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Chemical Profiling of Nutritional Value and Anti-Nutrient Content of Roselle (Hibiscus *sabdariffa* Linn.) Seed

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ABSTRACT

In this study a chemical analysis of *Hibiscus sabdariffa* Linn. seeds, was carried out. The proximate composition, anti-nutrients, and flavonoids content were determined by standard methods. Proximate analysis showed that the seeds contained a dry matter of 98.05 ±0.07%, 1.95 ±0.07% moisture, 21.84 ± 0.18% crude protein, the fat content was 6.50 ± 0.70%, Crude fiber content of the seed was 33.00 ± 1.41%, while the ash content was 6.00±0.00%. The crude lipid content of roselle was 6.50%. The concentrations of the anti-nutrient expressed in milligram per gram (mg/g) include tannin content which was 0.84 ± 0.02 mg/g, 3.08 ± 0.31 mg/g oxalate, phytate content was 0.96 ± 0.00 mg/g, and 2.49 ± 0.04 mg/g flavonoids. Roselle seeds are therefore a rich natural source of various phytochemicals, and can be exploited in the food, medical and pharmacology industries. The concentrations of the anti-nutrients are within the WHO recommended levels. However, they can effectively be lowered by boiling.

Keywords: Roselle seed, nutrients, anti-nutrients, proximate analysis.

INTRODUCTION

In the current decade, the demand for plant protein has been on the increase owing to health and environmental concerns as well as vegetarian trends among human and animal feeds. A wide range of plant species has been largely ignored over the years and generally considered as under-utilized minor crops [1]. Inadequate diet has been linked to the cause and severity of many diseases including cancer, heart disease and diabetes [2]. Among the ignored plants products are the seeds of *Hibiscus sabdariffa Linn*, commonly referred to as roselle seeds. This plant seeds contain protein as well as anti-nutrient compounds. Anti-nutrients are compounds that interfere with the absorption of vitamins, minerals, and other nutrients [3].

Anti-nutritional factors reduce the nutrient utilization and/or food intake of plants or plant products used as human foods. Plants evolved these substances to protect and prevent them from being eaten. However, if the diet is not varied, toxins may build up in the body to harmful levels. These anti-nutritional factors must be inactivated or removed, if values of food substances are to be fully maintained [4]. A variety of anti-nutrients such as oxalate and phytate, as well as toxic substances including, cyanide, nitrate, and phenols, have been reported present in many plants and vegetables. The cassava plant for instance is known to contain high levels of cyanide, a respiratory poison [5].

Consumption of vegetables in their fresh form which is believed to contain more micronutrients than processed vegetables becomes a major health concern because of the high levels of anti-nutrients and toxic substances that might be ingested with the associated health problems [4]. The anti-nutrients include compounds such as tannin, phytate, cyanide, oxalate and nitrate> Their presence in the body interferes with the absorption of minerals such as zinc, calcium and vitamin [6]. Polyphenols are example of anti-nutrient that can be beneficial depending on intake quantity. Similarly, flavonoids, another group of anti-nutrients found in healthy sources including tea, coffee, wine, and certain other whole plant foods [7].

Hibiscus anthocyanins, a group of phenolic natural pigments present in the dried flower of *Hibiscus sabdariffa* and Hibiscus rosa-sinensis, have been found to be cardio-protective [8], hypercholesterolemic [9], anti-oxidative [10] and hepatoprotective effects in animals [11]. Delphinidin 3-sambubioside, a Hibiscus anthocyanin, induces apoptosis in human leukaemia cells through oxygen reactive species-mediated mitochondrial pathway [12].

Roselle seeds are of different varieties and when cultivated in different geographical zones, with different soils, may produce different yields and chemical quantities [13]. Roselle seeds had been analysed and found to contain high amount of protein, dietary fiber, and minerals such as phosphorus, magnesium, and calcium. Singh *et al* [14] investigated roselle seeds in India and outlined the moisture content, crude protein, fat content, total dietary fiber, riboflavin, nicotinic acid, and the elemental composition therein. Sanders *et al* [15] characterized roselle seeds cultivated in the United States of America and concluded that the seeds consist of protein, moisture, lipids, carbohydrates, dietary fibers, and ash. Studies to investigate these parameters in the northern part of Nigeria on this plant are rare. Roselle seeds cultivated in Sudan savannah region of Nigeria have been rarely characterized and reported in literature.

Therefore, the present study was performed to investigate the nutritional value and anti-nutritional content of roselle seeds.

MATERIALS AND METHODS

Sample Collection and Identification

Hibiscus sabdariffa seeds were purchased from Friday market in Gadau, Itas/Gadau Local Government Area of Bauchi State, which is in Sudan savannah region of Nigeria. The seeds were identified at the herbarium of the Department of Biological Sciences, Bauchi State University, Gadau, Nigeria. Plates 1 (a) and (b) show the images of *hibiscus sabdariffa* plant and seeds, respectively.

Plate 1: (a) *Hibiscus sabdariffa* plant (b) *Hibiscus sabdariffa* seeds.

Sample Preparation

The seeds of *Hibiscus sabdariffa* plant were dried in an oven at 45 ^{0}C for 72 hours, and subsequently ground to fine powder (flour) using pestle and mortar and stored in a screw-capped plastic container in Biochemistry Department, Bauchi State University, Gadau, Nigeria.

Chemical Analysis of *Hibiscus sabdariffa* Seeds Flour

Determination of Crude Protein Content in Hibiscus sabdariffa Seed Flour by Kjehdahl method

Exactly 1.0 g of *Hibiscus sabdariffa* seeds powder was placed in a digestion flask, 5 g of Kjeldahl catalyst and 200 mL of concentrated H_2SO_4 were added to the flask. The flask was placed in an inclined position and heated gently until frothing ceased. Then, it was heated briskly until the solution became clear. The solution was cooled to room temperature and 60 mL of distilled water was added cautiously. The flask was immediately connected to the digestion bulb on the condenser with the condenser tip immersed in standard sulphuric acid,

while five drops of mixed indicator methyl red and methylene blue were added to receiver. The flask was rotated vigorously to mix the content followed by heating until all NH_3 was distilled. The receiver was removed, and the tip of the condenser washed. Subsequently, the excess standard acid distilled was titrated with standard NaOH [16].

Calculation of crude protein

The nitrogen content was calculated using the formular:

$$\mathrm{N}(\%\ \mathrm{w/w}) = (\text{volume of acid (ml)}\ \text{x molarity of acid (mol/l)}\ \text{x } 14(\mathrm{g/mol}))/(\text{weightof sample(g)}\ \text{x } 100)\ \text{x } 100 \quad (1)$$

The crude protein percentage (CP%) is calculated by multiplying nitrogen (N) percentage of feed by 6.25 as shown in equation 2:

$$\%\ \text{crude protein} = \%\mathrm{N}\ (\text{nitrogen}) \text{x } 6.25 \quad (2)$$

Determination of Ash Content in Hibiscus sabdariffa Seeds Powder

The crucible and lid were placed in the furnace at 550 ℃ overnight which ensured that all impurities on the surface of the crucible were burnt off. The crucible was cooled in desiccator to ambient temperature, the weights of the crucible and lid were determined. Then, 2.5 g of the sample was placed into the crucible and heated over low bunsen flame with the lid half covered. When fumes were no longer produced the crucible and lid were introduced into the furnace. The sample was subsequently heated at 550 ℃ overnight and cooled in a desiccator. The gray-coloured ash was weighed with the crucible and lid [17] .

Determination of Anti-nutrient Content

The anti-nutrient content of roselle (*Hibiscus sabdariffa*) seeds namely, tannin, oxalate, phytate and flavonoids were quantified and their concentrations expressed in milligram per gram (mg/g).

Determination of Tannin Content

The tannin contents were determined using the method reported by Mwanri et al [18]. The methanolic extract (1.0 ml) was mixed with vanillin hydrochloride reagent (prepared in methanol by combining equal parts 8% (v/v) hydrochloric acid and 4% (w/v) vanillin). After allowing the mixture to stand for 20 minutes at room temperature, absorbance at 660 nm was measured using a UV/Vis spectrophotometer (Jenway 6405). Absolute methanol was used as a control. The hydrolysable tannins were evaluated using equation 3 [19].

$$\text{Hydrolysable tannins } (\%) = (\mathrm{A} \times \mathrm{MW} \times \mathrm{V} \times \mathrm{DF})/\varepsilon_{\mathrm{mole}} \times \mathrm{w} \quad (3)$$

Where A= absorbance, MW= molecular weight of gallic acid (170.12 g/mol), V= volume of extract used, DF = dilution factor, ε_{mole} = 2169 mol/L (gallic acid constant), W = sample weight in g.The results were expressed as mg ascorbic acid equivalent (AAE) per g of dry extract (AAE/100 g).

Determination of Phytate Content

Phytate content was evaluated by method adopted from Mwanri et al [18]. In this design, 0.2 g of each powdered sample was weighed into a 125 mL Erlenmeyer flask. The phytic acid was extracted for 30 minutes with 50 ml of 3% Trichloroacetic acid (TCA) and 45 minutes of hand swirling. After centrifuging the suspension, a 10 ml aliquot of the supernatant was transferred to a 50 ml conical flask. By swiftly lowering the pipette, four millilitres of $FeCl_3$ solution were added to the aliquot. The mixture was heated in a boiling water bath for 45 min. After 30 minutes, two drops of 3% sodium sulphate were added to the 3% TCA extract and heated further. The supernatant was decanted after being centrifuged for 15 minutes. The precipitate was washed twice with 20 to 25 ml of 3% TCA, heated in a boiling water bath for 10 minutes, and centrifuged. After mixing, the precipitate was dispersed in 27 mL of water and 3 mL of 1.5 N NaOH. The volume was brought to approximately 30 ml with water and heated in boiling water bath for 30 min. The precipitate was filtered through Whatmann No. 2 paper, which was moderately retentive. The precipitate was washed with 70 ml hot water and the filtrate discarded. The precipitate was dissolved from the paper in a 100 ml volumetric flask with 40 ml 3.2 N of HNO_3. The filter paper was washed several times with water before being collected in the same flask, taking care not to exceed the 100 ml volume. The flask was cooled to room temperature and diluted with water to the mark.

A 5 ml aliquot was transferred to a second 100 ml volumetric flask and diluted to about 70 ml. 20 ml of 1.5 M KSCN was added and diluted to the mark, and the color was measured at 480 nm within 1 minute. A reagent blank was run with each set of samples. The phytate content of the sample was determined using the formula:

$$\text{Phytate content in µg/100 g sample} = \left(C \times \frac{E}{S} \times AV\right) \times 100 \quad (4)$$

Where, C = phytate concentration from standard graph, E = total extraction volume,

S = analytical sample taken, and Av = analytical volume.

Determination of Oxalate Content

Titration method as described in Mwanri et al [18] was adopted for the determination of the oxalate content. In 50 ml of distilled water, 2.0 g of powdered sample was heated. The heated sample was then treated with 0.3 M HCl. Prior to heating the mixture to 100 °C, 3 drops of methyl red indicator and NH_4OH solution were added to the cold filtrate. The mixture was set aside to cool and the filtrate heated again before adding 10 cm^3 of 10% $CaCl_2$ solution and leaving it to stand overnight. Whatman paper No.1 was used to filter the mixture. After filtration, the precipitate was washed to remove any remaining Ca^{2+} before being dissolved in diluted H_2SO_4 solution. The solution was heated before titrating with 0.05 M $KMnO_4$ solution for at least 30 seconds until a faint pink color persisted. The oxalate content was then determined by taking 1 ml of 0.05M KMnO4 as equivalent to 2.2 mg oxalate using the formula;

$$O = T_s \times M_d \times M_O \times 100/W_s \quad (5)$$

Where O = Oxalate concentration in mg/100 g, Ts = Volume of potassium permanganate used for sample, Md = number of moles of potassium permanganate reacted, Mo = number of moles of oxalate reacted, and Ws = sample weight.

Determination of Flavonoid Content

The aluminium tri-chloride assay technique [20] was used to determine flavonoids in methanolic extracts. To 1.0 ml of each sample, 1.0 ml of 2.0% (w/v) aluminium tri-chloride in ethanol was added and after 1 hour, absorbance was measured at 420 nm using a UV/Vis spectrophotometer (Jenway 6405). The flavonoid concentrations in the extracts were calculated using the catechin standard curve [20].

RESULTS AND DISCUSSION

Chemical Analysis of the Seeds of Roselle

Table 1 shows the results of chemical analysis of the seeds of *Hibiscus sabdariffa*. The composition of each parameter was expressed as percentage (%) composition. The phytochemical analysis revealed the presence and amount of ash content, dry matter, crude moisture content, crude protein, % fat, and crude fibre.

Table 1 Chemical analysis of the seeds of roselle.

plant sample	%Dry matter	% Moisture content	%Crude protein	%Fat	% Crude fibre	%Ash content
Roselle seed 1	98.10	1.90	21.97	6.00	34.00	6.00
Roselle seed 2	98.00	2.00	21.71	7.00	32.00	6.00
Average ($\bar{x}$)	98.05 ±0.07	1.95 ±0.07	21.84 ±0.18	6.50 ±0.70	33.00 ±1.41	6.00 ±0.00

Values are expressed as mean ±SD

The results from Table 1 reveal the presence of nutrients in roselle seed. The composition of each parameter was expressed as percentage (%) composition. The seeds contain a dry matter of 98.05%, 1.95 ± 0.07 % moisture, 21.84 ± 0.18% crude protein, the fat content was found to be 6.50 ± 0.70 %, Crude fiber content of the seed was 33.00 ± 1.41 %, while the ash content was to 6.00 ± 0.00%

The results in Table 1 also show that *Hibiscus sabdariffa* seed is rich in nutrients. The protein content determined in this work is on average of 22%. This value is higher than the 19.18% as reported for Benin Republic [21], whereas it is lower than the protein value of *Hibiscus sabdariffa* seed reported for Bangladesh at 28.18% [18] and 26.24% for *Hibiscus sabdariffa* seed in Egypt [22]. The protein value indicates that roselle seeds are good source of protein. Proteins are essential components of diet supplying adequate amounts of amino acids [23]. Furthermore, the value is in line with the protein content reported for pigeon pea [24]. The present study also agrees with other previous studies indicating that roselle seed (contain high amount of protein, dietary fiber, and minerals such as phosphorus, magnesium, and calcium [25].

The ash content of the roselle seed was found to be 6%. The level of ash content is quite high, which implies that the seed contain high mineral content. Roselle seeds are traditionally used in some parts of Northern Nigeria as pap which is given to woman immediately after birth to replenish lost blood. This suggests that the level of Fe and other elements associated with the roselle seed can boost blood levels.

The crude lipid content of this seed was observed to be 6.50%. The low lipid content of the plants agrees with the observation that vegetables are low lipid containing foods [26].

The fiber content value of the sample was 33.00%. This value obtained is also consistent with the average values reported for other legumes [27]. The presence of high crude fiber in food items decreases dry matter digestibility in animals, and this indicates that

the roselle seeds have good nutritive value of feed material [28]. The percentage dry matter of the seed product was 98%. This result indicates 2% moisture content in the preparation or processing of the roselle seeds.

Previous studies have shown that roselle seeds contain high amounts of protein and dietary fiber [29]. Literature shows that roselle seeds from Egypt contain 7.6% moisture, 34.0% protein, and 22.3% fat [30]. The seeds from Malaysia contain 9.9% moisture, 33.5% protein, 2.1% lipids, 7.5% ash, 18.3% dietary fibre [31]. The difference in the values of the nutritional content of roselle seeds obtained when compared to previous findings might be due to variety of the seeds, geographical factors, and the solvent use in the extraction.

Quantitative Anti-Nutrient Analysis of Roselle Seeds

Table 2 shows the results of the quantified anti-nutrient content of roselle seeds which include tannins, oxalates, phytates and flavonoids. The concentrations of the anti-nutrient are expressed in milligram per gram (mg/g). The mean tannin content was found to be 0.84 ± 0.02 mg/g, 3.08 ± 0.31 mg/g oxalate, phytate content was found to be 0.96 ± 0.00 mg/g, and 2.49 ± 0.04 mg/g flavonoid. The concentration of flavonoid and oxalate were relatively high while that of tannin and phytate low in comparison to roselle seed from WHO standard [36].

Table 2: Quantified anti-nutrient content of roselle seed.

plant sample	Tannin (mg/g)	Oxalate (mg/g)	Phytate (mg/g)	Flavonoid (mg/g)
Roselle seed 1	0.85	3.3	0.96	2.46
Roselle seed 2	0.82	2.86	0.96	2.49
Average ($\bar{x}$)	0.84±0.02	3.08±0.31	0.96±0.00	2.48±0.02

Values are expressed as mean ±SD

The result of the anti-nutrients analysis of the seed of *Hibiscus sabdariffa* as represented in Table 2 reveals the concentrations of tannins, oxalate, phytic acid and flavonoids. The results suggest that *Hibiscus sabdariffa* seed contains bioactive ingredients and anti-nutrients. The influence of anti-nutrients on metabolism is determined by their concentration in the diet [32].

Tannins and phytotoxins are reported to be toxic to animal and human tissue at high concentration and interfere with dietary iron absorption. They form complexes with protein and render it unavailable for absorption [33]. Owing to their heat stability, tannins decrease protein digestibility in animals, probably by either making protein partially unavailable or

inhibiting digestive enzymes and increasing faecal nitrogen [34]. Similarly, they have been reported to inhibit the activities of trypsin, chymotrypsin, amylase, and lipase [35]. According to WHO, phytate level in foods below 5 mg are safe for human consumption [36]. Consequently, the results in Table 2 indicate roselle seed may be safe for human consumption.

Table 2 revealed an average of 2.49 ± 0.04 mg/g of flavonoid. Flavonoids are widely distributed in plants fulfilling many functions. The widespread distribution of flavonoids, their variety and relatively low toxicity compared to other active compound (alkaloid) shows that animals ingest significant quantities in their diets. This class of plant secondary metabolites are commonly known for their anti-oxidant activity. However, flavonoids are reported to play a significant role in preventing cancers and cardiovascular disease [37].

CONCLUSIONS

The chemical profiling of roselle seeds carried out in this study showed that they contain high amount of crude protein, fiber, and dry matter. Roselle seeds are therefore a rich natural source of various phytochemicals, which can be exploited in the food and medical industries and in pharmacology. The seeds contain antinutrients such as flavonoids, tannins, oxalates, and phytate. The concentrations of the anti-nutrients determined in this study are within the WHO recommended levels and can be effectively lowered by boiling. These levels do not pose harmful effects to human and animal health. The seed of *Hibiscus sabdariffa* contains high amount of minerals as represented by the amount of ash content. The seeds of *Hibiscus sabdariffa* are highly nutritive and can be used for human and animal feed. It can therefore be concluded that cultivation of plant *Hibiscus sabdariffa Linn* be encouraged in view of the beneficial bioactive component of the seeds. However, a holistic approach will require chemical profiling of various parts of the plant including roots stem and leaves to effectively investigate and determine the dietary components of roselle plant as well as the antinutrient contents. Therefore, a more holistic approach and techniques in the evaluation of nutritional and antinutrient components of roselle plant are recommended for further studies.

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