

# Evaluation of the Physico-Chemical and Biochemical Characteristics of the Leaves of *Hibiscus cannabinus* L. Cultivated in Burkina Faso

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## Abstract

*Hibiscus cannabinus* L. is a leafy vegetable that contributes to improving the nutritional status of populations. Leaf production generates significant income in both rural and urban areas [1]. However, national reports on the state of plant genetic resources indicate a decline in cultivation and the loss of local knowledge related to its use, to such an extent that the nutritional value of the leaves remains unknown in Burkina Faso. Hence the need to explore the plant's nutritional potential through an evaluation of the nutritional value of the leaves, in order to revive interest in its cultivation. This study is situated within this context. The study involved 15 samples of powdered young leaves. These were dried in the shade and stored in the laboratory of the Plant Genetics and Breeding Team (EGAP). The determination of water, ash, lipid, protein, and carbohydrate content involved first taking a 5 g sample, and in accordance with the protocol of the respective standards, the content of these parameters was determined using appropriate formulas. The K, Mg, Ca, Fe, Zn, and Na content was measured by atomic absorption spectrophotometry using the AOAC digestion method quoted in [2] with strong acids and 0.5 g

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of ash from the complete incineration of each sample. Analysis of the results revealed significant variability between accessions, structured into four main groups, and interactions between certain agromorphological and chemical parameters. Based on the structure of this variance, two branches can be identified. The first major branch separates Group 1 into a mineral/protein pole. The second branch separates group 2 from groups 3 and 4, highlighting a marked divergence between genotypes with high structural vigor and genotypes exhibiting lower or early performance. These results can serve as a basis for breeders in choosing the parameters to integrate into the selection and improvement programs of *Hibiscus cannabinus* L.

## Keywords

Burkina Faso, *Hibiscus cannabinus* L., Physicochemical and Biochemical Composition, Leaves, Plant Genetic Resources

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## 1. Introduction

Malnutrition remains a major public health problem despite the efforts of organizations and governments. Worldwide, 805 million people, or more than one in eight people, suffer from chronic hunger [3]. This situation is mainly attributable to several deficiencies, including protein-energy malnutrition or undernutrition [4] [5]. According to global estimates, sub-Saharan Africa has the highest prevalence of undernutrition, with sparse progress in recent times. Faced with this situation, the use of local plants as leafy vegetables would be a promising approach to improve the nutritional status of populations if their nutritional potentialities are sufficiently explored and exploited. These local leafy vegetables occupy a place of choice because of their interesting properties such as rapid growth, ease of production and high nutritional value [6]. Indeed, these plants are important sources of vitamins, trace elements, proteins, fibers and carbohydrates [7]. They therefore contribute to improving the nutritional status of populations. In addition to their nutrient richness, local leafy vegetables are available, adapted to agro-ecological conditions and at low production cost [6]. They generate significant income in both rural and urban areas [1]. In Africa, particularly, they provide the essential part of nutritional and medicinal needs [8]. In Burkina Faso, the local dish, called “Kanzaga” prepared from fresh leaves of *H. cannabinus*, is a sauce with strong cultural identity among the Gurunsi [9]. Thus, this dish is increasingly popular during wedding and baptism ceremonies. However, the national reports on the state of plant genetic resources report that its cultivation and endogenous knowledge related to its use have become extinct to such an extent that the nutritional value of leaves remains unknown in Burkina Faso [10]. Therefore, it is imperative that the nutritional potentialities of the plant be explored through an evaluation of the nutritional value of the leaves, for a renewed interest in its cultivation. It is in this context that this present work, which aims to evaluate the physico-chemical and biochemical composition of the leaves of *Hibiscus cannabinus*. It will specifically

involve (i) determining the composition of micronutrients and macronutrients of the leaves and (ii) comparing the specific performance of the different accessions of *Hibiscus cannabinus* L studied.

## 2. Material and Methods

### 2.1. Genetic Material

The study took place from September 15 to September 30, 2025, at the National Public Health Laboratory (L.N.S.P) of Ouagadougou, which became the National Agency for Health Safety of the Environment, Food, Work and Health Products (ANSSEAT). It focused on 15 powder samples of young leaves of the different accessions of *Hibiscus cannabinus* L. studied. The young leaves were harvested in November 2018 in an experimental setup of 863.72 m<sup>2</sup>, managed at the experimental station of the Institute of Rural Development (IDR) in Gampèla, a locality located on the Ouaga-Niger axis at 12° 15' North latitude and 1° 12' West longitude. The climate is of the Sudano-Sahelian type with a long dry season from November to May and a short humid season from June to October [10]. In 2016, the experimental station recorded an annual rainfall of 974.3 mm between May and October. The soil is of the sandy-clay type with a water pH of 5.20. The analysis of this soil shows that it is composed of total nitrogen (0.046% - 0.071%), total phosphate (9.71 - 10.91 ppm) and total potassium (84.18 - 101.23 ppm) [11]. The experimental design was in incomplete randomized blocks with three (3) replications. Each block was subdivided into two (02) sub-blocks. The distances between the blocks and sub-blocks were respectively 1 m. Each sub-block was subdivided into four (04) elementary plots, each containing 8 rows spaced 0.8 m apart. Each row contained eight (08) hills spaced 0.6 m apart, from which young leaves were collected from four (04) plants randomly selected per row, forming the accession sample for physico-chemical and biochemical analyses. The five (05) agromorphological variables (FLD, PLH, SHD, WDL, WFL) were also measured at the flowering stage on these four randomly chosen plants per row (Table 1). The young leaves were previously shade-dried and stored in the laboratory of the Plant Genetics and Improvement Team (EGAP) at Joseph KI-ZERBO University from November 2018 to September 2025 in airtight packaging to prevent moisture reabsorption and in ventilated compartments, away from direct light, clean, and protected from pests, exclusively reserved for germplasm storage. The harvested young leaves come from seed accessions collected following an ethnobotanical survey in 2017 in five provinces of Burkina Faso, with the same codes being used to name the leaf accessions (Table 2).

**Table 1.** Agromorphological variables of *H. cannabinus* and measurement techniques.

Agromorphological variables	Measurement techniques
Flowering Date (FLD) [days]	By counting the number of days separating the sowing and the flowering of at least 50% of the plants along the entire row containing the accession

## Continued

<b>Plant Height (PLH) [m]</b>	Measured from the ground to the last leaf of the main stem using a well-marked ruler
<b>Shank Diameter (SHD) [cm]</b>	Measured at the neck using a caliper
<b>Weight of Fresh Leaves (WFL) [g]</b>	Evaluated by weighing fresh leaves from the four randomly chosen plants using an electronic balance model PL 601-L/00 from METTLER TOLEDO
<b>Weight of Dry Leaves (WDL) [g]</b>	Evaluated by weighing the dry leaves from the four randomly chosen plants using an electronic scale model PL 601-L/00 from the brand METTLER TOLEDO

**Table 2.** Accession codes and origins.

Accession codes	Provinces	Villages	Climate
KAT1	Kadiogo	Téyoko	Sudano-Sahelian
KOG2		Gambaga	Sudano-Sahelian
KOK1		Kagadguin	Sudano-Sahelian
KOK2		Kagadguin	Sudano-Sahelian
KOK3		Kagadguin	Sudano-Sahelian
KOW2	Kouritenga	Wolgo	Sudano-Sahelian
KOZ1		Zaogo	Sudano-Sahelian
KOZ3		Zaogo	Sudano-Sahelian
KZA3		Zakaré	Sudano-Sahelian
NAG2		Goumpia	Sudanian
NAT2	Nahouri	Tiakané	Sudanian
OUT1	Oubritenga	Tanguin	Sudano-Sahelian
SID2		Danfina	Sudanian
SIH1	Sissili	Bieha	Sudanian
SIS2		Sissili	Sudanian

Code = The first two initials of the province of origin of the accession + the first initial of the village + the order number of the sample in the locality.

## 2.2. Analysis of Physico-Chemical and Biochemical Parameters

The samples of the powder from the young leaves of *Hibiscus cannabinus* L, were analyzed to determine their physico-chemical and biochemical parameters such as water content, ash, potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), sodium (Na) and in lipids, proteins, carbohydrates. The water content was determined by differential weighing after passage in the oven at 1052°C according to standard [12] using the one-night method. The ash content was determined by differential weighing after passing the samples in the oven according to standard [13]. The lipid content of the samples was determined by the Soxhlet

method, according to [14]. Protein determination was carried out by the Kjeldahl method quoted in [2] according to standard [15]. The carbohydrate content was determined by calculating the difference proposed by [16]. The determination of the content of these parameters previously consisted of taking 5 g of the sample of the powder from the young leaves of *Hibiscus cannabinus* L and in accordance with the protocol of the respective standards, the content of the different parameters was determined by the formulas recorded in **Table 3**. The minerals were determined by atomic absorption spectrophotometry according to the digestion method of AOAC quoted in [17] using strong acids. Thus, an amount of 0.5 g of ash from the incineration of each of the powders of the samples was dissolved in 31 ml of a mixture consisting of perchloric acid (11.80 mol/l), nitric acid (14.44 mol/l) and sulfuric acid (18.01 mol/l). The mixture obtained was well shaken under the hood and then heated on a griddle until thick white fumes appeared. The reaction medium was then cooled on the bench for 10 minutes, then diluted in 50 ml of distilled water. It was brought to a boil again for 30 minutes, then cooled under the same conditions. The mixture was then filtered on the Whatman filter paper n°4 and the resulting filtrate was completed at the gauge mark of the flask (50 ml) with distilled water. The content of each mineral element was determined with a VARIAN AA.20 flame atomic spectrophotometer at a specific wavelength by comparison to standard solutions. The contents were expressed in mg/100 g of dry matter (DM).

**Table 3.** Formulas used.

Nº	Parameters	Formulas used
1	humidity (%)	$H (\%) = [Pe - (Pf - Pv)/Pe] \times 100$
2	ashes (%)	$C (\%) = (Pf - Pv/Pe) \times 100$
3	Lipid (%)	$MG (\%) = [Pf - Pv/Pe] \times 100$
4	protein (%)	$P (\%) = [N (\%) \times 6.25]$ ; $N (\%) = [0.014 \times 0.1 \times (Ve - Vb) \times 100]/Pe$
5	carbohydrates (%)	$G (\%) = 100 - [H (\%) + P (\%) + MG (\%) + C (\%)]$

Pe: test sample; Pf: final weight (basket + dehydrated sample, or crucible + calcined sample, or flask + fat); Pv: tare weight of the baskets, or crucibles, or balloons in g; Ve: fall of the cruet (sample), Vb: fall of the cruet (white), 0.1: sulphuric acid titre, 0.014: nitrogen molar weight  $\times 10^{-3}$ , 6.25: conversion factor to sulphuric acid equivalent.

### 2.3. Statistical Analyses of Data

The 2016 Excel spreadsheet was used for data entry and table management. The data collected was analyzed using the statistical software R version 4.0.3 (R Core Team 2020). Thus, the analysis of variance (ANOVA), followed by the Pearson correlation matrix were carried out in order to compare and establish the links between the agromorphological parameters of *Hibiscus cannabinus* with the physico-chemical and biochemical parameters of the leaves. The data were pre-

sented as a mean, the corresponding standard deviations and each experiment was carried out in a set of two distinct replicates ( $n = 2$ ) for physico-chemical parameters (water, ash, Fe, Mg, Ca, Zn, K, Na) and three distinct replicates ( $n = 3$ ) for the biochemical parameters (lipids, proteins, carbohydrates). In order to characterize the collected samples using their physico-chemical and nutritional profile, a principal component analysis (PCA) was carried out using only the parameters that could discriminate the accessions. Furthermore, a global thermal performance map was carried out with the aim of evaluating the specific performances of the accessions, which led to the grouping of individuals according to phenotypic and chemical similarity by Hierarchical Classification.

### 3. Results

#### 3.1. Variation of the Agromorphological Parameters of *Hibiscus cannabinus* L. and the Physico-Chemical and Biochemical Composition of the Leaves

The results of the analysis of variance (ANOVA) show a significant difference at the 1% threshold for the majority of the measured parameters ( $P < 0.0001$ ). Indeed, high ( $CV > 30\%$ ) and low ( $CV < 10\%$ ) coefficients of variation were recorded respectively for the weight of fresh leaves ( $CV = 47.32\%$ ) and for the height of the plant ( $CV = 8.29\%$ ). The results also reveal a variability in the physico-chemical and biochemical composition of the leaves (Table 4). High coefficients of variation ( $CV > 30\%$ ) were recorded for magnesium content ( $CV = 34.40\%$ ), sodium content ( $CV = 36.36\%$ ) and potassium content ( $CV = 36.16\%$ ). Moisture content ( $CV = 0.59\%$ ) and carbohydrate content ( $CV = 4.25\%$ ) provided low values ( $CV < 10\%$ ) of the coefficient of variation. As for the other characters, they presented average coefficients of variation. *Hibiscus cannabinus* leaves contain a high content of calcium (206.45 mg/g DM), potassium (101.83 mg/g DM), water (93.77 mg/g DM), carbohydrates (67.30 mg/g DM), magnesium (54.47 mg/g DM), protein (20.75 mg/g DM) and iron (12.41 mg/g DM). Moreover, the contents of sodium, lipid and zinc are low (3.95 mg/g DM; 3.51 mg/g DM and 2.34 mg/g DM respectively).

**Table 4.** Variation in agro-morphological, physico-chemical and biochemical parameters.

Variables	Min	Max	Mean	SD	CV (%)	P-value
FLD	59.916	87.500	75.744	7.851	10.365	<0.0001
PLH	2.233	2.950	2.604	0.216	8.297	0.0799 ns
SHD	2.591	3.766	3.041	0.314	10.323	0.8240 ns
WDL	35.600	72.033	58.748	9.650	16.426	<0.0001
WFL	424.600	1765.566	1187.020	561.802	47.328	<0.0001
Ca	126.305	262.227	206.454	47.514	23.014	<0.0001
Mg	26.630	106.797	54.470	19.287	35.409	<0.0001
Na	2.240	7.960	3.953	1.437	36.368	<0.0001

## Continued

Fe	5.905	15.720	12.416	2.680	21.586	<0.0001
Zn	1.190	3.363	2.344	0.569	24.311	<0.0001
K	45.025	149.575	101.832	36.830	36.167	<0.0001
Humidity	93.030	94.800	93.777	0.558	0.595	<0.0001
Ash	4.470	9.760	8.421	1.207	14.338	<0.0001
Lipid	2.620	6.960	3.516	0.994	28.280	<0.0001
Protein	15.225	23.450	20.755	2.647	12.754	<0.0001
Carbohydrate	61.930	72.995	67.307	2.862	4.253	<0.0001

Min: Minimum; Max: Maximum; SD: Standard Deviation; CV: Coefficient of Variation; FLD: Flowering Date; PLH: Plant Height; SHD: Shank Diameter; WDL: Weight of Fresh Leaves; WFL: Weight of Fresh Leaves; Ca: Calcium content; Mg: Magnesium content; Na: Sodium content; Fe: Iron content; Zn: Zinc content; K: Potassium content; Humidity: Water content; Ash: Ash content; Lipid: Lipid content; Protein: Protein content; Carbohydrate: Carbohydrate content.

### 3.2. Interaction between Agromorphological, Physico-Chemical and Biochemical Parameters

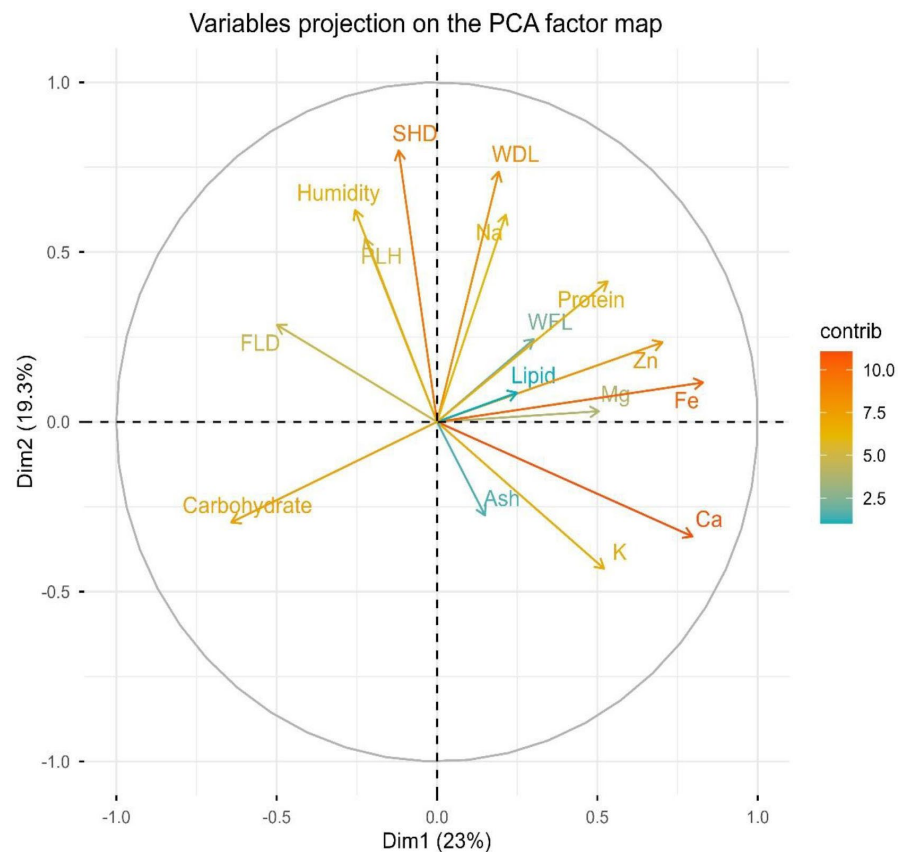
**Table 5.** Pearson correlation matrix between variables.

Variables	FLD	PLH	SHD	WDL	WFL	Ca	Mg	Na	Fe	Zn	K	Humidity	Ash	Lipid	Protein	Carbohydrate
FLD	1	-0.22	0.34	-0.15	-0.16	-0.41	-0.48	0.17	-0.24	-0.24	-0.25	0.48	-0.29	0.15	-0.05	0.12
PLH	-0.22	1	0.39	0.52	0.18	-0.44	0.22	0.18	-0.19	0.03	-0.43	0.29	0.16	-0.17	-0.15	0.13
SHD	0.34	0.39	1	0.45	0	-0.14	-0.12	0.6	-0.13	-0.06	-0.16	0.51	-0.29	0.11	0.29	-0.18
WDL	-0.15	0.52	0.45	1	0.3	-0.21	0.04	0.19	0.23	0.34	-0.26	0.23	-0.22	0.08	0.39	-0.3
WFL	-0.16	0.18	0	0.3	1	0.01	0.17	-0.22	0.28	0.34	-0.17	-0.05	-0.14	0.01	0.29	-0.21
Ca	-0.41	-0.44	-0.14	-0.21	0.01	1	0.3	0.06	0.57	0.36	0.72	-0.36	0.06	0.24	0.31	-0.4
Mg	-0.48	0.22	-0.12	0.04	0.17	0.3	1	0.19	0.32	0.33	0.17	-0.11	0.01	-0.04	0.19	-0.16
Na	0.17	0.18	0.6	0.19	-0.22	0.06	0.19	1	0.24	0.31	-0.05	0.39	0.04	0.02	0.31	-0.31
Fe	-0.24	-0.19	-0.13	0.23	0.28	0.57	0.32	0.24	1	0.88	0.45	0.15	0.07	0.28	0.24	-0.35
Zn	-0.24	0.03	-0.06	0.34	0.34	0.36	0.33	0.31	0.88	1	0.32	0.22	0.17	0.03	0.14	-0.21
K	-0.25	-0.43	-0.16	-0.26	-0.17	0.72	0.17	-0.05	0.45	0.32	1	-0.17	-0.03	-0.15	0.05	0.02
Humidity	0.48	0.29	0.51	0.23	-0.05	-0.36	-0.11	0.39	0.15	0.22	-0.17	1	-0.29	-0.06	-0.12	0.26
Ash	-0.29	0.16	-0.29	-0.22	-0.14	0.06	0.01	0.04	0.07	0.17	-0.03	-0.29	1	0.23	-0.3	-0.23
Lipid	0.15	-0.17	0.11	0.08	0.01	0.24	-0.04	0.02	0.28	0.03	-0.15	-0.06	0.23	1	0.01	-0.46
Protein	-0.05	-0.15	0.29	0.39	0.29	0.31	0.19	0.31	0.24	0.14	0.05	-0.12	-0.3	0.01	1	-0.81
Carbohydrate	0.12	0.13	-0.18	-0.3	-0.21	-0.4	-0.16	-0.31	-0.35	-0.21	0.02	0.26	-0.23	-0.46	-0.81	1

The analysis of the Pearson correlation matrix (**Table 5**) highlights numerous sig-

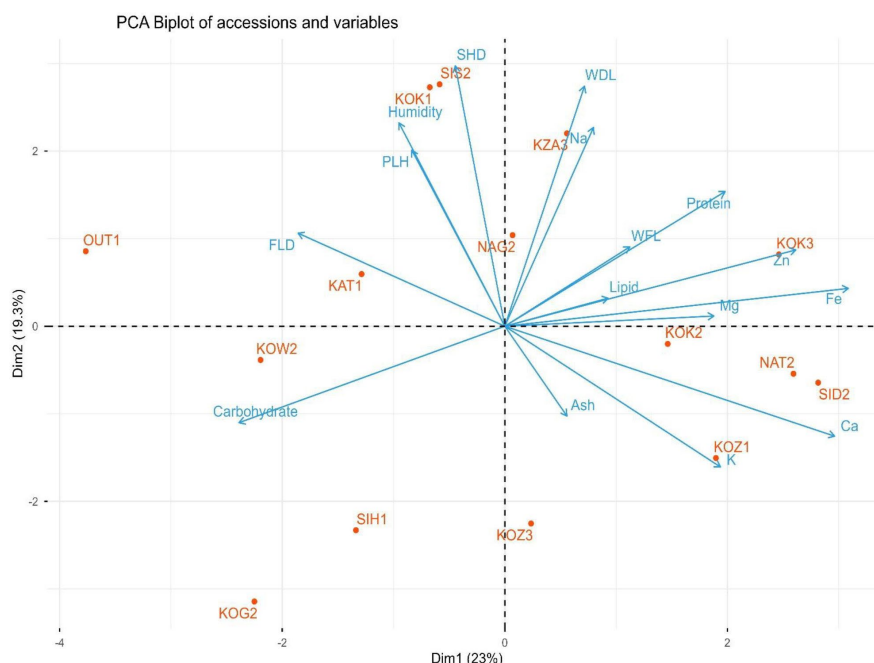
nificant correlations at the 1% threshold between the agromorphological parameters of *Hibiscus cannabinus* L., and the physico-chemical and biochemical parameters of its leaves. Indeed, the diameter of the stem is positively and strongly correlated with the sodium content of the leaves ( $r = 0.60$ ) but moderately correlated with the moisture content of the leaves ( $r = 0.51$ ). In addition, there is a strong positive correlation between calcium content, with the potassium content of *Hibiscus cannabinus* leaves ( $r = 0.72$ ) and a positive and highly significant correlation with iron content of the leaves ( $r = 0.57$ ). Moreover, there is also a positive and strong correlation between the iron content and the zinc content of *Hibiscus cannabinus* L. leaves ( $r = 0.88$ ). However, a negative and significant correlation is revealed between lipid content and carbohydrate content of the leaves ( $r = -0.46$ ). The latter (carbohydrate content) has a strong negative correlation with the leaf protein content ( $r = -0.81$ ).

### 3.3. Association of the Agromorphological, Physico-Chemical and Biochemical Parameters Studied, with the Axes



FLD: Flowering Date; PLH: Plant Height; SHD: Shank Diameter; WDL: Weight of Fresh Leaves; WFL: Weight of Fresh Leaves; Ca: Calcium content; Mg: Magnesium content; Na: Sodium content; Fe: Iron content; Zn: Zinc content; K: Potassium content; Humidity: Water content; Ash: Ash content; Lipid: Lipid content; Protein: Protein content; Carbohydrate: Carbohydrate content.

**Figure 1.** Projection of variables in plan 1 - 2 of the main component analysis (PCA).

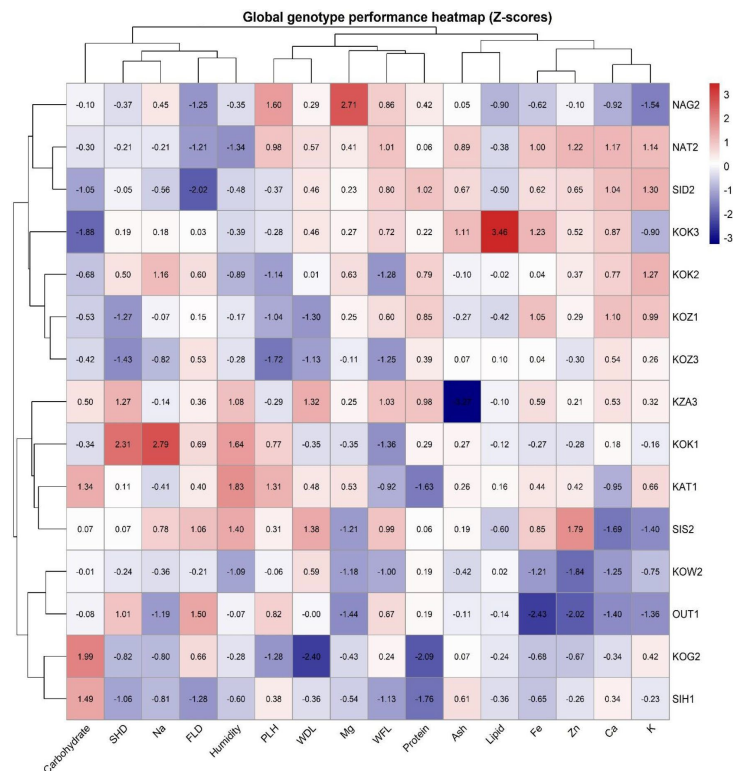


Code = The first two initials of the province of origin of the accession + the first initial of the village + the sample's order number in the locality. FLD: Flowering Date; PLH: Plant Height; SHD: Shank Diameter; WDL: Weight of Fresh Leaves; WFL: Weight of Fresh Leaves; Ca: Calcium content; Mg: Magnesium content; Na: Sodium content; Fe: Iron content; Zn: Zinc content; K: Potassium content; Humidity: Water content; Ash: Ash content; Lipid: Lipid content; Protein: Protein content; Carbohydrate: Carbohydrate content.

**Figure 2.** Statistical representativeness of accessions in the factorial plan 1 - 2.

The results of the Principal Component Analysis (PCA), illustrate the contribution and correlation of variables on the factorial 1 - 2 level, which explains a total of 42.3% of the variance (Axe1: 23%; Axe2: 19.3%). In the plane 1/2 (**Figure 1**), axis 1 with 23% of total inertia is strongly defined by a group of nutritional and mineral variables positively correlated to each other such as iron ( $r = 0.88$ ), calcium ( $r = 0.78$ ), zinc ( $r = 0.70$ ), potassium ( $r = 0.55$ ), protein ( $r = 0.55$ ), magnesium ( $r = 0.50$ ), lipid ( $r = 0.25$ ) and the weight of fresh leaves ( $r = 0.30$ ). The calcium, iron, potassium, zinc and protein content have the highest contributions on this axis, which negatively associates carbohydrate content ( $r = -0.68$ ) and flowering date ( $r = -0.50$ ). This axis characterizes accessions such as SID2, NAT2, KOK3, KOZ1 and KOK2 (**Figure 2**), having a good yield of fresh leaves rich in protein, lipid and minerals such as iron (Fe), calcium (Ca), zinc (Zn), potassium (K) and magnesium (Mg) essentially. On the other hand, axis 2 which totals 19.3% of the total variance strongly and positively associates the parameters stem diameter ( $r = 0.80$ ), dry leaf weight ( $r = 0.75$ ), moisture content ( $r = 0.70$ ), sodium content ( $r = 0.70$ ) and plant height ( $r = 0.55$ ). This axis shows a clear opposition between the parameters mentioned above and the carbohydrate content ( $r = -0.25$ ). It characterizes tall accessions with robust stems such as SIS2, KOK1, KZA3 and NAG2, having a good yield of water- and sodium-rich leaves (**Figure 2**).

### 3.4. Global Thermal Map Analysis of Accessions Performance



Code = The first two initials of the province of origin of the accession + the first initial of the village + the sample's order number in the locality. FLD: Flowering Date; PLH: Plant Height; SHD: Shank Diameter; WDL: Weight of Fresh Leaves; WFL: Weight of Fresh Leaves; Ca: Calcium content; Mg: Magnesium content; Na: Sodium content; Fe: Iron content; Zn: Zinc content; K: Potassium content; Humidity: Water content; Ash: Ash content; Lipid: Lipid content; Protein: Protein content; Carbohydrate: Carbohydrate content.

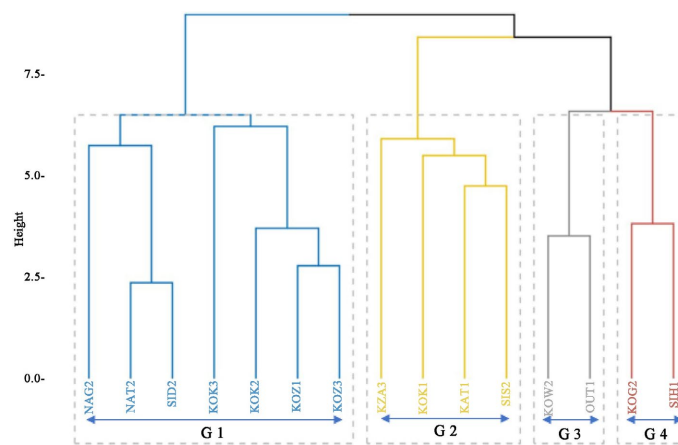
**Figure 3.** Global thermal map of accession performances.

The factual analysis of the global thermal performance map reveals a visual synthesis of standardized performances (Z-scores) for each genotype (accession) across all measured variables (Figure 3). Indeed, the variables are grouped by profile similarity. Essential minerals (Fe, Zn, Ca, K) are clustered together, as are vigor parameters (PLH, WDL, WFL). The carbohydrate content has a distinct distribution profile from other phytochemical variables. Regarding the specific performance of the accessions, NAG2 has a maximum magnesium content (Mg: 2.71). The accession KOK3 is distinguished by the highest lipid score (Lipid: 3.46), while KOK1 displays the highest values for sodium content (Na: 2.79) and stem diameter (SHD: 2.31). The highest levels of zinc and carbohydrates are expressed respectively, by the accessions SIS2 (Zn: 1.79) and KOG2 (Carbohydrate: 1.99). On the other hand, some genotypes showed very low ash, protein, iron, zinc and carbohydrate contents such as KZA3 (Ash: -3.27), KOG2 (Protein: -2.09), OUT1 (Fe: -2.43; Zn: -2.02) and KOK3 (carbohydrate: -1.88), respectively. In addition, the KOG2 and SIS2 genotypes exhibited low dry leaf weight (WDL: -2.40) and

late flowering (FLD:  $-2.02$ ), respectively. A hierarchical grouping of individuals allows dividing the 15 genotypes into several distinct groups according to their overall biological signature. A first upper group includes NAG2, NAT2, SID2 and KOK3, characterized by heterogeneous performance but globally positive on minerals. A second lower group includes KOG2 and SIH1, showing a predominance of negative values on the majority of nutritional parameters.

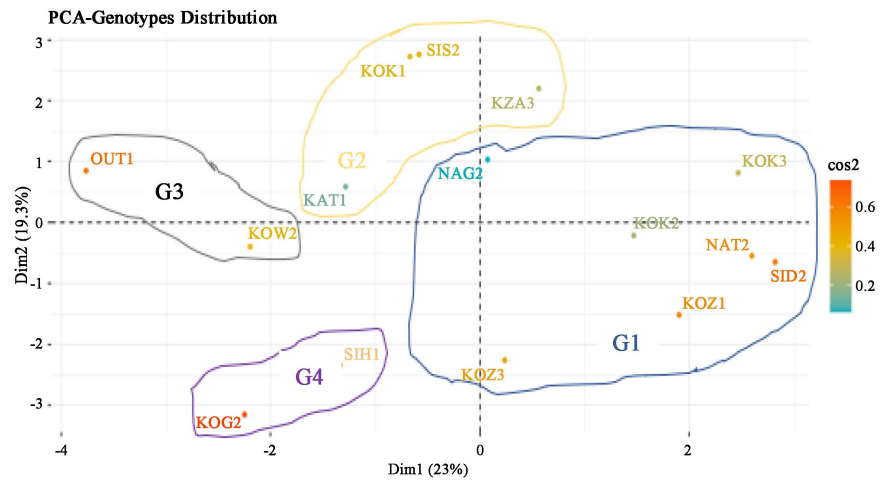
### 3.5. Organization of the Diversity of the 15 Accessions of *Hibiscus cannabinus* L.

The analysis of the dendrogram from the Hierarchical Classification of accessions allows grouping individuals by phenotypic and chemical similarity. The classification highlights four main groups, whose structure of genetic diversity is based on all agromorphological and chemical parameters (**Figure 4**). Group 1, consisting of seven accessions (NAG2, NAT2, SID2, KOK3, KOK2, KOZ1 and KOZ3) is the most abundant group in individuals with chemical similarity. This group separated from all other genotypes at the highest level of dissimilarity (Height  $> 8.5$ ). Group 2 brings together four individuals including KZA3, KOK1, KAT1 and SIS2. Groups 3 and 4, each composed of two individuals respectively KOW2, OUT1 and KOG2, SIH1; constitute the most restricted groups. The analysis of inter-genotype proximity relationships reveals strong similarities. Indeed, the genotype pairs with the shortest distances (most similar) are NAG2/NAT2 and KOG2/SIH1, with a branch height of less than 3.0. There are also moderate similarities in this case within group 1 where the KOK3/KOK2 subgroup joins the KOZ1/KOZ3 complex at a height of about 4.0. In group 2, KAT1 and SIS2 show greater proximity to each other than with KZA3 or KOK1. In connection with the structuring of variance, two phyla can be retained. The first major phylum separates group 1 as a mineral/protein pole. The second phylum separates group 2 from groups 3 and 4, highlighting a marked divergence between genotypes with high structural vigor and genotypes with lower or early performance (**Figure 5**).



Code = The first two initials of the province of origin of the accession + the first initial of the village + the sample's order number in the locality.

**Figure 4.** Dendrogram from the hierarchical classification of 15 accessions.



Code = The first two initials of the province of origin of the accession + the first initial of the village + the sample's order number in the locality.

**Figure 5.** Representation in the 1/2 plan of the AFD of accession groups from the CAH.

#### 4. Discussion

The analysis of the physico-chemical and biochemical composition of the leaves of *Hibiscus cannabinus* L. reveals the existence of a variability between accessions. The analysis of variance (ANOVA) significantly showed that all physico-chemical and biochemical parameters discriminate accessions ( $P < 0.0001$ ). Specifically, this variability concerns parameters related to mineral compounds (Mg, Ca, Fe, Zn, K and Na), organic substances (proteins, carbohydrates, lipids) and chemical parameters such as ash and water content. These parameters would be effective in the study of the genetic diversity of *H. cannabinus* cultivated in Burkina Faso. Similar variabilities were mentioned by [18] [19] on the main components of the essential oil of the leaves of *Hibiscus cannabinus* L. including E-phytol (28.16%), Z-phytol (8.02%), n-nonanal (5.70%), acetaldehyde benzene (4.39%), the (E)-2-hexenal (3.10%) and 5-methylfurfural (3.00%). High coefficients of variation ( $CV > 30\%$ ) were also recorded for magnesium content, sodium content and potassium content. According to [20], a high value of the coefficient of variation ( $CV > 30\%$ ) reflects a strong heterogeneity of the studied material. Thus, the accessions of *Hibiscus cannabinus* L. studied are highly heterogeneous for these three parameters. The different correlations provide information on the genetic association between various traits under specific environmental conditions. This could help in the formulation of selection methods within the framework of varietal improvement based on existing variability. Thus, the significant and positive correlations between stem diameter and leaf sodium content and leaf moisture characters indicate that cultivars that expressed high vigor by their diameter, are those that produce leaves rich in water and sodium. In addition, the strong positive correlation of calcium content with potassium and iron content suggests that *H. cannabinus* plants which have calcium-rich leaves also contain high levels of potassium and iron. Since iron content is strongly correlated with zinc content, it

would mean that a selection oriented towards calcium content would also involve that of zinc in addition to potassium and iron. However, the strong negative correlation of lipid content with the carbohydrate content of the leaves, which is strongly negatively correlated with protein content, shows that improving *Hibiscus cannabinus* for lipid content could indirectly be so for protein content. All these different correlations between agromorphological characters with physico-chemical and biochemical elements, constitute an indispensable tool for the enhancers in the choice of characters to be integrated into selection and improvement programs for nutritional elements in the leaves of *Hibiscus cannabinus* L. Indeed, when characters are positively correlated, the selection for improvement of one leads indirectly to that of the other. This is what would justify the grouping of positively correlated nutritional and mineral variables such as iron, calcium, zinc, potassium, protein, magnesium and lipid content, with accessions SID2, NAT2, KOK3, KOZ1 and KOK2 on axis 1 which totals 23% of inertia. These accessions are therefore essential leafy vegetables whose regular and sufficient consumption would greatly contribute to meeting the micronutrient needs for development and adequate protection against deficiency diseases. Indeed, regular consumption of vegetable proteins reduces the risk of long-term hypertension [21]. Lipids contribute significantly to the energy value and participate in the bioavailability of fat-soluble vitamins in the body [22]. A diet rich in potassium could help regulate blood pressure and support the proper functioning of the nervous system [23]. As for the calcium content, it could contribute to the daily intake recommended by the FAO which is 400 to 500 mg for adults [24]. The selection and improvement of these cultivars with high nutritional potential would be an effective means in the fight against poverty and malnutrition [25]. The high levels of mineral elements and organic substances recorded in the leaves of certain *Hibiscus cannabinus* L. cultivars would result from a combination of genetic, pedoclimatic, and physiological factors. They can be explained by efficient root absorption, active photosynthesis, and adaptation to rich soils, coupled with an inherent metabolic capacity to concentrate nutrients and metabolites. [26] also observed a positive correlation between leaf area and potassium content. All these different interactions between the agromorphological characters with the physico-chemical and biochemical elements, are an indispensable tool for breeders in the choice of parameters to be integrated into selection and improvement programs for biochemical and chemical elements in the leaves of *Hibiscus cannabinus*.

## 5. Conclusion

The study of the physico-chemical and biochemical composition of the leaves of *Hibiscus cannabinus* L. has highlighted the existence of variability within the studied accessions. The evaluation of physico-chemical and nutritional characteristics revealed that the accessions analyzed are rich in mineral elements and organic substances necessary for adequate protection against deficiency diseases and maintenance of the physiological balance of the organism. Interesting correlations be-

tween biochemical and morphological parameters were also noted. The study also revealed that a hierarchical grouping of individuals allows dividing the 15 genotypes into several distinct groups according to their overall biological signature. The first upper group, characterized by heterogeneous performance but globally positive on minerals and a second lower group, with a predominance of negative values on the majority of nutritional parameters. For a selection of vigorous genotypes rich in magnesium, lipid, carbohydrate, and zinc, the accessions KOK1, NAG2, KOK3, KOG2, and SIS2 are suitable. These data could be used in breeding and improvement programs of *Hibiscus cannabinus* L. as a leaf vegetable with the aim of contributing to the fight against nutritional deficiencies in Burkina Faso.

### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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