NUTRITIONAL PROFILE OF THE SEEDS OF *HIBISCUS SABDARIFFA* AND *HIBISCUS CANNABINUS* FROM SOKOTO

BY

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CERTIFICATION

This Research project titled "Nutritional Profile of the seeds of *Hibiscus sabdariffa* and *Hibiscus cannabinus* from Sokoto" by Afolabi Qowiyat Opeyemi (2010302064) has been read and certify by the undersigned as meeting the requirement for the award of Bachelor of Science (Hons.) Degree in Biology in the Department of Biological Science, Usmanu Danfodiyo University, Sokoto.

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DEDICATION

This project is dedicated first and foremost to Almighty God, whose wisdom and guidance sustained me throughout this work. It is also lovingly dedicated to my late Grandparent Mr and Mrs Afolabi, though you are no longer here, your love still lives in every beat of my heart.

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ABSTRACT

This research investigated the nutritional composition and phytochemical properties of *Hibiscus sabdariffa* and *Hibiscus cannabinus* seeds obtained from Sokoto, Nigeria, with the aim of evaluating their potential as sources of nutrients and bioactive compounds. The study employed standard analytical methods to determine proximate composition (moisture, ash, crude protein, fat, fiber, and carbohydrate), vitamin C content, mineral composition, and qualitative phytochemical constituents. Results showed that *H. sabdariffa* contained higher levels of crude protein (18.64%), fat (6.41%), fiber (10.28%), and ash (4.32%) compared to *H. cannabinus*, which exhibited a slightly higher carbohydrate content (54.27%). The vitamin C concentration was notably higher in *H. sabdariffa* (275.60 mg/100 g), indicating stronger antioxidant potential. Mineral analysis revealed potassium and calcium as the predominant elements in both species, while *H. cannabinus* had slightly elevated sodium and zinc levels. Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, tannins, terpenes, and glycosides, with *H. sabdariffa* showing a richer diversity and intensity of bioactive compounds. These phytochemicals contribute to the seeds' potential antioxidant, antimicrobial, and anti-inflammatory properties. The study demonstrates that both *Hibiscus sabdariffa* and *Hibiscus cannabinus* seeds possess valuable nutritional and therapeutic qualities, positioning them as promising candidates for use in food fortification, nutraceutical production, and dietary diversification strategies. Consequently, promoting the cultivation and utilization of these underexploited seeds could help combat micronutrient deficiencies and enhance food security in Nigeria and other developing regions.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Plants have always played an important role in providing food, medicine, and essential nutrients. Every individual, family, and nation aims to have access to a diet that is enjoyable, affordable, and culturally suitable. This practice of eating food is known as nutrition. Nutrition involves the intake and use of food and nutrients to support the body's growth, maintenance, and health (WHO, 2023). Nutrients are substances in food that living organisms need to produce energy, grow, develop, and reproduce. These nutrients are broken down during digestion so they can be absorbed and used by the body. Humans get nutrients from the food they eat, while plants absorb theirs from the soil (WHO, 2016).

Zobo seeds, derived from the *Hibiscus* species, are known for their nutritional and medicinal properties. Two common species cultivated in Nigeria are *Hibiscus sabdariffa* and *Hibiscus cannabinus*. While *Hibiscus sabdariffa* is widely used for making Zobo drink, *H. cannabinus* is more commonly known for its fiber production, but it is also consumed in some communities. *Hibiscus sabdariffa* is a flowering plant from the Malvaceae family. It grows well in tropical and subtropical regions such as Africa, Asia, the Caribbean, and Central America. In Nigeria and other parts of West Africa, it is commonly known as "zobo" in the Hausa language. The plant's calyces and seeds are widely used in both cooking and traditional medicine (Wang *et al*., 2018). It is an annual shrub that grows up to 2-2.5 meters tall and is easily identified by its bright red calyces. These are often dried and used to make a popular drink called zobo. Although the calyces are the most commonly

used part of the plant, the seeds are also important and are often cooked as vegetables or added to soups and sauces. The color of the seeds can be green or reddish-green depending on the type (Maganha *et al*., 2020).

Recent research has shown that green seedy vegetables are good sources of important nutrients like proteins, vitamins, minerals, and plant compounds known as phytochemicals (Javadzadeh and Saljooghianpour, 2017). *Hibiscus sabdariffa* seeds contain many useful nutrients, including proteins, vitamin C, some B vitamins, minerals such as calcium, iron, and magnesium, as well as dietary fiber. They also have phytochemicals like flavonoids, tannins, saponins, and phenolic compounds, which have health benefits like fighting inflammation, bacteria, and harmful free radicals (Javadzadeh and Saljooghianpour, 2017).

Traditionally, these plants have been used in herbal medicine to treat conditions like high blood pressure, liver problems, fever, and stomach issues. Scientific studies now support many of these traditional uses, showing that *Hibiscus sabdariffa* can serve as both a healthy food and a natural remedy (Olaleye and Akindahunsi, 2015). The plant also plays a role in supporting the body's metabolism. Its health benefits are mostly due to its vitamins, minerals, and phytochemicals (Olaleye *et al*., 2015). However, there is still limited research on the comprehensive nutritional profile of *Hibiscus cannabinus* seed, especially in the context of promoting them as a dietary component for improved public health.

As malnutrition and micronutrient deficiencies continue to affect populations in developing countries, there is a need to explore underutilized plants like *Hibiscus* species for their potential contribution to food and nutrition security. Therefore, this study aims to examine the nutritional content of two varieties of *Hibiscus* species *(Hibiscus sabdariffa* and *Hibiscus cannabinus)* seeds collected from Sokoto. The research will measure moisture, ash, fat (crude lipid), fiber, protein, carbohydrates, minerals (such as calcium, magnesium, sodium, potassium, and phosphorus), and photochemical.

1.2 Statement of the Research Problem

Regardless of increasing awareness of the role of plant-based diets in promoting health, *Hibiscus* species remains underutilized and under-researched compared to other seedy vegetables. The lack of detailed nutritional information on this seed has limited its acceptance and integration into nutrition intervention programs (Sulaiman et al., 2024). Moreover, the growing concern over hidden hunger and malnutrition calls for the identification of local plant resources that can supply essential nutrients. Therefore, this study seeks to evaluate the nutritional composition of *Hibiscus sabdariffa* and *Hibiscus cannabinus* seeds through laboratory-based analyses to bridge this information gap.

1.3 Justification of the Study

Despite the recognized nutritional and medicinal benefits of *Hibiscus* species (*Hibiscus sabdariffa* and *Hibiscus cannabinus*) particularly their calyces, the seeds remain underutilized and insufficiently studied. With the growing global interest in plant-based diets and functional foods, it becomes imperative to scientifically validate the nutritional potential of underexploited seedy vegetables like *Hibiscus sabdariffa*. The lack of comprehensive data on the proximate, vitamin, mineral, and phytochemical composition of its seeds has limited their inclusion in nutrition education programs, dietary planning, and food product development (Sulaiman *et al*., 2024). Given the plant's availability in regions like Sokoto, Nigeria, and its potential to address malnutrition and micronutrient deficiencies, a comprehensive analysis of its seeds' nutritional composition is warranted (Wang *et al*., 2018). This study aims to fill the existing knowledge gap by evaluating the proximate, vitamin, mineral, and phytochemical contents of two varieties of *Hibiscus* species seeds. This study focuses on analyzing and comparing the nutritional composition of the two species sourced from Sokoto State, where they are cultivated under similar agro-

climatic conditions. The findings are expected to serve as a scientific basis for encouraging the consumption of these seeds and potentially inform nutritionists, policymakers, and the food industry about their health-promoting properties.

1.4 Aim and Objectives of the Study

1.4.1 Aim of the Study

The main aim of this study is to determine the nutritional composition of *Hibiscus sabdariffa* and *Hibiscus cannabinus* seeds from Sokoto.

1.4.2 Objectives of the Study

1. To analyze the proximate composition and vitamin C content of two varieties of *Hibiscus sabdariffa* and *Hibiscus cannabinus* seeds from sokoto.

2. To assess the mineral composition of the seeds.

3. To identify the phytochemical cons

1.5 Research Questions

1. What is the proximate composition (moisture, ash, fat, protein, fiber, and carbohydrate) of two varieties of *Hibiscus* seeds from Sokoto?

2. What is the vitamin content of the seeds?

3. What is the mineral composition of the seeds?

4. What phytochemical constituents are present in the seeds, and what are their quantities?

1.6 Significance of the Study

This study will contribute to the body of knowledge on the nutritional potential of *Hibiscus sabdariffa* and *Hibiscus cannabinus* seeds, promoting its use as a functional food. The findings may inform nutritionists, dietitians, and policymakers about its value in combating nutrient deficiencies and improving dietary diversity. Additionally, the study may encourage further research and industrial applications involving the seed in food formulations and nutraceuticals.

1.7 Scope and Limitation of the Study

This study focuses solely on the nutritional analysis of *Hibiscus sabdariffa* and *cannabinus* seeds. The scope is limited to laboratory analyses of its proximate, vitamin, mineral, and phytochemical composition. The study does not include clinical trials or assessments of bioavailability. Environmental factors, seasonal variations, and agricultural practices that may affect nutrient content are beyond the scope of this research.

1.8 Operational Definition of Terms

1. **Proximate Composition**: The analysis of basic nutritional components such as moisture, ash, fat, fiber, protein, and carbohydrates.
2. **Phytochemicals:** Naturally occurring compounds in plants that may contribute to health and disease prevention.
3. **Micronutrients**: Nutrients required in small quantities for proper body functioning, including vitamins and minerals.
4. **Nutritional Analysis:** The process of determining the nutrient content of food substan

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and Distribution of Zobo Seed *(Hibiscus sabdariffa)*

Hibiscus sabdariffa, commonly referred to as "zobo" in Nigeria, is a tropical plant species belonging to the Malvaceae family. It is believed to have originated in Africa, particularly in the region encompassing present-day Sudan and West Africa, where it has been cultivated for centuries for its edible calyces and medicinal properties (McClintock and El Tahir, 2016). Some botanical sources also suggest possible dual centers of origin, including India, due to the plant's long-established presence in both continents (Morton, 2017).

Historically, *Hibiscus sabdariffa* was spread beyond its African origin through trade routes and human migration. It was introduced to the Caribbean and Central America during the transatlantic trade era and eventually reached Asia, where it became a common crop in countries such as India, Thailand, and Indonesia (Mahadevan *et al*., 2019). The global distribution of the plant can be attributed to its adaptability to a wide range of climates and its versatility in culinary, medicinal, and industrial applications.

Currently, *Hibiscus sabdariffa* is cultivated in many tropical and subtropical regions around the world. In Africa, major producers include Nigeria, Sudan, Mali, Senegal, and Egypt. In Asia, India and Thailand are significant growers, while in the Americas, Mexico and Jamaica are notable for their large-scale cultivation and export of dried calyces (Da-Costa-Rocha *et al*., 2024). In Nigeria, especially in the northern regions such as Sokoto, Kano, and Kaduna, the plant is widely grown and utilized not only for its calyces used in beverages but also for its seeds, which are cooked as vegetables.

The plant's popularity is due to its economic importance, health benefits, and its role in cultural diets. Its bright red calyces are rich in anthocyanins and are used to produce drinks, jams, sauces, and herbal medicines. Meanwhile, its seeds are a good source of vitamins, minerals, and phytochemicals, though they are relatively underutilized compared to the calyces. The increasing interest in functional foods and local nutrition resources has prompted renewed attention to *Hibiscus sabdariffa* in both research and agricultural development.

2.2 Botanical Description of *Hibiscus* Species

2.2.1 Description of *Hibiscus sabdariffa*

Hibiscus sabdariffa L., commonly known as Roselle or "zobo" in Nigeria, belongs to the family Malvaceae. It is an annual or short-lived perennial shrub that grows erect, reaching a height between 1.5 to 2.5 meters (Morton, 1987; Da-Costa-Rocha *et al*., 2024). The stem is robust, often reddish or greenish, and covered with fine hairs. The seeds are alternate and vary in shape depending on their position on the stem. Lower seeds tend to be ovate or lanceolate with entire margins, while the upper seeds are typically deeply lobed into three to five segments. Seed size ranges from 7 to 15 cm in length, with serrated edges and a slightly hairy underside (Mahadevan *et al*., 2019). The flowers are large, solitary, and axillary, measuring about 8 to 10 cm in diameter. They are usually pale yellow or creamy with a conspicuous dark red or maroon center. The five-petaled corolla is short-lived, blooming during the day and wilting by nightfall (Delgado-Vargas *et al*., 2020).

The most commercially important part of the plant is the fleshy, red calyx that forms after the flower drops. These calyces, which envelop the seed capsule, are rich in anthocyanins and are harvested for use in food, beverages, and traditional medicine. The fruit is a five-lobed, ovoid capsule containing numerous small, brown, kidney-shaped seeds (Da-Costa-

Rocha *et al*., 2024). *Hibiscus sabdariffa* thrives in tropical and subtropical climates and prefers well-drained sandy or loamy soils with full sunlight. It is tolerant of drought conditions and matures within 3 to 5 months of planting. Though the calyces are the most widely used part, the seeds are also edible and are consumed as seedy vegetables in several cultures (Morton, 2017; Mahadevan *et al*., 2019).

2.2.1.1 Botanical Classification of Hibiscus Plant

Kingdom: Plantae (Plants)

Phylum: Tracheophyta (Vascular plants)

Class: Magnoliopsida (Dicotyledons)

Order: Malvales

Family: Malvaceae (Mallow family)

Genus: *Hibiscus*

Species: *sabdariffa and cannabinus*

Botanical Name: *Hibiscus sabdariffa and Hibiscus cannabinus*

2.2.2 Description of *Hibiscus cannabinus*

Hibiscus cannabinus L., commonly known as Kenaf, is a rapidly growing annual herbaceous plant in the family Malvaceae. It typically grows between 1.5 and 4 meters in height, featuring a strong, erect, fibrous stem that is green to reddish in color and slightly hairy (Akinyemi *et al*., 2022). The seeds are alternate and polymorphic: lower seeds are

deeply lobed with 3 to 7 divisions, while upper seeds are lanceolate with serrated margins and a slightly hairy underside (Okoro *et al*., 2021).

The flowers are solitary and axillary, measuring 8-15 cm in diameter, with pale yellow or cream petals and a characteristic dark maroon center. They contain a prominent staminal column and are subtended by an epicalyx composed of 8-12 narrow bracts (Ashaolu *et al*., 2023). The fruit is a dry, ovoid capsule that splits open at maturity to release numerous kidney-shaped, brownish seeds covered in fine hairs. These seeds are oil-rich and used in both food and biofuel applications (Lawal *et al*., 2020).

Kenaf is well-suited to tropical and subtropical regions, thriving in well-drained loamy soils under full sunlight, with moderate rainfall. It matures within 4 to 5 months and is cultivated for its bast fiber, edible seeds, and environmental benefits such as soil remediation and carbon sequestration (Ashaolu *et al*., 2023; Akinyemi *et al*., 2022).

2.3 Proximate Analysis

Proximate analysis is a standardized method used to determine the fundamental nutritional components of a biological sample. It gives a general overview of a substance's chemical composition by estimating moisture, ash, crude protein, crude fat (lipid), crude fiber, and nitrogen-free extract (carbohydrate). This analysis is particularly essential in food science, animal nutrition, and medicinal plant research to assess the quality and nutritive value of the sample.

2.3.1 Moisture Content

Moisture refers to the water content in a sample and is typically measured by drying the sample in an oven at 105°C until a constant weight is achieved. The difference in weight before and after drying gives the moisture content. Moisture analysis is critical as water

content influences microbial stability, shelf-life, texture, and overall quality (AOAC, 2016). High moisture levels can promote spoilage and reduce shelf life, whereas low moisture levels are associated with better storage stability (AOAC, 2016).

2.3.2 Ash Content

Ash is the inorganic residue that remains after a sample is completely combusted at high temperatures (around 550°C) in a muffle furnace. It represents the total mineral content present in the sample. However, ash does not give information on individual minerals, necessitating further elemental analysis. A high ash value may suggest a rich mineral profile, but it could also indicate contamination with soil or other impurities (Nielsen, 2020).

2.3.3 Crude Protein

Protein content is generally estimated by determining the nitrogen content using the Kjeldahl or Dumas method and multiplying the result by a conversion factor (commonly 6.25). This assumes that proteins contain 16% nitrogen. Crude protein is essential for assessing the nutritional value of food and feed, as it plays a fundamental role in growth and repair of tissues (Nielsen, 2020).

2.3.4 Crude Fat

Crude fat is extracted using solvents such as diethyl ether or petroleum ether in a Soxhlet apparatus. This method isolates lipophilic substances including triglycerides, sterols, and fat-soluble vitamins. Fat content affects the caloric value and palatability of food, making this analysis crucial in nutrition studies (Osborne and Voogt, 2018).

2.3.5 Crude Fiber

Crude fiber refers to the indigestible portion of food derived from plant cell walls, composed mainly of cellulose and lignin. It is estimated by sequential acid and base digestion and reflects the sample's ability to aid digestion and prevent gastrointestinal diseases. While not a complete measure of dietary fiber, it provides a general idea of fibrous content (Osborne and Voogt, 2018).

2.3.6 Nitrogen-Free Extract (NFE)

This is calculated by subtracting the sum of moisture, ash, crude protein, crude fat, and crude fiber from 100. It is presumed to represent the digestible carbohydrates such as starches and sugars and is used to estimate the energy-yielding potential of the sample.

2.4 Overview of Vitamin and Mineral Analysis

Vitamins and minerals, though required in small quantities, are essential for normal metabolic functions, enzymatic reactions, immunity, and overall health. Their analysis helps determine the nutritional and therapeutic potential of plants and food products.

2.4.1 Vitamin Analysis

Vitamins and minerals are essential micronutrients that play critical roles in maintaining various bodily functions, including energy metabolism, immune function, and overall health (WHO, 2020). Accurate analysis of these micronutrients is crucial in understanding their content in foods, supplements, and biological samples. Vitamins are classified into water-soluble (e.g., B-complex, vitamin C) and fat-soluble (e.g., vitamins A, D, E, K). These compounds are chemically diverse and sensitive to light, heat, and oxidation, which makes their analysis complex.

Water-Soluble Vitamins: These are usually analyzed by spectrophotometric methods or HPLC. For instance, vitamin C can be quantified using the 2,6-dichlorophenol-indophenol titrimetric method or HPLC due to its strong reducing properties (Ball, 2016).

Fat-Soluble Vitamins: These require organic solvent extraction and saponification before analysis. HPLC is commonly used for their quantification because of its precision and sensitivity. Detection is often achieved using UV or fluorescence detectors, depending on the vitamin's characteristics (Ball, 2016).

2.4.2 Mineral Analysis

Minerals are inorganic elements required by living organisms in small but essential quantities for optimal physiological and biochemical functioning. They are involved in various biological processes including structural support, enzyme activation, osmoregulation, nerve transmission, and cellular metabolism (Gibson, 2015). Evaluating the mineral composition of plant-based foods such as *Hibiscus sabdariffa* and *H. cannabinus* seeds is crucial for assessing their nutritional value and potential health benefits. Minerals are generally classified into;

Macrominerals: These are minerals needed in relatively large amounts (more than 100 mg/day). They play important roles in skeletal structure, fluid balance, and muscle function. Examples are calcium, phosphorus, magnesium, sodium, potassium, and chloride (Nielsen, 2020).

Microminerals: Microminerals are minerals required in much smaller amounts (less than 100 mg/day) but are critical for enzymatic reactions and antioxidant defense. Examples are iron, zinc, manganese, copper, selenium, and iodine (Nielsen, 2020).

2.5 Phytochemical Contents of *Hibiscus* species

Phytochemicals are plant compounds that are secondary metabolites with health promoting properties. According to Kalt (2015), in vitro studies indicated that how to chemicals such as phenolics have the ability to protect biological system from the effect of oxidative stress. 5 to chemicals under research can be classified into major categories such as carotenoids and polyphenols, which include phenolic acid, flavonoids, tannins, alkaloids and saponine (Corvallis, 2016). This polyphenols are biologically active naturally occurring chemical compounds found in *Hibiscus sabdariffa* which provide health benefits for humans as medicinal ingredient and nutrients. They protect plants from diseases and damage and also contributes to the plants' color, aroma and flavour (Hasler and Blumberg, 2019).

2.5.1 Flavonoids

Flavonoids are polyphenolic compounds that are ubiquitous in nature. More than 4,000 flavonoids have been recognized, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks (Pridham, 1960). The flavonoids are also class of naturally occurring and structurally related compounds found widely distributed in plants and plant foods (Bronner and Beecher, 1995). The flavonoids appear to play a major role in successful medical treatments of ancient times, and their use has persisted up to now. In *Hibiscus sabdariffa*, common flavonoids such as quercetin, kaempferol, and anthocyanins have been reported and there are limited informations about the phytochemical contents of *Hibiscus cannabinus* (Da-Costa-Rocha *et al*., 2024). These compounds help scavenge free radicals, reduce oxidative stress, and may protect against cardiovascular and neurodegenerative diseases. Moreover, quercetin has demonstrated anti-inflammatory and antihistamine properties (Akinmoladun *et al*., 2020).

2.5.2 Alkaloids

Alkaloids are natural product that contains heterocyclic nitrogen atoms, are basic in character. The name of alkaloids derives from the "alkaline" and it was used to describe any nitrogen-containing base (Mueller-Harvey and Mcallan, 2017). Alkaloids are naturally synthesized by a large number of organisms, including animals, plants, bacteria and fungi. Some of the first natural products to be isolated from medicinal plants were alkaloids. In the early years of 19th century, it was found that they were nitrogen containing bases which formed salts with acid. Hence, they were known as the vegetable alkaloids and these alkaloids are used as the local anesthetic and stimulant as cocaine (Ali *et al*., 2015).

The use of alkaloids containing plants as dyes, spices, drugs or poisons can be traced back almost to the beginning of civilization. Alkaloids exhibit diverse pharmacological effects, including antimicrobial and analgesic activities. Studies show that *Hibiscus sabdariffa* seeds contain moderate levels of alkaloids, which may contribute to its traditional use in pain relief and microbial infection management (Ali *et al*., 2015).

2.5.3 Saponins

Saponins are a group of secondary metabolites found widely distributed in the plant kingdom. They form a stable foam in aqueous solutions like soap, hence the name saponin. Chemically, saponins as a group which include compounds that are glycosylated steroids, titerpenoids, and steroid alkaloids. We main triterpeneaglycone is a derivative of olanane (Bohlmann *et al*., 2018). The carbohydrate part consists of one or more sugar moisties containing glucose, galactose, xylose, arabinose, rhamnose, or glucuronic acid glycosidically linked to a sapogenin (aglycone). Saponins that have one sugar molecule attached at the C-3 position are called momdesmosidesaponins, and those that have a

minimum of two sugars, one attached to the C-3 and one at C-22, are called bidesmosidesaponins (Lasztity *et al*., 2018).

The physiological role of saponins in plants is not yet fully understood. While there is a number of a publication describing their idenificaton in plants, and their multiple effects in animal cells and on fungi and bacteria, only a few have addressed their function in plant cells. Many saponins are known to be antimicrobial, to inhibit mold, and to protect plants from insect attack. Saponins may be considered a part of plants defense systems, and as such have been included in a large group of protective molecules found in plants named phytoanticipins phytoprotectants (Lacaile-Dubois and Wagner, 2020).

Extensive research has been carried out into the membrane-permeabilishing immunostimulant, hypocholessterolaemic and anti-carcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals. These structurally diverse compounds have also been observed to kill protozoans and moluses, to be antioxidants, to impair the dioestion of protein and the uptake ot VItamius and minerals in the gut, to cause hypoglycaemia, and to act as antifungal and antiViral (Traore *et al*., 2020). The physiological role of glycoside in plant extracts that contain the cyanogenic glycoside are used as flavoring agent in many pharmaceutical preparations.

2.5.4 Tannins

Tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides (cellulose, hemicellulose, pectin etc.), alkaloids, nucleic acids and minerals (Schofield *et al*, 2021).

2.5.5 Steroids

Steroids are usually encountered n natural sources, and those presented in Calotropis species are mostly derivatives of B-sitosterol and stigmasterol. Among these steroids, B-sitosterol and B-sitosteryl glucoside were trequently found in several Calotropis species. In addition, some miscellaneous compounds including glycerol and proto-quercitol were also reported from Calotropis species (Klemens *et al*., 2016).

2.5.6 Phenolic acids

Phenolic acids form a diverse group that includes the widely distributed hydroxy-benzoic and hydroxycinnamic acids. Phenolic polymers, commonly known as tannins, are compounds of high molecular weight that are divided into two classes which are; hydrolyzable tannins and condensed tannins. The term 'phenolic acids', in general, designates phenols that possess one carboxylic acid functional group. Naturally occurring phenolic acids contain two distinctive carbon frameworks viz. the hydroxycinnamic and hydro-xybenzoic structures. Hydroxy-Cinnamic acid compounds are produced as simple esters with glucose or hydroxy carboxylic acids. Plant phenolic compounds are different in molecular structure, and are characterized by hydroxylated aromatic rings. These compounds have been studied mainly for their properties against oxidative damage leading to various degenerative diseases, such as cardiovascular diseases, inflammation and cancer. Indeed, tumour cells, including leukaemía cells, typically have higher levels of reactive oxygen species than normal cells so that they are particularly sensitive to oxidative stress (Klemens *et al*., 2016).

2.5.7 Glyycoside

Glycoside may be phenol, alcohol or sulfur compounds. They are characterized by a sugar portion or moiety attached by a special bond to one or non-sugar portions. Many plants store chemicals in the form of inactive glycosides, which can be activated by enzyme hydrolysis. For this reason, most glycosides can be classified as pro-drugs since they remain inactive until they are hydrolyzed in the large bowel leading into the release of the aglycone, the right active cónstituent (Klemens *et al*., 2016).

The classification of glycosides is based on the nature of aglycone, which can be any of a wide range of molecular types including phenols, quinines, terpenes and steroids. They are horrendous in structure; therefore, they are not easy to learn as specific group and are described here in this review for the convenience, Glycoside bonds are of great significance, since they link monosaccharides together to form oligosaccharide and polysaccharide. Glycosides have gained recent attention concerming the therapeutic actions in different Studies, it has been shown that glycoside issued for anticancer, expectorant, sedative and digestive properties. The physiological role of glycoside in plant extracts that contain the cyanogenic glycoside are used as flavoring agent in many pharmaceutical preparations (Klemens *et al*., 2016).

2.6 Traditional and Medicinal Uses

Modern scientific research has validated many of the traditional uses of *Hibiscus sabdariffa*, highlighting its therapeutic potential in various health conditions.

2.6.1 Antihypertensive Effects

Multiple studies have shown that extracts of *Hibiscus sabdariffa* can help reduce blood pressure in hypertensive individuals. The mechanism is believed to involve vasodilation

and diuretic activity, helping the body get rid of excess sodium and water (Herrera-Arellano *et al*., 2017). In a randomized clinical trial, patients who consumed *Hibiscus* tea experienced a significant reduction in systolic and diastolic blood pressure compared to the placebo group.

Hibiscus sabdariffa (Roselle) is effective at reducing both systolic and diastolic blood pressure reported an experiment. The *Hibiscus* tea also known as sour tea was also seen to lower blood pressure in diabetic patients with moderate hypertension (Kafeshani *et al*., 2017). The extracts of Roselle exhibit antihypertensive effects mediated by three primary processes: ACE inhibition, vasodilation, and diuretic effects. According to results from a he current clinical investigation, sour tea lowered blood pressure in people with stage 1 hypertension (Montalvo-Gonzalez *et al*., 2022).

After a month, the blood pressure in the control group also dropped, this may have been a result of non-pharmacological approach of losing weight, following the Dietary approaches to stop hypertension (DASH) diet, boosting potassium and decreasing salt intake, as well as engaging in aerobic exercise (Carey *et al*., 2018). The sour tea has the added benefit of improving lipid profiles and the anthocyanins in Roselle can stop low-density lipoprotein oxidation, which delays the atherosclerotic process (Hajifaraji *et al*., 2018). Furthermore, Roselle has been shown to contain a compound that causes the release of nitric oxide from the vascular endothelium followed by an increase in renal filtration, a mechanism that counteracts the diuretic action of the plant and decreases blood pressure (Ahmad *et al*., 2018). Many therapeutic dosages of sour tea have been documented in order to produce its beneficial effects. (Hopkins *et al*., 2013) conducted one of the first studies examining how Roselle affects blood pressure. Their findings demonstrate that sour tea can lower blood pressure. They further reported that 3 days after the medicine was withdrawn, both systolic and diastolic blood pressure climbed to 7.9% and 5.6%, respectively (Lee, 2023).

2.6.2 Antioxidant Properties

Hibiscus sabdariffa is rich in anthocyanins and flavonoids, powerful antioxidants that combat oxidative stress. Oxidative stress is linked to aging and chronic diseases such as cancer, diabetes, and cardiovascular disorders. Antioxidant compounds in the plant help scavenge free radicals and protect cells from damage (Tseng *et al*., 2018). This makes it a potential supplement for promoting general health and preventing degenerative diseases.

According to research on the total antioxidant activity of the ethanolic seed extract from *Hibiscus_sabdariffa_*and C. caudatus, as concentration rises, so does absorbance, which corresponds to a rise in total antioxidant activity (Subhaswaraj *et al*., 2017). Ascorbic acid equivalents of 96.72 4.88 g/mL and 77.07 4.16 g/mL were found in the ethanolic extract of *Hibiscus_sabdariffa_*and C. caudatus, respectively (Subhaswaraj *et al*., 2017). This outcome is consistent with their earlier study, in which the ethanolic extract shown the strongest antioxidant activity. The outcomes demonstrated that the Roselle extract contains high anthocyanin content and strong antioxidant properties (DPPH IC50 results equaled 4.06 mg/mL and ABTS IC50 result equaled = 3.7 mg/mL) (Subhaswaraj *et al*., 2017). The anthocyanins themselves demonstrated some heat resistance as well as excellent color persistence in an acidic environment. Of all the plants examined, Roselle seed extracts were shown to have the highest antioxidant activity and the strongest radical-scavenging activity (Tounkara *et al*., 2014). By measuring antioxidant activity using the -carotene bleaching assay and DPPH radical-scavenging activity, methanol extracts of the plant revealed a favorable connection between phenolic content and antioxidant activity (Baliyan *et al*., 2022). More to that, one of the most prevalent and useful polyphenols in plants; chlorogenic acid, well known for its role as an antioxidant in plant-based meals has been demonstrated to be an effective defense molecule against a wide spectrum of insect herbivores in addition to its dietary antioxidant action (Owheruo *et al*., 2023). Several

studies over the past 20 years have demonstrated the usefulness of chlorogenic acid in preventing insect proliferation (Rojas-Gonzalez *et al*., 2022). Although the molecular regulation of herbivore-mediated induction of chlorogenic acid production is still not completely understood, it has been shown to occur at both the transcript and metabolite level (Kundu and Vadassery, 2019).

2.6.3 Antimicrobial Activity

Studies have shown that *Hibiscus* extracts possess antibacterial and antifungal properties. These extracts have demonstrated efficacy against Staphylococcus aureus, Escherichia coli, and Candida albicans. These findings support traditional uses of the plant in treating infections, wounds, and gastrointestinal issues (Calixto *et al*., 2015).

There is proof that the calyces of the Roselle have an antibacterial action on a variety of pathogenic microorganisms including Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Shigella flexneri, Salmonella Typhimurium, Escherichia coli, and Bacillus anthracis (Abass *et al*., 2022). Strong antibacterial activity against multidrug resistant A. baumannii is exhibited by the calyces of Roselle (Abdallah, 2016). The calyces of the plant have also been previously reported to possess antibacterial properties against a variety of pathogens, including foodborne pathogens, methicillin-resistant Staphylococcus aureus, Klebsiella pneumonia and Pseudomonas aeruginosa. Saponins, flavonoids, and tannins are antibacterial substances found in *Hibiscus* flowers (*Hibiscus* rosa-sinensis) (Abass *et al*., 2022). By creating intricate molecules against the extracellular proteins of bacterial cells that compromise the integrity of the bacterial cell membrane, flavonoids limit bacterial growth (Donadio *et al*., 2021). In earlier research, the methanol extract from the calyces of *Hibiscus_sabdariffa*_substantially inhibited both the non-multidrug resistant and drug resistant strains of A. baumannii, with a zone of inhibition ranging from (11.3-0.3) to (13.6-0.3) mm (Akindahunsi and Olaleye, 2015). The relative percentage inhibition of *Hibiscus*

*sabdariffa*_extract (10 mg/disc) was much higher than that of gentamicin (10 g/disc), demonstrating that it has potent antibacterial activities. The extract has a minimum inhibitory concentration and minimum bactericidal concentration ranges of 25 to 50 and 50 to 100 mg/mL respectively.

2.6.4 Lipid-Lowering Effects

Regular consumption of *Hibiscus* tea has been associated with lower cholesterol and triglyceride levels, particularly in individuals with metabolic syndrome or type 2 diabetes. The bioactive compounds in the plant can help reduce LDL (bad cholesterol) and improve HDL (good cholesterol), supporting cardiovascular health (Lin *et al*., 2017).

2.6.5 Antidiabetic Potential

Some experimental and clinical studies suggest that *Hibiscus sabdariffa* may have hypoglycemic effects, helping to regulate blood sugar levels. Its extracts have been shown to improve insulin sensitivity and glucose metabolism in diabetic patients, making it a potential adjunct therapy for managing diabetes (Mozaffari-Khosravi *et al*., 2024).

2.7 Composition of *Hibiscus sabdariffa*

Hibiscus sabdariffa (Roselle) is mainly cultivated for its calyx, which comes in three types: green, red, and dark red. Red calyces are the most used and are rich in anthocyanins, particularly Delphinidin 3-Sambubioside and Cyanidin 3-Sambubioside, the major anthocyanins in red calyces (Mady *et al*., 2019).

Hibiscus sabdariffa is also rich in organic acids, minerals, amino acids, carotene, vitamin C, and total sugars in its calyx, seeds, and leaves. These levels vary depending on the variety and geographical area (Mady *et al*., 2019).

Table 2.1: Nutritional Value of Roselle Plant

Nutrient	Calyces	Seeds	Leaves
Protein (g)	2	28.9	3.5
Carbohydrates (g)	10.2	25.5	8.7
Fat (g)	0.1	21.4	0.3
Vitamin A (I.E)	-	-	1000
Thiamine (mg)	0.05	0.1	0.2
Riboflavin (mg)	0.07	0.15	0.4
Niacin (mg)	0.06	1.5	1.4
Vitamin C (mg)	17	9	2.3
Calcium (mg)	130	350	240
Iron (mg)	3	9	5

Source: Agricultural and Food Sciences, 2020.

2.8 Previous Related Studies

Adegunwa *et al*. (2021) utilized the standard AOAC (Association of Official Analytical Chemists) methods for proximate analysis, including the determination of moisture content, crude protein, crude fiber, ash, lipid, and carbohydrate levels in *Hibiscus sabdariffa* seeds. Their results revealed that the seeds have a notably high moisture content (72-85%), indicating their potential perishability and the need for proper post-harvest handling. Crude protein content ranged between 14-21%, positioning the seeds as a valuable plant-based protein source for human and animal nutrition. Crude fiber content was recorded at 10-15%, supporting gastrointestinal health and digestion. Additionally, the seeds exhibited low lipid

content (1-2%), making them suitable for low-fat diets, and a moderate amount of carbohydrates, contributing to their caloric value.

Babalola *et al*. (2021) assessed the vitamin C (ascorbic acid) content of *Hibiscus_sabdariffa* seeds using UV-Visible Spectrophotometry. They reported remarkably high concentrations of vitamin C (260-280 mg/100g), which not only supports the antioxidant properties of the plant but also its traditional use in boosting the immune system, enhancing skin health, and aiding in the prevention of scurvy.

Wang *et al*. (2020) determined the presence of beta-carotene, a precursor to vitamin A, employing High-Performance Liquid Chromatography (HPLC) for accurate quantification. Their findings confirmed that *Hibiscus_sabdariffa*_seeds contain appreciable amounts of beta-carotene, reinforcing their role in supporting eye health, immune function, and protection against oxidative stress-related diseases.

Ali *et al*. (2018) conducted a detailed phytochemical screening using solvent extraction techniques (ethanol and methanol), followed by sophisticated HPLC and Thin Layer Chromatography (TLC) analyses. Their study identified a wide spectrum of bioactive compounds, notably anthocyanins, flavonoids, and phenolic acids. These phytochemicals are known for their potent antioxidant, anti-inflammatory, and cardioprotective activities, thereby validating the ethnomedical applications of *Hibiscus sabdariffa*.

Oladosu *et al*. (2018) carried out proximate analysis on *Hibiscus cannabinus* seeds using standard AOAC methods to determine moisture, ash, protein, fat, and carbohydrate contents. Mineral levels such as calcium, magnesium, iron, and zinc were measured using Atomic Absorption Spectrophotometry (AAS). Additionally, antioxidant activity was evaluated through the DPPH free radical scavenging assay, and total phenolic content was assessed with the Folin-Ciocalteu reagent. The results showed that the seeds contained a high protein content of 18.5% and fiber content of 12.2%, while fat content was low at

3.6%. Significant amounts of minerals were found, including calcium at 420 mg per 100 g and iron at 6.2 mg per 100 g. The antioxidant tests revealed strong radical scavenging activity, indicating that the seeds have considerable potential health benefits related to oxidative stress reduction.

Ogunka-Nnoka and Mepba (2018) similarly confirmed the mineral richness of *Hibiscus sabdariffa* using Flame Photometry for the quantitative determination of sodium and potassium, and colorimetric assays for phosphorus estimation. Their analysis highlighted the high potassium content, important for maintaining fluid balance and proper muscle function, and phosphorus, essential for energy metabolism and bone health, thereby reinforcing the nutritional importance of the plant.

Mohamed *et al*. (2017) applied both gravimetric methods (for moisture and ash) and titrimetric analyses (for carbohydrates) to evaluate the nutritional profile of dried *Hibiscus sabdariffa* calyces and seeds. Their findings showed that dried samples are significantly rich in carbohydrates, contributing up to 60% of their dry weight. This high carbohydrate content underscores the energy-providing potential of *H. sabdariffa*, making it a promising supplement in energy-deficient diets, particularly in resource-limited settings.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the Study Area

Sokoto is a historic city located in the northwestern region of Nigeria. It serves as the capital of Sokoto State and is one of the oldest and most culturally significant cities in the country, being the seat of the famous Sokoto Caliphate founded in 1804 (Falola and Heaton, 2018). Sokoto is a major administrative, educational, and economic center in northern Nigeria. It hosts several important institutions, including Usmanu Danfodiyo University, Sokoto (UDUS), a Federal University known for its research and academic excellence across various disciplines (Usmanu Danfodiyo University, 2023). The city of Sokoto lies within the Sudan Savannah ecological zone and experiences a hot semi-arid climate, characterized by a long dry season and a short rainy season (NIMET, 2015). It is situated at an elevation of approximately 309 meters above sea level. Geographically, Sokoto is located at a latitude of 13.0059° N and a longitude of 5.2476° E, with GPS coordinates approximately 13° 0' 21.240" North and 5° 14' 51.360" East (Google Maps, 2024). Sokoto shares a border with the Republic of Niger to the north and is bordered by Zamfara State to the south and east, and Kebbi State to the west. The city and its surrounding areas are important for agriculture, livestock rearing, and traditional crafts, contributing significantly to the socio-economic life of the region (Ogundele, 2018).

3.2 Research Design

This research adopts a laboratory-based experimental design. It focuses on the nutritional and phytochemical analysis of two varieties of *Hibiscus* species (*Hibiscus sabdariffa* and *Hibiscus cannabinus*) seeds obtained from Sokoto State. The study involves sample

collection, preparation, and laboratory analysis using standard scientific procedures to evaluate the proximate composition, vitamin content, mineral composition, and phytochemical constituents of the seeds. The seeds used for this research were sourced from local markets and farms within Sokoto metropolis. Laboratory analyses were carried out at Chemistry Laboratory, Department of Biochemistry, Usmanu Danfodiyo University, Sokoto.

3.3 Population of the Study

The population for this study comprised two distinct varieties of *Hibiscus sabdariffa* and *Hibiscus cannabinus seed*s cultivated and marketed within Sokoto State. The varieties were identified based on their morphological features, such as seed coloration and size.

3.4 Sample Size and Sampling Technique

A purposive sampling technique was employed to select representative samples of the two varieties of *Hibiscus* seeds. A total of sixteen (16) samples were collected, eight (8) samples per variety to ensure sufficient replication and reliable statistical analysis. Fresh and healthy seeds were selected directly from farms and markets based on visual inspection for quality and maturity.

3.5 Experimental Methods Adopted

Proximate Composition (moisture, ash, crude fat, crude protein, crude fiber, and carbohydrate) determined using standard methods as described by the Association of Official Analytical Chemists (AOAC, 2019).

Vitamin vitamin C contents of the sample is determined through spectrophotometric methods.

Mineral Composition (e.g., calcium, iron, magnesium, potassium, and zinc) analyzed using Atomic Absorption Spectrophotometry (AAS).

Phytochemical Constituents (such as flavonoids, tannins, alkaloids, and saponins) identified and quantified using gravimetric methods as described by Harborne(1998), Sofowora (2008), and Trease and Evans (2002), with appropriate modifications.

All analyses were performed in triplicates to ensure precision and reproducibility.

3.6 Experimental Reagents and Apparatus

3.6.1 Materials and Experimental Reagents

The materials and reagents used for this study include: Fresh *Hibiscus sabdariffa* seeds (two varieties), Distilled water, Petroleum ether, n-Hexane, Sulfuric acid, Hydrochloric acid, Nitric acid, Boric acid, Sodium hydroxide, Wagner's reagent, Mayer reagen, Ammonium hydroxide, Phenolphthalein indicator, Ferric chloride, Folin-Ciocalteu reagent, Perchloric, Aceton, Diethyl ether, Ethanol and methanol, Aluminum chloride, Sodium carbonate, Standard vitamin and mineral solutions (metaphosphoric-acetic acid solution).

3.6.2 Apparatus

The equipment used includes; Analytical balance, Hot air oven, Muffle furnace, Soxhlet extractor, Kjeldahl apparatus, Spectrophotometer, Atomic Absorption, Spectrophotometer (AAS), Water bath, Desiccator, Blender, Crucibles, Beakers, conical flasks, filter papers, spatula, measuring cylinders, petri-dishes, and pipettes.

3.7 Sample Collection and Preparation

Seeds of two different varieties of *Hibiscus (H.sabdariffa* and *H. cannabinus)* were collected from local markets and farms within Sokoto metropolis using purposive sampling. The seeds were washed thoroughly with clean water to remove dirt and debris, then air-dried at room temperature for 7-10 days until constant weight was achieved. The dried seeds were ground into fine powder using an electric blender and stored in airtight containers (labelled A and B) at room temperature prior to analysis. The sample were taken to the Herbarium, Department of plant science, Usmanu Danfodiyo University for authentication where voucher specimen were deposited.

3.8 Analytical Procedures

3.8.1 Proximate Composition

Proximate analysis was conducted using the standard methods described by the Association of Official Analytical Chemists, 7th edition Arlington, Virginia (AOAC, 2019).

Moisture Content

The samples (2g each) were weighed into crucibles and the crucibles were then inserted into an oven at 105°C and allowed overnight. The crucibles were removed and inserted into a desiccator to cool for 5mins. Each sample was carefully removed from the desiccator and weighed. The %moisture content was calculated using;

$$\% \text{ Moisture} = \frac{\text{Loss in weight due to drying}}{\text{Weight of sample taken}} \times 100$$

Ash Content

The samples (2g each) were placed in empty crucibles and weighed. The crucible containing the sample was then heated in muffle furnace at 600°C for 5 hours to burn off all the organic matter. After the ashing period, the samples were placed into a desiccator gently to cool and weighed. The ash content was calculated using the following

$$\% \text{ Ash} = \frac{\text{Weight of ash}}{\text{Weight of sample taken}} \times 100$$

Crude Lipid

The samples (2g each) were measured into glass bottle. 20cm3 of n-Hexane was added. It was shaken thoroughly and the solvent solutions are allowed to settle for 24hrs. Empty petri-dishes were weighed. The oil was then carefully extracted into petri-dish and the solvent was allowed to evaporate and then weighed.

The percentage of crude lipid was calculated using the formula:

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

Crude Fibre

The samples (2g each) were placed in a conical flask, 20cm3 of distilled water and 20cm3 of 10% H2SO4 was added, it was fixed to boil for 30 minutes to maintain constant volume. The samples were filtered and rinsed with water. The samples were scrapped into a flask with the aid of spatula. 20cm3 of 10% NaOH was added and then placed on a heater again to boil for 30mins. The samples were filtered using a filter paper and ethanol was used to rinse the samples once again, it was allowed to drain and the residue was crapped into crucibles. The crucibles were then placed in an oven to dry at 105°C after which the weight was taken. The crucibles were then placed in muffle furnace to ash for 2 hours at 550°C and allowed to cool in desiccators and weighed again. Percentage fibre was then calculated using the following below;

$$\% \text{ Crude fiber} = \frac{\text{Weight after drying weight after ashing}}{\text{Weight of sample taken}} \times 100$$

Protein

The samples (0.5g) were weighed into 2 different distillation tubes and 10cm3 of Conc. H2SO4 was added into each tube followed by 40cm3 of distilled water to dilute the acid then kjeldahl tablet was added to each tube of the mixture to digest the inorganic matter present. The tubes were sent into the digestion chamber for digestion. From the digestion tubes, 10cm3 of the samples were measured respectively and added into the digestion flask followed by 20cm3 distilled water and then 20cm3 NaOH (40%) to make up the solution. The mixture was sent into the distillation chamber for the nitrogen content; to extract out ammonia present in the sample which will be evaporated into the boric acid indicator, before protein analysis. It was allowed for about 3 minutes, where $20cm^3$ of the boric acid indicator was placed into a flask and inserted beneath the distillation chamber used as the

receiver of the nitrogen extracted. Where the ammonia was liberated into the boric acid and changes the indicator's colour from pink to green. The green mixture with ammonia was titrated against 0.01M H_2SO_4 to end point, which give the actual amount of protein content in sample. The colour change was from green to pink and the end point and the titre values were recorded respectively. The crude protein was calculated using the following equations.

$$\% \text{ Nitrogen} = \frac{\text{Tv} \times \text{N} \times 0.014 \times \text{Dilution factor} \times 100}{\text{weight of sample (w)} \times \text{vol of aliqout}}$$

$$\% \text{ Protein} = \% \text{Nitrogen} \times \text{conversion factor (6.25)}$$

Where;

Tv = Titrate value (Volume in ml of tetraoxosulphate vi acid used), **N**= Normality (concentration) of the titrant, **w**= weight of the plant sample used, Dilution factor=Total volume of digest / aliquot volume used for distillation**,**100**=** To convert to percentage, **Volume of aliquot**= Volume of digest solution taken for distillation and titration.

3.8.2 Vitamin Analysis

Vitamin C (Ascorbic Acid)

Determined by 2,6-dichlorophenolindophenol titrimetric method (Omaye *et al*., 1979). 10g of the sample was homogenized with 50 ml of metaphosphoric-acetic acid solution. The extract was filtered and titrated with standardized dye solution until a light pink color persisted for 30 seconds. The vitamin C content was calculated using standard conversion factors.

3.8.3 Mineral Composition Analysis Procedures

Minerals were analyzed after wet digestion using a mixture of nitric, perchloric, and sulfuric acids (AOAC, 2019).

1g of the dried sample was digested in acid, filtered, and made up to 100 ml with deionized water.

The concentrations of calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), and potassium (K) were measured using Atomic Absorption Spectrophotometry (AAS), while sodium (Na) and potassium (K) were measured using a flame photometer.

Standards were prepared for each element to generate a calibration curve for concentration determination.

3.8.4 Phytochemical Analysis

Phytochemical analysis was carried out to determine the bioactive constituents present in the two varieties of *Hibiscus* species seeds. The procedures used include both qualitative and quantitative screening, following the methods of Mayer's and Wagner's Reagent Test, Sofowora (2008), and Trease and Evans (2002), with appropriate modifications.

Extraction Procedure (Preliminary Step)

1. Sample Preparation: The dried *Hibiscus sabdariffa* and *Hibiscus cannabinus* seeds were ground into fine powder using a mechanical grinder. Before analysis, the plant samples were prepared and extracted to obtain the crude extract.

2. Solvent Extraction: 50 grams of the powdered sample were soaked in 500 ml of 70% ethanol in a conical flask. The flask was covered and shaken intermittently and left to stand at room temperature for 72 hours.

3. Filtration and Concentration: The extract was filtered using Whatman No. 1 filter paper. The filtrate was concentrated in a water bath at 45-50°C to reduce the solvent volume and obtain a semi-solid crude extract. This extract was stored in a refrigerator at 4°C and used for both qualitative and quantitative phytochemical tests.

3.8.4.1 Qualitative Phytochemical Screening

The crude ethanol extract was subjected to standard phytochemical screening to test for the presence of the phytochemicals.

Test for flavonoids (Modified Shinoda Test)

3ml of the filtrated (extracts) are added into test tube and 1ml 10% NaOH was added into the extract. If a yellow color is developed, this indicates the possible presence of flavonoid compounds (Maheshwaran *et al*., 2024).

Test for Tannins (Ferric Chloride Test)

5% ferric chloride solution was added drop by drop into 3ml of the extracts in a test tube and the colored produced was noted. Condensed tannins usually give a dark green color. Hydrolysable tannins give blue-black color (Shaikh and Patil, 2020).

Test for Saponins (Frothing Test)

5ml of the extracts was placed in a test tube with 5ml of water and shaken strongly. The whole tube will be fused froth that lasts for several minutes show the presence of saponins (Yahaya *et al*., 2020).

Test for Alkaloids (Mayer's and Wagner's Reagent Test)

About 2ml of the extracts was stirred with 2ml of 10% aqueous hydrochloric acid. 1ml was treated with a few drops of Wagner's reagent and second 1ml portion was treated similarly with Mayer reagent. Turbidity or a reddish-brown precipitate with either of these reagents was taken as preliminary evidence for the presence of alkaloid (Maheshwaran *et al*., 2024).

Test for Glycosides (Molisch's Test)

Two milliliters of the extract were treated with two drops of Molisch reagent, and concentrated sulfuric acid was carefully added down the side of the test tube to form a separate layer. The appearance of a violet ring at the interface indicated a positive reaction for glycosides (Maheshwaran *et al*., 2024).

Test for Cardiac Glycosides (Keller–Killiani Test)

Two milliliters of the extract were mixed with 1 mL of glacial acetic acid containing a trace of ferric chloride, followed by the careful addition of 1 mL of concentrated sulfuric acid along the side of the test tube. The formation of a brown ring at the interface and a bluish-green coloration in the upper layer indicated the presence of cardiac glycosides (Trease and Evans, 2002).

Test for Steroids (Liebermann–Burchard's Test)

Two milliliters of the extract were mixed with 2 mL of acetic anhydride and carefully layered with 1 mL of concentrated sulfuric acid. The development of a bluish-green coloration confirmed the presence of steroids in the sample (Harborne, 1998).

Test for Resins

About 2 mL of the ethanolic extract was dissolved in 10 mL of distilled water. The formation of turbidity or a precipitate was considered a positive test for resins, suggesting the presence of resinous substances in the extract (Trease and Evans, 2002).

Test for Anthraquinones (Bornträger's Test)

Five milliliters of the extract were shaken with 5 mL of benzene and filtered. The benzene layer was then treated with 10% ammonia solution and shaken gently. The appearance of a pink or red coloration in the ammoniacal layer indicated the presence of anthraquinones (Harborne, 1998).

Test for Volatile Oils (Steam Distillation Method)

The presence of volatile oils was determined by steam distillation using a Clevenger apparatus. The powdered sample was boiled with water, and the distillate collected contained an oily layer with a characteristic aroma, indicating the presence of volatile oils (Trease and Evans, 2002).

3.9 Statistical Methods of Data Analysis

The data obtained were statistically analyzed using descriptive statistics (means and standard deviations) and inferential statistics (such as Analysis of Variance (ANOVA)) to determine significant differences between the two varieties. Data analysis was carried out using SPSS version 25.0 at a significance level of $p < 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Proximate Composition and vitamin C of the Seeds of *H. sabdariffa* and *H. cannabinus*

Table 4.1: Proximate Composition of *Hibiscus sabdariffa* and *Hibiscus cannabinus* Seeds. Results presented as %dry matter

Parameter	*H. sabdariffa*	*H. cannabinus*
Moisture	9.52 ± 0.15^{a}	8.89 ± 0.10^{a}
Ash	4.32 ± 0.09^{a}	3.98 ± 0.07^{a}
Crude Protein	18.64 ± 0.25^{a}	17.23 ± 0.22^{a}
Crude Fat	6.41 ± 0.12^{a}	5.89 ± 0.10^{a}
Crude Fibre	10.28 ± 0.18^{a}	9.74 ± 0.14^{a}
Carbohydrate	50.83 ± 0.36^{a}	54.27 ± 0.40^{a}
Available energy (k/cal)	335.57 ± 3.62^{a}	339.01 ± 3.89^{b}
Vitamin C (mg/100g)	275.60 ± 2.15^{a}	241.35 ± 1.98^{a}

Results presented as Mean ± SD of three replicates. Value in the table shows that the superscript should not be less than 0.05.

The proximate composition revealed that *Hibiscus sabdariffa* contained slightly higher values of protein, ash, fibre, and fat than *H. cannabinus*, while *H. cannabinus* showed a

marginally higher carbohydrate level. The higher protein content in *H. sabdariffa* suggests a better potential for body tissue development and repair. The elevated carbohydrate percentage in *H. cannabinus* indicates greater energy-providing capacity, while the moderate moisture content in both seeds implies good shelf stability. These findings align with Adegunwa *et al*. (2021), who reported similar trends in *Hibiscus* species analyzed in Nigeria.

The vitamin C content was higher in *H. sabdariffa* compared to *H. cannabinus*. This suggests that *H. sabdariffa* has a stronger antioxidant potential and may contribute more significantly to immune system enhancement and the prevention of oxidative stress. The values obtained in this study are comparable to those reported by Babalola *et al*. (2021), who noted high vitamin C levels in *Hibiscus sabdariffa* seeds.

4.1.2 Mineral Composition of the Seeds of *H. sabdariffa* and *H. cannabinus*

Table 4.2: Mineral Composition (mg/L, Mean ± SD) of *Hibiscus sabdariffa* and *Hibiscus cannabinus* Seeds

Mineral	*H. sabdariffa*	*H. cannabinus*
Calcium (Ca)	37.13 ± 0.77^{b}	36.40 ± 0.25^{a}
Iron (Fe)	3.14 ± 0.05^{a}	3.10 ± 0.05^{a}
Potassium (K)	466.60 ± 1.38^{b}	472.50 ± 0.7^{a}
Magnesium (Mg)	2.36 ± 0.00^{a}	2.37 ± 0.00^{a}
Sodium (Na)	1.64 ± 0.40^{b}	7.54 ± 0.04^{a}
Zinc (Zn)	1.19 ± 0.01^{a}	1.60 ± 0.01^{a}

Key: *H. sabdariffa = Hibiscus sabdariffa, H. cannabinus = Hibiscus cannabinus,* Mean ± SD = Mean value ± Standard Deviation. Values on the table shows that the superscript should not be less than 0.05.

The results show that potassium and calcium were the predominant minerals in both seed samples, with *H. cannabinus* recording slightly higher potassium and sodium contents. The presence of iron and zinc in both seeds indicates their potential contribution to blood formation and immune support. The mineral profile agrees with the findings of Oladosu *et al*. (2018), who observed similar concentrations in *Hibiscus* species cultivated in northern Nigeria.

4.1.3 Phytochemical Constituents of the Seeds of *H. sabdariffa* and *H. cannabinus*

Table 4.3: Qualitative Phytochemical Screening of *Hibiscus sabdariffa* and *Hibiscus cannabinus* Seeds Ethanol Extracts

Phytochemical	*H. sabdariffa*	*H. cannabinus*
Flavonoids	++	+
Alkaloids	++	+
Saponins	++	++
Tannins	+	+
Phenols	++	+
Terpenes	+	_
Steroids	+	_
Glycosides	+	+
Phenolic acid	_	_

***Key**: = Absent, + = Present in moderate amount, ++ = Present in high amount*

The phytochemical screening indicated that both *Hibiscus sabdariffa* and *Hibiscus cannabinus* contained significant quantities of bioactive compounds such as flavonoids, alkaloids, saponins, tannins, and phenols. However, *H. sabdariffa* showed stronger (++) reactions for most compounds, suggesting a richer phytochemical composition and higher therapeutic potential. The presence of these compounds implies antioxidant, antimicrobial, and anti-inflammatory properties, consistent with the reports of Da-Costa-Rocha *et al*. (2024) and Ali *et al*. (2018).

4.2 Discussion of Findings

The findings of this study revealed that both *Hibiscus sabdariffa* and *Hibiscus cannabinus* seeds are nutritionally rich, but with varying compositions across the analyzed parameters. The proximate analysis showed that *H. sabdariffa* recorded higher values for moisture (9.52 ± 0.15%), ash (4.32 ± 0.09%), crude protein (18.64 ± 0.25%), crude fat (6.41 ± 0.12%), and crude fibre (10.28 ± 0.18%) compared to *H. cannabinus*, which contained 8.89 ± 0.10%, 3.71 ± 0.08%, 17.23 ± 0.22%, 5.92 ± 0.10%, and 9.72 ± 0.14% respectively. In contrast, *H. cannabinus* showed a higher carbohydrate value (54.27 ± 0.40%) than *H. sabdariffa* (50.83 ± 0.38%). These variations indicate that *H. sabdariffa* may serve as a better source of protein, fat, and fibre for body growth and tissue repair, whereas *H. cannabinus* could be preferred as a high-energy food source due to its higher carbohydrate content. Similar varietal differences in nutrient distribution have been reported by Adegunwa *et al*. (2021) and Bello *et al*. (2019)**,** who attributed such differences in *Hibiscus* species to genetic and environmental influences.

The vitamin C analysis revealed that both species contained appreciable amounts of ascorbic acid, with *H. sabdariffa* having a slightly higher concentration (275.60 ± 2.15 mg/100 g) than *H. cannabinus* (241.35 ± 1.98 mg/100 g). The presence of vitamin C in both species indicates their potential as natural antioxidant sources that can help protect the body against oxidative stress, strengthen the immune system, and improve iron absorption. The slightly higher vitamin C level in *H. sabdariffa* supports its traditional use as an immune-boosting and refreshing beverage. These findings are consistent with the reports of Okoro and Adeyemi (2018) and Babalola *et al*. (2021)**,** who noted that *Hibiscus sabdariffa* is a good source of ascorbic acid and bioflavonoids that contribute to its antioxidant capacity.

The mineral composition analysis indicated that both seed varieties contained essential macro- and micro-minerals such as calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), sodium (Na), and zinc (Zn), though in varying amounts. The ANOVA results showed no significant differences ($p > 0.05$) in calcium and iron contents between *H. sabdariffa* (Ca: 37.13 ± 0.77 mg/L; Fe: 3.14 ± 0.05 mg/L) and *H. cannabinus* (Ca: 36.40 ± 0.25 mg/L; Fe: 3.10 ± 0.05 mg/L). However, significant differences ($p < 0.05$) were observed in potassium, magnesium, sodium, and zinc. *H. cannabinus* exhibited higher mean values for potassium (472.50 ± 0.71 mg/L), magnesium (2.37 ± 0.00 mg/L), sodium (7.54 ± 0.04 mg/L), and zinc (1.60 ± 0.01 mg/L) compared to *H. sabdariffa* (466.60 ± 1.38 mg/L, 2.36 ± 0.00 mg/L, 1.64 ± 0.40 mg/L, and 1.19 ± 0.01 mg/L respectively). These differences suggest that *H. cannabinus* may contribute more to electrolyte balance, enzymatic functions, and immune support due to its higher mineral content. These observations align with the findings of Oladosu *et al*. (2018) and Lawal *et al*. (2020)**,** who reported that varietal differences among *Hibiscus* species significantly affect mineral uptake and accumulation.

The phytochemical screening revealed that both *Hibiscus* species contained important bioactive compounds such as flavonoids, alkaloids, saponins, tannins, terpenes, steroids, glycosides, and phenolic acids, but with differing intensities. *H. sabdariffa* showed strong (++) reactions for flavonoids, alkaloids, saponins, and terpenes, moderate (+) presence for tannins, steroids, glycosides, and phenolic acids, while phenols were absent (–). In contrast, *H. cannabinus* exhibited high (++) levels only for saponins, moderate (+) levels for flavonoids, alkaloids, tannins, terpenes, and phenolic acids, and absence (–) of steroids and phenols. The higher occurrence of most phytochemicals in *H. sabdariffa* suggests that it has greater medicinal and antioxidant potential than *H. cannabinus*. These bioactive compounds are known for their protective roles against oxidative stress, microbial infection, and inflammation. This finding agrees with the works of Da-Costa-Rocha *et al*.

(2024) and Ali *et al*. (2018), who reported the rich phytochemical composition of *H. sabdariffa* responsible for its antioxidant and therapeutic effects. Collectively, the findings demonstrate that both *H. sabdariffa* and *H. cannabinus* possess valuable nutritional and medicinal properties, but differ significantly in nutrient and phytochemical compositions due to varietal and environmental influences.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

This study evaluated and compared the nutritional and phytochemical composition of two *Hibiscus* seed varieties *Hibiscus sabdariffa* and *Hibiscus cannabinus* to determine their relative nutritional and medicinal potentials. The analyses conducted included proximate, vitamin C, mineral, and phytochemical determinations, while Analysis of Variance (ANOVA) was used to assess statistical differences in mineral contents between the two varieties. The results revealed that *H. sabdariffa* contained higher levels of protein (18.64 ± 0.25%), fat (6.41 ± 0.12%), fibre (10.28 ± 0.18%), ash (4.32 ± 0.09%), and moisture (9.52 ± 0.15%), whereas *H. cannabinus* had a higher carbohydrate content (54.27 ± 0.40%) and superior mineral concentrations, particularly in potassium (472.50 ± 0.71 mg/L), magnesium (2.37 ± 0.00 mg/L), sodium (7.54 ± 0.04 mg/L), and zinc (1.60 ± 0.01 mg/L). The elevated protein, fat, and fibre content in *H. sabdariffa* suggests its potential as a functional food ingredient for supporting body tissue development, repair, and satiety. Conversely, the higher carbohydrate content in *H. cannabinus* seeds indicates a greater energy-providing capacity, making them suitable for inclusion in energy-dense food formulations. These findings are consistent with previous studies (Adegunwa et al., 2021; Bello et al., 2019), which reported similar nutritional variations in Hibiscus species and attributed differences to genetic, varietal, and environmental factors.

Vitamin C analysis indicated that *H. sabdariffa* seeds contained slightly higher ascorbic acid levels (275.60 ± 2.15 mg/100 g) than *H. cannabinus* (241.35 ± 1.98 mg/100 g), highlighting its stronger antioxidant potential. The presence of vitamin C in both seeds

reinforces their role in supporting immune function, reducing oxidative stress, and enhancing iron absorption, which is particularly important in populations susceptible to micronutrient deficiencies (Okoro & Adeyemi, 2018; Babalola et al., 2021).

Mineral profiling demonstrated that both species contained essential macro- and microelements, including calcium, iron, potassium, magnesium, sodium, and zinc. While calcium and iron showed no significant differences ($p > 0.05$), *H. cannabinus* exhibited significantly higher potassium, magnesium, sodium, and zinc contents ($p < 0.05$), suggesting its utility in promoting electrolyte balance, enzymatic activities, and immune function. These results align with reports by Oladosu et al. (2018) and Lawal et al. (2020), emphasizing that mineral uptake and accumulation can be influenced by varietal differences, soil composition, and environmental factors.

Phytochemical screening revealed the presence of bioactive compounds such as flavonoids, alkaloids, saponins, tannins, terpenes, steroids, glycosides, and phenolic acids in both species, although their intensities varied. *H. sabdariffa* showed stronger reactions (++) for most compounds, particularly flavonoids, alkaloids, saponins, and terpenes, while *H. cannabinus* exhibited moderate (+) or absent (-) levels for several compounds. This indicates that *H. sabdariffa* may possess superior therapeutic potential, with antioxidant, anti-inflammatory, and antimicrobial properties, corroborating the findings of Da-Costa-Rocha et al. (2024) and Ali et al. (2018).

5.2 Conclusion

The findings of this research have clearly demonstrated that both *Hibiscus sabdariffa* and *Hibiscus cannabinus* seeds are nutrient-dense and contain vital phytochemicals with significant nutritional and medicnal relevance. *H. sabdariffa*, being richer in protein, fat, fibre, and phytochemicals, can be classified as a high-protein and functionally bioactive seed with the potential for use in food fortification, nutraceuticals, and natural therapeutic products. Conversely, *H. cannabinus* demonstrated superior mineral and carbohydrate contents, making it a valuable energy-rich and mineral-enriched seed that can help combat micronutrient deficiencies and improve dietary balance.

The coexistence of both nutritional and phytochemical compounds in these seeds underscores their multifunctional roles in health maintenance, disease prevention, and industrial development. The results also highlight the impact of varietal and environmental factors on the compositional differences observed between the two *Hibiscus* species. Therefore, both *H. sabdariffa* and *H. cannabinus* have strong potential as sustainable raw materials for food, pharmaceutical, and agricultural industries. Their cultivation and processing should be encouraged as part of strategies to promote food diversification, enhance nutrition security, and support local economic empowerment.

5.3 Recommendations

1. Food scientists and nutritionists should incorporate *Hibiscus sabdariffa* and *Hibiscus cannabinus* seed flours into bakery, snack, and complementary food formulations to improve protein, fibre, and mineral intake among vulnerable populations.
2. Researchers should conduct further studies on the amino acid, fatty acid, and quantitative phytochemical profiles of both seeds to better understand their functional and pharmacological potentials.
3. Health practitioners and pharmacologists should explore the bioactive compounds, especially flavonoids, alkaloids, and saponins, from *H. sabdariffa* for the development of natural antioxidant, antimicrobial, and anti-inflammatory agents.
4. Agricultural extension officers should educate and encourage farmers to cultivate both *Hibiscus* species on a larger scale, given their nutritional, medicinal, and industrial significance.
5. The food and pharmaceutical industries should invest in processing technologies that can efficiently extract and utilize nutrients and bioactive compounds from *Hibiscus* seeds for industrial applications.
6. Government and policy makers should provide funding support, agricultural incentives, and technological training to local producers involved in *Hibiscus* seed cultivation and processing to enhance productivity and economic growth.
7. Nutrition awareness campaigns should be organized to promote public understanding of the nutritional and health benefits of *Hibiscus sabdariffa* and *Hibiscus cannabinus* as af

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