EVALUATION OF THE CHEMICAL COMPOSITION AND PHYTOCHEMICAL CONSTITUENTS OF TIGER NUT (*Cyperus esculentus*)

BY

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DEPARTMENT OF CHEMISTRY

FACULTY OF PHYSICAL SCIENCES

UNIVERSITY OF BENIN

APRIL, 2024.

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

APRIL, 2024.

CERTIFICATION

This is to certify that **Oghenero Faith Emumejakpo** an undergraduate student in the Department of Chemistry, Faculty of Physical Science, University of Benin, Edo state, with Matriculation Number **PSC1908734** satisfactorily completed this project work on her own as a partial fulfillment of the requirement for the award of Bachelor of Science Degree (B.Sc. honours) in Chemistry.

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DR. M.I. IMAFIDON (PROJECT SUPERVISOR)	DATE
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PROF. J.U.IYASELE. (HEAD OF DEPARTMENT)	DATE

DEDICATION

This project is dedicated to God for His mercy and protection, for the knowledge he has enabled me to acquire and for making this research work a success. Also, to my parents, Mr. and Mrs. Emumejakpo and my beloved siblings.

ACKNOWLEDGEMENT

My sincere gratitude goes to God Almighty for giving me the morale, courage and enthusiasm to embark on and complete this project work and for making my academic journey a success.

I wish to appreciate the University of Benin for giving me this great opportunity. My sincere appreciation goes to the Head of department, Prof. J.U. Iyasele and my lecturers for the knowledge imparted. Special thanks to Mr. Clinton for his immense assistance during my laboratory work.

It is pertinent at this juncture to appreciate the efforts of my project supervisor Dr. M.I. Imafidon for the thorough supervision, corrections and support towards making this work a success.

I sincerely appreciate the efforts of my parents Mr. and Mrs. Emumejakpo for bringing me up morally and academically. I must register my profound gratitude to my parents for their guide, moral and financial supports.

I am grateful for my siblings, Sarah, Joseph, David and Uncle Daniel who helped me in one way or the other throughout my undergraduate studies.

And to my dear friends and course mates, Daniel, Faith, Lucky, Sylvia, John, David, Glory, Phydeyliah who made my academic journey fun and enjoyable, thank you all. You’re one more reason for my joy.

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ABSTRACT

This study evaluated the chemical composition and phytochemicals present in tiger nuts as a potential alternative to synthetic drugs with side effects. The result for proximate analysis of tiger nut (*Cyperus esculentus*) showed carbohydrate 50.89±0.25, crude fibre 7.50±0.29, crude protein 4.57±0.08, ash 3.00±0.29, crude fat 26.53±0.18 and moisture 7.50±0.29. From the result, tiger nut contains high amount of carbohydrates and low moisture, indicating a good shelf life. Mineral analysis identified high levels of potassium (2565 mg/kg) and phosphorus (4914 mg/kg), along with significant amounts of magnesium (52 mg/kg) and iron (190 mg/kg). The phytochemical screening of tiger nut confirmed the presence of various bioactive compounds like glycosides, alkaloids, saponins, phenols, eugenols, terpenoids, and flavonoids, indicating potential health benefits. The absence of tannins and steroids suggests minimal anti-nutritional factors. These findings provide valuable data on the nutritional and bioactive profile of tiger nuts, supporting their exploration as a natural source of health-promoting compounds.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Tiger nut "*Cyperus esculentus Lativum*" belongs to Cyperaceae and it is known by other names like chufa, yellow nutsedge, earth almond and ground almond. This perennial crop is widely grown throughout Asia, East Africa, the Arabian Peninsula, and portions of Europe, especially Spain(Abdelkader *et al*., 2017). Tigernuts come in three varieties: brown, black, and yellow. The functional and physico-chemical characteristics of the different tigernutsvaries(Nina *et al*., 2019). Tigernut's rheological and functional qualities are significantly influenced by its moisture content(Gasparre and Rosell, 2019). Tigernut is an underutilized tuber rich in many critical elements like proteins, carbs, vitamins, minerals(Mohdaly, 2019), phytochemicals, oil and fiber(Ihenetu*et al*., 2021). Tiger nuts appear to have more potential use as food and industrial materials; they can be used to make milk or yogurt, flour, jam, beer, chocolate, edible oil, and soaps, among other products(Achoribo and Ong, 2017). Moreover, it defends the internal processes and prevents constipation and diarrhea(Maduka and Ire, 2018). Numerous nutritional and health benefits of the tuber have been observed(Bazine and Arslanoğlu, 2020).

1.1.1 BACKGROUD OF STUDY

Numerous plants are known to provide the nutrients and phytochemicals required for humans to live healthy lives. Researchers have shown that eating foods high in nutrients can improve health and lower the risk of developing certain conditions like diabetes, cancer, heart disease, and arthritis. It can also strengthen the immune system's ability to fight against infections and illnesses. The little tubers known as "tiger nuts" (*Cyperus esculentus*) have drawn interest lately because of their many culinary applications and possible health advantages. The tiger nut is a type of plant belonging to the cyperaceace family of sedges, which also includes spike rushes and bulrushes. It is a traditional food that is consumed throughout the world and provides essential nutrients. The distinctive composition of tiger nuts, which includes a high fiber content, lipids, and different bioactive components, is well-known. Because of the numerous minerals, vitality, and oleic acid it contains, tiger nuts have been utilized as a healthy plant for 4,000 years. In addition to having a lot of carbohydrates, primarily sucrose and starch, it also contains arginine, which is highly concentrated and releases the hormone that makes insulin(Bamishaiye and Bamishaiye, 2011).Tiger nuts have a number of nutritional and physiological benefits, including helping the body meet daily mineral requirements for potassium, phosphorus, and magnesium as well as their anti-inflammatory and antioxidant qualities. Because to its high content of phosphorus, potassium, calcium, magnesium, and iron, tiger nut tubers are beneficial for blood circulation, muscles, bones, tissue regeneration, and overall body growth (Mohdaly, 2019). Apart from its health advantages, tigernut tubers are known to contain 20–36% oil, which makes them a viable oil crop for the manufacture of biodiesel. The oil of the tuber was discovered to contain 18% saturated acid (palmitic and stearic acid) and 82% unsaturated acid (oleic and linoleic acid)(Zhang *et al.*, 1996).While it is shade-intolerant, the tiger nut plant can withstand high soil moisture levels. It grows best in moist sandy-loam soils, but it can also survive in the toughest clay(Bamishaiye and Bamishaiye, 2011). This study aims to assess the chemical and nutritional value of tiger nuts.

1.1.2 STATEMENT OF PROBLEM

Eating natural plants can help reduce the prevalence of health problems that affect both young and old individuals, such as obesity, heart disease, paralysis, cancer and mortality. In Nigeria, tigernuts are a well-liked and nutrient-dense tuber with a wide range of traditional applications. Tiger nuts are frequently used in Nigerian traditional medicine to aid with digestion, ease stomach aches, and occasionally to provide medication to patients without their prior knowledge of the components involved. As a chemist, studying plants is essential to ascertaining the general chemical makeup that confers health benefits and, consequently, to suggest strategies to reduce or eradicate the growing incidence of health problems

1.1.3 JUSTIFICATION/RELEVANCE OF STUDY

Some synthetic drugs have been banned from usage due to adverse effects accompanied with them after administration or prolonged usage. These drugs are meant to prevent or treat the diseases they are synthesized for but they go further to cause other health problems for the user. It has been proven that natural plant especially medicinal plants are as effective as synthetic drugs in preventing and combating these chronic diseases with little or no side effects. This research will investigate the nutrients content as it relates to the health benefits of tiger nut.

1.1.4 SCOPE OF WORK

Tiger nuts were bought from New Benin Market in Benin City, Edo State, Nigeria and were identified with herbarium number of UBH-C419 on April 9th, 2024 by Dr. H.A.Akinnibosun of the department of Plant Biology and Biotechnology, University of Benin. The tiger nuts were visually inspected, defective ones were removed manually and it was washed with clean water. They were sun-dried for five days and milled in a milling machine, sieved with sieve material to obtain a homogenous product (flour) which was kept in an air-tight container prior to further analysis i.e phytochemical, mineral and proximate analysis, in the laboratory.

1.1.5 AIM AND OBJECTIVES

AIM: The aim of this study is to evaluate the proximate, mineral elements and phytochemical constituents of tiger nut.

OBJECTIVES: The objectives of this study are to:

1. determine the proximate composition of tiger nut.
2. investigate the phytochemicals in tiger nut.
3. investigate the mineral content of tiger nut.

1.2 LITERATURE REVIEW

1.2.1 DESCRIPTION OF TIGER NUT PLANT

The tiger nut is an annual or perennial plant with stems that emerge from a tuber and reaches a height of 90 cm (3 feet).The plant is reproduced via seeds, creeping rhizomes and tubers(Stoller, 1981). Due to the clonal nature of the plant, it can take advantage of soil disturbances that is generated by either anthropogenic or natural sources(Renne and Tracy, 2006).The plant's stems have a triangular form, and the thin, 3–10 mm wide leaves are bearing them. The plant's spikelet is unique in that they have four dangling leaf-like bracts spaced 90 degrees apart, encircling a cluster of flat, oval seeds. They have 8–35 florets, are linear to narrowly elliptic, and are 5–30 mm long. The color shifts from straw to a golden-brown hue. Each plant has the capacity to yield up to 2420 seeds. The plant's incredibly tough and fibrous foliage is sometimes confused for grass. The roots are a tangled network of basal bulbs and small, spherical tubers linked to scaly rhizomes and thin roots. According to(Army, 2009), the tubers range in diameter from 0.3 to 2.5 cm and come in a variety of colors, including yellow, black, and brown. A single plant can yield hundreds to thousands of tubers in a single growing season. The tubers endure and resurface in the next spring as long as the soil temperature stays above 6 °C (43 °F). On the other hand, cold weather damages the basal bulbs, rhizomes, leaves, and roots(Stoller, 1981). As soon as the tubers germinate, several rhizomes emerge and grow to a basal bulb near the soil's surface. These basal bulbs serve as beginning places for the formation of above-ground stems and leaves, while simultaneously giving rise to fibrous roots below the surface. Because C. esculentus is self-incompatible, it requires cross-pollination and is dependent on wind pollination.

Figure 1.1: Pictorial representation of tigernut plant

Figure 1.2: Tiger nut tuber

1.2.2 CLASSIFICATION/TAXONOMY OF TIGER NUT

Kingdom— Plantae

Subkingdom —Tracheophytes

Super division — Angiosperms

Division — Magnoliophyta

Class — Liliopsida

Subclass — Monocotyledonae

Order — Cyperales

Family — Cyperaceae

Genus — Cyperus

Species — Cyperus esculentus

1.2.3 HISTORY OF TIGERNUT

Dating back to roughly 16,000 BC, wild chufa roots, a vital food source, were unearthed at Wadi Kubbaniya, north of Aswan, making Cyperus esculentus one of the earliest cultivated plants in prehistoric and Ancient Egypt. Cyperus esculentus was one of the first plants to be domesticated and was a major food(Nesbitt, 2005). Around 3000 BC, dry tubers also show up later in Predynastic graves. At that time, people ate tiger nut tubers roasted, cooked in beer, or as sweets made from crushed tubers and honey(Negbi,1992). During the Dynastic era, tigernut cultivation was limited to Egypt, despite the plant remaining a significant food source. At the tomb of the 15th-century BCE vizier Rekhmire, peasants were observed preparing and weighing tiger nuts to make votive cakes as sacrifices to the deity Amun(Miller, 2021).

The popularity of tigernuts has currently spread from Spain to the US, France, UK, Portugal, China, and other nations(Sánchez-Zapata *et al*., 2012).

1.2.4 CHEMICAL COMPOSITION OF TIGERNUT

Tiger nuts are incredibly nutrient-dense, offering nearly every necessary component for a child's or adult's healthy growth, according to numerous studies. Their contents range from 22.14–44.92% fats, 3.28–8.45% proteins, 23.21–48.12% starch, 8.26–15.47% fibers, and 1.60–2.60% ashes, indicating their high nutritious richness(Adel *et al*., 2015). Tiger nuts also include organic acids, alkaloids, and phenols, which are bioactive compounds(Nina *et al*., 2019).They are an excellent source of edible oils that are rich in monounsaturated fatty acids and have a nutritional value that is similar to that of olive oil(Roselló-Soto *et al*., 2018).

Tiger nuts are packed with a lot of fats (22.14–44.92%) and tiger nut oil also has vitamin C, vitamin E, and minerals like potassium, calcium, and magnesium, along with phenols(Ezeh *et al*., 2014). The antioxidants in this oil make it more stable against oxidation compared to other vegetable oils(Nina *et al*., 2021). It also has alkaloids, saponins, and tannins which have anti-bacterial and anti-inflammatory effects(Vega-Morales *et al.*, 2019). Tiger nut oil contains volatile compounds that contribute to its taste, and in roasted nut oil, there are 75 smell-related compounds making it flavorful(Lasekan, 2012).

Long-chain fatty acids (C16–C20) make up the majority of the fatty acids in tiger nut oil (73.83–76.16%), followed by polyunsaturated fatty acids(8.92–9.84%) and saturated fatty acids (14.60-17.12%)(Fabunmi *et al*., 2016). Minor acids include linolenic and palmitoleic acids, whereas oleic, palmitic, linoleic, and stearic acids are the principal ones(Edo *et al*., 2022). Oleic acid, the main fatty acid, has functional features like controlling blood lipids and decreasing cholesterol(Sobhani *etal*., 2018). According to an analysis of the lipid composition of tiger nut seed oils, triacylglycerols, which make up the majority of neutral lipids, account for 65.9% of the total lipid content. Phospholipids range in percentage from 1.4% to 3.1% and glycolipids from 5.6% to 6.9% of the total lipid composition.

Tiger nuts have a lot of straight-chain starch, and its textural features like hardness, elasticity, bonding, and chewiness are superior than those of maize and sweet potato starches(Akonor*et al.*, 2019, Builders *et al.*, 2013, Li *et al.*, 2017). Tiger nuts include two different types of polysaccharides: starch, which is a pure glucose polysaccharide, and heteropolysaccharides, which are glucose, fructose, and sucrose connected in an unidentified order. Tiger nut polysaccharides (TNPs) are mostly made up of glucose, mannose, galactose, and rhamnose; glucose makes up the majority of these water-soluble polymers. Research indicates that TNPs are composed of both linear and branched chains, with the branched chains including functional groups such as acyl, acetyl, and glycoside.

47.5% gluten, 31.8% albumin, 4.7% globulin, and 3.8% prolamin make up the protein found in tiger nuts(Agboola *et al.*, 2017). Other research, however, show differing findings; the greatest gluten content in tiger nut protein was found to be between 82% and 91%(Codina-Torrella, Güamis and Trujillo, 2015).

Research indicates the presence of small molecules or secondary metabolites, such as flavonoids, minerals, vitamins, and stigmasterol, that are responsible for biological activity in addition to macromolecular nutrients like oil, starch, and protein(Vega-Morales *et al.*, 2019). Notably, flavonoids with a variety of biological activities, including strong antioxidant, anticarcinogenic, anti-inflammatory, and antidiabetic properties, are found in myricetin and quercetin. Minerals including salt, potassium, calcium, iron, magnesium, zinc, copper, and phosphorus are also included in tiger nuts.

1.2.5 REPORTED USES OF TIGERNUT

1.2.5.1 CULINARY USES

Tiger nut tubers are used to make flour, starch, biscuits, and cakes in food goods. They can also be baked, fried, or roasted and eaten as a snack. Additionally, due to its unique sweetness, it could smell like cookies and ice cream(Gambo and Da'u, 2014). According to(Mohdaly, 2019) tiger nut oil is rated appropriate for usage as cooking oil. The caramel made from the malted tiger nut tubers can be used to color or taste various baked items. Moreover, it can be included in non-alcoholic malt drinks, dark beers, and condiment items(Bamishaiye and Bamishaiye, 2011). According to(Belewu and Abodunrin, 2007), it is beneficial while creating kunun, a traditional beverage in Nigeria. It may be advantageous to employ the chemical makeup and functional properties of flour generated from the brown and yellow tigernut types (*Cyperus esculentus*) in food formulation, as these research have been undertaken(Oladele and Aina, 2007). Furthermore, because of their inherent nutritional and health benefits, tigernuts are said to be a great cassava substitute in the baking industry(Ade-Omowaye*et al*., 2008).

1.2.5.2 PHARMACOLOGICAL USE ACCORDING TO RESEARCH

The bioactive compounds that are present in tigernut are responsible for the medicinal properties of tiger nut. The pharmacological use of tigernut include;

- APHRODISIAC PROPERTIES: It is well known that tiger nuts enhance men's libido. Tiger Nut tubers are referred to as "HabAl-zulom" in the Middle East, which means "the seeds of man," suggesting that the plant has aphrodisiac properties. The results of a 30-day trial on active and moderately active rats fed tiger nut meal at levels of 1 to 2 g/kg body weight per day show that yellow tigernut increases sexual desire by shortening the latency for arousal and intromission. Therefore, in comparison to the control group, a noteworthy rise in testosterone levels was seen in the rats given nut sedge over this evaluation period. The presence of zinc, vitamin E and C, and quercetin (flavonoids) in tigernuts may be the cause of these benefits(Allouh*etal.*, 2015). Quercetin, a secondary metabolite belonging to the flavonoid family, has been demonstrated to increase testosterone levels, improving sperm quality and reducing erectile dysfunction.

- CHOLESTEROL LOWERING PROPERTIES: Tigernut oil supplementation was observed to reduce cholesterol levels in albino rats by 6 weeks at dosages of 0.1 and 0.5 mg/kg, respectively, from 110.4 to 98.4 mg/dl and 88.1 to 110.4 mg/dl(Hassan, 2007). Tiger nut tubers have a cholesterol-lowering property that may help reduce the buildup of lipids, cholesterol, and other compounds in and on the walls of arteries, as atherosclerosis is associated with hypercholesterolemia. The biological effects of tiger nut oil were investigated in healthy, hypocholesterolemicrats(El-Naggar, 2016).
- ANTICANCER ACTIVITY OF TIGERNUT: The anticancer properties of tiger nuts against hela cells have been reported by(Nwosu *et al*., 2022). N-hexane extracts were evaluated at the concentration of ethanol (IC50 = 157.3 g/mL; IC50 = 350 μg/mL) in the MCF-7 breast cancer cell lines, and the investigation report states that no maximum anti-cancer effects were seen. The positive control, Actinomycin D, showed more activity than the ethanol extract at a concentration of 0.0087 μg/mL. Due to its significant flavone (5,7-dihydroxyflavone, or Chrysin) content, which has been demonstrated to be detrimental to MCF-7 cell lines, tiger nuts were assumed to have cytotoxic properties(Nwosu *et al*., 2022).
- ANTI-INFLAMMATORY AND ANTI-ARTHRITIC EFFECTS: An experiment using Swiss albino rats shown the potential of tiger nut oil to cure arthritis, prevent convulsions,

and reduce inflammation. In addition to reducing edema caused by carrageenan in a dose-dependent anti-inflammatory way, the oil decreased the extensor phase, the clonus phase, and the stupor phase linked with convulsions (convulsion generated in the rats with maximal electroshock, MES). Furthermore, following tiger nut oil dosages, the rats' formaldehyde-induced arthritis-related swelling in the back paw subsided, and the pain that accompanied it (as indicated by how long they licked the paw) stopped(Ezeh *et al.*, 2016). However, more investigation is needed to support claims that eating tiger nuts decreases joint and bone pain.

- ANTI-SICKLING ACTIVITY: When tiger nut extracts were examined in vitro for their anti-sickling properties, the gelatinization of hemoglobin S (Hbs) was dramatically reduced by both the methanol and aqueous extracts. On the other hand, the methanol extract shown better anti-sickling qualities. This study suggests that people with sickle cell disease receiving nutritionally based therapy may benefit from tiger nuts(Dash *et al.*, 2013). The plant's anti-sickling properties could be attributed to the presence of vital minerals like iron and phosphorus as well as vitamins C and E.
- ANTIMICROBIAL ACTION:(Asare *et al.*, 2020) assessed the antimicrobial efficacy of C. esculentus roots, inflorescence, and shoots in relation to traditional antibacterial drugs. When employed to treat Salmonella typhi (standard), the ethanol extract produced outcomes comparable to those of ciprofloxacin. The study indicates that the plant extract's strong antibacterial activity could aid in the creation of novel antibiotic therapies for S. typhi-related disorders.(Gambo and Da'u, 2014) have investigated the effect of tiger nut ethanol extract on Escherichia coli-induced endometritis in experimental mice. After seven days, there was a noticeable improvement in the reduced levels of

hemoglobin level (Hb) and packed cell volume (PCV), which are associated with endometritis, when compared to the initial findings. The opposing result of treatment with the extracts was a decrease in the elevated concentration of white blood cells (WBC) caused by bacterial invasion. The results observed in the group that received the extract treatment are similar to those of the group that received gentamicin as normal(Okwelle, 2020).

- HEPATOPROTECTIVE ACTIVITY: Tiger nuts have been shown in a study to have hepatoprotective effects on experimental rats' livers. The rats were injected via the intestine with carbon tetrachloride (CCl_4) to induce hepatotoxicity, which was demonstrated by significant elevations in lipid peroxides, alkaline phosphatase, and the liver marker enzymes aspartate and alanine transaminases. The experimental rats were given a 21-day pre-treatment before being given CCl_4, which resulted in a considerable drop in the level of liver serum enzymes(Onyibe*et al.*, 2021). Their results validate the tiger nut's reputation as an efficient liver tonic and suggest that they may prevent rats from liver damage.

1.3 CHEMICAL CONSTITUENTS OF PLANTS

Generally speaking, substances that are present in plants are called phytochemicals. The term "plant-chemicals" is a literal way to refer to phytochemicals. These plant-found non-nutritive chemical components, or phytochemicals, have several health advantages and the ability to stave against disease. According to(Nwozo *et al.*, 2023), the nutrients they contain are non-essential, which means they are not required for maintaining life. These substances are made by plants to ensure their own survival, and they are beneficial to human health when consumed(Rahim *et al.*, 2022). These chemical components protect plants against infections because of their antibiotic, antifungal, and antiviral qualities. Additionally, they are essential as UV-absorbing substances that protect plants from harmful radiation(Bourgaud*et al*., 2001). Depending on their function in plant metabolism, more than a thousand phytochemicals have been identified and classified as primary or secondary metabolites. Principal components of phytochemicals include amino acids, common sugars, chlorophyll, purines, and pyrimidines, which are present in proteins and nucleic acids. Secondary metabolites among phytochemicals comprise alkaloids, flavonoids, terpenes, phenolics, lignans, plant steroids, curcuminoids, saponins, and glucosides(Zahnit*et al*., 2022). Plant secondary metabolites, famous for their considerable biological activity, have been exploited in traditional medicine for ages, with the therapeutic effects of plants attributed to these molecules(Jamwal *et al*., 2018).

1.3.1 ALKALOIDS

Meissner, a German scientist, is credited with coining the term "alkaloid" in the year 1819. It comes from the Arabic name al-qali, which refers to the plant that was originally used to extract soda. Alkaloids are important secondary metabolites that have been used for medical purposes since they were discovered and used as early as 4,000 years ago. Alkaloids are low molecular weight secondary metabolites that make up around 20% of plant-based secondary metabolites. So far, around 12,000 alkaloids have been identified from numerous plant taxa(Zandavar and Babazad, 2023).

Alkaloids are a family of chemical compounds that are usually alkaline in nature and have at least one nitrogen atom in a heterocyclic ring(Ti *et al*., 2021). These compounds were first referred to as "alkaloid" (or "alkali-like") because they react with acids in a manner akin to that of inorganic alkalis, forming salts in the process. Alkaloids are chemically classified as amines, and as such, their names usually end in -ine. The majority of alkaloids are colorless, crystalline, nonvolatile substances that frequently have a bitter flavor when pure (Editors of Encyclopedia Britannica, 2023). Alkaloids play an essential role in both human medicine and in an organism's natural defense. In plants, alkaloids protect plants from predators and regulate their growth(Chik *et al*., 2013). Alkaloids are broadly classified into two classes depending upon whether the nitrogen is a part of a ring or not(Evans, 2009). These are;

1) Non-Heterocyclic Alkaloids or Atypical Alkaloids:

These are also occasionally referred to as biological amines or proto-alkaloids. In nature, these are less common. The nitrogen atom in these compounds is not a component of any ring structure.These include, for instance, taxol, erythromycin, colchicine, and ephedrine.

2) Heterocyclic Alkaloids or Typical Alkaloids(True Alkaloids):

In terms of structure, these contain nitrogen as a component of a cyclic ring system. In nature, these are more frequently encountered. Heterocyclic alkaloids can be further classified into fourteen classes according to the nitrogen-containing ring structure. They include;

a) Pyrrolidine e.g. Hygrine, Stachydrine.

b) Pyridine and Piperidine e.g. Lobeline, Nicotine, Piperine, etc.

c) Isoquinoline e.g. Morphine, Emetine, codeine, etc.

d) Indole or Benzopyrole e.g. Ergometrine, Vinblastine, Vincristine, etc.

e) Pyrrolizidine e.g. Senecionine, Symphitine,

Others are quinolizidines, tropanes, purines, piperidines, imidazoles, etc.

FUNCTIONS OF ALKALOIDS

Alkaloids have various effects on our health and are used for different purposes. They can work as antibiotics, poisons, stimulants, diuretics, astringents, anti-inflammatory agents, and anti-hypertensives. For instance, morphine helps relieve pain, quinine fights against malaria, and vincristine and vinblastine are used in cancer treatment. Alkaloids also help some plants defend against insects. Caffeine in coffee beans keeps certain bugs away, and berberine in tree bark is harmful to certain fungi. Alkaloids play a part in plant reproduction by producing hormones and pheromones. They are also used as natural pesticides because they are harmful to specific insects and fungi.

1.3.2. TERPENIOD (TERPENE)

Terpenes are categorized as secondary metabolites and are made up of five different types of jointed carbon isoprene units. Serving as main ingredients in essential oils, they exhibit structures with 2-methylbuta-1,3-diene (isoprene units) carbon backbones capable of building cyclic structures(Hyldgaard *et al*., 2012). The scent of plants and herbs is mostly determined by these aromatic chemicals. A significant structural difference exists between terpenes and terpenoids: terpenoids are a modified class of terpenes with different functional groups and an oxidized methyl group that can be changed or deleted at different places. Terpenes are simple hydrocarbons. The typical molecular formula for terpenes is (C5H8)n. Terpenoids are further categorized based on the quantity of isoprene units they contain(Perveen, 2018).

CLASSIFICATION BASED ON ISOPRENE UNITS PRESENT

1. Hemiterpene: consists of one isoprene unit e.g. isoprene itself and an oxygen derivative of isoprene e.g. prenol and isovaleric acid

2. Monoterpene: consists of two isoprene units e.g. limonene and carvone(a modified monoterpene),pine, etc.

3. Sesquiterpenes: consists of three isoprene units and are found in linear and cyclic forms as well as in form of lactose ring e.g. farnesenes, humulene

4. Diterpenes: consists of four isoprene rings e.g. texadiene (a precursor of Taxol) and cembrene

5. Sesterpenes: consists of five isoprene units e.g. geranylfarnesol

6. Triterpenes: consists of six isoprene units e.g. squalene

7. Tetraterpenes: consists of eight isoprene units e.g. lycopene

FUNCTIONS OF TERPENOIDS (TERPENES)

Terpenes are essential to plant life. Terpenes are used by certain plants to draw pollinators and by others to ward off predators such as insects and animals. Terpenes are utilized in agricultural insecticides due to their protective properties(Isman, 2000).Antimicrobial properties of terpenes against bacteria, including those susceptible and resistant to antibiotics, have been documented(Álvarez-Martínez *et al*., 2021).Terpenes have been linked to a number of health benefits, including the prevention and treatment of cancer, the reduction of inflammation, the function as antioxidants, the alleviation of allergies, and the provision of soothing and pain-relieving effects, according to numerous research(Zhao *et al*., 2016).

1.3.3 PHENOLICS

Plant shikimic acid and pentose phosphate undergo phenylpropanoid metabolization, which produces phenolic chemicals, which are categorized as secondary metabolites(Randhir *et al*., 2004).These substances, which range from simple phenolic molecules to more sophisticated forms like tannins, have benzene rings with one or more hydroxyl groups(Velderrain-Rodríguez *et al*., 2014). With over 8,000 identified phenolics, they are the most prevalent secondary metabolites in the kingdom of plants. These substances are frequently found in plant foods and contribute to their overall organoleptic qualities. For example, they interact with phenolics like procyanidin and saliva glycoproteins to impart an astringent and bitter taste to fruits and liquids(Dai and Mumper, 2010).

According to reports, the main sources of phenolic compounds in the human diet are fruits, vegetables, and beverages. This is because phenolic compounds have been linked to positive effects that come from eating fruits and vegetables, especially because of their antioxidant activity(Heimetal, 2002). These phenolic chemicals typically have something to do with the plant's defensive mechanisms. Nevertheless, phenolic metabolites are crucial for other functions as well, such as adding pigment for protection against herbivores, including appealing compounds to speed up pollination, and having antibacterial and antifungal properties(Alasalvar*etal*.,2001;Acamovic and Brooker,2005).

Flavonoids, tannins, phenolic acids, and less often occurring stilbenes and lignans are all considered phenolic substances. Of them, the most common polyphenols in human diets are flavonoids. Diphenyl propane, which consists of two benzene rings (rings A and B) connected by a linear three-carbon chain, is the carbon structure that unites them. One of the benzene rings and the center three-carbon chain may combine to produce a closed pyran ring (ring C). Based on the degree of oxidation of the core pyran ring, flavonoids are further divided into six subclasses: flavonols, flavones, flavanones, isoflavones, anthocyanidins, and flavanols (catechins and proanthocyanidins). More than 4000 flavonoids have been discovered in plants, and the catalog continues to expand(Harborne and Williams, 2000). This diversity is attributed to numerous substitution patterns, where primary substituents, such as hydroxyl groups, can undergo additional modifications, like glycosylation or acylation, resulting in highly complex structures. Common flavonoids include quercetin, a prevalent flavonol in onions and apples; catechin, a flavanol present in tea and various fruits; naringenin, the principal flavanone in grapefruit; cyanidin-glycoside, an anthocyanin abundant in berry fruits like raspberries; and daidzein, genistein, and glycitein, the primary isoflavones in soybeans.

Phenolic acids fall into two categories: those derived from benzoic acid, like gallic acid, and those from cinnamic acid, including coumaric, caffeic, and ferulic acid. Among them, caffeic acid is the most prevalent in many fruits and vegetables, commonly esterified with quinic acid, as seen in chlorogenic acid—the primary phenolic compound in coffee(D'Archivio*et al.,* 2007).

Tannins are an important class of polyphenols and two main types of tannins are commonly found in human diets: hydrolysable tannins and condensed tannins. The fundamental core of hydrolyzable tannins is glucose or another polyol esterified with either hexahydroxydiphenic acid (ellagitannins) or gallic acid (gallotannins)(Khanbabaee& Van Ree, 2001). Proanthocyanidins, another name for condensed tannins, are flavan-3-ol oligomers or polymers joined by an interflavan carbon bond. When heated in acidic alcohol solutions, they undergo acid-catalyzed oxidation and convert to anthocyanidins. Variations in the degree and pattern of methoxylation, glycosylation, and galloylation, as well as in the stereochemistry at the three chiral centers, interflavan linkage location and type, are the causes of the structural diversity(Kolečkář*et al*., 2008).

FUNCTION OF PHENOLIC COMPOUNDS

In the therapy of carbohydrate absorption conditions like diabetes, phenolic substances can prevent amylase from being absorbed(De Sales *et al.*, 2012). The benefits of phenolic compounds, including their anti-aging, anti-inflammatory, antioxidant, and antiproliferative properties, have been documented in several research. Furthermore, there exist pertinent antioxidant enzymes that counteract oxidants(Shukitt-Hale *et al.*, 2008; Moo-Huchin *et al.*, 2015). Phenolic compounds are bioactive ingredients that are used in cosmeceutical products(Soto *et al.*, 2015).

1.4 PROXIMATE ANALYSIS

This method entails the identification and measurement of many constituents, including ash, moisture, crude protein, crude fat, and crude fiber. An item's shelf life, nutritional value, and suitability for different industrial uses can all be ascertained using the data gathered from the proximate analysis. This approach, which addresses dietary and medical demands, has been used globally for more than 160 years to reduce specific healthcare issues(Ullah *et al.*, 2023). The chemical components studied under proximate analysis are moisture content, ash content, crude fat, crude fiber, crude protein and nitrogen free extract(carbohydrates). The last constituent (i.e. carbohydrates) are calculated based on the determination of the others via chemical reactions and experiments. There are standards used for carrying out the analysis, usually the Association of Analytical Chemist (AOAC) method. Ideally, when we test a food product for its basic components (moisture, protein, fat, ash, and carbs), these values, known as proximates, should sum up almost exactly to 100%. Any significant deviation from this suggests limitations in the testing methods. The slight variations in how each test is performed can accumulate and make the final picture of the food's composition less precise(Wikipedia).

1.4.1 PROXIMATE ANALYSIS OF TIGER NUT

Scientists analyzed tiger nut tubers to understand their nutritional profile. The tubers were mostly made up of carbohydrates (almost 46%), followed by a good amount of oil (over 30%). Interestingly, they had a decent amount of fiber (nearly 15%) and a moderate level of protein (around 5%). The moisture content, which impacts food spoilage and storage, was around 8.5%. Finally, the tubers had the lowest amount of ash, at about 2%(Arafat, 2019). The amount of water in a food, called its moisture content, plays a big role in how fresh it stays, how easy it is to store, and how long it lasts before spoiling. Among the main categories of nutrients are fats, proteins, carbohydrates, and minerals. Widely present in both natural and processed meals, carbohydrates are a very important nutrient. They range from complex polysaccharides like starch to simple monosaccharides like glucose, fructose, and galactose(Beare-Rogers *et al*., 2001). Triglycerides, the unit of fatty acids bonded to a glycerol backbone, are what are known as fats. Certain fatty acids must be consumed in the diet. In the body, they are not synthesized. Nitrogen atoms are present in protein molecules together with hydrogen, oxygen, and carbon. Amino acids that include nitrogen make up the basic building blocks of protein; certain amino acids are required because the body is unable to synthesis them. Through a process called gluconeogenesis, some amino acids can be converted to glucose(Churuangsuk&Kherouf, 2018). Crude fiber is the portion of feed that cannot be energetically utilized by the human body because it is made up of plant cell wall components that are often indigestible or hardly digestible. As demonstrated by tiger nuts, this unprocessed fiber found in most diets facilitates bowel movements and digestion. The inorganic residue that is left over after the organic material has burned up is known as the biological material's ash content. The ash content is significant because it provides an overview of the quantity of mineral elements in the sample. It is important to remember that the proximate composition of tiger nuts can alter because of both natural cultivar variations and environmental factors that may impact nutrient intake.

1.5 MINERAL ELEMENTS

Minerals are necessary to create and sustain life processes in both humans and plants, and they are also food components. Sometimes known as inorganic elements, these are substances that people, animals, and plants require in minimal quantities. For example, minerals aid in the growth of plants. These minerals are necessary for human growth and development, as well as being parts of bodily fluids and organs and giving the body its structure. Major and trace minerals are the two categories into which these minerals fall. While the major minerals, also referred to as macro minerals, are necessary in large quantities for human dietary requirements and are considered essential elements, e.g. calcium, magnesium, phosphorus, sodium, etc., the trace minerals, also referred to as micro minerals, are required in smaller quantities but are still vital, e.g. iron, zinc, copper, etc.(Asp, 2004).

1.5.1 TIGER NUT MINERALS

Using the techniques outlined by the Association of Official Analytical Chemists (AOAC), 2010, an analysis of the proximate and mineral content of tigernuts (Cyperus esculentus) was conducted. Potassium (K) was found to have the greatest value in the mineral analysis, followed by phosphorus (P), magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe), zinc (Zn), and copper (Cu) at the lowest levels (Suleiman, 2018). Below is a discussion of several of these tiger nut minerals, along with information on their sources, inadequacies, and uses.

1.5.1.1 POTASSIUM

The body's primary intracellular cation, potassium, is primarily engaged in the regulation of acid-base balance, membrane potential, and electrical excitation of muscle and nerve cells. Pork, beef, milk, cereal, veggies, fruits, and potatoes are some examples of foods high in potassium. Excessive use of potassium has been shown to provide protection against several medical conditions affecting the kidneys, bones, and cardiovascular system. Research has demonstrated that consuming more potassium also decreases blood pressure, may avoid chronic kidney damage, and may even have a good impact on decreasing stroke. Potassium deficiency of nutritional origin is rare because potassium can be found in a wide range of foods. However, due to the excessive consumption of processed foods, a significant population in the westernized region has been found to have a potassium deficiency, which may result in moderate chronic total body potassium depletion(Lanham-New *et al*., 2012).

1.5.1.2 MAGNESIUM

Magnesium has been identified as the fourth most abundant mineral in the human body, following potassium, sodium, and calcium. Notably, it also holds the distinction of being the second most frequent intracellular cation. This widespread presence within cells suggests a crucial role in various cellular processes (Schwalfenberg and Genuis, 2017). Magnesium can be found in a variety of foods, including whole grains, legumes, nuts, milk, meat, fish, and fortified foods like. Magnesium is also present in varying levels in tap, mineral, and bottled waters. A necessary mineral, magnesium (Mg^{2+}) is involved in numerous important metabolic processes, including the synthesis of proteins and nucleic acids, glycolysis, and energy production. In addition, it is critical for immunological, neuromuscular, oxidative, and bone growth processes. It helps to preserve calcium, sodium, and potassium homeostasis as well as electrolyte balance, all of which are necessary for stabilizing excitable membranes(National Institutes of Health, 2013).

1.5.1.3 CALCIUM

Calcium is the building block of bones and teeth, keeping these tissues strong, flexible, and rigid. This unique combination allows for smooth and regular physical movement. The bones contain 98% of the calcium in the body, which is used by the body to maintain calcium homeostasis as a source and storage for calcium. Dairy products like yogurt, cheese, and milk, as well as fortified grains and veggies like broccoli, cabbage, and other veggies, are good sources of calcium. A calcium deficit can weaken bones and cause osteoporosis, a condition marked by brittle bones and a higher chance of falling. In addition, rickets in children and several bone problems in adults, including osteomalacia or improper bone mineralization and softening, can be brought on by a calcium deficit(Institute of medicine, 2011).

Calcium is the key ingredient in bone, a mineralized connective tissue that provides our skeleton with both strength and structure. Calcium needs can change during the course of a person's life and proper dietary intake is crucial for bone formation and metabolism (Vannucci *et al.*, 2018). Additional benefits of calcium consumption for health include a decrease in blood pressure, a prevention of osteoporosis and adenomas, a reduction in pregnancy-related hypertensive problems, a decrease in cholesterol, etc.(Cormick and Belizán, 2019).

1.5.1.4 IRON

Dietary iron (Fe), is necessary for the maintenance of several critical human processes, including the development and maintenance of the immune system and the metabolism of cellular energy. Despite its significance, anemia caused by iron deficiency is the most common nutritional disorder in the world, affecting about 25% of the global population. However, the prevalence is much higher in certain populations, such as children (approximately 30-47%) and females (both pregnant and non-pregnant)(McLean *et al*., 2008). A healthy iron level is important for human energy metabolism, oxygen transport, and immune function, which enables the body to establish a suitable defense against invasive infections. Vegetable and animal sources of iron include cashews, spinach, green peas, beef, pig liver, and others(Weyh *et al*., 2022).

1.5.1.5 ZINC

After iron, zinc is the trace metal that is most widely distributed in the body. It can be found in many different meals, including cereals, chicken, cattle, and shellfish. Zinc, a mineral essential for human metabolism, works like a tiny machine in our bodies. It assists over 100 enzymes, making reactions run smoother. Zinc also helps fold proteins into their proper shapes and controls how our genes are expressed. Zinc supplementation may be useful in developing nations to prevent diarrhea and upper respiratory infections. Additionally, zinc and antioxidants may have a minimally beneficial influence on the rate at which age-related macular degeneration progresses. Wilson disease can also be effectively treated with zinc. Non-specific symptoms of zinc deficiency include reduced immunity, growth retardation, diarrhea, baldness, glossitis, and nail deformation(Saper, 2009).

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 APPARATUS

- UV/VIS Spectrophotometer
- Muffle furnace
- Soxhlet apparatus
- Hot air oven
- Desiccators
- Beakers
- Conical flask
- Test tubes
- Volumetric flask
- Crucibles
- Atomic Absorption Spectrophotometer
- Flame photometer
- Weighing balance
- Spatula and thong

2.2 REAGENTS

- Dragendroff’s reagent
- Ferric chloride
- Wagner's reagent
- Fehling's reagent

- Ammonia solution
- Sodium hypochlorite
- Sodium potassium titrate
- Potassium dichromate
- Alkaline sodium phenate solution
- Kjehdhal catalyst
- Hydrochloric acid
- Sulphuric acid
- Chloroform
- Sodium hydroxide
- Hexane
- Petroleum ether
- Distilled water

2.3 METHODOLOGY

2.3.1 SAMPLE COLLECTION AND PREPARATION

Fresh tiger nut tuber was bought from New Benin Market, Benin City, Edo State, Nigeria and was authenticated with herbarium number of UBH-C419 on April 9, 2024 by Dr. H.A. Akinnibosun of the department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Nigeria.

The tiger nut was sieved to remove dirt and was washed before sun-drying for five days. The dried sample was milled in a milling machine and was sieved with a sieve to get a fine powder. The powdered sample was then stored in a plastic airtight container for analysis.

2.3.2 PROXIMATE COMPOSITION ANALYSIS

The powder of tiger nut was subjected to proximate composition analysis and components analyzed were moisture, ash, protein, fat, crude fibre and carbohydrate. This was done using standard methods as described by the Association of Official Analytical Chemists(AOAC, 2005) and in triplicate.

2.3.2.1 DETERMINATION OF MOISTURE

Clean and dry crucible was weighed and the weight was recorded (W_1). 2g of the sample was weighed into the crucible (W_2). The crucible containing the sample was placed in an oven and the oven was set at 105^{o}c. The crucible containing the sample was allowed to stay in the oven for about three hours. It was allowed to dry until a constant weight (W_3) was obtained. This was done in triplicate.

The percentage moisture content was then calculated as follows;

$$\%\text{moisture} = \frac{\text{Loss in weight}}{\text{Weight of sample before drying}} \times 100 = \frac{W_2 - W_3}{W_1} \times 100$$

Where W_1 = initial weight of empty crucible

W_2 = weight of crucible + sample before drying.

W_3 = weight of crucible + sample after drying**.**

2.3.2.2 DETERMINATION OF ASH CONTENT

Crucibles were preheated to remove any form of moisture and they were placed in a desiccator to cool down before their weights were taken (W_1). 2g of tiger nut sample were weighed into the crucibles and their weights were recorded (W_2). The crucibles containing the sample were placed in a muffle furnace and ignited at a temperature of 550^{0}c which was maintained for three hours. The muffle furnace was then allowed to cool after which the crucibles were removed and put in a desiccator to cool. The weight of ash and crucible were taken (W_3). This was done in triplicate.

$\%ash = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100 = \frac{W_3 - W_1}{W_2 - W_1} \times 100$

Where W_1= Weight of crucible

W_2 = Weight of crucible + sample.

W_3 = Weight of crucible + ash.

2.3.2.3 DETERMINATION OF CRUDE FIBRE

2g of the sample was boiled under reflux in a 200ml solution containing 1.25g of sulphuric acid per 100ml of solution. The solution was filtered and washed with water and the filtrate was tested for acidity. The residue was washed continuously until the filtrate was no longer acidic. The residue was then transferred into a beaker and boiled with a 200ml solution of 1.25% NaOH. The solution was filtered and again washed. The final residue was filtered and washed thoroughly with hot water and twice with ethanol. The residue was dried in an electric oven and weighed after which it was incinerated cooled and weighed. The percentage crude fiber was calculated as follows:

$\%crude\ fiber = \frac{W_2 - W_3}{W_1} \times 100$

Where W_1= weight of sample used.

W_2 = weight of crucible + dried residue.

W_3=weight of crucible + ash.

2.3.2.4 DETERMINATION OF CRUDE FAT

The fat content determination was carried out using a Soxhlet apparatus.

2g each of the sample were wrapped in separate filter and place in a soxhlet reflux flask which is connected to a condenser on the upper side and to a previously weighed oil extractor flask containing 200ml 0f petroleum ether. The ether was set to boil and the vapour condensed into the reflux flask immersing the sample completely for extraction to take place on filling up the reflux flask siphons over carrying the oil extract back to the boiling solvent in the flask. The process of boiling, condensation and reflux was allowed to continue for 8hours after which the defatted sample was removed. The oil extract in the reflux was dried in the oven at 60^0 for 30min*utes* and then weighed. This was done in triplicate. The percentage fat was calculated as follow:

$$\%FAT = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

2.3.2.5 DETERMINATION OF CRUDE PROTEIN

Preparation of sample for digestion: 1g of the sample was weighed pinto micro-Kjeldahl digestion flask together with few anti bumps in granules. Two grams of catalyst mixture was added to the flask and then 10ml concentrated H_2SO_4 also added to each flask. The flasks were placed in inclined position on a heating mantle in a fume cupboard. Digestion was started at temperature of 30°C until frothing ceased and then heating was increased to 50°C for another 30min and finally at full heating (100°C) until a clear solution was obtained. Simmering was continued below boiling point for another 30 min to ensure complete digestion and conversion of nitrogen to ammonium sulphate. After digestion was completed, the sample was allowed to cool and then transferred quantitatively to 100ml volumetric flasks with washing and cooling to room temperature. Volume was made up to mark with distilled water.

Preparation of sample for absorbance reading: 5ml of the filtrate from the digest was transferred with the aid of a 10ml pipette into a 25ml standard flask. 2.5ml of the Alkaline Phenate was added and the solution shaken to mix properly. Then 1ml of Sodium Potassium Tartarate was added, shaken properly followed by the addition 2.5ml of sodium hypochlorite. There after the solution was made up to the 25ml mark with distilled water and the absorbance of the resultant solution measured with the aid of UV/visible spectrophotometer, at 630nm. This was done in triplicate. The Nitrogen standards were treated the same way with the sample.

$$\%N = \frac{\text{Instrument Reading x Slope Reciprocal x Color Vol. x Digest Vol.}}{\text{Weight of Sample x Aliquot Taken x 10000}}$$

%Crude Protein = %Nitrogen × 6.25

2.3.2.6 DETERMINATION OF CARBOHYDRATE CONTENT

The carbohydrate content determination of the tiger nut powder was calculated using the formula for food analysis and instrumentation:

%carbohydrate = 100 - %(protein + fat + ash + fibre + moisture content)

2.3.3 MINERAL ANALYSIS

Determination of minerals was done according to AOAC method. 2g sample was ashed in a furnace at the temperature of 550^{0}C and the ash of the sample was dissolved in 10ml of 0.1MHCl, filtered and made up with distilled water to the mark in a 100ml volumetric flask. This was used to determine the minerals Mg, Cu, Zn, Pb, Fe, Ca, and Mn by the use of atomic absorption spectrophotometer (AAS). Also, K and Na were determined with flame photometer while Phosphorus was determined using the spectrophotometer.

2.3.4 PHYTOCHEMICAL SCREENING

10 grams of the powdered tigernut samples were soaked in 50ml of distilled water for extraction. The extract was then filtered out with filter paper and used for the various qualitative screening for the presence of phytochemicals. Phytochemical screening was carried out on the aqueous extract of the tigernut powder using standard procedures to identify the constituents as described by(Shaikh and Patil,2020; Vishnu *et al*., 2019; Tiwari *et al*., 2001).

Test for alkaloid: 2ml of Drangendorff's reagent was added to 3ml of the extract.

Test for glycoside: To 2ml of filtrate, 3ml of chloroform is added and shaken. The chloroform layer is separated and 10% ammonia solution was added.

Test for phenolic compound: Few drops of 10% ferric chloride was added to 2ml of the extract

Test for tannins: Tigernut extract was dissolved in 5ml of distilled water .1% gelatin solution and 10% NaCl was added to the solution.

Test for saponin: 2ml of distilled water was added to 0.5g of extract and was shaken vigorously.

Test for flavonoids: 1ml of extract was taken and 10% of lead acetate was added.

Test for eugenols: 2ml of the extract was mixed with 5ml of 5% KOH solution. The aqueous layer was separated and filtered. A few drops of HCl were added to the filtrate.

Test for steroids: To 2ml of extract, 2ml of chloroform and 2ml of concentrated H_2SO_4 are added.

Test for terpenoids: 3ml of the extract was taken and 1ml of chloroform and 1.5ml of concentrated H_2SO_4 are added along the sides of the tube.

CHAPTER THREE

3. 0 RESULT AND DISCUSSION

The results obtained from this study are represented in the table below and the discussion for each result follows subsequently.

Table 1: Proximate composition of Tiger Nut (Cyperus esculentus)

NUTRIENT	COMPOSITION (%)
Moisture	7.50 ± 0.50
Crude Protein	4.57 ± 0.14
Crude Fat	26.53 ± 0.32
Crude Fiber	7.50 ± 0.50
Ash	3.00 ± 0.50
Carbohydrate	50.89 ± 0.44

Data are mean value ± standard deviation for triplicate determination

Proximate analysis was performed on tiger nut to determine the moisture, crude protein, crude fat, crude fiber, ash, and carbohydrate content and the result gotten was found to largely agree with that from literature (Arafat, 2019). The result from the proximate analysis of tiger nut (*Cyperus esculentus)* in percentage (%) as shown in Table 3.1, revealed that tiger nut is a good source of carbohydrates (50.89 ± 0.25%), followed by crude fat (26.53 ± 0.18%). This indicates that tiger nut is a potential source of energy. Moisture content was 7.50 ± 0.29%, indicating that tiger nut has a relatively low moisture content, making it shelf-stable. The amount of crude protein (4.57 ± 0.08%) and crude fiber (7.50 ± 0.29%) were found to be moderate. Tiger nuts are known for a decent amount of dietary fiber, which can be beneficial for digestion and gut health. The crude protein value gotten (4.57±0.08) is slightly lower than the usual range for tiger nuts according to literature. Compared to other nuts and seeds, tiger nuts generally have a lower protein content, and as such, the result aligns with that observation. Ash content, representing the mineral content, was 3.00 ± 0.29%.

Table 2: Mineral analysis of Tiger Nut (Cyperus esculentus)

PARAMETERS	CONCENTRATION (mg/kg)
Magnesium	52
Potassium	2565
Sodium	375
Phosphorus	4914
Calcium	23
Iron	190
Manganese	7
Copper	10
Zinc	55

The result of the mineral analysis in mg/kg contained in Table 3.2 revealed phosphorus to be the most abundant mineral followed by potassium which is known to be widely distributed and rarely deficient in diet. Sodium was discovered to be the next abundant mineral. Sodium and potassium function in liaison throughout the body. Potassium naturally balances the metabolic activity of sodium. The ratio of sodium to potassium (Na+/k+) in the diet is a significant predictor of hypertension than the amount of either one alone. From the data obtained the ratio of sodium to potassium (Na+/k+) was 0.15 which is within the range (Na+/k+ $\leq$ 1.0) advised by World Health Organization for the prevention of cardiovascular diseases and high blood pressure (World Health Organization, 2012). Magnesium is the next abundant mineral as stated in the table and it is necessary for diversity of cellular metabolic functions and sometimes has the ability to replace a portion of the body calcium(James, 2010). Magnesium is followed by calcium in terms of quantity, which is a vital mineral for healthy bones and teeth. Following these are the less abundant elements in mg/kg which are referred to as micronutrients like iron, which is necessary for its involvement in oxygen and electron transport, zinc which is vital for the creation of hormone. Also manganese and copper which are the least prevalent also act to execute their established biological tasks(Geleijnse *et al.*, 2004).

Table 3: Phytochemical screening of Tiger Nut (Cyperus esculentus)

PHYTOCHEMICALS	TEST METHOD	INFERENCE
Glycosides	General Test	+
Saponins	Frothing Test	+
Phenols	Ferric Chloride	+
Eugenols	K0H/HCL Test	+
Terpenoids	Salkowski Test	+
Steroids	Salkowski Test	–
Alkaloids	Drangendorff's Test	+
Flavenoids	Lead Acetate	+
Tannins	Ferric Chloride	_

Key: - Absent, + Present, ++ Largely Present

The results from the phytochemical screening of tiger nut showed in Table 3.3 revealed the presence of a diverse range of bioactive compounds. These include alkaloids, saponins, glycosides, phenolics, flavonoids, terpenoids, eugenols. Notably, tannins and steroids were absent. The presence of these various phytochemicals suggests potential health benefits for tiger nut consumption. Alkaloids, phenolics, and flavonoids are known for their antioxidant properties, while saponins and terpenoids possess potential anti-inflammatory and antimicrobial activities. Eugenol, another identified compound, contributes to antioxidant and pain-relieving effects.

These findings warrant further investigation to quantify the levels of these compounds and explore their specific biological activities and potential health applications. Overall, the presence of this array of phytochemicals highlights the potential of tiger nut as a source of bioactive compounds with promising health benefits.

Findings from this study are as follows;

- Tiger nut is a good source of energy due to its high carbohydrate (50.89%) and crude fat (26.53%) content.
- It has a relatively low moisture content (7.50%), making it shelf-stable.
- It contains a moderate amount of protein (4.57%) and fiber (7.50%), beneficial for digestion and gut health.
- The protein content finding might be a slight deviation, but it highlights the potential for variation within tiger nuts.
- Importantly, the ratio of sodium to potassium (0.15) falls within the World Health Organization's recommendation for preventing cardiovascular disease.

Contribution to knowledge is that regarding the ratio of sodium to potassium as a predictor of hypertension. The ratio of sodium to potassium (0.15) gotten from this study falling within the WHO recommendation for heart health is a positive finding compared to literature, where this ratio might not always be explicitly mentioned. As such, tiger nut is safe for consumption by all without risk of cardiovascular disease and high blood pressure.

CONCLUSION

This research work has revealed that *Cyperus esculentus* (tiger nut) is a good source of carbohydrate, crude fat and crude fibre. The high levels of crude fat and carbohydrate showed tiger nut to be a good source of energy. It has variable mineral content of phosphorus, calcium, magnesium, potassium and sodium. The low ratio of sodium to potassium particularly shows that tiger nut holds a promise to reducing cardiovascular problems. It also contains essential micro nutrients such as iron, copper, manganese and zinc all which recommends it for both domestic and industrial application as possible complementary food source. This obviously proved that tiger nut would have beneficial effect in the maintenance of heart function and prevention of cardiovascular diseases such as hypertension as well as other health related issues in both man and animals. The phytochemicals identified are responsible for the therapeutic functions of tigernut. As such, tiger nut benefits are relevant to maintaining healthy living.

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APPENDIX

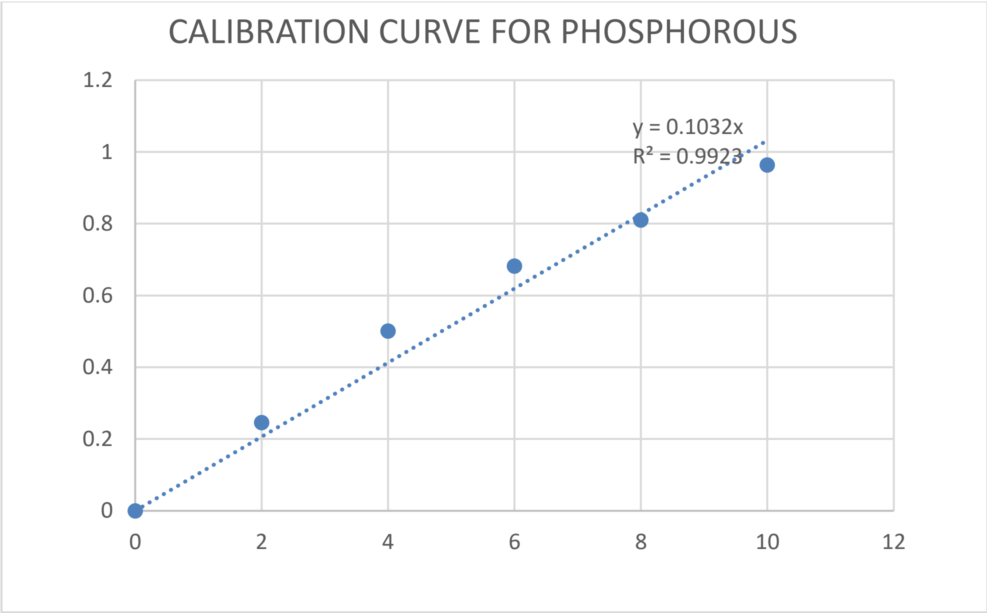
Preparation of phosphorus stock solution (100ppm)

Following standard to make 100 ppm Phosphorus stock solution, 0.439g of dried KH_2P0_4 was dissolved in deionized water then diluted to 1 L.

Using the dilution formula i.e. $C_1V_1= C_2V_2$, standard phosphorus solutions; 10ppm, 8ppm, 6ppm, 4ppm and 2ppm were prepared. These were then taken to be read in the uv/vis spectrophotometer and their absorbance recorded. The values gotten were used to prepare a standard curve. The prepared digest containing the sample was also taken to be read and its absorbance also recorded.

Table4: Concentration and absorbance values for phosphorus standard

Concentration (ppm or mg/L)	**Absorbance**
10	0.964
8	0.810
6	0682
4	0.501
2	0.246
0	0



Absorbance value of phosphorus in the sample from uv/vis spectrophotometer is 0.441.

Equation from Calibration curve;

$y = 0.1032x$

Where;

y is absorbance

x is concentration in ppm or mg/L

Since absorbance of phosphorus (y) is 0.441, then concentration of phosphorus (x) in mg/L can be calculated. Thus,

$x = y/0.1032$

$x = 0.441/0.1032$

$x = 4.27$mg/L

For other minerals, the digest was taken to be read with Atomic Absorption Spectrophotometer (AAS) for magnesium, calcium, iron, zinc, manganese, copper and Flame Photometer (FP) for potassium and sodium. The readings were given in mg/L.

Table 5: Concentration of tiger nut minerals in mg/L

Minerals	Concentration (mg/L)
Magnesium	1.05
Potassium	51.3
Sodium	7.50
Calcium	0.47
Phosphorus	4.27
Iron	3.80
Manganese	0.14
Copper	0.20
Zinc	1.10

For phosphorus;

$$\textbf{Concentration(mg/kg)} = \frac{\textbf{Concentration(mg/L) X Colour Volume (L) X Digest Volume(L)}}{\textbf{Aliquot taken(L) X Weight of Sample (kg)}}$$

Where;

Colour Volume = 11.5ml = 0.0115L

Digest volume=100ml = 0.1L

Aliquot taken = 1ml = 0.001L

Weight of sample=1g = 0.001kg

For other minerals;

$$\textbf{Concentration (mg/kg)} = \frac{\textbf{Concentration (mg/L) X Digest Volume(L)}}{\textbf{Weight of Sample (kg)}}$$

Where;

Digest volume = 0.1L

Weight of sample = 0.002kg.