International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(REVIEW ARTICLE)

Check for updates

Quantification of THC levels in different varieties of Cannabis sativa

Ravindra B. Malabadi [1,*], Sadiya MR [2], Kiran P. Kolkar [3], Lavanya L [4] and Raju K. Chalannavar [1]

[1] *Department of Applied Botany, Mangalore University, Mangalagangotri-574199, Mangalore, Karnataka State, India.*
[2] *Department of Biochemistry, JSS Medical College, Mysore- 570015, Karnataka State, India.*
[3] *Department of Botany, Karnatak Science College, Dharwad-580003, Karnataka State, India.*
[4] *Department of Biochemistry, REVA University, Bangalore -560064, Karnataka State, India*

International Journal of Science and Research Archive, 2023, 10(02), 860–873

Publication history: Received on 01 September 2023; revised on 12 November 2023; accepted on 15 December 2023

Article DOI: https://doi.org/10.30574/ijsra.2023.10.2.1029

Abstract

This literature review paper highlights and updates the THC quantification methods applied for the differentiation of Cannabis varieties and final Cannabis products as a part of the quality control measures. The quantification method also helps to differentiate between Medical *Cannabis sativa* (drug or marijuana) and Industrial *Cannabis sativa* L. (Hemp) since THC levels are different. Cannabis has been used for thousands of years for recreational, medicinal, or religious purposes and does not produce Δ9-tetrahydrocannabinol (THC). Tetrahydrocannabinolic acid (THCA) is produced by the cannabis plant as a precursor. The acidic residue of THCA undergo decarboxylation upon heating producing the psychoactive, Cannabinoid, Δ9-tetrahydrocannabinol (THC). A variety of analytical techniques have been developed for quantification and qualification of Cannabinoids and other compounds in Cannabis plant. The most common cannabinoid quantification techniques include color tests, testing gadgets, Cannabinoids direct ELISA Kit, thin layer chromatography (TLC), gas chromatography (GC) and high performance liquid chromatography (HPLC) followed by Fourier transform infrared spectroscopy (FTIR) and Nuclear magnetic resonance spectrometry (NMR). The lack of accurate reporting of THC potency can have impacts on medical patients controlling dosage, recreational consumers expecting an effect aligned with price, and trust in the industry as a whole. Therefore, quantification of final Cannabis product plays an important role in quality control measures. This literature review paper is developed as a part of Cannabis Science awareness programme since Cannabis with 2 different names (marijuana and hemp) is used as a medicine, food and psychotropic drug (THC).

Keywords: Cannabis; Color test; Direct ELISA Kit; Tetrahydrocannabinolic acid (THCA); High performance liquid chromatography (HPLC); Testing gadgets

1. Introduction

Cannabis sativa L. belongs to the family *Cannabaceae* was used as a medicine before the Christian era in Asia**,** mainly in India**,** China, Bhutan, Nepal, Afghanistan, Pakistan and Iran, and Persians [1- 25, 64]. Cannabis has been used for thousands of years for recreational, medicinal, or religious purposes [1- 25-64]. Cannabis is also a wild noxious weed with notorious psychoactive principle (THC) found growing in all the parts of India [1- 26]. Cannabis has a long history in India, recorded in legends and religion [1- 26]. It was 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- 25]. *Cannabis sativa* L., is classified into two types as Industrial *Cannabis sativa*, hemp (fibre or grain type) or Medical *Cannabis sativa* L.(drug or marijuana) based on its THC content. Industrial hemp and marijuana share the same species, *Cannabis sativa* L, but represent different varieties [1-26-64]. As such, there are genetic differences that lead to different chemical characteristics, which, in turn, lead to different uses [1-25]. Medical *Cannabis sativa* (drug or marijuana) contains very high levels of THC (above 0.3 to 38% of dry weight) [1- 26]. On the other hand Industrial *Cannabis sativa*

* Corresponding author: Ravindra B. Malabadi

L. (Hemp) contains very low levels of THC (0 to 0.3% of dry weight) [1- 25]. 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 [1-25-64]. The official discovery of Δ9-tetrahydrocannabinol (THC) is commonly attributed to Dr. Raphael Mechoulam affectionately referred to as the Godfather of Cannabis Science [1- 25-64]. Δ9-tetrahydrocannabinol (THC) was discovered in 1964 by Dr. Raphael Mechoulam and his colleagues at Israel's Weizmann Institute of Science [1- 25-60]. 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- 25].

The important characteristic of *Cannabis sativa* L. is the production of phytocannabinoids in abundance which are present in their acidic form in plant tissue [1- 27]. Phytocannabinoids are produced as a part of defensive mechanism in the trichomes on flower bracts of female inflorescences [1- 27-64]. The medicinal compounds from cannabis are mostly concentrated in the female flowers of this dioecious species. The so-called resin is the source of a wide variety of terpenoids and cannabinoids [1- 26, 55-60]. Cannabinoids are found in the resin produced by the trichomes which are widely distributed on both the male and female plants, however, are the most highly concentrated on the female flowers of the cannabis plant [1- 27, 55-60]. Female *Cannabis* 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) [27-60]. The two cannabinoids the most well known for their therapeutic properties are, Δ9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) [1- 27, 55-60]. THC and CBD are the neutral homologs of tetrahydrocannabinolic acid (THCA) and Cannabidiol acid (CBDA) respectively [1- 26]. 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- 27].

In recent years, the medicinal applications of *Cannabis sativa* L. have gained wider attention worldwide. Cannabis is a genus of annual flowering plant [1- 26]. Cannabis is often divided into 3 species—*Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis*—but there is significant disagreement about this, and some consider them subspecies of the same parent species [1- 26, 34-60]. Most of the Cannabis varieties on the market today are hybrids with both *Cannabis sativa* and Cannabis indica genetics [1- 26-64]. Hybrid refers to a strain created by combining both Cannabis indica and *Cannabis sativa* strains [1- 26]. Many commercial producers crossbreed Cannabis plants to develop new strains with specific characteristics [1- 26]. *Cannabis ruderalis* flowers as a result of age, not light conditions, which is called autoflowering [1- 26]. It is principally used in hybrids, to enable the hybrid to have the autoflowering property [1- 26, 34]. The medical and retail cannabis industry encourages the production of strains with high THC, as price is often contingent on THC potency [27-34-64].

There are thousands of *Cannabis* strains and cultivars with unique chemotypes on the legal market that are often categorized as *C. sativa*, *C. indica*, or hybrid based on purported effects and available ancestry information [27-34-64]. These terms are widely used in the industry, by online databases, by the community, and although the use of these terms is somewhat disputed [27-30, 34-60]. They will likely continue to be used as part of the common vocabulary for *Cannabis* products [27-30, 34-60]. Experts suggested that there more than 750 hybrid strains of as the Trusted Source of Cannabis, for example, Kush- Pure *Cannabis indica* or *Cannabis indica* hybrid [1- 60]. Afghan Kush, Hindu Kush, Green Kush, Purple Kush- Pure *Cannabis indica* [1-60]. Further, Bluberry Kush, Golden Jamaican Kush-*Cannabis indica* hybrid [1- 26]. Finally, Diesel Haze-Pure *Cannabis sativa* or hybrid 1- 26-60]. There are more than 750 strains of recreational Cannabis, the most with colourful names [1- 26-60]. Some are strains of *Cannabis sativa* and *Cannabis ruderalis* subspecies are crossbred hybrids [1- 26-34-60]. 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- 26-34-64].

In the following section, the methods for the quantification of THC levels in the final products has been highlighted and discussed different methods. The purpose of this literature review is to explore THC quantification techniques and subsequently suggested an optimal method for pharmaceutical grade quantification and quality of the preparations. This literature review paper is developed as a part of Cannabis Science awareness programme.

1.1. Differences between Cannabis and Hemp

Cannabis actually refers to the genus of the plant and encompasses both hemp and marijuana [1-59]. The USDA classifies both hemp and marijuana as *Cannabis sativa* L. The difference lies in the Δ9-tetrahydrocannabinol (Δ9- THC) levels in

each plant species [1-59]. Hemp is defined as material that has less than 0.3 percent (by dry weight) Δ9-THC [1-59]. Marijuana is defined as a material that exceeds 0.3 percent Δ9-THC threshold [1-59]. Δ9-THC as defined by the USDA includes Δ9-THC and tetrahydrocannabinolic acid (THCA) to indicate how much of the psychoactive component could be available to the consumer after decarboxylation [1-60]. Basically, the difference between hemp and marijuana comes down to an expression of THC or CBD (Cannabidiol) dominance within the plant species [1-60]. Hemp displays high CBD and low Δ9-THC expression, while marijuana has low CBD and high Δ9-THC expression [1-59]. A hemp variety of Cannabis may grow taller and faster, but it does not generate as much Δ9-THC, the psychoactive ingredient of marijuana [1-61]. Marijuana strains are rich in THC and CBD and are used for medical and recreational purposes [1-60]. Different strains of Cannabis are available, either pure Cannabis genus, including *C. sativa, C. indica,* and *C. ruderalis*, or hybrid varieties [1-61]. Chemotaxonomically, Cannabis is classified based on the phytocannabinoids (PCs) content, including THC and CBD in particular [1-60]. The most commonly grown Cannabis species are *C. sativa* and *C. indica*, where *C. ruderalis* is short and produces trace amount of THC and is thereby not commonly grown for medicinal or industrial purposes [1-60]. *C. sativa* is an energising strain with common psychoactivity, so it is known for being a 'head high', whereas *C. indica* is more commonly used for relaxation as a sedative and is regarded as 'body high or couch lock [1-61].

For growers and producers, THC content can determine the price point at which they can sell to wholesalers and dispensaries [1-60]. The difference between an 18.1 percent and a 21.3 percent flower could be the difference of more money. From a consumption standpoint, the higher the THC content, the more Δ9-THC is available to interact within the body's endocannabinoid system, specifically CB1 and CB2 receptors [1-60]. This interaction will induce the psychoactive effects attributed to Δ9-THC. However, this is not to conclude that the higher THC strains might find are better [1-60]. There is a certain point where the body of a normal consumer (a person that does not consume on a daily basis) cannot make the difference between the previously mentioned 18.1 percent and 21.3 percent strains [1-60]. The strain itself can also affect how a consumer experience the effects of the cannabis (e.g. calming and relaxing versus anxiety and paranoia) [1-60]. The effects of THC can also change with the different product consumption [1-60]. Smoking flower or extract material will have an immediate effect, peaking within 30-60 minutes [1-60]. Eating or drinking an infused product may take 20 minutes to 1.5 hours for initial effects and can last many hours [1-60]. Some studies have even shown that the body can metabolize Δ9-THC into 11-hydroxy-Δ9-THC for different psychoactive effects [1-60].

2. THCA into THC: Decarboxylation

- Cannabis plant does not make any Λ9-tetrahydrocannabinol (Λ9-THC) but plant produces tetrahydrocannabinolic acid (THCA) which is a non-intoxicating compound that can be converted into Λ9-tetrahydrocannabinol (Λ9-THC) through decarboxylation upon heating [27, 31-33]. This typically occurs in the presence of heat energy applied by the consumer. When lighter, vaporizer, or oven heats up Cannabis product, Tetrahydrocannabinolic acid (THCA) gets converted into THC [31-33]. Many people talk about "activated" vs. "inactive" THC. This is what they mean [31-33-60].
- Tetrahydrocannabinolic acid (THCA) is produced by the cannabis plant. With the application of heat energy, the acidic residue of THCA through decarboxylation producing the psychoactive cannabinoid THC [31-33-60]. Cannabis flower normally contains very low levels of THC [31-33]. The heat applied by consumer converts most of the THCA into THC [31-33-60].
- The process of turning tetrahydrocannabinolic acid (THCA) into THC is not 100% efficient [31-33]. Therefore, not every THCA molecule will be converted into a THC molecule, and at very high temperatures, some of the THC may degrade into CBN [31-33]. According to some of the laboratory studies for example at Steep Hill Labs which estimated that 75% is a representative upper limit for what fraction of THCA will end up as THC [31-33]. In that case, for every four molecules of THCA that get heated during consumption, only three get successfully converted to THC [31-33].
- Heating of Cannabis extracts at 200°C for five minute results in almost 100% decarboxylation of THCA to THC, without forming CBN [31-33].
- Decarboxylation of THCA to THC starts occurring at around 180°C [31-33]. As increase in the temperature above 180°C, other compounds like terpenes will begin to vaporize, each at a different temperature [31-33]. At even higher temperatures, started to get combustion [31-33]. This will affect not only levels of THC and other Cannabinoid, but also terpenes. Moreover, combustion can produce byproducts that may be hazardous to the health [31-33].
- Burning of Cannabis (not tested and validated in lab) at >700°C will probably result in a higher decarboxylation rate, but also more degradation of THCA to CBN and the production of potentially harmful byproducts [31-33].

In addition, temperatures that are too high led to the loss of terpenes, which are important entourage effect compounds [27, 31-33].

- It is shocking to discover that cannabis contains very little THC [27, 31-33]. The psychotropic cannabinoid actually comes being after exposure to heat [27, 31-33]. It exists in the form of THCA—a cannabinoid acid in raw flower buds [27, 31-33]. Also known as tetrahydrocannabinolic acid, THCA works in a unique way in the body [31-33]. It interacts with the endocannabinoid system, but it does not get high [31-33]. THCA features a heavier molecular mass than THC because it possesses an additional carboxyl group [31-33]. The molecule converts to THC when exposed to the heat of a bong bowl, vaporizer, or oven in a process called decarboxylation—the carboxyl group ejects, leaving THC behind [31-33].

2.1. Quantification of THC levels

As cannabis use becomes progressively accepted, it becomes increasingly important to quantify the cannabinoid profile and content of cannabis preparations to ensure the uniformity [1-27-33-48-60]. A variety of analytical techniques have been developed for quantification and qualification of Cannabinoids and other compounds in Cannabis plant [30-48-60]. Advances in analytical methods have also resulted in the detection of various compounds from cannabis extracts in the last decade (eg terpenes) [30-48-60].

The values of THCA and THC percentages are printed on the label of the Cannabis purchased products [31-33]. On the basis of these values, the actual THC dosage that will be present upon heating and inhaling will be calculated [31-33]. However, this can not be done directly, since THC is 87% of the mass that THCA and only 75% is actually converted from THCA to THC [27, 31-33]. Keeping that in mind, here is the equation to use to find out just how much THC will be present in the purchased puffs: (0.75 × 0.877 × % THCA) + % THC = Total THC in flower [31-33-60]. Total THC was calculated using the industry standard equation: THC TOTAL = THC + (THCA x 0.877) which determines total THC when THCA and THC are separated during chromatography [27-60].

2.2. Equation To Determine Total THC Level

As mentioned above, THCA and THC have a slightly different molecular mass—THC is 87.7% of the mass of THCA [31-33]. As THCA converts to THC, the total weight of the newly formed cannabinoid makes up less of the total dried weight of the herb [31-33]. Next step is to consider the efficiency of conversion. Not every molecule of THCA will be converted to THC during decarboxylation [31-33]. An estimated 75% of the cannabinoid acid will make the switch [31-33-60]. On top of that, some THC will degrade into CBN under these harsh conditions [31-33-60]. Based on these variables, the following formula accounts for the imperfect conversion of THCA and takes the difference in molecular mass into consideration:

$$\mathbf{(0.75 \times 0.877 \times \%\ THCA) + \%\ THC}$$

For example:

Many raw flowers and extracts are labeled with precise percentages that display both THC and THCA levels [31-33-60]. A THC content of 0.82% and a THCA content of 17.4% equal a total THC value of 18.2%? which is not a correct value [31-33]. There are a few variables need to consider to determine the true THC value of a particular strain [31-33].

$$\mathbf{(0.75 \times 0.877 \times 17.4) + 0.82 = 12.26\ \%}$$

2.3. Calculation of THC and CBD Levels

Cannabis plants do not synthesize THC or CBD; instead, they synthesize the cannabinoid acids THCA and CBDA, which are made from a common precursor and must be decarboxylated (e.g. by heat energy) to yield the phytocannabinoids THC and CBD [31-33, 58-60]. Thus, cannabis products, especially flower samples, contain mainly THCA and/or CBDA, as well as small levels of THC and CBD that resulted from spontaneous decarboxylation during the cultivation process [31-33, 58]. Total THC or CBD levels, in units of percent of dry weight, are typically calculated as:

$$\mathbf{Total\ CBD = (0.877 \times CBDA) + CBD\ (1)}$$

$$\mathbf{Total\ THC = (0.877 \times THCA) + THC\ (2)}$$

Where THCA and THC refer to the percent dry weight concentration of each cannabinoid present in a cannabis product, and 0.877 is the scaling factor accounting for the difference in molecular weight between THCA and THC [31-33, 58-60].

"Total THC" or "Total CBD" refers to this value, which is the maximum potential THC or CBD content of a cannabis product, assuming 100% decarboxylation of cannabinoid acids [31-33, 58-60]. This is the standard way of reporting "total" cannabinoid levels for legal cannabis products in Washington, USA [58]. Unless otherwise noted, "THC levels" or "total THC levels" refers to this value (similar for CBD levels) [31-33, 58-60].

3. Detection of Cannabinoids: Colour Chart Test Kits

At-home color chart test kits come with a container and fluid that customer can place a small sample of flower[31-33, 58-60]. After shaking the sample, the solution will turn a color that can be matched to the provided chart to determine the levels of THC [31-33, 58-60]. Colour chart kits test for both CBD and THC. They required only a small pinch of material to get a reading, and are compatible with flower, extracts, and oils [31-33, 58-60]. Take a sample and place it into the container along with the test fluid**.** Give it a shake and wait a couple of minutes. The solution will turn a specific colour [31-33, 58-60]. Compare the colour with the chart provided to determine the cannabinoid content [31-33, 58-60]. As they are cheaper than other tests, colour chart kits do not offer the most detailed results. Some kits only test for THC levels of up to 20%, and CBD quantities of up to 2.5% [31-33, 58-60].

3.1. Cannabis Colour tests

Colour tests for cannabis are among the most specific colour tests available (only a few plants such as henna, nutmeg, mace and agrimony give false-positive results) [49-51]. However, a positive colour test only provides an indication of the possible presence of cannabis-containing material and not a definitive identification of cannabis [31-33, 58-60]. It is therefore, mandatory for the analyst to confirm such results by the use of additional, typically more discriminative techniques. For example, a laboratory may allow a combination of a colour test, thin-layer chromatography and microscopy for cannabis plant material for positive identification, provided that at least three cannabinoids are identified by TLC [49-51].

3.1.1. Fast Corinth V salt test

On a filter paper

- **Reagent A:** Petroleum ether.
- **Reagent B**: Fast Corinth V salt (1% w/w in anhydrous sodium sulphate).
- **Reagent C**: Sodium bicarbonate (1% w/w aqueous solution).
- **Fast Corinth V salt =** Dichlorozinc; 2-methoxy-5-methyl-4-(4-methyl-2-nitrophenyl) diazenylbenzenediazonium; dichloride
  = Azoic diazo component 39
  = $C_{15}H_{14}N_5O_3 \cdot 0.5\ ZnCl_4$
- **Method**: Fold two filter papers laid on top of each other into quarters and open them partly to form a funnel. Further place a small amount of pulverized sample into the centre of the upper paper [49-51]. Add two drops of reagent A allowing the liquid to penetrate to the lower filter paper [49-51]. Discard the upper filter paper and allow the lower filter paper to dry [49-51]. Add a very small amount of reagent B to the centre of the filter paper and then add two drops of reagent C [49-51].
- **Results:** A purple red coloured stain at the centre of the filter paper is indicative of a cannabis containing product [49-51]. THC, CBN and CBD yield the same [49-51]. This is a practical advantage in a field test reagent for samples of different age or origin [49-51].

3.1.2. Fast Blue B salt test

- **Reagent A**: Petroleum ether
- **Reagent B:** Fast Blue B salt (1% w/w diluted with anhydrous sodium sulphate).
- **Reagent C**: Sodium bicarbonate (10% w/w aqueous solution).
- **Method:** Fold two filter papers laid on top of each other into quarters and open them partly to form a funnel [49-51]. Place a small amount of pulverized sample into the centre of the upper paper [49-51]. Add two drops of reagent A allowing the liquid to penetrate to the lower filter paper [49-51]. Discard the upper filter paper and allow the lower filter paper to dry [49-51]. Add a very small amount of reagent B to the centre of the filter paper and then add two drops of reagent C [49-51].
- **Results:** A purple red coloured stain at the centre of the filter paper is indicative of a cannabis containing product [49-51]. This colour is a combination of the colours of the different cannabinoids which are the major components of cannabis: THC = red, CBN = purple, CBD = orange [49-51]. Fast Blue B salt keeps very well when

stored in a refrigerator [49-51]. But when kept at room temperature, it tends to deteriorate with time and the powder becomes a solid rock (especially in warm regions) [49-51].

3.1.3. Rapid Duquenois test (Duquenois-Levine test)

- **Reagent A:** Acetaldehyde (A1) (0.5 ml (A1) and 0.4 g (A2) in 20 ml ethanol).
    - :Vanillin (A2). Solution must be stored at a cool dark place and discarded if it assumes a deep yellow colour [49-51].
- **Reagent B:** Concentrated hydrochloric acid [49-51].
- **Reagent C**: Chloroform [49-51].
- **Method**: Place a small amount of the suspect material in a test tube and shake with 2 ml reagent A for one minute [49-51]. Add 2 ml of reagent B and shake the mixture. Allow to stand for ten minutes. If a colour develops, add 2 ml of reagent C, mix gently [49-51].
- **Results:** If the lower (chloroform) layer becomes violet coloured this indicates the presence of a cannabis product[49-51]. This test is not as sensitive as the two filter paper tests above [49-51].

4. Testing gadgets

Testing gadgets (the most expensive type are mainly purchased by cultivators) were found very expensive [49-51]. They provide an accurate reading of cannabinoid levels [49-51]. Most testing gadgets are connected to a smartphone device, where the cultivator or customer provided a chart of cannabinoid percentages after the test is performed as per the instructions [49-51].

Testing gadgets are the premium option when it comes to at-home THC testers [26-51-60]. These devices save a lot of time, but will cost more [26-51-60]. This is an investment geared toward growers, breeders, and those who really care about the cannabinoid content of their buds [26-51-60]. These handheld cannabis testing devices link up to smartphones via Bluetooth [26-51-60]. Place a sample of flower into the device, and use phone app to select the "flower" setting [26-51-61]. Wait 3–5 minutes while the analyser works as magic [26-51-60]. Next, see a chart pop up that gives a reading for THC, CBD, and CBN, as well as a host of different terpenes [26-51-60]. This app will also suggest other strains available with similar compositions [26-51-61].

4.1. GC and HPLC Methods

The most common cannabinoid quantification techniques include gas chromatography (GC) and high performance liquid chromatography (HPLC) [26-51-60]. GC is often used in conjunction with mass spectrometry (MS) or flame ionization detection (FID) [26-51-60]. The major advantage of GC is terpenes quantification. However, for evaluating acidic cannabinoids it needs to be derivatised [26-51-60]. 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 [26-51-60]. Based on the information presented in this review, the ideal cannabinoid quantification method is HPLC-MS/MS for the cannabinoids [26-51-60].

4.1.1. Gas chromatography (GC)

Gas chromatography (GC) is one of the most commonly used chromatographic methods in quantitative cannabinoid analysis [26-51-60]. Gas chromatography is typically completed in under 20 min at up to 300 °C and makes use of stationary phases with low polarities, such as 5% diphenyl- and 95% dimethyl polysiloxane [26-51-60]. It is important to note that the total quantity of cannabinoids in a sample is the sum of the acidic and neutral components [26-51-60]. Because gas chromatography requires high column temperatures, the acidic cannabinoids undergo decarboxylation during transit through the column [26-51-60]. Thus, acidic cannabinoids cannot be determined unless they are derivatized prior to analysis [26-51-60]. GC is normally coupled with mass spectrometry (MS) or flame ionization detection (FID) to detect and quantify cannabinoids [26-51-60]. Experience has shown that one-dimensional gas chromatography does not provide enough resolution to analyse complex cannabinoid mixtures [26-51-60]. Two-dimensional gas chromatography (GC × GC) has been found to be preferable over one dimensional GC for analyzing complex mixtures, such as cannabis extracts, in that it reveals more sample components [26-51-60]. GC-MS is often employed for cannabinoid quantification [26-51-60]. However, quantification of cannabinoids via GC requires a derivatization step to avoid the decarboxylation of acidic cannabinoids [26-51-60]. Performing GC without derivatization requires the calculation of total cannabinoid content from a combination of acidic and neutral cannabinoid content which can be an uncertain process [26-51-60].

4.1.2. High-Performance Liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC) is a commonly used liquid chromatography (LC) technique in quantitative cannabinoid analysis [26-51-60]. The most common columns used in HPLC consist of C18 stationary phases [26-51] and methanol with 0.1% formic acid or water with 0.1% formic acid as mobile phases [26-51-60]. C18 columns have high resolution and can differentiate between cannabinoids [26-51-60]. The use of formic acid in the mobile phase provides better peak shape than other mobile phases and improved resolution in the chromatographic analysis [26-51-60]. One of the study ran two HPLC assays to identify major and minor cannabinoids [26-51-60]. Extract profiling was based on the main cannabinoid (THC, CBD, CBG, and CBN) quantification and the presence of acids and flavones [26-51-60]. In this research, they found good resolutions of THCA, CBGA, CBDA, THCVA, THC, CBG, CBD, and THCV by HPLC [26-51-60]. Different detection techniques can be used in conjunction with High Performance Liquid Chromatography (HPLC) to analyze cannabinoids [26-51]. Common detection methods include mass spectrometry (MS) and ultraviolet (UV) absorbance (190 to 400 nm) [26-51-60]. UV detection is much less expensive and more straightforward than MS detection [26-51-60]. Acidic cannabinoids showed absorption peaks at around 270 nm and 310 nm while neutral cannabinoids showed absorption peaks at about 220 nm [26-51]. HPLC can differentiate between acidic and neutral cannabinoids, unlike GC [26-51-60].

4.2. TLC (Thin layer chromatography)

One of the study used the TLC method both for polar and non-polar systems [26-51]. They used reversed-phase silica gel plates and normal phase silica gel plates for nonpolar and polar systems respectively [26-51]. For more accurate results, they used Fast-Blue B salt (4-benzoylamino-2, 5- diethoxy benzene diazonium chloride hemi salt) which is a suitable coloring agent for visualization of cannabinoids at TLC plates [26-51]. Fast-Blue B can determine acetylcholinesterase, α- and β-glucosidase activity by changing to different colours which come from reacting of FBB with various compounds [26-51-60]. However, the colors depend on the concentration of constituents [26-51]. As a result, they found that TLC is useful in rapid screening of many samples for the existence of cannabinoids [26-51]. However, its performance is lower compared to other separation methods and the reproducibility of TLC depends on some parameters such as relative humidity [26-51-60]. TLC test kits not only showed levels of THC, but other minor cannabinoids, too [26-51-60]. With this test, the customer can use a liquid to saturate the flower [26-51-60]. This process separates individual cannabinoids, which are then sprayed with a dye to reveal the percentage of each one [26-51-60].

4.2.1. Thin-Layer Chromatography (TLC) Test Kits

TLC test kits are more expensive, and this test will test between 20–25 samples [26-51-60**].** TLC testing offers much more accurate readings, and also tests for minor cannabinoids including CBN, CBG, and THCV [26-51-60]. To conduct these tests, mix 0.1g of flower, edible, or extract with the test fluid [26-51-60]. Use the pipette provided to add small quantities of the mixture onto the coated glass plate [26-51-60]. Position the plate into the additional jar of test fluid and watch the fluid wick up the plate [26-51-60]. This process detects the properties of individual cannabinoids and separates them [26-51-60]. Next, spray the plate with the dye to reveal coloured spots [26-51-60]. Compare the size of the dots to the template provided to figure out the quantities of each cannabinoid within increments of one percent [26-51-61].

4.3. Fourier transform infrared spectroscopy (FTIR)

Hazekamp et al., (2005) measured cannabinoids with FTIR [26-51]. They added KBr to the ethanolic solution of cannabinoids followed by vacuum ethanol evaporation because KBr does not showed any absorption spectrum in IR region [26-51]. Additionally, KBr has a 100% transmission window in the range of wave number at the FTIR spectroscopy [26-51-60]. The IR spectra were measured in the range of 500 to 4000 cm^{-1}[26-51-60]. Compared to UV spectra, IR spectra presented more absorbance peaks [26-51-60]. Another study showed the existence of carbonyl and ester groups by the FTIR peak at 1775 and 1725 cm^{-1} in composite samples of cannabis extract [26-51-60].

4.4. Nuclear magnetic resonance spectrometry (NMR)

Another alternative to GC and HPLC is NMR [26-51-60]. NMR is accurate reproducible and unlike GCand HPLC, NMR is not sensitive to impurities, such as chlorophyll or lipids present in the sample [26-51-60]. Another study developed a method for cannabinoid quantification using ^{1}H-NMR that does not required chromatographic purification and has a 5-min final analysis time [26-51-61]. In this study, they analysed singlets in the δ 4.0–7.0 range in the ^{1}H-NMR spectrum and found that their technique was appropriate for the quantification of CBDA, THCA, CBG, CBGA, and possibly other cannabinoids as well [26-51, 61]. One of the major advantages of this technique is that reference standards are not required meaning that this method can quantify cannabinoids that lack pre-existing reference standards [26-51], and

therefore, cannot be analysed by other techniques [26-51-60]. Although the results from NMR are promising, one major disadvantage to NMR is that high resolution instruments are very expensive [26-51-60, 61].

5. Method for the Estimation of THC

- For estimation of THC (Tetrahydrocannabinol), female flowers of Cannabis plant were picked and Cannabis flowers were dried at 100º C for 30 minutes.
- 1 g of dried flowers were soaked in 100 ml of Petroleum ether and gently mixed by stirring for 2-3 minutes. Then solution was kept in Shaker at 200 rpm at room temperature for overnight.
- The solution is filtered through Charcoal to clarify the solution. The filtrate was again kept overnight at 4ºC to precipitate waxes.
- The solution was filtered again with whatman filter paper no. 52 to remove precipitated waxes. The solution was extracted with a 5 % solution of Na_2SO_3 by separating funnel.
- Extracted Petroleum ether was then concentrated to yield THC.
- After dilution, O.D. value of the solution was measured in combinations through 275 nm wavelength in a spectrophotometer against blank.
- All the spectrophotometer readings were recorded for the quantification of THC levles.

5.1. ELISA Detection

For THC estimation, the Cannabinoids Direct ELISA Kit were used. The Cannabinoids Direct ELISA Kit is a specific and sensitive in vitro test to detect the presence of cannabinoids in samples such as flower buds, whole blood, serum, plasma, and urine. Λ9 -THC (a member of the cannabinoid family) is the primary psychoactive ingredient of marijuana. Cannabinoid metabolites appear in urine two to four hours after exposure to marijuana smoke and may persist for days (up to thirty). Thus a urine assay reasonably serves to detect cannabis use even though a considerable period may have elapsed since smoking or ingestion of marijuana (Cannabinoids (THCA/CTHC) ELISA Kit, Catalog Number SE120020; Sigma-Aldrich).

The Cannabinoids Direct ELISA Kit is based upon the competitive binding to an antibody of enzyme labelled antigen and unlabeled antigen, in proportion to their concentration in the reaction mixture. A 10 mL aliquot of a diluted unknown specimen is incubated with a 100 mL dilution of enzyme (Horseradish peroxidase) labelled carboxy THC (THCA) derivative in microplate wells, coated with fixed amounts of oriented high affinity purified polyclonal antibody. The wells are washed thoroughly and a chromogenic substrate is added. The color produced is stopped using a dilute acid stop solution and the wells read at 450 nm. The intensity of the color developed is inversely proportional to the concentration of drug in the sample. The technique is sensitive to 1 ng/ml (THCA/CTHC) ELISA Kit, Catalog Number SE120020; Sigma-Aldrich). The THC Direct ELISA Kit avoids extraction of urine or blood sample for measurement. It employs a polyclonal high affinity, purified carboxy THC antibody (THCA/CTHC) ELISA Kit, Catalog Number SE120020; Sigma-Aldrich).

- **Phosphate Buffer Saline pH 7.0 (0.01 M):** 0.1377 g of Na_2HPO_4 and 0.23 g of NaH_2PO_4 dissolved in 200 ml of sterile distilled water. The pH of solution is adjusted to 7.0 and volume is made up to 250 ml by distilled water.
  - The 100 mg dried leaves soaked in 50 ml PBS, and then solution was kept in shaker at 200 rpm for overnight. Solution was filtered through charcoal.
  - Appropriately 10 µl diluted standards were added to each well. This step was done in duplicate.
  - 10 µl of diluted specimens added in duplicated to each well.
  - 100 µl of the enzyme Conjugate added to each well and the sides of the plate holder tapped to ensure proper mixing.
  - ELISA Plate was incubated for 60 minutes at room temperature in the dark, after addition of enzyme conjugate to the last well.
  - The wells were washed 6 times with 300 µl distilled water.
  - Wells were inverted and vigorously slap dry on absorbent paper so that all residual moisture was removed.
  - 100 µl of substrate reagent added to each well sides of plate holder tapped to ensure proper mixing.
  - Followed by incubation for 30 minutes at room temperature in the dark.
  - 100 µl of stop solution added to each well, to change the blue colour to yellow.
  - Absorbance measured at a dual wavelength of 450 nm for the quantification of THC levles.

5.2. THC Potency

As *Cannabis* becomes more widely used in retail and medicinal contexts, it is important that consumers are presented with accurate information about the THC potency of what they are consuming [27-58-60]. Legal *Cannabis* products in the United States are required to report THC potency (total THC %by dry weight) on packaging [27]. However, concerns have been raised that reported THC potency values are inaccurate [27-57-60]. Multiple studies have demonstrated that THC potency is a primary factor in determining pricing for *Cannabis* flower, so it has an outsized role in the marketplace [27-57-60]. Reports of inflated THC potency and "lab shopping" to obtain higher THC potency results have been circulating for some time [27-60]. The lack of accurate reporting of THC potency can have impacts on medical patients controlling dosage, recreational consumers expecting an effect aligned with price, and trust in the industry as a whole [27-57-60]. As the legal cannabis market continues to grow, it is essential that the industry moves toward selling products with more accurate labelling [27-58]. A study conducted by Schwabe et al., (2023) analyzed THC potency using HPLC in 23 samples from 10 dispensaries throughout the Colorado Front Range, USA and compared the results to the THC potency reported on the packaging [27-57-60]. Average observed THC potency was 14.98 +/- 2.23%, which is substantially lower than recent reports summarizing dispensary reported THC potency [27-60]. The average observed THC potency was 23.1% lower than the lowest label reported values and 35.6% lower than the highest label reported values [27-60]. Overall, ~70% of the samples were more than 15% lower than the THC potency numbers reported on the label, with three samples having only one half of the reported maximum THC potency [27-61].

According to the study conducted by Schwabe et al., (2023), the experimental results demonstrated that retail *Cannabis* flower THC potency is significantly inflated in samples purchased in Colorado, USA [27]. Given the numerous recent reports and lawsuits questioning THC potency reporting, it is likely that this is an industry wide problem [27]. Additional studies should be conducted in other USA states and with larger sample sizes to confirm Schwabe et al., (2023) experimental findings [27]. Schwabe et al., (2023) study also mentioned that although have no power to change the current system, they hope highlighting this issue and educating consumers will affect the change needed to remedy inflated potency of flower products [27]. Addressing this discrepancy will be required both changes to the regulatory system and consumer awareness that reported THC potencies are frequently inflated [27]. It is currently unclear how many consumers are aware of issues with THC potency reporting and how it might impact their purchasing decisions [27]. Accurate label reporting is essential to ensure consumer trust [27]. If consumers cannot trust THC potency reporting, they may also question the reliability of other testing results, such as a lack of pesticides, moulds, and other contaminants [27, 54, 58].

- **Sample Collection**– According to the study conducted by Schwabe et al., (2023) it has been documented that THC potency varies in flowers located on the top, middle and lower branches on the same plant as well as the timing of plant development [27]. Colorado, USA has developed guidelines for how flowers should be sampled [27, 53], but there is limited to no enforcement of these guidelines [27]. If growers are not randomly selecting flowers from throughout plants for testing, THC potency results may not be indicative of the entire batch [27].
- According to the study conducted by Schwabe et al., (2023), the testing instrumentation used in this study is the most common method used in Colorado, USA with all ten current testing labs advertising HPLC as the method to quantify the THC % by dry weight of cannabinoids tested in each sample [27]. Therefore, the methodology used in this study should not impact the observed decrease in THC content of samples [27].
- **Sample Degradation**–THC is known to degrade if not stored correctly. Ross and ElSohly [26, 27, 52] found that when stored at room temperature, THC potency decreased by 16.6% (±7.4) after one year, and up to 41.4% (±6.5) after four years [27-57]. Furthermore, when exposed to light at room temperature, THC is almost 100% degraded after four years [26, 53-57]. However, when THC degrades, it is converted to Cnnabinol (CBN) which was not observed in sizeable quantities in the samples used in this study, indicating the lower potency in the observed versus reported values were not due to age or poor storage conditions [26, 52-57].
- The three most used extraction techniques by the cannabis industry are alcohol extraction, hydrocarbon extraction, and supercritical carbon dioxide (CO_2) extraction [20- 52-57]. As with most things in life, there are pros and cons for each of these methods. Alcohol extraction uses ethanol and can be performed cold, hot, or at room temperature [26, 52-57]. It remains one of the most efficient extraction methods. However, the hot ethanol often extracts unwanted chlorophyll and plant waxes and required clarification or correction steps [26, 52-57]. The cold process reduces these contaminants at the price of longer extraction times [26, 52-57]. Room temperature ethanol extraction splits these differences [26, 52-57]. Hydrocarbon extraction uses butane or propane, solvents with much lower boiling points than ethanol [26, 52-57]. These solvents extract more of the plant terpenes, which provide more flavour, aroma and are therefore better for vape oils or oral tinctures [26-52-57]. However, like the alcohol method, scaling up to large batches is difficult and presents significant safety hazards [26, 52-57].

- Many USA states allowing medical and recreational cannabis use have set standards for laboratories to obtain a representative sample of the batch of cannabis to be tested [33]. This includes training guidelines for sampling staff, sampling plans for different types of cannabis products (e.g. flower, extracts, infused products), maximum batch weights or sizes, and a minimum required sample for the laboratory sampler to take [33]. These guidelines required a percentage of the batch to be obtained as a representative sample [26, 52-57-60]. In Oregon, USA, testing required to obtain a minimum 0.5 percent of the total batch weight for flower material to be tested. With a maximum batch size of 15 pounds, that equates to a maximum 34.2 gram sample of flower material to be sampled from throughout the batch and returned to the lab for testing [25, 26, 52-57-60].
- Cannabinoid testing, sometimes referred to as potency testing, can be done on either an HPLC (high performance liquid chromatography) or GC (gas chromatography) [27, 33-61]. Most laboratories have transitioned to HPLC analysis as this provides the laboratory with more reliable results for the acidic cannabinoids like THCA and CBDA [27, 33-60]. Currently, there are not standard methods for this analysis or any analysis in the cannabis testing sphere [33-60]. The lack of standardization stems from cannabis being a fairly new legal industry in the United States, coupled with laboratory competition and hesitancy to share knowledge [33-60]. Therefore, laboratory is looking to overcome these hurdles and work with groups like AOAC and ASTM to help to build standard methods and implement them in the industry [33-60].
- Total THC includes Δ9-THC and THCA to indicate how much of the psychoactive component could be available to the consumer after decarboxylation [33]. This is calculated using the following equation:

Total THC = [Δ9-THC] + [THCA x 0.877]

This equation assumes 100 percent decarboxylation of THCA into the active Δ9-THC cannabinoid [33]. In its acid form, THCA has no psychoactive effect. A lot of challenges comes from the matrix itself [33]. Variability in tested values can be observed in a lot of infused products, and even flower material [33]. Some infused products, like drinks, are difficult to infuse due to the hydrophobicity of the THC or CBD molecules [33-60]. With other products such as gummies, homogenization and consistency are difficult to achieve within and across batches [33]. The laboratory does a lot of consulting with clients to help them dial in their process to provide consumers with consistent products [33-60].

6. Conclusion

Cannabis has been used for thousands of years for recreational, medicinal, or religious purposes. Cannabis has a long history in India, recorded in legends and religion. However, due to the presence of psychoactive molecules, Λ9-tetrahydrocannabinol (Λ9-THC) and Λ8-tetrahydrocannabinol (Λ8-THC), the Cannabis cultivation and its use is restricted/regulated in many countries. The medicinal compounds from cannabis are mostly concentrated in the female flowers of this dioecious species. Cannabis plant does not make any Λ9-tetrahydrocannabinol (Λ9-THC) but plant produces tetrahydrocannabinolic acid (THCA) which is a non-intoxicating compound that can be converted into Λ9-tetrahydrocannabinol (Λ9-THC) through decarboxylation upon heating. A variety of analytical techniques have been developed for quantification and qualification of Cannabinoids and other compounds in Cannabis plant. As THCA converts to THC, the total weight of the newly formed cannabinoid makes up less of the total dried weight of the herb. At-home color chart test kits come with a container and fluid that customer can place a small sample of flower. Colour tests for cannabis are among the most specific colour tests available. The most common cannabinoid quantification techniques include Cannabinoids direct ELISA Kit, thin layer chromatography, gas chromatography (GC) and high performance liquid chromatography (HPLC) followed by Fourier transform infrared spectroscopy (FTIR) and Nuclear magnetic resonance spectrometry (NMR). As *Cannabis* becomes more widely used in retail and medicinal contexts, it is important that consumers are presented with accurate information about the THC potency of what they are consuming. The lack of accurate reporting of THC potency can have impacts on medical patients controlling dosage, recreational consumers expecting an effect aligned with price, and trust in the industry as a whole. Multiple studies have demonstrated that THC potency is a primary factor in determining pricing for *Cannabis* flower, so it has an outsized role in the marketplace. Accurate label reporting is essential to ensure consumer trust. If consumers cannot trust THC potency reporting, they may also question the reliability of other testing results, such as a lack of pesticides, moulds, and other contaminants.

Compliance with ethical standards

Acknowledgments

We would like to thank and acknowledge, Karen Viviana Castaño Coronado, Chief Communications Officer (CCO) and CO-Founder of LAIHA (Latin American Industrial Hemp Association), and CEO- CANNACONS, Bogota, D.C., Capital

District, Colombia for thoughtful discussions, critical comments, supporting, promoting, encouraging and appreciating this research work. We also thank all the members of LAIHA for supporting and encouraging this research work.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Malabadi RB, Kolkar KP, Chalannavar RK. *Cannabis sativa*: Ethnobotany and phytochemistry. International Journal of Innovation Scientific Research and Review. 2023; 5(2): 3990-3998.

[2] Malabadi RB, Kolkar KP, Acharya M, Chalannavar RK. *Cannabis sativa*: Medicinal plant with 1000 molecules of pharmaceutical interest. International Journal of Innovation Scientific Research and Review. 2023; 5(2):3999-4005.

[3] Malabadi RB, Kolkar KP, Chalannavar RK. *Cannabis sativa*: Industrial hemp (fiber type)- An Ayurvedic traditional herbal medicine. International Journal of Innovation Scientific Research and Review. 2023; 5 (2): 4040-4046.

[4] Malabadi RB, Kolkar KP, Chalannavar RK. Medical *Cannabis sativa* (Marijuana or Drug type); The story of discovery of Δ9-Tetrahydrocannabinol (THC). International Journal of Innovation Scientific Research and Review. 2023; 5 (3):4134-4143.

[5] Malabadi RB, Kolkar KP, Chalannavar RK. Δ9-Tetrahydrocannabinol (THC): The major psychoactive component is of botanical origin. International Journal of Innovation Scientific Research and Review. 2023; 5(3): 4177-4184.

[6] Malabadi RB, Kolkar KP, Chalannavar RK. *Cannabis sativa*: Industrial Hemp (fibre-type)- An emerging opportunity for India. International Journal of Research and Scientific Innovations (IJRSI). 2023; X (3):01-9.

[7] Malabadi RB, Kolkar KP, Chalannavar RK. Industrial *Cannabis sativa* (Hemp fiber type):Hempcrete-A plant based eco-friendly building construction material. International Journal of Research and Innovations in Applied Sciences (IJRIAS). 2023; 8(3): 67-78.

[8] Malabadi RB, Kolkar KP, Chalannavar RK, Lavanya L, Abdi G. *Cannabis sativa*: The difference between Δ8-THC and Δ9-Tetrahydrocannabinol (THC). International Journal of Innovation Scientific Research and Review. 2023; 5(4): 4315-4318.

[9] Malabadi RB, Kolkar KP, Chalannavar RK, Lavanya L, Abdi G. Hemp Helps Human Health: Role of phytocannabinoids. International Journal of Innovation Scientific Research and Review. 2023; 5 (4): 4340-4349.

[10] Malabadi RB, Kolkar KP, Chalannavar RK, Lavanya L, Abdi G. *Cannabis sativa*: Botany, cross pollination and plant breeding problems. International Journal of Research and Innovations in Applied Science (IJRIAS). 2023; 8 (4): 174-190.

[11] Malabadi RB, Kolkar KP, Chalannavar RK, Lavanya L, Abdi G, Baijnath H. Cannabis products contamination problem: A major quality issue. International Journal of Innovation Scientific Research and Review. 2023;5(4): 4402-4405.

[12] Malabadi RB, Kolkar KP, Chalannavar RK, Lavanya L, Abdi G. Medical *Cannabis sativa* (Marijuana or drug type): Psychoactive molecule, Δ9-Tetrahydrocannabinol (Δ9-THC). International Journal of Research and Innovations in Applied Science. 2023; 8(4): 236-249.

[13] Malabadi RB, Kolkar KP, Chalannavar RK, Mondal M, Lavanya L, Abdi G, Baijnath H. *Cannabis sativa*: Release of volatile organic compounds (VOCs) affecting air quality. International Journal of Research and Innovations in Applied Science (IJRIAS). 2023; 8(5): 23-35.

[14] Malabadi RB, Nethravathi TL, Kolkar KP, Chalannavar RK, Mudigoudra BS, Lavanya L, Abdi G, Baijnath H. *Cannabis sativa*: Applications of Artificial Intelligence and Plant Tissue Culture for Micropropagation. International Journal of Research and Innovations in Applied Science (IJRIAS). 2023; 8(6): 117-142.

[15] Malabadi RB, Nethravathi TL, Kolkar KP, Chalannavar RK, Mudigoudra BS, Abdi G, Baijnath H. *Cannabis sativa*: Applications of Artificial intelligence (AI) in Cannabis industries: In Vitro plant tissue culture. International Journal of Research and Innovations in Applied Science (IJRIAS). 2023; 8 (7): 21-40.

[16] Malabadi RB, Kolkar KP, Brindha C, Chalannavar RK, Abdi G, Baijnath H, Munhoz ANR, Mudigoudra BS. *Cannabis sativa*: Autoflowering and Hybrid Strains. International Journal of Innovation Scientific Research and Review. 2023; 5(7): 4874-4877.

[17] Malabadi RB, Kolkar KP, Chalannavar RK, Munhoz ANR, Abdi G, Baijnath H. *Cannabis sativa*: Dioecious into Monoecious Plants influencing Sex Determination. International Journal of Research and Innovations in Applied Science (IJRIAS). 2023; 8(7): 82-91.

[18] Malabadi RB, Kolkar KP, Chalannavar RK, Abdi G, Munhoz ANR, Baijnath H *Cannabis sativa*: Dengue viral disease-Vector control measures. International Journal of Innovation Scientific Research and Review. 2023; 5(8): 5013-5016.

[19] Malabadi RB, Nethravathi TL, Kolkar KP, Chalannavar RK, Mudigoudra BS, Abdi G, Munhoz ANR, Baijnath H. *Cannabis sativa*: One Plant-One-Medicine for many diseases-Therapeutic Applications. International Journal of Research and Innovations in Applied Science (IJRIAS). 2023; 8(8): 132-174.

[20] Malabadi RB, Nethravathi TL, Kolkar KP, Chalannavar RK, Mudigoudra BS, Abdi G, Munhoz ANR, Baijnath H. Fungal Infection Diseases- Nightmare for Cannabis Industries: Artificial Intelligence Applications International Journal of Research and Innovations in Applied Science (IJRIAS). 2023; 8(8):111-131.

[21] Malabadi RB, Kolkar KP, Chalannavar RK, Baijnath H. *Cannabis sativa*: Difference between Medical Cannabis (marijuana or drug) and Industrial hemp. GSC Biological and Pharmaceutical Sciences. 2023; 377-381.

[22] Malabadi RB, Kolkar KP,Chalannavar RK, Acharya M, Mudigoudra BS. *Cannabis sativa*: 2023-Outbreak and Re-emergence of Nipah virus (NiV) in India: Role of Hemp oil. GSC Biological and Pharmaceutical Sciences. 2023; 25(01):063–077.

[23] Malabadi RB, Kolkar KP, Chalannavar RK, Acharya M, Mudigoudra BS. Industrial *Cannabis sativa*: Hemp-Biochar-Applications and Disadvantages. World Journal of Advanced Research and Reviews. 2023; 20(01): 371–383.

[24] Malabadi RB, Kolkar KP, Chalannavar RK, Vassanthini R, Mudigoudra BS. Industrial *Cannabis sativa*: Hemp plastic-Updates. World Journal of Advanced Research and Reviews. 2023; 20 (01): 715-725.

[25] Malabadi RB, Kolkar KP, Chalannavar RK. Industrial *Cannabis sativa*: Hemp oil for biodiesel production. Magna Scientia Advanced Research and Reviews. 2023; 09(02): 022–035.

[26] Lazarjani MP, Torres S, Hooker T, Fowlie C, Young O, Seyfoddin A. Methods for quantification of Cannabinoids: A narrative review. Journal of Cannabis Research. 2020; 2:35 https://doi.org/10.1186/s42238-020-00040-2.

[27] **Schwabe** AL, Johnson V, Harrelson J, McGlaughlin ME. Uncomfortably high: Testing reveals inflated THC potency on retail Cannabis labels. PLoS ONE. 2023; 18(4): e0282396.

[28] Pollio A. The name of Cannabis: A short guide for nonbotanists. Cannabis and Cannabinoid Research. 2016 Oct 1; 1(1):234–8.

[29] McPartland JM. *Cannabis sativa* and Cannabis indica versus "Sativa" and "Indica". In *Cannabis sativa* L.-botany and biotechnology 2017 (pp. 101–121). Springer, Cham.

[30] McPartland JM, Small E. A classification of endangered high-THC cannabis (*Cannabis sativa* subsp. indica) domesticates and their wild relatives. PhytoKeys. 2020; 144:81.

[31] Sumpter L, Payá G. How To Test the THC and CBD Content of Your Weed - RQS Blog (royalqueenseeds.com). 20th July 2021.

[32] Jikomes N. How to measure THC and CBD content in weed. How to Measure THC & CBD Levels in Cannabis Products | Leafly. March 22, 2017 and updated on 28th July 2020.

[33] Dotzert M. THC Testing in Cannabis and Hemp. Lab Manager's Michelle Dotzert speaks with Ellen Parkin, quality assurance officer at Juniper Analytics, LLC. Jun 11, 2020.

[34] Schwabe AL. A multifaceted approach to address variation in *Cannabis sativa*. PhD Dissertation, University of Northern Colorado. 2019.

[35] Aizpurua-Olaizola O, Omar J, Navarro P, Olivares M, Etxebarria N, Usobiaga A. Identification and quantification of cannabinoids in *Cannabis sativa* L. plants by high-performance liquid chromatography-mass spectrometry. Anal Bioanal Chem. 2014;406(29):7549–60.

[36] Berman P, et al. A new ESI-LC/MS approach for comprehensive metabolic profiling of phytocannabinoids in Cannabis. Sci Rep. 2018;8(1):1–15.

[37] Brighenti V, et al. Development of a new extraction technique and HPLC method for the analysis of non-psychoactive cannabinoids in fiber-type *Cannabis sativa* L.(hemp). J Pharm Biomed Anal. 2017;143:228–36.

[38] Casiraghi A, et al. Extraction method and analysis of cannabinoids in cannabis olive oil preparations. Planta Med. 2018;84(04):242–9.

[39] Ciolino LA, Ranieri TL, Taylor AM. Commercial cannabis consumer products part 1: GC-MS qualitative analysis of cannabis cannabinoids. Forensic Sci Int. 2018; 289:429–37.

[40] Citti C, Ciccarella G, Braghiroli D, Parenti C, Vandelli MA, Cannazza G. Medicinal cannabis: principal cannabinoids concentration and their stability evaluated by a high-performance liquid chromatography coupled to diode array and quadrupole time of flight mass spectrometry method. J Pharm Biomed Anal. 2016;128:201–9.

[41] Dussy FE, Hamberg C, Luginbuhl M, Schwerzmann T, Briellmann TA. Isolation of Delta9-THCA-A from hemp and analytical aspects concerning the determination of Delta9-THC in cannabis products. Forensic Sci Int. 2005; 149(1):3–10.

[42] Hazekamp A, Choi YH, Verpoorte R. Quantitative analysis of cannabinoids from *Cannabis sativa* using 1H-NMR. Chem Pharm Bull. 2014;52(6):718–21.

[43] Hazekamp A, Simons R, Peltenburg-Looman A, Sengers M, van Zweden R, Verpoorte R. Preparative isolation of cannabinoids from *Cannabis sativa* by centrifugal partition chromatography. J Liq Chromatogr Relat Technol. 2009; 27(15):2421–39.

[44] Leghissa A, Hildenbrand ZL, Schug KA. A review of methods for the chemical characterization of cannabis natural products. J Sep Sci. 2018a;41(1):398–415.

[45] Leghissa A, et al. Detection of cannabinoids and cannabinoid metabolites using gas chromatography with vacuum ultraviolet spectroscopy. Sep Sci Plus. 2018c;1(1):37–42.

[46] Namdar D, et al. Variation in the compositions of cannabinoid and terpenoids in *Cannabis sativa* derived from inflorescence position along the stem and extraction methods. Ind Crop Prod. 2018;113:376–82.

[47] Namdar D, et al. LED lighting affects the composition and biological activity of *Cannabis sativa* secondary metabolites. Ind Crop Prod. 2019;132:177–85.

[48] Pellati F, et al. New methods for the comprehensive analysis of bioactive compounds in *Cannabis sativa* L.(hemp). Molecules. 2018;23(10):2639.

[49] Faubert Maunder, M.J. de. UNODC, Two simple colour tests for cannabis 1969.

[50] Andrew Holmes. Personal communication. 2007.

[51] Laboratory and Scientific Section United Nations Office on Drugs and Crime Vienna. Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products (Revised and updated). MANUAL FOR USE BY NATIONAL DRUG ANALYSIS LABORATORIES. UNITED NATIONS, New York, 2009. UNITED NATIONS PUBLICATION Sales No. E.09.XI.15 ISBN 978-92-1-148242-3.

[52] Ross SA, ElSohly MA. CBN and Δ 9-THC concentration ratio as an indicator of the age of stored marijuana samples. Bulletin on Narcotics. 1997; 49(50):139–139.

[53] Zamengo L, Bettin C, Badocco D, Di Marco V, Miolo G, Frison G. The role of time and storage conditions on the composition of hashish and marijuana samples: A four-year study. Forensic Science International. 2019; 1; 298:131–7.

[54] Oldfield K, Ryan J, Doppen M, Kung S, Braithwaite I, Newton-Howes G. A systematic review of the label accuracy of cannabinoid-based products in regulated markets: is what's on the label what's in the product?. Australasian Psychiatry. 2021; 29(1):88–96.

[55] Schwabe AL, Hansen CJ, Hyslop RM, McGlaughlin ME. Comparative Genetic Structure of *Cannabis sativa* Including Federally Produced, Wild Collected, and Cultivated Samples. Frontiers in Plant Science. 2021: 2098.

[56] Smart R, Caulkins JP, Kilmer B, Davenport S, Midgette G. Variation in cannabis potency and prices in a newly legal market: Evidence from 30 million cannabis sales in Washington state. Addiction. 2017; 112(12):2167–77.

[57] Cascini F, Aiello C, Di Tanna G. Increasing delta-9-tetrahydrocannabinol (Δ-9-THC) content in herbal cannabis over time: systematic review and meta-analysis. Current Drug Abuse Reviews. 2012; 5 (1):32–40.

[58] Jikomes N, Zoorob M. The cannabinoid content of legal cannabis in Washington State varies systematically across testing facilities and popular consumer products. Scientific REPORTS. 2018; 8:4519 | DOI:10.1038/s41598-018-22755-2.

[59] Zoorob MJ. The frequency distribution of reported THC concentrations of legal cannabis flower products increases discontinuously around the 20% THC threshold in Nevada and Washington state. Journal of Cannabis Research. 2021; 3:6 https://doi.org/10.1186/s42238-021-00064-2.

[60] AL-Ubeed HMS, Bhuyan DJ, Alsherbiny MA, Basu A, Vuong QV. A comprehensive review on the techniques for extraction of bioactive compounds from medicinal Cannabis. Molecules. 2022; 27: 604. https://doi.org/ 10.3390/molecules27030604.

[61] Dresen S, Kneisel S, Weinmann W, et al. Development and validation of a liquid chromatography-tandem mass spectrometry method for the quantitation of synthetic cannabinoids of the aminoalkylindole type and methanandamide in serum and its application to forensic samples. J. Mass Spectrom. 2011; 46:163–171.

[62] Malabadi RB, Sadiya MR, Kolkar KP, Chalannavar RK. Biodiesel production via transesterification reaction. Open Access Research Journal of Science and Technology. 2023; 09(02): 010–021.

[63] Malabadi RB, Sadiya MR, Kolkar KP, Chalannavar RK. Biodiesel production: An updated review of evidence. International Journal of Biological and Pharmaceutical Sciences Archive. 2023; 06(02): 110–133.

[64] Niloy N, Hediyal TA, Vichitra C, Sonali S, Chidambaram SB, Gorantla VR, Mahalakshmi AM. Effect of Cannabis on Memory Consolidation, Learning and Retrieval and Its Current Legal Status in India: A Review. Biomolecules. 2023; 13: 162. https://doi.org/10.3390/ biom13010162.