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Nutritional Valorization and Chemical Composition of Seeds of *Hibiscus sabdariffa sabdariffa* from Benin

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Abstract *Hibiscus sabdariffa sabdariffa* is a non-timber forest product found in sub-Saharan Africa and used in both pharmacopoeia and food. The present study aims to evaluate the chemical composition and the nutritional potential of *Hibiscus sabdariffa sabdariffa* from Benin. Thus, the moisture content and volatile matter has been determined according to ISO 665:2000; the ash following the standard ISO 749:1977, the proteins according to ISO 5983-1:2005; the total carbohydrates by the difference formula; the oil content according to ISO 734:2015; the content of mineral and trace elements by atomic absorption spectrophotometry and secondary metabolites following staining and precipitation reactions specific to each metabolite family. The results revealed that the seed of *Hibiscus sabdariffa sabdariffa* contains 95.27% of dry matter; 6.33% of ash; 19.18% of proteins; 56.16% of carbohydrates; 13.6% of oil and provides 423.76 kcal/100g. As secondary metabolites, saponosides, alkaloids, coumarins and reducing compounds were identified. The content of mineral and trace elements showed 61.70 mg/kg of manganese, 1.91% of potassium, 199.16 mg/kg of iron, 4663.60 mg/kg of magnesium, 178.07 mg/kg of sodium and 0.24% of calcium. These results therefore reflect a high nutritional potential of the seeds of *Hibiscus sabdariffa sabdariffa*, favorable to the establishment of a viable agro-food chain for this NTFP already commonly used by the people of Benin.

Keywords: *nutritional characterization, Hibiscus sabdariffa sabdariffa, seeds, Benin*

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1. Introduction

For a very long time, plant species have been used by humans for various purposes [1]. Indeed, the rich and diverse African flora plays a big role in Africa economy. Thus several species of *Hibiscus* are used as ornamental and/or medicinal plants. In the genus *Hibiscus*, the plant species *Hibiscus sabdariffa* is a non-timber forest product (NTFP) found in tropical and subtropical regions of the world [2], which is used in pharmacy and in feeding. Known as "bissap", *Hibiscus sabdariffa* is a shrub of the Malvaceae family which two varieties are commonly encountered: this is *Hibiscus sabdariffa* variety *sabdariffa* whose calyxes are red and *Hibiscus sabdariffa* variety *altissima* which is endowed with green calyxes [2]. Previous studies on the calyxes of the variety *sabdariffa* showed their richness in anthocyanins, and their antioxidant, anti-inflammatory and anti-cancer properties [3,4]. In Benin, previous recearch [5,6,7] have demonstrated the use of this seed as a dietary supplement on the one hand, and as a ferment in the production of traditional condiments (*yanyanku and Ikpiru*). The seeds of this plant species have a good scientific interest because they still are underutilized oilseeds. This under-utilization is a huge shortfall in Benin's GDP as the oilseed market is growing. Indeed global oilseed consumption increased from 392.0 Mt in 2013 to 444.5 Mt in 2018 with an increase of 13.4% over the 2013 level. Also, forecasts of 489.6 Mt for 2022 indicate an increase in world oilseed demand of 24.9% compared to the 2013 level [8]. In view of the above, the main objective of this study is to contribute to the establishment of a viable agri-food chain for this NTFP, through the agro-food valorization of seeds produced by this plant species.

2. Material and Methods

2.1. Harvesting and Pre-conditioning of Plant Material

The plant material consists of the seeds *of Hibiscus sabdariffa sabdariffa* harvested in December 2017 in Lobogo, on the Valley farm in the department of Mono (Benin). After the harvest, the calyxes were shelled; the seeds were separated from the hulls and then dried for one month at 25°C, away from UV light. These dried seeds were ground into powder using an electric grinder and the resulting powder was stored in closed bottles with airtight closing, under a hood for later use.

2.2. Methods

2.2.1. Physical Description of the Seeds

Parameters such as a shape, color and size of seeds of *Hibiscus sabdariffa sabdariffa* were evaluated by a visual description. In addition the average weight of the seeds was determined by weighing using a precision balance.

2.2.2. Nutritional Potential of *Hibiscus sabdariffa sabdariffa* Seeds from Benin

In order to know the nutritional potential of the seeds of *Hibiscus sabdariffa sabdariffa*, different Nutritional parameters of these seeds were evaluated according to different standard methods summarized as below:

2.2.2.1. Moisture Content and Volatile Matter of *Hibiscus sabdariffa sabdariffa* Seeds

The moisture content and volatile matter content was determined according to ISO 665: 2000 [9,10]**.** Ten grams (10 g) of powder were weighed in a previously dried and tared crucible. The crucible containing the sample was kept in an oven at 103 ± 2°C for 48 hours. After cooling in a desiccator, the crucible was weighed again. This operation was repeated until a constant mass was obtained between two consecutive weightings and a reduction of the drying time. The moisture content and volatile matter (Tw) as a percentage of the sample mass was obtained by equation (1).

$$Tw = \frac{m1 - m2}{m1 - m0} \quad (1)$$

T_w: moisture content and volatile matter in % (m/m) of dry matter
m_0: mass of the vacuum crucible (g).
m_1: mass of the crucible containing the sample before drying (g).
m_2: mass of the crucible containing the sample after drying (g).

2.2.2.2. Protein Content

The nitrogen content was obtained by the KJELDAHL method from the micro-distillation of the mineralized material of the sample digested with sulfuric acid in the presence of a selenium-based catalyst according to ISO 5983-1: 2005 [11,12]. This micro-distillation was done by steam distillation in the presence of a normal solution of sodium hydroxide. In order to obtain the nitrogen content of the sample, the distillate collected in boric acid was titrated with sulfuric acid in the presence of methyl red (2). The nitrogen content obtained was multiplied by 6.25 to obtain the sample protein content expressed in % of dry matter (3).

$$N\% = \frac{1,4 * V * N * d}{100 * p} \quad (2)$$

N: Nitrogen content (%)
V: volume of the sulfuric acid solution used during the titration (ml).
N: normality of the sulfuric acid solution used during titration
d: dilution of the mineralizer.
p: mass of the sample (mg)

$$\%Proteins = \%N * 6.25. \quad (3)$$

2.2.2.3. Extraction of Vegetable Oil

The extraction of the vegetable oil was carried out with hexane using a Soxhlet for 6 hours at 69°± 1C. After the extraction, the solvent was recycled in a rotavapor and the crude vegetable oil obtained was dried in an oven for 20 min at 103°C, cooled in a desiccator for 30 min and weighed. The extracted oil was then packaged in dark bottles for later use [13,14,15]. The seed oil content expressed as a percentage (m/m) of dry matter was obtained by equation (4).

$$T_o = \frac{m_2 - m_1}{m_0} * 100 \quad (4)$$

m_0: mass of the sample (g).
m_1: mass of the empty vacuum flask (g).
m_2: mass of empty flask + oil (g).
m_2 - m_1: mass of oil obtained (g).
T_0: vegetable oil content (% m/m).

2.2.2.4. Total Carbohydrate Content

The total carbohydrate content was determined according to the difference formula (5) [16]:

$$Total - carbohydrate(\%) = 100 - (\%moisture + \%protein + \%fat + \%ash). \quad (5)$$

2.2.2.5. Energetic Value

The energetic value was determined by calculation (6), according to the Coleman (1970) formula and using the Atwater and Rosa (1899) coefficients [16].

$$E(Kcal/100g) = (4 * \%proteins) + (4 * \%carbohydrates) + (9 * \%lipids). \quad (6)$$

2.2.2.6. Ash Content

The ash content has been evaluated following the standard ISO 749: 1977 [17]. The ash was obtained from the seed powder by incineration at 550°C ± 15°C, in a muffle furnace until a constant mass was obtained. Crude

ash content (T_a) expressed in % (m/m) of dry matter was obtained using equation (7):

$$Ta = \frac{m3 - m1}{m2 - m1} * 100 \quad (7)$$

T_a: ash content (% m/m) dry matter
m_1: mass of the empty crucible (g).
m_2: mass of the crucible + sample (g).
m_3: mass of the crucible + ash (g).

2.2.2.7. Mineral and Trace Elements Content

Five (05) grams of the sample were cremated following the procedure indicated above. The ash obtained was dissolved in 5 ml of hydrochloric acid (6N) and then evaporated on a hot plate at 125°C. The resulting residue was dissolved and recovered with HNO_3 (0.1M) in a 100 mL flask. This solution was used to obtain the content of the minerals by spectrophotometer Atomic Absorption [18,19]. The results are expressed in relation to the dry matter.

2.2.2.8. Total Phenols Content

The total phenols content was assessed by spectrophotometry of Molecular Absorption, using the Folin-Ciocalteu reagent [20,21]. Thus 3 ml of distilled water were mixed with 50 µl of extract and then with 250 µl of Folin-Ciocalteu reagent and 750 µl of 7% Na_2CO_3. After 8 min, 950 µl of distilled water was added and the absorbance measurement was made after two hours at 765 nm. A white without extract was used as a reference. Calibration curve obtained with gallic acid at different concentrations in the same conditions as for the sample was used to evaluate the content of total phenols. The results are expressed in mg equivalent of Gallic acid per gram of dry matter.

2.2.2.9. Phytochemical Screening

Determination of secondary metabolites was made by staining and precipitation reactions specific to each metabolite family. 97960094.

3. Results and Discussion

3.1. Physical Characteristics of *Hibiscus sabdariffa sabdariffa* Seeds

Photo 1. Seeds of Hibiscus sabdariffa sabdariffa

In each calyx of *Hibiscus sabdariffa* is located a large number of small seeds enclosed in hulls. At maturity, the hulls open to let the seeds appear. These seeds *Hibiscus sabdariffa sabdariffa* are ovoid, brown and have an average weight of 7.2 mg each.

3.2. Nutritional Characteristics of Seeds of *Hibiscus sabdariffa sabdariffa*

The results of the nutritional characterization of the seeds of *Hibiscus sabdariffa sabdariffa* are summarized in Table 1 to Table 6.

3.2.1. Moisture Content and Volatile Matter

The seeds of *Hibiscus sabdariffa sabdariffa* have a moisture content and volatile matter of 4.73% thus a dry matter rate of 95.27% (Table 1). This value is close to the recommended content (≤4% w/w DM) for desiccated coconut [22]; less than the maximum level (9% w/w DM) accepted for peanut seeds [23], nuts and shea (8% w/w DM) [24], seed cotton (≤11% w/w DM) [25], dry soybean [26] and sesame [27] which is 7% w/w of DM. This water content is also higher than that obtained (3.98%) by [7] on the seeds of *Hibiscus sabdariffa sabdariffa* from Benin.

Table 1. Moisture content and volatile matter of *Hibiscus* seeds *sabdariffa sabdariffa*

Parameter	Amount	[23]	[22]	[24]	[7]
Moisture content and volatile matter (%) DM	4.73	≤9	≤4	≤8	3.98

The low value of the moisture content and volatile matter from the seeds of *Hibiscus sabdariffa sabdariffa* obtained in the current study is an indicator for their good conservation by the low occurrence of alterations reactions, on one hand and an indicator for a good oil yield [28] on the other hand. The differences observed [7] data would be related to the harvest period and the drying conditions.

3.2.2. Protein Content of *Hibiscus sabdariffa sabdariffa* Seeds

The crude protein content of *Hibiscus sabdariffa sabdariffa* seeds obtained in this study is 19.18% w/w DM. This value is higher to that obtained (13.0%) with the seeds of the *Hibiscus sabdariffa sabdariffa* from India [29], but lower than the value (23.25%) obtained with the seeds from Saudi Arabia [30] (Table 2). The content obtained in the current work, meets the requirements of sesame and cotton seed protein content which is ≥15% w/w DM [25,27] but is lower than the dry soy protein content requirement which is 40% w/w of DM [25,26].

Table 2. Protein content of *Hibiscus sabdariffa sabdariffa* seeds

Parameter	Amount	[29]	[30]
Protein content (%) w/w DM	19.18	13.0	23.25

This high protein content obtained from seeds of *Hibiscus sabdariffa sabdariffa* shows that they are excellent protein sources and justify their use as a substitute for meat and in the production of ferment for traditional condiments such as *yanyanku* and *Ikpiru* [6].

3.2.3. Oil Content of *Hibiscus sabdariffa sabdariffa* Seeds

Soxhlet extraction with hexane gave a seed oil yield of *Hibiscus sabdariffa sabdariffa* of 13.6%. The oil content is less than those obtained with the samples tested respectively in Ivory Coast (24.53%) [31]; in Nigeria (17.85%) [32]; in Benin (14.39%) [7]; and in Saudi Arabia (19.36%) [30]. However it is located in the range of 8.9 to 29.5% found by [33] for the seeds of the natural species of *Hibiscus spp.* In addition, the oil content of the seeds of *Hibiscus sabdariffa sabdariffa* is close to that of the cottonseed which is 10-16% [34].

According to its oil content, like cottonseeds, *Hibiscus sabdariffa sabdariffa* seeds occupy the penultimate position in the list of major oilseeds, just in front of corn. Global production of cottonseed oil is low (3.3%) and only 3% of this world production is traded internationally. Nevertheless its price on the market is roughly the same as those of sunflower and corn oils, and a little more expensive than palm oil and soy. Because of its composition, cottonseed oil is similar to other food oils and its price depends on the availability of different oils on the market and their commercial policies [34]. In view of the foregoing, it would be wise, like the cottonseed oil, to study *Hibiscus sabdariffa sabdariffa* oil with a view to its worldwide valuation.

3.2.4. Total Carbohydrate Content of *Hibiscus sabdariffa sabdariffa* Seeds

The carbohydrate content is 56.16%. This content is higher than the one obtained (37.3%) with seeds from India [29], and those from Saudi Arabia (21.7%) [30]. This content is also two times higher than that required for grilled cashew nuts [35]. This high content denotes the richness of the seeds of *Hibiscus sabdariffa sabdariffa* in carbohydrates.

3.2.5. Energy Value of *Hibiscus sabdariffa sabdariffa* Seeds

The energy value obtained is 423 Kcal/100g. This energy value is related to the high levels of the seed of *Hibiscus sabdariffa sabdariffa* in protein, carbohydrates and lipids, making it a good dietary supplement.

3.2.6. Ash Content of Seeds of *Hibiscus sabdariffa sabdariffa*

The seeds of *Hibiscus sabdariffa sabdariffa* have an ash content of 6.33% (Table 3).

Table 3. Ash content of seeds of *Hibiscus sabdariffa sabdariffa*

Parameter	Amount	[7]	[36]
Ash content (%)	6.33	5.64	6.70

This ash content is higher than those obtained on the samples analyzed in Benin (5.64%) [7] but closed to that obtained with seed from Sudan [36]. The ash content obtained in this study is higher than the recommended rate (≤2.5% w/w) for roasted cashew kernels [37] and for desiccated coconut [22] on one hand, and to the one required (≤2.0% w/w) for sesame on the other hand [27].

The high ash content of the seeds of *Hibiscus sabdariffa sabdariffa* from Benin is an indicator for their richness in minerals and trace elements, essential for the survival and proper functioning of the body.

3.2.7. Mineral and Trace Elements Content of *Hibiscus sabdariffa sabdariffa* Seeds

The analysis of the mineral composition reveals that magnesium, iron, sodium and manganese are the major mineral constituents of the seeds of *Hibiscus sabdariffa sabdariffa* of Benin while potassium, calcium and nitrogen are present in low quantity (Table 4). The richness of *Hibiscus sabdariffa sabdariffa* seeds in inorganic mineral was also noticed with seeds from India and Cameroon [38,39].

Table 4. Mineral composition of *Hibiscus sabdariffa sabdariffa* seeds from Benin

Mineral	Amount	[7]	[39]	RDI [40]
Nitrogen	30.6 mg/g	7.5 mg/g	Nd	Ne
Potassium	19.1 mg/g	14.1 mg/g	19.25 mg/g	Ne
Calcium	2.4 mg/g	9.3 mg/g	3.20 mg/g	750-1000 mg/j
Sodium	0.17807 mg/g	0.231 mg/g	Nd	Ne
Magnesium	4.6636 mg/g	3.8 mg/g	4.6436 mg/g	360-420 mg/j
Manganese	0,0617 mg/g	0.043 mg/g	0.076 mg/g	2.5-2.8 mg/j
Iron	0.1991 mg/g	0.1285 mg/g	0.1145 mg/g	6-16 mg/j

Nd: Not determined; Ne: Not existing; RDI: Recommended daily intake from Nutritional references for adult women and men in the French population [40].

The richness of the seeds of *Hibiscus sabdariffa sabdariffa* in these different minerals gives it a place of choice in the human and animal food. In fact, a diet rich in potassium reduces the risk of hypertension [41]; Calcium and phosphorus are minerals present in large amounts in the body structure and in the bones [42]. Iron deficiency has been associated with developmental and cognitive behavior problems [43]. In addition, manganese is an essential trace element for normal growth and development [44].

3.2.8. Bioavailability of the Nutrients Contained in the Seeds of *Hibiscus sabdariffa sabdariffa*

In order to evaluate the bioavailability of nutrients, the maximum total phenol content of the seeds was evaluated using three different types of extracts (ethanolic, aqueous and hydroethanol). The results expressed in milligram of Gallic acid equivalent per gram of dry matter (mg GAE/g DM) are presented in Table 5.

Table 5. Total phenol content in the seeds of *Hibiscus sabdariffa sabdariffa*

Extracts	Total phenols (mg GAE/g DM)
ethanolic	0.104
Aqueous	0.203
Hydroethanolic	0.166

The total phenol content of the aqueous extract of the seeds of *Hibiscus sabdariffa sabdariffa* (0.203 mg GAE/g DM) is greater than that of the hydroethanolic extract (0.166 mg GAE/g DM) and that of the ethanolic extract (0.104 mg GAE/g DM). The low value of the total phenol content obtained in the present study allows the

bioavailability of the nutrients contained in the seeds of *Hibiscus sabdariffa sabdariffa* from Benin and confirms their food use in several areas.

3.2.9. Phytochemical Screening

Various secondary metabolites have been identified in seed of *Hibiscus sabdariffa sabdariffa* as shown in Table 6.

Secondary metabolites identified in the seeds of *Hibiscus sadariffa sadariffa* are the saponosides, alkaloids, coumarins and reducing compounds. Flavonoids, tannins, anthocyanins, leuco-anthocyanins, anthraquinones, mucilages and quinones are absent.

Table 6. Secondary metabolites identified in the seeds of *Hibiscus sabdariffa sabdariffa*

Secondary metabolites	*Hibiscus sabdariffa sabdariffa*
Alkaloids	+
Flavonoids	-
Tannins	-
Anthocyanins	-
Leuco-anthocyanins	-
Anthraquinones	-
Mucilages	-
Saponosides	+
Coumarins	+
Quinones	-
Reducing Compounds	+

+: presence;-: absence.

4. Conclusion

This study was conducted to contribute to the food and agro-economic valorization of the seeds of *Hibiscus sabdariffa sabdariffa*. The results showed that the seeds of *Hibiscus sabdariffa sabdariffa* are not very prone to alterations and can be preserved over a long period. In fact, the low moisture and volatile matter content of the seeds (4.73%) militate in favor of their good quality of conservation and their appreciable yield in oil. The high protein content of the seeds of *Hibiscus sabdariffa sabdariffa* (19.18%) makes them a good alternative to meat, and favors its use as ferments in the production of traditional condiments. With an ash content of 6.33%, these seeds contain an appreciable level of mineral. These results are complemented by high contents in total carbohydrate (56.16%) oil (13.6%) and secondary metabolites, which make them a good supplement to resolve and/or prevent certain deficiencies in rural populations from developing countries such as Benin.

Competing Interests

The authors declare that they have no competing interests.

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