ISOLATION AND CHARACTERIZATION OF BUTANOL EXTRACT OF *HIBISCUS SABDARIFFA* CALYXES

(Zobo calyxes)

BY

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DEPARTMENT OF CHEMISTRY

FACULTY OF PHYSICAL SCIENCES

UNIVERSITY OF BENIN

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APRIL 2024

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A PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, FACULTY OF PHYSICAL SCIENCE, UNIVERSITY OF BENIN, BENIN-CITY, IN PARTIAL FULFILMENT FOR THE REQUIREMENTS OF THE AWARD OF BACHELOR OF SCIENCE (BSc) IN CHEMISTRY.

APRIL 2024

CERTIFICATION

This is to certify that this project was carried out by Ozeh Onoriode Sonia, PSC1908690 of the Department of Chemistry, Faculty of Physical Science, University of Benin, Benin City, Nigeria.

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DEDICATION

This project is dedicated to Almighty God. And to the loving memory of my dad, Mr. Benard Ozeh, whose guidance and wisdom continue to inspire me to reach new heights.

ACKNOWLEDGEMENT

My earnest appreciation goes to God Almighty and also to my project supervisor Dr. O. Iyekowa, for his invaluable materials and academic support during the course of this study. I also appreciate my course advisers, especially my 400L course adviser, Dr. A. Aiwonegbe, for his leadership during my stay in the university.

With deep sense of respect, I also wish to acknowledge the invaluable role of the head of department, Prof. Julius Iyalsele and other lecturers in the department for their wonderful support and dedication to their passion.

Special appreciation to my loving mother Mrs. Felicia Ozeh for her immense support, understanding, the struggle she went through to make this milestone a reality and her unconditional love toward me.

I feel a deep sense of gratitude to Dr. Thompson, Mr. Muyor, Aunty Sally, and my entire family for their involvement, sacrifices and concerns towards my academics.

I am also grateful to my friends Prestige, Faith, Iyanuoluwa, Abel, Emmanuel and my project colleagues, Loveandpeace, Peter, Lucky, Marvelous, John and others for their immense support, dedication and cooperative collaboration during this research work. God bless you all.

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ABSTRACT

The study focused on the extraction, phytochemical screening, isolation, and characterization of Hibiscus Sabdariffa. The cold extraction was conducted to extract the plant sample using methanol as solvent. The crude extract was concentrated in a rotary evaporator. A portion of the extract was screened for phytochemicals while the other portion was subjected to vacuum liquid chromatography (VLC) , using solvent systems of varying polarity ;from Hexane, Ethyl acetate , Methanol ,and Butanol of increasing polarity respectively .A Brown precipitate was obtained after the concentration of Butanol extract and characterization was done by High Performance Liquid Chromatography (HPLC). Saponins, alkaloids, and terpenes were detected. However, from the HPLC myrtilin, Linalool, lycopene, where present amongst others. The study shows that H. Sabdariffa contains useful phytochemicals which are implicated as medicinal uses.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Plants have been known to humans since the beginning of time, and they have served a wide range of functions throughout history. Early humans learned to distinguish between plants that could be used medicinally and those that had specific pharmacological effects in their quest for food and efficient pain relief. Many plants are now used as medicines as a result of the growing relationship between humans and plants. As our understanding of how to treat illnesses grew, so did traditional medicine still being used by about 80% of the world's population, our knowledge of how to treat illnesses has expanded along with the number of novel medications that have been developed. These medications are an essential part of human health care systems in many regions (Aschwandanden, 2001) and claim that it is a vital component of the global biodiversity (Lambert *et al*., 2005). Eighty percent of the world's population lives in developing countries, where over four billion people mostly rely on plant-based medications (Bodeker, 2005) and (Ekor, 2014). Conventional medicine is practiced not only in developing countries but all over the world. Over the past 20 years, industrialized countries have seen a significant increase in the use of herbal medicine and natural therapies (Van and Carvalheiro, 2013). Many pharmaceutical products contain other semi-synthetic

pharmacologically active substances that are produced by pharmaceutical companies using medicinal plants as raw material (Cragg and Newman, 2005). Thirty percent of medications sold globally are made up of substances originating from plants (Calixto, 2019). Though mostly used as a vegetable, Hibiscus sabdariffa, or Roselle, has also long been used medicinally, particularly as an alternative therapy (Tahmina, 2021). Before modern medicine was developed, traditional medicine was the application of medical knowledge that had been passed down through generations in various societies (WHO 2008). It is referred to in different countries as alternative, complementary, or indigenous medicine.

Traditional medicine is defined by the World Health Organisation (WHO) as "the whole of the knowledge, skills, and practices based on theories, beliefs, and experiences indigenous to different cultures, whether explicable / not, used in the prevention, diagnosis, improvement, or treatment of physical and mental illness." Traditional knowledge, which included medical applications, was developed over generations in a variety of societies before the advent of modern medicine. It is known as alternative medicine, complementary medicine, or indigenous medicine in many other countries.

1.1.1 BACKGROUND OF STUDY

Many different types of plants with therapeutic qualities are referred to as medicinal plants. These plants are a rich source of chemicals that can be utilized to create new drugs, according to (Rasool, 2012). Different kinds of seeds, roots, leaves, fruit, skin, flowers, or even the entire plant are among the parts of medicinal plants that can be used. Most medicinal plant parts contain active compounds that are used as medicinal agents because they have direct or indirect therapeutic effects. Certain substances, known as active compounds, are produced and stored in the bodies of these plants and have physiological effects on living things (Philipson, 2001). Medicinal plants are used as raw materials to make medications that provide people with reasonable and effective healthcare. However since the human body is unable to produce phytochemicals, which are good for our health, all plants produce them (Martinez *et al.,* 2008). Furthermore abundant in nutrients that are essential for sustaining a healthy body are vitamins, minerals, and biomolecules found in plants. It has been noted that the metabolites found in many plants give them pharmacological effects. Plant metabolites are organic substances that fall into two categories: primary and secondary metabolites. Organic substances known as primary metabolites, which are good for the body's growth and development, include glucose, starch, polysaccharides, protein, lipids, and nucleic acid. Secondary metabolites produced by plants include tannins, alkaloids, flavonoids, saponins, terpenoids,

steroids, glycosides, and volatile oils, among others. Plants are highly effective in treating various diseases due to the presence of secondary metabolites. Phytochemicals are substances with pharmacological activity. Among these are alkaloids, which have diuretic, antispasmodic, antimalarial, and analgesic properties; terpenoids, which have antiviral, anthelmintic, antibacterial, anticancer, and antibacterial, anti-inflammatory properties; glycosides, which have been shown to have antifungal and antibacterial properties; phenols and flavonoids, which have been shown to have antioxidant, anti-allergic, and antibacterial properties; and saponins, which are said to have anti-inflammatory, antiviral, and plant defense activities. Extracts of hibiscus (Hibiscus sabdariffa) contain a high number of polyphenolic compounds, such as anthocyanins, protocatechuic acid, and other polyphenols, significantly reducing potential.

1.1.2 STATEMENT OF PROBLEMS

Hibiscus Sabdariffa is an essential herbaceous plant especially the Calyces which is beneficial for numerous health issues such as high blood pressure, laxatives, and reduction in sugar and fats in the blood. The possible Economic problem of this plant is the regulation of the other substituents used in making the soft drink majorly known as zobo drink which usually results in chest burn after consumption, and the experimental problem is the characterization of the sum of numerous constituents of zobo leaves.

1.1.3 RELEVANCE OF STUDY

Due to the rich profile constituents of Hibiscus Sabdariffa, a significant experimental study was carried out on the phytochemical determination and characterization of the ethyl acetate extract of Hibiscus Sabdariffa.

1.1.4 SCOPE OF STUDY

The scope of this work covers the Isolation and examination of Hibiscus sabdariffa with different solvents using methanol, water, hexane, and ethyl acetate ranging from polar to non-polar.

1.1.5 AIM OF STUDY

The aim of this study is to isolate and characterize the extract of ethyl acetate of Hibiscus Sabdariffa.

1.1.6 SPECIFIC OBJECTIVES

To achieve this aim, some specific objectives were put into consideration;

1. Collection, drying, and pulverizing of Hibiscus Sabdariffa (Roselle).
2. Extraction of the powdered sample of Hibiscus sabdariffa (Roselle) leaves.
3. phytochemical screening of Roselle leaves.
4. Isolation of Roselle extract with different solvents.

5. Chromatographic extraction of Roselle extract.
6. Characterization of the ethyl acetate fraction of the plant.

1.2 LITERATURE REVIEW

The genus Hibiscus and family Malvaceae comprise the perennial shrub known as hibiscus. There are over 275 species in the genus Hibiscus, which are extensively dispersed throughout the globe and are indigenous to tropical and South Asian regions. The majority of hibiscus species are grown as decorative plants (Lowry, 1976). All year long, they produce spectacular flowers with brilliant colors. There are several cultivars with single or double flower colors peach, red, white, pink, and orange (Gilman, 1999). The morphology of flowers indicates that hummingbirds and sunbirds, which are drawn to blooms that produce nectar, are responsible for pollination.

SPECIES OF HIBISCUS

The most common and few species of Hibiscus will be outlined below:

◆ HIBISCUS ROSA SINENSIS:

India is probably where hibiscus rosa-sinensis originated. Old Moorish (Arab) people think it came from Spain. Many others contend that Hibiscus rosa sinensis is not a natural herb but rather a group of artificial hybrids. The term "hibiscus," which means "white" or "marshmallow," is the root of the word "hibiscus," which comes from greed. (Khan *et al*., 2017). A perennial shrub

with tap roots is called Hibiscus rosa-sinensis. The dimensions of its leaves range from 2 to 5 cm in length and 3 to 12 cm in width. The leaves are just lanceolate or ovate, whole at the base and coarsely serrated at the edges or tip. Flowers are pentamerous, complete, pedicillate, and actinomorphic. Corolla, which is three inches in diameter and has five petals, (Rao *et al*., 2014). There are several kinds with varying corolla sizes and colors. Fruit is a 3-centimeter-long capsule that occurs very infrequently (Ross, 2003). The ideal growing conditions for Hibiscus rosa sinensis are slightly acidic, well-drained soils. It uses completely decomposed organic matter in sandy soils to keep the soil aerated, drain properly, and retain its water-holding capacity. Full daylight is necessary for plants since partial light prevents them from blossoming. They can, however, withstand some shade.

- HIBISCUS SABDARIFFA:

A member of the Malvaceae family, Hibiscus sabdariffa is sometimes referred to as roselle and is a nutritious and therapeutic herb. It's an annual summer shrub that grows straight and usually branches out. It has a deeply piercing taproot. Its huge, short-peduncled blooms have a dark center, and its leaves range in color from green to crimson (Abou-Sreea *et al*., 2022). The reddish-brown, dried petals (calyces) of Hibiscus sabdariffa are used to manufacture the aqueous extract called zobo. Originating in Malaysia and India, the plant is widely farmed in numerous tropical countries in both hemispheres (Ogundapo

et al., 2014). is an erect, annual shrub that has branches at the base of its reddish or green, mostly unbranched stem. With little tubercles, the stem is either glabrous or somewhat hairy. The leaves are prickly; the upper ones are palmately 3-5 lobed, while the lower ones are oblong and undivided (Qi *et al*., 2005). The calyx is connected at the base with the epicalyx, and the enormous yellow blooms with a dark crimson eye. The fleshy capsules are ovoid-shaped and contain numerous seeds (Juhi and Ela, 2014).

◆ HIBISCUS SYRIACUS:

Numerous colloquial names for H. syriacus differ by location, region, and nation. Although the lovely name "Rose of Sharon" was probably given for the same reason, it is now believed to have Chinese origins. Another name for it that's used in some places is Shrubby Althea. With stunning solitary or double blooms in shades of white, blue, or mauve, this plant reaches a height of about 2.7 meters. Similar to hollyhocks, they emerge in the leaf axils. Like other Hibiscus species, it has been widely planted, even up to Ontario, and given a variety of descriptive names (Cowen, 1950). H. syriacus is a significant species in the genus that is utilized in beverages in Asian nations and has significant commercial and medicinal value (Kumar and Pandey, 2011). The height of the shrub or small tree is 4 meters. Stems: upright or ascending, sparsely to moderately hairy in youth, globous or almost so as they get older. The petiole of leaves has abundant hair on the adaxial side. Inflorescence in the axil of the

distal leaves with single flowers or a few blooms in clusters. Flowers on a horizontal, ascending, or occasionally double pedicel A pair of 0.9-2.2 cm long, minutely, thickly, stellate-hairy epicalyx bracts, measuring 1.5 cm in length (Tropicos, 2021).

1.2.1 TAXONOMY/CLASSIFICATION OF HIBISCUS SABDARIFFA

Botanical classification (Osman, 2011)

Kingdom: Plantae (Plants)

subkingdom: Tracheobionta (Vascular plants)

super division: Spermatophyta (Seed plants)

Division: Magnoliophyta (Flowering plants)

class: Magnoliopsida (Dicotyledons)

subclass: Dilleniidae

order: Malvales

Family: Mavaceae (Mallow family)

Genus: Hibiscus L. (Rosemallow)

Species: Hibiscus sabdariffa

1.2.2 BOTANICAL CLASSIFICATION OF HIBISCUS SABDARIFFA

A lovely annual or perennial herb is Roselle. This native edible medicinal plant is a dicotyledonous herbaceous shrub. It has a deep-penetrating tap root and can reach heights of 2.4 metres or more (Alarcon et al., 2007). Its smooth cylindrical stems range in colour from dark green to red (Maganha et al., 2010). The leaves are 7.5–12.5 cm long, alternating, green, and have long petioles and reddish veins. According to Mahadevan et al. (2009), the flowers are hermaphrodites and are pollinated by insects.The red calyx of the plant is made up of five sizable sepals, an epicalyx, and bracteoles surrounding the base (Heinrich et al., 2014).When the capsule ages and dries out, it splits open and turns brown. Every capsule has 22–34 kidney-shaped seeds that range in length from 3 to 5 mm, are coloured from light to deep brown, and have tiny, thick hairs covering them (Riaz et al., 2018).Acidic extracts can be found in the calyx, stems, and leaves (Heinrich et al., 2014).

1.2.3 MEDICINAL USES OF HIBISCUS SABDARIFFA

According to (Luvonga *et al.,* 2010), the roselle plant has 68.7% carbohydrates, 14.6% crude fiber, 12.2% ash, and other nutrients in their study. Numerous minerals, including magnesium and potassium, are abundant in the plant. Notable concentrations of vitamins, such as pyridoxine, niacin, and ascorbic acid, were also found. (Nmahadevan and Pradeep, 2009) documented the physicochemical components of H. sabdariffa's fresh calyxes and leaves and variable content. The herb is purportedly useful as a purgative, antihypertensive, antibacterial, astringent diuretic, and therapy for cancer, abscesses, cough, debility, dysuria, scurvy, and fever (Olawale, 2011). Usually sweetened with sugar to taste, the extract is also sometimes flavored with artificial tastes like strawberry vanilla or pineapple juice, or with natural flavors like lime juice and pineapple juice, as well as spices like ginger hot pepper. Usually, individual tastes dictate how sweet the extract is (Ogundapo *et al.*, 2014).

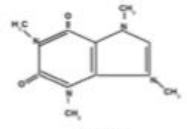
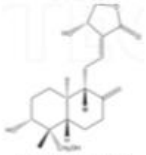
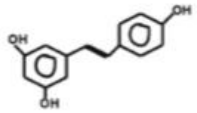
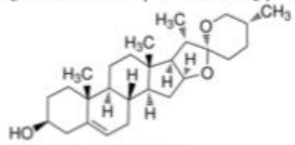
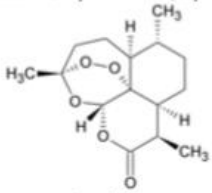
According to reports from past research, Hibiscus sabdariffa has a lot of ascorbic acids (Aganbi *et al.,* 2017). H. sabdariffa is low in sugar and rich in calcium, iron, riboflavin, niacin, and other nutrients, according to (Builders *et al.,* 2010) and (Teye *et al.,* 2019). The claimed medicinal advantages of H. sabdariffa are believed to be caused by the presence of anthocyanins, a colored byproduct of the flavonoid pathway. The anthocyanins found in H. sabdariffa have been shown to have antioxidant activity, which offers protection against

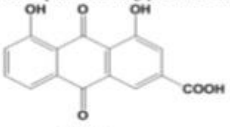
atherosclerosis and cancer. They also have something to do with better effect on cholesterol and liver preservation. According to (Carvajal-Zarrabal, 2012), there is evidence that the antioxidant capacity of this particular species is significantly more active than average, suggesting that differences within the same species are produced by the sort of soil effect on its ash and mineral content. For a long time, liver damage, pyrexia, and hypertension have been treated with this herbal tea, even though the pharmacological components are unclear.

1.3 PHYTOCHEMICAL (BIOACTIVE CHEMICAL) CONSTITUENTS IN MEDICINAL PLANTS

Plants (Hibiscus Sabdariffa) synthesize secondary metabolites which include alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, volatile oils, etc. Phytochemicals can be classified based on their chemical composition.

Table 1.1: Classification of phytochemicals

S. No.	Phytochemicals	Chemical structure	Example
1.	Alkaloids	Nitrogen atom in heterocyclic rings  Caffeine	Morphine, caffeine, Berberin, codeine [3]
2.	Glycosides	Derived from carbohydrates and noncarbohydrates molecules.  Andrographolide	Amygdalin, gentiopicrin, and rographolide, polygalin, Cinnamyl acetate [4]
3.	Polyphenoles (Flavonoids, Phenolics Tannins)	Aromatic aliphatic ring containing phenols  Resveratrol	Quercetin, reservaratrol kaempferol and quercitrin, caffeic acid, flavones, rutin, naringin, hesperidin and chlorogenic, tannic acid, gallic acid and ellagic acid [5]
4.	Saponins	Sugar attached to triterpene or steroid aglycone  Diosgenin	Diosgenin and hecogenin [6]
5.	Terpenes (Carotenoids, steroids)	Long unsaturated aliphatic chains (isoprene units)  Artemisinin	Artemisinin, α-carotene, β-carotene, lycopene, lutein and zeaxanthin [7]

6.	Anthraquinones	Derivatives of phenolic and glycosidic compounds  Rhein	Rhein, salinos poramide and luteolin [8]

1.3.1 ALKALOIDS

Alkaloids are organic chemicals that are found in nature. They are primarily found in plant sources, such as sea algae, and are sometimes found in animals, such as the toxic secretions of ladybugs, fire ants, and toads. They are primarily found in berries, bark, fruits, roots, and leaves of plants that produce seeds. Alkaloids frequently have a heterocyclic ring with at least one nitrogen atom. Since they are fundamental in nature, they are referred to as alkaloids (alkali-like). Alkaloids have a remarkable physiological effect on animals, including humans. These are the active ingredients in many therapeutic plants and medications made from plants. Their distinct physiological actions and diversity of structure set them apart from other groups of natural products. Numerous substances utilized by humans for both therapeutic and recreational purposes are found in nature as alkaloids, such as strychnine, atropine, caffeine, nicotine, morphine, codeine, cocaine, and so forth. Many alkaloids have also been found to have naturally existing receptors in humans and other animals, indicating that alkaloids may have evolved to play a role in physiological processes. Alkaloids are end products of several biosynthetic routes, most of which begin with common amino acids like lysine, ornithine, tyrosine, and tryptophan. They are relatively stable molecules. Although most of these compounds are colorless, several colored alkaloids have also been recorded, such as the yellow color of berberine, the copper-red color of sanguinarine salt, and the red color of

betanidin (Kokate *et al*., 2005). These are crystalline solids with a bitter flavor, a ring structure, and distinct melting points. They can be found in plants in a free state, as salt or N-oxides, and are infrequently found as glycosides (Biswas and Sharia, 1978; Tanahashi *et al*., 2000; Kashidaba *et al*., 2000). Most alkaloids contain oxygen in addition to carbon, hydrogen, and nitrogen. Some are oxygen-free, such as nicotine from tobacco and cocaine from hemlock. With their salts, however, this is frequently not the case; for example, strychnine hydrochloride is more soluble in organic solvents than in water. The free bases are sporadically soluble in water but readily soluble in organic solvents. The majority of alkaloids have optical activity, usually because of the presence of tertiary nitrogen in their structures.

CLASSIFICATION OF ALKALOIDS

True alkaloids: These alkaloids share a heterocyclic ring containing nitrogen and are derived from amino acids. They have strong biological activity and are quite reactive in the environment. They combine with acid to generate water-soluble salts, many of which are crystalline in character. Except for nicotine, which is a dark liquid, almost all real alkaloids have a bitter taste and are solid (Aniszewski, 2007).

- Pseudoalkaloid:

This type of alkaloid contains a nitrogen atom, which is derived from an amino acid but is not part of the heterocyclic ring system. L-tryptophan and l-

tyrosine are the main precursors of this type of alkaloid. This minor group is structurally composed of simple alkaloids. Yohimbine, mescaline, and hordenine are the main alkaloids of this type. They are used in various health disorders, including mental illness, pain, and neuralgia (chini *et al.,* 1992). In

- Pseudo alkaloid:

Pseudo-alkaloids' basic carbon skeleton is not derived directly from amino acids; rather, they are linked to amino acid pathways through an amination or transamination reaction with precursors or postcursors of amino acids (Dewick, 2002; Jacobke and Jeschkeit, 1992). Precursors that are not amino acids can also yield pseudo-alkaloids. Acetate or phenylalanine can be the source of them. Common examples of pseudoalkaloids are capsaicin, caffeine, and ephedrine.

1.3.2 GLYCOSIDES

Glycosides can be alcohol, sulfur compounds, or phenol. They are distinguished by having one or more non-sugar proteins bound to sugar proteins through a unique connection. Numerous plants store compounds as inactive glycosides that can be hydrolyzed by enzymes to become active (Polt, 1995). Because they are inert until they are digested and release aglycogen, the active component, most glycosides can therefore be categorized as prodrugs. Glycosides are classified according to the type of glycogen they include, which can range from phenols, quinines, terpenes, and steroids, among other molecular types. Since

their structures vary, it is challenging to investigate these glycosides as a distinct category. The role of glycosides is crucial because they join monosaccharides to create polysaccharides and oligosaccharides (Levy and Tang., 1995; Newman *et al*., 2008).

1.3.3 SAPONINS:

Natural substances called saponins are found in large quantities in every cell of legume plants. A complex and chemically varied category of chemicals, saponins get their name from their capacity to produce stable, soap-like foams in aqueous solutions. Chemically speaking, a carbohydrate moiety is joined to a triterpenoid or steroid via saponins. Because of their many characteristics, both positive and negative, saponins are generating a lot of attention. According to clinical research, these health-promoting substances, called saponins, also have the effect of lowering cholesterol and strengthening the immune system, which helps shield the human body from cancer. Saponins diminish blood glucose response, blood lipid levels, and cancer risks. A diet rich in saponins can be used to treat hypercalciuria in humans, prevent dental caries, and inhibit platelet aggregation (John Shi *et al*., 2004). It can also be used as a countermeasure against acute lead poisoning.

1.3.4: Phenols

Secondary metabolites known as phenolic compounds are produced by plants via the pentose phosphate, shikimate, and phenylpropanoid pathways (Randhir *et al*., 2004). One of the most prevalent classes of phytochemicals, these substances have a significant impact on plant morphology and physiological processes. These substances are crucial for development and reproduction because they shield organisms from infections and predators (Bravo, 1998). Most phenolic chemicals are derived from plants and are classified as secondary products. They have an aromatic ring with a hydroxyl substituent. The chemical composition of plant phenolics varies; some are soluble only in organic solvents, while others are soluble in water as glycosides and carboxylic acids. (Taiz and Zeiger, 1991) identified insoluble polymers as another class of phenolics. 1.3.5: TANNINS

Phloroglucinol, a polyphenol present in a wide variety of terrestrial and marine plants, is known as plant tannin. For a long time, plant tannins have been added to animal production (Xiao *et al*., 2018). Proteins are bound by bitter-tasting organic molecules called tannins. The following contains tannins: wine, tea, and cannabis. Leather is also treated with tannins to increase its durability. Oak trees naturally provide tannins for this function, while synthetic tannins are more commonly utilized (Wikipedia). Plant tannins have a great affinity for protein, according to research by (Aboagye *et al*., 2019), and adding plant tannins to

ruminants in the right amounts can benefit them nutritionally. Other physiological effects of tannins have been documented, including the production of liver necrosis, lowering of serum lipid levels, acceleration of blood coagulation, and modification of immune responses. (Chung *et al*., 1998.)

1.4 Extraction: using specific solvents and a systematic procedure, extraction is the process of isolating the medicinally active combination of numerous naturally occurring active chemicals that are often found in plant material (tissues), (Handa, 2006). It can also be described as treating the plant with solvent, in which the medical components dissolve while the majority of the plant remains insoluble. The goal of any extraction process is to separate the plant's soluble chemical products while leaving the insoluble residue behind (Azwanida, 2015). The result is a rather complex mixture of components that are either liquid, semi-solid or powdered (after the water has been removed) and are intended for internal and /or external uses. The basis for extraction is the solutes's and other compound's solubility differences as well as that of the stabilizing solvent (Omeroglu *et al.,* 2019).

TYPES OF EXTRACTION METHODS

1.4.1 Maceration

To obtain plant extracts, this procedure involves soaking coarse or powdered plant materials in a solvent in a closed, stopped container. The mixture is then let to stand at room temperature for two to three days, stirring often. In order to

prevent solvent evaporation at air pressure, a sealed extractor is utilized. The goal of the procedure is to break down and soften then plant's cell walls I order to release the soluble phytoconstituents. The mixture is then pressed or strained by filtration or decantation after a specific time (Azwanida, 2015, Handa *et al*., 2008). Maceration is simplest and still widely used procedure. The extraction procedure in this stationary process works on principle of molecular ensures dispersal of the concentrated solution accumulation around the particles' surface and brings fresh solvent to the surface of particles for further extraction (Zhang and li, 2010).

Digestion

This type of maceration involves applying a low amount of heat during the extraction step of the maceration. There is increased efficiency in the use of menstruum (a solvent or mixture of solvent used for extraction), since temperature does not change the active components of plant material. It is used when the moderately elevated temperature is not objectionable and the solvent efficiency of the menstruum is increased thereby (Pandey and Tripathi, 2014). The most used temperature are between 35 and 40^{o}c, although it can rise to no higher than 50^{o}c. The plant part to be extracted is placed in a container with the pre-heated liquid to the indicated temperatures, is maintained for a period that may vary between half an hour to 24 hours, shaking the container regularly.

This process is used for the herbal materials or plant parts that contain poorly soluble substances or polyphenolic compound (Husain *et al.*, 2019).

Decoction

The current process involves boiling the plant material in water to obtain plant extracts. Heat is transferred through convention and conduction, and the choice of solvents will determine the type of compound extracted from the plant material (Azwanida, 2015). The sample is boiled in a specified volume of water for a defined time (15 to 60 minutes). It is then cooled, strained, filtered and added enough water through the drug to obtain the desired volume. This method is suitable for extracting thermostable (that does not modify with temperature) and water-soluble compounds, hard plant materials and commonly resulted in more oil-soluble compounds than maceration.

Percolation

It is conducted by passing the boiled solvent through the plant material at a controlled and moderate rate (e.g 5-7 drops per min) until the extraction is complete before evaporation. The concentrated plant extracts are commonly collected at the bottom of the vessel. To obtain a significant amount of extract, successive percolations can be performed by refilling the percolator is mostly used to extract active compounds in the preparation of tinctures and fluid extracts. Its major disadvantage is that large volumes of solvents are required,

and the procedure can be time-consuming and may require skilled persons (Husain *et al.,* 2019).

USES OF EXTRACTION

In the chemistry lab, extraction is used for several purposes. It is a key technique for separating chemicals from plant sources. Compounds are transferred from one liquid to another by extraction, making it easier to control or concentrate them. Additionally, it makes it possible to remove specific mixture components.

CHAPTER TWO

MATERIALS AND METHODS

2.1.1: MATERIALS

Rotary evaporator

weighing balance (digital)

Separatory funnel

Thin layer chromatographic plates (TLC)

Stirrer

Retort stand and clamp

wlhatman’s filter paper

Beaker

Funnel

Test tubes

measuring cylinder

silica gel

mortar and pestle

PLANT SAMPLE:

- Hibiscus Sabdariffa

2.1.2: Reagents

The reagents used are of analytical grade.

n-hexane, methanol, butanol, ethylacetate, distilled water, Sulphuric Acid(H_2SO_4), 10% Lead Acetate, 20% Sodium Hydroxide (NaOH), Dilute Hydrochloric Acid (HCL), 10% $FeCl_3$, 90% Ethanol, 5% KOH, Acetic anhydride, Dragendoff (potassium bismuth iodide salt), Wagner's reagent, picric acid.

2.1.3: EQUIPMENT

Electric blender

Heating mantle

weighing balance

Rotary evaporator

High Performance Liquid chromatography (HPLC) spectrometry

2.2: METHODS

2.2.1: collection of samples

The dried calyxes of Hibiscus Sabdariffa were purchased from uselu market at Egor local government area.

The identification of the dried calyxes was done by prof. J.J Bamidele (a taxonomist in the department of plant Biology and Biotechnology, UNIBEN).

SAMPLE TREATMENT

The dried calyxes were washed properly from dirt and air dried in the lab for 28 days and pulverized for extraction process.

2.2.3 EXTRACTION

The powdered calyxes of hibiscus Sabdariffa of about 300.18grams, was packed in a 1000ml beaker and soaked for three days with 1000ml of methanol (maceration). Filtration was done to separate out the filtrate, and concentration was done by a Rotary evaporator to get the extract.

2.3 PHYTOCHEMICAL SCREENING OF HIBISCUS SABDARIFFA EXTRACT

The phytochemical and bioactive screening of the plant Hibiscus Sabdariffa were performed using the standard procedures of Sofowora (1993), Trease and Evans (1989), as well as Odebiyi and Sofowora (1978).

2.3.1 TEST FOR GLYCOSIDES

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

2.3.2: TEST FOR SAPONINS:

0.5g of the plant extract was shaken with water in a test - tube and observed for frothing. Saponin Rein Weiss. (supplied by Merck) was used as standard.

2.3.3: Test for flavonoids

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

(i) To portion A: 10% Lead acetate solution was added in few drops. A yellowish precipitate is indicative of a positive result.

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

2.3.4 Test for phenolic compounds

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

2.3.5 Test for Tannins

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

(i) To about 2 drops of the filtrate, ferric chloride (FeCl3) solution was added; formation of a bluish precipitate is required for hydrolysable tannin.

(ii) To about 5 drops of the filtrate, 2ml dilute HCI was added and boiled for 5 minutes. Red precipitate is required for condensed tannin.

2.3.6 Test for Eugenols

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

2.3.7 Test for Steroids

2ml of acetic anhydride was added to 0.5g plant extract in 2ml of dilute H_2SO_4. A colour change from violet to blue or green is required for the presence of steroids.

2.3.8 Test for Terpenoids (Salkowski test)

5ml of each extract was mixed in 2ml of chloroform and 3mls of conc. H_2SO_4, 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

2.3.9 Test for alkaloids

Dragandoff's, Wagner's reagent and Picric acid were med to test for alkaloids. About 1ml each of the plant extract was transferred into three different lest tubes labeled A, B and C

(i) To portion A: 2mls of Dragendoff's reagent (made of a mixture of Potassium Bismuth lodide salt) was added. Reddish brown precipitate is required for a positive test.

(ii) To portion B: 2mls of Wagner's reagent was added. Reddish brown precipitate is indicative of a positive test.

(iii) To Portion C: 2mls of Picric acid was added. A yellowish precipitate is a positive test.

2.4 DETERMINATION OF RETENTION FACTOR (RF) BY THIN LAYER CHROMATOGRAPHY

A precoated TLC plates were cut to size of 8cm length by lcm width and used at varying solvent systems of methanol, N- hexane and ethyl acetate. The RF value were calculated from the distance moved by the compound against the solvent front.

2.5 ISOLATION BY VACUUM LIQUID CHROMATOGRAPHY

The methanol crude extract was mixed with silical gel (particle size 200- 425 mesh) as the solid phase and butanol as the mobile phase based on the RF value of . A light yellow phase obtained was concentrated to recover the pure isolate. The weight of the oily substances was obtained to be **0.36g.** The isolated oil was further sent for analysis using HPLC.

CHAPTER THREE

RESULT AND DISCUSSION

3.0 Results

3.1 Extraction

The result of the percentage yield and color of the extract are shown below;

Mass of powdered sample =300.18g

Mass of extract= 53.36g

percentage yield =mass of extract /mass of powdered sample. * 100

53.36g/300.18g * 100 = 17.78%

3.2 PHYTOCHEMICAL SCREENING OF THE PLANT EXTRACT

CONSTITUENTS	TEST	Butanol extract
GLYCOSIDES	GENERAL TEST	+
SAPONINS	Frothing	+
PHENOLICS	Ethanol/Ferric chloride test	-
ALKALOIDS	Purim acid	+
TERPENOIDS	Salkowski Test	+
STEROIDS	Acetic acid /H2SO4 Test	-
EUGENOL	Ethanol /Ferric chloride Test	+
FLAVONOIDS	Lead Acetate	+
TANNINS	Ferric chloride	+

+ = present

- = Absent

3.3 THIN LAYER CHROMATOGRAPHY

TLC is a chromatographic technique used tin the desperation of constituents in an extract. For this to be done, the Retention factor (Rf) values are calculated and these give direction to air. It is the best isolation method of a particular component in an extract.

Rf = Distance moved by compound / Distance moved by solvent

This was done using

- Methanol Extract
- 100% Methanol
- 100% Hexane
- 1:1 (Methanol , Ethyl acetate)
- 100% Butanol
- 100% Eyhyl acetate
- 70:30 (%) Hexane / Ethyl acetate

solvent sample: Hibiscus Sabdariffa

Table 1: Rf and color reaction of Hibiscus Sabdariffa in 100 % methanol.

Origin	Colour	Solvent dist/ TLC
0.0	Red	0.00
3.0	purple	0.395
4.5	Light yellow	0.563

Table 2: Rf and Colour reaction of Hibiscus Sabdariffa in 100% Hexane

Origin	Colour	Solvent dist / TLC
0.0	Red	0.0
0.5	Green	0.5/ 8=0.0.625
1.4	Light purple	1.4/ 8=0.175

Table 3: Rf and Colour reaction of Hibiscus Sabdariffa in 50:50 (%) Methanol/ Ethyl acetate

Origin	Colour	Solvent dist / TLC
0.0	Red	0.0
4.0	Dark purple	4/8= 0.5
5.0	Light yellow	5/8= 0.625

Table 4: Rf and Colour reaction of Hibiscus Sabdariffa in 100% Ethyl acetate

Origin	Colour	Solvent dist/TLC
0.0	Red	0.0
2.5	Light yellow	2.5/ 8= 0.3125

Table 4: Rf and Colour reaction of Hibiscus Sabdariffa in 70:30(%) Hexane / Ethyl Acetate

Origin	Colour	solvent dist/ TLC
0.0	Red	0.0
0.5	Light yellow	0.5/ 8=0.0625

Table 5: Rf and color reaction of Hibiscus Sabdariffa in 100 % Butanol

Origin	Colour	Solvent dist/ TLC
0.0	Red	0.00
4.0	Dark purple	0.495
5.5	Dark yellow	0.663

3.4 HPLC RESULTS

Table 6 HPLC profile of Butanol extract of Hibiscus Sabdariffa

Name of Compound	Retention time	Peak area
BETA CARYOPHYLLENE	1.266	562.3820
GALIC ACID	2.750	1706.1570
GAMMA –TOCOPHEROL	4.450	527.0050
CATECHIN	5.466	124.5610
PROTOCATECHUIC ACID	6.483	204.8600
B-IONONE	7.033	71.9155
PHYTOENE	7.950	100.7250
LYCOPENE	9.350	87.7320
MYRTILLIN	12.166	2033.0015
LINALOOL	13.700	934.3970
DELPHINIDINE	14.600	305.7540
DAPHNIPHYLLINE	15.700	294.0955
TETRAHYDROXYCHALCONE	17.616	454.0275
SABDARETINE	19.683	87.9300
CHRYSANTHENIN	20.233	75.9640
HIBISCETINE	11.050	7069.9670

DISCUSSION

The dried calyxes of Hibiscus Sabdariffa underwent cold extraction in 1000ml of methanol, and concentration was done too by the Rotary evaporator at 50•c. The Calculated percentage yield of the concentrated crude extract, was found to be 17.78%.

The phytochemical constituents present in the plants, are responsible for its antimicrobial, anti-fungi, antioxidants activities. Isolation was done to on the secondary metabolites (phytochemical constituents) and identification of its polarity was possible. The phytochemical Screening of Hibiscus Sabdariffa proved to contain Glycosides, Terpenoids, flavonoids, Eugenols, saponins and to not contain steroids and Phenolics.

TLC Results showed Rf values in favor with 100% Methanol solvent system due to polarity, and the reverse for 100% Hexane due to its unpolar nature. The Rf values calculated for the solvent systems showed its highest value for Methanol and its least value for Hexane. This is done by dividing the distance moved by compound, over the distance moved by solvent. The polar nature of the extract is the major factor, because it causes a more attraction with the mobile methanol phase, and least attraction with the Hexane mobile phase, affecting the Retention time along the stationary phase.

HPLC Results showed abundance in Hibiscetine, Myrtillin, Linalool, Beta - Caryophyllene, with Hibiscetine being the most present.

Hibiscetine is one of the major flavonoids found in Hibiscus Sabdariffa, its phytocconstituents have been found to contain anti-Oxidant activities against free radicals that causes oxidative stress in the body.

Linalool are natural terpenes alcohols found in flowed and spice food. It uses in manufacturing industry (soap, household products, food adictives and insecticides) due to its fragrance and its antimicrobial properties (Wikipedia*).*

Myrtillin is an anthocyanin (water - soluble flavonoids) .it is a 3 - glycoside of delphinidins found in green plants, black currants, etc., and are very potent antioxidants (*Wikipedia).*

CONCLUSION

Hibiscus Sabdariffa is a peculiar medicinal plant with numerous pharmacological uses. This peculiarity is attributed to secondary metabolites such as glycosides , Eugenols, Alkaoids, Flavonoids , which are present in the plant extract . More so, sabdaretine an alkaloid was also detected by the HPLC and this suggest the plant extract is a medicinal plant.

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