Nigerian Journal of Pharmaceutical Sciences
Vol. 24, No2, 2025, ISSN: 0189-823X

PHARMACOGNOSTIC PROPERTIES OF *CANNABIS SATIVA* L. (CANNABACEAE) SAMPLES COLLECTED FROM VARIOUS REGIONS OF NIGERIA

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ABSTRACT

Cannabis sativa (*C. sativa*) belonging to the family Cannabaceae and commonly referred to as 'marijuana', is one of the most used illicit drugs in Nigeria. There is significant level of *C. sativa* cultivation and trafficking in Nigeria. To date, pharmacognostic properties of *C. sativa* material confiscated and cultivated in various regions of Nigeria have not been reported. This study aimed to determine the pharmacognostic properties of *C. sativa* grown in various regions of Nigeria. This study used *C. sativa* sample of unknown origin confiscated from traffickers in Kaduna State (A) and five samples seized from farms (cultivated) in Kaduna (B), Adamawa (C), Edo (D), Enugu (E) and Ondo (F) States of Nigeria. Standard, validated techniques were used to evaluate pharmacognostic parameters including morphological, organoleptic, microscopic, physical and phytochemical properties of six matured *C. sativa* samples, comprising leaves and flowering parts. All the six samples conformed to the general characteristics of *C. sativa* and showed no significant morphological and organoleptic differences. Quantitative microscopy and other physico-chemical analyses produced similar results. Phytochemical screening yielded carbohydrate, cardiac glycoside and phenols in all samples. Anthraquinones, flavonoids, tannins and tritepenoids were present in most samples. Saponin was absent in all samples. Colour tests and TLC confirmed the presence of cannabinoids in all samples. Phytochemical profile and TLC fingerprints of the sample confiscated in Kaduna State (A) and that cultivated in Ondo State (F) were very similar, suggesting a common chemical identity of both. This study showed that cannabis grown in various regions of Nigeria shared many important features. The result forms important baseline records for further studies of cannabis confiscated and cultivated in various regions of Nigeria with possible applications in medicine, forensics and law enforcement.

Keywords: cannabinoids, *C. sativa*, pharmacognostic, phytochemical, thin layer chromatography

INTRODUCTION

Cannabis sativa L. (*C. sativa*) is illicit in many countries, yet it is one of the most widely used plants for both recreational and medicinal purposes. It is a monospecific plant belonging to the Cannabaceae family (Elsohly & Gul, 2014). In Nigeria, *C. sativa* is commonly known as "Marijuana", "Indian hemp" and "igbo" (Braji, 2023). Identification of cannabis material could be done with either one or a combination of methods such as organoleptic, macroscopical, microscopical, physicochemical or chemical analysis. (Raman *et al*., 2019). Over 750 compounds have been reported in *C. sativa* and the best known and the most specific class of cannabis constituents is tetrahydrocannabinoid (THC) (Dos Santos *et al*., 2019; Stefkov *et al*., 2022). Cannabinoids are specific to cannabis and are used as markers for the identification of *C. sativa* material. Chemical analysis of *C. sativa* for forensic

purposes can be done with fresh plant material or dried parts (Sirikantaramas *et al.*, 2004). Chemical analysis of *C. sativa* could be presumptive or confirmatory. Presumptive tests include Fast Corinth V salt test, Fast Blue B salt test and Rapid Duquenois test (Srivastava & Yadav, 2013). Confirmatory tests for cannabis include thin layer chromatography (TLC), gas chromatography (GC), liquid chromatography (LC), deoxyribonucleic acid (DNA) analysis and their variants, among others.

A few countries have legitimatized the use of *C. sativa*. It however, remains banned in many others such as Nigeria despite enormous documented economic and medical values (UNODC, 2022a). This has left a gap in knowledge of cannabis grown and trafficked in Nigeria. The pharmacognostic studies of *C. sativa* are therefore necessary to ensure that the plant is always correctly identified and/or its presence in other materials is confirmed. The aim of this paper therefore, is to determine the pharmacognostic properties of *C. sativa* grown in various regions of Nigeria. The objectives of the paper include the determination of the morphological, organoleptic, microscopical, physical and phytochemical properties of the *C. sativa* sample from various regions of Nigeria. The outcome of the study would bridge the knowledge gap and provide more information on the *C. sativa* landraces in the country and provide baseline data for further studies.

MATERIALS AND METHOD

Plant Collection and Identification

Plant material (leaves and flowering parts) of mature *C. sativa* were collected from 5 states of Nigeria namely Kaduna (North West), Adamawa (North East), Edo (South South), Enugu (South East) and Ondo (South West). Being a banned substance, the sample collection was done through the National Drug Law Enforcement Agency (NDLEA). Each of the five NDLEA State Commands released 750g of cannabis from samples seized during raids of cannabis farms by its operatives within the previous three months (June – August 2023). In addition, a second sample was collected from Kaduna from among those confiscated from traffickers along Kaduna – Zaria Road. All samples were identified as *C. sativa* by the designated NDLEA officers.

Preparation of Plant Material

Collected samples were visually sorted to remove extraneous materials and labeled as shown in Table 1. Specimen were taken from each of the 6 samples and kept aside for organoleptic examination and qualitative microscopy. The remaining samples were further dried at room temperature for seven days and hot air oven at about 40^{o}C for about 30 minutes, until the leaves become brittle. The dried samples were then pulverized and sieved to get consistent powders. For quantitative microscopy, few mature seeds from each of the samples were planted in separate pots. The resultant seedlings were allowed to grow for six to eight weeks under humid and moderate sunshine conditions. Mature fresh leaves were then cut and used for quantitative microscopy.

Extraction of Plant Materials

The extraction of the plant material was achieved by percolation technique using two different solvent systems, ethanol (95 %) and acetonitrile/water (ACN/H2O (1:1)). Ethanol crude extracts (Ee) were used for general phytochemical tests. While ACN/H_2O crude extracts (Ae) were used for cannabinoids studies (Zivovinovic *et al*., 2018). The extracts were labeled as shown in Table 1.

Table 1. *C. sativa* Samples Collected from Various Parts of Nigeria and their Extracts using Ethanol and ACN/H_2O as Solvents

Serial	Source	Region	Sample code	Ethanol extract	ACN/H_2O (1:1) extract
1.	Confiscated in Kaduna	Unknown	A	EeA	AeA
2.	Cultivated in Kaduna State	North West Nigeria	B	EeB	AeB
3.	Cultivated in Adamawa State	North East Nigeria	C	EeC	AeC
4.	Cultivated in Edo State	South South Nigeria	D	EeD	AeD
5.	Cultivated in Enugu State	South East Nigeria	E	EeE	AeE
6.	Cultivated in Ondo State	South West Nigeria	F	EeF	AeF

Determination of Organoleptic and Macroscopic Properties of Leaf of *C. sativa*

Organoleptic properties of cannabis were determined using the primary senses of sight, touch and smell as described by Alchimia (2015). Also, detailed macroscopy of the leaves was studied and authenticated (UNODC, 2009).

Determination of Physical Properties and Phytochemical Contents of *C. sativa*

Various standard methods were used to determine the physical and phytochemical properties of the *C. sativa* samples from various regions of Nigeria. Qualitative and quantitative microscopy of fresh leaves of the samples were done using the methods described by Evans (2002). Similarly, physical constants of the samples were determined using few grammes of powdered plant material (PPM) (Kokate, 1994). Furthermore, the techniques described by WHO (1998), Kokate (1994) and Sofowora (1993) were used to determine the Chemo-microscopical and phytochemical constituents of the *C. sativa* samples.

Tests for Cannabinoids

Presumptive tests for cannabinoids were done using Fast Blue B salt test and Rapid Duquenois test and development of characteristic colours indicated the presence of cannabinoids (UNODC, 2009). TLC using hexane-ethyl acetate-acetic acid (4: 1: 0.1) as mobile phase was used to confirm the presence of cannabinoids (Nakkliang *et al.*, 2022).

RESULTS

Organoleptic and Macroscopic Properties of *C. sativa*

All samples were received at different levels of dryness from the NDLEA (Plate I). The samples were generally greenish brown in colour. The bigger stems felt hard, while the smaller ones were soft and the material becoming doughy at the flowering tip. The stems break without snapping, indicating presence of outer fibre. The leaves become brittle when fully dried. The dried plant materials were matte with dry to dirty appearance. When held close to the nostrils, the materials emitted from sweet musky to mildew to earthy smells. Both mature and immature seeds were present. Matured seeds were brown with mottled designs resembling tortoise-shell. Each matured seed is hard, slightly ellipsoid in shape, smooth and measures about 3 to 5 mm long. The immature seeds were cream coloured, soft and usually wrapped in very thin, clear bract (Plate II). There were no major distinguishing features among the seeds from different samples.

Plate I. Samples of *C. sativa* received from NDLEA

Plate II. Cannabis seeds (immature seeds on the left and matured seeds to the right)

The leaf is palmate and attached to the stem by a petiole measuring about 2 to 6 cm. When well developed, most of the leaves have 5 - 7 lanceolate leaflets, with the central leaflet usually longer and wider than the rest. Each leaflet measures from 3 - 15 by 0.2 - 2 cm. The leaf margins are serrated with the teeth directed towards the tips. The veins of the leaf run obliquely from the midrib to the tips of the serrated edges. The abaxial (lower) leaf surfaces are pale green in colour while the adaxial (upper) surfaces have darker shade. See Plates III and IV.

Plate III. Immature (A) and flowering (B) plants of *C. sativa* growing in pots at ABU Zaria

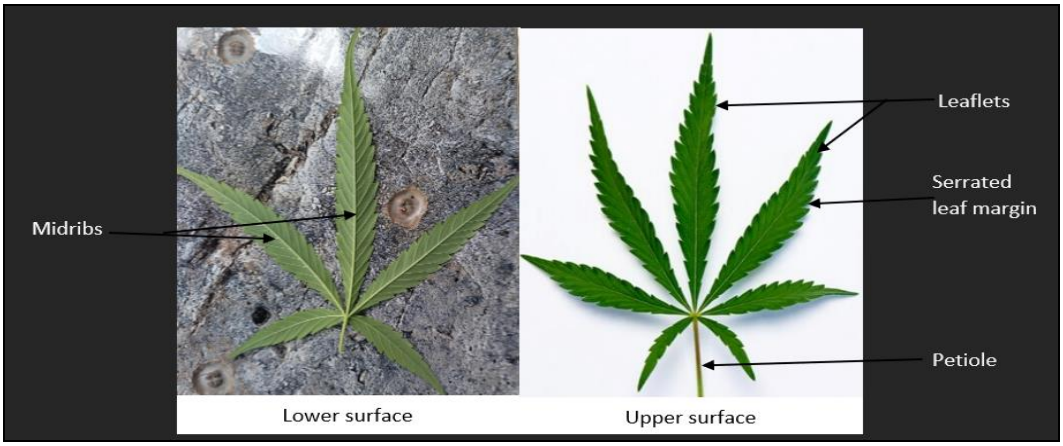

Plate IV. Major macroscopic features of fresh leaf of *C. sativa*

Microscopic Properties of the Leaf of *C. sativa*

Qualitative microscopy of the leaf showed the leaf surface is amphistomatic, with upper surface showing cells with striations. Fine hairs were present on both surfaces, which were covered by single layer of epidermis. Anomocytic stomata (Plate V) and epidermal cells were obvious. The leaves have mostly unicellular trichomes with both glandular and non-glandular forms present. The characteristic claw-shaped cystolithic trichomes were also observed mostly in the lower epidermal layers (Plate VI). TS of the midrib of the leaf showed upper and lower epidermal layers covered in fine layer of cuticle. The mesophyll showed distinct palisade layer and spongy cells while the midrib has collenchyma cells between the epidermal cells. The vascular bundle was at the centre and is made up of phloem and xylem and surrounded by Sclerenchyma tissue (Plate VII).

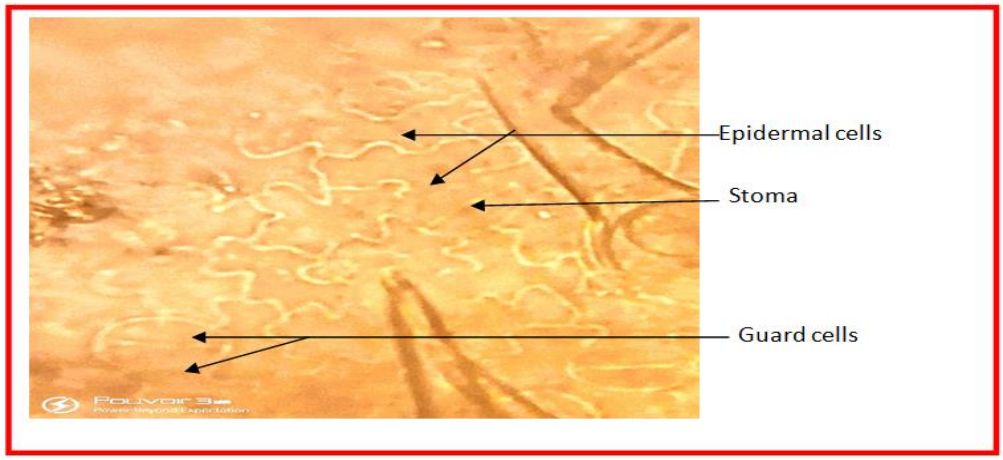

Plate V. Microscopic features from the leaf of *C. sativa s*howing anomocytic stomata (x400)

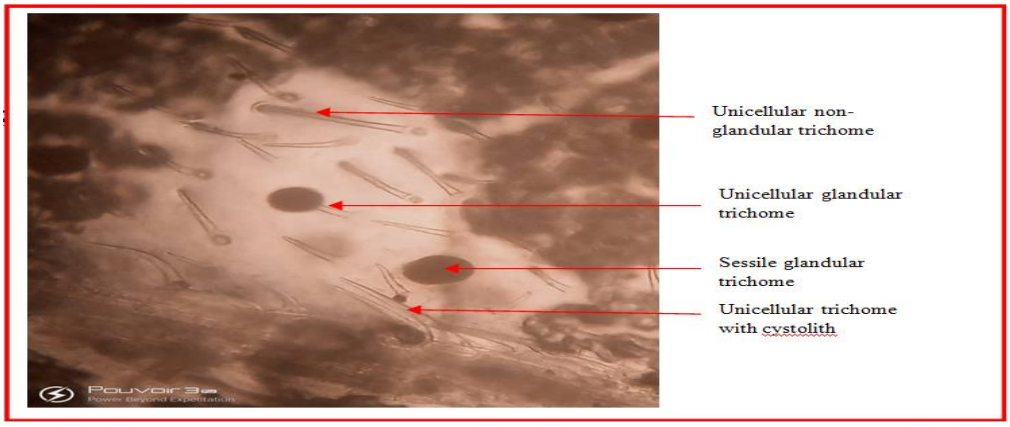

Plate VI. Various types of trichomes of *C. sativa* (x400)

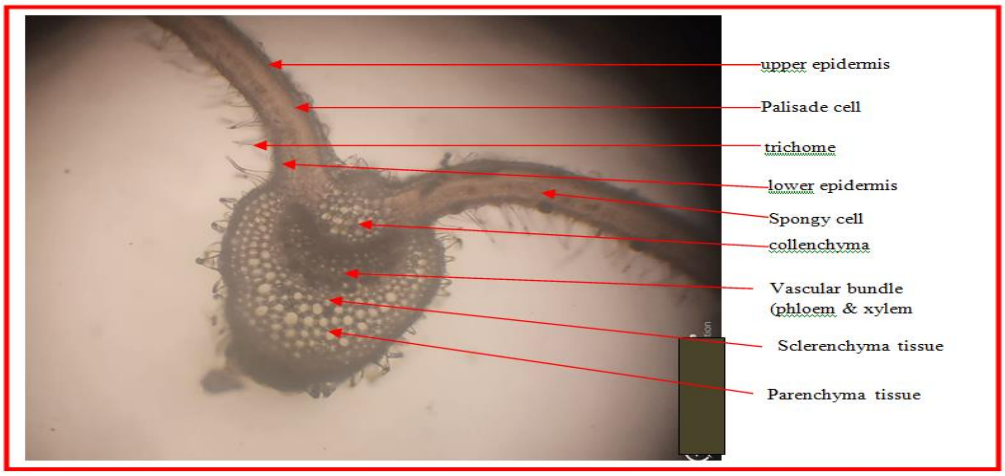

Plate VII. TS of *C. sativa* leaf (x400)

Quantitative microscopy of the leaf showed that Sample D had the lowest stomata number of 19.4 ±1.14, while Sample A had the highest number of 23.4 ±1.14 with a mean of 21.5 ±1.75. All the samples had relatively even values across other parameters. There were no significant differences in the quantitative microscopy of the samples. Quantitative microscopy of the leaf is as shown in Table 2.

Table 2. Result of Quantitative Microscopy of Leaf of Six *C. sativa* Samples Collected from Various Regions of Nigeria

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 ±090	**21.5 ±1.75**
Stomata Index (%)	18.0±0.86	17.1±0.57	17.6±0.75	18.1±0.74	16.9±0.30	17.4 ±0.44	**17.5 ±0.46**
Palisade Ratio	6.3±0.31	7.1±0.29	6.2 ±0.14	6.9 ±0.57	6.3 ±0.60	6.8 ±0.70	**6.6 ±0.41**
Vein Termination Number	13.6±0.89	12±1.41	13.6 ±0.90	12.6 ±0.55	13.0 ±0.71	13.4 ±0.55	**13.0 ±0.64**
Vein Islet Number	4.6±0.55	4.0±0.71	4.2 ±0.45	4.6±0.55	4.4 ±0.55	3.6 ±0.55	**4.2 ±0.39**

The value is shown as mean ± SD. (SD = Standard deviation). Each sample was repeated 5 times.

Chemo-Microscopical Features of *C. sativa*

Chemo-microscopical analysis of the powdered cannabis material showed that all the samples contained starch, lignin, tannins and calcium oxalate crystals. Calcium carbonate, cellulose, mucilage and cutin were also present.

Physical constants of leaf of *C. sativa*

The powdered drug showed percentage moisture content levels ranging from low 4.1 ±0.17 (Samples A and F) to 15.3 ±0.34 (Sample E). The mean alcohol soluble extractive value (**9.2 ±1.3**) was lower than the mean water-soluble extractive value (**11.9±1.39**). Physical constants of the powdered drug are as shown in Table 3.

Table 3. Result of Evaluation of Physical Constants of Powdered Plant Material of Six *C. sativa* Samples Collected from Various Regions of Nigeria

Physicochemical Parameter	Value (% w/w)						
	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Mean
Moisture Content	4.1 ±0.17	4.6 ±0.23	4.6 ±0.23	9.1 ±0.19	15.3 ±0.34	4.1 ±0.17	**7.00±4.52**
Total Ash Value	5.3 ±0.29	6.3 ±0.29	6.3 ±0.29	7.5 ±0.00	7.2 ±0.29	7.0 ±0	**6.6 ±0.77**
Acid insoluble ash (AIA)	4.0 ±0.00	4.3 ±0.29	4.3 ±0.29	5.7 ±0.29	4.8 ±0.29	3.8 ±0.29	**4.5 ±0.67**
Water soluble ash (WSA)	2.2 ±0.29	2.1 ±1.59	3.0 ±0.00	3.0 ±0.00	2.2 ±0.29	3.5 ±0	**2.7 ±0.59**
Alcohol soluble extractive value (ASEV)	8.3 ±0.58	9.0 ±0.00	9.0 ±0.00	11.7 ±0.58	8.0 ±0.00	9.0 ±0.00	**9.2 ±1.3**
Water soluble extractive value (WSEV)	11.3 ±0.58	12.3 ±0.58	12.3±0.58	14.3 ±0.58	10.7 ±0.58	10.7±1.15	**11.9±1.39**

The value is shown as mean ± SD. (SD = Standard deviation). Each sample was done 3 times.

Phytochemical Constituents of *C. sativa* Samples Collected from Various Regions of Nigeria

Phytochemical screening of the ethanolic extract yielded carbohydrate, cardiac glycoside and phenols in all the samples. Anthraquinones and flavonoids were absent in Samples EeB and EeC; tannins (absent in Samples EeC and EeE) and tritepenoids (absent in Sample EeE). Saponin was absent in all the samples.

Presence of Cannabinoids in *C. sativa* Plant Material

Result of presumptive screening for cannabinoids in the six *C. sativa* samples indicated the presence of cannabinoids in all except Sample C. However, confirmatory test for cannabinoids in the ACN/H_2O extracts using TLC confirmed the presence of the compound in all the samples as shown in Plate VIII. The retention factor (R_f) values for all the samples ranged from 0.08 to 0.7 with three R_f values (0.38, 0.57 and 0.63) common to all of them. Table 4 shows the R_f Values of the six *C. sativa* samples

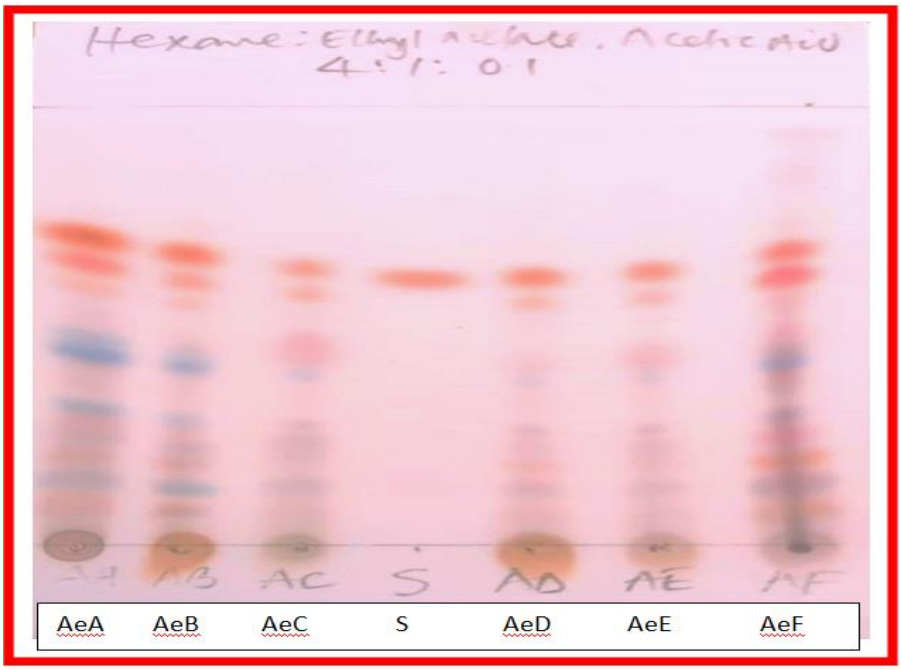

Plate VIII. Picture of TLC plate of *C. sativa* samples and standard cannabidiol (S) developed in hexane: ethyl acetate: acetic Acid (4: 1: 0.1) sprayed with P-anisaldehyde and sulphuric acid

Table 4. Rf Values of TLC of Six *C. sativa* Samples Collected from Various Regions of Nigeria and Standard Cannabidiol

Spot	**R_f values of samples**						
	AeA	**AeB**	**AeC**	**AeD**	**AeE**	**AeF**	**Cannabidiol**
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						

Solvent system: Hexane: Ethyl acetate: Acetic Acid (4: 1: 0.1)
Spraying reagent: P-anisaldehyde and sulphuric acid

DISCUSSION

One of the biggest challenges to this study was the collection and transportation of the cannabis plant material from various parts of Nigeria to the study location. This is due to the fact that the plant remains banned in Nigeria due to its association with crime, chaos and conflict (Marwa, 2021). The difficulty in legal possession and movement of cannabis across Nigeria is in keeping with its classification as a banned narcotic drug (Chouvy, 2019 and UNODC, 2022b). However, despite the tight control of cannabis; it is easily trafficked and widely available in most parts of Nigeria. This has prompted the call for its legalization in Nigeria, as has been done by many other countries (Okorocha, 2019). The enormous economic and medicinal values of cannabis make this call plausible. However, Marwa (2021) insists that the harmful effects of cannabis consumption in Nigerian society far out weights its value.

The cannabis material received from NDLEA State Commands in the sampled states (Kaduna, Adamawa, Edo, Enugu and Ondo) were in dried condition. According to UNODC (2022a) report, cannabis is cultivated in all states in Nigeria. Cannabis in Nigeria is mostly trafficked as dried plant material comprising the upper stems, leaves and flowering parts at the tips of the plant. In some climes, *C. sativa* provides food and oil from its seeds, fibre for rope, fabric from its stems and drugs from its leaves and flowers (Ranalli & Venturi, 2004). However, in Nigeria, it is mostly smoked and also added to cooked foods and eaten for its psychotic effect (Braji, 2023). The samples used in this research were received in the state cannabis is trafficked and consumed in Nigeria. The appearance, colour, feel and smell of the *C. sativa* samples resembled the organoleptic properties of cannabis described by Alchimia (2015). The immature seeds were whitish and indistinguishable from other types of seeds. However, the mature seeds had similar size, shape and most importantly, the characteristic 'tortoise-shell' striplings as reported in UNODC (2009), making them easy to identify.

Macroscopic and microscopic examination of the fresh leaves showed palmate shape appearance and the characteristic presence of cysolithic and glandular trichomes conformed to those described by Clarke and Merlin (2013) and UNODC (2022b). Further qualitative microscopy showed that all the samples shared the same properties of anomocytic stomata and similar arrangement of tissues in the transverse section of the leaf. Generally, as expected, the outcome of the evaluated organopleptic, macroscopic and qualitative microscopic properties showed that all the samples were similar and indistinguishable. According to UNODC (2009), the development of the cannabis plant such as branching and height depend on environmental and hereditary factors. This therefore means that since all the plants used in the macroscopic and microscopic studies of the leaf were planted under same condition at ABU, any observed differences are very likely due to heredity. This is in line with the observation of Zuk-Golaszewska and Golaszweski (2018) that morphological traits of cannabis are mostly influenced by cultivation regimes. Jackson and Chakraborty (2023) also observed that genetic differences of *C. sativa* samples are expressed more in the various phytochemicals than in morphological appearance. Quantitative microscopy parameters showed mean values of stomata number 21.5±1.75, stomata index 17.5±0.46, palisade ratio 6.6±0.41, vein termination number 13.0±0.64 and vein islet number 4.2±0.39. The individual samples showed random differences among them but generally remained within acceptable and expected ranges (WHO, 2011 and Nakkliang *et al*., 2022). UNODC (2022a) showed that most cannabis in Nigeria is cultivated under similar conditions. This follows that the quantitative microscopy of *C. sativa* samples grown in Nigeria are similar and cannot be used to distinguish them or determine the source of the plant. Evaluation of chemo-microscopical features of the six *C. sativa* samples did not yield any surprise. All the samples had starch, lignin, tannins, calcium oxalate crystals, calcium carbonate, cellulose, mucilage and

cutin conforming to the general characteristics of cannabis. Chemo-miscroscopy assessment is an important measure in quality control of herbal drugs (WHO, 1998).

The outcome of physical constants evaluations showed that all fell within acceptable limits of quality control of drugs, except Sample E, whose moisture content was 15.3 %. This is higher than the recommended upper limit of 12 % (WHO, 2011). The high moisture content of Sample E could be due to poor storage following preparation, initial moisture content of the raw material or environmental factors during harvest (Muyumba *et al.,* 2021). Excess moisture content of drugs could suggest the buyer may be paying a high price for unwanted water but also that the drug has been prepared incorrectly. High moisture content could lead to breakdown of important constituents due to enzyme activity or microbial attacks (WHO, 1998). The general physical constants results of this study however, was similar to the outcome in Thailand, where Nakkliang *et al*. (2022) showed that physico-chemical parameters of *C. sativa* leaves collected from different geographic regions were all within acceptable ranges. A closer comparison with the result of Nakkliang *et al*. (2022) shows that total ash value (14.36 %), water soluble extractive value (23.04 %) and ethanol soluble extractive value (11.10 %) obtained from samples in Thailand were higher than those in this study with mean total ash value of 6.6 %, water soluble extractive value of 11.9 % and alcohol soluble extractive value of 9.2 %. The lower total ash value of this study showed that cannabis grown in Nigeria had lower inorganic matter (impurities, phosphates, silicates and carbonates) when compared to Thailand (Nakkliang *et al*., 2022). Both studies however, point to the fact that water had more extractive power over alcohol in cannabis extraction. It has been stated that water extracts more constituents than alcohol but most plant active constituents are extracted by ethanol (Obi & Onuoha, 2000). A comparison of the mean of the results of the six samples used in this study showed that the moisture content of Samples D (9.1 %) and E (15.3 %) were outliers, being much higher than the average (6.9 %) for the parameter. The consistent elevated values for Sample D distinguish it from other samples. Consequently, elevated physical constant values could be used to distinguish Sample D (cultivated in Edo State) from other *C. sativa* plants grown in Nigeria.

Phytochemical screening of the ethanolic extract yielded carbohydrate, cardiac glycoside and phenols in all the samples. Saponin was absent in all the samples. The results from this study are similar to those of Nakkliang *et al*. (2022) and Sharma *et al*. (2022) in Thailand and India, respectively. Recent studies have indicated that over 750 compounds are present in *C. sativa*. These include phytochemicals such as sesquiterpenes, flavonoids, steroids and nitrogen-containing compounds, as well as, cannabinoids (Dos Santos *et al*., 2019 and Stefkov *et al*., 2022). A comparison of the phytochemical constituents of the six cannabis samples in this study did not show any particular pattern, except for Samples A and F that had same phytochemical profiles. Similar phytochemical pattern may indicate closer relationship between both samples when compared to others. In a study to establish the genetic basis of chemical composition of cannabis, Jackson and Chakraborty (2023) identified some genes expressed through chemicals possessing links to a set of properties. It showed a chain of genes – chemicals – properties and relationship. Another study in China indicated that flavonoids and cannabinoids could be potential geographical markers for distinguishing various cultivars (Liu *et al*., 2022). Unfortunately, the outcome of this study could not lead to categorical statement as above on the influence of geographical location within Nigeria on cannabis chemical constituents. This is probably due to the low number of samples evaluated and the test repeats. As a result, it is safer to assume that the six cannabis samples in this study have more in common phytochemically and comparatively indistinguishable from each other.

The target of cannabis identification tests is usually the cannabinoids, which are unique to cannabis plant (Nakkliang *et al*., 2022; Dos Santos *et al*., 2019 and Stefkov *et al*., 2022). Jackson and Chakraborty (2023) further confirmed that cannabis is the sole botanical producer of the terpenoid, cannabinoids. For this study, ACN/H2O (1:1) was chosen for the extraction of cannabinoids over the standard method of methanol/chloroform (MeOH/CHCl3 (9:1)) (Patel *et al*., 2017; Crime, 2013 and De Backer *et al*., 2009 in Zivovinovic *et al*., 2018) because it has been shown to extract more cannabinoids. ACN/H2O (1:1) also has better compatibility with starting conditions of the HPLC method. Furthermore, higher aqueous content renders this extraction system more environmentally friendly (Zivovinovic *et al*., 2018). This study used different methods to test for cannabinoids in the six *C. sativa* samples. These include colour tests (Fast Blue salt and Dequenois-Levine tests) and TLC. Rousell (2012) and Hazekamp *et al*. (2005) observed imprecise outcome of various tests for cannabinoids and suggested that various tests are required to reinforce each other. A summary of both presumptive screening and confirmatory tests showed that all samples had cannabinoids. TLC cumulatively separated 15 compounds among all the 6 samples, yielding 12 spots/compounds in Samples A and F, 10 in Sample B, seven in Samples C and D; and five in Sample E. Three of the compounds found in TLC were common to all the samples. The results of the colour and chromatographic tests showed the weaknesses of each technique. Malabadi *et al*. (2023) and Lazerjani *et al*. (2020) elaborated the many advantages and disadvantages of various methods for testing cannabinoids including colour test, TLC, HPLC-FTIR, NMR, etc. To mitigate the shortcoming of each of the techniques, many approaches are being adopted by cannabis researchers. One of the innovative techniques was the modification of a silica gel TLC plate by impregnating it with silver ions (Ag (I)) in the lower third portion, which enabled digital chromatographic separation of strongly retained CBD analogues and poorly retained THC analogues within minutes. The results obtained correlated to those of HPLC-UV. This modified TLC technique has advantages of short analysis time, high throughput, low solvent consumption, ease of interpretation and portability, among others (Huang *et al*., 2022). Earlier, Turner and Mahlberg (1984) showed that dried cannabis leaves yielded more cannabinoids than fresh leaves using HPLC.

It is generally accepted that cannabis, which has been known to man for over 4,700 years remains an evolving plant. It is a dynamic product with gradually changing prevalence, cultivation, potency, public health and legislative concerns (Sznitman *et al*., 2008). However, the outcome of this study showed that *C. sativa* samples conformed to the global trend and shared similar pharmacognostic properties, albeit with slight deviations as indicated by the TLC fingerprint. With more studies, better understanding and application of knowledge backed by appropriate government policies, *C. sativa* could be turned to a force for good. The Nigerian Government could join the rest of the world by taking a second look at cannabis plant and its enormous potentials as a drug, raw material and revenue generator, as against a tool for criminals. This study, although constrained by government policy and low sample size could be the basis for more robust study of *C. sativa* in Nigeria.

CONCLUSION AND RECOMMENDATIONS

Pharmacognostic studies of six samples of *C. sativa* collected in various regions of Nigeria showed that organopleptic, macroscopic, qualitative and quantitative microscopic properties of all the samples were similar and indistinguishable. Evaluated physical constants of the six cannabis samples were similar, with the exception of sample from Edo State (Sample D) with elevated values. Phytochemical screening yielded carbohydrate, cardiac glycoside and phenols in all the samples. Saponin was absent in all the samples. The six cannabis samples shared high similarity

phytochemically. Cannabinoids were present in all the samples and TLC separated 15 compounds among all the samples. Generally, the unique chromatographic fingerprints from TLC can be used to distinguish the samples. It is recommended that more researches should be conducted on pharmacognostic properties of *C. sativa* using larger sample sizes in order to establish standard parameters for cannabis grown in Nigeria

Funding and Conflict of Interest

The study was fully funded by the researchers. The researchers hereby declare no conflict of interest in this study.

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