Journal of Biological Sciences and Bioconservation
Volume 7, Number 1, 2015
ISSN: 2277-0143

# CHEMICAL ANALYSIS OF CANNABIS; REVIEW

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## ABSTRACT

Cannabis is an annual dioecious plant, known by the Name cannabis sativa L and belongs to the member of the family Cannabaceae. $\Delta^9$-tetrahydrocannabinol (THC), cannabinol (CBN) and Cannabidiol (CBC) are among the major chemical components of cannabis but the presence of cannabinol (CBN) in the sample can indicate that the sample is not fresh. Since $\Delta^9$-tetrahydrocannabinol (THC) is known to oxidize to cannabinol (CBN) over time, it is assumed that the higher the amount of CBN in a sample the older the sample and the rate at which $\Delta^9$ tetrahydrocannabinol degrade to cannabinol per year is seven per cent. This work comparatively reviewed the quantification methods of cannabis which includes Presumptive test, thin layer chromatography, Supercritical fluid chromatography with atmospheric pressure, chemical ionisation mass spectroscopic detection, High-performance liquid chromatography and Gas Chromatography–Mass Spectrometry. Because of GC-MS accuracy, linearity, quickness, efficiency, reproducibility, precision and resolution in quantification of cannabis, it is chosen to be the best analytical method used in the quantification of cannabis as it was also recommended by United Nation Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products.

**Keywords:** Chemical, Analysis, Cannabis, $\Delta^9$-tetrahydrocannabinol, Cannabidiol

## INTRODUCTION

Cannabis sativa L belongs to the member of the family Cannabaceae, it is one of the oldest cultivated plant, mainly use for fabric and rope from the fibre stem, and oil production from the seed. It was also been used as psychoactive drug due to presence of $\Delta^6$-tetrahydrocannabinoid. The name cannabis sativa L was first use in Linnaeus General plantarum in 1753; moreover the family of cannabis sativa L has only one species of tremendous diversity in cultivation and found in wild environment because of the presence of psychoactive compounds in

cannabis plant. [1,2] Cannabis sativa L is an annualdioecious herb, that is, it has both male and female plant.[3] It originate from Eastern and Central Asia, gender difference in cannabis can easily be differentiated at their flowering stage due to the fact that the structure of their flower is different, the female plant is preparable because they produce more glandular trichome and are therefore richer in cannabinoid than male plant.[4]Cannabis products are the most widely trafficked drugs worldwide, accounting for 65 per cent of all global seizure cases (1.65 million cases) in 2006.[5] 5,200 metric tons of herb and 1,000 metric tons of resin were seized in 2006. Practically all countries in the world are affected by cannabis trafficking. Similarly, cannabis also remains the most widely used drug worldwide, with an estimated 166 million people having used cannabis in 2006.[5]Cannabis has been used for over 5000 years starting from both central and northeast Asia, Cannabis (marijuana) is abused throughout the world because of the presence of psychoactive cannabinoids such as $\Delta^9$-tetrahydrocannabinol [6].

Figure 1 . Pictures of cannabis leave [5]

Cannabis plant can be identified microscopically when finely fragmented by its variety of glandular and non-glandular hair. Glandular hair is found in all of part the body and they are responsible for secretion of the resin which contains the psychoactive constituent of the plant. Cannabis contains 483 natural compounds among which six (6) are identified last decade. [7] Majority of this compounds are classified as hydrocarbon, sugar, terpenes, flavonoids and miscellaneous

compounds all in total of 413.[8] Seventy of these compounds are classified as cannabinoids, mostly contain 21 carbon atom but some of them contain less than 21 carbon atoms all of which are included in the seventy 70 compounds of cannabis[8]. $\Delta^9$-tetrahydrocannabinol is a compound found in cannabis which is responsible for its pharmacological activity, it has a double chiral centre and naturally occurring THC contain trans configuration, the psychoactive component of cannabis $\Delta^9$-THC can not only be found in the plant but it can also be form during smoking by decarboxylation of acidic component of cannabis, THC is in every part of cannabis plant but high concentration of it, is found in the female flower head (sinsemilla) the potency of cannabis depend on the nature of growing condition and seeds[9,10].

Non glandular trchomes magnified image[5]

Glandular trichomes magnified image[5]

Generally there are two type of cannabis, the fibre type (hemp)which is mainly cultivated for fibre production and contain less than 0.3% of $\Delta^9$-tetrahydrocannabinol and more cannabidiol then the drug type that is cultivated for narcotics namely marijuana which contain 2-8% of $\Delta^9$-tetrahydrocannabinol[11]

**Side effect and Metabolites of Cannabis**

Cannabis is the most widely used illicit substance, disorder in cannabis users is more common than all other illicit drug use disorders, quitting cannabis is very difficult to its users, it has many negative affect among which is difficult to abstain. Anxiety/ sensitivity and introversion/hopelessness, are common type of

negative affect that are systematically related to cannabis users, but there is contradiction between the literatures agreeing on anxiety as the only negative effect related to cannabis users[13,14] $\Delta^9$-tetrahydrocannabinol is one of the important compound in cannabis and the compound of interest in forensic and pharmacology. $\Delta^9$-tetrahydrocannabinol is metabolized by the liver first to Hydroxy- $\Delta^9$-tetrahydrocannabinol which is active, then to the inactive 11-nor-$\Delta^9$-tetrahydrocannabinol Carboxylic acid (THC-A or THC-COOH). Later can found in the urine sample long (may be months) after the use of cannabis.[15]The psychoactive component of cannabis which is $\Delta^9$-tetrahydrocannabinol (THC) tends to oxidize to cannabinol (CBN) over time; the presence of CBN in a cannabis sample indicates that the sample is not fresh. It is presumed that the higher the amount of CBN, the older the sample.[16]

**Mode of Identification**

Identity and confirmation is a critical part of forensic toxicology and provides the foundation for all subsequent quantitative results, interpretations, and finally court reports and testimonies. Most approach use to identify or detecting drug use is the examination of drugs, biological fluids and tissues for drugs and/or metabolites. This laboratory approach demands analytical technologies capable of accurate identification and quantification, high specimen throughput and cost effectiveness. For instance determination the cause of accidents or deaths that is drug-related in criminal justice, identification of drug abuse in workplace, drug treatment and emergency room settings, and detection of use of performance-enhancing drugs in competitive sports. This report reviewed the recommended analytical technologies used in the analysis of cannabis [17,18] . There are many methods used to identify cannabis products but all this depend on the nature of the cannabis product, hash oil and resin are identify base on the evidence of presence of $\Delta^9$-tetrahydrocannabinol, cannabidiol and their oxidized product, identification of herbal cannabis is based on its morphological characteristics only.[5]

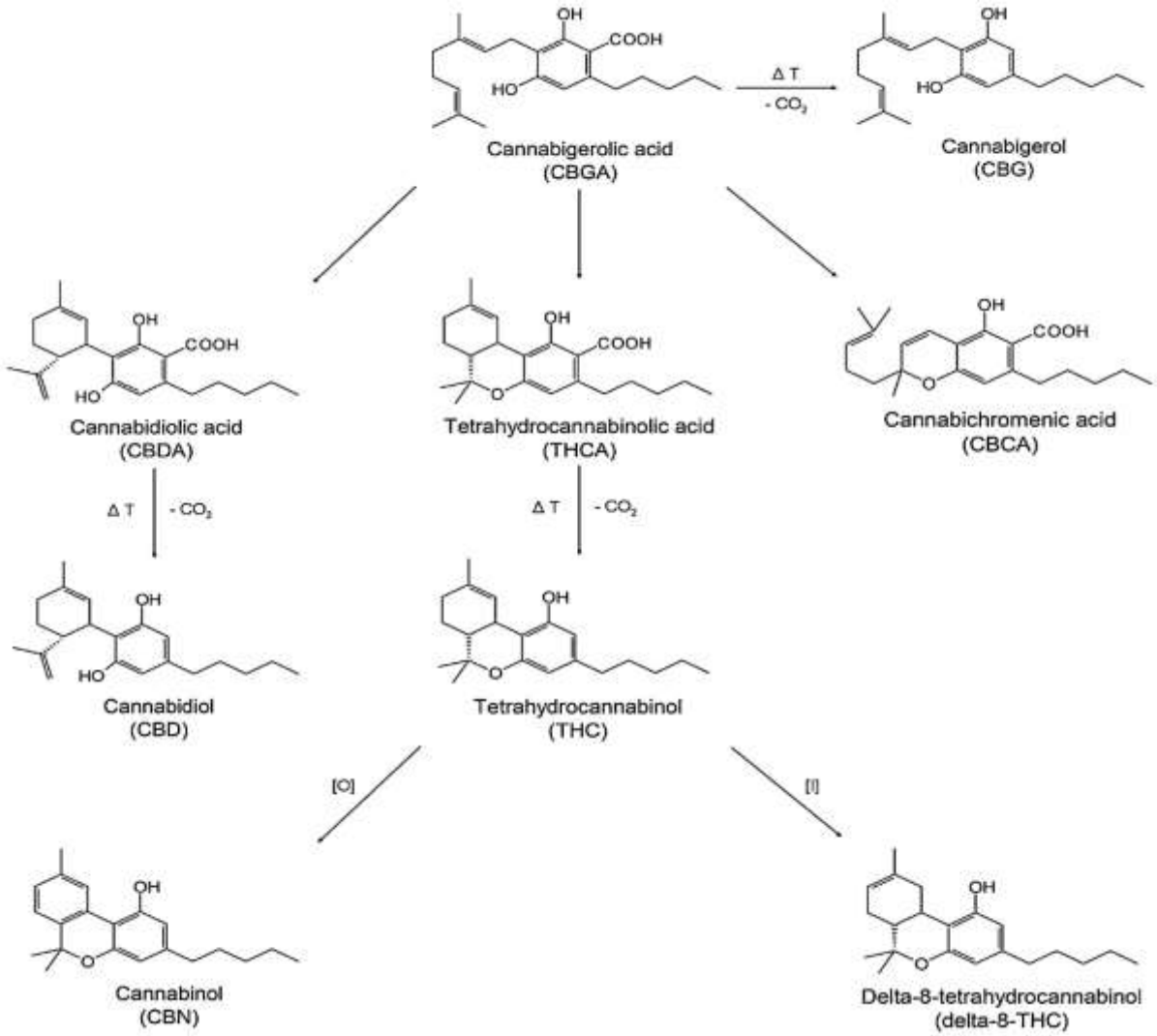
Cannabis sativa is mainly identified on the basis of its morphological features and this is always achieved by using microscope to identify the structures on the surface of the plant, namely the trichomes. Most of the futures used to identify cannabis microscopically are palmate leaves with serrated edges, four corners and square stem. Furthermore microscopically, three forms of trichome are observed, namely the glandular trichomes, unicellular trichomes and cystolithictrichomes. All these trachomes structures are found all over the body of the cannabis but glandular trichomes is mostly associated with the flower structures; the cystolithictrichomes has oxalic acid crystals which is

visible within their bases. Any plant found with all of the trichomes mention above together is definitively identified as *Cannabis sativa*, because no any other plant possess all the futures mentioned[5,19,20]. For the cannabis material in which all the morphological characteristics are not present, or destroyed the only alternative is to identify the material base on the phytochemical content. Which involves the identification of the principle drugs which characterize the plant, namely $\Delta^9$-tetrahydrocannabinol, cannabidiol and cannabonol for bulk samples, the identification is usually done by GC–MS, HPLC,TLC, and supercritical fluid chromatography with atmospheric pressure, chemical ionisation mass spectroscopic detection.[19,20]

**Compound and Structures**

Many attempt have been made from the end of eighteenth century to early nineties to determine the structures of the compounds of cannabis but all failed, until 1960, Mechoulam and his associates carried a long successful research by isolating, purifying and determining the structures of the compounds of cannabis some of which are cannabigerol,cannabichromene, cannabicyclic, their carboxylic derivatives and $\Delta^9$-tetrahydrocannabinol which is one of the component of the cannabis.

**Structures**




**Structures of the Compounds of Cannabis**[27]

Due to the presence of two chiral centres in (THC) molecular structure can exist in almost four different stereoisomers, the natural existing configuration is (3R,4R)-trans isomer which was established in 1967.[12]

## BACKGROUND AND DISCUSSION

### Presumptive Tests for Cannabis

Presumptive tests for cannabis products are test used check the presence (or otherwise) of (THC) in a material. There are many methods that can be used in cannabis presumption test which include Immunoassays, Ion mobility spectrometry (IMS), Duquenois– Levine test and thin layer chromatography. Immunoassay can be used in seconds to trace drug substances in a sample but this analysis is hardly used due to the fact that the analysis has not much power

proof and is costly. Tetrahyrocannabinoid can be identify using Ion mobility spectrometry but the problem from humidity and heroin signal separation have been noted, that is why it cannot be used for identification again.[5]

Duquenois– Levine test can also be used in presumption identification of cannabis it involve a reaction between a test reagent and cannabis to form a colour product, in this analysis both negative and positive control test are needed. The positive control is required to show that the test is working, provision of reference colour for which the sample can be tested and reference time for which the colour change of the reaction will be expected, the negative control is needed to show that colour change in the reaction is due to the presence of cannabis in the sample. Duquenois–Levine Test reaction is usually carried out in a small test tube with the use of three to four kind of reagent which include a acetaldehyde, ethanol, chloroform and concentrated hydrochloric acid, this form of presumption test or analysis is normally use in identification of phenolic compounds in cannabis.[1,5,21,22,]All the analytical techniques mentions above are used in identification of cannabis in a sample but cannot be used in quantification.

**Thin Layer Chromatography Approach**

Thin layer chromatography is method used for the qualitative and semi-quantitative analysis of cannabis using a different stationary phases (TLC plates) and solvent systems. TLC is another presumption analysis method which provides a rapid, easy and cost-effective means for analysis of cannabis materials. Cannabinoids normally oxidized and so should be prepared for TLC in solvents which are stable, Ethanol were used as a solvent considering its solubility in cannabinoid and their carboxylic acid, thin layer chromatography is among the specific colour test methods used to analyse and indicate the presence of cannabis material from a given sample, the colour test only indicate the presence of cannabis in a sample therefore there is a need for definitive identification of cannabis. Thin layer chromatography is useful method on preliminary comparison between the samples, most of the result obtained depend on laboratory condition which include temperature, humidity and other parameters which also include age and quality of the material use [1,5,23]. Colour test in thin layer chromatography is one of the simplest procedure used to analyse the presence of THC in a sample, there are three forms of colour test which include. Fast Corinth V salt test, fast blue salt test and Rapid dequenois test, the stated analysis are carried out with colour developing reagent, TLC sheet, vial mobile reagent and extract retention. [5,44] Colour change in technique said above indicate the presence of cannabis in the analyte, when

colour changes to purple in the case of fast Corinth V salt test, that indicate the presence of $\Delta^9$-THC, CBN and CBD. For fast blue salt test there is variation in the colour change of the components, red colour indicate the presence of THC, purple indicate CBN and orange indicate CBD.[5]TLC colour test is being used to presume presence of THC and its component.

**Supercritical Fluid Chromatography with Atmospheric Pressure Chemical Ionisation Mass Spectroscopic Detection**

This is a novel method used in identification of cannabis product, the cannabinoid are separated using this method in eight minutes, supercritical fluid chromatography (SFC) coupled to atmospheric pressure chemical ionization-mass spectroscopic (APCI-MS) detection does not need derivatization before the analysis and has a short time analysis than most method analysis of cannabis compound, it also has good resolution and definitive identification. The sequence of retention time are 4.19ng for cannabinol, $\Delta^8$-tetrahydrocarnabinol 4.67ng, $\Delta^9$-THC 5.19ng and CBN 6.98ng and detector respond ware also 0.55ng, 1.20ng, 0.69ng and 2.10 following above sequence respectively. Identification was carried out through the use of combination deuterated internal standard and mass spectra.[24] Analysis was carried out by B. Backstrom *et al* 1997 and the result of the analysis show that SFC-APCI-MS method of analysis can be used in analyzing THC samples without difficulty and it is sensitive for the analyzing both trace and bulk samples, Supercritical fluid chromatography with atmospheric pressure chemical ionisation mass spectroscopic detection (SFC-APCI-MS) does not create too much volume of waste that require disposal and this method of analysis can also be used to analyses a bulk sample without difficulty and is sufficiently sensitive method of analysis[24]. SFC-APCC-MS has only one disadvantage in the identification of cannabinoids, at low level (about 40mg) matrix recovery is only about 90%, and looking at the differences in matrix materials, recovery of cannabinoids from hashish is double that of cannabis due this fact matrix has to be consider before choosing SFE for quantification of cannabis and its metabolites.[45,46]

**Gas Chromatography–Mass Spectrometry**

One of the most frequent methods used in identification of cannabis product is gas chromatography–mass spectrometry (GC–MS). The identification depends on the presence of $\Delta^9$-THC, CBD and CBN in the sample, and to some extent the presence of isomers of the active component of cannabis. There are conditions for extraction and quantification of these cannabis components CBD, $\Delta^9$-THC and CBN which are variables such as number of extract, extraction time and response to the surface analysis-RSA, then derivatization. When using gas

chromatography in analysis of cannabinoid derivatization must be employed, if not the carboxylic acid compounds will decompose.[5,25] due to the fact that they are thermally labile. They are many reasons why derivatization of cannabinoids sample is necessary [1,5,25]. Derivatization is necessary because the carboxylic and hydroxide entities of THC can form hydrogen bonding in gas phase which will result in poor chromatogram. These entities or compounds can also be absorbed on to chromatographic system which will also lead to poor chromatographic performance, the shape, peak and concentration will be low, $\Delta^9$-THC carboxylic acid thermally decarboxylate to form $\Delta^9$-THC.[1] Derivatizing a THC sample allowed determination of both $\Delta^9$-THC and its acid, it can also improve the limit of detection and quantification in a low concentration of analyte.[1]

In gas chromatography the extracts obtained can be analyzed by different forms of detection, Gas chromatography-flame ionization detection (GC-FID), Gas chromatography-mass spectrometry (GC-MS) scan mode and GC-MS Selected Ion Monitoring (SIM) mode. In all form of detectors mention above, researches shown that Gas chromatography-mass spectrometry GC-MS provide the highest result in all GC analysis. In GC method for quantification of cannabinoids component there are chromatographic parameters need to be put in to consideration such as selectivity, precision, linearity, accuracy (98-99%), limit of quantification (LOQ) and limit of detection (LOD).With respect quantification of cannabis GC is selected due to its quickness, efficiency and reproducibility[25]. Limit of detection (LOD) is statistical valid lowest amount of analyte that can be detected in a standard free of matrix. The acceptable way of calculating LOD is as a multiple of the peak-to-peak noise (typically stated as 2 or 3 × noise). Statistically (LOD) related to the valid lowest measureable peak response that can be detected above typical system noise under the conditions of analysis. Noise can be determined by using a clean standards and analytical systems. Limit of detection is normally based on the analyte selective response that is response per concentration per time, using either peak height or area and the background noise. It can be both chemical and electronic noise, but mainly focuses on the higher frequency noise when doing noise determinations for calculating LOD.[47]

S. A. ROSS *et al* (1999) carried out analysis to determination the age of a cannabis sample in forensic work, since it has been known that $\Delta^9$-THC is chemically unstable and will oxidize to CBN over a period time or chemically altered, presence of CBN in cannabis sample indicates that the cannabis sample is not fresh. It is assumed that the higher concentration the cannabinol CBN, in

the cannabis sample the older the sample. The result of this analysis correlates between the amount of CBN and THC with respect to the age of cannabis samples. The different cannabis samples stored at room temperature, these samples were first analyse immediately after harvesting then year after for up to four years

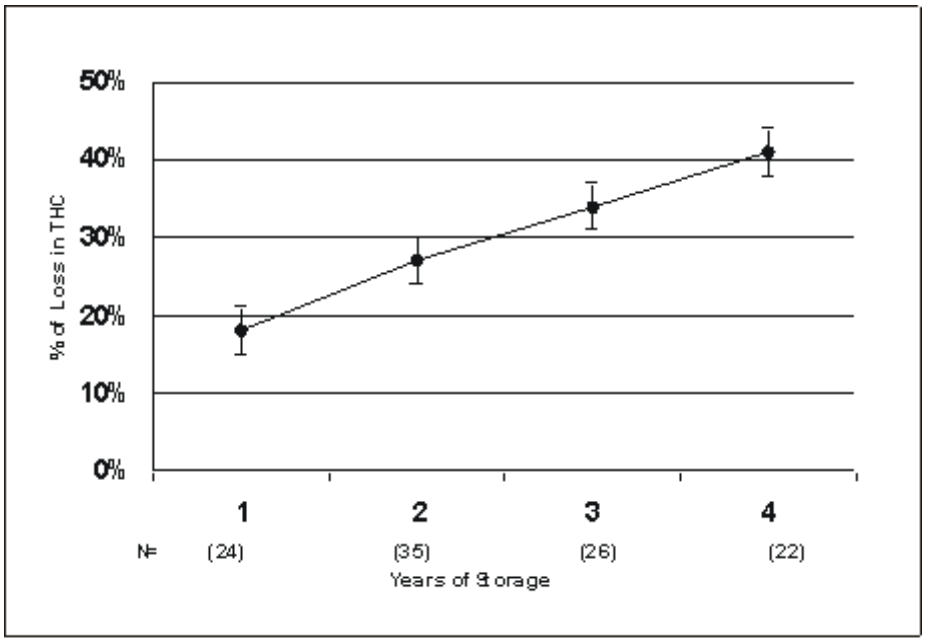



**Figure 3.Relationship between the Percentage Loss of THC and Years of Storage [16]**

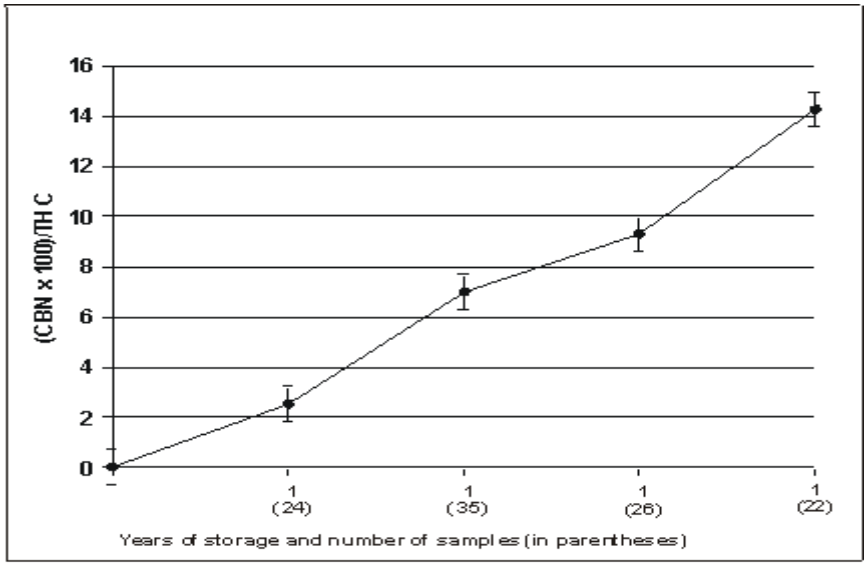



**Figure 4.Relationship between the percentage ratio of CBN to THC and years of storage [16]**

The figures above show the relationship between the percentage loss of THC and the storage time. Beside by clear looking there is a difference in the percentage loss at each data point (standard deviation of 6.5-7.6 per cent), the percentage loss of $\Delta^9$-tetrahydrocannabinol THC was proportional to the storage time. "On the average, the concentration of THC in the plant material decreased by 16.6% ±7.4 of its original value after one year, 26.8% ±7.3 after two years, 34.5% ±7.6 after three years and 41.4% ±6.5 after four years"[16]. Many efforts have been made to create a relationship between storage time and the concentration of THC and CBN. It was analysed that the percentage ratio of CBN to THC at a time predict the age of the plant material. The percentage ratio of $\Delta^9$ –THC to CBN of all the samples stored at different time ( one, two, three and four years individually) are found to be 2.5 ±0.9, 6.7 ±1.4, 9.4 ±1.7 and 14.2 ±1.2 for all the samples stored for one, two, three and four years respectively.[16]

After the analysis there were numbers of observations made. First, it was noted that not all the $\Delta^9$-THC was directly converts to can CBN, it was suggested that there were other intermediates in that process, CBN does not exist in the fresh cannabis and carefully dried cannabis and then finally $\Delta^9$ tetrahydrocannabinol appears to degradation at a higher rate for the first year than the following years. Based on the studies carried out by S. A. ROSS *et al* 1999, conclusion was drawn that only seven percent of $\Delta^9$-THC degrade to CBN per year, it is believe that the rate at which $\Delta^9$-THC is loss in the original sample is the function of initial concentration, the faster the degradation, the higher the concentration of THC on the sample per year.[16,26,27,28]

**High-Performance Liquid Chromatography (HPLC)**

HPLC as a comparative technique used in analysis of cannabis, it has many advantages over other techniques used in the analysis of cannabis. HPLC does not require the sample analysed to be volatile, it does not require any pre-treatment of the sample prior to analysis, such as derivatization, it can also be automated and can be used quantitatively.[1,29]The carboxyl group of cannabinoids is not very stable and is easily lost as $CO_2$ under influence of heat or light, which result in the corresponding neutral cannabinoids, THC, CBD and cannabigerol (CBG).[29,30,31] All These component are formed during harvesting, drying, storage, heating and smoking of cannabis plant. Cannabis is known to oxidize due to many variables some of which includes stages of growing, harvesting, processing, heat and storage. The most common degradation Product of aged cannabis is cannabinol (CBN), which is produced by degradation of

tetrahydrocannabinoid (THC)[32,33] In order to determine neutral cannabinoids, High Performance Liquid Chromatography (HPLC) gives us the opportunity to determine the original composition of the cannabinoids in the plant by direct analysis. There is no decomposition of the cannabinoids during analysis by HPLC.[29,34,35] There are numerous analytical conditions established in the Laboratories for quantification of cannabis by HPLC which give higher value (18.96, 98%) than those used in quantifying cannabis by order techniques[29,36] Analysis was carried out by Benjamin. D.B *et al* 2012 to determine the total THC content of cannabis, the plant was cultivated under controlled environmental conditions, and sampling ware performed each week to determine total THC (THCA + THC), total CBD (CBDA + CBD and CBN quantitatively whereas $\Delta^8$-THC was qualitatively determined.[19] Samples were analyzed by high-performance liquid chromatography with diode array detection (HPLC-DAD), the detector was chosen due to the thermal conversion of acidic cannabinoids that occurs in gas chromatography (GC). With high-performance liquid chromatography HPLC, samples are not heated. There for acidic and neutral cannabinoids are detected with their true content.[37,38]

Based on the result of the analysis mention above $\Delta^9$-THC content was determine at different developmental stages during growth three sets of cannabis plants were analysed, namely set A, B and C.[37] In set A germinated plant was analyse at different developmental stages, although age of the plants was not known precisely. Fig 5 shows the result of the analysis of estimated comparison between concentration and weeks of post germination, (the time as the number of weeks). After each week a newly shoots were analyse and total concentration of THC was recorded, THC content increased at early vegetative state with the increase in every week. It was also notice that the content of cannabinoid was stable before the flowering. The total THC content was higher in set B and C than in first week (A) due to the fact that clones contain the same content of THC as the mother plant. During the reproductive stage the total content of THC was stable but increases with the age of the plant and reached the highest level in week five and six. The highest total of THC concentrations were 18.91 ± 0.86%, 19.52 ± 3.02%, and 22.50 ± 2.65% in sets A, B, and C, respectively. At the end of analysis $\Delta^8$-THC was never detected in the analyte which show that drying and storage methods were appropriate, small amount of CBD was also detected.[37]

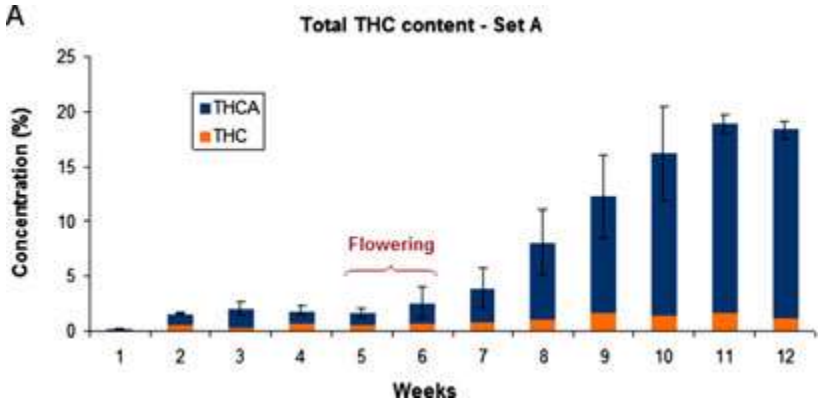



Figure 5 [37]

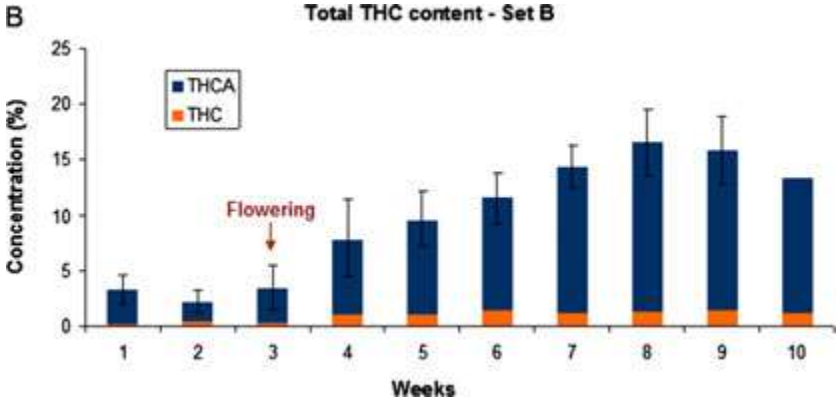



Figure 6 [37]

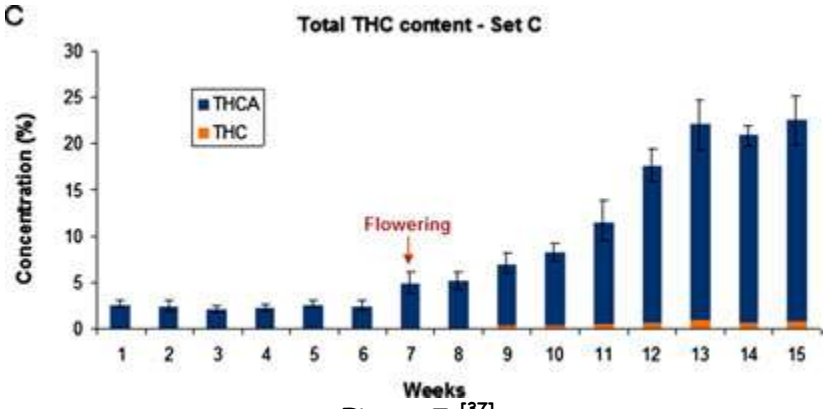



Figure 7 [37]

The results of this analysis show that the high-THC concentration can be determined at a young age in cannabis plant and is stable throughout the life of the plants. Moreover it was also noted that high THC concentrations observed in this study likely represent the strong potency of cannabis varieties cultivated.

## DISCUSSION

$\Delta^9$-THC, CBN and CBC are among the major chemical component of cannabis but presence of CBN in the sample indicates that the sample is not recently harvested. Stability of cannabinoid in cannabis material is influenced by age, light, temperature and also oxygen availability. The stability of acidic and neutral cannabinoids differs, with the acidic species being more susceptible to degradation. Since $\Delta^9$-THC is known to oxidize to CBN over time, it is Assume that the higher the amount of CBN in a sample the older the sample [16,37]. As mention earlier many techniques have been used in the identification of cannabis but were unsuccessful due to problems such as dark resolution. Gas Chromatography (GC) is the most commonly used method for the analysis of cannabis products, especially in forensic chemistry [32, 34, 35,39]. Moreover some the stated techniques were unsuccessful due to one reason or the other. Immunoassays is the fastest technique use in cannabis identification, it can identify cannabis within a seconds but the analysis is not reliable because it can only be use as presumptive test, it does not have a power to proof. Levine-duquenois can only be used to presume the presence of phenolic compound of cannabis it cannot be used to identify cannabis.[1,5,21,22,]. Most of these stated techniques can only be used as presumptive or spot test in the analysis of cannabis, they can only identify the presence of cannabis but cannot be used to quantify or analyse the component of cannabis.

Thin layer chromatography is among, the recommended methods for the identification and analysis of cannabis and cannabis products but it has its own limitation. Thin layer chromatography technique provides a rapid, easy and cost-effective means for analyzing cannabis materials but cannabinoids normally oxidized and so the analyte need to be prepared for TLC in solvents which are stable, Ethanol can be used as a solvent considering the solubility of cannabinoid and their carboxylic acid but yet the result of the Thin layer chromatography also indicate only the presence of cannabis in the sample, still there is need for definitive identification of the compound.[1,5,23] Thin layer chromatography is mostly used in preliminary comparison between the sample, the result obtain depend on laboratory condition.[1] TLC is another analytical technique that can be used as presumptive test due to the fact that it can only identify cannabis from the sample but cannot analyse cannabis itself. Supercritical fluid chromatography (SFC) coupled to atmospheric pressure chemical ionization-mass spectroscopic (APCI-MS) and High-performance liquid chromatography (HPLC), does not need derivatization before the analysis on like Gas Chromatography (GC). Most of the analytical technique used in analysis of cannabis have few advantages over other Gas Chromatography technique used in

the analysis of cannabis, gas Chromatography in the analysis of cannabis require a derivatazation of the sample prior to analysis, the sample used in the analysis require to be thermally stable and volatile . But HPLC and SFC-APCI-MS does not require the sample to be volatile, they does not require any pre-treatment of the sample prior to analysis, such as derivatizatio.[1, 24, 29]

In gas chromatography Derivatization is necessary because it allow determination of both the $\Delta^9$ -tetrahydrocannabinol and its carboxylic acid, to some extent, derivatization will improve the limits of determination and quantification.[1] HPLC and GCMS can be used to determine the age of cannabis, by consideration parameters used in chromatographic techniques such as selectivity, precision, linearity, accuracy, limit of quantification (LOQ) and limit of detection (LOD).With respect to quantification of cannabis GCMS is more efficient than HPLC due to its quickness, efficiency and reproducibility.[25] When cannabis are extracted into organic solvent and stored at room temperature where light rays can reach it, the degradation of both neutral and acidic THC increases in the sample but the degradation of neutral THC occurred faster in the extracts. When the extracts are stored in the dark no degradation of neutral THC seems to occur. Based on the analysis carried out by S. A. ROSS *et al* 1999 in forensic laboratory they suggested that improper storage of cannabis material may result in inconsistent analytical results. As soon as cannabis material is extracted into organic solvents, it is recommended to use light protective containers, such as brown glass to avoid the influence of sunlight so that degradation of cannabinoids should only have minor or no effect on the analytical results [40,41,42]Analysis has been carried out by the use of gas chromatography and liquid chromatography to determine the age of a cannabis in forensic work, since it has been known that $\Delta^9$-tetrahydrocarnabinol THC is known to oxidize to cannabinol CBN over a period time, presence of cannabinol CBN in cannabis sample indicates that the cannabis sample is not newly harvested. It is assumed that the higher concentration the cannabinol CBN, in the cannabis sample the older the sample. The result of this review correlates between the amount of CBN and THC with respect to the age of cannabis samples. This analysis, analyses the different cannabis samples stored at room temperature, these samples were first analyse immediately after harvesting and then years after.[16,37]

High Performance Liquid Chromatography (HPLC) gives us the opportunity to determine the original composition of the cannabinoids in the plant by direct analysis. There is no decomposition of the cannabinoids during analysis by HPLC, This is achieved by numerous analytical conditions established in the

Laboratories for quantification of cannabis by HPLC which give higher value than GC in the quantification of cannabis.[29,36]Using HPLC three sets of plants were analyse and compare in terms of cannabinoids content, the most important difference in the total THC content was seen at the early development stage between sets A, B and C. In the case of drug-type cannabis clones, the maximum THC content was (0.2%) and it exceeded at the developmental stage. On the other hand seedlings contain very low total THC concentration right after the germination. The THC level gradually increased during development for both the plant with high increase in drug type plant.[1] At the end of their analysis they agreed on pacifico's postulation which says" that no plant intended for the effective production of THC and marijuana will develop a low THC/CBD ratio typical of fiber hemp, irrespective of the development stage; vice versa, no fiber-type plant will show a high THC/CBD ratio typical of drug plants whatever the moment of the analysis".[43] There is no clear resolution from their result. Conclusively, in order to achieve a perfect and a result with clear resolution analysis must be carried out using GC-MS as recommended by many literatures[1,5,16] and also GC-MS is the United Nation Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products[5]

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**Reference** to this paper should be made as follows: M. Musa *et al*, (2015), Chemical Analyses of Cannabis; Review. *J. of Biological Science and Bioconservation,* Vol. 7, No. 1, Pp. 122 – 140.