

Functional Nutrients and Antioxidant Activity of *Cannabis sativa L.* cultivated in Ondo State, Nigeria

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ABSTRACT

This study aimed to evaluate of the nutritional composition, phytochemical content, and antioxidant activity of different parts *Cannabis sativa L.* cultivated in Ondo State, Nigeria. Leaves, stems, roots, seeds, and the composite blend of the whole plants were analyzed for proximate composition using standard analytical procedures, for saponins, alkaloids, oxalates, tannins, phenols, and flavonoids using spectrophotometric techniques and for antioxidant capacity using DPPH radical scavenging and ferric reducing antioxidant power assays. seeds exhibited the highest lipid content (36.44%), leaves contained the highest protein concentration (19.63%) while stems and roots were rich in fibre (45.76% and 47.13%, respectively). Leaves exhibited the highest phenol concentration (8.25 mg/100 g), and roots showed the highest alkaloid levels (6.92%). The composite showed the highest DPPH inhibition (91.55%) and stems showing the highest reducing power (142.93 mg/100 g). These findings indicate marked variation among plant tissues and reveal that *Cannabis sativa* cultivated in Ondo state, as a source of nutrients and bioactive compounds with potential nutraceutical, functional food, and pharmaceutical applications. These findings contribute valuable data toward the safe utilization and valorization of *Cannabis sativa* as a multipurpose medicinal plant. Further studies on compound isolation, toxicity and safety are recommended.

Keywords: *Cannabis sativa*, antioxidant activity, proximate, phytochemicals, nutraceutical potential

INTRODUCTION

Cannabis sativa L., a member of the Cannabaceae family, is a highly versatile plant with a rich history of use for medicinal, industrial, and nutritional purposes worldwide [1]. Historically, the plant has been cultivated for its durable fibers (hemp), edible seeds, and its psychoactive and therapeutic properties derived from a diverse array of chemical compounds [2,3]. While its recreational use has been the subject of legal and social debate, scientific interest has expanded to include its nutritional and functional attributes.

Beyond the pharmacological properties, *Cannabis sativa* is increasingly recognized as a nutritional valuable plant. Its seeds are recognized for their high nutritional quality and widely referred to as a complete protein source, containing all essential amino acids required for human nutrition, and significant levels of polyunsaturated fatty acids such as α linolenic (omega-3) and linoleic (omega-6) fatty acids in an ideal ratio [4]. In addition, various plant parts, including leaves and seeds, provide dietary fiber, minerals and other macronutrients that contribute to their potential as functional food ingredients [5, 6].

Beyond its nutritional value, *Cannabis sativa* is characterized by a complex phytochemical composition. More than five hundred (500) compounds have been identified, including over one hundred (100) cannabinoids such as Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD) which are the most studied cannabinoids [7, 8]. In addition, the plant contains several other classes of bioactive compounds including flavonoids, terpenoids, lignanamides, and alkaloids, which contribute to its distinctive aroma and therapeutic effects [9].

Many of these compounds possess antioxidant properties capable of neutralizing reactive oxygen species and mitigating oxidative stress [10, 11]. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and antioxidant defenses, plays a critical role in the pathogenesis of chronic diseases, including cardiovascular disorders, cancer, and neurodegenerative conditions [12-14]. Plant-derived antioxidants, particularly phenolics and flavonoids, are known to mitigate oxidative damage by scavenging free radicals and inhibiting lipid peroxidation [15, 16]. Previous studies have demonstrated that *C. sativa* extracts exhibit significant antioxidant activity attributable to these compounds [17, 18]. However, the phytochemical and nutritional composition of *Cannabis sativa* is not uniform: it is influenced by

several factors, including the plant's genotype, the cultivation method, plant part and environmental conditions such as climate and soil composition [9, 19]. These environmental variables can significantly affect the biosynthesis and accumulation of phytochemicals within plant tissues.

Consequently, the biochemical composition of *Cannabis sativa* may vary considerably depending on geographic location and cultivation conditions [20, 22].

In Nigeria, *Cannabis sativa* is widely cultivated in spite of regulatory restrictions, with southwestern states of Nigeria especially Ondo State serving as a major hub for its cultivation. The distinct agroecological characteristics of this region may contribute to biochemical profiles in locally grown plants [23]. Nevertheless, scientific data on the nutritional and phytochemical composition of *C. sativa* cultivated in Nigeria remain limited, as most existing studies originate from Europe, North America, and Asia.

Given this knowledge gap, the present study aimed to evaluate the proximate composition, phytochemical constituents, and antioxidant properties of different anatomical parts of *C. sativa* cultivated in Ondo State, Nigeria. The findings provide baseline data that may support the development of functional foods, nutraceuticals, and plant derived antioxidants from Ondo State *Cannabis sativa* strain.

MATERIALS AND METHODS

Samples collection and preparation

The *Cannabis sativa* L. whole plant were obtained from Nigeria Drug and Law enforcement Agency, Akure, Ondo state headquarters with approval reference number NDLEA/DPRS/11/VOL.1/42. The plants sample was separated into the various plants part such as leaves, seeds, stems, roots and a composite sample was prepared by combining proportional quantities of all anatomical parts. Samples were washed with distilled water to remove debris, extraneous matter and freeze dried using lyophilizer (Drawell scientific instruments, China) at – 57 °C under reduced pressure. The dried samples were ground into powder and kept in airtight screw capped containers and refrigerated at 4 °C prior to analysis.

Proximate analysis

The proximate composition of the samples was determined as described by the Association of Official Analytical Chemists [24]. The parameters evaluated included moisture content, crude fibre, crude fat, ash contents, carbohydrate calculated by the difference and the nitrogen was determined using Kjeldahl method as described by Johnbull *et al* [25] and the percentage crude protein calculated by multiplying with a 6.25 converting factor. This collectively provides an assessment of the nutritional composition of the plant materials.

Phytochemical determination

Phytochemical analysis was conducted to quantify bioactive constituents, such as tannins, saponins, alkaloids, flavonoids, and phenolic compounds which are known to contribute to biological and antioxidant properties of plant materials.

Determination of tannins

Tannin contents was determined using the Folin-Ciocalteu method as described by Iwuozor *et al* [26] with slight modifications. Samples (5 g) were extracted using 70% acetone and centrifuged. The supernatant was then reacted with Folin-Ciocalteu reagents and sodium carbonate. The absorbance was measured at 725 nm, and tannin concentration calculated using a calibration curve prepared from tannic acid standard

Determination of saponins

About 5 g of sample was extracted using 20% aqueous ethanol followed by solvent partitioning with diethyl ether and butanol. The residue obtained after evaporation was dried to constant weight and expressed in unit percentage (Equation 1) as described by Radha Kumar *et al* [27].

$$\text{Saponin (\%)} = \frac{\text{Weight of residue}}{\text{Weight of Original Sample}} \times 100 \quad (1)$$

Determination of alkaloids

The alkaloid content was determined by extraction with 10% acetic acid in ethanol followed by precipitation using ammonium hydroxide using 5 g of sample. The precipitate obtained was

filtered, dried, and weighed, and the percentage alkaloid content was calculated (Equation 2) as described by Nazir *et al* [28].

$$\text{Alkaloid (\%)} = \frac{\text{Weight of precipitate}}{\text{Weight of Original Sample}} \times 100 \quad (2)$$

Determination flavonoids

The total flavanoid content was determined using spectrophotometric method as described by Kasote *et al* [29]. It was quantified using rutin as the standard.

Determination of total phenolic compound

Total phenolic content was determined using the Folin–Denis method. Absorbance was measured at 725 nm, and concentrations were calculated using tannic acid calibration curves [30].

Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical-Scavenging Activity

The free radical scavenging activity was evaluated using the method described by Gulcin and Alwasel [31]. The DPPH assay was used to evaluate the free radical scavenging capacity of the extracts based on their hydrogen-donating ability. Sample extracts were mixed with DPPH solution and incubated in the dark for 60 min. The absorbance was measured at 517 nm, and scavenging activity was calculated as shown in Equation 3:

$$\text{DPPH scavenging activity (\%)} = \frac{A_{\text{blank}} - A_{\text{sample}} \times 100}{A_{\text{blank}}} \quad (3)$$

Where A is the absorbance.

Ferric reducing power assay

Ferric reducing power assay was determined using method described by Landry *et al.* [32]. The FRAP assay was performed to assess the reducing power of the extracts. The samples were reacted with phosphate buffer and potassium ferricyanide, followed by trichloroacetic acid and ferric chloride. The absorbance was measured at 700 nm, with higher absorbance indicating stronger reducing power.

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of the composite blends, leaves, seeds, stem and root of *Cannabis sativa* L. cultivated in Ondo state, Nigeria is presented in Table 1.

Table 1: Proximate composition (mean value) of *Cannabis sativa* L. leaves, stems, root and seeds and a composite blend of all tissues.

Sample	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	Carbohydrates (%)
Composite	10.36	14.34	15.95	27.18	21.48	10.68
Leaves	9.66	17.12	19.63	31.55	7.91	14.14
Seed	7.72	5.73	19.16	36.44	29.50	1.45
Stem	6.41	6.90	8.72	23.69	45.76	8.52
Root	5.59	5.93	9.25	21.16	47.13	10.94

The results showed a marked variation in the nutritional composition across the different plant tissues which highlight the diverse nutritional potential of this plant.

The moisture content ranged from 5.59% (Root) to 10.36% (Composite) indicating relatively low water activity and suggesting good storage stability. The composite sample had the highest moisture, likely reflecting the combined moisture levels of its constituent parts. Lower moisture content is generally desirable for storage stability as it inhibits microbial growth and enzymatic activities [33]. These values are consistent with previous study that reported that cannabis leaves and aerial parts contain 6–11% moisture [34].

Ash content, an indicator of the total mineral content, was highest in the leaves (17.12%) and the composite (14.34%). the seed (5.73%), stem (6.90%), and root (5.93%) had considerably lower ash content. This suggests that the leaves are particularly rich in inorganic minerals. The values obtained in this study are higher compared with Vonapartis *et al.* [35], who reported ash values ranging from 5.1 to 5.8 g 100 g⁻¹ in hemp seeds from ten different *cultivars*. This may be due to environmental conditions such as climate and soil composition [9]. Ash content reflects the inorganic residue and is indicative of mineral richness. The high ash content observed in the leaves

is consistent with findings in other plant species where leaves are primary sites for mineral accumulation [36].

Protein content was notably high in the leaves (19.63%), seed (19.16%), supporting their nutritional relevance and the composite (15.95%). the stem (8.72%) and root (9.25%) had lower protein values which reflect their structural rather than metabolic functions [19]. Proteins are involved in almost all bodily functions. These macromolecules perform diverse biological functions such as signaling proteins, enzymes, and are engaged in essential cellular processes like transcription and DNA replication [37]. The high protein content in leaves and seeds reinforces the potential of *Cannabis sativa* as a valuable protein source, particularly the seeds which are well-known for their complete amino acid profile [4]. This aligns with global interest in plant-based proteins with reports that *Cannabis sativa* seeds are a valuable protein source [38]. This makes cannabis promising for food fortification and malnutrition alleviation.

The fat content was remarkably high across all samples, with the seed having the highest at 36.44%, followed by leaves (31.55%), composite (27.18%), stem (23.69%), and root (21.16%). The very high fat content in the seeds is expected, as cannabis seeds are renowned for their rich oil content, predominantly healthy unsaturated fatty acids like omega-3 and omega-6 (39). This aligns with a similar report by Siano *et al.* [18], who recorded that whole hemp seeds contain approximately 25–35% (by weight) of lipids and 20–25% crude proteins. The fat content in other parts, especially leaves, suggests a broader potential for lipid extraction or as a source of energy.

Fiber content showed an interesting distribution. The root (47.13%) and stem (45.76%) were exceptionally high in fiber, reflecting their structural role in the plant. the seed also had a high fiber content (29.50%). in contrast, the leaves had a much lower fiber content (7.91%), and the composite was moderate (21.48%). This high fiber in roots and stems supports their traditional use in hemp industries for textiles and building materials [40, 41]. The high fiber concentration in seeds contributes to digestive health benefits when consumed [42]. Similar fiber levels have been reported in *Cannabis sativa* stems by Audu *et al* [5].

The stem (8.52 %) and root (10.94%) had comparable levels to the composite (10.68%). The leaves had a higher carbohydrate content (14.14%). Generally, carbohydrates contribute to the energy yield of plants [4].

Phytochemical composition

The phytochemical composition of the different plant parts is presented in Table 2.

Table 2: The mean value of phytochemical analysis of the whole *Cannabis sativa* L. Plant and dried composite of (leaves, stems, root and seed)

Sample	Saponin (%)	Alkaloids (%)	Oxalate (mg/g)	Tannin (mg/100 g)	Flavonoids (mg/100g)	Phenol (mg/100g)
Composite	10.13	4.25	5.46	1.33	1.43	3.48
Leaves	8.07	4.59	9.34	2.21	2.02	8.25
Seed	9.65	1.74	1.80	0.54	0.79	0.21
Stem	5.43	3.01	3.15	1.22	1.43	ND
Root	6.23	6.92	4.53	1.17	1.44	2.74

The analysis revealed the presence of various bioactive compounds, including saponins, alkaloids, tannins, phenolics, and flavonoids, across all plant tissues. The concentration of saponin was found to be (10.13 %) in the composite sample which was the highest when compared with other parts with seed (9.65%), and leaves (8.07%). The stem (5.43%) and root (6.23%) had lower levels. Saponins are known for their foaming properties and various biological activities, including anti-inflammatory and cholesterol-lowering effects [43]. Their presence, particularly in the composite and seeds, suggests the plant potential application in nutraceutical formulations and therapeutic potential of *Cannabis sativa*.

The results showed the presence of alkaloids in all samples, with the root having the highest concentration (6.92%), followed by leaves (4.59%) and composite (4.25%). the seed (1.74%) had the lowest. Alkaloids are class of secondary metabolites that have been found in *C. sativa* and isolated from leaves, stems, pollen, roots, and seeds, according to other reports [17]. Alkaloids are a diverse group of compounds with pharmacological activities, often acting on the central nervous system [44]. Their higher concentration in the root and leaves suggests these parts might have more pronounced pharmacological effects related to alkaloid content. This is consistent with work by Andre *et al.* [17], who reported the presence of alkaloid in cannabis roots. The oxalate levels

varied considerably, the leaves showed a remarkably high oxalate content (9.34 mg/g), followed by the composite (5.46 mg/g) and root (4.53 mg/g), the seed (1.80 mg/g) and stem (3.15 mg/g) had lower concentrations. High oxalate levels in leaves can be a concern as oxalates can bind with essential minerals like calcium, reducing their bioavailability and potentially contributing to kidney stone formation if consumed in large quantities [45]. However, oxalates are common in leafy plants and are not harmful in moderate intake [46, 47]. This finding suggests that direct consumption of raw cannabis leaves might require consideration of oxalate content.

Tannin content was highest in the leaves (2.21 mg/100 g), followed by the composite (1.33 mg/100 g), stem (1.22 mg/100 g), and root (1.17 mg/100 g). The seed had the lowest concentration (0.54 mg/100g). Tannins are polyphenolic compounds known for their astringent properties and antioxidant activity [48,49]. Their presence contributes to the protective mechanisms of the plant and may offer health benefits.

Phenol content varied among the different parts of the plants. The leaves had the highest phenolic content (8.25 mg/100g), followed by the composite (3.48 mg/100g) and root (2.74 mg/100g) while the seed had the lowest phenolic content (0.21 mg/100g). The values of the phenolic content measured in stem quantitated was below the detectable limit, which may reflect methodological limitations. Phenolic compounds are well-known for their strong antioxidant and anti-inflammatory properties [50]. The higher concentration in leaves suggests they are a richer source of these beneficial compounds. Flavonoids were most abundant in the leaves (2.02 mg/100g) and the composite (1.43 mg/100 g) with the root also containing (1.44 mg/100 g), stem contains (1.43 mg/100 g), while the seed had the lowest concentration (0.79 mg/100g).

Flavonoids are powerful antioxidants with diverse pharmacological activities, including anti-cancer and cardiovascular protective effects [51].

The leaves appear to be the primary source of flavonoids in *Cannabis sativa*. These findings support cannabis medicinal potential [52], particularly in its aerial parts. The distribution of phytochemicals in this study highlights the biochemical diversity of *Cannabis sativa* and supports our hypothesis that different plant part could serve distinct functional and pharmacological roles.

Antioxidant activity

The antioxidant capacity of the samples was evaluated using DPPH and Ferric Reducing Antioxidant Power (FRAP) scavenging assays as presented on Table 3.

Table 3: The mean value of antioxidant activities of the whole *Cannabis sativa* L. plant and dried blend of (leaves, stems, root and seed)

Sample	FRAP (mg/100 g)	DPPH (%)
Composite	127.96	91.55
Leaves	110.49	83.73
Seed	117.82	84.64
Stem	142.93	87.64
Root	ND	83.55

The FRAP assay demonstrated that stems exhibited the highest reducing power (142.93 mg/100 g), indicating a strong electron-donating capacity. The strong antioxidant activities observed in this study can be attributed to the presence of phenolic compounds and flavonoids, which are known to act as reducing agents, hydrogen donors, and metal chelators [15]. This finding is consistent with previous studies indicating that different parts of *Cannabis sativa* have varying antioxidant potentials due to their phytochemical profiles. For instance, Andre *et al.* [17] reported that *Cannabis sativa* stems and seeds contain phenolic acids and flavonoids, which contribute to their redox activities. Furthermore, Namdar [53] found that plant parts such as inflorescences and stems possess high antioxidant capacity, supporting the high FRAP value of the stem sample in this study.

In the DPPH assay, the composite showed the highest scavenging activity (91.55%), indicating strong free radical neutralizing potential. This was followed by stem (87.64%), seed (84.64%), leaves (83.73%), and root (83.55%). The high value in the composite could be attributed to the synergistic effect of combining different parts of the plant, each contributing a variety of antioxidant compounds including flavonoids, phenolics, and terpenes. According to Casedas *et al.* [54], the presence of cannabidiol (CBD) and other phytocannabinoids appeared to contribute to the antioxidant activity of the cannabis extract. Additionally, the physicochemical composition of

the plants may change the antioxidant profile, depending on the conditions of the harvest and cultivar. The antioxidant potency observed in this study was higher with findings by Siano *et al.* [18], who observed strong DPPH activities in seeds, oil and flour of the edible fedora cultivar hemp (*Cannabis sativa* L.). Free radicals, are highly reactive chemicals that can harm cells, DNA, and other biomolecules, can be neutralized by antioxidants [55,56]. This study demonstrates that various parts of *Cannabis sativa*, especially the stem and composite, possess good antioxidant properties. The results support the potential of *Cannabis sativa* not only as a medicinal plant but also as a source of natural antioxidants that can be utilized in nutraceutical, food preservation, and therapeutic formulations.

CONCLUSION

The comprehensive evaluation of the nutritional, phytochemical, and antioxidant profiles of *Cannabis sativa* L. cultivated in Ondo State, Nigeria, reveals its vast potential as a functional and medicinal plant. Each plant part exhibited a unique composition that can be harnessed for specific nutritional or therapeutic purposes. Leaves and seeds were rich in proteins and lipids, while stems and roots possessed high amounts of dietary fiber. The phytochemical screening further confirmed the presence of health-promoting compounds such as flavonoids, saponins, tannins, and phenols, predominantly concentrated in the aerial parts, especially leaves and composites. Notably, the high antioxidant activities observed particularly in the stem and composite samples demonstrate the plant's capacity to scavenge free radicals and reduce oxidative stress, validating its traditional and emerging roles in ethnomedicine and modern therapeutics. These results not only reinforce the nutraceutical and pharmacological potential of *Cannabis sativa* but also suggest a pathway for the valorization of its various plant parts like the antioxidant rich stem and composite fractions into functional food, nutraceutical and pharmaceutical product development.

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Ethical Approval

The present study involved only plant materials (*Cannabis sativa* L.) obtained from Nigeria Drug and Law enforcement Agency, Akure, Ondo State headquarters with approval reference number NDLEA/DPRS/11/VOL.1/42. No human participants, clinical samples, or experimental animals were involved in this research. Therefore, ethical approval and informed consent were not required and clinical trial number not applicable.

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