

Full Length Research Paper

Determination of tetrahydrocannabinol and cannabidiol contents in *Cannabis sativa* L. samples in Togo using gas chromatography-mass spectrometry

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***Cannabis sativa* L. is a widely used recreational drug in Togo, especially among young people. However, little is known about its chemical composition in Sub-Saharan Africa, and specifically in Togo. This study aimed to determine the levels of phytocannabinoids, specifically Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD), in cannabis samples collected from various towns in Togo using gas chromatography coupled to mass spectrometry (GC-MS). Thirteen cannabis samples were extracted with hexane using maceration and ultrasound to isolate phytocannabinoids, and a quick 24-min gas chromatographic separation method was used to analyse the extracts. THC, CBD, and cannabinol (CBN) were the major components identified in the samples. THC content ranged from 37.73±0.31 to 87.9±1.30% for maceration and from 39.09±2.17 to 84.54±0.80% for sonication, while CBD content varied from 1.36±0.27 to 4.07±0.23% for maceration and from 1.79±0.12 to 5.03±0.90% for sonication. These results indicate that cannabis consumed in Togo has high concentrations of THC and that maceration and sonication is more likely to extract THC and CBD, respectively. The findings could provide important information for the authorities in Togo to assess the extent of THC exposure among cannabis consumers in the country.**

Key words: *Cannabis sativa* L., cannabidiol, phytocannabinoids, tetrahydrocannabinol, solvent extraction.

INTRODUCTION

Cannabis sativa L. (cannabis) is an annual plant that has been used for human consumption and traditional medicine since ancient times (Clarke and Merlin, 2016; Jabeen et al., 2010). The plant originated in Central Asia and subsequently spread to Asia, Europe, and America. Today, it is found in almost all African countries, including

Togo.

Cannabis is a highly pollinated plant that can cross with several varieties. More than 400 chemical compounds have been identified in cannabis, with over 70 of them being phytocannabinoids or cannabinoids. These terpenophenolic compounds are largely responsible for

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the biological activities of the plant (ElSohly and Slade, 2005).

Δ^9 -tetrahydrocannabinol (Δ^9 -THC), commonly known as THC, is the primary compound that exhibits biological and psychoactive activities of the plant. It has the highest psychoactivity and is responsible for euphoria and altered sensory perception. THC also has analgesic, anti-inflammatory, appetite stimulating, antiemetic, and antispasmodic properties. Another major cannabinoid with great therapeutic potential is cannabidiol (CBD). It is a non-psychoactive isomer of THC, with anti-inflammatory, anticonvulsant, anxiolytic, analgesic, neuroprotective, anticancer, and antioxidant properties (Rodriguez-Almaraz and Butowski, 2023; Weiss et al., 2023).

There are two primary groups of *C. sativa* varieties: those with THC as the primary molecule, known as "marijuana," and those with CBD as the primary compound, known as "hemp". Chemical analysis is critical to classify a *C. sativa* variety. Marijuana is typically prohibited and used for recreational purposes, while hemp is used for therapeutic purposes. Chronic THC use has been associated with serious side effects, including tachycardia, cognitive deficits, anxiety, paranoia, chronic psychosis, and drug dependence (Favetta, 2023; Hasan, 2023). Studies suggest that CBD has a modulating effect on the psychoactivity of THC, thereby mitigating its adverse psychological effects (Cunha et al., 1980; Machado et al., 2011; Pintori et al., 2023). It is, therefore, essential to understand the composition of these two molecules in the cannabis consumed in a country.

In recent years, there has been growing interest in the therapeutic effects of CBD, and efforts have been made worldwide to breed cannabis varieties that are rich in this molecule. Nowadays, plants that contain up to 25% total CBD and less than 1% total THC are being selected (Chandra et al., 2019; Karthik et al., 2014). Once the chemical composition has been certified to confirm a low THC content, some countries allow the marketing of cannabis. The legal status of cannabis varies considerably from country to country. For example, in the Netherlands, all varieties of cannabis are marketable. In Italy, the cultivation and sale of non-psychoactive fiber-type varieties of hemp with total THC levels below 0.2% are permitted (Mirošničenko, 2019). Similarly, the states of Washington and Colorado in the US have legalized marijuana for recreational use, and several other states have legalized it for medical use. If the sale of marijuana is allowed for recreational and medicinal purposes, nearly \$9 billion could be generated for the United States (Evans, 2013). Developing countries could benefit from this financial windfall if they develop skills in chemical analysis of these plants.

A recent study conducted in Togo revealed that around 10% of motorbike taxi drivers in the country regularly use cannabis (Salifou et al., 2021; Soedje et al., 2022).

Additionally, a report published by the Ministry of Security indicated that nearly 20% of young people

between the ages of 12 and 24 have used cannabis at least once. It is worth noting that despite being illegal, cannabis is the most commonly consumed drug in Togo, accounting for 95.6% of all drugs used in the country (Ekouevi et al., 2013). Despite the high prevalence of cannabis use in Togo, there is a lack of available data on the chemical composition of *C. sativa* consumed in Togo. The primary objective of this study is to contribute to the understanding the detailed cannabinoids composition of consumed cannabis in Togo. This will be achieved by analyzing cannabinoids levels in collected samples from different parts of the country using gas chromatography-mass spectrometry (GC-MS) and two extraction methods.

MATERIALS AND METHODS

Chemicals and instruments

Hexane used for sample extraction was of high-performance liquid chromatography grade. BRANSON 2510 sonicator (New York, USA), a BUCHI rotary evaporator system (Flawil, Swiss), a precision balance and an Agilent Technologies 7890B GC-MS apparatus were used for extraction assays and chemical composition determination.

Plant material

As the sale and consumption of all cannabis derivatives are prohibited in Togo, we had to obtain authorization from the Ministry of Security to carry out this work. The plant material used in this study comprised of cannabis samples purchased from distributors in 13 localities where consumption is prevalent in Togo (Table 1). The cannabis samples were used as purchased, without separating the leaves from the flowers, to be as close as possible to consumers' intake. All samples were authenticated and identified at the Laboratory of Botanic and Vegetal Ecology, Department of Botany, Faculty of Sciences, University of Lomé (FDS, UL).

Plants solvent extraction

Extraction of cannabinoids was done using two methods: maceration and ultrasonic assisted extraction. All collected cannabis samples were grounded into powder using a mortar and pestle before use. The yields of bioactive compounds were determined in relation to the dry mass of the extracts. Every cannabis sample was extracted in triplicate.

Maceration

A mass $m_0 = 1\text{g}$ of the powder is placed in an Erlenmeyer flask to which 10 mL of hexane is added. The mixture was shaken for 5 min and then left to sit for three days (72 h). Afterward, the mixture is filtered using a glass funnel fitted with a sintered filter and filter paper. The filtrate is concentrated to dryness using a rotary evaporator, yielding a sticky resin, which is stored at -4°C for further analysis.

Ultrasonic assisted extraction (Sonication)

An ultrasonic bath with 100 W ultrasonic power, 63 W heating

Table 1. The sampling locations of cannabis and their respective regions of origin. These regions are identified as the areas with the highest consumption of cannabis in Togo.

Regions	Population	Locations (city or neighbourhood)
Maritime	3,258,300	Ahanou Kope, Nukafou, Agoè Kididjan, Agoè deux Lions, Agoè Zongo, Gbossime, Totsi, Tsévié Boloumodji, Tsévié Hetchavie
Plateaux	1,705,300	Atakpamé
Kara	957,600	Kara Ville and Kara Zongo

power, and 42 kHz operating frequency was used for the experiments. The cannabis powder samples (1 g) were weighed and introduced into 10 mL of hexane, sonicated for 30 min at 45°C, decanted, and filtered through a glass funnel sintered with cotton and Whatman N°1 filter paper. The filtrates were concentrated to dryness using a rotary evaporator to give a sticky resin, which was stored at -4°C until analysis.

Gas-chromatography-mass spectrometry analysis

For GC-MS analysis, extracts obtained were solubilized in hexane to a stock concentration of 10 mg/mL. Working solutions of each sample were prepared at a concentration of 50 mg/L from the stock solutions by dilution with hexane and immediately analysed by GC-MS. Samples were analysed in triplicate and a total of 78 chromatograms were obtained.

A gas chromatograph (Agilent Technologies 7890B) equipped with a mass selective detector (Agilent Technologies 5977B) was utilized for the analysis with a 30 m × 0.25 mm × 0.25 µm column containing 5% phenylmethylpolysiloxane (HP-5MS). The injection was performed in splitless mode, and a sample volume of 1 µL was injected. The oven temperature was set to 100°C for 1 min, followed by an increase of 10°C/min until 260°C, which was held for 8 min giving a total analysis time of 24 min. Helium gas (99.999% purity) was used as the carrier gas at a flow rate of 1 mL/min. The injector, transfer line, and ion source temperatures were set at 280, 250, and 250°C, respectively, while the quadrupole was maintained at 150°C. Ions produced at 70 eV were recorded at 1.6 scan/s in full scan mode with a mass range of 50-500 m/z.

Cannabinoids identification

The GC-MS data were analysed by integrating each individual peak from the resolved chromatogram and normalizing the area with respect to the corrected total area of the chromatogram. Areas of the compounds were normalized to 100, and the percentages of the identified compounds were calculated. The peaks were then scrutinized for their mass spectra, and subsequently identified using the NIST08 library following deconvolution with an automated mass spectral deconvolution and identification system (AMDIS).

Statistical analyses

Results from solvent extraction yields and chromatographic areas were processed using GraphPad Prism 8 software and expressed as mean ± SEM (Standard Error Mean). Differences were considered significant at the 5% level ($p < 0.05$).

RESULTS

In this study, 13 cannabis samples were collected from

various consumption locations across Togo to investigate their chemical composition. Two solvent extraction techniques, utilizing hexane, to obtain the phytocannabinoid compounds: Traditional maceration and ultrasound-assisted extraction. The analysis of extracts was conducted using a 24 min gas-chromatographic method with a mass detector.

The yields obtained through ultrasound-assisted extraction, which lasted for only 30 min, was significantly different when compared to the 3 days required for maceration ($p < 0.0001$). The yields ranged from $3.2 \pm 0.4\%$ to $6.6 \pm 0.1\%$ for maceration and from $3.9 \pm 1.2\%$ to $6.5 \pm 0.2\%$ for ultrasound-assisted extraction across the different samples.

Utilizing a 24 min analytical method, cannabinoids were successfully separated from extracts. Figure 1 illustrates the chromatogram from cannabis plant parts under investigation. A total of 13 cannabinoids were identifiable in the analyzed samples (as shown in Table 2), with the prominent compounds comprising tetrahydrocannabinol (Δ^9 -THC), cannabidiol (CBD), and cannabinol (CBN) (as illustrated in Figure 2). However, the cannabinoid precursors such as THC (tetrahydrocannabinolic acid (Δ^9 -THCA)) and CBD (cannabidiolic acid (CBDA)), respectively, were not detected. Upon normalizing the chromatographic areas to 100 for the different constituents, the relative percentages of THC and CBD in each extract were determined (Figures 3 and 4). The levels of cannabinoids exhibited variation based on the sample collection locality and the extraction method. For maceration, the THC level ranged from 37.73 ± 0.21 to $87.90 \pm 1.30\%$, while for ultrasound, it varied from 39.09 ± 2.13 to $84.54 \pm 0.80\%$.

Furthermore, there was a significant difference in the THC percentages between the two extraction methods (Figure 3A) ($P < 0.0001$). On average, the highest total amount of THC was obtained with maceration (74.03%), compared to 71.10% with sonication. Upon sample analysis, it became evident that the concentration of CBD was significantly lower in comparison to THC (Figure 4). However, the CBD level varied based on the extraction methods, ranging from $1.36 \pm 0.27\%$ to $4.07 \pm 0.23\%$ for maceration and from 1.79 ± 0.12 to $5.06 \pm 0.89\%$ for ultrasound. Furthermore, a notable disparity in the levels of the two extraction methods was observed ($P < 0.0001$).

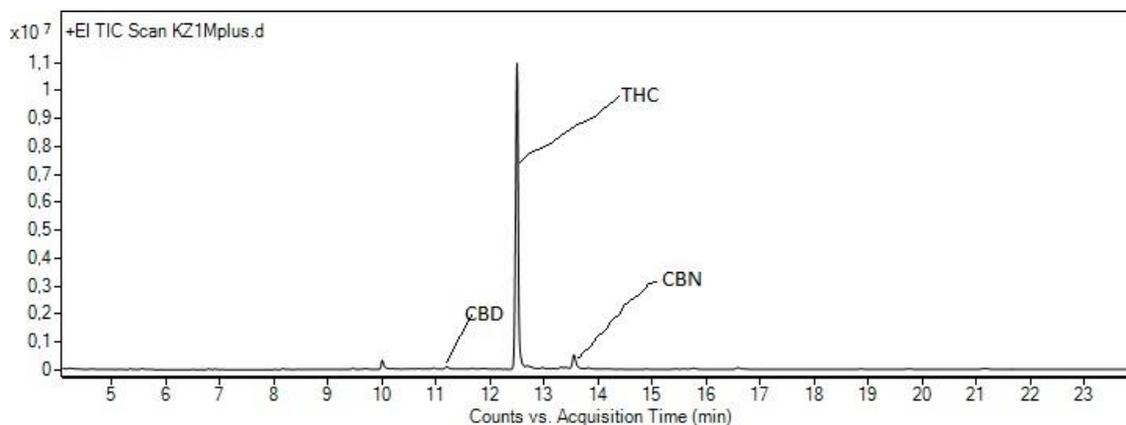


Figure 1. Gas-chromatography mass spectrometry (GC-MS) chromatogram of a *C. sativa* cannabinoids extraction from a locality of Togo. This chromatogram shows that THC is largely the most abundant compound in analysed cannabis samples.

Table 2. Cannabinoids identified in all the samples in order of retention time.

No.	Retention time (min)	Compound name
1	9.691	DELTA.8-Tetrahydrocannabinol
2	10.006	Tetrahydrocannabivarin
3	10.211	Cannabichromene
4	10.532	Cannabispiran
5	10.673	Cannabivarin
6	11.178	Cannabidiol (CBD)
7	11.673	Cannabicumaronone
8	12.527	DELTA.9-Tetrahydrocannabinol (THC)
9	13.282	Methoxy-THC
10	13.31	Cannabigerol
11	13.567	Cannabinol
12	15.091	Hydroxy- δ 9-tetrahydrocannabinol
13	15.575	Homotetrahydrocannabinol

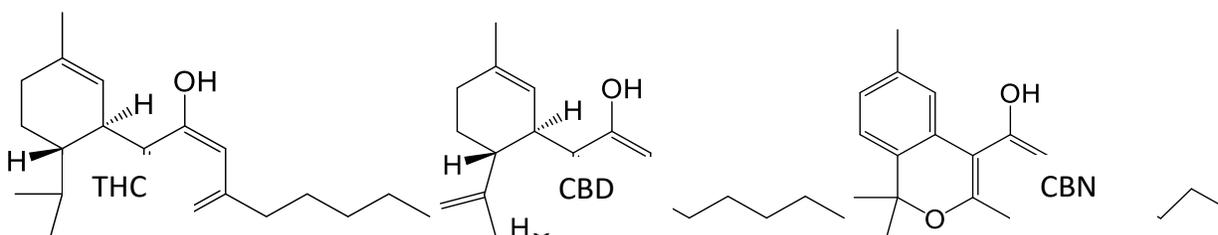


Figure 2. Chemical structures of tetrahydrocannabinol (Δ^9 -THC), cannabidiol (CBD) and cannabinol (CBN).

DISCUSSION

The high extraction speed during ultrasonication can be attributed to the mechanical effect. This phenomenon

occurs when cavitation bubbles collapse on the surface of the plant material, resulting in the breakdown of cell walls, thereby enhancing mass transfer and increasing the contact area between the solvent and the material

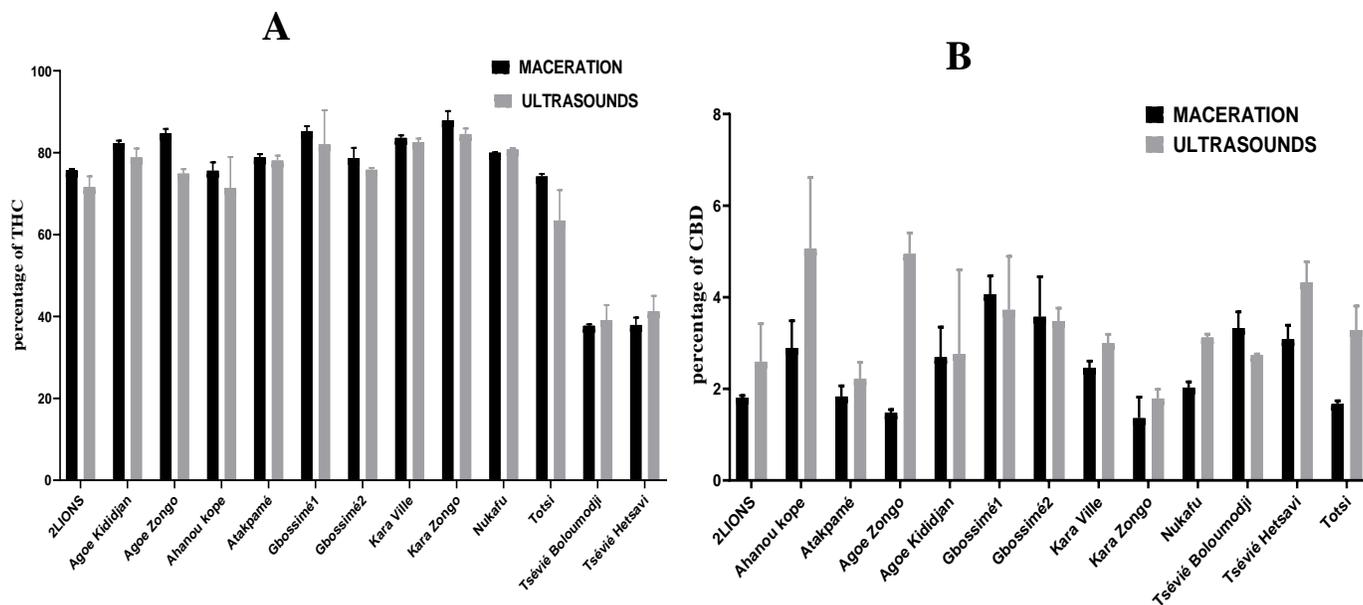


Figure 3. Comparison of THC (A) and CBD (B) contents of all samples for both extraction methods. Values are expressed as mean \pm SD (n=3).

(Asghari et al., 2011; Dranca and Oroian, 2016). Consequently, time can be saved by utilizing sonication in the extraction process.

The analysis revealed the absence of Δ^9 -THCA and CBDA in the extracts. Typically, in *Cannabis sativa* plants, most phytocannabinoids, such as Δ^9 -THC and CBD, exist in the form of their acidic precursors (Δ^9 -THCA and CBDA). When exposed to high temperatures, these acidic precursors undergo decarboxylation to form Δ^9 -THC and CBD. The absence of these acids in the samples may be attributed to the heating process in the GC-MS injector and oven during analysis (Dussy et al., 2005). With the high levels of THC observed, it can be concluded that all the cannabis samples consumed in Togo exhibit the characteristics of recreational or marijuana-type cannabis, primarily due to the elevated THC levels in the samples (Figure 4) (Spitzer-Rimon et al., 2019).

Prior studies have compared four extraction methods, including maceration, sonication, Soxhlet, and supercritical solvent extraction, and reported that maceration extracts more THC than sonication (Rožanc et al., 2021). The disparity in THC levels between the two extraction methods is likely due to the varying extraction times, with maceration requiring 72 h and ultrasound-assisted extraction only taking 30 min. Our results emphasize that maceration over several days should be the preferred method for extracting THC when targeting this compound.

On the average, ultrasound-assisted extraction yielded a higher CBD content compared to maceration, with values of 3.31 and 2.48%, respectively. The variation in

CBD levels between the two methods may be attributed to the increased temperature, up to 45°C. It has been reported (De Vita et al., 2020) that CBD content increases with temperature and reaches an optimum at 65°C. Additionally, the choice of extraction method can influence the amount of CBD extracted due to the affinity of the CBD molecule for specific extraction techniques (Azmir et al., 2013; Ötles and Kartal, 2016).

Our findings align with previous studies that have reported ultrasound as the most effective method for CBD extraction compared to microwave-assisted extraction and maceration (De Vita et al., 2020). However, it is noteworthy that other studies have reported maceration to be more efficient than ultrasound in extracting CBD (Rožanc et al., 2021). This discrepancy may be attributed to differences in the solvents employed in the respective studies, with Rožanc et al. using methanol, while hexane was utilized as the solvent in this study. Given CBD diverse therapeutic effects, including neuroprotection, antiepileptic properties, anxiolysis, antipsychotic effects, anti-inflammatory properties, analgesia, and anticancer activity, our research contributes valuable insights into an efficient extraction method for this molecule.

The THC levels obtained in all extracts, as illustrated in Figure 4, exceeded those of CBD in both maceration and ultrasound-assisted extraction. These THC levels surpassed the legal limits for therapeutic use, which are set at 0.2, 0.3, and 1% for the EU, Canada, and Switzerland, respectively. A previous study on recreational cannabis with red crosses found THC levels ranging from 85.1% to 86.4% (Leos-Toro et al., 2020).

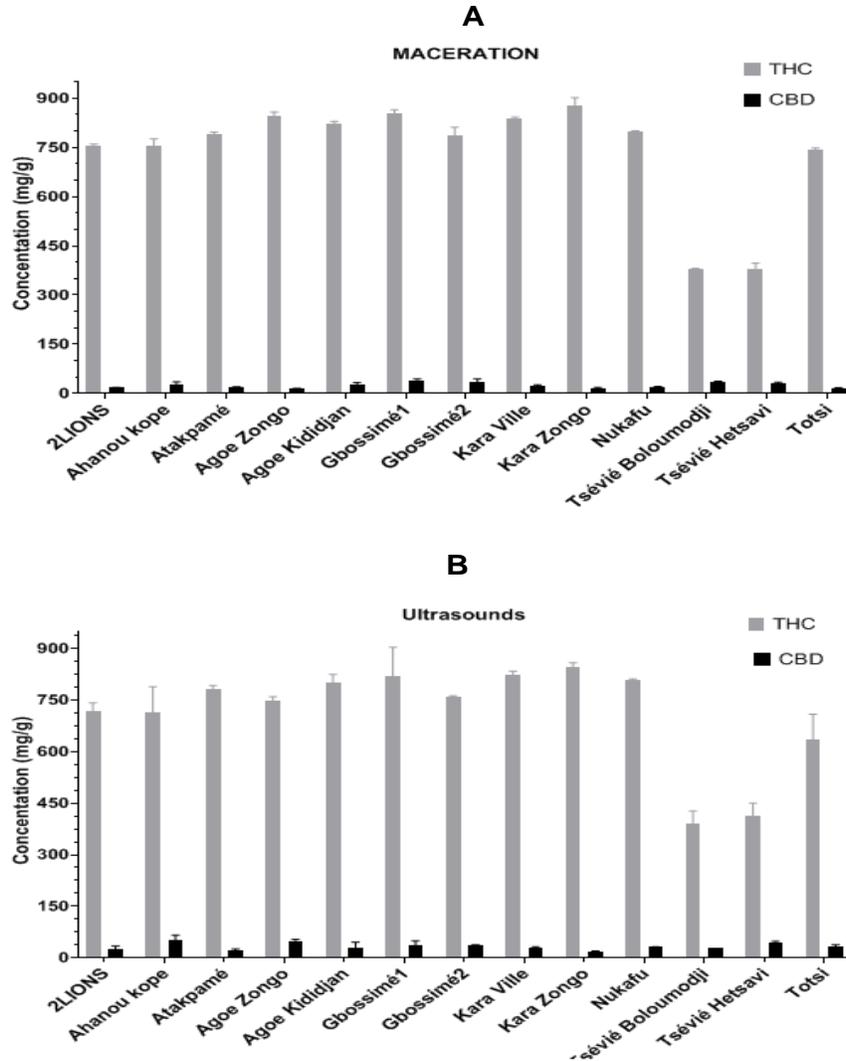


Figure 4. THC (A) and CBD (B) levels in samples for two extraction methods: Maceration and Ultrasound extraction. Values are expressed as mean \pm SD (n=3).

Additionally, the Canadian Drug Committee reported that the use of apolar solvents such as hexane can result in THC levels as high as 90% in some cannabis varieties (Fischer et al., 2021). Studies by Chandra on the evolution of THC levels in cannabis in the US and Europe from 2008 to 2017 identified THC levels ranging from 73 to 83% in samples provided by the UK police (Chandra et al., 2017). This research uncovered similar THC levels, indicating that illegal consumers of cannabis in Togo are exposed to the same high doses of the psychotropic compound. To the best of our knowledge, this study represents the first report of its kind from a West African country.

The disparity in THC levels among the samples was statistically significant ($P < 0.0001$) and can be attributed to the origin of each sample and the type of cannabis variety consumed in each area. The elevated THC levels

found in all analyzed cannabis samples raise concerns, as there is a well-established positive association between high THC levels and adverse health effects. Previous studies have demonstrated that THC can induce psychotic-like symptoms in healthy volunteers, and these effects are dose-dependent, suggesting that products with higher concentrations of THC may pose a greater health risk for consumers (Morrison et al., 2009). THC acts as a non-selective partial agonist of both CB1 and CB2 receptors, leading to a range of effects, including appetite stimulation, analgesia, and neural inhibition, when bound to CB1 (Bhattacharyya et al., 2017). In a study involving adult males with low cannabis use, an oral dose of 15 mg THC was found to impair episodic memory and increase error rates in learning 2 h after administration (Curran et al., 2002). Based on the results of this study, it can be estimated that as little as

0.77 and 0.26 g of samples from Tsévié Boloumodji and Kara Zongo, respectively, could contain a dangerous equivalent amount of 15 mg of THC. Another study demonstrated that a single oral dose of 10 mg THC in healthy volunteers was associated with symptoms such as anxiety, dysphoria, positive psychotic symptoms, physical and mental sedation, intoxication, and an increased heart rate 2 h after administration (Martin-Santos et al., 2012). In this context, only 0.58 and 0.17 g of samples from Tsévié Boloumodji and Kara Zongo, respectively, could induce these effects in consumers.

Cannabis is frequently linked to traffic accidents, including fatal ones (Stoecker et al., 2018). THC impairs learning and information acquisition, reduces attention, and increases reaction time, all of which can have negative impacts on driving (Khiabani et al., 2006). Controlled driving simulation studies have demonstrated a relationship between THC blood concentration and driving performance (Lenné et al., 2010). THC blood levels ranging from 2-5 ng/mL were associated with substantial impairment of driving performance (Hartman and Huestis, 2013) and an increased risk of accidents. Considering that approximately 10% of motorbike taxi drivers in Togo use cannabis (Salifou et al., 2021) and cannabis use has been identified as one of the contributing factors to the high number of recorded accident cases (7392 in 2021, with 680 deaths, 68% of which were caused by motorcycles), it is imperative to address the issue of cannabis use and driving in Togo. This national analysis, being the first of its kind on the illegal cannabis chemical composition, could serve as a valuable starting point for decision-making authorities.

Conclusion

The results of the present study showed that three-day maceration in solvents is better for extracting THC, while sonication promotes CBD extraction. Furthermore, it was demonstrated that cannabis consumed in Togo harbors alarmingly elevated levels of THC, which heightened the associated risks of exposure to this compound. This report presents a simple and reproducible gas chromatography-mass spectrometry analysis of cannabinoids and could contribute to a better control of the consumption of *C. sativa* L. in West Africa after a study with larger samples.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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