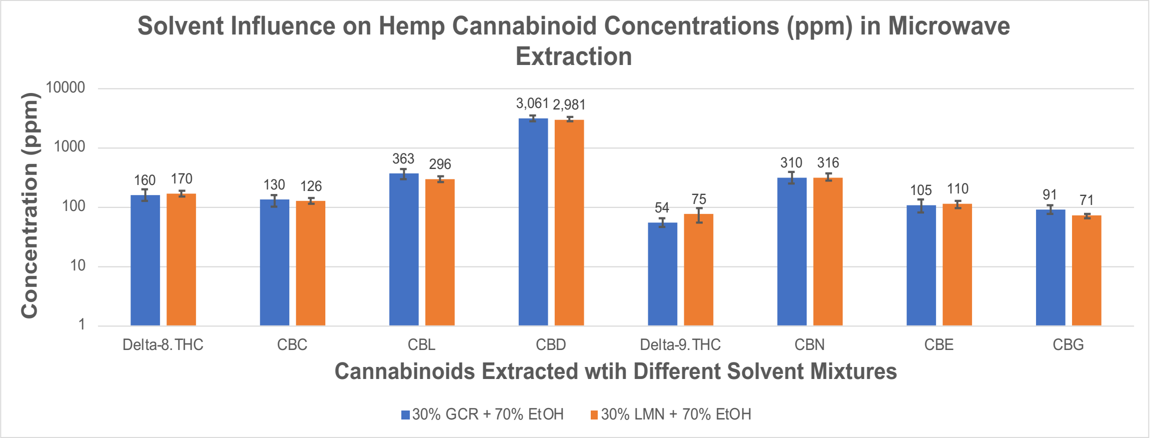
Appendix B. Logarithmic plot of cannabinoid concentrations (ppm) versus extracted cannabinoids in different solvent mixtures containing 40% ethanol using microwave extraction.



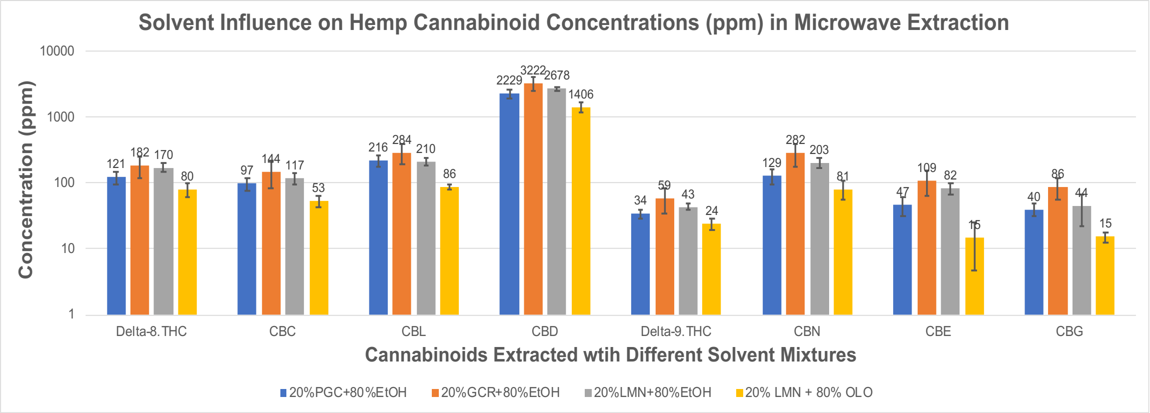
Appendix C. Logarithmic plot of cannabinoid concentrations (ppm) versus extracted cannabinoids in different solvent mixtures containing 50% ethanol using microwave extraction.



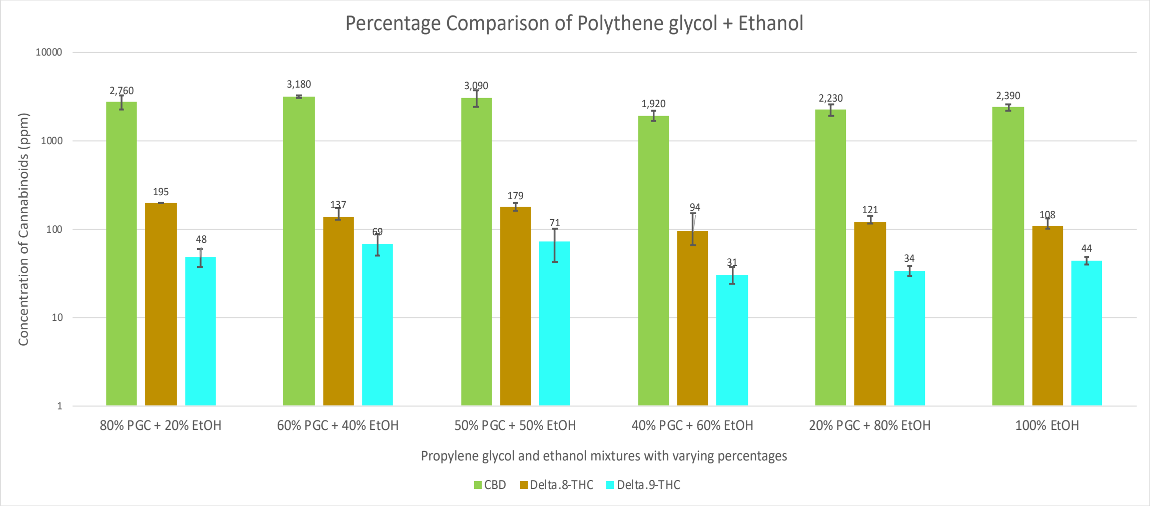
Appendix D. Logarithmic plot of cannabinoid concentrations (ppm) versus extracted cannabinoids in different solvent mixtures containing 60% ethanol using microwave extraction.



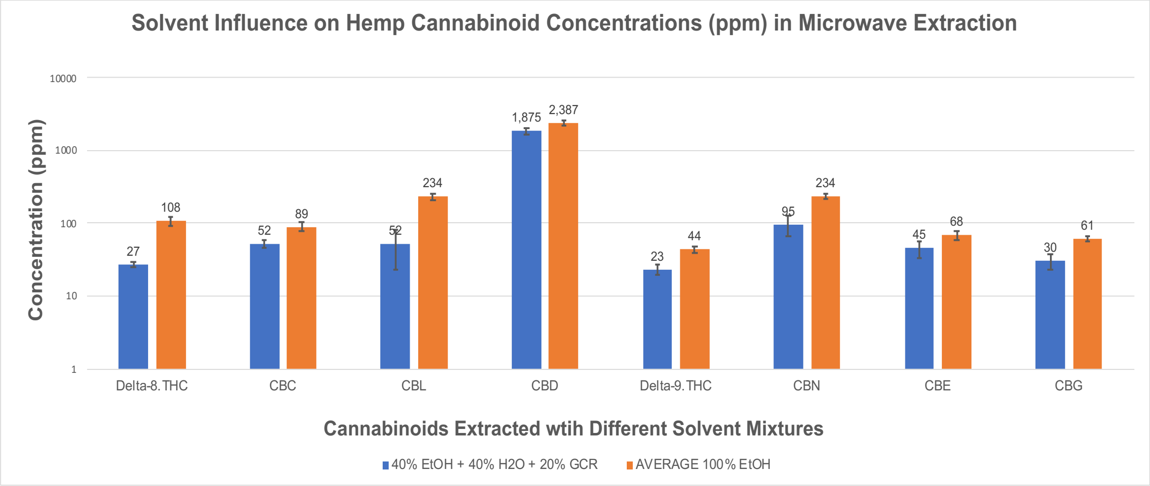
Appendix E. Logarithmic plot of cannabinoid concentrations (ppm) versus extracted cannabinoids in different solvent mixtures containing 70% ethanol using microwave extraction.



Appendix F. Logarithmic plot of cannabinoid concentrations (ppm) versus extracted cannabinoids in different solvent mixtures containing 80% ethanol using microwave extraction.



Appendix G. Microwave-assisted Extraction Logarithmic plot of Cannabinoid Concentrations (ppm) Versus Propylene glycol + Ethanol Solvent Mixture at Varying Percentages.



Appendix H. Logarithmic plot of cannabinoid concentrations (ppm) versus extracted cannabinoids in 40% ethanol + 40% H_2O + 20% glycerol solvent mixture using microwave extraction.