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Original Article

Phytoconstituents Determination of *Cannabis sativa* Plant Extracts

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ABSTRACT

Background: *Cannabis sativa* has garnered significant attention due to its complex phytochemistry and diverse pharmacological properties. Given the rising interest in medicinal cannabis, understanding its physicochemical properties is crucial for drug development, quality control, and abuse prevention. This study aimed to analyze its phytoconstituents, with a focus on the isolation and profiling of cannabinoids.

Methods: Fresh *C. sativa* leaves were macerated in ethanol to obtain a crude extract, which underwent phytochemical screening to detect alkaloids, flavonoids, cardiac glycosides, terpenes, steroids, and resins. Thin-layer chromatography (TLC) was performed using a *n*-hexane/diethyl ether (8:2) ratio as the mobile phase to separate cannabinoids, with visualization under UV light. Column chromatography further purified the extract, and subsequent TLC confirmed cannabinoid-rich fractions. Hydrogen peroxide-modified TLC was employed to assess oxidation effects on cannabinoid stability.

Results: Phytochemical screening confirmed the presence of alkaloids, flavonoids, terpenes, and resins, while saponins and tannins were absent. TLC analysis revealed distinct Rf values for tetrahydrocannabinol (THC = 0.94) and cannabidiol (CBD = 0.90), with color differentiation indicating successful separation and a more polar nature of CBD. Column chromatography yielded enriched fractions, validated by TLC. Hydrogen peroxide exposure altered Rf values of 0.78 to 0.8, suggesting oxidative degradation. The study identified THC and CBD as dominant markers, alongside minor cannabinoids, reinforcing *C. sativa's* complex chemical profile. These findings give clues to further research into standardized extraction protocols and stability testing to optimize medicinal applications of the plant and its phytoconstituents.

Conclusions: This study highlights the efficacy of TLC and column chromatography for cannabinoid isolation and profiling. The presence of THC, CBD, and other bioactive compounds underscores *C. sativa's* dual therapeutic and psychoactive potential.

Key words: *Cannabis sativa*, cannabinoid, thin-layer chromatography, phytoconstituents, oxidative stability

INTRODUCTION

Cannabis sativa (Cannabis), commonly referred to as marijuana (**Figure 1**), is a psychoactive plant-derived substance with both medicinal and recreational applications. [1–3] Traditional medical uses include alleviating chemotherapy-induced nausea and vomiting, managing chronic pain, muscle spasms, arthritis, and migraines, as well as

Figure 1: Leaves of *Cannabis sativa* (marijuana).

stimulating appetite in HIV/AIDS patients and treating severe epilepsy and multiple sclerosis. [4–6]

However, cannabis use carries potential health risks. Acute effects include anxiety, panic attacks, cognitive impairment, and increased accident susceptibility. Δ9-Tetrahydrocannabinol (THC) (**Figure 2A**) is frequently detected in impaired drivers. [7,8] While emergency cannabis-related presentations often involve polydrug use, cannabis alone can precipitate adverse reactions. The practice of adulterating cannabis with opioids such as heroin or fentanyl exacerbates psychoactive effects and overdose potential. [9,10] Chronic, heavy use has been associated with detrimental impacts on multiple organ systems, including the liver, lungs, and cardiovascular system, particularly in individuals with pre-existing conditions like hepatitis C. [11] Prenatal cannabis exposure has been correlated with neurodevelopmental consequences in offspring, including increased risks of depression, hyperactivity, and attention deficits. [12,13] Respiratory complications such as chronic bronchitis are also reported among long-term smokers, as well as transient elevation in myocardial infarction. [14,15]

Previous chemical analyses have identified 483 distinct compounds in cannabis, including at least 65 cannabinoids. [16,17] THC (primary psychoactive constituent) interacts with central nervous system receptors involved in pain modulation, memory formation, and sleep regulation. [18,19] Other significant phytocannabinoids include cannabidiol (CBD; **Figure 2B**), cannabinol (CBN) (**Figure 2C**), β-caryophyllene (**Figure 2D**), and cannabigerol (CBG; **Figure 2E**). [20,21] THC and CBD serve as key analytical markers, with THC mediating psychotropic effects and CBD, anxiolytic and sedative properties. [22] Administration methods encompass inhalation (smoking/vaporization), oral ingestion, and concentrated extracts. [23]

CBD, a predominant phytocannabinoid in medicinal cannabis, exhibits broad therapeutic applications in seizure suppression, anti-inflammation, anxiety reduction, and antiemetic effects. Emerging evidence suggests additional promise in managing schizophrenia and various neurological conditions. [24–26] CBN, formed through THC degradation, possesses modest psychoactive properties with therapeutic potential for sleep induction, anticonvulsant, and nausea mitigation. [27,28] The sesquiterpene β-caryophyllene mediates anti-inflammatory responses through selective CB2 receptor activation, a mechanism distinct from classical cannabinoids. [29] CBG,

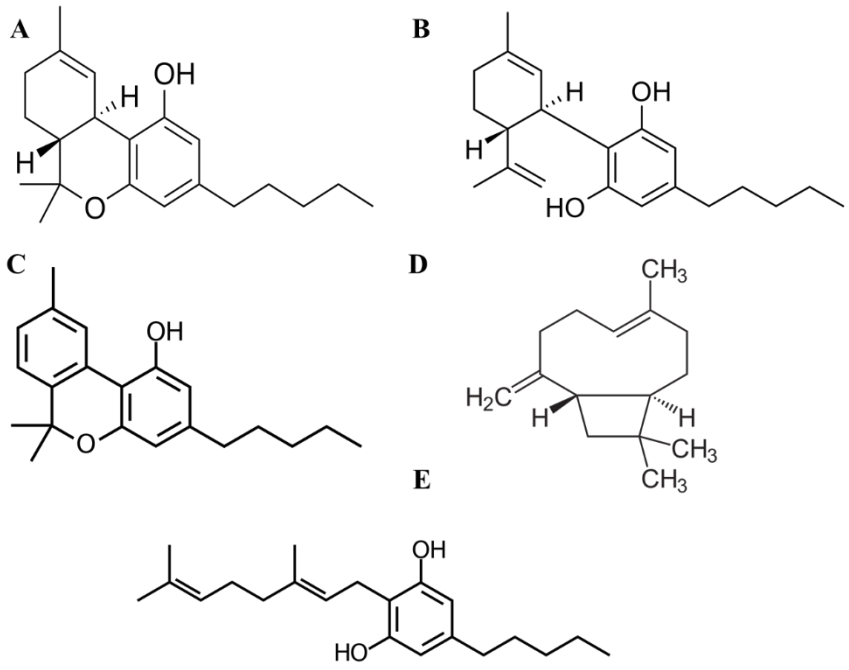

Figure 2: Chemical structures of (A) tetrahydrocannabinol, (B) cannabidiol, (C) cannabinol, (D) *beta*-caryophyllene, (E) cannabigerol.

while devoid of psychoactivity, shows particular promise in ocular therapeutics, with intraocular pressure-lowering effects. [30,31]

Thin-layer chromatography (TLC) is a fundamental analytical method that exploits differential affinities of compounds between a stationary phase and a mobile phase to achieve separation. [32–34] The TLC technique offers exceptional versatility, enabling simultaneous analysis of multiple samples on a single plate, which proves particularly valuable for screening purposes, including drug quantification and water quality assessment. The use of fresh stationary phase for each analysis minimizes cross-contamination risks. TLC demonstrates superior performance compared to paper chromatography, providing faster separation times, enhanced resolution, improved quantitative capabilities, and flexibility in adsorbent selection. [35–38] Complementing this, column chromatography employs a tubular system containing either a solid stationary phase or a liquid-coated support. In packed column configurations, the stationary material occupies the entire column volume, whereas open tubular columns feature a stationary phase lining the inner walls, creating an unobstructed central channel for mobile phase flow. [39] Component separation occurs based on differential migration rates, with retention times serving as key analytical parameters. The present study focuses on characterizing cannabinoid profiles in *C. sativa* plants while developing physicochemical approaches to quantify THC concentrations through targeted conversion into alternative cannabinoid forms. This current study aimed to analyze its phytoconstituents, focusing on cannabinoid isolation and profiling.

MATERIALS AND METHODS

Reagents

All solvents used in the experiment: ethanol, *n*-hexane, diethyl ether, conc H_2SO_4, and hydrogen peroxide were obtained from JHD Chemical Industry. All reagents were of analytical grade.

Plant collection

A sample of *C. sativa* was collected from Professor H. Obianwu of the Department of Pharmacology and Therapeutics, Faculty of Basic Medical Science, Niger Delta University. It was identified and authenticated by Professor K. Ajibesin of the Department of Pharmacognosy and Herbal Medicinal, Niger Delta University, Bayelsa state, where a voucher specimen was deposited for future reference, with herbarium number: G152412. The study was approved by the research and ethics committee of the Faculty of Pharmacy, Niger Delta University, Nigeria.

Plant extraction: Maceration

The study was designed as a quantitative validation analysis and conducted between 2022 and 2024 at Amassoma, Niger Delta University. The collected plant material underwent drying and pulverization to produce a fine powder. A measured quantity of 70 g of this powdered material was placed in an appropriate container. The material was then subjected to ethanol extraction by adding 700 mL of ethanol solvent and allowing the mixture to macerate for 72 hours (3 days) with periodic agitation. Following maceration, the mixture was filtered at ambient temperature and pressure using standard filtration apparatus. Approximately 600 mL of the resulting filtrate was subsequently chilled overnight to facilitate lipid separation, followed by cold filtration. The filtrate was then concentrated to approximately 100 mL using a water bath. The final ethanolic extract was stored at room temperature for subsequent analytical procedures. Phytochemicals were determined following standard protocols (**Table 1**). [40]

TLC and column chromatographic analysis

A mobile phase consisting of *n*-hexane/diethyl ether (8:2 v/v) was prepared by mixing 15 mL of the solvents for the preliminary TLC analysis of the *C. sativa* crude extract. Uniform capillary tubes were used to apply sample spots onto the marked TLC plates, which were then air-dried for 10 minutes. The plates were carefully immersed in the solvent tank, ensuring the mobile phase contacted the base of the plates for chromatographic development. Following development, the plates were removed using forceps, air-dried in a vertical position, and examined under UV light. For enhanced visualization, the plates were sprayed with concentrated hydrochloric acid and heated at 110°C for 15 minutes in an oven to develop characteristic bands.

For Column Chromatography, the ethanol fraction (5.0 g dry weight) was dissolved in *n*-hexane and uniformly mixed with silica gel (60-120 mesh) to form a homogeneous slurry, which was then dried. The chromatographic column was packed with silica gel to approximately three-quarters of its height using *n*-hexane as the packing solvent, after which the prepared sample mixture was carefully loaded onto the column. Elution was first performed with 200 mL of pure *n*-hexane to collect the non-polar fraction, followed by 200 mL of *n*-hexane/diethyl ether (8:2 v/v) for the moderately polar fraction. The *n*-hexane eluate was left to evaporate under ambient laboratory conditions for 24 hours. Based on preliminary TLC analysis, fractions 1 to 4 exhibiting similar migration patterns were pooled into three distinct combined fractions, which were subsequently re-chromatographed using the same column conditions. Throughout the process, all eluates were collected in separate beakers and analyzed by TLC to assess separation efficiency and fraction purity.

The extract was also subjected to gas chromatography-mass spectrometric analysis using GC-MS-QP2010SE Shimadzu, Japan, which gave different isolates.

Statistical analysis

All statistical analyses were performed, including simple calculations of frequency, Rf values, and percentages, with Microsoft Excel version 2019, utilizing one-way ANOVA. Colors were detected using UV light and iodine stain, as well as visual inspection.

RESULTS

The current study isolated important phytoconstituents from *C. sativa*, a very popular medicinal plant used in various medicinal and recreational purposes globally. The crude extract was subjected to TLC analysis using different solvent systems and compared with standard THC, CBD, CBN, and CBN oil (**Table 2**).

Table 1: Phytochemical analysis of crude extract.

Test	Procedure
Alkaloids	To 2 mL of petroleum ether, crude leaf extract of cannabis in a test tube, a few drops of Dragendorff's reagent were added. The mixture was observed for a color change, typically from orange to deep orange.
Saponin	10 mL of distilled water was mixed with 2 mL of the petroleum ether crude leaf extract of cannabis in a test tube. The solution was shaken vigorously for 1 minute and left to stand for 30 seconds. Then, three drops of olive oil were added, and the mixture was observed for a dark brown coloration.
Tannins (ferric chloride test)	Tannins were detected by adding three drops of 10% ferric chloride ($FeCl_2$) to 2 mL of the petroleum ether crude leaf extract of cannabis diluted with 4 mL of distilled water. The mixture was observed for reddish precipitate formation.
Flavonoid (lead acetate test)	To test for flavonoids, 2 mL of 10% lead acetate solution was added to 2 mL of the petroleum ether crude leaf extract of cannabis in a test tube. After standing for 10 seconds, the mixture was observed for a yellowish coloration and precipitate formation.
Cardiac glucosides (Keller-Killiani test)	Cardiac glycosides were identified by adding 2 mL of glacial acetic acid and 1 drop of ferric chloride ($FeCl_2$) to 1 mL of the petroleum ether crude leaf extract of cannabis. Then, 1 mL of concentrated sulfuric acid (H_2SO_4) was carefully introduced along the test tube wall, and the formation of a brown ring at the interface was observed.
Terpenes and steroids (Burchard test)	Terpenes and steroids were tested by adding 1 mL of anhydrous acetic acid to 2 mL of the petroleum ether crude leaf extract of cannabis. Concentrated sulfuric acid (H_2SO_4) was carefully added down the test tube side, and a reddish color change with interface formation was observed.
Balsams	Balsams were detected by mixing 2 mL of alcoholic ferric chloride with 2 mL of the petroleum ether crude leaf extract of cannabis. The mixture was briefly warmed over a Bunsen flame for 5 seconds and observed for a reddish-brown color change.
Volatile oils	Volatile oils were identified by adding 0.1 mL of dilute sodium hydroxide (NaOH), followed by 0.5 mL of hydrochloric acid (HCl) to 2 mL of the petroleum ether crude leaf extract of cannabis. After standing for 5 seconds, a light blue color change and precipitate formation were observed.
Resins	Resins were tested by adding 2 mL of acetic anhydride to 2 mL of the petroleum ether crude leaf extract of cannabis. Three drops of concentrated sulfuric acid (H_2SO_4) were carefully added, and a violet color change was observed.

Table 2: Rf values of *n*-hexane/diethyl ether (8:2) and hydrogen peroxide solvent systems.

Spots	Sample distance (cm)	Solvent distance (cm)	Rf value	Color observed
n-Hexane/diethyl ether solvent system				
Ref sample (THC)	7.6	8.1	0.94	Pink
Ref sample (CBD)	7.6	8.1	0.90	Yellow
Ref sample (C. oil)	7.8	8.1	0.96	Light brown
Ref sample (CBN)	7.6	8.1	0.95	Light pink
Crude extract 1	7.6	8.1	0.94	Pink
Crude extract 2	7.9	8.1	0.98	Yellow
Hydrogen peroxide solvent				
Crude extract (A1)	7.0	9.0	0.78	Faint pink
Crude extract (A2)	7.0	9.0	0.78	Faint pink
Crude extract (1A)	7.2	9.0	0.80	Brown
Crude extract (1B)	7.2	9.0	0.80	Brown

Rf: distance traveled by the compound(sample)/distance traveled by the solvent.

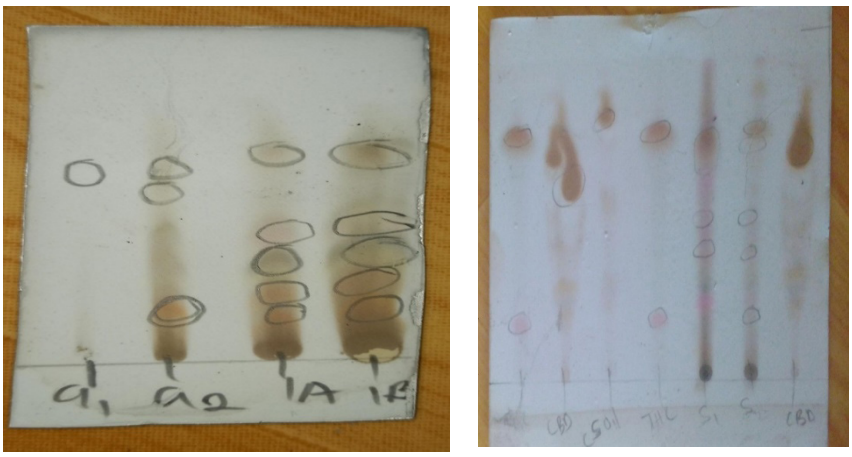

Figure 3: Thin-layer chromatography analysis of different *C. sativa* fractions.

The distance travelled by each sample was visually observed after being subjected to a UV lamp or iodine spray, ranging from pink, yellow, brown, and so on (**Figure 3**).

Following the TLC analysis, phytochemicals were isolated, and the presence of different phytoconstituents, including alkaloids, cardiac glycosides, terpenes, steroids, resins, and flavonoids, was observed based on their differential analysis (**Table 3**).

Furthermore, the extract was subjected to advanced Gas chromatography-mass spectrometry (GC-MS) analysis, and CBD and (B) tetrahydrocannabinol were quantified from the *C. sativa* sample (**Figure 4**).

DISCUSSION

TLC, using *n*-hexane/diethyl ether (8:2), effectively separated cannabinoids, with Rf values of 0.94 for THC and 0.90 for CBD. These Rf values of these cannabinoids suggest that THC is more non-polar, followed by CBD under the tested conditions, highlighting a limitation of TLC in distinguishing structurally similar compounds without additional visualization techniques such as UV or derivatization (**Table 2**). [41–44] The pink spot (THC) and yellow spot (CBD) align with their distinct chemical properties, while the light brown spot (Rf 0.96) may represent CBN, an oxidation product of THC (**Figure 3**).

Column chromatography further resolved the crude extract into distinct fractions, with pooled eluates showing consistent TLC profiles. This reproducibility supports the method's utility for preparative isolation, though the broad Rf range (0.90–0.98) in crude extracts indicates co-elution of non-target compounds. Hydrogen peroxide-based TLC yielded lower Rf values (0.78–0.8), likely due to oxidative degradation of cannabinoids, which underscores the instability of these compounds under harsh conditions (**Table 2**). This observation has practical implications for storage and processing, as oxidation could diminish therapeutic potency. [45,46]

The phytochemical screening of *C. sativa* extracts revealed a complex profile of bioactive compounds. [47] The presence of alkaloids (detected via Dragendorff's test) aligns with previous reports of nitrogen-containing secondary metabolites in cannabis, though their specific identities warrant further characterization. [48] Flavonoids, indicated by the lead acetate test, are known for their antioxidant and anti-inflammatory effects, which may contribute to the plant's neuroprotective properties. [49] The strong positive

Table 3: Phytochemical analysis of cannabis extract.

Phytochemicals	Reagents	Observation	Inference
Alkaloids	Dragendorff's	Orange	+++
Saponin	Distilled water and olive oil	Dark brown	–
Flavonoids	Lead acetate	Light yellow	++
Tannins	Ferric chloride	Reddish	–
Cardiac glycosides	Keller Killiani	Reddish brown	+++
Balsam	Ferric chloride	Reddish brown	–
Terpenes	Burchard	Reddish brown	+++
Steroids	Burchard	Reddish brown	+++
Resins	Acetic anhydride	Violet	+++
Volatile oils	Dilute sodium hydroxide and hydrochloric acid	Light blue	–

+++ = significantly present, ++ = moderate, – = absent.

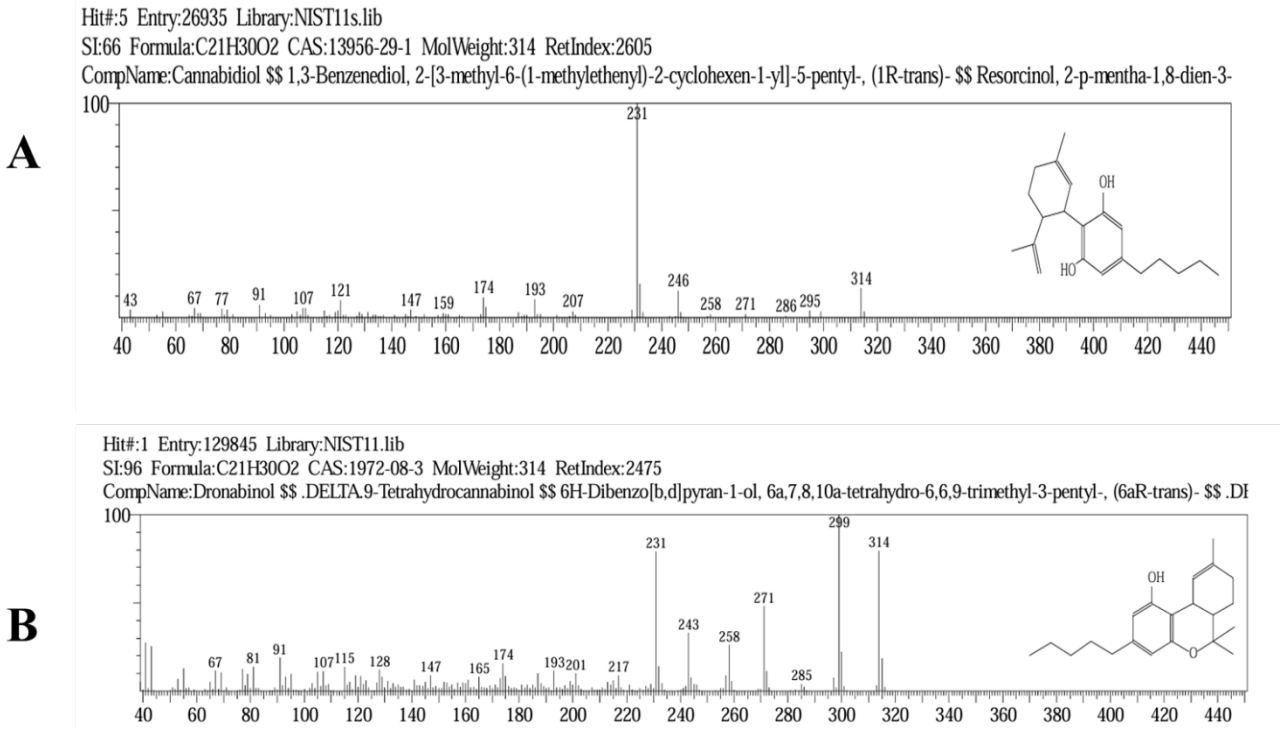

Figure 4: Gas chromatography-mass spectrometry (GC-MS) spectra of (A) cannabidiol (CBD) and (B) tetrahydrocannabinol (THC).

result for cardiac glycosides (Keller-Killiani test) is particularly intriguing, as these compounds are not typically emphasized in cannabis research (**Table 3**). [50–53] Their presence suggests potential cardiovascular effects that merit deeper investigation, especially given their historical use in treating heart conditions.

The Burchard test confirmed terpenes and steroids, supporting *C. sativa's* role in modulating the endocannabinoid system and producing anti-inflammatory effects. [54] β-caryophyllene, a prominent cannabis terpene, is a known CB2 receptor agonist, which could explain the observed reddish coloration in the test. [29,55] Resins, detected via acetic anhydride and sulfuric acid, are likely linked to the sticky trichomes of cannabis flowers, which are rich in cannabinoids and terpenes (**Table 3**). [56] The absence of saponins and tannins may reflect the extraction solvent's polarity (petroleum ether), which preferentially isolates non-polar compounds like THC and CBD over polar saponins or tannins.

While the study confirmed the presence of major cannabinoids, the detection of cardiac glycosides and the absence of volatile oils diverge from some literature. [57,58] For example, volatile oils such as limonene and pinene are often reported in steam-distilled extracts but may not partition into petroleum ether. [59] Similarly, the absence of tannins contrasts with studies using aqueous or alcoholic extracts, emphasizing the impact of solvent choice on phytochemical profiles.

The GC-MS spectrum of CBD, identified by its molecular formula $C_{21}H_{30}O_2$, a molecular weight of 314, and a retention index of 2605. Key fragment ions in the spectrum include m/z 43, 67, 77, 91, 107, 121, 147, 159, 174, 193, 207, 246, 258, 271, 286, 295, and the molecular ion peak at m/z 314. The structure of CBD consists of a resorcinol core with a pentyl side chain and a p-mentha-1,8-dien-3-yl substituent, contributing to its characteristic fragmentation pattern. While the GC-MS spectrum of THC shares the same molecular formula ($C_{21}H_{30}O_2$) and weight (314) as CBD, it has a lower retention index of 2475. The fragmentation pattern of THC is more complex, with a prominent cluster of peaks between m/z 230 and 260, including m/z 231, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, and 261 (**Figure 4**). This pattern is typical of THC's dibenzopyran (chromene) ring system, which includes a pentyl side chain and methyl groups. Both CBD and THC have identical molecular formulas but exhibit different mass spectral profiles due to their distinct structural features. CBD's spectrum shows simpler fragmentation with prominent lower m/z peaks, while THC's spectrum displays a more complex series of fragments in the higher m/z range, reflecting its rigid dibenzopyran core. The difference in retention indices (CBD: 2605, THC: 2475) suggests that CBD is less polar or interacts differently with the chromatographic stationary phase compared to THC. These spectral differences are crucial for analytical applications, such as differentiating between CBD and THC in cannabis testing, forensic analysis, or pharmaceutical quality control.

While the current study was able to quantitatively determine the presence of different significant phytochemicals in *C. sativa*, especially THC and CBD, the therapeutic utilization of these constituents was not assessed.

CONCLUSIONS

This study identified major phytoconstituents in *C. sativa*, validating THC and CBD as primary biomarkers. TLC and column chromatography proved effective for cannabinoid

separation, though oxidative conditions (hydrogen peroxide) may degrade compounds. The presence of terpenes, steroids, and resins underscores the plant's therapeutic potential, while variability in detected compounds warrants further optimization of extraction protocols. These findings contribute to the scientific basis for *C. sativa's* medicinal use and highlight the need for standardized analytical methods to ensure consistency in phytochemical profiling. Future research should explore the potential therapeutic applications and pharmacological mechanisms of lesser-studied constituents like cardiac glycosides and optimize oxidation-resistant isolation techniques.

AUTHORS' CONTRIBUTION

Each author has made a substantial contribution to the present work in one or more areas, including conception, study design, conduct, data collection, analysis, and interpretation. All authors have given final approval of the version to be published, agreed on the journal to which the article has been submitted, and agree to be accountable for all aspects of the work.

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None.

CONFLICT OF INTEREST

None.

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