

UNIVERSITY OF GHANA
SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES
DEPARTMENT OF CHEMISTRY



**THE EFFECT OF PLANT AGE ON CANNABINOIDS CONTENT OF CANNABIS
SATIVA GROWN IN THE EASTERN REGION OF GHANA**

BY

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**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN
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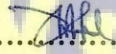
DECLARATION

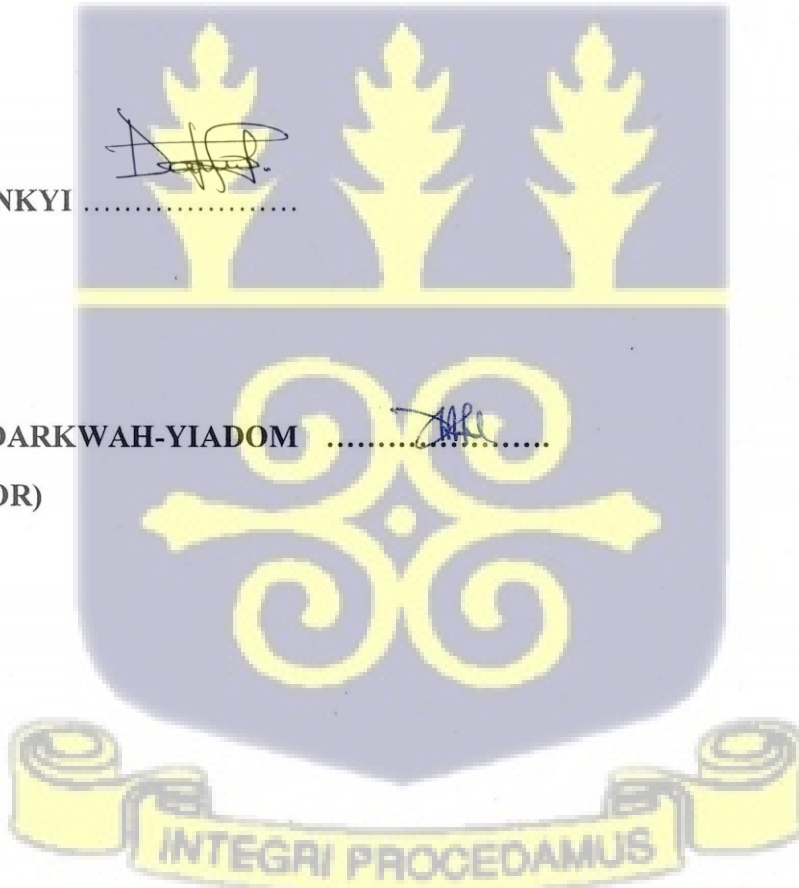
I ERNESTINA ADJEI MENSAH, author of this dissertation, hereby certify that this dissertation is my original work, completed under the supervision of Dr. Enoch Dankyi and Dr. Martin Adarkwah-Yiadom. I have properly cited all references to others' works and have not submitted it for any other degree or award.

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ABSTRACT

The long-standing criminalization of cannabis has greatly limited research into its properties and uses. Recently, advancements in cannabis research have led to global reforms that allow for various applications, including recreational, medicinal, and industrial uses. In Ghana, cultivation of cannabis with less than 0.3% Δ^9 -THC on dry weight basis has been legalized for industrial and medicinal purposes. As a result, analysing locally grown cannabis has become essential to identify and quantify its constituents in accordance with the law. This study aims to explore how plant growth affects the cannabinol content of local cannabis cultivars in Ghana. Cannabis samples cultivated from seeds obtained with permit from Narcotics Control Commission were assessed to determine their usefulness as local cultivars for industrial hemp. For each sample, the THC, CBD and CBN concentrations were determined monthly for four (4) months at the Ghana Standards Authority using High Performance Liquid Chromatography. The results indicate that cannabinoid concentrations vary significantly, primarily due to the age of the plant. THC content was between the ranges of 0.03% - 0.65% from month 1 to month 4 respectively. THC content increases with the age of the plant, but the trend for the concentration of CBD was generally linear. The concentration of CBD between the first month and fourth month were 0.056% and 0.069% respectively. The CBN concentration was negligible at the outset, but exhibited a marked increase to 0.043% by the second month. This upward trend continued, with concentrations reaching 0.051% and 0.061% in the third and fourth months, respectively. The results indicate that cannabinoid concentrations vary significantly, primarily due to the age of the plant. While the THC content increases with the age of the plant, similar increases were not observed for other cannabinoids like CBD and CBN.

DEDICATION

I dedicate this work to my husband and children, Shadrack, Marjorie, Lloyd, Jason and Justin, and other family members for their support and encouragement.



ACKNOWLEDGMENTS

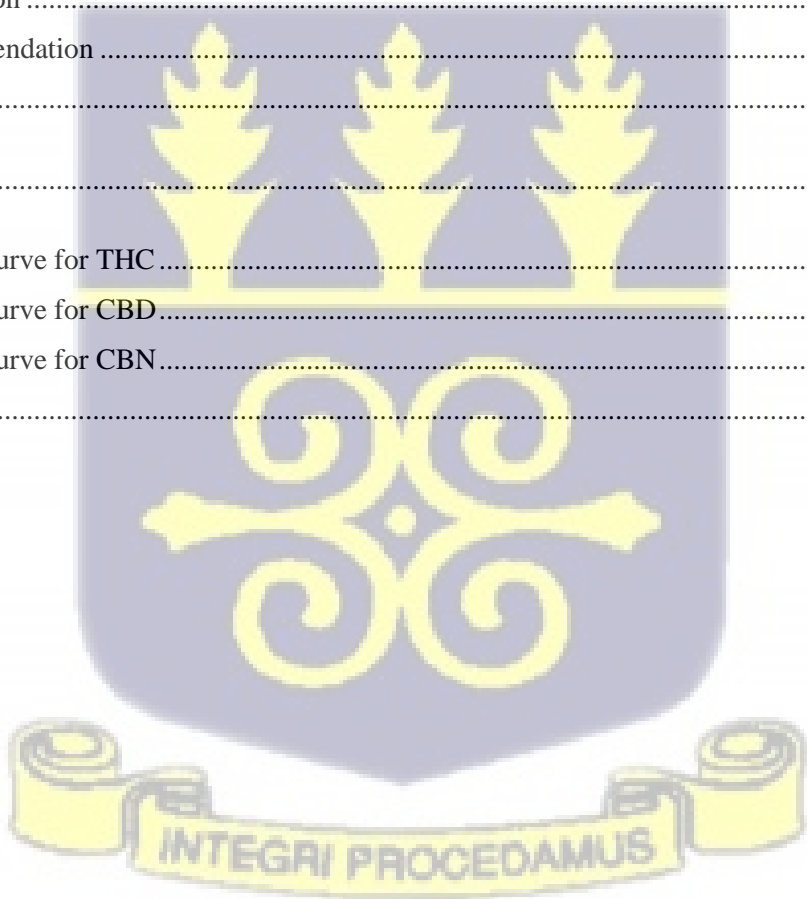
I am eternally grateful to God for his unwavering love, kindness and abundant mercy that have guided me every step of the way.



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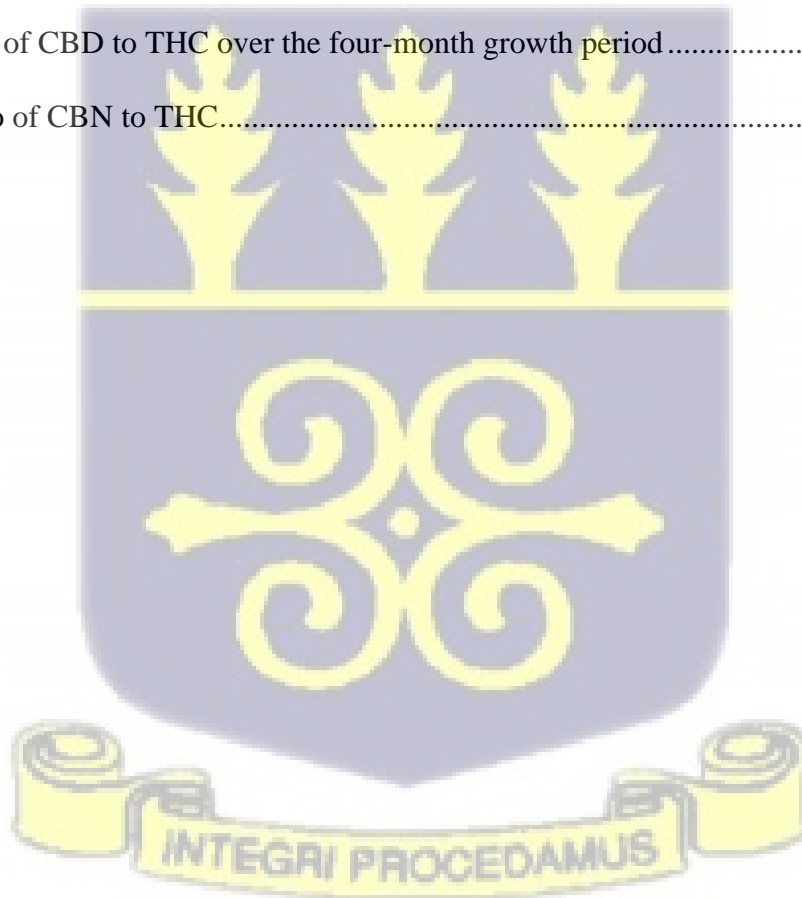
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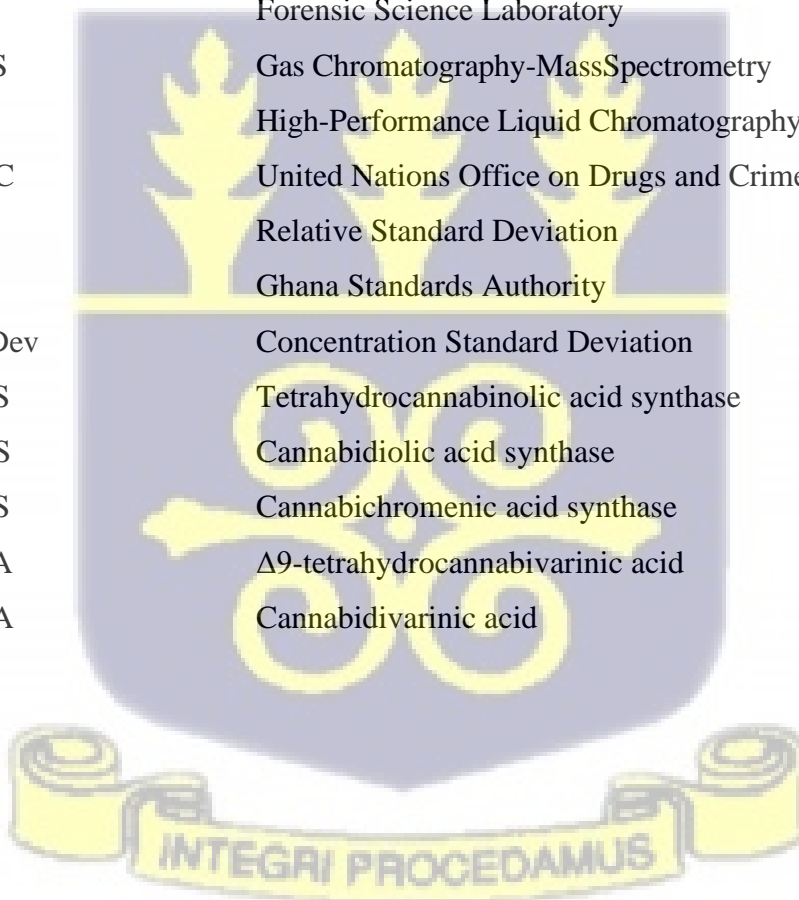
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LIST OF ABBREVIATIONS

Δ^9 -THC/THC	Delta-9-tetrahydrocannabinol
CBN	Cannabinol
CBD	Cannabidiol
Δ^9 -THC AA	Δ^9 -tetrahydrocannabinolic acid A
CBL	Cannabicyclol
CBE	Cannabielsoin
CBND	Cannabinodiol
CBT	Canabitrinol
CBDA	Canabidiolic acid
CBGA	Cannabigerolic acid
FSL	Forensic Science Laboratory
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High-Performance Liquid Chromatography
UNODC	United Nations Office on Drugs and Crime
RSD	Relative Standard Deviation
GSA	Ghana Standards Authority
Conc STDev	Concentration Standard Deviation
THCAS	Tetrahydrocannabinolic acid synthase
CBDAS	Cannabidiolic acid synthase
CBCAS	Cannabichromenic acid synthase
THCVA	Δ^9 -tetrahydrocannabivarinic acid
CBDVA	Cannabidivarinic acid



CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The term "cannabis" was coined by Carolus Linnaeus in 1753, derived from Latin words referring to the plant's characteristics. It is known by various names in different regions and cultures. Some of which include hashish, Indian hemp, Ganja, joint, dope, sinsemilla, etc (Hourfane et al., 2023). As one of the earliest plants cultivated by humans, *Cannabis sativa* has long been valued for its wide-ranging uses – spanning industrial, medicinal, and agricultural applications. However, its legalization has remained a subject of global controversy over the years (Farrelly et al., 2023). Although numerous conspiracies have emerged to explain its long-standing prohibition, scientific investigations have identified its principal psychoactive component, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), which has been shown to induce acute psychotic symptoms and impair episodic memory (Bridgeman & Abazia, 2017). The term *cannabis* typically refers to cannabis strains with high concentrations of Δ^9 -THC – commonly referred to as drug-type cannabis, owing to their intoxicating properties (Hasan, 2023). In contrast, *hemp* refers to cannabis strains used primarily for industrial and medicinal purposes and characterized by very low levels of THC (fibre-type cannabis).

There are two main factors that affects the content of THC in a cannabis plant. These are the genetic factors and the environmental factors. The effect of genetic factors on THC content in cannabis is significant because cannabinoid production is primarily under genetic control, even though environmental factors can modulate expression. Different cultivars (fibre, grain, CBD-dominant) have different biomass and seed yield potentials. CBD-rich cultivars tend to produce higher cannabinoid concentrations, while fibre cultivars produce more biomass but lower

cannabinoids (De Meijer et al., 2003). Some cultivars have a higher tendency to approach or exceed 0.3% THC, especially CBD-dominant ones (Aina et al., 2025). The cannabinoid synthase genes primary determinant of THC content by encoding for THCAS and CBDAS which converts CBGA → THCA, and CBDA respectively. THCA and CBDA are later converted to THC and CBD respectfully (Walsh et al., 2021). Allelic variations of THCAS and CBDAS influence the amount of THC (De Meijer et al., 2003). Mutations or deletions in THCAS gene lead to near-zero THC production (Shoyama et al., 2012). Marker-assisted breeding targets these alleles for low-THC hemp compliance (Staginnus et al., 2014). An important consideration for cannabinoid biosynthesis is the interaction between plant genotype and developmental stage. Rates of trichome maturation differ among cultivars and influence both the timing and extent of THC versus CBD synthesis (Punja et al., 2023). This suggests that different genotypes may reach peak cannabinoid accumulation at varying ages, emphasizing the age-genotype relationship in cannabinoid productivity.

The THC content in *Cannabis sativa* is influenced by environmental factors, though genetics set the upper and lower limits. Environmental conditions mainly affect THC expression, synthesis rate, and cannabinoid ratios during growth and maturation. The environmental factors include light intensity and photoperiod, temperature, soil, nutrient and plant age. High light intensity increases photosynthesis, leading to more precursor molecules for cannabinoid synthesis (Danziger & Bernstein, 2021). Artificial lighting spectrum (e.g., more UV and blue light) can increase THC concentration (Danziger & Bernstein, 2021). The optimal temperature range: 25–30°C (day), 15–20°C (night) for cannabinoid synthesis. High temperature (>32°C) increases evaporation, plant stress sometimes higher THC (stress response) (Holweg et al., 2025). Low temperature (<15°C): slows metabolism, reduces THC accumulation. Severe heat or cold → enzyme denaturation,

trichome damage. Nitrogen promotes vegetative growth; excessive N can delay flowering → risk of higher THC due to prolonged cannabinoid synthesis (Struik et al., 2000). Phosphorus and Potassium are essential for energy transfer and enzyme function resulting in high yields (Malík et al., 2025). Micronutrients such as magnesium, calcium and iron influence enzyme activity in biosynthetic pathways. Magnesium is a co-factor necessary for photosynthesis (Malík et al., 2025). THC levels increase with plant maturity (De Backer et al., 2012; Yang et al., 2020). Plant age was taken as the main experimental variable, since it integrates cumulative development; whereas soil content, light intensity and nutrition in the environment are frequently changing factors that can be barely standardized under Ghanaian cultivation conditions. Cannabinoid levels, in particular Δ^9 -THC, have been demonstrated to increase in a presumably predictable fashion during the maturation of the plant; for instance, De Backer et al. (2012) reported THC quantity increasing from <0.2% in seedlings to multi-per cent levels during the first few weeks of growth. This allows to disentangle biosynthetic changes due to aging from any potential environmental control (De Backer et al., 2012). THC content is very low at germination and before flowering. The THC content then peaks at peak at late harvest. Delaying harvest beyond the legal compliance window increases THC risk.

Cannabis prohibition has varied by country, with one of the earliest recorded bans occurring in 1378 under the Emir of the Joneima (Crocq, 2020). Since then, cannabis legislation has undergone significant changes worldwide, with an increasing number of countries adopting more tolerant policies. Many governments cite public health and safety, along with the reduction of drug-related criminal activity, as the main objectives for legalizing cannabis (Hall, 2020).

The Alcohol, Drugs and Addictive Behaviours Unit of the United Nations Office on Drugs and Crime (UNODC) reports that the annual prevalence of cannabis use stands at 2.5% of the global

population – significantly higher than the 0.2% recorded for both cocaine and opiate users (UNODC, 2020). Between 2011 and 2021, the number of cannabis users worldwide increased by 23%, a trend partly attributed to global population growth.

In 2018, Africa accounted for 19% of the total quantity of cannabis seized globally, the second-highest proportion after the Americas (UNODC, 2020). Studies by Onaolapo et al. (2022) further indicated that about 60% of all cannabis resin seized in 2018 originated from Africa, reflecting increased use within the region. The continent's cannabis prevalence rate stands at 6.3% among individuals aged 15–64, markedly higher than the global average. Notably, West and Central Africa recorded the highest prevalence, estimated at 9.3%. Countries within West Africa are frequently cited as areas of origin, transit, and departure for *Cannabis sativa* on the continent.

In Ghana, data on cannabis prevalence remain limited. Asante, (2019) estimated that approximately 5.3% of adolescents in the country use cannabis. Other research has established a correlation between cannabis use and suicidal behaviour among Ghanaian students (Tetteh et al., 2020). Ibrahim (2019) recommended implementing large-scale educational campaigns to raise awareness of the associated risks.

Cannabis is categorized into two main phenotypes; drug type and fibre type cannabis. because These categories reflect different chemical compositions, genetic traits, and intended uses, which have major implications for agriculture, industry, medicine, and law. This is very helpful because most countries forbid the possession of the drug type of *Cannabis sativa*, and the legal evaluation requires a strict clear method of recognising plant material as either drug or non-drug type, even in cases where immature specimens are confiscated. Each plant has a prominent cannabinoid derivative that defines it throughout its whole growth phase and can be used with confidence for forensic purposes involving drug-suspected material in very young plants. Fiber-type plants are

tall, with long stalks and few branches to maximize fiber yield. Drug-type plants are shorter, bushier, optimized for flower production where cannabinoids concentrate. These differences stem from selective breeding for different purposes, creating genetically distinct populations. Fiber types are cultivated for industrial products: textiles, paper, ropes, bio-composites, building materials. Drug types are cultivated for recreational and medicinal cannabinoids: THC-rich for psychoactive use, CBD-rich for therapeutic purposes.

Ghana has historically criminalized cannabis use. The Pharmacy and Narcotics Act, 1961 (Act 64), was enacted to regulate and restrict cannabis due to its perceived harmful effects on the population, including veterans (Pharmacy and Drugs Act, 1961.). This Act prohibited the ownership, cultivation, consumption, and trafficking of narcotic substances. Ghana's commitment to international conventions, such as the 1961 Single Convention on Narcotic Drugs, was fulfilled through this legislation. Following its ratification of the 1988 UN Convention on Narcotic Drugs and Psychotropic Substances, Ghana passed the Narcotic Drug Sanctions, Enforcement, and Control Act, 1990 (PNDC Law 236), which introduced stricter penalties and amended earlier laws. Under Part I of PNDCL 236, it became illegal to import, export, possess, cultivate, manufacture, supply, use, or administer any narcotic substance without a valid license (Mensah & Adu-Gyamfi, 2019). Cannabis, along with its extracts, tinctures, and resin, was listed under the First Schedule of this law. In March 2020, the Narcotics Control Commission Bill (2019) was passed by Ghana's Parliament, legalizing the cultivation of cannabis for medicinal and industrial purposes (Agboli, 2024).

Agyepong, (2019) reported that seized cannabis samples in Ghana had high concentrations of Δ^9 -THC, ranging from 0.93% to 15.14%, and cannabidiol (CBD) levels between 0.88% and 1.19%. These values exceed the legal THC threshold of 0.3% (on a dry weight basis) set for industrial or

medicinal cannabis under the Narcotics Control Commission Act, 2020. Compared to this legal limit, the THC content of local cultivars – often exceeding 0.9% – would necessitate their destruction, resulting in the loss of livelihoods for many growers and reduced economic benefits for the nation. Consequently, all cannabis seeds cultivated for legal purposes would have to be imported to meet regulatory standards. Against this backdrop, further research is essential to examine how environmental factors, including plant age, influence the THC content of local cannabis strains. Such data could serve as valuable reference material for informed policymaking.

1.2 Problem Statement

The THC content in seized drugs in Ghana is always high that is above 0.9% as indicated earlier by a study by Agyepong in the University of Ghana. Bruci et al., (2012) also indicated that illicitly cultivated cannabis in Albania is of high potency which can reach as high as 12.13%. Many factors affect the cannabinoid content in cannabis; environmental factors such as soil type, nutrient availability, temperature as well as cultivation practices or light exposure. The legalization has to do with production of cannabis has to do with growing cannabis so as not to be abused by the youth. As a result, plant age is one of the strongest factor that will let us know whether the plant has reach its legal limits for cultivation for the country to earn foreign exchange. The average Ghanaian farmer may not be able to build a greenhouse to control factors such as temperature and light exposure. Factors such as soil type and nutrients may also be out of the control of the average farmer. However, when it comes to harvesting at the right time so as not to exceed legal limits farmers will be able to do so.

The age of the cannabis plant is crucial because THC content increases as it matures. Careful control of the plant's age and precise timing of the harvest are necessary to achieve the right percentage yields for different uses (like hemp), which in turn, ensures export viability, generates

revenue for both farmers and the state, and allows for regulation to prevent abuse. The alternative strategy is to cultivate cannabis plants with low THC content also known as hemp but research on seized cannabis plant indicates that these hemp plants are rare in Ghana. Plant age is therefore the critical determinant when intending to use cannabis as hemp. Based on existing evidence, this study hypothesizes that Δ^9 -THC and related cannabinoids will increase significantly as plant age advances, while the relative chemotypic profile remains stable. Studies have demonstrated substantial age-related increases in THC, including rapid escalation during the flowering period (De Backer et al., 2012; Yang et al., 2020). With new regulations emerging in Ghana that set a 0.3% THC limit for industrial cultivation (IDPC, 2025), it is important to determine the precise growth stage at which local cultivars rise above this value to help inform compliant production.

I hypothesize that cannabinoid content increases with the plant's age, while the characteristic cannabinoid profile remains constant throughout the entire growth period. If this is correct, harvesting cannabis before it reaches the legal THC limit would allow farmers to remain compliant with the law. Furthermore, timely harvesting would help farmers earn income and increase Ghana's foreign exchange earnings. The resulting data could provide the basis for enhancing the agronomic potential of hemp while ensuring compliance for law enforcement purposes.

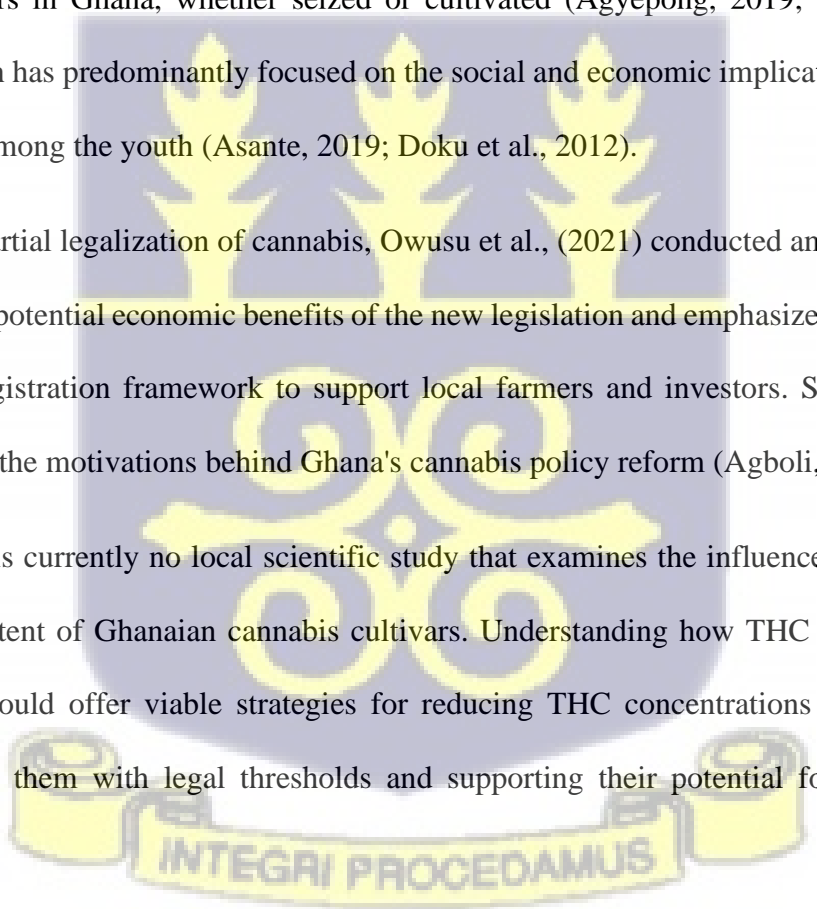
The last several decades have seen a significant change in the medicinal cannabis landscape (Hossain & Chae, 2024). Once widely outlawed and stigmatized, cannabis plant is now significantly gaining the recognition for its potential therapeutic benefits, which is evident by a growing body of scientific research (Asante, 2019). In Ghana, however, the legalization of cannabis remains a contentious issue. The country maintains strict penalties for drug possession and use, even in minor cases. Although the Narcotics Control Commission Act, 2020 (Act 1019) permits the cultivation of cannabis for medicinal and industrial purposes, this is tightly regulated.

Unlicensed possession or cultivation remains illegal. Under the Act, any cannabis plant with a Δ^9 -THC concentration exceeding 0.3% on a dry weight basis is classified as drug-type and prohibited for legal use in Ghana (Narcotics Control Commission Act, 2020).

The cannabis plant contains numerous cannabinoids, with Δ^9 -THC, cannabidiol (CBD), and cannabinol (CBN) being the most prominent. The concentrations of these cannabinoids are influenced not only by genetic factors but also by environmental conditions such as soil type, nutrient availability, and temperature (Shiponi & Bernstein, 2021). Despite the growing interest in medicinal cannabis, there is limited scientific data on the cannabinoid composition of local cannabis cultivars in Ghana, whether seized or cultivated (Agyepong, 2019; Quansah, 2022). Existing research has predominantly focused on the social and economic implications of cannabis use, especially among the youth (Asante, 2019; Doku et al., 2012).

Following the partial legalization of cannabis, Owusu et al., (2021) conducted an electronic study highlighting the potential economic benefits of the new legislation and emphasized the importance of creating a registration framework to support local farmers and investors. Similarly, a 2023 review explored the motivations behind Ghana's cannabis policy reform (Agboli, 2024).

However, there is currently no local scientific study that examines the influence of plant age on the Δ^9 -THC content of Ghanaian cannabis cultivars. Understanding how THC levels vary with plant maturity could offer viable strategies for reducing THC concentrations in local strains, thereby aligning them with legal thresholds and supporting their potential for industrial and medicinal use.



1.3 Aim of the study

This study aims to investigate how the cannabinoid profile of local cannabis cultivars changes over time during growth, with the ultimate goal of identifying the optimal harvest period that ensures compliance with the statutory Δ^9 -THC threshold for legal cultivation.

1.3.1 Specific Objectives

- i. To quantify and analyse changes in Δ^9 -THC, CBD, and CBN concentrations in local cannabis cultivars across a defined period using HPLC.
- ii. To determine the phenotype index of cultivated cannabis samples in order to distinguish between drug-type and fibre-type cannabis using the phenotypic index ratio.



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Genus Cannabis

In systematic classification, cannabis belongs to the division of Angiosperms, class: dicotyledon and order: Urticales. Originally native to Central Asia, cannabis genus is the botanical name given to the leafy, flowering plants within the Cannabaceae plant family (Hasan, 2023). Now transported and grown throughout the world, this genus contains three mainly recognised species namely *Cannabis indica*, *Cannabis ruderalis* and *Cannabis sativa*. Each of these species have its own chemical characteristics, distinctive look and uses (Hourfane et al., 2023).





Cannabis ruderalis

Cannabis hybrid

Figure 2.1: Differences in physical characteristics between *C. sativa*, *C. indica* and *C. ruderalis*

2.1.1 Chemical Composition of Cannabis

More than 500 chemical compounds have been isolated from *Cannabis sativa* L. to date (Atakan, 2012). According to Fordjour et al (2023), the total number of compounds that have been identified and isolated from the cannabis plant is 566. These comprise of over 18 classes of distinct secondary metabolites. The majority of these substances are abundant in the flowers and leaves, with 125 identified as cannabinoids, 198 as non-cannabinoids, and 120 as terpenes, making up a total of 443 compounds. Female cannabis plants' distinct aroma, attributed to terpenes like pinene, limonene, linalool, and borneol – also possess properties that repel insects and inhibit nearby plant growth (Fordjour et al., 2023). The glandular trichomes of the cannabis plant secrete a resinous substance that functions as an advanced defense system against insects and demonstrates potential as both an antibiotic and antifungal agent (Tanney et al., 2021). The trichomes excrete a sticky resin that collects in droplets at the tip of each hair and they are harvested to produce the drug cannabis

(Tanney et al., 2021). As they bind to receptors in the body, cannabinoids exert their effect on the body which varies according to the route of administration (Lucas et al., 2018).

2.1.2 Phytocannabinoids

Phytocannabinoids are bioactive natural products (terpenoids) that are found in some flowering plants (Gülck & Møller, 2020). *Cannabis sativa* has yielded a range of cannabinoids, categorized into distinct types including CBG, CBC, CBD, Δ^9 -THC, Δ^8 -THC, CBL, CBE, CBN, CBND, CBT, and others. These cannabinoids are primarily alkyl-type, characterized by a 10-carbon monoterpene unit and a 5-carbon pentyl chain. The main constituents are Δ^9 -THC, CBD, CBC, CBG, and their respective acidic forms: Δ^9 -THCA, CBDA, CBCA, and CBGA. Most phytocannabinoids in nature occur in acidic forms, known as 'pre-cannabinoids,' as biosynthetic pathways produce these compounds. These acidic forms can spontaneously break down or convert through oxidation, decarboxylation, and cyclization, especially during isolation, largely due to the instability of alkylic cannabinoids like Δ^9 -THC. While *Cannabis sativa* is the primary source of phytocannabinoids, other organisms can also synthesize these compounds, according to research (Gülck & Møller, 2020). Some of these organisms includes liverworts (Marchantiophyta, or 'hepatics'), fungi from the genus *Albatrellus* (Albatrellaceae), and certain flowering plants including *Rhododendron* species (Ericaceae) and *Helichrysum umbraculigerum* Less. (Asteraceae) (Gülck & Møller, 2020).

Legare et al., (2022) highlights the varying biological effects of cannabinoids: Δ^9 -THC can stimulate appetite and combat nausea, while CBD shows promise in reducing seizures and inflammation. Compounds like flavonoids, stilbenoids, and terpenoids may work synergistically with phytocannabinoids, contributing to the "entourage effect," (Jin et al., 2020).

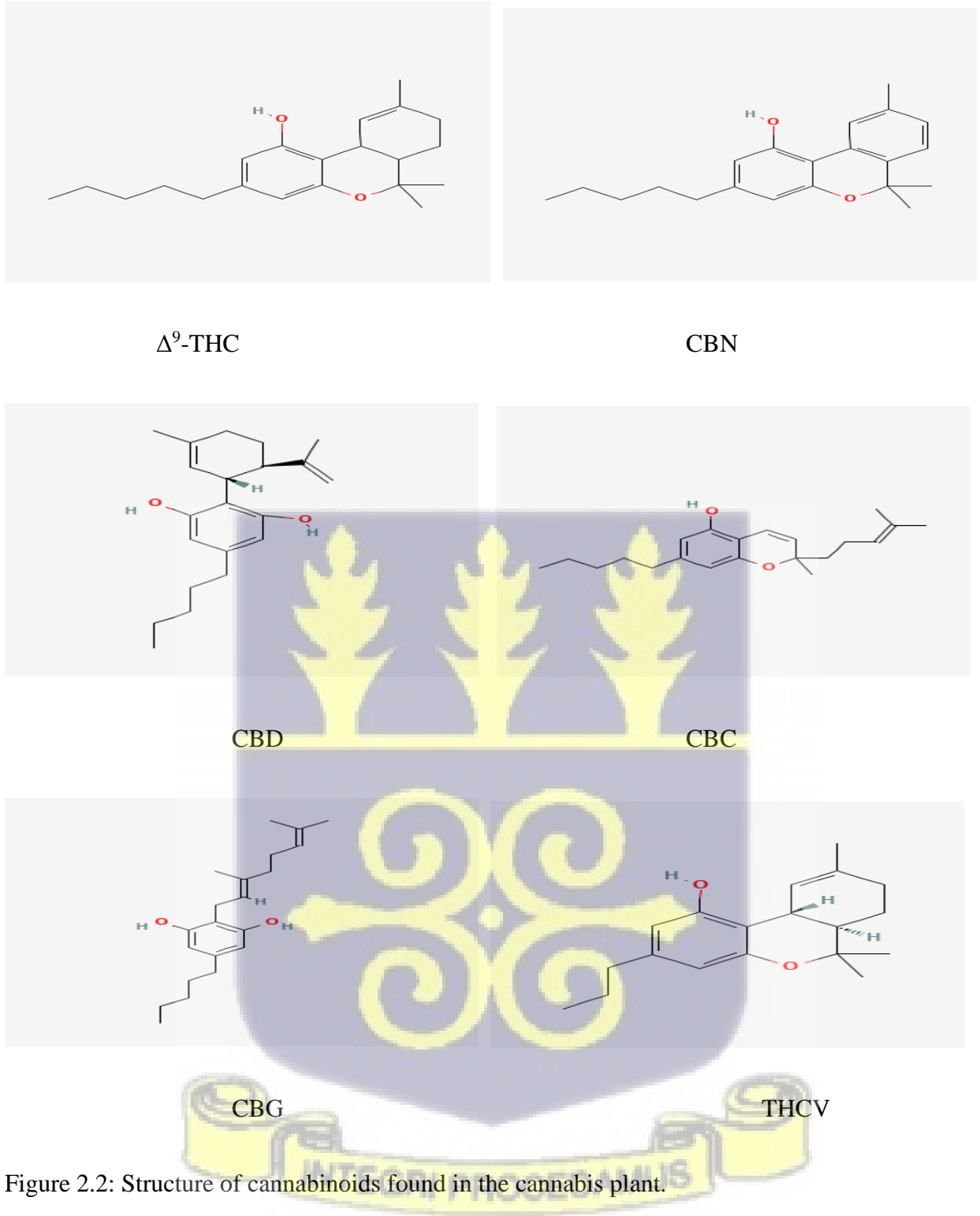


Figure 2.2: Structure of cannabinoids found in the cannabis plant.

2.1.3 Δ^9 -THC

Δ^9 -THC is one of the main psychoactive constituent of the cannabis plant (Atakan, 2012). Δ^9 -THC produces myriads of effects when taking into the body which includes dose-dependent mannerism, hypoactivity, hallucinations, hypothermia, sleep, spatial, epilepsy verbal short-term memory impairment and psychiatric disability. Yet, it has become the most relevant constituent in the cannabis plant due to its pharmacological importance (Farrelly et al., 2023), However, the accepted medicinal applications are restricted due to the unwanted psychoactive effects it can also produce hence rendering it toxic in some jurisdictions (Tanney et al., 2021). Dronabinol (trade name Marinol®) and Nabilone (trade name Cesamet™) which are orally administered are synthetically produced Δ^9 -THC that has been approved by the United States FDA in 1985. These drugs were approved for the treatment of anorexia or lack of appetite, improve weight loss in AIDS patients and reduce nausea and vomiting in patients undergoing chemotherapy (Legare et al., 2022).

The molecular formula for THC is $C_{21}H_{30}O_2$ which though can also represent multiple isomers. The commonly known isomer is the Δ^9 -THC isomer with commonly known chemical name as (-)- $\text{tran}\Delta^9$ -tetrahydrocannabinol (Lin et al., 2022) The main precursor of Δ^9 -THC is Δ^9 -THC AA (Shoyama et al., 2012). The reaction is shown in the figure below:

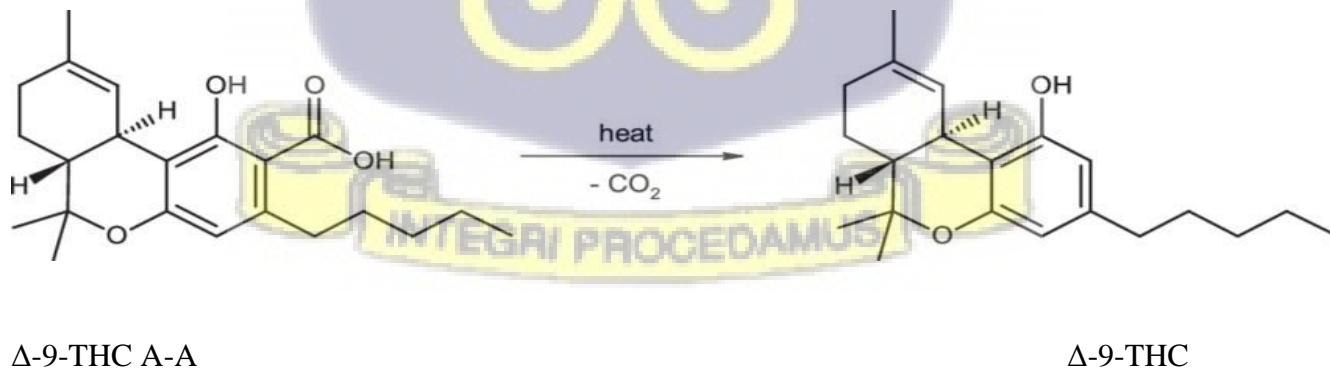


Figure 2.3: Chemical structures for the decarboxylation reaction of Δ^9 -THC A-A

2.1.4 Cannabidiol (CBD)

Cannabidiol accounts for over 40% of an extract of cannabis (Legare et al., 2022). It was isolated in 1940 by Roger Adams and was discovered that CBD was not psychoactive (Aguillón et al., 2021). CBD is the second major compound found in cannabis. In contrast to THC, CBD has no independent effect on memory, body temperature, or locomotor activity. On the other hand, Legare et al., (2022) noted that when the dose of CBD is higher, CBD can increase the effect of lower doses of Δ^9 -THC by increasing the level of CB1 receptor activity in the hippocampus and hypothalamus.

2.1.5 Cannabinol (CBN)

CBN, was discovered in 1896 and noted to be the first cannabinoid to be ever discovered (Pollastro et al., 2018). Although Δ^9 -THC and CBD have been the greatly researched cannabinoids; CBN has been noted for inducing sleep hence recognised as a putative 'sleep-enhancing' cannabinoid (Arnold et al., 2024). CBN is produced as an oxidative (reaction) metabolite of Δ^9 -THC. Hence CBD accumulates in aged cannabis materials (leaves, stalk and flowers) as a result of exposure to atmospheric oxygen, light, and heat. Due to the non-enzymatic oxidation of THC to CBN, CBN has significant high concentrations in older cannabis plant rather than young cannabis plant material. This is important since it has been anecdotally stated that aged cannabis plant material can promote sleep. According to cannabis legend, CBN is the active ingredient noted for the potent soporific effects of aged cannabis. (Pollastro et al., 2018). CBN is frequently termed the 'sleepy cannabinoid,' with assertions of significant pro-sleep effects comparable to those of traditional sleep medications like benzodiazepines. In fact, several makers of CBN isolation products sell them as sleep aids.

2.2 Conceptual framework

The framework illustrates how plant age influences enzymatic activity, which in turn affects cannabinoid biosynthesis and compound transformation, all under the constraint of legal THC threshold regulations. The framework further posits that increasing plant age drives enzymatic shifts in cannabinoid biosynthesis, mediated by developmental regulation of key enzymes such as THCAS and CBDAS. Sampling at defined age intervals and quantifying cannabinoids via HPLC operationalizes this theoretical relationship. The framework integrates growth stage, biochemical activity, and resulting cannabinoid concentrations while aligning these biological processes with Ghana's regulatory benchmark of 0.3% Δ^9 -THC for industrial hemp (IDPC, 2025).

Independent Variable: Plant Age

Mediating Variable: Enzymatic Activity (Synthases)

Process: Cannabinoid Biosynthesis and Transformation

Dependent Variable: Cannabinoid Profile (THC/CBD levels)

Regulatory Constraint: Legal THC Threshold



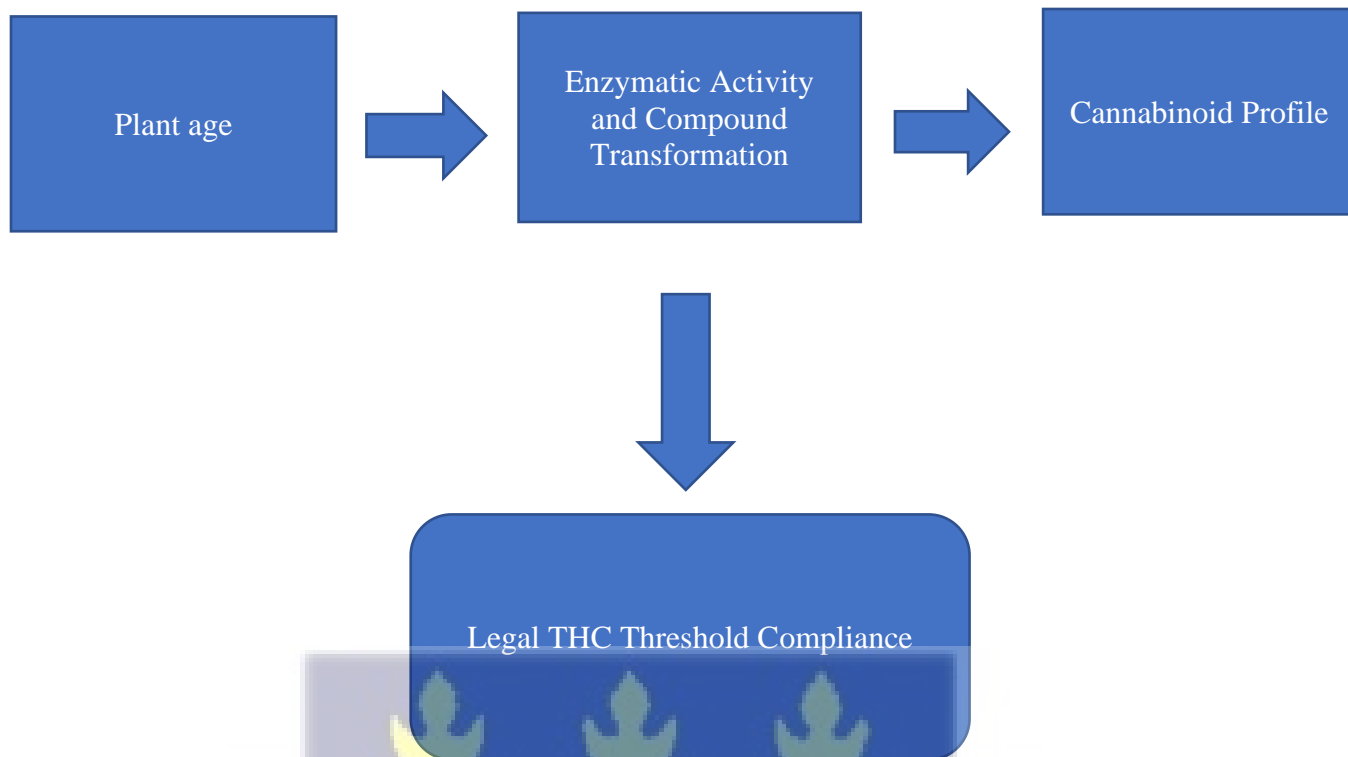


Figure 2.4 Conceptual framework of study.

2.2.1 Biosynthesis of cannabinoids

Phytocannabinoids are synthesized and stored in the part of the plant known as glandular trichomes (Walsh et al., 2021). The glandular trichomes are found in highest density in the female flowers of the plant. The two enzymatic pathways located in two separate sites within the glandular trichomes which are involved in synthesis of the cannabinoids. The first enzymatic pathway which occurs in the cytoplasm of the gland cells leads to the production of olivetolic acid (OLA) from hexanoic acid. Hexanoic acid is enzymatically converted to hexanoyl-CoA by the enzyme hexanoyl-CoA synthetase enzyme. Hexanoyl-CoA is then also enzymatically converted to OLA in a reaction involving three malonyl-CoA molecules. The reaction is made possible by the enzymes polyketide

synthase (PKS) and olivetolic acid cyclase (OAC). The second enzymatic pathway occurs in the plastidial organelles and it involves production of geranyl diphosphate (GPP) using the mevalonate-dependent isoprenoid (MEP) pathway (Hanuš et al., 2016). CBGA, the precursor of phytocannabinoids containing a pentyl side chain, is then synthesized from the GPP prenylation of olivetolic acid; a reaction catalysed by olivetolate geranyltransferase (GOT)

The three oxidocyclases THCAS, CBDAS, and CBCAS are in charge of creating structural variation among the cannabinoids downstream in the pathway (Singh et al., 2021). CBGA is stereo-selectively cyclized by these enzymes to produce THCA, CBDA, and CBCA, respectively. Alongside the pentyl-alkyl-cannabinoids that are prevalent in plants, propyl-alkyl-cannabinoids, such as THCVA and CBDVA, have also been identified in cannabis plants from specific regions. All of the cannabinoids are produced in plants as carboxylic acids, which can be thermally decarboxylated to provide their neutral forms.

2.2.2 The effect of plant age on enzymatic activity and compound transformation

Cannabinoid biosynthesis in cannabis is highly dependent on the plant's developmental stage. Plant age significantly influences enzymatic activity and the subsequent transformation of cannabinoids throughout the growth cycle. This relationship is crucial for determining optimal harvest times and cannabinoid profiles in both drug-type and fiber-type cannabis (van Bakel et al., 2011). As the plant transitions to the pre-flowering and early flowering stages, trichome development initiates a surge in enzymatic activity (Sirikantaramas et al., 2005). At this stage, CBGA, is synthesized and converted to THCA, CBDA, and CBCA by their respective synthases. During peak flowering, enzyme activity reaches its maximum in glandular trichomes, leading to the highest accumulation of cannabinoid acids (Tanney et al., 2021). In the late flowering stage

and senescence, enzymatic activity declines as metabolic processes slow down, resulting in reduced synthesis of new cannabinoids.

Cannabinoids are initially produced in their acidic forms (such as THCA and CBDA). Over time, and especially after harvest, these compounds undergo decarboxylation, transforming into neutral cannabinoids such as THC and CBD, either enzymatically or through exposure to heat and light (Shoyama et al., 2012). In overripe or senescent plants, cannabinoids like THC may further oxidize into CBN (cannabinol), reducing psychoactive potency while increasing degradation products (De Backer et al., 2012). Longer maturation can also cause shifts in the overall cannabinoid profile (Tipparat et al., 2012). Early harvests typically yield higher ratios of precursor cannabinoids, while delayed harvests favour more degradation products. Therefore, harvest timing is critical for controlling the chemical composition of cannabis (Massuela et al., 2022)

2.2.3 The legal THC threshold as a regulatory limitation.

Cannabis regulation worldwide is heavily influenced by the psychoactive potential of tetrahydrocannabinol (THC). Legal frameworks set strict thresholds for THC concentration, particularly in industrial hemp, to distinguish it from drug-type cannabis and control its use. These thresholds directly impact cultivation practices, harvest timing, and compliance strategies for growers. The legal THC threshold refers to the maximum allowable THC concentration in cannabis plants, usually measured as a percentage of dry weight. The most widely recognized limit is 0.3% THC, established by the 2018 U.S. Farm Bill for industrial hemp (Abuhasira et al., 2018). However, thresholds vary globally: the European Union enforces a 0.2% limit, while some countries, such as Switzerland and Uruguay, allow up to 1.0% THC in hemp (European Monitoring Centre for Drugs and Drug & Addiction, 2023).

This regulatory distinction aims to prevent industrial hemp from being used as a psychoactive substance while permitting its cultivation for fibre type cannabis and CBD extraction. The limit is based on research indicating that cannabis with THC levels below these thresholds lacks intoxicating effects (Malabadi et al., 2023) Compliance is complex because THC concentration increases as plants mature, especially during late flowering. Environmental factors, genetics, and stress conditions can accelerate THC biosynthesis (Walsh et al., 2021). Even cultivars classified as hemp can exceed the legal threshold if harvest is delayed beyond the recommended stage . Once THC surpasses the legal limit, crops may be classified as drug-type cannabis and must often be destroyed, resulting in financial losses (Abuhasira et al., 2018).

2.3 Cannabis and Hemp

The cannabis plant can be classified into two different types based on the concentration of Δ^9 -THC found in the cannabis plant. These classifications are hemp and cannabis (Malabadi et al., 2023). Cannabis also known as medical cannabis contains very high levels of Δ^9 -THC (> 0.3 to 38% of dry weight) and planted in controlled environmental conditions (usually greenhouses) for the mainly to harvest the female flowers which are unfertilized (Malabadi et al., 2023). On the other hand, hemp, which is cultivated outdoors in big agricultural fields for the produce materials such as seed, plant fibre, and oil, has very low quantities of Δ^9 -THC (Malabadi et al., 2023). Thus, hemp and cannabis are different in terms of the amount of Δ^9 -THC that their respective cannabis strain contain. The primary ingredient in cannabis that gives it its psychotropic qualities is Δ^9 -THC. Despite the conventional definition of hemp being less than 0.3%, some states in the US define hemp as having no more than 0.5% Δ^9 -THC. In addition, some of the states and Canada has set a limit threshold of 10 parts per million (ppm) for Δ^9 -THC residue in hemp-based goods, such as grain, flour, and oil. (Malabadi et al., 2023). Nonetheless, due to the presence of psychoactive

molecules, Δ^9 -THC cannabis cultivation and consumption are limited or controlled in many nations.

The profile and morphology of the plant, which are influenced by both genetics and environmental factors, distinguish cannabis from hemp. In terms of genetics, hemp generates the BD allele, which codes for CBDA synthase, whereas medicinal cannabis contains the BT allele, which codes for tetra-hydrocannabinolic acid synthase. (Vasantha Rupasinghe et al., 2020). Compared to hemp, the transcriptome of female cannabis flowers is continuously expressed along the whole THC synthesis process. (van Bakel et al., 2011). This means that many medical cannabis samples produce up to 10% THC, whereas the majority of hemp samples have a total THC content of 0.3% or less.

Medical cannabis is specially planted controlled conditions (greenhouse) in order to produce primarily female flowers that are not fertilized with very high concentration of Δ^9 -THC which can be above 0.3 % to 38% of dry weight. Its acidic precursor, tetrahydrocannabinolic acid (THCA) also adds on to this concentration when it gets converted further into THC through decarboxylation (Gülck & Møller, 2020). This variety is used to cure a number of health disorders including glaucoma, multiple sclerosis, epilepsy, posttraumatic stress disorder and others.

Industrial Cannabis Sativa L. (hemp), on the other hand, has very low concentration of THC (0 to 0.3% of dry weight) and high CBD and it's grown in the open basically to produce fibre, seeds and oil. Products derived from hemp and indeed the hemp plant materials itself can found in the agriculture, construction, automotive, paper, textiles, food and even recycling markets to name just a few (Vasantha Rupasinghe et al., 2020).

2.3.1 Phenotypic index

The potency of cannabis products denotes the concentration of the principal psychoactive constituent, Δ^9 -THC. In 2008, European Monitoring Centre for Drugs and Drug & Addiction (2023) noted that, the average Δ^9 -THC concentration of cannabis resin in several European nations varied from 3% to 16%, whilst the mean concentration of herbal cannabis materials varied between 1% to 10%. There was an average concentration of sinsemilla in Norway of 12%, which is much lower than the over 16% concentration found in the Netherlands and the United Kingdom. In Germany, the average concentration of THC has been steady at around 8%. United Nations Office on Drug and Crime (2020) study in 2006 indicated that two-thirds of herbal cannabis seizures in Switzerland had THC levels between 2% and 12%. Additionally, a investigation on cannabis concentration in France found an increase in herbal cannabis THC levels from 7% to 13% between 2009 and 2015, and an increase for cannabis resin from 10% to 23% during the same period (Dujourdy & Besacier, 2017). According to the findings of Bruci et al. (2012), the concentrations of THC found in cannabis materials collected from different places in Albania ranged from 1.07% to 12.13%. Similarly, a study in Northern Thailand found THC levels ranging from 0.72% to 1.48% (Tipparat et al., 2012). Cannabis has become more potent in recent years due in part to the rising practice of indoor cannabis plant cultivation using seeds from high-THC strains. (Atakan, 2012).

In addition to assessing THC content, cannabis may be categorised into fiber-type and drug-type based on specific ratios of the percentage content of its principal cannabinoids. (Fetterman et al., 1971) and Small & Beckstead, (1973) are credited with creating two noteworthy classifications of cannabis. Fetterman et al., (1971) established the phenotypic index as the ratio (% THC + % CBN)/% CBD, which is shown in Table 2.1.

Table 2. 1 Table of description of phenotype index

Phenotype	$R = \frac{\%THC + \%CBN}{\%CBD}$	Class
Phenotype 1	Greater than 1	Drug type cannabis
Phenotype 2	Less than 1	Fibre type cannabis

Diverse cultivars of cannabis can have dramatically diverse phenotypic characteristics, such as the various kinds of cannabinoids and the amounts of those cannabinoids that are present in the plant. Certain cannabis plants are known to be incapable of synthesizing some cannabinoids (Kojoma et al., 2006). This is typically attributable to a deletion factor that obstructs the metabolic pathway preceding THCA. Because there are no upstream precursors in the biosynthetic route in these plants, CBD and THC cannot be synthesized (Cascini et al., 2019). The genetic sequences of the enzymes responsible for catalysing THCA, THCA synthase (THCAS) and that responsible for catalysing CBDA, CBDA synthase (CBDAS) earlier described by Sirikantaramas et al., (2005). There are a lot of similarities between the THCAS and CBDAS gene sequences (87.9% similarity). It has also been documented in the literature that certain cannabis plant kinds can be distinguished from non-drug types using four single-nucleotide polymorphisms (SNPs) on the THCAS gene (Rotherham & Harbison, 2011).

Staginnus et al. (2014) identified three qualitative chemotypes (chemical phenotypes), using the ratio CBD/THC and they proposed that these variations resulted from variations in the genetic sequences of THCAS. De Meijer et al., (2003) found a locus (B) with two co-dominant alleles (BT and BD). The phenotype that is led by THC is caused by the homozygous BT/BT genotype, while the phenotype that is led by CBD is caused by the BD/BD genotype. The intermediate phenotype

is caused by the heterozygous genotype (BT/BD). Furthermore, qualitative chemotype as demonstrated by Staginuss et al., (2014) obeyed the Mendelian law of inheritance, whereas the total concentrations of the cannabinoids was a quantitative feature.

Study by Cascini et al., (2019) discovered substantial genetic differences between two subgroups of Cannabis in two genes that regulate THC buildup in this plant species, allowing for differentiation between drug and fibre types. One of the first new aspects of this work is the investigation of both the THCAS and CBDAS genes. In fact, because their generated proteins fight for the same substrate, the participation of both genes can result in a greater and more robust differentiation between drug and fibre variants. The discovery of 18 amino acid changes in the arrangement of the sequences of high-THC and low/absent-THC accessions, however, represents another unique aspect. For the purpose of gaining an understanding of the enzyme's functioning, this knowledge is absolutely necessary. In this situation, four amino acid changes appeared to reduce THCAS enzymatic activity in fiber-type cannabis plants, one of which was harmful. Additionally, in high-THC accessions, a shortened protein and an inoperable enzyme are caused by the stop codon at position 195 which comes early in the genetic sequence and the 4 base pair deletion in the CBDAS sequence that results in a frame-shift. The high-affinity enzyme produced by CBDAS would preferentially metabolise the intermediate substrate CBGA, presumptuous of the fact that the THCAS-encoded protein could still be active in fiber-type genotypes. Drug-type genotypes exhibit the reverse pattern, with THCAS functioning and the CBDAS protein being totally inert. Even though THCAS as an enzyme has relatively low affinity for its substrates, it was nonetheless able to convert the substrate CBGA under these circumstances. The chemical analysis of cannabis verified and corroborated both of these findings at the metabolic level.

2.4 The Use of Cannabis in Ghana

Documentation on domestic drug consumption in Ghana as compared to reports on its emergence as a transit hub for international trade has been significantly minimal. With virtually no data to work with, policymakers have long ignored this growing phenomenon and hence no effective interventions have been implemented to address it (Doku et al., 2012).

Substances commonly used or abused in Ghana include cannabis, heroin, cocaine, tramadol or even a cocktail of heroin and cocaine (Affinnih, 1999). A Consultative Committee to Combat Drug Menace in Schools was set up in September 2019 to undertake nationwide survey of drug use among high school students. From the survey, it was reported that 65 % used drugs to have good time with their friends, 54 % wanted to experiment to see what it is like, 20 % to 40 % used it to alter their moods, to feel good, to relax, to relive tension and to overcome boredom and problems.

When used by students, cannabis impairs cognitive development and makes it difficult for the learner to learn and recall things learned (Asante, 2019). Vehicular accidents caused by cannabis-induced driving is on the increase and in long time users, cannabis dependence syndrome may develop which is characterized by the addict's inability to control its use (Chow et al., 2019). The classification of cannabis as a dangerous narcotic drug due to its psychoactive properties obstructs its ability to evolve into useful and much-needed medicines. With a rational approach followed by an unbiased, well-conducted research, the right distinction can be made and the medicinal value of cannabis will be much appreciated.

2.5 Legal Status of Cannabis Globally and in Ghana

In recent years, most countries have changed their laws regarding cannabis use. These recent developments with regards to cannabis regulations have triggered calls to amend or review the

current treaty framework of the United Nations. For example, when it comes to therapeutic uses, Latin America leads in the promotion and adoption of policies that gives access to cannabis legally (European Monitoring Centre for Drugs and Drug & Addiction, 2023). The report also mentioned that Mexico now allows the use of cannabis for scientific and medical purposes whereas Uruguay permits these in addition to industrial and recreational uses. Twenty-one (21) states in the US have legislated to allow the use of cannabis for medicinal purposes and over forty-four (44) licensed producers of cannabis have been authorized by the Ministry of Health in Canada (Malabadi et al., 2023). Anti-drug laws in Africa have been described as being quasi-legal depicting the vagueness of these laws and their flexibility (Carrier & Klantschnig, 2018). Notwithstanding the enforcement of legal constraints on cannabis cultivation and use throughout Africa over the twentieth century, cannabis remains pervasive and provides several farmers with a source of income.

Ghana has also enacted the Narcotics Control Commission 2020 (Act 1019) into law on 11 May 2020. Section 43 of the Law legalized the cultivation of cannabis with not more than 0.3% Δ -9-tetrahydrocannabinol (Δ -9-THC) on a dry weight basis for industrial purposes, for obtaining fiber and seed and for medicinal purposes (Narcotics Control Commission Act, 2020). This in effect refers to the legalization of only the variety known as industrial hemp with very low THC, much the same as the level that was made legal for cultivation in the U.S 2018 Farm Bill. Recreational use of cannabis remains illegal in Ghana.

2.6 Instrumentation

Quantifying phytocannabinoids in cannabis matrices (inflorescence, extracts, edibles, and oils) is dominated by chromatographic techniques, with orthogonal spectroscopic methods increasingly used for screening and reference measurements. The most common cannabinoid quantification techniques include gas chromatography (GC) and high-performance liquid chromatography

(HPLC). GC is often used in conjunction with mass spectrometry (MS) or flame ionization detection (FID). Even though cannabinoid profiling has been well described, few studies have so far reported on age-dependent chemical variation in Ghanaian cannabis. Elsewhere, as in the case of De Backer et al. (2012), THC has been reported to increase significantly from early vegetative stage and late vegetative phase into flowering but this remains unknown for Ghanaian cultivars. Analytically, gas-chromatography (GC) has a decarboxylation of cannabinoid acids effect at high temperature settings and derivatization is needed in most cases (Lazarjani et al., 2020). HPLC by comparison facilitates the concurrent analysis of acidic and neutral cannabinoids with no need for heat induced decarboxylation (Lazarjani et al., 2020). These merits supported its application for the measurement of THC, CBD and CBN in the present work.

Gas chromatography (GC) is a widely employed method for cannabinoid quantification due to its speed and resolution capabilities (Hazekamp et al., 2005). Operating at high temperatures (up to 300 °C), GC separates cannabinoids using low-polarity stationary phases; however, these conditions cause acidic cannabinoids to decarboxylate, necessitating derivatization for accurate measurement (Leghissa et al., 2018). Derivatization preserves structure, enhances volatility, and improves peak shape. GC is commonly coupled with mass spectrometry (MS) or flame ionization detection (FID) for identification and quantification (Citti et al., 2016). While MS provides structural information through analyte fragmentation and library matching, FID is often preferred for cost-effective quantification because it uses inexpensive authentic standards, unlike MS, which requires costly deuterated standards (unavailable for all cannabinoids) (Citti et al., 2016).

HPLC is a commonly used liquid chromatography (LC) technique in quantitative cannabinoid analysis. HPLC is the most widely used method for cannabinoid quantification, especially because it measures both neutral and acidic cannabinoids (like THC vs THCA, CBD vs CBDA) without

thermal degradation (Pourseyed Lazarjani et al., 2020). Unlike gas chromatography, HPLC operates at ambient or moderately elevated temperatures, thereby preserving the native structure of carboxylated cannabinoids, which is essential for potency labelling and regulatory compliance. PLC separates cannabinoids based on polarity and hydrophobic interactions using a reversed-phase system, typically with a C18 stationary phase (Citti et al., 2016). Cannabinoids are resolved under a gradient elution of polar (water with an acidic modifier such as formic acid or ammonium formate) and non-polar solvents (methanol or acetonitrile). Detection is commonly performed at 220 nm using a UV or diode array detector (DAD), while LC-MS/MS is used for enhanced selectivity and lower detection limits in complex matrices (Pourseyed Lazarjani et al., 2020).



CHAPTER THREE

3.0 METHODOLOGY

3.1 Overview

The chapter described the methodology of the research which is usually described as materials and method. In this chapter, the methods used which includes study site, growing and collection, reagents and chemicals, colour test and analysis are discussed. The study was intended to ascertain the percentage concentration of the cannabinoid, Δ^9 -THC, CBD and CBN in cannabis samples. Seeds were cultivated over a period of four months. The content of the cannabinoids was analysed using High-Performance Liquid Chromatography (HPLC) (Waters e2695 HPLC Separations Module, United States).

3.2 Study Site, Growing and Collection

Ghana cultivates cannabis in all regions for both local recreational use and international export, according to World Drug Report, 2020. For this study, seeds were taken from seized materials of cannabis from three different sites across Ghana. These sites are in the Eastern region, Volta Region and Brong Ahafo Region. This was done after acquiring a permit for cultivation from the Narcotics Control Commission, Ghana.

Fifty (50) dispensers were filled with garden soil for planting and forty (40) seeds were cultivated on 17 June 2023. However, only seeds from Eastern region grew successfully even after multiple attempts, hence seven (7) samples from the region were chosen for further analysis. These samples were taken for four months consecutively on the 17th day of each month and sent to the laboratory for analysis.

3.3 Reagents and Chemicals

Among the chemicals and reagents employed in the studies were: methanol (Park Scientific Limited, UK), chloroform (Park Scientific Limited, UK), acetonitrile (Park Scientific Limited, UK), Fast Blue B Salt (Glentham Life Sciences, UK) and distilled water.

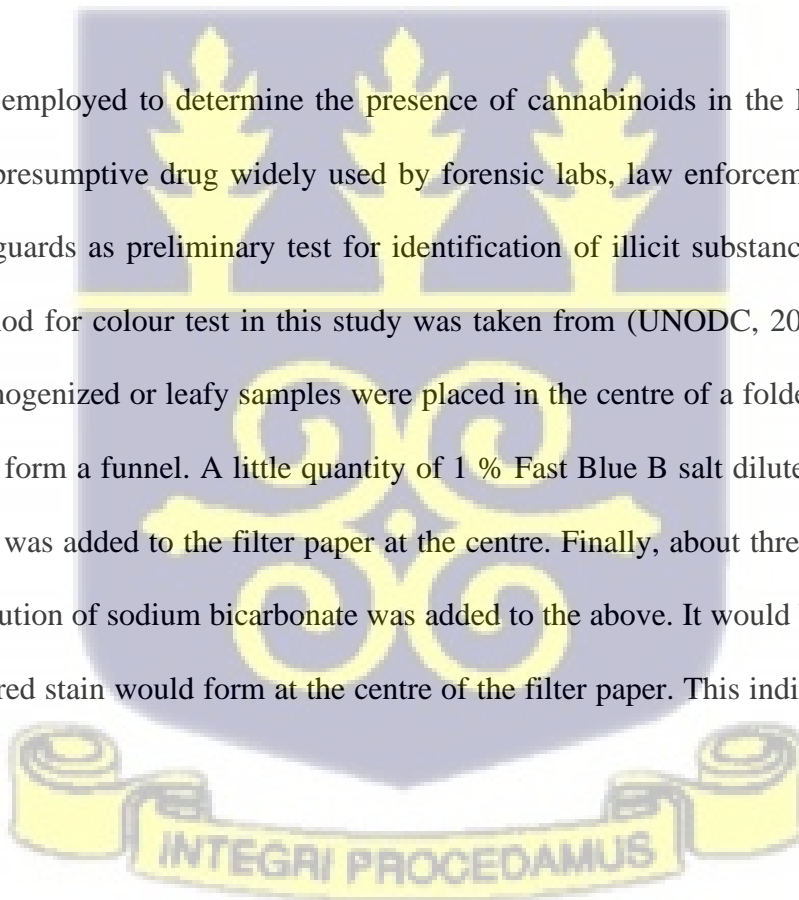
3.3.1 Reference Standards

Reference standards for THC, CBD and CBN was obtained from Absolute Standards, United States.

3.4 Colour Test

Colour test was employed to determine the presence of cannabinoids in the leafy sample. The colour test is a presumptive drug widely used by forensic labs, law enforcement agencies, and border security guards as preliminary test for identification of illicit substance. (Gorziza et al., 2023). The method for colour test in this study was taken from (UNODC, 2022). Two to three drops of the homogenized or leafy samples were placed in the centre of a folded filter paper and partly opened to form a funnel. A little quantity of 1 % Fast Blue B salt diluted with anhydrous sodium sulphate was added to the filter paper at the centre. Finally, about three drops of a 10 % w/w aqueous solution of sodium bicarbonate was added to the above. It would be observed that a purple red coloured stain would form at the centre of the filter paper. This indicates the presence of cannabinoids.

3.5 Analysis



3.5.1 Sample Treatment

In order to eliminate any moisture and render the samples suitable for grinding, the cannabis samples were desiccated in an oven (Thermo Fisher Scientific, United States) at a temperature of at 40°C for 60 minutes and homogenized using a blender (Memmert GmbH + Co. KG, Germany). The sample was stored in a dark polythene bag to avoid light and degradation of the cannabis content.

3.6.2 Extraction

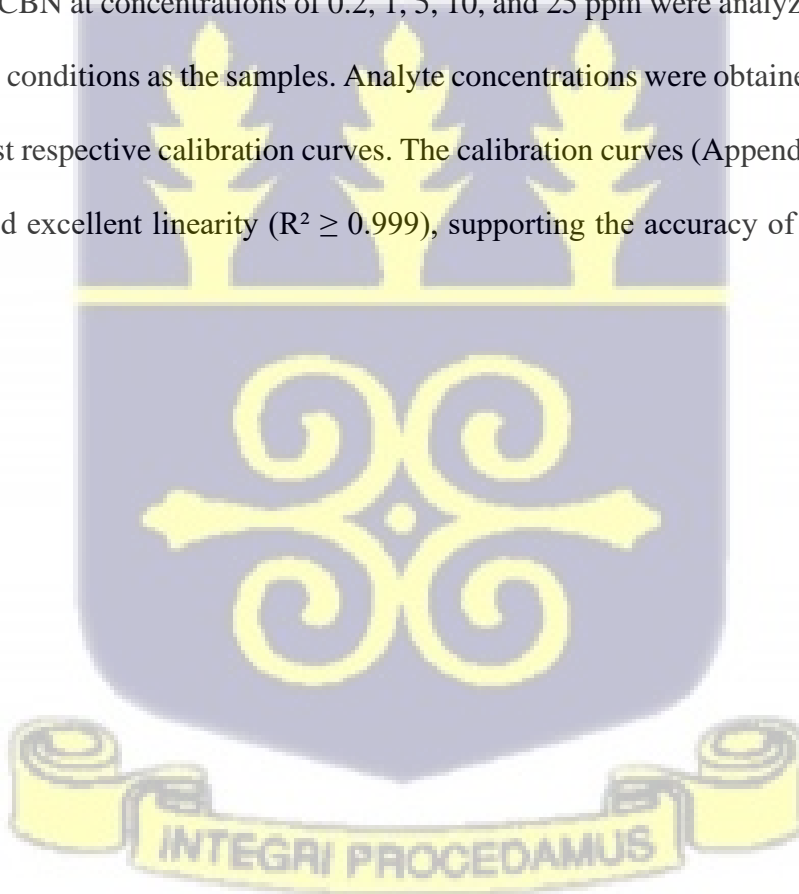
This study used the method of extraction as used by Hädener et al. (2019) with slight modification. A 500 mg powder was weighed into a 50 ml centrifuge tube. An amount of 5 ml methanol/Chloroform (90/10 v/v) was added into the centrifuge tube. The sample was then vortexed for 5 minutes and sonicated on an ultrasonic water bath (WiseBath, Witeg Labortechnik GmbH, Wertheim, Germany) for 15 minutes. For complete decarboxylation, 200 µL of the sample was pipetted into an autosampler vial. The extract was allowed to evaporate under steam of air. For total THC, the sample was heated at 210 °C in an oven. The residue was then reconstituted in 1 mL methanol.

3.6.3 HPLC Conditions

The cannabinoid concentrations were analysed using a high-performance liquid chromatography (HPLC) system (Waters e2695 HPLC Separations Module) in a spectrum mode. The HPLC system is coupled with a quaternary pump, auto sampler, column compartment, and a fluorescence detector (2475 FLR Detector). Chromatographic separations were conducted with a Synergi 4µ Hydro-RP 80A column (Phenomenex, Torrance, CA, United States), with a length of 250 mm and

internal diameter of 2.1 mm, employing acetonitrile and 0.1% (v/v) formic acid in a ratio of (75:25), at the isocratic mode, with a flow rate of 1.0 ml/minute and run time of 15 min under 25°C. 10 µl of the extract that was dissolved in methanol was injected unto the HPLC. An individual calibration curve of 0.2, 1, 5, 10, 25 ppm for THC, CBD and CBN was used to quantify the cannabinoid content in the extract. ~~was Quantification was based on analytical standards: THC, CBD, and CBN from Sigma Aldrich (Germany) obtained at the Forensic laboratory of Ghana Standards Authority.~~

Cannabinoid quantification was performed using external calibration. Standard solutions of Δ^9 -THC, CBD, and CBN at concentrations of 0.2, 1, 5, 10, and 25 ppm were analyzed under the same chromatographic conditions as the samples. Analyte concentrations were obtained by interpolating peak areas against respective calibration curves. The calibration curves (Appendix A, Figures 6.1–6.3) demonstrated excellent linearity ($R^2 \geq 0.999$), supporting the accuracy of the quantification process.



CHAPTER FOUR

4.0 RESULTS

4.1 Colour test

Cannabis was identified by a purple-red stain in the middle of the filter paper, indicating the need for further extraction procedures. The colour obtained is a combination of the colours of the different cannabinoids which were the major components of cannabis: THC = red, CBN = purple, CBD = orange.

4.2 Trend Analysis of the concentration of cannabinoids

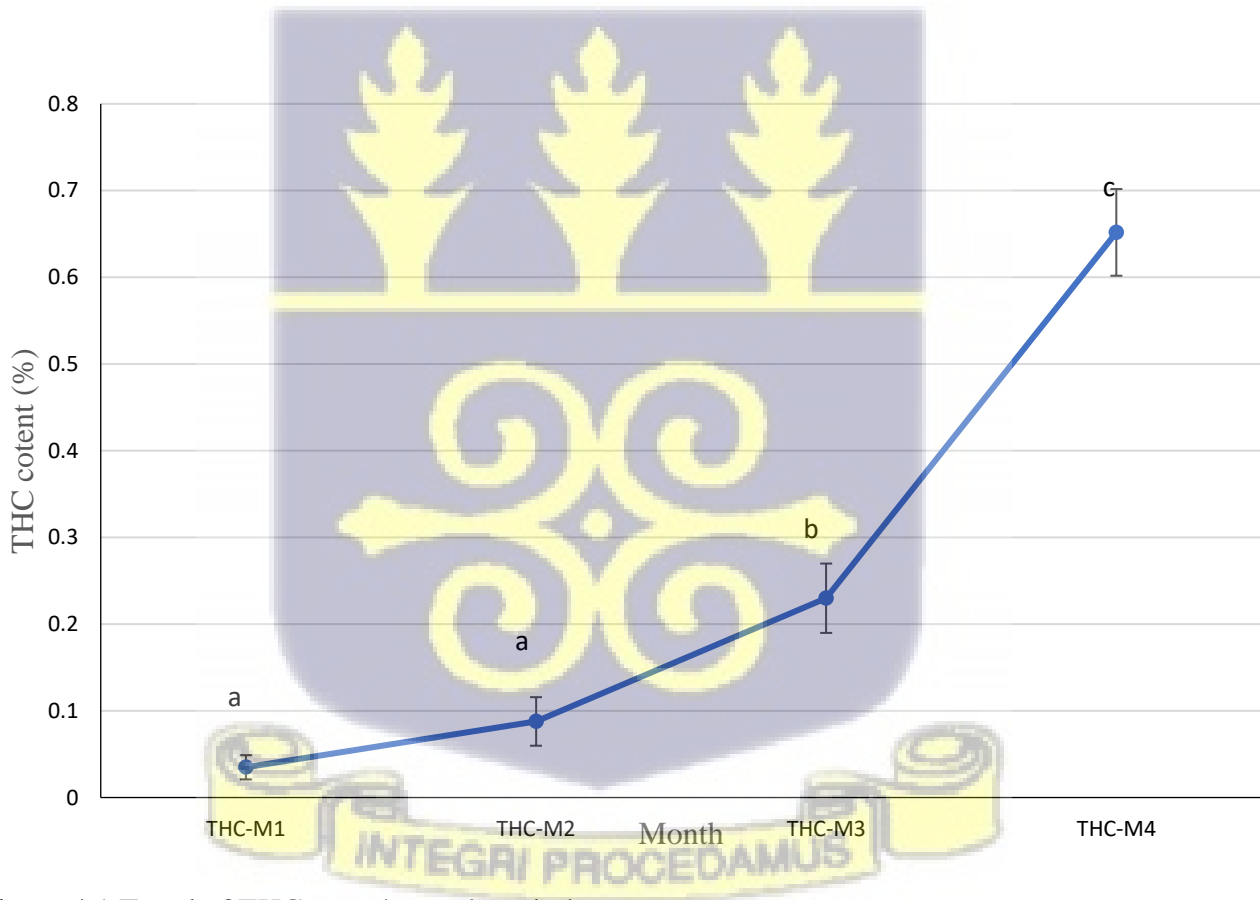


Figure 4.1 Trend of THC over 4-month period

Different superscripts on the error bar are significantly different ($p < 0.01$) from each other.

The figure 4.1 shows trend of THC content over the 4-month period of study. It can be seen that the first month (M1) recorded the least (0.03%) whilst the fourth month recorded the highest (0.65%). The range of THC content within the four-month period was 0.03% - 0.65%. The percentage of THC for the third month was higher than the legal limit of 0.3% dry weight basis of the cannabis plant.

Figure 4.2 shows the trend in CBD concentration over the 4-month period of growth.

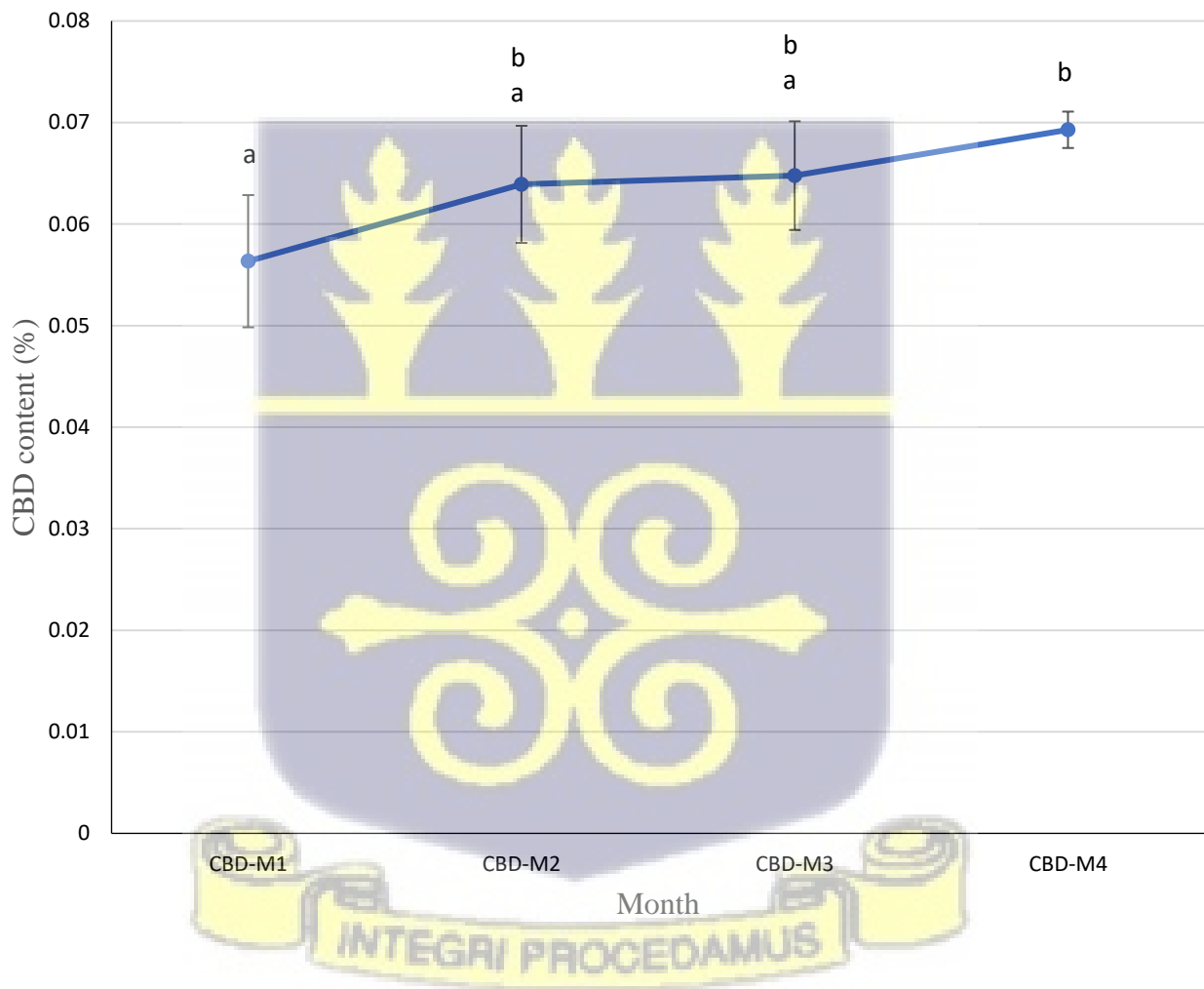


Figure 4.2 Trend of CBD over 4-month period
Different superscripts on the error bar are significantly different ($p < 0.01$) from each other.

From Figure 4.2 the concentration of CBD had a very slow rise across the 4-months period. The concentration of CBD rose from the first month (0.056%) to the second month (0.064%). The concentration remained slightly constant from the second to the third month (0.064%) and increased again in the fourth month (0.069%).

Figure 4.3 shows the concentration of CBN in the four-month growth period.

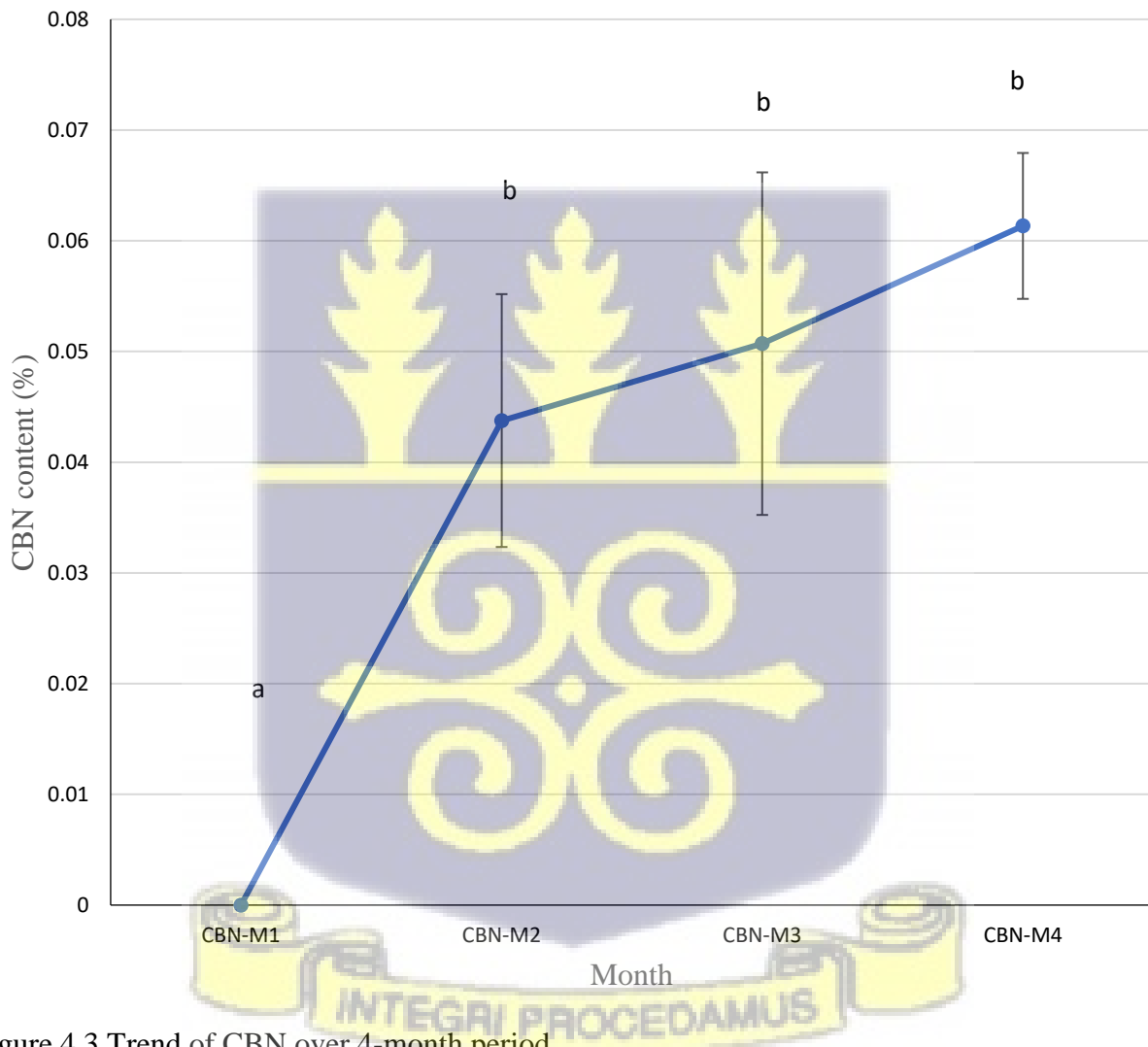


Figure 4.3 Trend of CBN over 4-month period
Different superscripts on the error bar are significantly different ($p < 0.01$) from each other.

CBN levels started near zero in month 1, then rose to 0.043% in month 2, followed by gradual increases to 0.051% in month 3 and 0.061% in month 4.

Figure 4.4 shows the average concentration of THC, CBD and CBN over the 4-month period

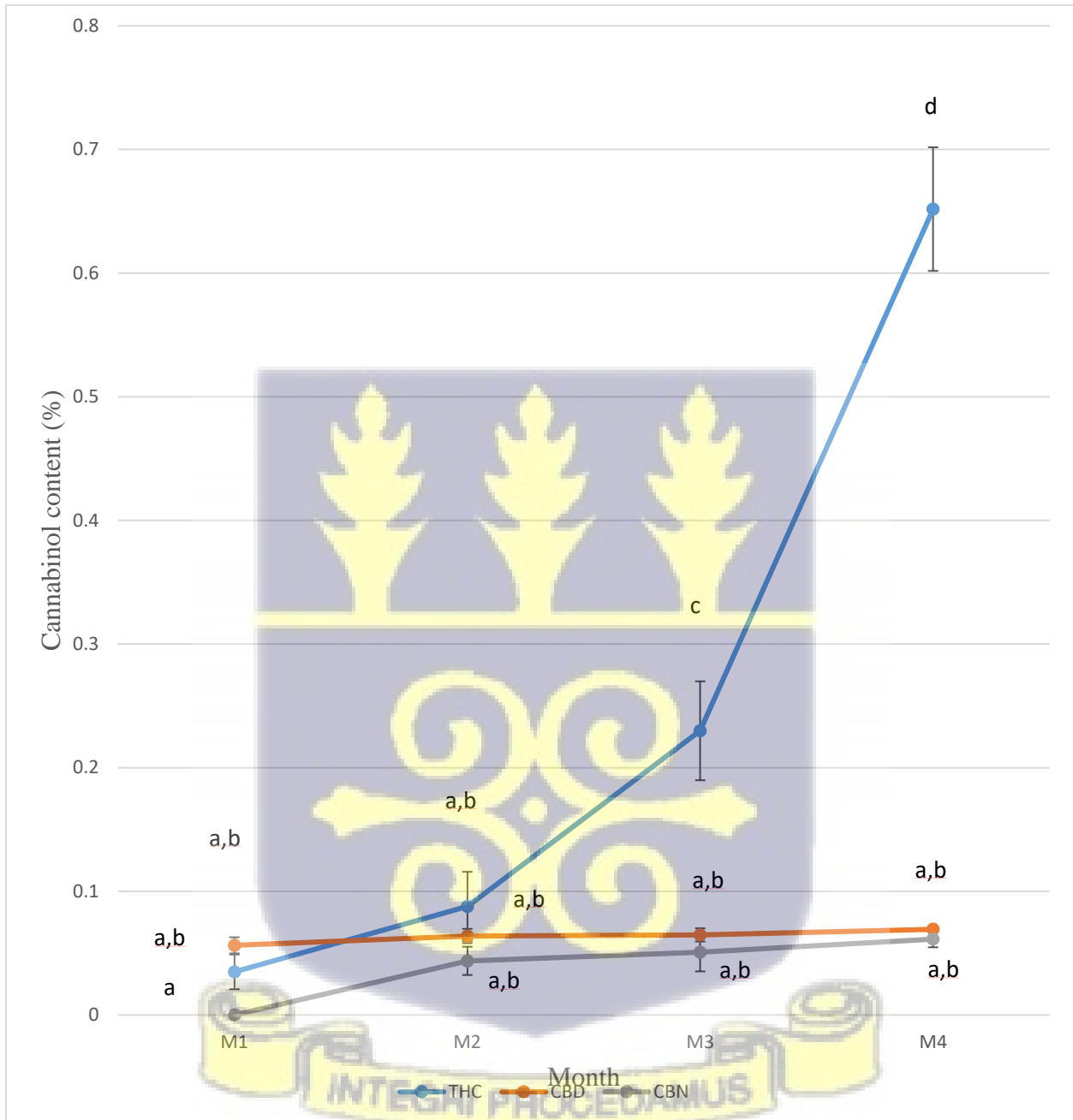


Figure 4.4 Average concentration of THC, CBD and CBN over the 4-month period. Different superscripts on the error bar are significantly different ($p < 0.01$) from each other.

THC levels show a gradual increase from month 1 to 2, followed by sharp rises from month 2 to 3 and again from month 3 to 4. The CBN content exhibits a gradual upward trend from month 1 to month 3, followed by a notable decline in month 4. The CBD content was relatively unchanged from the first to the fourth month.

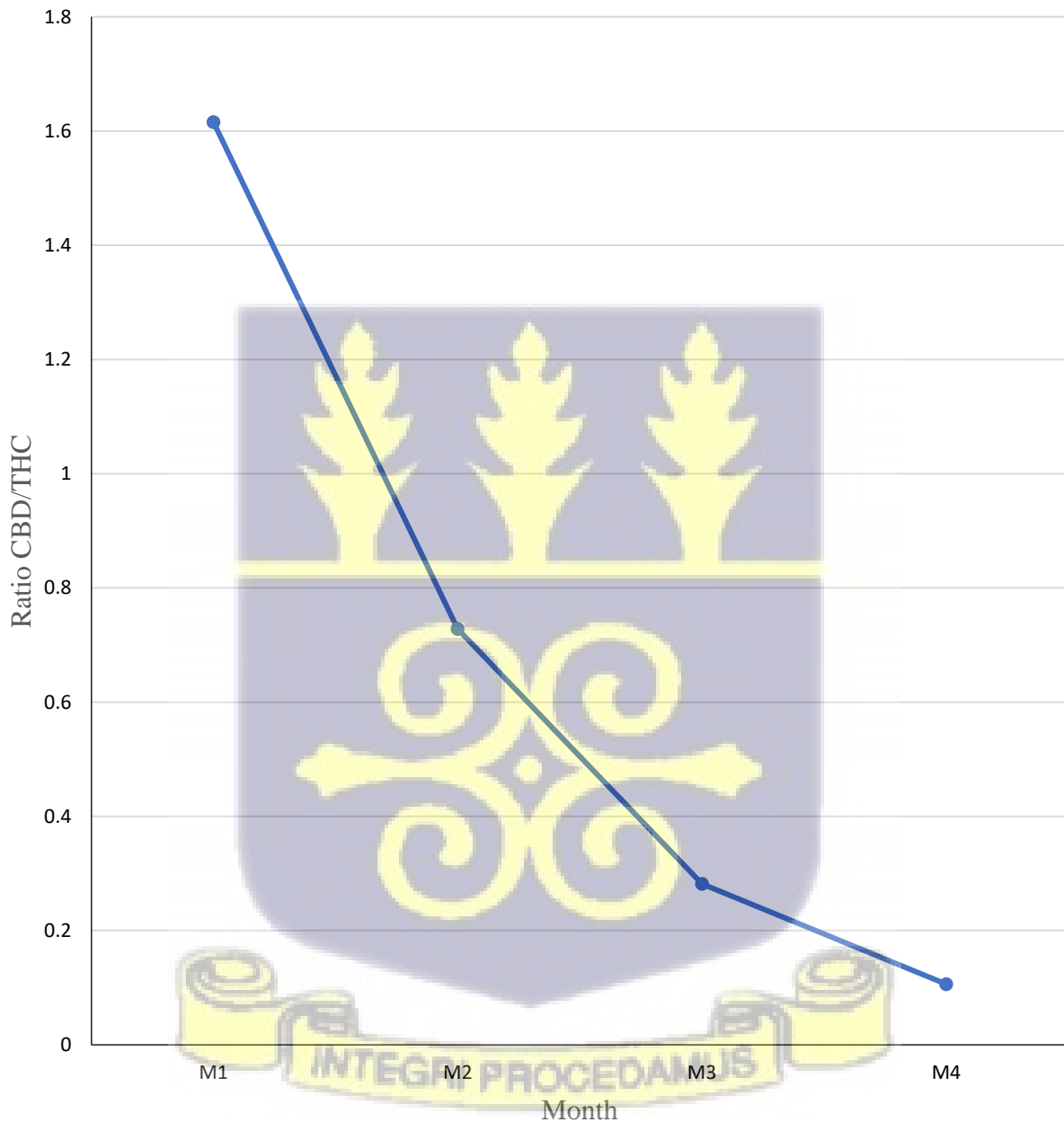


Figure 4.5 Ratio of CBD to THC over the four-month growth period

The ratio of CBD to THC decreased from the first month, M1 (1.62) to the fourth month, M4 (0.11). The ratio of CBD to THC in the second month, M2 is 0.72. The ratio of CBD to THC in the third month, M3 was 0.28.

Figure 4.6 shows the ratio of CBN to THC over the four-month growth period

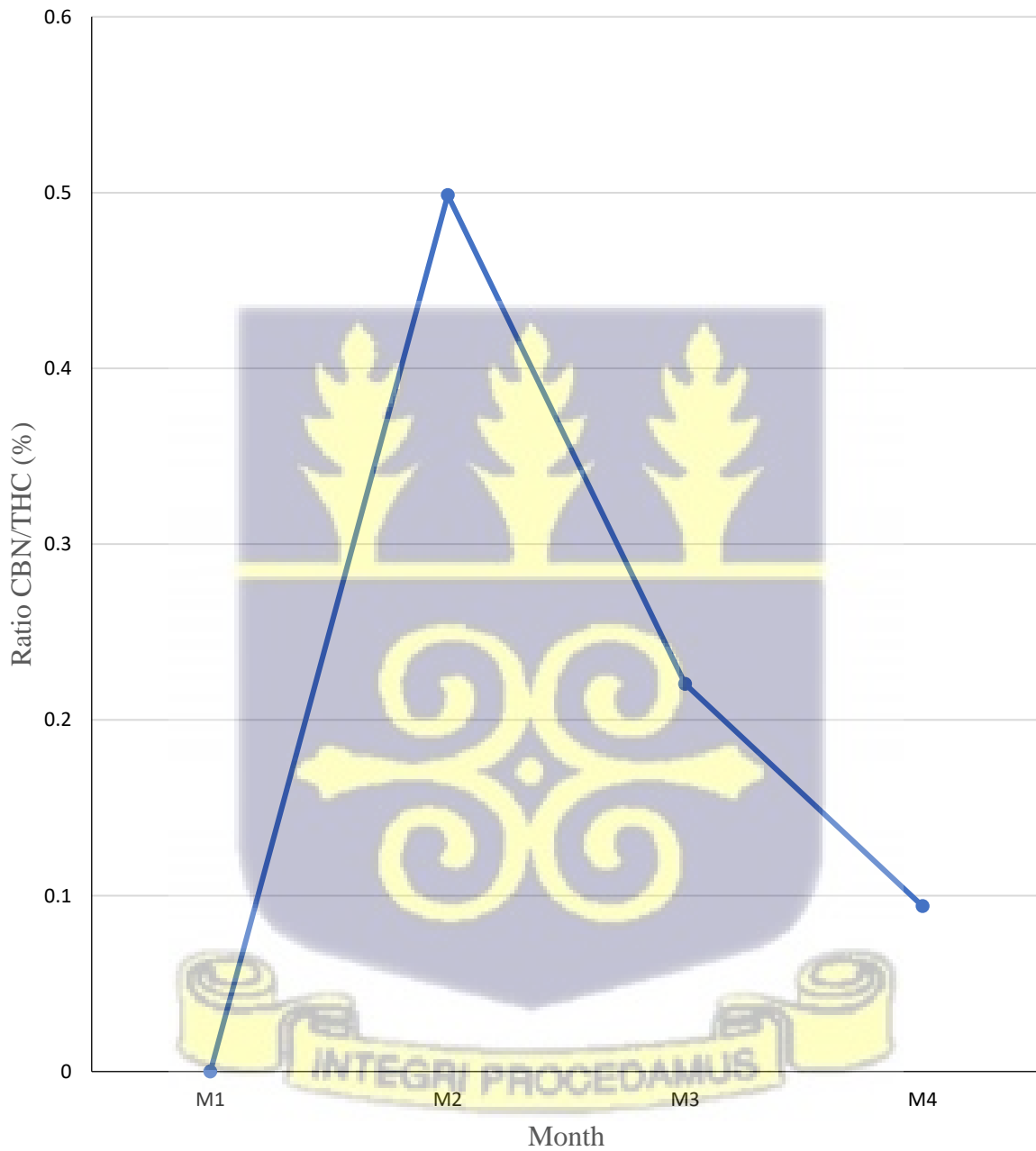


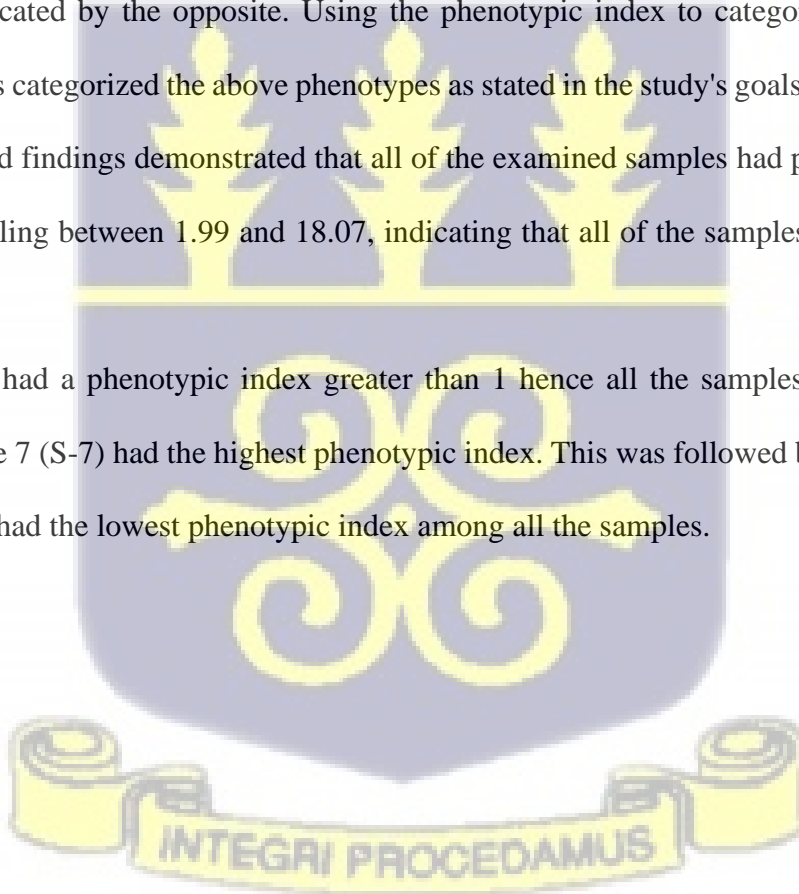
Figure 4.6; Ratio of CBN to THC

From the ratio of CBN to THC in the first month, M1 was approximately zero. The second month, M2 had the highest ratio, 0.50. The ratios decreased from M2 to M4 (0.094).

4.3 Phenotype Index of samples analysed

Using the ratio of the overall THC concentration to the CBD content, Fetterman et al. (1971) determined the drug type and fibre type of cannabis. The ratio is known as the phenotype index. The phenotype index is calculated by add total content of THC and CBN the dividing the sum by the total content of CBD. The phenotypic index is the name given to the ratio. Phenotype I, or drug type cannabis, is indicated by a phenotype index larger than 1, while phenotype II, or fibre type cannabis, is indicated by the opposite. Using the phenotypic index to categorise the examined cannabis samples categorized the above phenotypes as stated in the study's goals. As shown in Fig. 4.7, the computed findings demonstrated that all of the examined samples had phenotypic indices larger than 1, falling between 1.99 and 18.07, indicating that all of the samples were of the drug type cannabis.

All the samples had a phenotypic index greater than 1 hence all the samples are of drug type cannabis. Sample 7 (S-7) had the highest phenotypic index. This was followed by sample S-5 and sample S-6. S-2 had the lowest phenotypic index among all the samples.



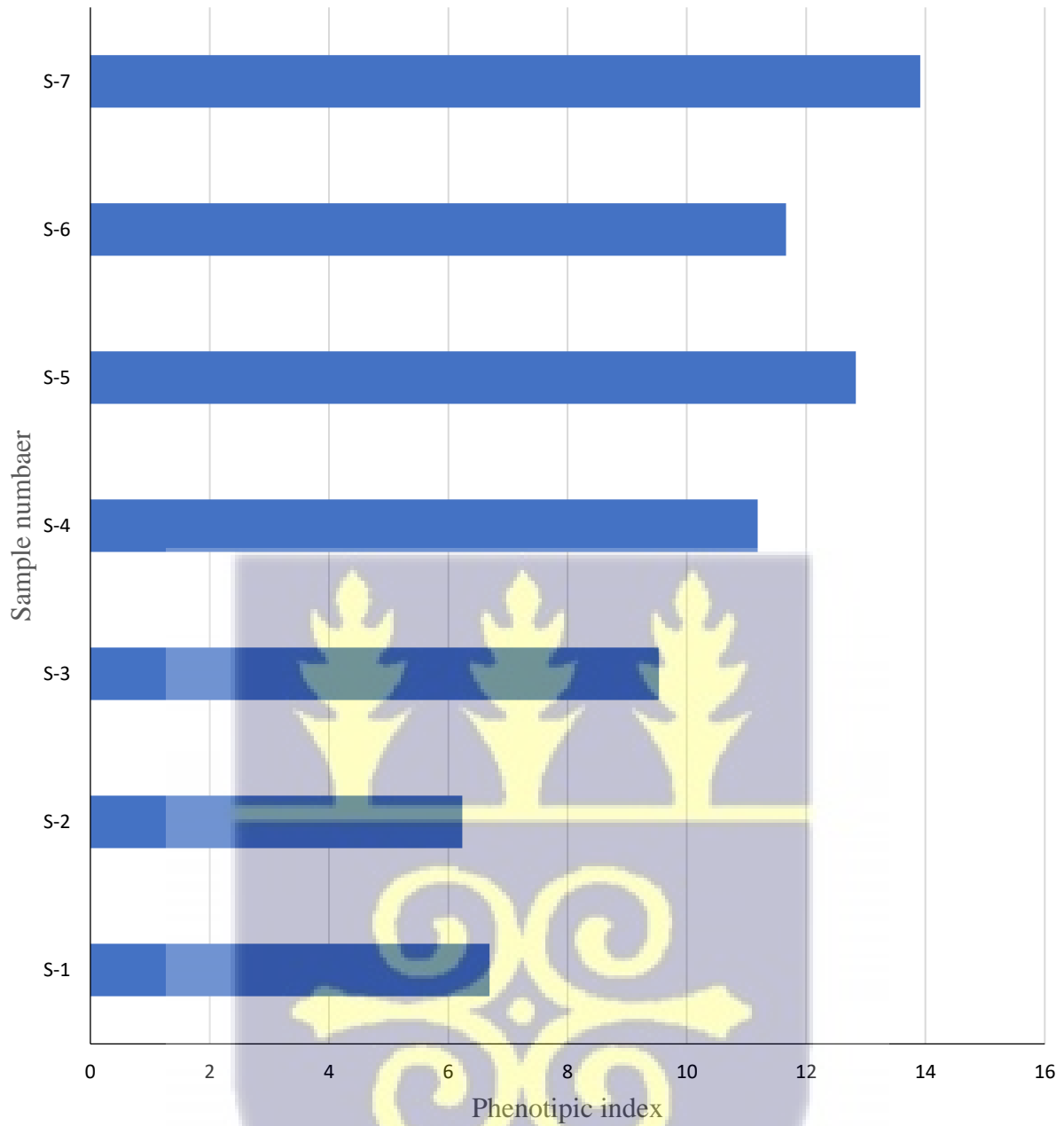
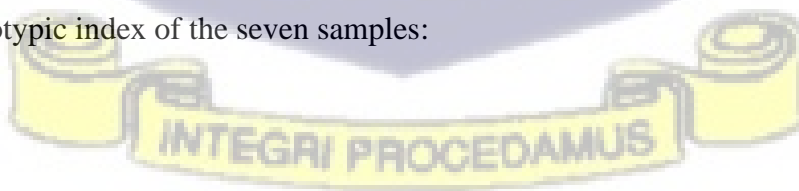


Figure 4.7 Phenotypic index of the seven samples:



CHAPTER FIVE

5.0 DISCUSSION

In this study, seeds from three different regions in Ghana—Eastern Region, Bono Region, and Volta Region were planted; however, only the seeds from the Eastern Region successfully germinated. The seeds were grown on three separate occasions to verify germination, yet each time only the Eastern Region seeds sprouted. The lack of germination in the other regions may be attributed to environmental factors. Bruci et al., (2012) indicated that aspects such as temperature and humidity can significantly influence cannabis growth. Additionally, the viability of seeds plays a crucial role in the germination process, which can be influenced by the growing environment and the various hemp varieties found in Ghana (Malabadi et al., 2023). The application of fertilizers and adherence to good farming practices are also essential; otherwise, stress may be induced in the hemp plants (Spano et al., 2022). Differences in germination success across regions may reflect variability in seed viability and microclimatic conditions, as local temperature, humidity, and soil factors can influence early plant development (Bruci et al., 2012). Even under semi-controlled environments, minor fluctuations in environmental parameters may affect metabolite production and growth characteristics.

Figure 4.1 provides analysis of the total THC concentration based on plant age. As the plants mature, there is a notable increase in the total THC content, with the highest concentration observed in the fourth month of growth. There is no significant difference ($P=0.353$) in THC levels at a 95% confidence interval between the first and second months. However, significant differences emerge when comparing the THC content from the second month with the contents of the third ($P = 0.002$) and fourth months ($P < 0.001$), as well as between the third and fourth months ($P < 0.001$) themselves. THC content surged 151% from month one to two, another 151% from

month two to three, and a sharp 183% increase from month three to four. Notably, most samples were near the 0.3% THC (dry weight basis) legal limit by the third month. However, by the fourth month, all samples exceeded this legally permissible threshold, highlighting a significant concern regarding compliance with regulations. Sandhu et al., (2022) observed that THC concentrations in most cannabis varieties surpassed the legal limit of 0.3% at various points during the growing season. Linder et al., (2022) similarly reported that THC levels exceeded this legal threshold approximately four weeks into the growth cycle. This observation stands in contrast to the findings of De Backer et al., (2012), who identified the highest total THC content occurring during the sixth week post-flowering, suggesting important variations across different studies and growth conditions. Furthermore, research by Spano et al., (2022) indicated that THC content peaks during the flowering stage, which corresponds with the second month of growth. This assertion further emphasizes the divergence of this study from others in the field. Despite these differing conclusions, the overarching trend of increasing THC concentration with plant age aligns with the research conducted by Mariotti et al., (2016), who also documented a growth-related rise in THC levels. THC concentrations exhibited a significant age-associated rise, with minimal differences between months 1 and 2 ($p = 0.353$), followed by pronounced increases from month 2 to month 3 (151%) and from month 3 to month 4 (183%) ($p < 0.001$). These findings align with prior research showing THC escalation during flowering and maturation (Sandhu et al., 2022; Linder et al., 2022; De Backer et al., 2012; Spano et al., 2022).

These results hold relevance for Ghana's regulatory environment, where industrial hemp cultivation is limited to plants containing $\leq 0.3\%$ Δ^9 -THC (NACOC Act, 2020; IDPC, 2025). The data indicate that harvesting before the end of the third month may help maintain compliance, whereas delaying into the fourth month is likely to exceed legal thresholds. This provides

actionable guidance for growers, regulators, and policymakers regarding harvest scheduling and cultivar selection.

Figure 4.2 indicated the age analysis of CBD. The concentration of CBD for all the months were below 0.1% did not vary much within the months in the study. There is no significant difference in CBD content at 95% CI between the first and second month ($P = 0.106$) and between the first and third month ($P = 0.074$). There is also no significant difference in CBD content at 95% CI between the second and third month ($P = 0.850$) and the second and fourth month ($P = 0.244$). However, there was a significant difference between the first and the fourth month ($P = 0.008$). There was a 13% increase in CBD content from month 1 to month 2, 1% increase from month 2 to month 3 and 6% increase from month 3 to month 4. The concentration of CBD showed no age dependent correlation. This result is similar to Mariotti et al. (2016) where CBD concentration does not show any age dependency. The result is similar to Massuela et al., (2022) where CBD concentrations were less than 0.1% and a similar plateau was observed in CBD concentrations. Shiponi & Bernstein, (2021) also showed similar though the concentration of CBD were significantly higher, the plateau trend with time could be seen. Linder et al., (2022) also noted that CBD continued to increase, reaching its greatest concentration at 6 weeks. The trend observed was due to the fact that the samples were analysed immediately they were harvested. It is known that THC breaks down upon storage to CBD (Jaidee et al., 2022). The effect of other environmental factors was such as temperature and light exposure in negligible. THC degrades in the presence of light to CBD. The linear progression of the CBD graph indicates lack of conversion of THC into CBD. CBD levels remained consistently low ($\leq 0.1\%$), consistent with reports of limited CBD fluctuation over growth stages (Mariotti et al., 2016; Massuela et al., 2022). A modest but

significant increase was observed between months 1 and 4 ($p = 0.008$). Prolonged light exposure → THC oxidizes to CBN (cannabinol), which is much less psychoactive.

Figure 4.3 indicated the age analysis of CBN. There is no significant difference in CBN content at 95% CI between the second month and the third month ($P = 0.633$) and second month and the fourth month ($P = 0.233$). There is also no significant difference in CBN content at 95% CI between the third month and the fourth month ($P = 0.633$). The first month showed no detectable CBN concentration. There was a 15% increase in CBN content from the month 2 to month 3 and 20% increase from month 3 to month 4. This result agreed with Mariotti et al. (2016) where there was also no age-dependent correlation with the concentration. CBN is a non-enzymatic oxidation by-product of THC and is most commonly an artefact found after prolonged storage, especially at higher temperatures. Therefore, these results were expected based on its biosynthetic pathway, because the content of this compound increases with the age of the cannabis plant up to a maximum in the peak budding stage, reaching a plateau, followed by a decrease during the onset of senescence (Mariotti et al., 2016). The concentration of CBN was higher than those from Jin et al., (2020) who detected CBN concentration at less than 0.01%. The very low concentration of CBN indicates that there was minimal degradation. The non-breakdown of THC to CBN could be as a result of determination of the cannabinol content right after harvest. CBN levels rose slightly toward month 4, reflecting its formation as an oxidative product of THC (Pollastro et al., 2018). Figure 4.4 indicate the age analysis of THC, CBD and CBN. The conversion of THC to CBN was prominent at high-temperature conditions (Jaidee et al., 2022). Therefore, minimum transformation of CBD, CBN, and Δ^9 -THC was achieved by using a lower temperature (refrigeration at 4°C) and analysing samples within a short time after harvest (Gülck & Møller, 2020).

Figure 4.5 shows the ratio of CBD to THC over the four-month growth period. The ratio of CBD-to-THC can be used for the distinction of fibre-type cannabis (hemp) from drug-type cannabis (cannabis) (De Backer et al., 2012). In fibre-type cannabis, CBD is the major cannabinoid and the CBD content in these cases is generally higher than the total THC content (Mariotti et al., 2016). The low CBD ratio in this study suggests a high THC concentration. The legal threshold will always limit the greatest CBD concentration that producers can achieve because THC concentrations surpass 0.3% prior to maximum CBD production.

The cannabis samples were categorized into different chemotypes based on the ratio of CBD, THC, and CBN, using a classification system recommended by the UNODC. (Mariotti et al., 2016). Ghana's new law may require forensic labs to test for cannabis chemotypes with high CBD content and distinguish between drug-type and fibre-type cannabis. Figure 4.6 shows the ratio of CBN to THC over the four-month growth period. The ratio of CBN to THC serves as a marker for degradation, as THC breaks down into CBN over time due to oxidation. (Ambach et al., 2014). The ratio of CBN to THC was less than 1.0% which means high THC content which indicates low transformation of CBN to THC. In this study all samples had very low CBN to THC ratio which indicates that the THC in the samples are still intact and have not undergone any chemical changes. Figure 4.7, the CBD to THC ratio can help distinguish between cannabis plants grown for fibre and those grown for medicinal or recreational use.(Ambach et al., 2014). Hemp typically has more CBD than THC. CBD can counteract some of THC's effects and has calming properties. In this study, all samples had more THC than CBD by the fourth month, indicating they're likely drug-type cannabis. The cannabis samples in this study would be illegal to grow in many places because they contain high levels of THC, which is characteristic of drug-type cannabis.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Accurate knowledge of cannabinoid composition, particularly the concentrations of Δ^9 -tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) is crucial for the scientific understanding of cannabis plants, the development of cannabinoid-based therapeutics, and effective law enforcement and policy regulation. This study has shown that cannabinoid levels vary substantially with the age of the plant, especially for THC. Over a four-month period, THC concentrations increased significantly from 0.04% to 0.7%, while CBD and CBN levels remained relatively stable, at approximately 0.075% and 0.044%, respectively.

These findings underscore the dynamic nature of cannabinoid biosynthesis in cannabis and highlight the importance of harvest timing as a key determinant of compliance with legal THC thresholds. The accumulation of THC with plant maturity suggests that premature or delayed harvesting could have serious implications for both legal cultivation and potential medicinal applications.

Moreover, the study confirms that cannabinoid concentrations are influenced not only by age but also by a range of environmental and biological factors. Variables such as light intensity, temperature, and moisture conditions, as well as intrinsic plant characteristics – including phenological stage, sex, and the specific part of the plant harvested—all contribute to variations in cannabinoid profiles.

Taken together, these insights offer a scientific basis for developing cultivation guidelines tailored to the Ghanaian context. They support the need for future research into genotype-environment interactions and call for regulatory frameworks that accommodate local cultivars. Ultimately, this

knowledge is vital for informing sustainable cannabis policies, ensuring quality control, and maximizing both public health and economic benefits under Ghana's evolving legal landscape.

6.2 Recommendation

Based on the findings of this study, it is recommended that local cannabis farmers harvest their plants at or before the three-month growth stage to ensure compliance with Ghana's statutory THC limit of 0.3% on a dry weight basis. Harvesting beyond this period may result in THC concentrations that exceed the legal threshold, potentially disqualifying the product for industrial or medicinal use under current legislation. Adhering to this recommendation will help safeguard the livelihoods of farmers and promote lawful cannabis cultivation.

Furthermore, it is strongly recommended that additional research be conducted on cannabis cultivars from other regions of Ghana, including the Western, Ashanti, Ahafo, and Northern regions. Such studies would help verify whether similar patterns of cannabinoid development exist across different agroecological zones. This would not only enhance the understanding of regional variations in cannabinoid biosynthesis but also inform region-specific cultivation strategies.

Finally, to enrich the body of knowledge on cannabis production and usage in Ghana, further investigations should be carried out on seized or cultivated samples across the country. Expanding research efforts will support evidence-based policymaking, improve monitoring and regulatory frameworks, and contribute to the responsible development of the country's cannabis sector.



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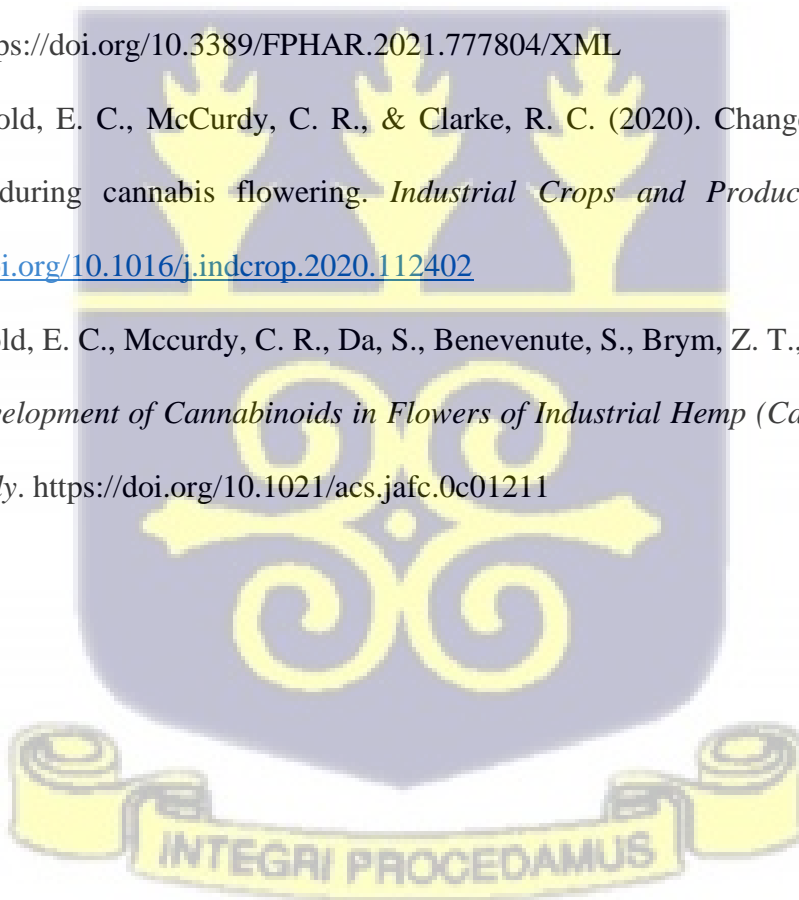
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Appendix A

Calibration curve for THC

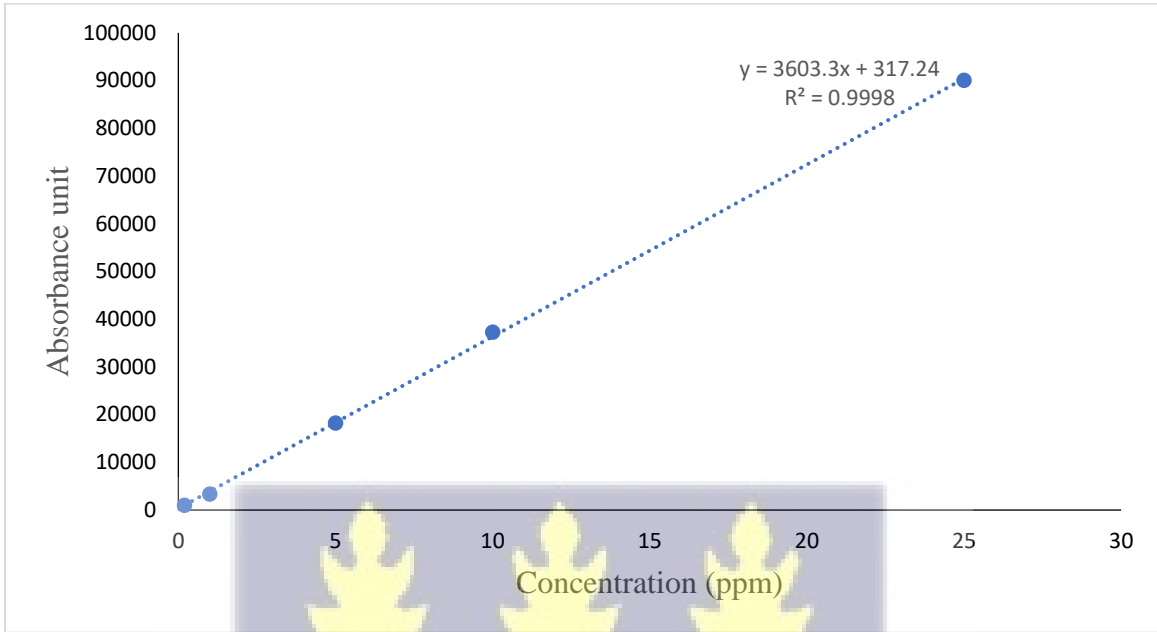


Figure 6.1 Calibration curve for THC

Calibration curve for CBD

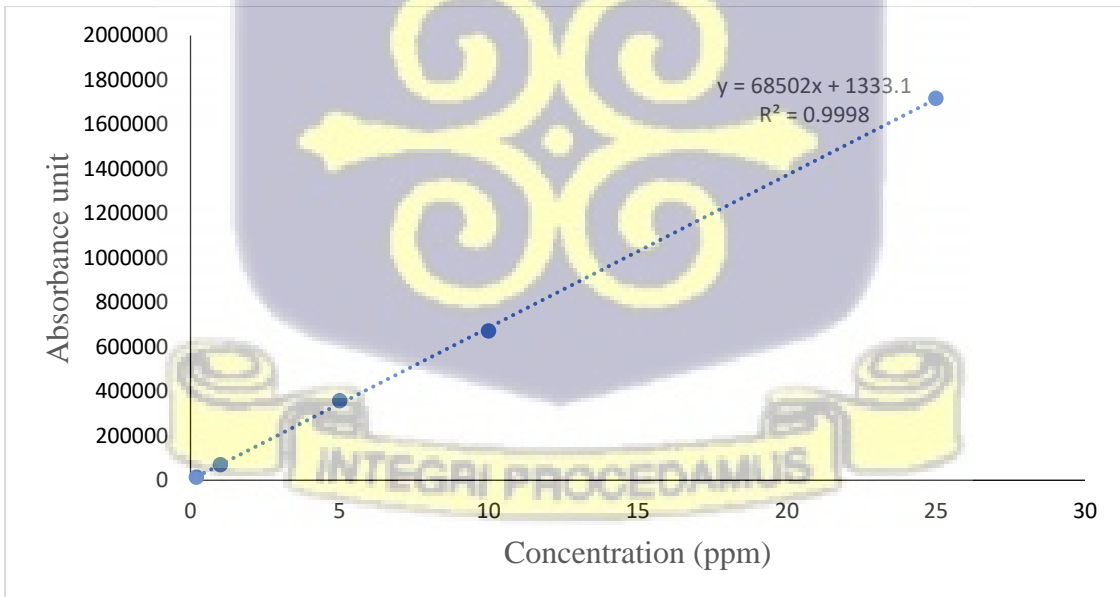


Figure 6.2 Calibration curve for CBD

Calibration curve for CBN

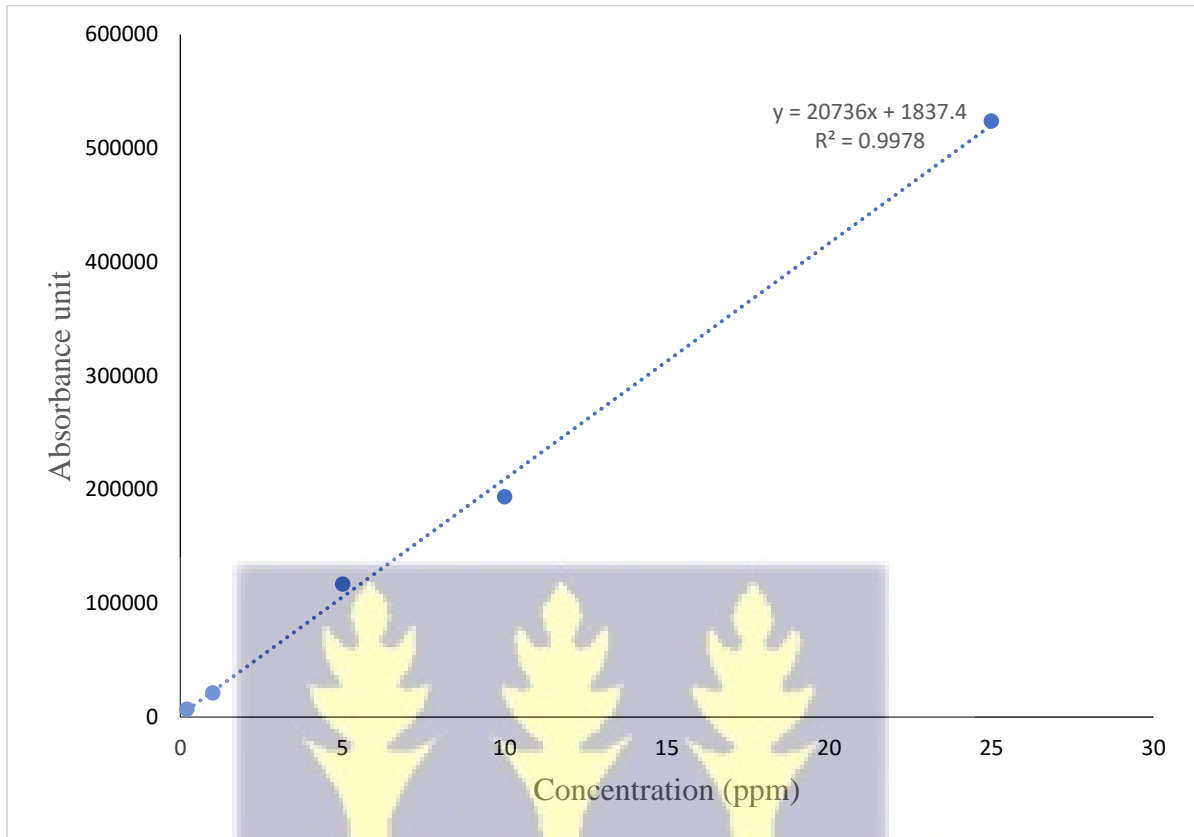
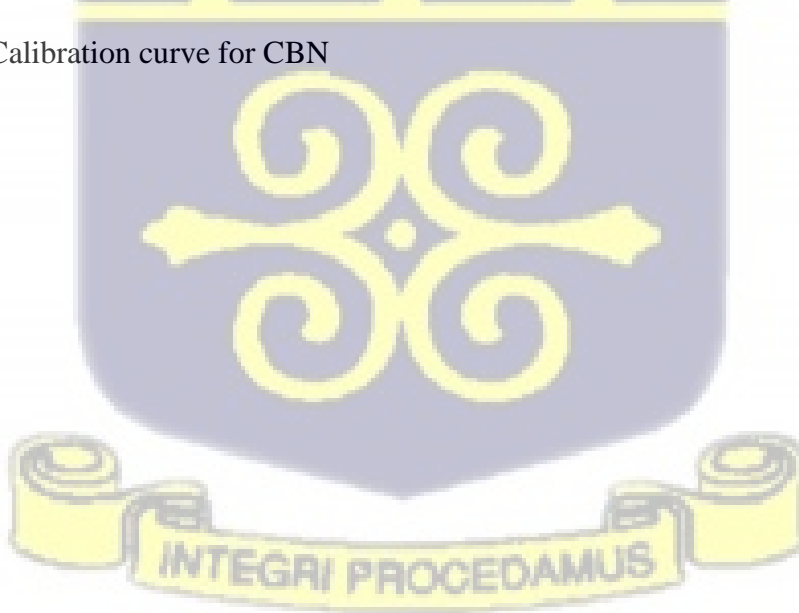


Figure 6.3 Calibration curve for CBN



APPENDIX B

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NARCOTICS CONTROL COMMISSION

PMB, Cantonments
Accra - Ghana
Location: 6 Airways Avenue
Airport Residential Area

Digital Address: GA-037 2475

Our Ref. No: NCC/ORI/200/10/04/VOL.1/64

Date: 05 SEPTEMBER 2022

ENOCK DANKYI (PHD)
SUPERVISOR AND EXAMINATIONS OFFICER
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF GHANA

RE: PERMISSION TO UNDERTAKE CANNABIS RESEARCH FOR POSTGRADUATE STUDIES

Your letter dated 19 July 2022 on the above subject refers.

2. Please be informed that, the requisite regulations providing for the issuance of licence/permit to undertake research on cannabis in the country are not yet in place. However, considering the significance of the research work, the Commission is willing to assist in the conduct of the research.
3. In this regard, you are to liaise with the Acting Director for Research, Policy and Planning at the Commission, Principal Narcotics Control Officer (PNCO) Sylvester Koomson who could be contacted on 0202021136.
4. Should you be ready to pick samples/nurse the cultivars for your study, the Commission would grant you security clearance to that effect for a period, when duly notified in writing.
5. Accept our best regards.

Yours faithfully,

SYLVESTER KOOMSON
AG. DIRECTOR, RESEARCH, POLICY & PLANNING
FOR: DIRECTOR-GENERAL

INTEGRI PROCEDAMUS

RESTRICTED

Page 1 of 1