



Comparative Studies of Proximate Composition of Three Species in Calyces of *Hibiscus sabdariffa* Linn

¹Abdulazeez A., ¹Ibrahim I.L., ^{1,2}Mohammad Y.S., ¹Gimba A. and ³Nuhu A.

¹Department of Chemistry, Ibrahim Badamasi Babangida University, Lapai, Nigeria

²Department of Food Science and Technology, Ibrahim Badamasi Babangida University, Lapai, Nigeria

³Faculty of Agricultural Sciences, Ibrahim Badamasi Babangida University, Lapai, Nigeria

ABSTRACT

Hibiscus sabdariffa Linn belong to the family malvaceae. It is an annual herbaceous sub- shrub with more than 300 species which are distributed in tropical and sub-tropical regions around the world. Most hibiscus species are used as ornamental plants, medicinal values; among them is *Hibiscus sabdariffa* commonly named as 'Red sorrel' and 'Roselle'. *Hibiscus sabdariffa* (calyces) have been found to be rich in vitamin, antioxidant and minerals. Therefore, the aim of this work is to determine the proximate composition in calyces of *H. sabdariffa*. Three samples of calyces were collected, identified as light green, bright red and dark red. They were washed with distilled water and air dried for two weeks and grounded to uniform powder using wood type of mortar and pestle. Proximate analysis was carried out on dried sample of the *Hibiscus sabdariffa*. The ash contents of the light green, bright red and dark red calyces ranged between 10.00-10.60%, moisture, crude fiber, crude fat, crude protein, and carbohydrate ranged between 9.00-11.00%, 12.00-13.54%, 0.32-0.62%, 7.70-7.88% and 56.90-60.55%. The calyces of *Hibiscus sabdariffa* if consumed in sufficient amount would contribute greatly towards supplementing human nutritional requirements for normal growth and adequate protection against infection associated to the malnutrition.

Keywords: Proximate composition, Distillation, Uniform powder, Human nutrition

INTRODUCTION

Plant constituents, as the word implies, are the individual chemicals from which plants are made. These constituents are organic in nature and synthesized in plants by the activity of individual cells. The process by which these complex organic chemical constituents are formed, utilizes simple substances like enzymes by biosynthesis (Ahmed, 2007).

Essentially, several compounds, which are pharmaceutically and medicinally important, are derived from plant sources. However, the medicinal value depends on the nature of its constituents, the active constituents are those chemical substances which are solely responsible for therapeutic activity of plants. A large number of theories (Ahmed, 2007).

Have been proposed as to why these compounds are formed in plants. It is likely that many of them are synthesized as part of chemical defense mechanism to protect the plant. On the other hand, chemical constituents present in plants

that do not possess any definite therapeutic value are known as inactive constituents. The formation of the various active and inactive constituents of plants involves various metabolic pathways. Hence, the inactive plant constituents are termed primary plant metabolites, whereas the active plant constituents are termed secondary plant metabolites (Ahmed, 2007).

The contribution of medicinal plants to sciences in the Ethno medicine for curing diseases has been interestingly on the increase and consumer demand have promoted the development of herbal products as dietary supplements. In view of these; interest, herbal medicines have a prominent role to play in the pharmaceutical and health concern especially in 21st century.

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Address Correspondence to:

abdoolrah@yahoo.com,

Roselle belongs to *malvaceae* family. It is an erect, mostly branched, annual shrub (Qi *et al.*, 2005) or herbaceous (Falusi, 2007) or an annual herbaceous shrub (Mahadevan *et al.*, 2009). The plant is about 3.5m tall and has a deep penetrating taproot. It has a smooth or nearly green to red stems; according to Udom *et al.*, (2001), there are three common varieties of roselle grown in Nigeria; two of these varieties have red calyces while one has green calyces.

Leaves are alternate, 7.5- 12cm long, green, glabrous with reddish veins and long or short petioles. Leaves of older plants are simple; lower leaves are palmately divided 3 to 5 or even 7-lobed and the margins are toothed.

Flowers, borne singly in the leaf axils are up to 12.5cm wide, yellow or buff with a rose or maroon-eye and turn pink as they wither at the end of the day. The typically red calyx, consist of 5 large sepals with a collar (epicalyx) of 8-12 slim, pointed bracts (or bracteoles) around the base; they begin to enlarge at the end of the day, 3.2 to 5.7cm long and fully enclose the fruit.

The fruit is a velvety capsule, 1.25 to 2cm long, which is green when immature. 5-valved with each valve containing 3 to 4 seeds. The capsule turns brown and splits open when mature and dry. Seeds are kidney-shaped, light-brown, 3-5mm long and covered with minute stout and stellate hairs (Julia, 1987).

Many parts of the plant are also claimed to have various medicinal values. They have been used for such purposes ranging from Mexico through Africa and India to Thailand. Roselle is associated with traditional medicine and is reported to be used as treatment for several diseases such as hypertension and urinary tract infections (Dinae *et al.*, 2010). There is currently insufficient evidence to demonstrate any beneficial effect of Roselle on raise blood pressure (Ngamjarus *et al.*, 2010) or on blood lipid lowering (Kuriyan *et al.*, 2010). *Hibiscus Sabdariffa* has shown in vitro antimicrobial activities against *E. Coli* (Fullerton *et al.*, 2011). A recent review stated that specific extracts of *Hibiscus Sabdariffa* exhibit activities against atherosclerosis, Used to treat respiratory problems, in colds, indigestion and to cure circulation disorders, liver disease, cancer (Chang *et al.*, 2006), diabetes and other metabolic syndromes (Lin *et al.*, 2011).

The most exploited part of a roselle plant is its calyces that are obtained by removing the calyces or petal of the flower from it capsules containing the seeds. Calyces are used for the preparation of herbal drink, cold and warm beverages jams and jellies. The brilliant red colour and unique flavour gives it valuable products (Morton, 1987). Calyces from the roselle flower, which are very high in vitamin C are processed to produce juices. Duke and Atchley (1984) reported that every 100g of fresh calyx contains 2.85µg vitamin D, 0.04mg vitamin B1, 0.6mg vitamin B2, and 0.5mg vitamin B complex. Furthermore, other studies have reported that it is good in reducing hypertension (Amin *et al.*, 2008).

METHODOLOGY

Sample Collection and Preparation.

The plant was collected from Ibrahim Badamasi Babangida University, Lapai, farm, Niger State, Nigeria and identified at the Herbarium section of the Department of Biological Science of Ibrahim Badamasi Babangida University, Lapai, as *Hibiscus sabdariffa*. The calyces were air dried for two weeks and ground to uniform powder using wood type of mortar and pestle. Proximate analysis were carried out on dried sample of the *Hibiscus sabdariffa* calyces.

Proximate Analysis

Proximate analysis involves the estimation of the main components of food (moisture content, crude lipid, crude protein, fat, ash and crude carbohydrate) using standard methods of analysis.

Determination of Moisture Content

The method of AOAC (1990) using hot air drying oven was used. Empty clean dish was dried in an oven at a temperature of 105⁰C for one hour and cooled in a dessiccator. Two grammes of the samples was put into the dish and heated in an oven for 24 hours. The dish was then removed from the oven, cooled in a desiccator and weighed. Moisture content was then calculated using equation below

$$(\%) \text{ Moisture content} = \frac{\text{Loss in weight}}{\text{Sample weight}} \times 100$$

Determination of Ash Content

The method of AOAC (1990) was adopted to determine the percentage of ash content. Two grammes each of dried samples were weighed into a pre-heated and cooled crucibles and incinerated in a muffle furnace (Lenton, England) at 600°C for thirty minutes. The flask was then cooled and the ash was weighed. The percentage of ash content was calculated using equation below.

$$(\%) \text{ Ash} = \frac{\text{Weight of ash}}{\text{Weight of dry sample}} \times 100$$

Determination of Crude Fibre

Two grams of the ground sample were weighed and placed in 1litre conical flask containing 200cm^3 of 1.25% H_2SO_4 and boiled gently for thirty minutes. The content were filtered and the residue was scrapped back into the flask with spatula. 200cm^3 of 1.25% NaOH was added and allowed to boil gently for 30 minutes. The content were filtered and washed thoroughly with hot distilled water. The precipitate was rinsed once with 10% HCl and twice with ethanol. The content was allowed to dry and the residue were scrapped into a crucible and dried overnight at 105°C in a hot oven. It was then removed and cooled in a desiccator. The sample was then weighed and ashed at 600°C for ninety minutes in a furnace. This will be finally cooled in a desiccator and weighed again (AOAC, 1990).

The percentage crude fibre was calculated using equation below.

$$(\%) \text{ Crude fibre} = \frac{\text{Weight loss}}{\text{Weight of sample}} \times 100$$

Determination of Crude Lipid

The method of AOAC (1990) using Soxhlet extractor was adopted for crude lipid determination. Two grams each of the dried samples will be weighed into a porous thimble, and it is mouth covered with cotton. The thimble was then placed in an extraction chamber which was suspended above a receiving flask containing petroleum ether (B.P $40-60^{\circ}\text{C}$). The flask was heated on hot mantle and the oil extracted. The extraction continued for eight hours after which the thimble will be removed from the soxhlet and the apparatus was

reassembled and heated on water bath or solvent recovery. The flask containing the crude oil was then disconnected, cleaned up and placed in an oven at 100°C for thirty minutes. The flask was then cooled in a desiccator. The percentage of crude lipid was calculated using equation below.

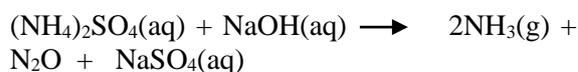
$$\% \text{ of Crude Lipid} = \frac{\text{Weight of oil extracted}}{\text{Weight of sample}} \times 100$$

Determination of Crude Protein

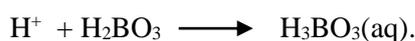
The method of AOAC (1990) was adopted using Micro-Kjeldahl apparatus. The technique was based on the transformation of protein nitrogen and other nitrogen compounds other than nitrate and nitrite into ammonium sulphate by acid digestion with strong acid usually, Conc. H_2SO_4 . The stages involved are:



The ammonia present in the digest with the help of sodium hydroxide was distilled out and collected into a receiving flask containing boric acid indicator which changes colour from pink to green.



The nitrogen content was then estimated by titrating the content in the receiving flask with standard acid (H_2SO_4).



The amount of H^+ consumed in the reaction is equivalent to the amount of nitrogen present in the sample.

Although the assumption is not entirely valid. The protein contained in plant sample in term of nitrogen content is from 13-18%.

Procedure

Two grams of the dried sample were weighed into kjeldahl digestion flask and a catalyst mixture was added (NaSO_4 , CuSO_4 and Selenium oxide in 10:5:1 ratio) followed by 10cm^3 of concentrated tetraoxosulphate (iv) acid. The content in the flask was then heated in the Kjeldahl digestion until the digestion is completed (approximately one and half hour). The flask was cooled, the contents diluted with

10cm³ distilled water, filtered into a 100cm³ volumetric flask and made up to the mark with distilled water. 10cm³ of the aliquot was taken into the digestion flask and 20cm³ of 45% NaOH solution will be added. The content was diluted to about 200cm³ with distilled water and distilled using Micro-kjeldahl distillation apparatus. The distillate was receiving into receiving flask containing 10cm³ boric acid solution indicator. After the distillation, the distillate was titrated with 0.0014N HCl to the end point. The crude protein is then calculated using the equation below:

$$\text{Crude protein (\%)} = \frac{TV \times C \times 0.0014 \times V1}{W \times V2} \times 100$$

Where

TV = Titre value of the acid

C = Concentration of acid used.

V1 = Volume of the distilled water used for diluting the digest

V2 = Volume of the aliquot used for titration

W = Weight of the sample used

F = Protein multiplication factor = 0.0014

Determination of Carbohydrate (By Difference).

The method of James (1995) was used where the total amount of carbohydrate in the sample were obtained by calculation using percentage weight difference. This involved subtracting the percentage sum of the food nutrients, % crude protein, % crude lipid, % crude fibre, and % ash from 100% dry weight.

Percentage carbohydrate was calculated using equation below:

$$\text{Carbohydrate \%} = 100 - (\text{crude protein} + \text{crude lipid} + \text{crude fibre} + \text{ash}).$$

Table 1: Proximate Analysis of Hibiscus sabdariffa calyces (Light green, Bright red and Dark red).

	Light green calyces (%)	Bright red calyces (%)	Dark red calyces (%)
Ash content	10	10.6	10.56
Fat content	0.62	0.32	0.4
Crude Fibre	12	13	13.54
Protein content	7.83	7.7	7.8
Moisture content	9	11	10.72
Carbohydrate	60.55	57	56.9

DISCUSSION

Proximate Analysis

The proximate analysis of *Hibiscus sabdariffa* calyces (Light green, Bright red and Dark red) are presented in figures 1 – 3. The moisture contents were found to be 9.00%, 11.00% and 10.72%, respectively. The low value indicates general decrease in the tendency of microbial attack on the calyces since water is a good medium for microbial growth (Lee, 2005). The ash contents were 10.00%, 10.60% and 10.56%, respectively and this is an indication of the level of inorganic chemical present in the sample and account for its potency of been attacked by insects (Lee, 2005). The crude fibre contents were 12.00%, 13.00% and 13.54% respectively.

Since fibre value indicates the degree of digestibility of food in animals. This value as compared with the minimum standard of 10.00% is corroborated by recommendation of consumption after every meal as it helps in human digestion (Oyeleke *et al.*, 2006). Non-starchy vegetables like this plant are reported to be a very rich source of dietary fibre and therefore are employed in the treatment of diseases such as obesity, high blood pressure, diabetes, cancer and gastrointestinal disorders (Agostoni *et al.*, 1995). The crude fat contents in this study were found to be 0.62%, 0.32% and 0.40%, this values accounts for the fact that the plant is recommended for patient with high obesity and arteriosclerosis. This abnormalities

are ascribed to high fat content in food consumed by patients (Lee, 2005). The crude protein contents were found to be 7.83%, 7.70% and 8.8%, respectively. These values are not surprising as the plant syrup have always been recommended for patients that are diabetic. The carbohydrate contents in this study were found to be 60.55%, 57.00% and 56.90%, respectively. This is in-tune with recent studies with respect to chemical content in of *Hibiscus sabdariffa* families (Lee, 2005).

Conclusion

The present study has shown that the *Hibiscus sabdariffa* calyces (Light green and Bright red) studied within limited experimental results have high content of carbohydrate with low fat content, crude protein and crude fibre. The ash contents were of moderate values. The result suggest that the calyces if consume in sufficient amount would contribute greatly towards supplementing human nutritional requirements for normal growth and adequate protection against diseases arising from data generated from this study and other studies in past.

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