



Chemotypic Variation of Nigerian *Cannabis sativa* Across Five Geopolitical Zones

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Abstract

Cannabis sativa L. remains Nigeria's most widely cultivated and consumed illicit drug, yet its chemical characterization has remained largely unexplored due to legislative restrictions. This study presents the first comprehensive pharmacognostic and chemotypic evaluation of *C. sativa* samples collected from five geopolitical zones of Nigeria through the National Drug Law Enforcement Agency (NDLEA). Samples from Kaduna (North West; farm-cultivated and trafficker-confiscated), Adamawa (North East), Edo (South South), Enugu (South East), and Ondo (South West) were subjected to comparative pharmacognostic analysis including macroscopic/microscopic evaluation, physicochemical standardization, phytochemical screening, and cannabinoid profiling using Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), and Gas Chromatography-Mass Spectrometry (GC-MS).

Results revealed significant chemotypic variation among regions while maintaining core pharmacognostic identity. Quantitative microscopy parameters remained within acceptable ranges (stomata index 16.98-18.09%; palisade ratio 6.15-7.1) with no statistically significant regional differentiation. However, physicochemical constants showed marked variation, with the Edo sample exhibiting consistently elevated values distinguishing it from others. Phytochemical screening demonstrated uniform presence of carbohydrates, cardiac glycosides, and phenols across all samples, while flavonoids, tannins, and anthraquinones showed variable distribution. Saponins were uniformly absent.

Cannabinoid profiling revealed the most striking chemotypic diversity. HPLC quantification using cannabidiol (CBD) reference standard showed CBD concentrations ranging from 17.41 mg/g (Ondo) to 30.54 mg/g (Edo). GC-MS confirmed the presence of Δ^9 -tetrahydrocannabinol (THC) in samples from multiple regions, with the Ondo sample exhibiting the most diverse profile containing CBD, cannabichromene (CBC), THC, and cannabinol (CBN). The trafficker-confiscated sample from Kaduna showed close phytochemical and chromatographic similarity to the Ondo sample, suggesting potential southern origin of cannabis trafficked to northern Nigeria. This study establishes baseline pharmacognostic data for Nigerian *C. sativa* and demonstrates that regional chemotypic variation can be exploited for forensic source attribution. The findings have significant implications for law enforcement, medicinal cannabis development, and evidence-based policy formulation in Nigeria's evolving drug control landscape.

Keywords: *Cannabis sativa*, chemotype, pharmacognosy, Nigeria, cannabinoids, geographic

INTRODUCTION

The genus *Cannabis* presents one of the most fascinating and controversial chapters in the history of medicinal plants. For over four millennia, *Cannabis sativa* L. has accompanied human civilization, serving as a source of fibre, food, medicine, and sacrament (Alamgir, 2017). The first recorded reference to cannabis consumption appears in the Chinese *Shennong pên Ts'ao ching* circa 2700 BC, where it was recommended for soothing pain (Elhendawy et al., 2018). From ancient Greek and Indian medical traditions to modern pharmacopoeias, cannabis has maintained its relevance as a therapeutic agent of remarkable versatility.

Yet, the Twentieth Century witnessed a dramatic reversal in cannabis's fortunes. The plant became ensnared in international drug control treaties, classified as a Schedule I narcotic with "no accepted medical use" and high abuse potential. This classification effectively criminalized cannabis research, creating vast knowledge gaps that persist to this day (Duvall, 2014; Chouvy, 2019). Nowhere is this knowledge deficit more pronounced than in Nigeria - a country identified by the United Nations Office on Drugs and Crime as one of Africa's largest cannabis producers and consumers (UNODC, 2022). The paradox could not be starker. Nigeria's National Drug Law Enforcement Agency (NDLEA) dedicates substantial resources to identifying and destroying cannabis farms, intercepting traffickers, and prosecuting offenders (Braj, 2023). The 2018 national drug use survey estimated that 10.6% of Nigerian adults - approximately 10.6 million people—had used cannabis in the preceding year (UNODC, 2019). Cannabis cultivation has been documented across numerous states, particularly in the humid forest zones of Ondo, Edo, Ekiti, and Cross River (Veldman, 2021). Yet, despite this widespread presence, not a single comprehensive scientific study has characterized Nigerian *C. sativa*'s pharmacognostic properties or chemical composition.

This knowledge gap carries profound consequences. Forensically, successful prosecution of drug offenses requires definitive identification of seized material as *C. sativa* - a determination currently made without benefit of locally-relevant reference data. Medically, the global trend toward cannabis legalization for therapeutic purposes has created a multi-billion-dollar industry, with researchers actively collecting germplasm from African landraces without benefit-sharing agreements (Chouvy, 2019). Economically, Nigeria risks losing its genetic heritage to foreign commercial interests while forgoing potential revenue from a regulated cannabis industry. Scientifically, the international community lacks understanding of how tropical cultivation conditions shape cannabis chemotypes, as most research has focused on temperate-zone cultivars. Pharmacognosy, the study of medicines derived from natural sources, provides the ideal framework for addressing these gaps. Classical pharmacognostic evaluation encompasses macroscopic and microscopic characterization, physicochemical standardization, phytochemical screening, and

chromatographic profiling (WHO, 2011). These tools, applied comparatively across geographic regions, can reveal whether Nigerian cannabis exists as a uniform chemotype or exhibits region-specific variation with forensic and medicinal implications.

The present study therefore undertook the first systematic pharmacognostic investigation of *C. sativa* samples collected from five Nigerian geopolitical zones. We sought to determine: (1) whether Nigerian cannabis maintains consistent pharmacognostic identity across regions; (2) whether physicochemical parameters vary geographically; (3) what phytochemical constituents characterize Nigerian *C. sativa*; and (4) whether cannabinoid profiles differ sufficiently to enable forensic source attribution. The answers to these questions will provide essential baseline data for law enforcement, medicinal plant research, and evidence-based drug policy in Nigeria.

Materials and Methods

Plant Material Collection

Six samples of *Cannabis sativa* L. were obtained through official authorization from the National Drug Law Enforcement Agency (NDLEA) Headquarters, Abuja. Samples were collected from five states representing different geopolitical zones of Nigeria:

- **Sample A:** Confiscated from traffickers along Kaduna-Zaria Road, Kaduna State (source/origin unknown). Obtained July 2023. Voucher No: NDLEA/KDSC/NHQ/VOL.VIII/42.
- **Sample B:** Cultivated and seized from a farm in Kaduna State (North West Nigeria). Obtained July 2023. Voucher No: NDLEA/KDSC/NHQ/VOL.VIII/43.
- **Sample C:** Cultivated and seized from a farm in Adamawa State (North East Nigeria). Obtained August 2023. Voucher No: NDLEA/ADSC/NHQ/VOL.1.
- **Sample D:** Cultivated and seized from a farm in Edo State (South South Nigeria). Obtained August 2023. Voucher No: NDLEA/EDSC/NHQ/VOL.1.
- **Sample E:** Cultivated and seized from a farm in Enugu State (South East Nigeria). Obtained August 2023. Voucher No: NDLEA/ENSC/NAF/VOL.1.
- **Sample F:** Cultivated and seized from a farm in Ondo State (South West Nigeria). Obtained August 2023. Voucher No: NDLEA/ODSC/NHQ/VOL.1.

All samples originated from raids conducted between June and August 2023, ensuring freshness and minimizing post-harvest degradation. Due to the illicit status of cannabis in Nigeria, samples were transported as hand luggage by authorized security personnel after courier services refused to handle controlled substances.

Sample Preparation

Collected samples were visually sorted to remove extraneous materials and air-dried at room temperature for 5 days, followed by hot air oven drying at 40°C for 30 minutes until leaves became brittle. Dried samples were pulverized using a mechanical grinder and sieved to obtain consistent particle size. Powdered samples were stored in airtight containers at 4°C until analysis.

For quantitative microscopy, selected seeds from each sample were planted in pots at the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria. Seedlings were grown for 6 - 8 weeks under humid, moderate sunshine conditions, and mature fresh leaves were harvested for microscopic examination.

Macroscopic and Organoleptic Evaluation

Detailed macroscopic examination of leaves, stems, and seeds was conducted according to UNODC (2009) guidelines. Organoleptic properties - including appearance, color, texture, odor, and touch were assessed using primary senses as described by Alchimia (2015).

Quantitative Microscopy

Stomatal number, stomatal index, palisade ratio, vein-islet number, and veinlet termination number were determined using camera lucida techniques as described by Evans (2002). For each parameter, five replicate measurements were performed and mean values calculated.

Stomatal index was calculated using the formula: $SI = \frac{S}{S+E} \times 100$, where S = number of stomata per unit area, and E = number of epidermal cells in the same area.

Physicochemical Analysis

Physicochemical parameters were determined according to WHO (2011) guidelines and Kokate (1994). Parameters evaluated included:

- **Moisture content:** 3g powdered sample heated at 105°C to constant weight
- **Total ash value:** 2g sample incinerated in muffle furnace at ≤450°C
- **Acid-insoluble ash:** Total ash boiled with 25mL dilute HCl, insoluble matter collected and ignited
- **Water-soluble ash:** Total ash boiled with 25mL water, insoluble matter collected and ignited
- **Alcohol-soluble extractive value:** Maceration with 95% ethanol for 6 hours, followed by 18-hour standing
- **Water-soluble extractive value:** Maceration with chloroform water (0.25% v/v) for 6 hours, followed by 18-hour standing

All determinations were performed in triplicate, and mean values ± standard deviation calculated.

Chemo-microscopical Analysis

Chemo-microscopical examination of powdered samples was conducted using specific reagents according to WHO (1998) methods:

Starch was present in starch grains with the characteristic blue-black colouration with iodine solution. Lignified elements (xylem vessels, sclerenchyma fibers) showed pink colour with phloroglucinol-HCl. Tannin was present as cell inclusion showed greenish-black coloration with ferric chloride. Calcium oxalate crystals (rosette and prismatic forms) dissolved in hydrochloric acid without effervescence. Calcium carbonate was found (in cystolithic trichomes) producing effervescence with hydrochloric acid. Cellulose cell walls stained blue with chlor-zinc-iodine. Mucilage stained red with ruthenium red. Cutinized and suberized cell walls stained orange-red with Sudan red.

- **Starch:** Iodine solution (blue-black coloration)
- **Lignin:** Phloroglucinol + HCl (pink coloration)
- **Tannins:** 5% FeCl₃ (greenish-black coloration)
- **Calcium oxalate:** HCl (dissolution of crystals)
- **Calcium carbonate:** HCl (effervescence)
- **Cellulose:** Chlor-zinc-iodine solution (blue coloration)
- **Mucilage:** Ruthenium red (red coloration)
- **Cutin/suberin:** Sudan red (orange-red coloration)

Phytochemical Screening

Ethanol extracts (95% ethanol) of each sample were prepared by percolation. Phytochemical screening was conducted using standard methods (Evans, 2002; Sofowora, 1993; Kokate, 1994) for:

- **Carbohydrates:** Molisch test
- **Alkaloids:** Dragendorff's and Wagner's tests
- **Flavonoids:** Sodium hydroxide test, zinc-HCl test
- **Tannins:** Ferric chloride test, phenazone test
- **Phenols:** Lead acetate test
- **Saponins:** Frothing test, haemolysis test
- **Anthraquinones:** Borntrager's test
- **Cardiac glycosides:** Keller-Kiliani test
- **Steroids and triterpenes:** Salkowski test, Lieberman-Burchard test

Cannabinoid Extraction and Analysis

Acetonitrile/water (1:1 v/v) was selected as the extraction solvent based on its demonstrated superiority over conventional methanol/chloroform for cannabinoid recovery, its compatibility with HPLC mobile phases, and reduced environmental toxicity (Zivovinov et al., 2018). Extraction was done by percolation technique.

Thin Layer Chromatography (TLC)

TLC was performed on pre-coated silica gel 60 F₂₅₄ aluminum plates (Merck) using hexane:ethyl acetate:acetic acid (4:1:0.1 v/v/v) as mobile phase. Plates were visualized under UV light (254 and 366 nm) and after spraying with p-anisaldehyde-sulfuric acid reagent. Cannabidiol reference standard (Cerilliant, Sigma-Aldrich) was co-chromatographed for comparison.

High Performance Liquid Chromatography (HPLC)

HPLC analysis was conducted using an Agilent 1260 Infinity system equipped with ZORBAX SB-C18 column (2.1 × 150mm, 3.5µm). Isocratic elution with water:acetonitrile (20:80 v/v) at 0.5 mL/min was employed, with UV detection at 240nm. CBD quantification was achieved using a calibration curve (10-100 mg/L). Due to inability to source THC reference standard in Nigeria, THC could not be directly quantified.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was performed on an Agilent Intuvo 9000 GC system coupled with 5977B MSD. Separation employed a DB-5 MS column (30m × 320µm × 0.25µm) with helium carrier gas. Temperature program: 50°C (2 min) → 250°C at 20°C/min (2 min hold) → 300°C at 20°C/min (3 min hold). Compound identification was achieved by comparison with NIST17 Mass Spectral Library.

Statistical Analysis

Quantitative data were expressed as mean ± standard deviation. One-way Analysis of Variance (ANOVA) followed by t-Test for pairwise comparisons was used to assess statistical differences among samples, with significance set at $p \leq 0.05$.

Results

Macroscopic and Organoleptic Characteristics

All samples were received as dried plant material comprising upper stems, leaves, flowering tops, and seeds. The material was generally greenish-brown in color, with larger stems feeling hard and smaller stems soft, becoming doughy at flowering tips. Stems broke with difficulty, indicating presence of outer fibers. Dried leaves were brittle, and the material exhibited a matte, dry to dirty appearance. Organoleptic assessment revealed odors ranging from sweet musky to mildew to earthy.

Mature seeds were brown with characteristic "tortoise-shell" mottled patterns, ellipsoid, smooth, measuring 3-5 mm long. Immature seeds were cream-colored, soft, and wrapped in thin, clear bracts. Seeds from all samples germinated successfully when planted.

Leaves exhibited typical *C. sativa* morphology: palmately compound with 5-7 lanceolate leaflets, serrated margins with teeth directed toward tips, and veins running obliquely from midrib to serrations. Abaxial surfaces were pale green; adaxial surfaces darker. Petiole length ranged from 2-6 cm, leaflet dimensions from 3-15 × 0.2-2 cm.

Microscopic Characteristics

Qualitative microscopy revealed amphistomatic leaves with anomocytic stomata distributed on both surfaces. The upper epidermis showed cells with striations. Fine hairs covered both surfaces, with unicellular trichomes predominating. Both glandular and non-glandular trichomes were present, including characteristic claw-shaped cystolithic trichomes primarily on lower epidermis (Plate 1).

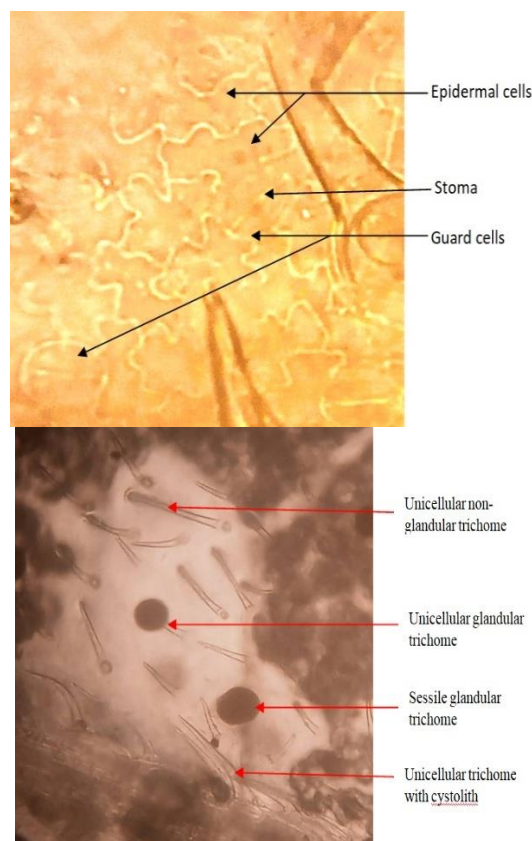


Plate 1: Microscopic features of *C. sativa* leaf: A) Anomocytic stomata (×400); B) Cystolithic trichome (×400); C) Glandular trichomes (×100); D) Non-glandular trichomes (×100).

Transverse sections through the midrib showed upper and lower epidermis covered by thin cuticle, distinct palisade and spongy mesophyll layers, collenchyma between epidermal cells, and central vascular bundle (phloem and xylem) surrounded by sclerenchyma tissue.

Quantitative Microscopy

Quantitative microscopy parameters showed variation among samples while remaining within expected ranges (Table 1). Stomata number ranged from 19.4±1.14 (Sample D) to 23.4±1.14 (Sample A). Stomatal index ranged from 16.98±0.30% (Sample E) to 18.09±0.74% (Sample D). Palisade ratio varied from 6.15±0.14 (Sample C) to 7.1±0.29 (Sample B). Veinlet termination number ranged from 12±1.41 (Sample B) to 13.6±0.89 (Sample A). Vein islet number ranged from 3.6±0.55 (Sample F) to 4.6±0.55 (Samples A and D).

ANOVA revealed statistically significant differences among samples for stomata number and palisade ratio ($p \leq 0.05$), while stomatal index, veinlet termination number, and vein islet number showed no significant differences. t-Test analysis indicated that the significant differences in palisade ratio were primarily attributable to Sample B (North West farm) with the highest value (7.1), while stomata number differences were randomly distributed with no single sample exerting predominant influence.

Table 1: Quantitative Microscopy Parameters of Six *C. sativa* Samples

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Mean
Stomata Number	23.4±1.14	20.2±1.13	23.4±0.55	19.4±1.14	22.4±0.55	20.4±0.90	21.53±1.75
Stomatal Index (%)	18.03±0.86	17.11±0.57	17.62±0.75	18.09±0.74	16.98±0.30	17.41±0.44	17.54±0.46
Palisade Ratio	6.25±0.31	7.1±0.29	6.15±0.14	6.95±0.57	6.3±0.60	6.8±0.70	6.59±0.41
Veinlet Termination No.	13.6±0.89	12±1.41	13.6±0.90	12.6±0.55	13±0.71	13.4±0.55	13.03±0.64
Vein Islet Number	4.6±0.55	4±0.71	4.2±0.45	4.6±0.55	4.4±0.55	3.6±0.55	4.23±0.39

Values expressed as mean ± SD; n=5 replicates

Physicochemical Constants

Physicochemical parameters showed considerable variation among samples (Table 2). Moisture content ranged from 4.1±0.17% (Samples A and F) to 15.33±0.34% (Sample E). Notably, Sample E (Enugu) exceeded the WHO-recommended upper limit of 12%, suggesting improper drying or storage conditions.

Total ash values ranged from 5.33±0.29% (Sample A) to 7.5±0% (Sample D). Acid-insoluble ash ranged from 3.83±0.29% (Sample F) to 5.67±0.29% (Sample D). Water-soluble ash values were uniformly low (2.17±0.29% to

3.5±0%). Alcohol-soluble extractive values ranged from 8±0% (Sample E) to 11.67±0.58% (Sample D). Water-soluble extractive values ranged from 10.67±0.58% (Samples E and F) to 14.33±0.58% (Sample D).

ANOVA revealed statistically significant differences among samples for all parameters except water-soluble ash. t-Test analysis consistently identified Sample D (Edo) as the primary contributor to significant differences, with its elevated values across multiple parameters distinguishing it from other samples.

Table 2: Physicochemical Constants of Six *C. sativa* Samples

Parameter (% w/w)	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Mean
Moisture Content	4.1±0.17	4.57±0.23	4.57±0.23	9.11±0.19	15.33±0.34	4.1±0.17	6.97±4.52
Total Ash	5.33±0.29	6.33±0.29	6.33±0.29	7.5±0	7.17±0.29	7±0	6.6±0.77
Acid-Insoluble Ash	4±0	4.33±0.29	4.33±0.29	5.67±0.29	4.83±0.29	3.83±0.29	4.5±0.67
Water-Soluble Ash	2.17±0.29	2.08±1.59	3±0	3±0	2.17±0.29	3.5±0	2.65±0.59
Alcohol-Soluble Extractive	8.33±0.58	9±0	9±0	11.67±0.58	8±0	9±0	9.17±1.3
Water-Soluble Extractive	11.33±0.58	12.33±0.58	12.33±0.58	14.33±0.58	10.67±0.58	10.67±1.15	11.94±1.39

Values expressed as mean ± SD; n=3 replicates

Chemo-microscopical Features

Chemo-microscopical analysis revealed uniform presence of all tested constituents across the six samples (Table 3). Starch, lignin, tannins, calcium oxalate crystals, calcium carbonate, cellulose, mucilage, and cutin/suberin were detected in all samples, confirming the similarity of cellular inclusion profiles regardless of geographic origin.

Table 3: Chemo-microscopical Features of Six *C. sativa* Samples

Constituent	Reagent	Observation	A	B	C	D	E	F
Starch	Iodine	Blue-black	+	+	+	+	+	+
Lignin	Phloroglucinol-HCl	Pink	+	+	+	+	+	+
Tannins	5% FeCl ₃	Greenish-black	+	+	+	+	+	+

Calcium Oxalate	HCl	Dissolution	+	+	+	+	+	+
Calcium Carbonate	HCl	Effervescence	+	+	+	+	+	+
Cellulose	Chlor-zinc-iodine	Blue	+	+	+	+	+	+
Mucilage	Ruthenium red	Red	+	+	+	+	+	+
Cutin/Suberin	Sudan red	Orange-red	+	+	+	+	+	+

Phytochemical Profiles

Phytochemical screening of ethanolic extracts revealed both uniform and variable constituents across samples (Table 4). Carbohydrates, cardiac glycosides, and phenols were detected in all six samples. Tannins were present in Samples A, B, D, and F, but absent in C and E. Alkaloids were detected in all

samples except Sample D. Steroids and triterpenes were present in all samples except Sample E. Flavonoids were detected in Samples A, D, E, and F, but absent in B and C. Anthraquinones showed variable distribution, present in A, D, E, and F but absent in B and C. Saponins were uniformly absent from all samples.

Notably, Samples A (trafficker-confiscated, Kaduna) and F (Ondo cultivated) exhibited identical phytochemical profiles, suggesting closer chemical relationship between these two samples than with others.

Table 4: Phytochemical Constituents of Six *C. sativa* Samples

Constituent	Test	A	B	C	D	E	F
Carbohydrates	Molisch	+	+	+	+	+	+
Anthraquinones	Borntrager's	+	-	-	+	+	+
Saponins	Frothing	-	-	-	-	-	-
Cardiac Glycosides	Keller-Killiani	+	+	+	+	+	+
Tannins	FeCl ₃	+	+	-	+	-	+
Alkaloids	Dragendorff's	+	+	+	-	+	+
Steroids/Triterpenes	Salkowski	+	+	+	+	-	+
Flavonoids	NaOH	+	-	-	+	+	+
Phenols	Lead Acetate	+	+	+	+	+	+

Cannabinoid Profiles

Thin Layer Chromatography

TLC analysis revealed complex cannabinoid profiles varying considerably among samples (Table 5). Total resolved compounds ranged from 5 (Sample E) to 12 (Samples A and F). The cannabidiol reference standard produced a single spot at R_f 0.61, and all samples exhibited a corresponding spot at R_f 0.61-0.63, confirming CBD presence in all six samples.

Three compounds were common to all samples, with R_f values of approximately 0.38, 0.57-0.58, and 0.61-0.63 (CBD). Samples A and F showed the greatest similarity, sharing nine spots with identical R_f values, consistent with their identical phytochemical profiles.

Table 5: TLC R_f Values of Six *C. sativa* Samples

Table 5: TLC R_f Values of Six *C. sativa* Samples

Spot	A	B	C	D	E	F	CBD
1	0.08	0.08	-	-	-	0.08	-
2	0.14	0.13	0.13	-	-	0.15	-
3	0.21	0.19	-	0.19	-	0.20	-
4	-	0.23	0.23	0.23	-	-	-

5	0.25	-	0.26	0.26	0.26	0.25	-
6	0.28	0.29	-	-	-	0.29	-
7	0.32	-	-	-	-	-	-
8	0.37	0.38	0.38	0.38	0.38	0.38	-
9	0.43	0.42	-	0.43	0.43	0.42	-
10	-	-	0.45	-	-	0.46	-
11	0.47	-	-	-	-	0.48	-
12	0.58	0.57	0.57	0.56	0.57	0.58	-
13	0.63	0.61	0.63	0.63	0.63	0.63	0.61
14	-	0.67	-	-	-	0.67	-
15	0.70	-	-	-	-	-	-

High Performance Liquid Chromatography

HPLC analysis provided superior resolution, detecting 15-25 compounds per sample (Table 6). Sample B showed the highest number of resolved peaks (25), followed by Sample F (22), Samples A and C (20 each), Sample D (19), and Sample E (15). Eight compounds were common to all six samples, as indicated by matching retention times across all chromatograms.

CBD content, quantified using the calibration curve ($R^2 = 0.999$), showed considerable variation (Table 7). Sample D (Edo) contained the highest CBD concentration at 30.54 mg/g, followed closely by Sample E (Enugu) at 29.52 mg/g. The lowest CBD content was found in Sample F (Ondo) at 17.41 mg/g, followed by Sample A (trafficker-confiscated) at 18.65 mg/g.

Table 6: Summary of HPLC Peak Distribution

Parameter	A	B	C	D	E	F
Total Peaks	20	25	20	19	15	22
CBD RT (min)	4.31	4.33	4.36	4.38	4.39	4.33
Common Compounds	8	8	8	8	8	8

Table 7: Quantitative CBD Content by HPLC

Sample	Origin	CBD Content (mg/g)
A	Trafficker-confiscated (Kaduna)	18.65
B	Kaduna State (North West)	25.56
C	Adamawa State (North East)	20.78
D	Edo State (South South)	30.54
E	Enugu State (South East)	29.52
F	Ondo State (South West)	17.41

Gas Chromatography-Mass Spectrometry

GC-MS analysis identified 82-100 compounds per sample, with variable success in cannabinoid detection (Table 8). Sample A yielded 82 compounds and confirmed THC (RT 23.532 min, 99% match) and CBN (RT 24.013 min, 99% match). Samples B and C, despite yielding 100 compounds each, showed no detectable cannabinoids by GC-MS—a finding that contrasts with positive TLC and HPLC results, suggesting concentrations below GC-MS detection limits or thermal degradation issues.

Sample D confirmed THC (RT 29.174 min, 99% match) and CBN (RT 29.689 min, 98% match). Sample E confirmed only CBN (RT 29.666 min, 98% match). Sample F exhibited the most diverse cannabinoid profile, with CBD (RT 27.887 min, 50% match), CBC (RT 28.133 min, 25% match), THC (RT 29.163 min, 98% match), and CBN (RT 29.678 min, 99% match) identified. The lower match percentages for CBD and CBC suggest low concentrations or co-elution with interfering compounds.

Table 8: GC-MS Cannabinoid Identification

Sample	Total Compounds	Cannabinoids Identified	RT (min)	Match Quality
A	82	THC, CBN	23.532, 24.013	99%, 99%
B	100	None detected	-	-
C	100	None detected	-	-
D	100	THC, CBN	29.174, 29.689	99%, 98%
E	100	CBN	29.666	98%
F	100	CBD, CBC, THC, CBN	27.887, 28.133, 29.163, 29.678	50%, 25%, 98%, 99%

Discussion

Pharmacognostic Identity of Nigerian *Cannabis sativa*

The macroscopic and microscopic characteristics observed in this study confirm that all six samples belong to *Cannabis sativa* L. The presence of anomocytic stomata, cystolithic trichomes, and the characteristic tortoise-shell seed patterning align perfectly with established descriptions of the species (UNODC, 2009; Raman et al., 2019). Importantly, these features were consistent across all geographic regions, confirming that Nigerian cannabis maintains the fundamental pharmacognostic identity of *C. sativa* regardless of cultivation location.

The quantitative microscopy parameters provide the first baseline data for Nigerian *C. sativa*. The stomatal index values (16.98-18.09%) fall within ranges reported for cannabis in other tropical regions (Nakkliang et al., 2022). The statistical analysis revealing significant differences in stomata number and palisade ratio suggests that some quantitative microscopic features may respond to

environmental conditions, as all plants for microscopy were grown under identical conditions at Zaria. Sample B's significantly higher palisade ratio (7.1) compared to others may indicate underlying genetic differences in the Kaduna North West landrace, as environmental factors were controlled during cultivation.

The uniform chemo-microscopical profile across all samples (Table 3) demonstrates that the cellular inclusion characteristics of cannabis include starch, lignin, tannins, calcium oxalate, calcium carbonate, cellulose, mucilage, and cutin which are constitutive features unaffected by geographic origin. This uniformity reinforces the reliability of these features for species identification in forensic contexts.

Physicochemical Variation as Regional Marker

The physicochemical parameters revealed more pronounced regional variation than microscopic features. Of particular interest is Sample D (Edo), which consistently exhibited elevated values across multiple parameters (moisture content 9.11%, total ash 7.5%, acid-insoluble ash 5.67%, alcohol-soluble extractives 11.67%, water-soluble extractives 14.33%). Statistical analysis confirmed that Sample D accounted for most of the significant differences observed among samples.

This finding suggests that Edo, South South region may produce cannabis with distinct physicochemical characteristics. Several factors could contribute to this distinctiveness: (1) soil composition in Edo's forest zones may differ from other regions, affecting mineral uptake reflected in ash values; (2) cultivation practices unique to Edo farmers might influence extractable constituents; (3) the Edo landrace may possess genetic characteristics promoting higher accumulation of extractable compounds.

The elevated moisture content of Sample E (Enugu, 15.33%) exceeding the WHO-recommended 12% upper limit indicates improper post-harvest handling. High moisture content predisposes herbal drugs to enzymatic degradation and microbial contamination (WHO, 1998). This finding has practical implications for NDLEA seizures, suggesting that some confiscated cannabis may be of poor quality due to inadequate drying, potentially affecting both its street value and evidentiary integrity.

The generally higher water-soluble extractive values (mean 11.94%) compared to alcohol-soluble values (mean 9.17%) align with observations from Thailand (Nakkliang et al., 2022) and confirm that water extracts more material from cannabis than alcohol. However, this does not imply therapeutic superiority, as many bioactive constituents including cannabinoids are more efficiently extracted by organic solvents.

Phytochemical Chemotypes

The phytochemical screening revealed both core constituents uniformly present across all samples and variable constituents defining distinct chemotypes. The uniform presence of carbohydrates, cardiac glycosides, and phenols suggests these compounds are essential to cannabis metabolism regardless of

growing conditions. The complete absence of saponins across all samples confirms previous reports (Nakkliang et al., 2022; Sharma et al., 2022) and suggests saponin absence may be a chemotaxonomic marker for *C. sativa*.

The variable distribution of flavonoids, tannins, anthraquinones, alkaloids, and steroids/triterpenes defines at least three distinct chemotypes among the six samples:

- **Chemotype I (Samples A and F):** Characterized by presence of all tested constituents except saponins. These two samples—the trafficker-confiscated Kaduna sample and the Ondo cultivated sample showed identical phytochemical profiles, suggesting they may represent the same or closely related landraces. The similarity implies that cannabis trafficked in northern Nigeria may originate from Ondo or chemically similar southern populations.
- **Chemotype II (Samples B and C):** Characterized by absence of flavonoids and anthraquinones, with Sample C additionally lacking tannins. These northern samples (Kaduna farm and Adamawa) form a distinct chemotypic cluster.
- **Chemotype III (Samples D and E):** Characterized by presence of flavonoids and anthraquinones but with Sample D lacking alkaloids and Sample E lacking steroids/triterpenes. These southern samples (Edo and Enugu) show intermediate profiles.

The concept of chemotypes - chemically distinct populations within a single plant species—has been well-established in cannabis research (Hillig, 2004; Hazekamp & Fisedick, 2012). Traditionally, cannabis chemotypes have been defined primarily by cannabinoid ratios, particularly THC:CBD. Our findings extend this concept to include broader phytochemical profiles, suggesting that Nigerian cannabis exhibits phytochemical diversity beyond the cannabinoid spectrum.

Cannabinoid Chemotypes

The cannabinoid profiles revealed the most striking chemotypic variation and have the most direct forensic implications. The detection of cannabinoids in all samples by TLC and HPLC, despite GC-MS failures for Samples B and C, underscores the importance of using complementary analytical techniques in forensic casework. The failure of GC-MS to detect cannabinoids in Samples B and C despite positive TLC and HPLC results, likely reflects either low cannabinoid concentrations near detection limits or thermal degradation of acidic cannabinoids in the GC injector (Dussy et al., 2005).

The quantitative CBD data (Table 7) reveal a north-south gradient, with southern samples (Edo 30.54 mg/g, Enugu 29.52 mg/g) showing higher CBD content than northern samples (Kaduna farm 25.56 mg/g, Adamawa 20.78 mg/g). This pattern aligns with UNODC observations that southern Nigeria's humid climate favors cannabis cultivation (UNODC, 2022) and may reflect environmental modulation of cannabinoid biosynthesis. The high CBD content of southern

samples has potential medicinal implications, as CBD possesses documented therapeutic properties including anticonvulsant, anti-inflammatory, and anxiolytic effects (Joca, 2024; Russo, 2011).

The GC-MS identification of THC in Samples A, D, and F confirms that Nigerian cannabis is predominantly drug-type rather than fiber-type. The presence of CBN - a degradation product of THC in all four samples where cannabinoids were detected by GC-MS indicates varying degrees of post-harvest aging. CBN forms through non-enzymatic oxidation of THC during storage (UNODC, 2009), and its presence suggests that seized samples may have been stored for some time before analysis.

Sample F (Ondo) exhibited the most diverse cannabinoid profile, containing CBD, CBC, THC, and CBN. The presence of CBC, a non-psychoactive cannabinoid with anti-inflammatory and antidepressant properties (ElSohly & Slade, 2005) adds to the medicinal potential of Ondo cannabis. The relatively low match percentages for CBD (50%) and CBC (25%) suggest either low concentrations or the presence of isomeric forms not perfectly represented in the NIST library.

Forensic and Policy Implications

The unique chromatographic fingerprints generated by TLC, HPLC, and GC-MS for each sample have direct forensic applications. Law enforcement agencies could potentially:

1. **Link seizures to geographic origins:** By comparing unknown seizure profiles with regionally-characterized reference data, investigators could infer cultivation regions, aiding in mapping trafficking networks.
2. **Identify common sources:** Matching profiles among separate seizures would suggest common supply chains, supporting conspiracy charges.
3. **Distinguish drug-type from fiber-type:** THC presence in multiple samples confirms drug-type classification relevant for prosecutions.
4. **Detect adulteration:** Profiles inconsistent with reference patterns might indicate sample adulteration or mixing.

The similarity between Sample A (trafficker-confiscated in Kaduna) and Sample F (Ondo cultivated) across phytochemical profiles, TLC patterns, and HPLC fingerprints strongly suggests that cannabis trafficked in northern Nigeria originates from southern cultivation regions. This finding, if confirmed by larger studies, would provide actionable intelligence for NDLEA operations, enabling targeted interventions along known trafficking routes.

The high CBD content of southern Nigerian samples raises important policy questions. As global trends move toward cannabis legalization for medical purposes (Chouvy, 2019; Sabaghi, 2024), Nigeria possesses potentially valuable germplasm with significant CBD content. The current prohibition regime prevents systematic evaluation and development of these genetic resources, potentially ceding economic opportunities to foreign interests who may collect

and patent Nigerian landraces without benefit-sharing (Chouvy, 2019).

The ongoing debate regarding cannabis policy in Nigeria (Marwa, 2021; Okorocha, 2019) would benefit from scientific evidence such as that generated by this study. While enforcement agencies emphasize the social harms of cannabis abuse, the medicinal and economic potential cannot be ignored. Evidence-based policymaking requires accurate data on the nature and variability of Nigerian cannabis, which this study begins to provide.

Limitations and Future Directions

This study, while pioneering for Nigeria, has several limitations that should inform interpretation and guide future research:

1. **Sample size:** Six samples cannot capture the full diversity of Nigerian cannabis. Larger sampling campaigns are urgently needed.
2. **Lack of THC reference standard:** The inability to source THC reference standard in Nigeria precluded calculation of CBD:THC ratios, the primary determinant of cannabis chemotype classification.
3. **Single growing season:** Samples represent only one harvest period. Multi-year studies are needed to establish stable regional characteristics.
4. **Unknown genetic background:** Without corresponding genetic data, we cannot definitively separate environmental from genetic contributions to observed chemotypic variation.

Future research should address these limitations through: expanded sampling; establishment of THC reference standards in Nigerian laboratories; correlation of chemical profiles with genetic data; pharmacological evaluation of Nigerian cannabis extracts; and investigation of seasonal and annual variation.

Conclusion

This first comprehensive pharmacognostic study of *Cannabis sativa* from five Nigerian geopolitical zones reveals that while the species maintains consistent microscopic and chemomicroscopical identity across regions, significant chemotypic variation exists in physicochemical parameters, phytochemical constituents, and cannabinoid profiles.

Three major findings emerge with particular significance:

1. **Regional chemotypic variation enables forensic differentiation.** Each sample exhibited unique chromatographic fingerprints, suggesting that chemical profiling can support source attribution in forensic casework.
2. **Southern Nigerian cannabis shows high cannabinoid content.** Samples from Edo (30.54 mg/g CBD) and Enugu (29.52 mg/g CBD) contain substantial cannabinoid concentrations with potential medicinal applications.
3. **Trafficking patterns may be inferred from chemical similarity.** The close relationship between the trafficker-confiscated Kaduna sample and the

Ondo cultivated sample suggests that cannabis consumed in northern Nigeria may originate from southern cultivation regions.

These findings provide essential baseline data for law enforcement, medicinal plant research, and evidence-based drug policy in Nigeria. As global attitudes toward cannabis continue to evolve, Nigeria stands at a crossroads: the current prohibition regime maintains the status quo but forecloses economic and medicinal opportunities; evidence-based reform could unlock the potential of Nigerian cannabis while addressing legitimate public health concerns. The scientific foundation provided by this study should inform whatever path Nigeria chooses.

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