PHYTOCHEMICAL AND MINERAL COMPOSITION OF WATER EXTRACTS OF HIBISCUS SABDARIFFA (zobo)

BY

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CERTIFICATION

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DEDICATION

This project is dedicated to our parents because without their prayers we were unable to complete this project. Our parents gives strength and courage to complete this project effectively.

ACKNOWLEDGMENT

I give thanks and praise to Almighty God for His infinite mercy, guidance, and strength throughout this journey. Your divine providence has been my source of inspiration, comfort, and hope. I am grateful for the blessings, wisdom, and resilience You've provided, enabling me to navigate challenges and achieve my goals.

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ABSTRACT

This work was carried out to investigate the chemical properties of the water extract of zobo (*Hibiscus sabdariffa*) drink using the modern atomic absorption spectrophotometer. Zobo drink is a popular drink in the Nigeria, especially in the northern part of Nigeria. The result of the analysis indicated that the drink is high in vital mineral nutrients required for the healthy growth by humans and for the proper metabolic processes to be adequately maintained. Zobo drink is high, as indicated in the result, in iron – a major component of the human haemoglobin, when compared with other leaf like the popular vegetable leaf known as awa (*Piper methysticum*).

CHAPTER ONE

INTRODUCTION

Hibiscus sabdariffa is a species of hibiscus, native to the old world tropics, used for the production of bast fiber and as an infusion (herbal tea). The plant is an annual or perennial herb or woody-based sub-shrub, growing up to 2-2.5 m (7-8 ft) tall. The leaves are deeply 3-5 lobed, 8-15 cm long arranged alternatively on the stems. The flowers are 8-10 cm in diameter, white to pale yellow with a dark red spot at the base of each petal and have a stout fleshy calyx at the base 1-2 cm wide, enlarging to 3-3.5 cm, fleshy and bright red as the fruit matures. It takes about 6months to mature. The plant is widely cultivated for its strong fibers and it is well known for its edibility and medicinal properties, though the calyx is the most frequently used portion of the plant, the leaves and seeds are often made into salads, curries and potherbs. They are rich in vitamins, natural carbohydrate, protein, tannins, gums and other antioxidants including minerals. The chemistry of the calyx revealed that per 100 g, it contained 49 calories, 84.5% water, 1.99 protein, 0.1 g fat, 12.3 g total carbohydrate, 2.3 g fiber, 1.2 g ash, 1.72 mg calcium, 57 mg phosphorus, 2.9 mg iron, 300 g vitamin A equivalent and 14mg ascorbic acid.

Hibiscus sadariffa has common names such as “Roses of anthea’ “African mallow”, ‘Roselle’, “Rose mallow’, Indian sorrel’, “Flore de Jamaica” and Jamaica tea. Hibiscus sabdariffa is known as “Roselle” in Australia, ‘Tengamoran’ in Asam, ‘Gongura’ in Hindi, ‘Krajeab’ in Thailand, “Bissap” in Senegal, Guinea Bissau, Mali, Burkina Faso, Ghana, Benin, Niger, Congo and France. ‘Wonjo’ in Gambia, ‘Torosh’ in Iran, ‘Sorrel’ in Carribean, ‘Karkade’ in Egypt, ‘Asam Paya’ in Malaysia, “Luoshen Hua’ in Chinese, ‘Lamanda’ in Zambia and “Zobo’ in Nigeria. The fruits are surrounded by enlarged fleshy calyces containing 22-34 seeds per capsule. The seeds are dark brown in colour, 4-6cm long and about 0.025g in weight, grow up to 2 meters and leaves vary in shape and size. There are more than 200 species in all over the world. Hibiscus sabdariffa is cultivated for leaf, seed, fleshy calyx or fiber. The plant is grown in all parts of the world; it is a native to India but was introduced to other parts of the world such as Central America, West Indies and Africa. It is highly cultivated and distributed in the northern part of Nigeria because of favourablenclimate. Hibiscus sabdariffa is found in tropical and subtropical regions of the world, it is cultivated for its fiber with characteristics similar to those of hemp or jute which is used to make cloth, rope and in Polynesia, grass skirts. Hibiscus sabdariffa plant

parts such as seed, stem, leaves and calyces are harvested from late December to February. The harvest is timed according to ripeness of the seed. The fleshy calyces are harvested after the flower has dropped but before the seedpod has dried and opened. The more time the capsule remains on the plant after the seeds begin to ripe, the more susceptible the calyx is to sores, sun cracking and general deterioration in quality.

Hibiscus sabdariffa ‘Zobo’ drink has a shelf of life normally 24 to 48 hours after which it begins to deteriorate. It is best preserved by refrigeration which will control the micro- organism reaction on the drink . Due to the ubiquitous nature of micro-organisms, the level of contamination can be reduced if proceeded under standard hygienic conditions and at low temperatures to prevent the multiplication of pathogenic micro organisms that can cause infection to the consumers. Delicate hibiscus flower requires special handling during and after preservation. The blossoms are fragile and cannot tolerate the pressure from conventional flower pressing. The use of desiccants and silica crystals helps wick away moisture from the petals while still protecting the shape and colour of the bossom of the plant.*(Steyerberg, E.W. 2004)*.

1.1 STATEMENT OF PROBLEM

Today, people are troubled because of the way various diseases and sickness are springing up. The only seemed major way out is to visit modern hospital for both prevention and control. This is treatment using synthetic drugs which are very much expensive, and most time with negative side effect. And not readily available.

1.2 JUSTIFICATION OF STUDY

Several studies undertaken on use of plant-derived compounds as alternatives to synthetic drugs have established that hibiscus sabdariffa has medicinal values. The activities are linked to the type of phytochemical and mineral present in the plant. It was on the basis of this, that this study was taken with the aim and objectives stated below.

1.3 AIMS OF RESEARCH

The research aimed at monitoring the phytochemical and mineral composition of water extracts of *hibiscus sabdariffa* (zobo).

1.4 OBJECTIVE OF RESEARCH

The objectives of the research were to

i. collection and preparation of sample
ii. determine the phytochemical analysis
iii. determination of mineral content
iv. Investigate acute toxicity

CHAPTER TWO

LITERATURE REVIEW

2.1 SCIENTIFIC CLASSIFICATION OF *ROSELLE* (*Hibiscus Sabdariffa*).

Kingdom: *Plantae*

It is an Angiosperm, Eudicots and Rosids

Order: *Malvales*

Family: *Malvaceae*

Genus: *Hibiscus*

Species: *Sabdariffa*

Binomial name: *Hibiscus Sabdariffa*

The *Roselle* is known as the *Rosella* fruit in Australia. It's close relative, Hibiscus Cannabinus is also known as meshta on the Indian subcontinent, Tengamora in Assam, Gongura in Telugu, lalchatni or Kutrum in Mithula.

Mathipuli in kerala. Chin baung in Burma, krajeab in Thailand, Bissapin Senegal, Guinea Bissau, Mali, Burkina Faso, Ghana, Benin and Niger, Congo and France. Wonjo in Gambia, Zobo in western Nigeria. Yoruba's in Nigeria call the white variety Isapa, Zoborodo in Northern Nigeria, Chaye-Torosh in Iran, Karkade in Egypt, Saud, Arabia and Sudan; Omutete in Namibia, Sorrel in the Caribbean and in latin America, Flor de Jamaica in Mexico, Saril in panama, rosella in Indonesia, asam paya in Malaysia, Luoshen Hua in Chinese. In Zambia the plant is called Lumanda in Gbemba, Katolo in Kikaonde and, wusi in chilunda *(Chau, et al, 2000).*

2.1.1 Effect on smooth muscle

The aqueous extract of *H. Sabdariffa* calyces inhibited the tone of various isolated muscle preparations that included rabbit aortic strip (Obiefuna *et al*., 1994)and rat ileal strip (Salah *et al*., 2002). The extract also rhythmically contracted rat uterus, guinea-pig tracheal chain and rat diaphragm. The mechanism of action of *H. Sabdariffa* aqueous extract on smooth muscles is not certain but appears to be mediated through calcium channels, possibly generated by constituents such as quercetin and eugenol (Salah *et al*., 2002). However, further studies of the mechanistic aspects of the extract on smooth muscles are warranted.

2.1 .2 Blood pressure lowering effect

The effectiveness of an aqueous extract of Roselle on mild to moderate hypertension was investigated in many

researches. Aqueous extract of Rosellewas as effective as captopril in treating mild to moderate hypertension and there is no adverse effect with the treatment, confirming the effectiveness and safety of the extract. Even though the possible mechanism(s) of action of Roselle extract is

not investigated, daily consumptionof an aqueous Roselle extract resulted in decrease in systolic and diastolic blood pressure.

2.1.3 Anti diabetic activity

Extracted the polyphenolic components of Roselle and studied their effect in a type II diabetic rat model (high fat diet model). Studied revealed anti-insulin resistance properties of extract at a dose level of 200mg/kg, and reduction in hyper glycaemia and hyper insulinemia. The extract was found effective in lowering serum cholesterol, triacylglycerol, the ratio of low density lipoprotein/high-density protein (LDL/HDL), and also (AGE) formation and lipid per oxidation. Intestinal α-glycosidase and pancreatic α-amylase help in digestion of complex carbohydrates present in the food into bioavailable monosaccharide and plays an important role in postprandial hyperglycaemia; therefore inhibition of

these enzymes has been reported as an effective mechanism for the control of postprandial hyperglycaemia.

Hibiscus acid (hibiscus- type (2S,3R)-hydroxycitric acid lactone) have been shown as a potent inhibitor of pancreatic α-amylase and intestinal α-glucosidase and pancreatic α-amylase activity. In another study, Adisakwattanaet al., conducted an *in vitro* study and reported Roselle extracts as an effective inhibitor of pancreatic α-amylase.

2.1.4 Anti helmentic and anti microbial effects

Roselle is known for its antibacterial, antifungal and anti-parasitic actions. Oil extracted from seeds of Roselle has been shown to have an *in vitro*

inhibitory effect on *Bacillus anthracis* and *Staphylococcus albus*. Aqueous and ethanol extracts were also found to be effective against *Schistosoma mansoni* and other microorganisms. Afolabi et al., demonstrated the antibacterial effect of hibiscus extract on *Streptococcus mutans*, a bacterium from oral cavity. In a similar study, antibacterial potential of hibiscus was also observed on *Campylobacter* species. An ethanol extract of the dried leaves of Roselle reduce aflatoxin formation and have *in vitro* inhibitory effect against some fungi.

2.1.5 Anti-oxidant effect

Protective property of a compound to inhibit the oxidative mechanisms by scavenging reactive oxygen and free radicals is known as antioxidative activity. It protects lining organelles from premature cell damage and reduces ageing. A large number of *invitro* and *invivo* studies have shown that Roselle calyxes contain potent antioxidant. According to Augustine, both the whole aqueous and anthocyanin-rich extracts of Roselle are effective antioxidant. Studies have also highlighted that poly-phenolic acid, flavonoids and anthocyanins which are found in Roselle are potent antioxidants.

2.2 *HIBISCUS SABDARIFFA* PLANT PROFILE

The economic uses of the plant are as garden ornamentals and potherbs, used locally in Africa as source of dye and timber and as medicinal plant and drink. In Africa, the water extract of *Hibiscus sabdariffa*is taken as a hot or cold drink. The drink is known as Wanjo in the Gambia, 'Zobo' in Nigeria, Karkadayin Egypt and Sudan and Omutete in Namibia. The plant is highly cultivated in the northern part of Nigeria probably because of the favourable climate. Hibiscus (Hibiscus spp.) has common names including: Rose of Althea, African mallow, rose mallow, *roselle*, Indian sorrel, flor de Jamaica, Agua de Jamaica, Jamaica tea

Cultivation Range: Found in tropical and subtropical regions throughout the world. Parts of the plant used include the flowers, whole, dried and cut, or powdered.

Preparations: Hibiscus is not generally used in Western herbal medicine, although it does have pharmacological effects.

The powdered flowers are sometimes used in topical applications or when making natural cosmetics. The vibrant flowers make a flavorful and attractive addition to cake tops, puddings, salads, sorbets and teas, hot or iced. Hibiscus is a member of the Malvaceae family, or the mallow family of flowering plants. There are more than 200 species, all of which are known for their colorful, showy blooms. One species, Kenaf (Hibiscus cannabinus), is cultivated for its fiber with characteristics similar to those of hemp or jute, which is used to make cloth, rope and, in Polynesia, grass skirts. This species is also used to produce Kenaf paper, which originated in ancient Egypt and is still manufactured today in India, Bangladesh, Indonesia and elsewhere.

Hibiscus rosa-sinensis is used in Mexico as a dieting aide. The herb is a source of hydroxycitric acid, also known as hydroxycut, which is used in some supplement formulas to promote weight loss. There may be some merit to this claim since some animal-based studies have shown that this substance deters the conversion of dietary carbohydrates into fat.

However, this effect has only been observed when following calorie-restricted diets designed to reduce total intake of fats, carbohydrates and protein, and not in low-carb diet plans. Tea made from the flowers may lower blood pressure and low-density cholesterol levels, but studies on these effects are inconclusive and ongoing.

Hibiscus has a diuretic and laxative effect, especially the powdered form. Prepared and applied topically as a wash, the plant is reputed to be effective against inflammatory skin disorders, most notably eczema.

2.3 Constituents of *Hibiscus sabdariffa*

The plant's constituents, among others include the cyanidins, pectin, citric acid, hydroxycitric acid, malic acid, tartaric acid, mucilage. Contraindications: This includes any side effect(s) resulting from the preparation of any of the Hibiscus species. The dried flowers should be used within six months. Avoid use if there is a history of gallstones, gallbladder disease or liver disease. *(Steyerberg, E.W. 2004)*.

2.4 DESCRIPTION OF *HIBISCUS SABDARIFFA*

Hibiscus has more than three hundred species distributed in tropical and subtropical regions around the world and are used as ornamental plants. Research on have shown that some species of Hibiscus possess certain medicinal properties of which *Hibiscus sabdariffa* is one. *Hibiscus sabdariffa* is commonly named as “red sorrel” or “roselle”. Even though permeable soil is the best, Roselle can adapt to a variety of soil in a warmer and more humid climate.

Hibiscus sabdariffa, a member of Malvaceae family, is a known medicinal plant with a worldwide fame and the plant can be found in almost all warm countries such as India, Saudi Arabia, Malaysia, Indonesia, Thailand, Philippines, Vietnam, Sudan, Egypt and Mexico. Roselle is mainly cultivated to be consumed and the main producers of Roselle blossoms are Egypt, Sudan, Mexico, Thailand and China. Other hibiscus varieties are planted for their fibers they produce.

2.5 ORIGIN OF *HIBISCUS SABDARIFFA*

There is a big argument about the origin of Roselle among different scholars. Cobley suggested Roselle is a native plant of West Africa and from there it was carried to other parts of the world such as Asia and

America, whereas in others opinion, Roselle was originated from India and Saudi Arabia.

2.6 VARIETIES OF *HIBISCUS SABDARIFFA*

Among numerous verities of Hibiscus, *Hibiscus altissima* and *Hibiscus sabdariffa* are the commonest and better introduced. *Hibiscus altissima* is branchless plant with yellow flowers and red or green colored calyxes. Though this species is not used for food, this plant is more economically important than *Hibiscus sabdariffa* because of its high fiber content. The other distinct type *Hibiscus sabdariffa* or "Roselle" grows in a bush with many branches. The flowers of Roselle are axillaries or in terminal racemes, the petals are white with reddish center at the base of the stamina column and this species is widely used as food

2.7 COMPOSITION OF *HIBISCUS SABDARIFFA*

Roselle is mainly cultivated for its calyx, which is of three types: green, red and dark red. The red calyxes are the most used are characterized by their concentration anthocyanin. Delphinidin 3-Sambubioside and Cyanidin3-Sambubioside are the major anthocyanin. Roselle is also rich in organic acids, minerals, amino acids, carotene, vitamin C and total sugar in its calyx, leaves and seeds at variable levels depending on the

variety and geographical area. According to Manita–mishra a number of compounds have also been isolated and characterized from Roselle including flavonoids, anthocyanidins, triterpernoids, steroids and alkaloids. Nutrient contents of different part of *Hibiscus sabdariffa* per 100 gram are clearly stated in table below.

Nutrients	**Calyxes**	**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]	150	350	240
Iron [mg]	3	9	5

2.8 HERBAL MEDICINE AND PLANTS

Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness *(Craig, 1999).*

Herbs refer to not only the herbaceous plants but also to bark, roots, leaves, seeds, flowers and fruits of trees,

shrubs and woody vines. *Hibiscus sabdariffa* (Linn) (family *Malvaceae*), is an annual dicotyledonous herbaceous shrub popularly known as 'Gongura' in Hindi or 'PulichaKeerai' in Tamil (Kuriyan et al., 2010).This plant is well known in Asia and Africa and is commonly used to make jellies, jams and beverages (Kuriyan *et al*., 2010).

In folk medicine, it has been used to treat hypertension (Haji and Haji, 1999), inflammatory disease (Dafallah and al-Mustafa, 1996) and cancer (Chewonarin *et al*., 1999). The flowers of*Hibiscus sabdariffa* contain anthocyanins, flavonoids and polyphenols (Lin *et al*., 2007). Studies have highlighted the role of polyphenolic acid, flavonoids and anthocyanins that may act as antioxidants or have other mechanisms contributing to the cardio protective actions. *(Crawford et al., 1998; Rimm and Stamfer, 2000).*

2.9 PREPARATION OF 'ZOBO' MADE FROM *ROSELLE* (*HIBISCUS SABDARIFFA*).

The Zobo drink is prepared by boiling the dry calyces of *Hibiscus sabdariffa* in water for about 10-15 mins from which the pigment or flavour embedded is extracted.

After extracting the filterate may be taken as hot tea or allowed to cool and packaged in plastic sachet containers then taken as a refreshing drink

when chilled. The sharp taste of the raw extract is usually sweetened with sugar cane or granulated sugar, pineapple, orange or other fruits depending on choice. The sweetness of Zobo drink does not last long due to spoilage by microbial activities. It life shelf is approximately twenty-four hours following production if not refrigerated.
(Omemu, *et al*, 2006).

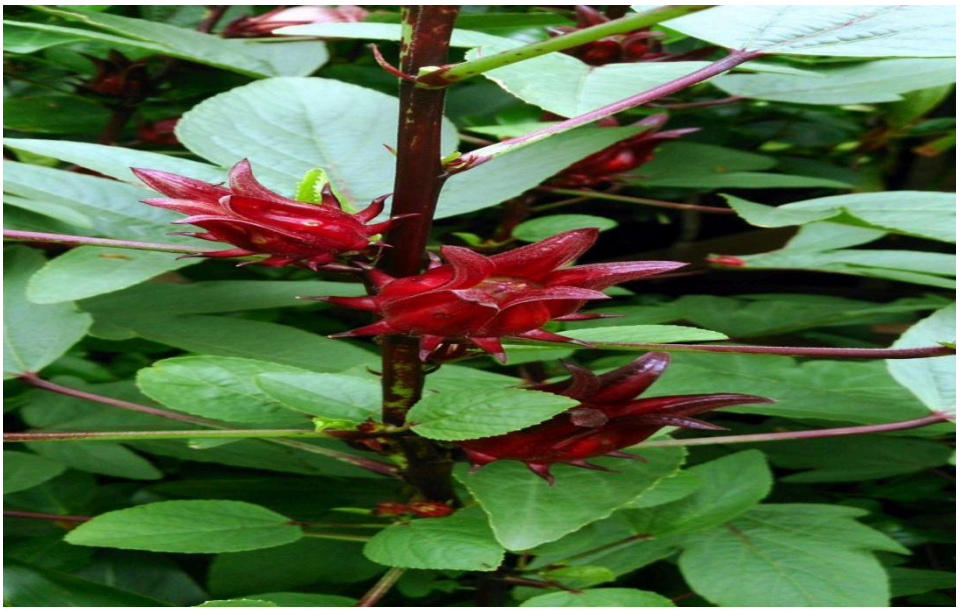
In countries like Indian *Roselle* calyces are utilized in producing refreshing beverages, jellies, jam sauces and food preserves. (Clydescale, *et al*, 1970). The fleshy, swollen red calyces and the flowers are used to colour and season other food as well as in preparation of a fruit drink called 'Zobo' in Northern central states of Nigeria, which is gaining wide spread acceptance across the country. It is also used as thickner and flavourants in soups (Ibrahim, *et al*, 1971). Primarily, the plant is also used for the production of bast fibre from the stem of the plant and as an infusion.
The plant is considered to have antihypertensive properties. The fibre may be used as a substitute for jute in making burlap *(Chau, et al, 2000)*. It has been used in folk medicine as a diuretic, mild laxative and treatment for cardic and nerve diseases and cancer *(Mohammed, et al, 2002)*.

The red calyces of the plant are increasingly exported to America and Europe where they are used as food colourings. It is found in market in some places in France as flowers or syrup.

The green leaves are used like a spicy version of spinach. They give flavour to the Senegalese fish and rice dish (thiėboudieume). In myanmar, the green leaves of *Roselle*, are the main ingredient in making chin baung Kyaw Curry. In East African the calyce in fusion called “Sudan tea” is taken to relieve coughs. *Roselle* juice with salt, pepper, arafetida and molasses, is taken as a remedy for biliousness.

The heated leaf are applied to cracks in the feet and on boils and ulcers to speed maturation. A lotion made from leaves is used on sores and wounds. The seeds are said to be diuretic and tonic in action and the brownish yellow seed oil is claimed to heal sores on camels. In India, a decoction of the seeds is given to relieve dysuria strangury and mild cases of dyspepsia. Brazillians attribute stomachic, emollient and resolutive properties to the bitter roots.

DIAGRAM OF *HIBISCUS SABDARIF*



CHAPTER THREE

METHODOLOGY

3.0 MATERIALS

The materials used for this analysis included the following:

Soxhlet Extractor

Atomic Absorption Spectrophotometer (AAS)

Ultra Violet Spectrophotometer (UV)

Distilled water

Test tubes

Beakers

Weighing balance

Hibiscus sabdariffa (Zobo leaf)

3.1 EXTRACTION PROCEDURE/PLANT MATERIALS

3.1.1 Plant Materials

The samples of *Hibiscus sabdariffa* L. leaves were collected in July 2010 in Enugu State of Nigeria. The plant was identified by Mr. Ozioko, A. at Nsukka, Enugu State.

3.1.2 Extract Preparation

The samples of *Hibiscus sabdariffa* L. leaves were collected in July 2010 in the Enugu State of Nigeria. The plant was identified by Mr. Ozioko, A.

at Nsukka, Enugu State. The *Hibiscus sabdariffa* L. leaves were washed thoroughly anddried under room temperature. The extraction of *Hibiscus sabdariffa* L. leaves was done using fifty grams (50g) of the ground leaf sample in soxhlet extractor with distilled water in Department of Biochemistry,Caritas University, Amorji-Nike, Enugu State, Nigeria. The recycling of the solvent (distilled water) was allowed to be repeated for complete extraction. The slurry extracts were then poured into evaporating dish to evaporate the solvent in the extract over the water bath at the temperature of 80ºC - 95 ºC and a yield of 17ml of crude extract was obtained. *(Steyerberg, E.W. 2004)*.

3.2 ATOMIC ABSORPTION SPECTROPHOTOMETRIC DETERMINATION

The water extract of the *H. sabdariffa* was later read in the atomic absorption spectrophotometer to determine the actual concentration of mineral elements present in the leaf extract.

3.3 PHYTOCHEMICAL ANALYSIS

The water extract of *Hibiscus sabdariffa* L. were subjected to preliminary phytochemical screening to identify the chemical constituents.

Traditional medicine plays a significant role in the healthcare of the people in developing countries, including Ethiopia, and medicinal plants provide a valuable contribution to this practice (Tesfahuneygn and Gebreegziabher 2019). In this review, around 33 medicinal plants species were identified from published articles. The different parts of the plant such as root, leaves, and fruit, in which these different parts have many traditional values, pharmacological uses, and phytochemical constituents were mentioned. From few medication values of plant parts, to treat rheumatism, madness, snakebite, chest pain, jaundice chest pain, malaria, headache, cough, etc. All the medicinal plants are shown in the table form with the scientific name, families, local name, and importance. Most plants were reported and investigated in Ethiopia. As reported by many authors, some medicinal plants with their scientific name, family, local name and their importance are shown in Table 23, and these plant species listed in this review were often used by the people in Ethiopia.

Phytochemicals

Analysis of the phytochemical properties of the medicinal plants used to show and isolate the drug, lead compounds and components from the parts of the plant. The unique biological activity of the plants can be identified by their phytochemicals properties. Most parts of the plants used for the analysis of the phytochemical properties were leaves, roots,

stem barks, and fruits. In this review, medicinal plants were investigated for phytochemical constituents of ethanol, methanol, chloroform, acetone, hexane, petroleum ether, ethyl acetate, and aqueous (water) extraction of different phytochemicals.

In this review, the most published articles recognized the presence of of phytochemical components in the plants was indicated by the positive sign (+) and the absence of phytochemical components in the plants, by the negative sign (−) .

3.3.1 Tannins

400 g of AOLP was soaked in 2000 ml 70% ethanol, shaken for six hours, then left undisturbed for another 48 hours

before filtering t hrough Whatman No 1 f ilter paper. Using a rotary evaporator, the AOLP ethanolic extract was vacuum

condensed a t 35-40°C.

200 g of leaf powder was immersed in 1000 ml 70% ethanol, vibrated continuously for six hours before being left

undisturbed for another 48 hours and filtered using W hatman No 1 f ilter paper.

The Folin-Ciocalteau technique [18] was used to determine total tannins.

In a volumetric flask (100 ml), 1 ml of the leaf ethanolic extract was

diluted with 49 ml distilled water, 1.7 ml 75% ethanol, 0.1 ml metaphosphoric acid, 1.0mol/ml Na2CO3 (10 ml), and 2.5 ml Folin-Ciocalteu. The mixture was thoroughly blended and left at room temperature for 15 minutes. Following that, the absorbance of standard solution and leaf extract was measured in a spectrophotometer at 680 nm against a blank. As a reference, the standard curve (R2 = 0.9972) was used, and the total tannin content of the sample was expressed as tannic acid (TA) mg TA/g DW.

.3.3.2 Alkaloids

The gravimetric method [22] was used to determine the alkaloid content of the leaf sample. 5 g of the AOLP was distributed in 50 ml of acetic acid solution in ethanol (10%, w/v). The mixture was subjected to vibration and left undisturbed for about 240 minutes before it was sieved. The filtrate was lessened to a quarter of its initial volume on a hot plate. The alkaloids were then precipitated by adding drops of concentrated ammonium hydroxide. The filter paper was used to filter the precipitate, which was then washed with a 1 percent ammonium hydroxide solution. The precipitate was then oven-dried for half an hour at 60°C before being transferred to desiccators and reweighed until it

reached a constant weight. The weight of the alkaloids was calculated as a percentage of the sample weight.

3.3.3 Flavonoids

Surana et al. [19] method for determining the flavonoids content of leaf samples was followed. In a test tube containing 0.50 ml of leaf powder extract, 0.1ml aluminium chloride solution, 1.50 ml methanol, 0.1 ml potassium acetate solution, and 2.8 ml distilled water were added. Both extract and rutin standard dilutions (10-100 g/ml) sample blanks were made the same way, but with distilled water instead of aluminium chloride solution. After that,

the solutions were filtered through Whatman filter paper (No. 1). Absorbance ratios were measured at 510 nm against blanks. Then, total flavonoid content was determined as equivalent to 1 mg rutin per gram of the ethanolic leaf extract.

3.3.4 Saponins

The vanillin and concentrated sulfuric acid colourimetric method described in [21] was used for quantifying saponin.

The 0.1 ml extract was mixed with 0.5 ml ethanol (50%, w/v), 4.0 ml sulfuric acid (77% w/v), and 0.5 ml of freshly prepared vanillin solution (8% w/v). The mixture was allowed to cool to room temperature before

being heated in a water bath to 60 °C for 15minutes. A UV/Vis spectrophotometer was used to detect the absorbance at 545 nm. The total saponin content in each sample was determined using a tea saponin calibration curve and represented as mg tea saponin equivalent per g (TSE/g DW).

.3.3.5 Phenols

The Folin-Ciocalteau method [20] was used to determine the total phenolic content of the leaf sample. In 50 µL of leaf extract or standard solution, 250 µL of Folin-Ciocalteau reactive was added. This combination was kept at room temperature for 5 minutes in a dark environment. A 750 µL 7 percent Na2CO3 solution was added at the end of this period. Distilled water was used to dilute the mixture to 5 mL. After that, the mixture was kept at room temperature in a dark environment for 120 minutes to react. The absorbance of the samples and standards were measured at 760 nm. Instead of 50 µL extract, an 80 percent methanol solution (50 µL) was added to the blank solution. The total phenolic content was determined using a calibration curve using gallic acid equivalent standards.

.3.3.6 Phytate

The phytate in the leaf samples was quantified using the anion exchange methods described by [23]. The previous AOLP filtrate (0.2-1.0 ml) was

diluted with distilled water to a final volume of 1.4 ml, then 1.0 ml ferric ammonium sulfate solution containing 50 µg Fe was added and adequately mixed. The test tubes were then sealed and immersed in a boiling water bath for 20 minutes. Then, 5 ml amyl alcohol was added to the test tube once it had cooled to room temperature, followed by 0.1 ml of ammonium thiocyanate solution (100 g/l). The contents of the test tubes were immediately mixed using inversion and shaking. The colour intensity in the amyl layer was measured with a spectrophotometer at 465 nm against an amyl alcohol blank precisely 15 minutes after ammonium thiocyanate application and brief centrifugation at low speed. Since ferric ions complexed with phytate at pH 1-2 cannot combine with thiocyanate ion to form the characteristic pink complex, the absorbance at 465 nm in the amyl layer is inversely linked to the phytate anion concentration.

3.4 ACUTE TOXICITY STUDY

The lethal dose (LD50) of the plant leaf extract was determined by the method of Lorke (1983)using 13 rats. In the first phase rats were divided into 3groups of 3 rats each and were treated with the water extract of the leaf at doses of 10,100 and 1000 mg/kgbody weight intraperitoneal. They were observed for 24h for signs of toxicity. In the second phase 4 rats were divided into 4 groups of 1 rat each and were also treated with the

aqueous extract at doses of 1000, 1600, 2900 and5000 mg/kg bodyweight,

intraperitoneally (i.p). The median lethal dose (LD50) was calculated using the second phase. (*Ajabonna, O.P. 1996)*.

CHAPTER FOUR

RESULTS

4.0 PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical analysis of the aqueous seed extract of *Hibiscus sabdariffa*L.revealedthe presence of varying amount of alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, cardenoledes, flavonoids and phlobatanins in the concentrations shown in the table.

Table

Phytochemical screening of aqueous extract of *Hibiscus sabdariffa*L. seeds.

Extract Constituents	**Concentrations**
Tannins	✚
Alkaloids	++
Flavonoids	+

Saponins	+++
Phenol	++
Phytate	+

+++ = High concentration

++ = Moderate concentration

+ = Low concentration

- = Not detected

Table 4.2

Mineral compositions (AAS results) of leaves *Hibiscus sabdariffa* (zobo) water extract.

S/N	**Unit (mg/g)**
Ca	0.25
Mg	0.027
Na	0.30
K	0.051
Mn	0.00013

Fe	0.50
Cu	0.0096
Zn	5.35

The result shown above in Table 4.2 indicate that the water extract of zobo drink (*Hibiscus sabdariffa*) contains healthy concentration of essential mineral ions, which are highlighted as stated above.

4.1 MAGNESIUM:

This is the third most abundant mineral in the human body, approximately seventy percent of which is located in the bone. It is essential for healthy bones and teeth. It is also critical for maintenance of cell membrane potential and works very synergistically with the amino acid, taurine. It is involved in maintaining nerve-muscle interaction. It regulates heartbeat. It helps utilize B complex and vitamin C and E, bone

growth, and the function of all muscles, especially including the heart. It appears to be associated with the regulation of the body temperature, and is essential for conversion of blood glucose into energy. The single most important and most common mineral deficiency is probably magnesium. Like calcium, magnesium must be constantly supplied to maintain

optimal function. Magnesium doesn't have an active transport, but depends entirely on dietary intake and healthy intestinal lining for absorption, and can be absorbed throughout the entire small intestine and even in the colon. Low intakes of magnesium, or loss of ability of the intestinal tract to absorb magnesium due to intestinal inflammation or disease, can result in a variety of problems such as muscle twitching or tremors, weakness irritability and restlessness, depression, and weak bones.

Deficiency can result in agitation, anxiety, and hallucination, as well as a variety of physical problems. Other possible reasons for a deficiency include; kidney or parathyroid disease, high blood pressure. *(Steyerberg, E.W. 2004).*

4.2 POTASSIUM

An essential mineral that helps regulate sodium and water balance. It is very important in growth, nerve function, and proper PH of the body. Involved in the conversion of glucose to glycogen and in many enzyme reactions in general cell metabolism. One of the critical minerals in maintaining a normal heartbeat, as well as nerve conduction, production of energy and synthesis of nucleic acids and proteins.

4.3 IRON

An essential mineral, whose major function, in combination with protein and copper, is in the manufacture of hemoglobin, which transport oxygen throughout the body. Myoglobin, found in muscle tissue, also contains iron and assist in the transfer of oxygen into the muscle. Iron is important for protein metabolism and immune system. It works closely sync with calcium and copper. Iron deficiency can result in anemia, which can produce symptoms such as depression, irritability, fatigue, loss of attention span, and insomnia. One study found that nearly half of all pre-menopausal women and a third of all children do not get enough iron, so supplementation in these groups could have a significant impact on the frequency of depression and other disorders.

From 15 to 30 mg a day is a good maintenance dose. On the other hand, excessive iron can lead to toxicity, especially in men, as they do not lose the mineral through menstruation. Thus, men shouldn’t supplement with iron unless under a physician’s direction.

4.4 COPPER

An essential trace mineral, imported in the formation of hemoglobin and red blood cells, and involved in many enzymes, protein metabolism, and synthesis of phospholipids, including the production of myelin sheaths. It

helps the antioxidant effect of vitamin C, and is essential in the production of elastin, a component of muscle fiber. Is also necessary for proper bone formation and production of RNA and melanin.

Copper aids the conversion of nutrient to energy.

4.5 CALCIUM

The most abundant mineral in the body, most of which occurs in the bones, teeth and connective tissues. Calcium requires magnesium, phosphorus, and vitamins A,C and D for adequate metabolism. It is essential for maintaining the PH of the blood and regulating cell membrane potential. it helps in patients suffering from insomnia and irregular heartbeats, and is essential for preventing high blood pressure. Calcium aids in blood clotting. Depressed individuals often have excessive calcium level; particularly those with bipolar disorder. When these patients recover, their calcium levels usually return to normal. Depression can also occur in cases of calcium deficiency, long before the appearance of physical deficiency symptoms. Calcium also works with magnesium to maintain balance, or homeostasis, in the body.

4.6 SODIUM

An essential mineral found predominantly in blood serum, and extracellular fluid, as well as in bone. It functions synergistically and

inversely with potassium to assist in PH balance, and helps keep other blood minerals soluble. It is essential for hydrochloric acid production and very involved in overall adrenal function. Sodium and potassium generally determine the body's electrolyte balance, which regulates water levels. Eating a lot of salty food (sodium) disrupt this balance this not only produces high blood pressure, but also affects neurotransmitter levels.

4.7 MANGANESE

A trace mineral important in many enzymes and the metabolism of many vitamins as well in the synthesis of fatty acids and cholesterol, protein,carbohydrates, and fat metabolism , as well as sex hormone production, bone and connective tissue, health, and nerve and brain function.

4.8 ZINC

An essential trace mineral, particularly involved in the immune system, nervous system, digestive, and reproductive systems. It is involved with a wide variety of enzymes necessary for metabolism. It is also a major component of insulin. It is essential in the formation of protein, general growth and development, synthesis of DNA(genetic code), and all

healing. Helps regulate blood level of vitamin A. zinc deficiencies frequently lead to depression, since this minerals is essential to many processes related to brain function. In addition to irritability, mental slowness, and emotional disorders, zinc deficiency can produce changes in taste and smell sensations, a loss of appetite, reduced immune function, and rough skin. These symptoms are particularly common among older people and in women, especially those with eating disorders. An excellent treatment for anorexia and bulimia uses high doses of zinc, beyond the recommended daily values of 15 to 30 mg. These ions are abundant in zobo and its constant and persistent intake replenishes the lost ones in the biological system- leading to the maintenance of a healthy life.

4.9 DISCUSSION

The phytochemical analyses of Hibiscus sabdariffa (Zobo flower) showed the presence of some phytonutrients and some nutrients. The phytonurtient otherwise known as phytochemical present on the plant sample are flavonoids, glycosides, phytosterols and tannis while the macronutrients are proteins, fats and oil and reducing sugar and carbohydrates. The role of Hibiscus sabdariffa in the folk medicine has been attributed to the treatment of abscesses, bilious conditions, cancer, cough, debility, dyspepsia, fever, heart ailments, more especially in cardiovascular diseases, cancer, allegic reactions etc. flavonoids are most

commonly known for their antioxidant activity, however it is now know that the health benefit they provide against cancer and heart diseases are the result of other mechanism (Lotito and Frei, 2006). Flavonoids have been referred to as nature's biological response modifiers because of strong experiment evidence showing their inherent ability to modify the body's reaction to allegens, viruses and carcinogens. They also show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities, these effects have also been attributed to the action of zobo flower *(Kushi et al., 2006)*.

Flavonoids like gossypetin and anthocyanin have effects on decreasing the viscocity of blood, reducing blood pressure and stiulating intestinal peristalsis (Perry, 1980). Studies have showed that the specific flavonoids contained in Hibiscus sabdariffa F. are gossypetin and anthocyanin which make the plant very useful in the treatment of some ailments (Wikipedia, 2006). From results of this study the presence of the flavonoids agreed that of previous studies. This agreement concern the fact that flavonoids are poorly absorbed by human body (less than 5%) and most of what is absorbed is quickly metabolized and excreted, the action of excreting the flavonoids from the body induces the phase II enzymes that gets rid of carcinogens and mutagens that causes diseases like cancer, arteriosclerosis and other cardio vascular diseases *(Slavin, 2003)*.

The anti-microbial activity of Hibiscus sabdariffa could also be attributed to the presence of tannins found on the analyses carried. Tannins have anti-microbial activity by precipitating protein content of the outer wall of the microbes, thereby forming complex with the proteins and stop their activities. Also tannins have an antioxidants activity which helps in mopping up the free radicals that causes oxidative damages of the cell which results in many known diseases. The study also revealed the presence of phytosterols, which play major role in inhibiting the intestinal absorbption of cholesterol. Studies showed that zobo F. contains β-sitosterol as one of the phytosterols. B-sitosterol has been used in lowering serum cholesterol levels in hypercholesterolemic individuals *(Hicks and Moreau, 2001)*. This can attributed to use of Hibiscus sabdariffa F. in the treatment of arteriosclerosis which occurs as a result of increase in cholesterol levels.

CHAPTER FIVE

CONCLUSION

Hibiscus sabdariffa or “Roselle” is medicinal plant with a worldwide fame. Roselle, having various medically important compounds called phytochemicals, is well known for its nutritional and medicinal properties. Seeds, leaves, fruits and roots of the plant are used as food and herbal

medicine. Extracts from Roselle plays a crucial role in treating different medical problems including many cardiovascular disorders and cancer but further researches are required to know its exact mechanism of action and to formulate food products using Roselle with locally grown food items. Obesity is a growing problem, affecting not only adults but also children. The effectiveness of Roselle extract for metabolic disorders like type II diabetes should be examined further, as previous clinical studies have shown encouraging effects on hyperlipidemia and hypertension, conditions strongly correlated with type II diabetes or metabolic syndrome.

A lot have been known and researched on the effects of *Hibiscus sabdariffa* extracts on various parameters in the metabolic processes. This

work focused mainly on the mineral properties present in the water extract of zobo drink (*Hibiscus sabdariffa*). Biological processes and every enzyme require cofactors and coenzymes to function optimally. Most of these cofactors are mineral elements. Complex mineral elements are not synthesized by the mammalian metabolic system, however they can be sourced through the nutrients requirements of such mammals.

Zobo drink is a major popular drink in Nigeria. The results of this research work show that the plant material (*Hibiscus sabdariffa*) is high enough in essential nutrients required for optimal performance of health and the maintenance of good health together with the reduction of aging. It is, therefore, encouraged to increase the intake of zobo drink.

The physico-chemical properties of Zobo was analysed which showed low acidic content of the Zobo, which is normal and unharmful to the health. In the microbiological properties, micro organisms where isolated as shown in tables 1-5, but the presence of *Staphylococcus aureus, Escherichia and Aspergillus sp* is pathogenic to human health.

The presence of these poisonous micro organisms may be due to contamination by the materials used in producing the Zobo juice and improper or lack of sterilization of the materials used and the low acid content of the Zobo which cannot inhabit the presence of these Microorganisms.

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