# PHYTOCHEMICAL SCREENING AND ACUTE TOXICITY OF ETHANOL EXTRACT OF *HIBISCUS SABDARIFFA* STEM IN MICE


**BY**

**AROSE GIFT ODION**

**PSC2008064**

**DEPARTMENT OF CHEMISTRY**

**FACULTY OF PHYSICAL SCIENCE**

**UNIVERSITY OF BENIN**

**BENIN CITY**



**FEBRUARY 2025**

# PHYTOCHEMICAL SCREENING AND ACUTE TOXICITY OF ETHANOL EXTRACT OF *HIBISCUS SABDARIFFA* STEM IN MICE

**BY**

**AROSE GIFT ODION**

**PSC2008064**

**A PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.SC HONOURS) IN CHEMISTRY**

**FEBRUARY, 2025**

## CERTIFICATION

This is to certify that this research project was carried out by **AROSE GIFT ODION** with matriculation number **PS2008064** under the supervision of **DR. O. IYEKOWA** in the department of chemistry, faculty of physical sciences, university of Benin, Benin City, Edo state.

_____ _____

**AROSE GIFT ODION** **DATE**

(Project Student)

_____ _____

**DR. O. IYEKOWA.** **DATE**

(Project supervisor)

_____ _____

**PROF E. E. I. IRABOR** **DATE**

(Head of Department)

## DEDICATION

I dedicate this project to God Almighty, whose grace, strength, and wisdom have guided me throughout this journey.

I also dedicate this work to my lovely parents, Mr. and Mrs. Odion, whose unwavering love, support, and sacrifices have been the starting point of my success. Your encouragement has been my biggest motivation.

## ACKNOWLEDGMENT

First and foremost, I give thanks to God Almighty for His wisdom and guidance, from the beginning till the end of this work, and for granting me the strength needed to complete this work.

I am very grateful to my parents, Mr. and Mrs. Odion, for their constant support, both financially and emotionally, their encouragement, and love for me. They have been my constant source of motivation.

To my siblings, Precious, Odion, and Omon, I am grateful for your support, understanding, and words of encouragement throughout this journey.

I appreciate my project supervisor, Dr. Iyekowa, for his patience and guidance throughout this research, and for giving me the opportunity to carry out this research under his tutelage.

Finally, my gratitude goes to everyone who, in one way or another, contributed to the success of this project. Your encouragement and support mean the world to me.

## TABLE OF CONTENTS

## LIST OF PLATES

## LIST OF TABLE

## ABSTRACT

*Hibiscus sabdariffa* L. (Roselle) is a medicinal plant grown in different countries, including India, Africa, Thailand and Mexico. It is known as zobo in Nigeria, Jamaica flowers, Sorrel and Karkade (in Egypt), and is a member of the Malvaceae family. It can be used as a colorant for foods, flavoring for sauces, jellies, marmalades and soft drinks.

The study researched the phytochemical constituents and acute toxicity profile of the ethanol extract of *Hibiscus sabdariffa* stem in mice. Phytochemical screening was done using standard and qualitative methods to identify the presence of bioactive compounds. The acute toxicity assessment followed OECD guidelines, where mice were given increasing doses of the extract, and mortality was recorded. The phytochemical evaluation showed the presence of Glycosides, flavonoids, terpenoids, alkaloids, saponins, and phenolic compounds, which are known for their therapeutic benefits. The acute toxicity study showed no mortality at doses up to 1600 mg/kg, while a slight toxicity effect (16.66% mortality) was observed at 2900 mg/kg. These results suggest that the ethanol extract of Hibiscus sabdariffa stem is relatively safe at moderate doses and contains bioactive compounds that may contribute to its therapeutic potential.

# CHAPTER ONE

# INTRODUCTION AND LITERATURE REVIEW

## 1.1 INTRODUCTION

For many years, medicinal plants have played an important part in human healthcare as the main source of healing chemicals in many different civilizations. Old societies have long used herbal medicine, commonly referred to as phytotherapy, a word used by French physician Henri Leclerc (Weiss, 1988).These plants are valued for their abundance of bioactive chemicals, which have served as a basis for the synthesis and development of new drugs (Hassan, 2012).The important contributions of plant-derived chemicals to current drug therapy are highlighted by remarkable instances such as the extraction of quinine from the cinchona tree and aspirin from willow bark.Due to their ease of access and cultural acceptance, medicinal plants continue to play an important role in primary healthcare in many poor countries (Srivastava & Singh, 2020). According to estimates from the World Health Organization, almost 80% of people worldwide get their medical care from traditional plant-based remedies. Alkaloids, flavonoids, tannins, and terpenoids are chemical substances of medicinal plants that have a range of healing qualities and are frequently associated with their effectiveness (Hoffmann, 2003). Deep learning technology has improved the grouping and acceptance of medicinal plant species, leading to the discovery of new drug therapy agents (Chanyal *et al*, 2022). Ethnobotanical surveys, which record traditional knowledge and identify plant species with potential medicinal applications, also contribute to drug discovery efforts (Teklehaymanot & Giday, 2007).

In spite of their global use, medicinal herbs and their preparations must be safe, of high quality, and effective.Rigorous and organized scientific validation are required to successfully mix traditional treatments into modern healthcare systems (Hassan, 2012).This research is

aimed at the phytochemical screening and acute toxicity of ethanol extract of *Hibiscus sabdariffa* stem in mice.

### 1.1.1 BACKGROUND OF STUDY

*Hibiscus sabdariffa,* often known as *roselle*, is a member of the Malvaceae family and is acknowledged by various names all over the word, including Indian sorrel, Jamaica sorrel, and karkade (Sanders *et al*, 2020). This plant is famous for its vibrant red calyces, which are made use of in the preparation of beverages, culinary dishes, and traditional medicines. Because it contains organic acids including citric, malic, and tartaric acids as well as beta-carotene, vitamin C, proteins, and carbohydrates, *H. sabdariffa* has a significant nutritional profile (GSC Biological and Pharmaceutical Sciences, 2024). These elements aid both its prospects as a useful food ingredient and its health-promoting qualities.

Several kinds of bioactive substances, such as polyphenols, anthocyanins, polysaccharides, and organic acids, have been found in *H. sabdariffa* through plant derived research(GSC Biological and Pharmaceutical Sciences, 2024). These elements have been linked to a number of health merits, including antibiotic,anti-inflammatory, and antioxidant properties. Traditionally, *H. sabdariffa* has long been used to treat diverse illnesses. For example, research suggests that it may reduce blood pressure, and it has been used to treat hypertension (Wahabi *et al,* 2007). Additionally, studies have examined if it has abnormal low levels of lipid in blood, which point to a potential function in lipid control (Haji Faraji & Haji Tarkhani, 1999).

Modern studies have focused on the mode of action behind *H. sabdariffa's* medicinal properties. For Example, El-Nashar *et al* (2024) looked at the production of nanoliposomes for the skin delivery of H. sabdariffa extracts with the goal of improving skin health and combating aging. H. sabdariffa is becoming increasingly popular worldwide, not just for its

health benefits but also for its workability as a beverage and food ingredient. Its high anthocyanin content gives it a characteristic red hue, making it a natural pigment source for the food sector (Sanders *et al*., 2020).

*Roselle* thrives in warm, humid regions with a well-distributed annual rainfall of 150 to 200 cm. The ideal temperature range for growth is 15°C to 27°C (vikaspedia). The plant prefers well-drained loamy soils rich in organic matter with a pH of 6.0 to 7.5. While it may succeed in a variety of soil types, including sandy and heavy clays, enough drainage is needed to avoid water logging (Hibiscus sabdariffa: health and benefit, cultivation and precaution). Roselle may be planted as a part of a multi-cropping system, it is rather simple to grow, requires a lot of process, and is able to withstand drought (Food and Agriculture organization).

The calyces of *Hibiscus sabdariffa* have an abundance of nutrients and essential minerals such as iron, manganese, magnesium and copper, which are important for human health.

In Nigeria, *Hibiscus sabdariffa* is commonly known as *zobo*. It is a widely known drink in Nigeria and the rest of West Africa. It is formed from the dried calyces of the *Hibiscus sabdariffa* plant which are high in nutrients and phytochemicals. The drink is famous for its brilliant red colour, tangy and slightly sour taste, and it is popular both when hot and cold (Health Guide, 2023). It is prepared by rinsing dried hibiscus petals completely to remove impurities, then boiled with water, ginger, clove, and pineapple for about 25 minutes to remove the flavors and nutrients (Dobby's signature, 2021).

After boiling, the mixture was tightened to remove solids in it, and sugar was added for taste and placed in the freezer until it became cooled before serving (All Nigerian Foods, 2021).

### 1.1.2 STATEMENT OF PROBLEM

The last decade has seen a notable increase in interest in the use of herbal medicines. Almost 50% of the medicines we use today are gotten directly from plants, and 25% of prescription drugs arise from tropical plants. 80% of the world's population according to WHO rely mostly on traditional medicines made from plants, particularly for primary health care necessity. The idea based on tradition that medicinal herbs are natural or close to nature are always safe has not been true of late. The upgrade of technology has allowed the scientists to detect small amounts of dangerous and toxic chemicals in these herbs and recognize or assess potentially hazardous effects of some of the herbs used in traditional medicines since centuries.This draws attention to the need for a wide study to back up traditional claims and guarantee safe application in modern medicine (Haq 2004).

Diseases have been reported as a limiting factor to the production of Roselle worldwide. Many fungal and few bacterial diseases of Roselle have been reported from various parts of the world including Nigeria, and these include damping-off, vascular wilt, leaf spot, stem and foliar blight, leaf, stem, fruit and root rot (Attah *et al,* 2021). An incidence was reported by ogunsola et al, of leaf blight, leaf spot,stem wilt, flower decay and leaf discolouration in roselle plants cultivated in northern Nigeria. In Taiwan,wilt disease of *Roselle* is common however, the causative agent remains unknown. The stems of wilted roselle are browned, slightly constricted, and covered by white aerial hyphae, suggesting that potential pathogens may originate from soil.( Wang *et al*, 2021).

This study aims to identify the major bioactive components in the phytochemical screening of *hibiscus sabdariffa,* which have different effects on the body.

### 1.1.3 RELEVANCE OF STUDY

Health research, drug development, growth of the economy, and systenance of the environment all depend on the study of medicinal plants. Their safe, efficient, and good usage while protecting the diversity of life is assured by ongoing research. Growth in the study of medicinal plants will improve healthcare systems all over the world and offer natural alternatives for manmade medications.

Because of *Hibiscus sabdariffa's* medicinal, nutritional, and financial advantages, research on the plant is important. Research into its healing properties and plant nutrients may result in new medications, useful foods, and better public health drive. Its uses in business and medicine will be made better by more research on the system of action, drug availability, and long-term safety.

This study provides a rundown on the health benefits of the stem of *hibiscus sabdariffa.*

### 1.1.4 SCOPE OF STUDY

The study covers the phytochemical screening and acute toxicity of ethanol extract of *Hibiscus sabdariffa* stem in mice, including physical and behavioral evaluation, and monitoring likely or future toxic effects to show its medicinal properties.

### 1.1.5 AIM

The aim of the study is to assess the phytochemical composition and acute toxicity of ethanol extract of *Hibiscus sabdariffa* stem in mice to determine its effect on living organisms, safety and potential for medical use.

### 1.1.6 SPECIFIC OBJECTIVES

To achieve the aim above, the following specific objectives are set to:

1. collect, dry and pulverize *Hibiscus sabdariffa* stem

2. extract the Roselle stem of *Hibiscus sabdariffa* using ethanol as the solvent with soxhlet extraction

3. identify the phytochemical parts present in the ethanol extract of the stem of *Hibiscus sabdariffa* using standard method

4. to check the acute toxicity profile of the ethanol extract in mice

## 1.2 LITERATURE REVIEW

### 1.2.1 HIBISCUS SABDARIFFA

*Hibiscus sabdariffa L.*(roselle) Which belongs to the Malvalceae family is commonly cultivated in many countries, including Jamaica, Egypt, Nigeria, Mexico, Thailand among others. This plant is often used in traditional medicines, being high in phytochemicals like polyphenols especially anthocyanins, polysaccharides and organic acids thus having high potential in modern medicinal uses. (Riaz & Chopra 2018)

In general, there are two types of roselle, the first *Hibiscus altissima,* they are farmed for having jute-like fiber, and the second, *Hibiscus sabdariffa* presents short and bushy shrubs. The most frequently cultivated of them is *Hibiscus sabdariffa*. It is identified by having a herbaceous shrub, with smooth, rod-shaped, and typically red stems. The leaves are green with lengths that differ between 7.5 and 12.0 cm. Its flowers are up to 5 inches or 12.5 cm wide, yellow and may turn pink as they wither. Its calyx, stems, and leaves are acidic and have a blueberry-like taste (Izquierdo-Vega *et al,* 2020).

Even though it may resist poor soils, *Hibiscus sabdariffa* grows well in most drained soils. The plant needs 4-8 months of growth, a minimum temperature of 20°c at night, 13 hours of sunlight, and a monthly rainfall of 5-10 inches (130-250 mm), during the first few months to prevent early blossoming. Rain or high humidity during harvest and drying process can reduce calyces quality and production. The quality of *Hibiscus sabdariffa* is dictated by seed stock, local growing states, harvest timing, post-harvest treatment, and most importantly, drying. It is typically grown as a supplement crop and is a subject to fungal, viral, bacterial attacks, as well as insects. A single plant produces about 1.5kg of fruit, which is about 8t/ha. Yields of leaves may be about 10t/ha (Eco crop 2007).

**Plate1: Hibiscus sabdariffa plant**

### 1.2.2 TAXONOMY OF HIBISCUS SABDARIFFA

**Kingdom:** plantae

**Phylum:** magnoliophyte

**Class:** magnoliopsida

**Sub class:** Dilleniidae

**Order:** malvales

**Family:** Malvalceae

**Genus:** Hibiscus

**Subject:** Hibiscus sabdariffa L.

**Botanical name:** Hibiscus sabdariffa

### 1.2.3 USES OF HIBISCUS SABDARIFFA AND SOME PARTS OF THE PLANT

The use of *hibiscus sabdariffa* has been involved in culinary, medicinal concerns, as a basis of cosmetics and on botanical and floral aspects. In the culinary aspect, the fresh or dried calyces and the flower pods of *Hibiscus sabdariffa* are used for the making of hot and cold drinks, tea, fermented beverages, wines, jams, jellies, ice cream, chocolates,and cakes . Different cultures have traditionally consumed drinks prepared with hibiscus sabdariffa. For instance, in Egypt, calyces are used to make “cacody tea” and fermented beverages, while in Sudan and Nigeria, they are boiled and sugar is added to produce a beverage known as Karkade or Zobo. The calyces are mainly used as a colorant and flavoring for rum in west India. In Mexican cuisine, the flower is used in the drink known as jamaica water or Jamaican tea, as well as different typical dishes. Research shows that in Sudan, Malaysia, China and

Africa, the leaves are ingested raw or cooked, like a vegetable, while the seeds are ground, roasted and used to produce oils or serve as an alternative for coffee. As for its use in cosmetology, Malaysians often use the oil from their seeds to make scrubs and soaps (Izquierdo-Vega *et al,* 2020). It is a commercially cultivated species valued for its fibre, which serves as a substitute for jute in the production of textiles, clothing, linen, fishing nets, ropes and other similar products (Clydesdale *et al,* 1979).Roselle is a versatile ingredient in folk medicines. It is prized for its mild laxative effect, ability to increase urination, relief during heatwaves, treatment of foot cracks, nausea, sores and wounds. In Sudan, *Roselle* has been used for centuries in traditional medicine to soothe sour throat and promote wound healing. In African folk medicine, Roselle leaves are used for their, antibacterial, soothing nature, Fever-reducing, high-urine output,and sedative properties and as a soothing cough remedy, whereas in India, leaves are ointment on blisters or boils (Singh *et al*, 2017).

The calcium content in Roselle protects teeth by strengthening the jawbone promoting a tight teeth alignment where bacteria cannot stay, therefore ensuring a rich calcium diet before the teeth and gums start giving trouble is advisable. Its intake should be high, especially at young ages, to ensure children grow up naturally with strong teeth. Sexual health issues can also be cured with the healthy addition of phosphorus into the body, so issues like low libido and erectile dysfunction can be improved by having enough supply of phosphorus in your system which Roselle possesses (Sylvia 2016).

### 1.2.4 THE LEAF

The leaves of *Hibiscus sabdariffa,* usually known as roselle, are used for many purposes such as culinary, medicinal, and industrial purposes.

The juice obtained from these leaves has been used in the treatment of the redness of the eye. Additionally, the leaves have been applied as a plaster to address sores and ulcers. In Senegal,

they are used as a remedy for scurvy treatment, as a cooling medium to reduce fevers, a substance that soothes the skin, a sedative and increases urine production (Ajiboye et al, 2024).

Recent studies have looked into the medicative properties of *Hibiscus sabdariffa* leaves. For instance, extracts from the leaves that are rich in flavonoids have shown potential in the protection of the heart from diseases in diabetic rats, implying a role in reducing diabetes-induced heart failure (odigie *et al*, 2003).Furthermore, the leaves are consumed in different forms, such as in cold drinks and herbal teas, owing to their antioxidant properties. Research indicates that the leaves possess important antioxidant activity, associated to their health benefits (Gulcin *et al*, 2010).

**Plate 2: Hibiscus sabdariffa leaves**

### 1.2.5 THE CALYX

Calyx is also known as natal sorrel. Its clear flavour and beautiful red colour makes it a valued food item.

The calyx of *Hibiscus sabdariffa* is a fleshy, cup-shaped structure that surrounds the developing fruit and serves an important function in guarding the seeds, attracting pollinators, and playing a role to the plant's economic and medical value.

The calyx of *Hibiscus sabdariffa* is made up of five to six big, bright red sepals that join at the base to form a tubular or funnel-shaped structure (Mohammed & Abood, 2021).The exterior surface of the calyx is covered in secretory and protective hairs that defend it against herbivores and environmental stress (Ali *et al*, 2020). The calyx is high in anthocyanins, flavonoids, and organic acids, which give it its clear deep red color and sour flavor (Chumsri *et al*, 2019).

In addition to preserving the ovary and seeds, *hibiscus sabdariffa* calyx is widely used in food and beverages, herbal medicine, and drugs sector because of its high preservative and an Soothing characteristics. Because of its high vitamin C content, it is used in the cosmetic industry to make skincare products, shampoos, and anti-aging lotions (Wang *et al*, 2021).It is used in foods that have health benefits beyond their nutritional value, which supports general wellness, in both classic and modern day herbal therapy (Hopkins *et al,* 2018). Because of their tendency to prevent the growth of bacteria, the calyx residues and extracts are being researched for possible usage as natural insecticides (El-Sherif & Sarhan, 2018).

The calyx of Hibiscus sabdariffa is utilized to make nutrient-rich herbal tea, boosting high levels of antioxidants and vitamin C, providing several health benefits such as reducing blood pressure and promoting liver health (McKay & Blumberg, 2006).

Dried calyx is used to make a tasty beverage, and it is also used to make tea, jelly, marmalade, ice cream, sorbets, butter, pies, sauces, tarts, and other desserts (Mohammed *et al*, 2012).The fresh calyces are eaten raw in salads. The calyx is cooked and used as flavoring in cake, jellies, soups, sauces, pickles and pudding.

**Plate 3: calyx of Hibiscus sabdariffa**

### 1.2.6 THE SEED

Seeds are typically kidney shaped, light brown in color, 3-5 mm in length and covered with minute stout and stellate hairs. The Fruit has a tart, fruity flavor similar to cranberries, rhubarb and red currants.( Sylvia 2016).The seed oil can be used in reducing high blood pressure and utilized in beverages, traditional medicine and pharmaceuticals (Futuless *et al,* 2010).

**Plate 4: Roselle seeds**

## 1.3 PHYTOCHEMICAL SCREENING

Phytochemicals derived from the Greek word ‘phyton’ meaning plant, refers to naturally occurring plant compounds that can have either beneficial or adverse effect on human health. Medicinal plants used to treat diverse diseases and conditions owe their therapeutic properties to the presence of phytochemicals. Some of the important phytochemicals include alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, etc. which are distributed throughout various parts of the plants, contributing to its therapeutic value (Shaikh & Patil, 2020).

### 1.3.1 GLYCOSIDES

Glycosides are a diverse group of compounds that occur naturally in which a sugar molecule (glycone) is bonded to a non-sugar molecule (aglycone) via a glycosidic bond.

Glycosides, one of the several components found in these plants, have made out themselves as an interesting class of substances with big potential to treat disease and help healing take place. Glycosides can be gotten from both plant and animal sources. Glycosides, which have

a special chemical structure that consists of a sugar molecule (glycone) and a non-sugar aglycone, have drawn a lot of attention for their diverse bioactivities and possible use in modern day medicine.

Glycosides are excellent chances for battling diseases that have developed resistance to drug because of their distinctive chemical structures that allow them to engage with particular microbial targets (Riaz *et al*, 2023).

### 1.3.2 ALKALOIDS

Alkaloids are a diverse group of naturally occurring organic compounds distinguished by the presence of at least one nitrogen atom, often within a heterocyclic ring. They are produced by a wide range of organisms, including plants, bacteria, fungi, and animals. Alkaloids are valued for their outstanding biological activities and have played vital roles in traditional and modern medicine.

Plant alkaloids constitute one of the largest groups of natural products, exhibiting a vast array of chemical entities. Alkaloids contain an enormous class of approximately 12000 natural products (Bribi 2018).

The term “alkaloid” was initially applied to N containing compounds (compounds containing nitrogen) of plant origin that have a clear basic nature, now this term is used more broadly. Unlike other classes of natural compounds, alkaloids display limitless structural diversities with the presence of an N atom in their molecular framework, contributing to their remarkable variability

### 1.3.3 FLAVONOIDS

Flavonoids are a broad group of natural substances, exhibiting diverse phenolic structures, and are found in fruits, roots, stems, bark, vegetables, grains, flowers, tea and wine. These natural products have been recognized for their positive impacts on human health, prompting efforts to isolate and utilize their therapeutic properties. Flavonoids have become an essential component in various applications, including pharmaceutical, medicinal product, cosmetic and nutritional supplements. This is attributed to their ability to prevent cancer development, reduce swelling and redness and prevent or delay damage caused by unstable molecules (panche 2016).

### 1.3.4 SAPONINS

Saponins are surface active sterol or triterpene glycosides. They occur in a large number and a wide variety of plants but only about 28 of these are used as food by man. The most commonly eaten of these are peanuts, chickpeas, soybeans and spinach. The presence of saponins in plant extracts is readily indicated by their activity to destroy red blood cells and ability to form stable foams in aqueous solution.

Although saponins have antibiotic activity and are toxic to insects and fish, they appear to be practically non-toxic to man, remaining within the gastrointestinal tract. Dietary saponins, either isolated or as saponin-containing food plants, lower plasma cholesterol levels in several mammalian species. They are therefore likely important in human diets to reduce the risk of coronary heart disease (Oaken full 1981).

### 1.3.5 TANNINS

Tannins are complex chemical compounds derived from phenolic acids, also known as tannic acid. They are categorized as phenolic compounds which are found in various plant species across the globe, regardless of climate. They are large molecules that readily form bonds with protein cellulose, starches, and minerals, resulting in the formation of substances are insoluble and resistant to decomposition. Tannins are abundant in species of coniferous trees and several families of flowering plants. (United State Department of Agriculture USD).

Tannins are mostly found in the bark of trees, fruits, leaves, buds, roots, wood, seeds, stem, and plant gall. In all of these plant structures, tannins help safeguard the individual plant species. For instance, tannins stored in tree barks protect the tree against bacterial and fungal infections. Many bud scales on woody plants contain tannins to shield the delicate inner leaf tissues from being consumed. In many seed plants, the initial sets of leaves from a germinating seed are also high in tannins offering protection. (USDA 2000).

Unripe fruits typically have high levels of tannins, which discourages premature consumption by animals, allowing the seeds to be fully mature and viable for dispersal. As the fruit ripens the tannin content decreases, making them more appealing to consumers. Tannins are also present in coffee, tea, red wine and beer. Notably, the initial bitter taste associated with red wine is due to tannins present in the wood of oak barrels used in the aging process..

### 1.3.6 TERPENOIDS

Terpenoids, also referred to as isoprenoids, represent the most abundant and structurally diverse natural products found in many plants (Ludwick *et al*, 2017).

Terpenoids are widely found in nature, with various structures and a wide variety. To date, more than 50 000 terpenoids have been discovered in nature, primarily isolated from plants.

Some terpenoids play a crucial role in plant growth and development, such as gibberellin, a plant hormone regulating plant development and carotenoids which participates in photosynthesis. Additionally, some terpenoids mediate plant-environment interaction such as, participating in plant defense systems. Many volatile terpenoids such as menthol and perillyl alcohol serves as essential raw materials for spices, flavorings, and cosmetics.There are also certain terpenoids with important economic value, being employed as pesticides and industrial raw materials (Yang *et al*, 2020).

### 1.3.7 STEROIDS

Steroids are complex four-ringed organic molecules that serve many roles and functions in multicellular organisms.Steroids are sometimes referred to as secondary plant metabolites, because the distribution of certain classes, such as the cardiac aglycones and the alkamines, is restricted to a few plant families.However, it is becoming increasingly evident that all plants contain steroids of some kind and that they are vitally important cell constituents (Heftmann 1975**)** Steroids play a role as essential hormones in plants as well as in animals. Plants produce numerous steroids and sterols, some of which are recognized as hormones in animals (Bishop & koncz 2002).

### 1.3.8 EUGENOLS

Eugenol is a naturally occurring compound found in various plants, such as basil, nutmeg, lemon balm, and cinnamon. However it is primarily extracted from clove plants( *Eugenia aromaticum or Eugenia caryophyllata*) from which it derives its name. When extracted, eugenol appears as a clear yellow liquid that smells strongly of clove. It can also be used in products such as perfume and clove-flavored cigarettes. In medical application, eugenol is used as an antiseptic and an anesthetic. It is believed to provide pain relief when applied to skin or other injured body parts as well. Eugenol is also commonly used as an additive in

clove cigarettes. While eugenol has various applications, it may be considered dangerous, as excessive intake can be detrimental. Potential side effects include, rapid heartbeat, nausea, convulsion and dizziness (Pavithra 2014).

### 1.3.9 PHENOLICS

Phenolics are hydroxyl groups (-OH) containing a class of chemical compounds where the (-OH) bonds directly to an aromatic hydrocarbon group (Walton *et al*, 2003). Among phytochemicals, phenolic compounds have been extensively researched due to their diverse health benefits. Phenolic compounds occur mostly as soluble conjugates and insoluble forms,

covalently bound to sugar moieties or cell wall structural components. Absorption mechanisms for bound phenolic compounds in the gastrointestinal tract greatly depend on the liberation of sugar moieties. Food processes such as fermentation, malting, thermoplastic extrusion or enzymatic, alkaline and acid hydrolysis occasionally assisted with microwave or ultrasound have potential to release phenolics associated to cell walls (Acosta-Estrada *et al*, 2014)

## 1.4 EXTRACTION

Phytochemicals can be separated from plant materials using various extraction methods. The most commonly used conventional methods include maceration, percolation, infusion, digestion, decoction, hot continuous extraction (Soxhlet extraction) e.t.c

Extraction involves separating desired compounds from raw materials using selective solvents. It is a crucial step in various industries, including, pharmaceuticals, food processing, herbal medicine, and chemistry to obtain bioactive compounds, essential oils, and other valuable substances.The resulting plant-derived products are relatively complex mixtures of metabolites, available in various forms, such as, liquid, semi-solid state or (after removing the

solvent) in drug powder form, and are intended for oral or external use.

### 1.4.1 SOLVENTS USED FOR EXTRACTION

#### 1.4.1.1 WATER

water is used to remove highly polar molecules such as organic acid and alkaloids. It's a cheap, non-toxic, non-flammable substance that dissolves many materials. Acidic and alkaline water can help some substances dissolve. Its drawbacks include increasing bacterial or mold growth, making preservation difficult. Water's high boiling point causes hydrolysis and requires more heat and time to concentrate (Sinha *et al*, 2022).

#### 1.4.1.2 ACETONE:

It is a polar solvent used to extract both hydrophilic and lipophilic molecules, it is water miscible, volatile, and has low toxicity. It aids in tannin, phenolic compounds and saponin extraction (Singha *et al,* 2022).

#### 1.4.1.3 ALCOHOL

The extraction of polar secondary compounds is aided by alcohol. It's a polar hydrophilic organic solvent that's miscible in water. It's harmless, self-preserving, and only requires a small amount of heat to concentrate. Alcohols such as ethanol, methanol, and acetone are commonly employed in extraction (Singha *et al*, 2022)

#### 1.4.1.4 CHLOROFORM

Chloroform is a lipophilic organic solvent nonpolar in nature used to extract nonpolar compounds like fats, volatile oils, terpenoids, tannins, and flavonoids. It is colorless and possesses a sweet smell. It is soluble in alcohol but not in water. Though it is well absorbed and metabolized in the body, its toxicity, flammability, volatility and reduced permeability in plant cells are some of the constraints faced while the application (Singha *et al*, 2022).

#### 1.4.1.5 IONIC LIQUIDS

They are known as green solvents and can replace the disadvantages of volatile organic solvents. Its strong polarity, thermal stability, and low vapor pressure make it easier to confine, recover, and recycle. It is non-flammable and extremely miscible in water and other solvents. Their strong ionic nature also helps speed up numerous reactions (Singha *et al*, 2022).

#### 1.4.1.6 ETHER:

Nonpolar solvent. It is tasteless, soluble in water, and possesses a low boiling point. It is very stable and used in the extraction of alkaloids, terpenoids, coumarins, and fatty acids**.**

### 1.4.2 METHODS OF EXTRACTION

#### 1.4.2.1 MACERATION

In this process, menstruum (a liquid solvent used to extract medical properties from plants) is poured over finely powdered drug material, like leaves, stem bark, or root bark, until it is covered completely. The container is then closed and kept for three days. The contents are regularly swirled until the soluble matter is dissolved and if placed in a bottle, it is shaken to ensure thorough extraction (Abubakar & Haque, 2020). The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing.

#### 1.4.2.2 INFUSION

In this process, the plant material is ground into a fine powder and kept in a clean container. After that, extraction solvent is saturated and stored on the surface of the drug material for a short period. This method is good for extracting bioactive components that are easily soluble. Furthermore, it is a good way to prepare fresh extract before using it. The solvent to sample

ratio is usually 4:1 or 16:1 depending on the intended use (Abubakar & Haque, 2020)

#### 1.4.2.3 DIGESTION

Digestion is a type of maceration that prefers to be extracted at a low temperature. The drug material digests when heated, enhancing the solvent efficiency. It is preferred for pharmaceuticals whose components do not degrade when exposed to relatively high temperatures. The heat was utilized to lower the viscosity of the extraction solvent and facilitate the removal of secondary metabolites during the extraction process. This method works well with easily soluble plant materials (Abubakar &Haque, 2020)

#### 1.4.2.4 DECOCTION

In this process, the crude drug is boiled in a specified volume of water for a specific duration. It is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heat stable constituents. A similar process is employed in the preparation of Ayurvedic extracts called “quath” or “kwath”. The initial ratio of crude drug to water is fixed, ranging from 1:4 to 1:16. The volume reduced to one-fourth its original volume by boiling during the extraction procedure. Then, the concentrated extract is filtered and used either directly or processed further.

#### 1.4.2.5 PERCOLATION

This procedure is the most frequently used for extracting active ingredients in the production of tinctures and fluid extracts. A percolator (a cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with a suitable amount of the specified menstruum and allowed to stand for approximately 4 h in a sealed container, after which the mixture is packed into the percolator, and the top is sealed.

The process continues with the addition of more menstruum to create a shallow layer above

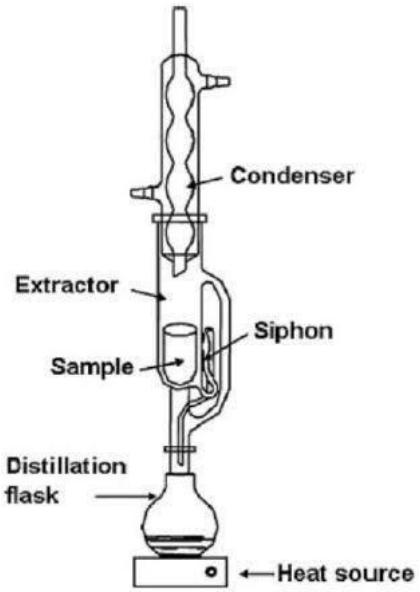
the mixture, which is then allowed to macerate in the closed percolator for 24 hours. The percolator's outlet is then is opened allowing the liquid to drip slowly. More menstruum is added as required, until the collected liquid (percolate) reaches about three-quarters of the required volume of the finished product. The marc is then pressed to extract any remaining liquid which is added to the percolate. Sufficient menstruum is added to achieve the required volume.

Finally, the combined liquid is clarified by filtration or by standing followed by decantation.

#### 1.4.2.6 HOT CONTINUOUS EXTRACTION (SOXHLET)

In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus . The extraction solvent in flask A is heated, and its vapors condense in condenser D. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into flask A.

This continuous process is repeated until a drop of solvent from the siphon tube evaporates without leaving a residue. The advantage of this method, compared to previously described methods, is its ability to extract large amounts of drugs using a small quantity of solvent. This results in substantial economic benefits, including time, saving, energy efficiency and reduced financial costs. At small scale, it is employed as a batch process only, however it becomes much more economical and viable when implemented as a continuous extraction procedure for medium or large scale operations.




**Soxhlet Extraction**

## 1.5 ACUTE TOXICITY

Acute toxicity describes the adverse effects that occur within a short period, after exposure to a substance, typically within a 24 hour period. This exposure can occur through various means, including oral ingestion, dermal exposure, or inhalation. The intensity of these adverse effects depends on various factors such as, the substance's inherent toxicity, the dose or exposure level, and the exposure route.

The toxicity of a substance is often quantified using metrics such as, the median lethal dose ($LD_{50}$) and the median lethal concentration ($LC_{50}$). The $LD_{50}$ represents the lethal dose that causes mortality in 50% of a test population, which is expressed in milligrams of substance per kilogram of body weight (mg/kg). The $LC_{50}$ denotes the concentration of a substance in air or water that proves fatal to 50% of the test organisms, usually measured in parts per million (ppm) or milligrams per liter (mg/L).

# CHAPTER TWO

# MATERIALS AND METHODOLOGY

## 2.1 MATERIALS

Hand Gloves

Nose mask

Conical flask

Sample bottles

Pipette

Cotton wool

5ml syringes

Stirrer

Whatman’s

filter paper

Beakers

Flat bottom

flasks

Funnels

Test tubes

Measuring cylinder

**2.2 REAGENTS**

Ethanol

Acetic anhydride

Dilute HCl

Picric Acid

10% Ferric Chloride

5% KOH solution

Conc.$H_2SO_4$

10% Lead Acetate

Glacial Acetic

20% NaOH

Dilute $H_2SO_4$

Ferric Chloride

Distilled Water

Ferric Acid

## 2.3 EQUIPMENTS

Electric Blender

Heating Mantle

Retort Stand

Clamp

Electrical weighing balance

Water bath

## 2.4 METHODS

### 2.4.1 COLLECTION OF PLANT SAMPLE

The dried hibiscus sabdariffa were purchased at uselu market in Egor local government area in Edo state. The plant was identified by prof. J.F. Bamidele, a taxonomist in the department of plant Biology and Biotechnology, university of Benin.

### 2.4.2 EXTRACTION OF THE BIOACTIVE PHYTOCHEMICAL

The dried stem were grinded in a blender, weighed and placed in a bag(Tyndall).The bag is then placed in the soxhlet extractor, 500ml of ethanol was poured into the round bottom flask.The condenser is placed on the soxhlet extractor and the round bottom flask is heated using the heating mantle. As the ethanol in the round bottom flask heats up, it evaporates into the condenser which cools the vapor causing it to drip into the soxhlet extractor where it enters Roselle stem. When the soxhlet extractor fills to a certain level it siphons back into the round bottom flask. This process continues for 8 hours.

At the end of the extraction the solvent in the round bottom flask now contains the bioactive

compounds. The bag containing the Roselle stem was removed from the soxhlet extractor. The solvent in the round bottom flask was removed by evaporation into the condenser which enters the soxhlet extractor until it got to a certain certain level where the extractor is removed and the solvent in it is poured into a beaker, this continues till all the solvent in the round bottom flask was removed leaving behind the plant extract.

### 2.4.3 DETERMINATION OF THE PERCENTAGE YIELD OF EXTRACT

The percentage yield of extracts were determined and calculated by using the following method: percentage Yield= $\frac{Extract\ weight}{Original\ Sample\ Weight}\ X\ 100$

### 2.4.4 PHYTOCHEMICAL SCREENING OF THE PLANT EXTRACT

Phytochemical screening of the extract were done using standard procedures by (sofowora, 1993), Trease and Evans 1989 as well as odebiyi and sofowora 1978

#### 2.4.4.1 TEST FOR GLYCOSIDE

1ml of the extract is dissolved in 1ml of glacial acetic containing one drop of ferric acid solution. This was under-layered with 1ml of conc.$H_2SO_4$. A brown ring is required for the presence of glycoside.

#### 2.4.4.2 TEST FOR ALKALOIDS

About 1 ml each of the plant extract was transferred into a test tube, 2mls of picric acid was added, a yellow precipitate is a positive test.

#### 2.4.4.3. TEST FOR SAPONINS

0.5 ml of the plant extract was shaken with water in a test tube and observed for frothing.

Saponin rein wess( supplied by Merck) was used as standard.

#### 2.4.4.4 TEST FOR PHENOLICS COMPOUNDS

5ml of 90% ethanol was added to 1ml of the plant extract. In addition, 1 drop of 10% Fecl3 was added, a pale yellow coloration is indicative of a positive test.

#### 2.4.4.5 TEST FOR EUGENOLS

2ml of the extract was mixed with 5 ml of 5% KOH solution. The aqueous layer was separated and filtered.Few drops of dilute HCl were added to the filtrate. A pale yellow colouration is indicative of a positive test.

#### 2.4.4.6 TEST FOR STEROIDS

2ml of acetic anhydride was added to 0.5g of plant extract in 2 ml of dilute H2SO4. A colour change from violet to blue or green is required for the presence of steroids.

#### 2.4.4.7 TEST FOR TERPENOIDS

5 ml of each extract was mixed in 2 ml of chloroform and 3 ml of conc.H2SO4 was carefully added down the side of the inner wall of test tube to form a layer.A reddish brown colouration of the inter-phase is required for the presence of Terpenoids.

#### 2.4.4.8 TEST FOR FLAVONOIDS

2ml of the extract was boiled in 10 ml of distilled water and filtered. The filtrate was divided into two different portions A and B of 5ml each.

**To portion A:** 10% lead acetate solution was added in a few drops. A yellowish precipitation is indicative of a positive test.

**To portion B:** 5ml of 20% NaOH and a few drops of dilute HCL were added to the solution. Formation of a colourless solution is indicative of a positive test.

### 2.4.4.9 TEST FOR TANNINS

To 2ml of the extract, 10 ml of distilled water was added and boiled for 5mins and filtered into halves.

To about 2 drops of the filtrate, ferric chloride (Fecl3) solution was added: formation of a bluish precipitate is required for hydrolyzable tannin

To about 5 drops of the filtrate, 2ml dilute HCL was added and boiled for 5 mins. Red precipitate is required for condensed tannin.

## 2.5 ACUTE TOXICITY TEST

24 mice weighing (27-36) were purchased for acute toxicity test.All animals were kept in cages and were left to adjust in the animal house of the department of anatomy, faculty of basic medical sciences, university of benin for 14 days. The animal houses were well ventilated while pellet feed and water were made available to the rats.At the end of the 14 days, the animals were grouped A, B, C, D.

Group A received 5ml of water which is diluent for the extract while group B, C, D were given 1000, 1600 and 2900 of the stem extract respectively. Observation was made for the first 4 hours (for immediate effect) and then 24 hours ( for delayed effect) after the extract was given for acute effects such as weakness, drowsiness, aggressiveness, food refusal, weight loss.

The rats were monitored for weeks to see if any delayed effect would emerge. Ethanol was used for the toxicity test.

The acute toxicity test was conducted by new approach to Acute Toxicity Testing according to Igbe et al. (2010).

# CHAPTER THREE

# RESULTS, DISCUSSION AND CONCLUSION

## 3.1 PHYSICAL CHARACTERISTICS AND PERCENTAGE YIELD OF THE EXTRACTS

The results of the percentage yield and extract and physical characteristics of the extracts are shown in table below below:

**Table 3.1: Percentage yield and physical characteristics**

| **Plants** | **Yield%** | **Colour** |
|---|---|---|
| Hibiscus sabdariffa stem | 6.89% | Dark red |

## 3.2 PHYTOCHEMICAL SCREENING OF THE EXTRACT

**Table 3.2: The bioactive Phytochemical present in the extracts**

| **S/N** | **Phytochemical constituents** | **Results** |
|---|---|---|
| 1 | Glycosides | + |
| 2 | Alkaloids | + |
| 3 | Saponins | + |
| 4 | Phenolics | + |
| 5 | Eugenols | - |
| 6 | Steroids | - |
| 7 | Terpenoids | + |
| 8 | Flavonoids | + |
| 9 | Tannins | - |

+ = Present

- = Absent

The phytochemical analysis of the sample revealed the presence of various bioactive compounds, such as, glycosides, alkaloids, saponins, phenolics, flavonoids and Terpenoids, while eugenols, Tannins and steroids were absent. These findings indicate that the plant material may possess significant medicinal properties.

**Glycosides** are known for their role in heart health and may help with inflammation and infections. **Alkaloids** are commonly found in medicinal plants and can have pain-relieving, antibacterial, and even cancer-fighting properties. The presence of alkaloids has been reported in various parts of Hibiscus sabdariffa, contributing to its medicinal applications.. **Saponins** are naturally occurring compounds that provide several health benefits which includes, lowering cholesterol levels, boosting immune system, and reducing inflammation. Research by okerere *et al*, (2015) emphasizes the association between saponins and various health benefits, such as enhanced immune system and reduced cancer risk.

The presence of **phenolics** are particularly significant due to their potent antioxidant properties, which shield the body from damage caused by free radicals.

**Terpenoids**, another compound found in the plant, are known for their potential anti-inflammatory and antimicrobial effects.

**Flavonoids** are a group of natural substances with variable phenolic structures. They are known for their antioxidant, anti-inflammatory, and anticancer activities.

The absence of **eugenols**, **steroids**, and **tannins** suggests that the sample may lack certain properties associated with these compounds. Eugenols are known for their antiseptic and analgesic properties, steroids for their anti-inflammatory effects, and tannins for their astringent and antimicrobial activities.

### 3.3 ACUTE TOXICITY TEST

**Table 3.3: Oral administration of stem of** *Hibiscus sabdariffa*

| **Group** | **Doses mg/kg** | **Period of sign observation (hr)** | **Signs of toxicity observed** |
|---|---|---|---|
| A | DW(5ml/kg) | 24 | No death, no shallow breathing, no raised tail, no paw licking, no salivation |
| B | 1000 | 24 | No death, no shallow breathing, no raised tail, no paw licking, no salivation |
| C | 1600 | 24 | No death, no shallow breathing, no raised tail, no paw licking, no salivation |
| D | 2900 | 24 | No death, slight dullness in some animals in the first 4 hours |

**Table 3.4: Oral acute toxicity results of ethanol stem extract of** *Hibiscus sabdariffa*

| **Group** | **Doses mg/kg** | **Number of lethality** | **Percentage mortality** |
|---|---|---|---|
| Control | DW(5ml/Kg) | 0/6 | 0 |
| Ethanol | 1000 | 0/6 | 0 |
| Ethanol | 1600 | 0/6 | 0 |
| Ethanol | 2900 | 1/6 | 16.66 |

DW= distilled water

$LD_{50} \leq 1$ mg/kg (Extremely toxic); 1 mg/kg $\leq LD_{50} \leq 50$ mg/kg (Highly toxic) 50 mg/kg $\leq LD_{50} \leq 500$ mg/kg (Moderately toxic)

500 mg/kg $\leq LD_{50} \leq$ 5000 mg/kg (Slightly toxic)

5000 mg/kg $\leq LD_{50} \leq$ 15000 mg/kg (Non-toxic or harmless). Hodge and Sterner scale in Unuigbe et al. (2021).

From the Hodge and Sterner scale, some levels of toxicity were recorded for ethanol extracts which gave 16.66% mortality after the 24 hours for the acute toxicity test.

The results indicate that the extract exhibited a relatively low toxicity level within the tested dose range.The control group, which received distilled water (DW) at 5 ml/kg, exhibited no mortality, confirming that the experimental conditions themselves did not contribute to any lethal effects. Similarly, ethanol extract at doses of 1000 mg/kg and 1600 mg/kg did not result in any deaths, suggesting that these concentrations are safe in mice.

However, at the highest tested dose of 2900 mg/kg, one out of six mice succumbed, resulting in a mortality rate of 16.66%. This indicates that at very high doses, the extract may begin to exhibit toxic effects. Despite this, the relatively low mortality at 2900 mg/kg suggests a high margin of safety.

Overall, these findings suggest that the ethanol extract of Hibiscus sabdariffa stem has a relatively low acute toxicity in mice, as doses up to 1600 mg/kg were completely non-lethal, and even at 2900 mg/kg, mortality remained low.

## CONCLUSION

The phytochemical screening of Hibiscus sabdariffa stem extract identified various bioactive compounds, which may be responsible for its pharmacological properties. These compounds include flavonoids, tannins, alkaloids, saponins, and phenolic compounds, which are known for their antioxidant, anti-inflammatory, and therapeutic effects.The acute toxicity study indicated that the extract has a high safety margin, as no mortality was observed at doses up to 1600 mg/kg in mice. However a slight toxicity effect was observed at 2900 mg/kg, resulting in 16.66% mortality rate, suggesting that extremely high doses may pose some risks.This suggests that the extract is relatively safe for consumption at moderate doses.

## REFERENCES


Abubakar, A. R., & Haque M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of pharmacy & bioallied science,* 12(1), 1-10.

Acosta-Estrada, B., Gutierrez-urine, J., & serna-saldiver, S. (2014). Bound phenolics in foods: A review. *Food chemistry*, 152, 46-55.

Ajiboye, B. O., Famusiwa, C. D., Ajaesowuonu, J. F., Chibueze, O. P., Adejumo, A. A., Onikanni, S. A., Ajeigbe, K. O., Ajuwon, O. R., & Oyinloye, B. E. (2024). Flavonoid-rich extracts of Hibiscus sabdariffa leaf attenuate cardiomyopathy in streptozotocin-induced rats. *Comparative clinical pathology*, 33, 927-941.

Ali, H. M., El-kholy, M. A., & Ahmed, H. A. (2020). Microscopic and phytochemical characterization of Hibiscus sabdariffa calyx. *Journal of medicinal plants research*, 14(2), 45-53.

All Nigerian Foods.( 2021). *Zobo drink.*

Attah, D. D., Sanyinna, Y. M., Isayaku, N. T., Kele, M. K., Ridwan, N. A., & Emmanuel, B.

A. (2021). Proximate composition and parasitic contamination of Hibiscus sabdariffa seed cake( Roselle seed cake). *A soup condiment produced by North- western community, Nigeria,* 8(4), 18-31.

Bishop, G. J., & Koncz csaba (2002). Brassinosteroids and plant steroid hormone signaling. *The plant cell*, 14, S97- S110.

Bribi Noureddine (2018). Pharmacological activity of Alkaloids. Asian journal of botany, 1(1), 1-6.

Chanyal, S., Semwal, V. B., & Kumar, R. (2022). Deep learning for medicinal plant specie, classification and recognition: A review. *Frontiers in plant science,* 13, 1286088.

Chumsri, P., Sirichai, W., & Puwastien, P. (2018). Anthocyanin and organic acids contents in Hibiscus sabdariffa calyx extracts. *Food chemistry*, 276, 43-49.

Clydesdale, F. M., Main, J. H., & Francis, F. J. (1979). Roselle ( Hibiscus sabdariffa L.) anthocyanin as colorants for beverages and gelatin desserts. *Journal of food protection,* 42(3), 204-207.

Dar, R. A., Shahnawaz, M., Ahanger, M. A., & Majid, I. (2023). Exploring the diverse bioactive compound from medicinal plants. *The journal of phytopharmacology.* 12(3), 189-195.

Dobby's signature.(2021). How to make Nigerian Zobo.

El-Nashar, H. A. S., El-feky, G. S., & El-banna, M. (2024). Molecular mechanisms and enhanced functions of Hibiscus sabdariffa L. extract- loaded nanoliposomes for combating skin aging. *Journal of pharmaceutical sciences,* 10(1), 1-14.

El-sheriff, F., & sarham, M. (2018). Developmental anatomy of the floral organs of Hibiscus sabdariffa L. Botanical studies, 59(1), 23-31.

Eco crop (2007). Hibiscus sabdariffa var. Sabdariffa. In food and agriculture of the UN

Food and Agriculture organization. *Hibiscus food and Agriculture.*

Futuless, K. N., Kwaga, Y. M., Clement, T., Aneja, K. R., Joshi, R., & Sharma, C. (2010). Effect of sowing date on calyx yield components of Roselle (Hibiscus sabdariffa L.). *New York science journal*, 3(11), 1-4.

GSC biological and pharmaceutical sciences. (2024). Hibiscus sabdariffa L. (Traditional medicines and food ingredients). *GSC biological and pharmaceutical sciences*, 29(2), 114-122.

Gulcin, I., Huyut, Z., Elmastas, M., & Aboul-Enein, H. Y. (2010). Radical scavenging and antioxidant activity of taric acid. *Arabian journal of chemistry*, 3(1), 43-53

Haji-Faraji, M., & Haji-Tarkhani, A. H.( 1999). The effect of sour tea ( Hibiscus sabdariffa) on essential hypertension. *Journal of Ethnopharmacology*, 65(3), 231-236.

Haq Inamul (2004). Safety of medicinal plants. *Pakistan journal of medical research,* 43(4), 203-210.

Hassan, R. (2012). Medicinal plants ( importance and uses). *Pharmaceutical analytical acts*, 3(10), e139

Heftmann Erinch (1975). Functions of steroids in plants. *Phytochemistry*, 14(4), 891-901

Hibiscus sabdariffa: Health benefits, cultivation and precautions

Hoffman, R. (2003). Medical Herbalism: The science and practice of herbal medicine.

*Healing arts press.*

Hopkins, A. L., Crown, M. J., & Evans, S. M. (2018). Effects of Hibiscus sabdariffa calyx extracts on blood pressure: A systematic review. Phytomedicine, 50, 24-33.

Izquierdo-Vega, J. A., Arteaga-Badillo, D. A., Sanchez-Gutierrez, M., Morales-Gonzalez, J., Vargas-Mendoza, N., Gomez-Aldapa, C., Castro-Rosas, J., Delgado-Olivares, L., Madrigal-Bujaidar, E., & Madrigal- Santillan, E. (2020). Organic acids from roselle (Hibiscus sabdariffa L.): A review of it's pharmacolgical effects. *Biomedicines,* 8(5),

100.

Ludwiczuk, A., Skalicka- Wozniak, K., & Georgiev, M. I. (2017). Terpenoids. *Pharmacognosy*, 233-266

McKay, D. L , & Blumberg, J. B. (2006). A review of the bioactivity and potential health benefits of Hibiscus sabdariffa L. tea. (Roselle). *Phototherapy research,* 20(5), 370-375 Mohamed, B. B., Sulaiman, A. A., & Dahab, A. A. (2012). Roselle Hibiscus sabdariffa L.) in Sudan, cultivation and their uses. *Bulletin of environmental pharmacological and life sciences*, 1(6), 48-54.

Mohammed, M. A., & Abood, A. A. (2021). Structural and functional attributes of the hibiscus sabdariffa calyx: A review. *Plant science journal*, 39(4), 321-335.

Oakenfull David (1981). Saponins in food: A review. *Food chemistry,* 7(1), 19-40

Odebiyi,O.O., & Sofowora, E. A (1978). Phytochemical screening of Nigeria medicinal plants 1. *Lioychia* 4:232

Odigie, I. P., Ettarh, R. R., & Adigun, S. A. (2003). Chronic administration of aqueous extract of Hibiscus sabdariffa attenuates hypertension and reverse cardiac hypertrophy in 2K- 1C hypertensive. *Journal of Ethnopharmacology,* 86( 2-3), 181- 185

Ogunsola, K. E., Ogunfunmilayo, A. O., oluitan, J. A., Kazeem, S. A., Folorunsho, D. O , & Ibrahim, S. ( 2016). Incidence and distribution of Roselle (Hibiscus sabdariffa L.) Diseases of Quarantine importance in six northern states of Nigeria. *Nigerian journal of plant protection*, 30(1), 46-59

Okereke, C. N., Iroka, F. C., & Chukwuma, M. O. (2015). Phytochemical analysis and medicinal uses of Hibiscus sabdariffa. *International journal of Herbal medicine.* 6(2).

Panche, A.N., Diwan, A. D., & Chandra, S. R. (2016). Journal of Nutritional science, 5, e47.

Pavithra, B., (2014). Eugenol: A review. *Journal of pharmaceutical sciences and research,* 6(3), 153.

Riaz Ghazala & Chopra rajini (2018). A review on Phytochemistry and therapeutic uses of Hibiscus sabdariffa L. 102, 575-586

Riaz, T., Akram, M., Laila, U., Zainab, R., Khalil, M. T., Iftikhar, M., Ozdemir, F. A., Solowski, G., Altable, M., Sfera, A., Ibrahim,H. K., & Parmar, P. (2023). Therapeutic application of glycosides obtained from medicinal plants. 10(8), 30-38

Sanders, M., Ayeni, A., & Simon, J. (2020). Comparison of yield and nutrition of Roselle ( Hibiscus sabdariffa) genotypes in central new Jersey. *Journal of medicinally active plants*, 9(4), 242-252.

Shaikh, J. K., & Patil, M. (2020). Qualitative tests for preliminary Phytochemical screening: A review. *International journal of chemical studies.* 8(2). 603-608

Singh Pragya, Khan mahejibin, & Hailemariam Hailu. (2017). Nutritional and health importance of Hibiscus sabdariffa: A review and indication for research needs. 6(5): 00212

Sinha, D., Mukherjee, S., & Chowdhury, S. (2022). Methods of extraction of phytochemicals. *Isolation, characterization and therapeutic applications of natural bioactive compounds*. 250-279

Sofowora. A. (1993). Medicinal plant and Traditional medicines in Africa. Spectrum Books limited Ibadan. 289

Srivastava, S. K., & Singh, N. K. (2020). General overview of medicinal and aromatic plants: A review. *The journal of medicinal plant studies*, 8(5), 82-87

Sylvia (2016). Roselle facts and health benefits.

Teklehaymankt, T., & Giday, M. (2007). An ethnobotanical survey of medicinal plants used by people in zegie peninsular, north western Ethiopia. *Journal of ethnobiology and ethnomedicine*, 3,12

Trease, G. E., & Evans, W. C. (1989). *Pharmacognosy.* 15th Edition published by sanders, 142:333-337

Unuigbe, C. A, Unula, C. F., Aiwonegbe, A., Uadia, A. O., Akhigbe,I., Asakitikpi E., & Ogbeide, O. K. (2021). Bioactive chemical constituents, acute toxicity and 1,1-diphenyl - 2- pictylhydrayl radical scavenging activity of *polyalthialongifoliaroot. GSC Biopharm Sci.* 14(1): 018-026

Wahabi, H. A , Alansary, L. A., Al- Sabban, A. H., & Glasziuo, P. (2007). The effectiveyof Hibiscus sabdariffa in the treatment of hypertension: A systematic review. *Journal of Ethnopharmacology,* 114(2), 218-223.

Wang Chi-Wei, Yu Yu-Hsiang, Wu Chu-Yao, Feng Ru-Ying, Tandon Kshitij, Chen Ying-Lien, Tang Sen-Lin. (2021). Detection of pathogenic and Beneficial microbes for roselle wilt diseases. *Front microbial.* 12: 756100

Wang, L., Zhang, W., & Li. Y. (2021). Antioxidant and anti-inflammatory activities of Hibiscus sabdariffa calyx extracts: A review. *Phototherapy research,* 35(5), 765-778

Weiss, R. F. (1988). Herbal medicine. *Beacon field publishers*.

Yang Wenqiang, Xu Chen, Yanli Li, Shaofen Guo, Zhen Wang, Xiuling Yu. (2020). Advances in pharmacological activities of Terpenoids. *Natural product communications*, 15(3).