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MICRONUTRIENTS AND ANTINUTRIENTS OF ZOBO CALYCES OF THE FLOWER PLANT (*HIBISCUS SABDARIFFA*).

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ABSTRACT

The flower calyx of *Hibiscus sabdariffa* (Roselle), commonly known as Zobo, is in high demand globally because of its polyphenol content, which is concentrated in the flower calyx. The dried calyces, which may be a good source of carbohydrate, dietary fiber, vitamins, minerals, and bioactive substances such as organic acids, phytosterols, and polyphenols, are of commercial significance to Roselle growers. After the minerals value of the calyces were cleansed, liberated of foreign materials and crushed with a mixer grinder the antinutrient and micronutrient (minerals and vitamins) contents of Zobo calyces of the floral plant *Hibiscus sabdariffa* were assessed using established procedures. Calcium (Ca^{2+}), potassium (K^{+}), copper (Cu^{2+}), iron (Fe^{2+}), magnesium (Mg^{2+}), manganese (Mn^{2+}), and phosphorous (P^{+}) were found in the calyces sample by the micronutrients test. Calcium (Ca^{+}) had the highest concentration and zinc (Zn^{2+}) had the lowest concentration in the calyces of *Hibiscus sabdariffa*, with 4698.348 mg/kg and 0.76 mg/kg, respectively. The presence of fat-soluble vitamin D, as well as two water-soluble vitamins B and C was also discovered. Vitamin D levels were determined to be 18.26 mg/100g, 4.11 mg/100g for vitamin B_1, and 13.24 mg/100g for vitamin C. Antinutrients such phenol, glycosides, saponin, tannin, terpenes, and steroids were also tested, with results ranging from 0.187 percent to 0.0013 percent. The results revealed that the calyces contain a significant amount of micronutrients, vitamins, and antinutrients. Roselle Calyces drinks should be consumed on a regular basis because they are safe, natural, and nutritional.

Keywords: Antinutrients, micronutrients, minerals, vitamins, calyces, Roselle, *Hibiscus sabdariffa*, Zobo

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INTRODUCTION

Roselle (*Hibiscus sabdariffa L.*), an annual or biennial plant belonging to the family Malvaceae (Onyeukwu *et al.*, 2023). It is commonly called 'red sorrel' or 'roselle.' Roselle is mainly cultivated for its calyx, which can occur in the green, red and dark-red pigment forms. The green type is the one used in cooking isapa soup. It has hermaphrodite flowers with five petals and a short peduncle. Calyx diameter is 2.5–3.5 cm and length is 4.2–7.2 cm. The flowers are light yellow to pink, red, orange, and purple red with a reddish center at the base of the stamina column (Baigts-Allende *et al*., 2023).

It is known by a variety of vernacular names in various Countries, including Roselle, Karkade, Zobo, Bissap, Congo, and others; however, Roselle is the most common name Riaz *et al*., (2020).

Zobo is a typical Nigerian street food prepared from the calyces of the *Hibiscus sabdariffa* flower. The flower calyx is known as (Roselle) highly demanded globally due to its positive health effects of the polyphenols content Nkumah, (2015). The seeds, leaves, fruits, and roots of the Roselle plant are all used in food and herbal medicine. The plant is an annual or perennial herb or woody-based that can grow up to 2-2.5 m (7-8 ft) tall. The leaves are 8-15 cm long, 3-5 lobed and alternately arranged on the stalks Nkumah, (2015). The flowers are 8-10 cm in diameter, white to pale yellow with a dark red mark at the base of each petal, with a strong fleshy calyx 1-2 cm wide at the base, widening to 3-3.5 cm as the fruit matures, and meaty and bright.

Hibiscus sabdariffa) is a species of flowering plant in the genus *Hibiscus* native to Africa, most likely West Africa (Onyeukwu *et al.*, 2023) or most likely native to Central and West Africa grown around the world. The part of the plant that is edible is the calyces of the Roselle flower which can be used to make a variety of jams, sauces, and teas.

Roselle belongs to Malvaceae family. It is an erect, mostly branched, annual shrub. Stems are reddish in color and up to 3.5m tall. Leaves are dark green to red, alternate, glabrous, long petiolate, palmately divided into 3–7 lobes, with serrate margins. Flowers are red to yellow with a dark center containing short-peduncles (Qi *et al*., 2005).

The flowers have both male and female organs. Seedpods are enclosed in their red, fleshy calyces which are commonly used for making food and tea. It is known by a variety of vernacular names in various countries, including Roselle, Karkade, Zobo, Bissap, Congo, and others; however, Roselle is the most common name Riaz *et al*., (2020). The plant is frequently cultivated for its stem, leaves, and calyces.

The seeds are high in protein, can be roasted and brewed like coffee, or ground and added to soups and salads. The nutrient-rich calyces can either be stored frozen or dried for making cordials, punches, and jams. The calyces can also be used to add color and flavor

to herb teas. Be sure to harvest calyces before they turn brown on the plant and separate them from the seeds before using them in recipes (Suhaili and Manshoor, 2022).

Many parts of Roselle including seeds, leaves, fruits and roots are used in various foods. Among are the fleshy red calyces which are most popular. They are used fresh for making wine, juice, jam, jelly, syrup. gelatin, pudding, cakes, ice cream and flavors and also dried and brewed into tea, spice, and used for butter, pies, sauces, tarts, and other desserts. The calyces possess pectin that makes a firm jelly (Qi *et al*., 2005). The young leaves and tender stems of Roselle are eaten raw in salads or cooked as greens alone or in combination with other vegetables and/or with meat. They are also added to curries as seasoning. They have an acid, rhubarb-like flavor. The red calyces contain antioxidants including flavonoids, gossypetine, hibiscetine and sabdaretine. The fresh calyces are also rich in riboflavin, ascorbic acid, niacin, carotene, calcium, and iron that are nutritionally important. The seeds, are high in protein, can be roasted and ground into a powder then used in soups and sauces. The roasted seeds can be used as a coffee substitute. The young root is edible, but very fibrous (Qi *et al*., 2005).

Roselle is used in many folk medicines. It is valued for its mild laxative effect and for its ability to increase urination, attributed to two diuretic ingredients, ascorbic acid and glycolic acid. Because it contains citric acid, it is used as a cooling herb, providing relief during hot weather by increasing the flow of blood to the skin's surface and dilating the pores to cool the skin Islam, (2019). The leaves and flowers are used as a tonic tea for digestive and kidney functions. The heated leaves are applied to cracks in the feet and on boils and ulcers to speed maturation. The calyces and seeds are diuretic, laxative and tonic. The ripe calyces, boiled in water, can be used as a drink to treat bilious attacks. A lotion made from roselle leaves is used on sores and wounds Islam, (2019).

The plant is also said to be antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, purgative and re-solvent. It is used as a folk remedy in the treatment of abscesses, bilious conditions, cancer, cough, debility, dyspepsia, fever, hangover, heart ailments, hypertension, and neurosis according to Mungole and Chaturvedi, (2011).

Dried calyces may be a good source of carbohydrate, dietary fiber, vitamins, minerals, and bioactive substances such as organic acids, phytosterols, and polyphenols, which are important to Roselle Riaz *et al*., (2020).

It is grown for fibers as well as thick and fleshy green, red (the most common), or red dark calyxes.

This plant has been used since ancient times, primarily in the preparation of hot and cold beverages with antioxidant properties that aid in the treatment of chronic diseases, such as

antihypertensive, anti-hyperlipidemic, anti-inflammatory, anti-bacterial, and anti-cancer properties Peredo Pozos *et al*., 2020.

Hibiscus sabdariffia is a high-value tropical plant. Its calyces have been proposed for use in the food industry as food colorants, emulsifiers for carbonated drinks, jam manufacturing, juices, and natural food colorants. The calyces also contain a lot of anthocyanin, ascorbic acid, and hibiscus acid. It is water soluble, with a bright and appealing red color and a sour and pleasing acidic taste that aids digestion Imam *et al*., (2013). This plant also has diuretic properties, as well as intestinal antiseptic and moderate laxative properties. It is also used to treat heart and neurological issues, as well as high blood pressure and clogged arteries Azza *et al.,* (2011). It is used in folk medicine to treat hypertension Balarabe, (2019).

It is made by heating water with the calyces. A dark red extract with a sour taste is obtained after further filtration. This can be flavored with sugar, spices like garlic and ginger, or fruits. The beverage contains a lot of vitamins and minerals. Zobo is a low-cost beverage produced by indigenous women that generates cash and aids in poverty alleviation. Due to its short shelf life, the drink is not widely distributed despite its low cost and nutritional value Ezearigo *et al*., (2014).The maturation period lasts approximately 6 months.

The purpose of this research is to determine the Micronutrient composition (calcium, magnesium, sodium, potassium, phosphorus, iron, zinc, cadmium, and copper), vitamin (water and fat soluble), and Antinutrient composition which are phenol, glycosides, saponin, tannin, terpenes, and steroids of Zobo calyces (Hibiscus sabdariffa).

Figure 1: Zobo (*Hibiscus sabdariffa*)

MATERIALS AND METHODS

Sample Collection

Hibiscus sabdariffa dried calyces was obtained from a local market called Oba Adesola Market, Lusada, Ogun State, Nigeria.

Sample processing

After cleaning and removing visible non-calyces materials, the Roselle calyces were sun dried for two weeks. The samples were dried before being crushed into a fine powder and stored in clean, properly labeled polythene bags for analysis.

Quantitative analyses of antinutrients

Saponin determination by (Obadoni and Ochuko, 2001)

20 g of each grounded sample was put into a conical flask and 100 cm^3 of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4 h. with constant stirring at about 55°C. The mixture was then filtered and the residue was again extracted with another 200 ml 20% ethanol. The combined extract was reduced to 40 ml on a hot water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel, added 20 ml diethyl ether in it followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in oven, weighed and saponin content was calculated as percentage.

Determination of total phenols by spectrophotometric method by Edeoga *et al*., (2005).
2 g of each plant sample was defatted with the help of 100 ml of diethyl ether using a soxhlet apparatus for 2 h. The fat free sample was boiled with 50 ml of ether for 15 min for the extraction of phenolic component. 5 ml of the extract was pipetted into a 50 ml flask and 10 ml distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added in it. The samples were made up to mark and left to react for 30 min. Colour was developed and its absorbance was measured at 505 nm.

Tannin determination by Van-Burden and Robinson (1981) method

500 mg of the sample in each case was taken in a plastic bottle and 50 ml of distilled water was added. Then it was shaken in a mechanical shaker for 1 h, and filtered in a 50 ml volumetric flask made up to the mark. 5 ml of the filtrate was pipetted out into the test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M $K_4Fe(CN)_6$ (Potassium ferrocyanide). The absorbance was measured at 120 nm within 10 min.

Terpenoids were extracted using the method of Siddiqui *et al*. (2011). One gram of the finely ground dried plant samples was incubated in ethanol–water (9:1) (2 ml×10 ml) on a

mechanical shaker for 5h at ambient temperature and filtered through a double layer of Whatman no. 1 filter paper. The filtrate was evaporated to dryness, resulting in a dark-coloured extract. The dried crude extract was weighed, and 100mg of dried extract was redissolved in ethanol–water (9:1) and brought up to 10 ml. The solution was filtered through a 0.22µm membrane filter and the clear filtrate was analysed using high-performance liquid chromatography (HPLC).

Total Steroid Content Determination by Panchal Mital and Jha, (2021).

Solvent extraction

By using Soxhlet extraction method, crude plant extract was prepared. In a thimble 10 gm of powdered plant material was loaded and 300 ml solvents were also extracted independently. As solvent, water, Acetone and Chloroform was used till the solvent changed to colorless, the process of extraction sustained for 24 hours, in siphon tube of an extractor. Then in a beaker extract were taken. Then at 30- 40℃ till all the solvent was evaporated, kept and heated the extract on hot plate. The extract obtained was used for steroid quantitative analysis.

1 ml of test extract of Steroid solution was transferred into 10 ml volumetric flask, Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2ml) were added followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5ml). The mixture was heated in a water bath maintained at 70±2°C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Estimation of cardiac glycoside contents

Kedde's reagent (A solution of glycosides is treated with a small amount of Kedde reagent (Equal volumes of a 2% solution of 3, 5 dinitrobenzoic acid in menthol and a 7.5% aqueous solution of KOH were mixed): Three (3ml) of the plant extract and 3ml of freshly prepared Kedde's reagent were mixed together. The absorbance of mixture was measured after 2.5 min at 560 nm. The difference between the intensity of the colors of the experimental and blank (distilled water and Kedde's reagent) were proportional to the concentration of the glycoside.

Percentage of phenols, saponins, tannins, terpenes, and glycosides used in this analysis were calculated as follows:

$$\frac{100}{W} \times \frac{AU}{AS} \times C \times \frac{VF}{VA} = \%\ \text{constituent}$$

Where:

W = Weight of sample analyzed

AU = Absorbance of test sample

AS = Absorbance or concentration of standard solution

VF = Total volume of filtrate analyzed

VA = Volume of filtrate analyzed

C = Total volume of extract.

Micronutrients and Macronutrients composition

Mineral contents (Ca, Mg, K, Fe, and Na) were determined using an Atomic Absorption Spectrophotometer according to AOAC, (2005), while (Cu, Mn, and Zn) were determined using the inductively coupled plasma technique mass spectrometry (ICP-MS) as described by (Riaz *et al.*, 2020) with minor modifications.

Vitamins studies

Vitamin contents (Vitamin B, C, and D) were determined using an Atomic Absorption Spectrophotometer according to AOAC, (2005), while (Cu, Mn, and Zn) were determined using the inductively coupled plasma technique mass spectrometry (ICP-MS) as described by (Riaz *et al.*, 2020) with minor modifications.

RESULTS

Quantitative analyses of antinutrients *Hibiscus sabdariffa* dried calyces:

The top minerals in the calyx's research were potassium, calcium, sodium, manganese, and iron, with 14047.02 mg/kg, 4698.348 mg/kg, 2838.75 mg/kg, 538.936 mg/kg, and 154.411 mg/kg, respectively. The remaining minerals were present in progressively lower levels in the calyx's sample, with the exception of Lead, Nikel, and chromium, which were not present.

Table 1: Micro and Macro minerals of *Hibiscus sabdariffa* dried calyces in mg/ kg

Mineral element	**Concentration (mg/kg)**
Zinc	0.76
Cadmium	9.485
Nickel	0
Iron	154.411
Lead	0
Chromium	0
Copper	3.075
Calcium	4698.348

Magnessium	14.341
Manganese	538.936
Sodium	2838.75
Potassium	14047.02
Phosphorous	36.46

Table 2: Vitamins composition of *Hibiscus sabdariffa* dried calyces in mg/100g and µg/100g

Vitamins	**concentration in mg/100g**	**concentration in µg/100g**
Vitamin C	13.24	
Vitamin D		18.26
Vitamin B_1	4.11	

Quantitative composition of antinutrients of *Hibiscus sabdariffa* dried calyces

Table 3 shows the findings of the quantitative phytochemical study of antinutrients.

The sample was examined for terpenes, glycosides, saponin, steroids, tannins, and phenols. Phenol was found to be the most abundant component, whereas tannin was found to be the least abundant.

Table 3: Phytochemical composition of *Hibiscus sabdariffa* dried calyces (%)

Components	**Values (%)**
Tannin	0.0013
Terpenes	0.0021
Steroids	0.0032
Glycosides	0.068
Saponnin	0.096
Phenol	0.187

DISCUSSION

The presence of secondary metabolites in the form of phytochemicals, vitamins, and essential minerals was discovered in *Hibiscus sabdariffa* extracts. The chemicals that are physiologically active have curative powers Nkumah, (2015).Tannins, saponins, glycosides,

phenols, and terpenes are among the phytochemicals extracted qualitatively. Table 3 displays the quantitative analysis results in low percentages.

Phenol, saponin, glycosides, steroids, tannin, and terpenes are listed in decreasing order as a result of phytochemical analysis.

Steroids are of great importance and interest in pharmacy due to their interaction with chemicals such as sex hormones.

Tannins are astringent, which promotes wound healing and prevents deterioration (Imam *et al.,* (2013). The most commonwere glycosides (0.068 percent) and saponin (0.0 96 percent), with phenol (0.187 percent) being the most prevalent.

The least prevalent were Tannin, terpenes and steroid (0.00217%, 0.0013% and 0.0032%). Tannins are polyphenols that can be found in a wide range of plants. They have been connected to blood clotting speed, blood pressure reduction, immune response modulation, and plasma lipid reduction. Glycosides tend to change biological activity by helping to strengthen cardiac muscle contractions. Saponnin is an anticancer agent Yildirim and Kutlu, (2015), preventing the growth of fungi, yeasts, bacteria, and viruses while lowering the mutagenic activity of a number of mutagens.

The Zobo calyces phenol content was lower than previous values of Azza, *et al.,* (2011) in *H. sabdariffa.* Tannin levels were also higher than those reported by Chinatu, *et al*., (2016) in *H. sabdariffa* calyces. The aqueous extract of *Hibiscus sabdariffa* calyces is consumed as a hot or cold beverage in Africa. The leaves and calyces are used in a variety of local recipes as vegetables Obiefuna *et al.,* (1994).

The mineral makeup of dried Roselle calyces is shown in Table 1. Sodium, potassium, calcium, magnesium phosphorus, iron, zinc, and cadmium are all present.

The calyx of the *H. sabdariffa* plant contains more potassium (K), calcium (Ca), sodium (Na), Manganese (Mn) and Iron content, with values of 14047.02mg/kg, 4698.348mg/kg, 2838.75mg/kg, 538.936 mg/kg, and 154.411mg/kg, respectively, according to the present data. Essential minerals such as sodium, calcium, potassium, and phosphorus have been shown to be equivalent to published data Balarabe, (2019).

Calcium, magnesium, potassium, and sodium are all nutrients that the body needs (Hassan *et al*., 2011).The concentration at which they are required by the body on the other hand varies. Potassium is a critical mineral that is required for both cellular and electrical function. Magnesium also helps maintain potassium levels in the cells, although the sodium-potassium

balance is just as finely tuned as the calcium-phosphorus or calcium-magnesium balances Izah *et al*., (2015).

Fruits and vegetables are high in potassium, calcium, magnesium, and sodium while being low in salt. Increased calcium, magnesium, and potassium intake, as well as a decrease in sodium intake, reduce the risk of hypertension, or high blood pressure, according to Izah *et al.,* (2015). Potassium, like sodium, aids in the regulation of blood and tissue water and acid-base balance.

Zinc is required for the formation of bone and teeth, whereas iron is required for the formation of blood. Zinc also aids calcium absorption in the bones and is considered an important nutrient for the immune system Salami and Afolayan, (2020).

The *H. sabdariffa* calyx is also rich in carotene, riboflavin, anthocyanins, ascorbic acid, niacin, and vitamin C. The sour taste of Roselle is related to ascorbic acid content and boiling of Roselle for 10 minutes reported to increase the vitamin C but more boiling time will decrease vitamin C Tahir *et al*., (2021).

The current tendency is that eating Roselle calyces will aid in the creation of healthy bones and teeth, as well as being beneficial to blood formation. Na is needed to maintain bodily fluid osmotic equilibrium and pH, muscle modulation and nerve irritability, glucose absorption control, and adequate protein retention throughout growth. Similar amounts of these minerals had previously been found in plant minerals. Fe and Zn concentrations were sufficient for metabolic function Azza *et al*., (2011).

Human Fe requirements are 10-15 mg for children, 18 mg for women and 12 mg for adults on a daily basis. Zinc plays an important role in human nutrition. Because of its role in nucleic acid metabolism and protein synthesis, zinc deficiency causes stunted development and delayed sexual maturation. As a result, adequate mineral and trace element consumption, such as iron, zinc, and calcium, is critical for supporting optimal health, growth, and development in newborns and young children. The enzyme cytochrome oxidase, which is important in energy metabolism, contains Fe and Cu Azza *et al*., (2011).

Calcium is a coordinator among inorganic elements; for example, excess K, Mg, or Na in the body can be corrected by Ca, and a sufficient amount of Ca in the diet can help with Fe use. The Roselle calyces have high Calcium value 4698.348mg/kg respectively. Mg maintains the electrical potential in nerves by activating various enzyme systems. Variety, location, and environmental circumstances all have an impact on plant mineral content Azza *et al*., (2011).

Vitamins are micronutrients that serve as body protectors and must be obtained through food. The calyx of *H. sabdariffa* has high levels of vitamin D (18.26µg/100g), often known as the

sunshine vitamin. Vitamin study found that the calyx of *H. sabdariffa* is high in vitamins D, B_1, and C, which all play vital roles in human health Onyegeme-Okerenta *et al.*, (2017).

Humans receive the water-soluble vitamin C from their diet. Vitamins help to maintain a healthy diet. Ascorbic acid, also referred to as vitamin C is an essential micronutrient required for normal metabolic function of the body, has a number of health advantages Khan *et al*., (2006). The prevention of some DNA-damaging free radicals, which could hasten aging and contribute to the emergence of heart disease and other illnesses, as well as oxidative damage, improves aspects of cardiac health such as hypertension, reduces the risk of severe respiratory conditions such as asthma, promotes wound healing, scurvy prevention and treatment, common cold therapy, immune system boosting, lowers the risk of lead toxicity and cancer prevention, and treats cataracts Izah *et al*., (2015).

Vitamins B complex and C are important sources of antioxidants and provide an adequate amount of vitamins for the synthesis of enzymes required for optimal health.

The calyx is the most important part of the plant because it contains the valuable components that influence the quality of the product, namely: Anthocyanin is responsible for the color, flavor (organic acid), and scent of Balarabe (2019). Calyx is also high in malic acid, anthocyanins, ascorbic acid, and minerals. Plant primary and secondary metabolites in the form of Steroids, phenolics, alkaloids, pigments, vitamins and more were discovered to be the dietary ingredients contributing to these plant materials' protective benefits Balarabe, (2019).

The most significant soluble antioxidant that shields cells from damage in an aqueous environment is vitamin C, according to Izah *et al.,* (2015). Proteins, calcium, and vitamin C all promote bone development Chinatu *et al.,* (2016).These emphasize how crucial Roselle calyx is to the general health of Nigerians.

CONCLUSION

The well-known medical plant *Hibiscus sabdariffa*, sometimes called "Roselle or Zobo," includes a number of essential nutrients. The calyces of the *Hibiscus sabdariffa* are used to make the traditional Nigerian beverage Roselle. People from various socioeconomic levels consume it. Given its abundance in macro- and micronutrients, Roselle holds great promise for improving socioeconomic development and livelihoods through the production of a range of food products with added value.

Hibiscus sabdariffa is a plant that contains phytochemicals, nutrients, minerals, and vitamins that have a multitude of health benefits. According to the findings, *H. sabdariffa* calyx is a good source of important minerals (potassium and calcium), as well as vitamins C, B_1, and D. As a result of the current study's findings, we can assume that these plants can serve as

mineral constituents to animals and people through eating and drinking. The antinutrient composition, vitamins and micronutrient components of the locally grown Roselle (*Hibiscus sabdariffa L.*) were determined in this study and the findings and conclusions matched those of other studies. Drinks made from Roselle calyces should be consumed regularly as it is safe, natural and nutritious.

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