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EVALUATION OF PHYTOCHEMICAL AND ANTIOXIDANT VITAMINS A, C, AND E COMPOSITIONS OF CHIA SEED AND TIGER NUT PROCURED FROM NNEWI, ANAMBRA STATE, NIGERIA

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ABSTRACT

Salvia hispanica (Chia Seed) also known as chia seed, is an annual herbaceous plant, originally from Southern Mexico. Chia seeds have become one of the world's most recognizable foods based on their nutritional properties and medicinal values.*Cyperus esculentus L.* (Tiger Nut) is a valuable food for nourishment with significant health benefits, they are rich in vitamins and minerals. In this study, phytochemical and antioxidant vitamins A, C and E compositions of Chia Seed and Tiger nut procured from Nnewi, Anambra State were evaluated using standard methods. Phytochemical and bioactive contents of the samples were determined using Gas chromatography- flame ionization detector while antioxidant vitamins (A, C, E) were determined using Pearson method. Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences (SPSS) version 26. Level of significance was set at $p<0.05$. The results of this study revealed that chia seed and tiger nut possess good quantity of antioxidant phytochemicals such as quercetin (6.86 ± 0.00 and 0.83 ± 0.00)ppm, nobeletin(1.21 ± 0.00 and 0.15 ± 0.00)ppm, resveratrol (0.32 ± 0.00 and 2.04 ± 0.00)ppm, vanillic acid (0.14 ± 0.00 and 18.28 ± 0.00)ppm respecvtively and antioxidant vitamins A (13.28 ± 0.02 and 25.00± 0.02)mg/l, C (117.65 ± 0.25 and 109.09±0.21)mg/kg and E (19.73± 0.02 and 31.49± 0.02)mg/l respectively. In conclusion,the results of this study revealed that chia seed and tiger nut possess good quantity of antioxidant phytochemicals and antioxidant vitamins and therefore could be very useful in neutralizing the free radicals normally generated in some disease conditions,hence in management of oxidative stress.

Keywords: Phytochemicals, Antioxidants, Vitamins, Chia Seeds and Tiger nut

INTRODUCTION

Chia seeds, derived from *Salvia hispanica L.*, are small edible seeds that have gained international recognition as a functional food due to their high content of omega-3 fatty acids, protein, and dietary fiber. Taxonomically, chia belongs to the Lamiaceae family, commonly known as the mint family, and is native to southern Mexico and Guatemala (Silva *et al*., 2022). It is a flowering herbaceous plant with a short-day photoperiod and a growing interest due to its agronomic adaptability and nutritional value (Jamboonsri *et al*., 2020).

The classification of chia has been supported by morphological, botanical, and molecular analyses. *Salvia hispanica L.* is classified within the order Lamiales, family Lamiaceae, and genus Salvia, which includes more than 900 species worldwide (Souza *et al*., 2021). Modern molecular studies using DNA barcoding and simple sequence repeats (SSR markers) have been employed to distinguish chia from closely related species and to confirm its genetic stability (Silva *et al*., 2022). The consistent seed morphology, coupled with molecular data, provides a solid framework for its classification.

Chia seed cultivation is expanding globally due to its ability to adapt to various climates and soils. Originally grown in Mesoamerica, it is now cultivated in South American countries like Argentina, Bolivia, and Peru, and has expanded to Australia and some regions of North America (Martínez-Cruz *et al*., 2020). The plant thrives in subtropical and temperate regions with well-drained soils, preferring a pH of 6.0 to 8.5 and elevations between 500 and 2,000 meters above sea level (Porras-Loaiza *et al.,* 2023). It is sown during the spring when soil temperatures reach optimal levels for germination and can be grown under rainfed or irrigated conditions depending on the region.

Agronomic practices for chia cultivation include direct seeding at a depth of about 1–2 cm and row spacing of 60 to 80 cm to ensure adequate sunlight and air circulation. Chia is considered a low-input crop; it requires minimal fertilization and is relatively pest-resistant, which makes it suitable for organic farming systems (Souza *et al*., 2021). However, weed control during the early stages is essential, as young chia plants are less competitive until they reach maturity. Crop rotation and minimal tillage are recommended to maintain soil fertility and reduce disease risk (Ramírez-Moreno *et al*., 2021).

The harvesting of chia seeds occurs when the plant reaches physiological

maturity, typically between 90 and 120 days after sowing. The plant is ready for harvest when its flowers dry and the seed capsules turn brown or gray, indicating full seed maturity (Porras-Loaiza *et al.,* 2023). Manual harvesting is still common in traditional farming regions; however, mechanical harvesting is increasingly adopted in large-scale operations for efficiency. After harvesting, the seeds are separated using threshing and sieving techniques, followed by cleaning and drying processes to reduce moisture levels and prevent fungal contamination.

Proper post-harvest handling is critical to maintain chia seed quality. After separation from plant material, seeds are dried to a moisture content of less than 10%, which helps extend their shelf life and reduce the risk of spoilage (Ramírez-Moreno *et al*., 2021). They are then stored in cool, dry conditions, ideally in airtight containers to preserve their nutritional properties. Mechanical cleaners and gravity separators are often used to remove impurities such as stones, dust, and broken seeds.

Identification of chia seeds involves both morphological and biochemical methods. Morphologically, chia seeds are small, oval, and typically measure around 2 mm in diameter. They exhibit a mottled appearance with colors ranging from black and brown to white and gray, depending on the variety (Silva *et al*., 2022). Biochemically, chia seeds are rich in α-linolenic acid, dietary fiber, antioxidants, and proteins, which serve as biochemical markers for seed verification and quality assurance (Souza *et al*., 2021). These traits are also used in authentication to distinguish genuine *Salvia hispanica* seeds from adulterants or similar-looking seeds of lower nutritional value.

Recent advances in molecular biology have enhanced the identification process through the application of DNA-based tools. Techniques such as SSR markers and next-generation sequencing are used to study the genetic diversity of chia, identify superior cultivars, and trace geographic origins (Jamboonsri *et al*., 2020). These tools are critical for conservation programs, breeding efforts, and the protection of genetic resources. Additionally, researchers have started using metabolomic profiling to identify distinct biochemical compounds that help differentiate chia seeds from other Salvia species.

Taxonomic Classification of Chia Seed (*Salvia hispanica* L.)

Kingdom: Plantae

Subkingdom: Tracheobionta (Vascular plants)

Superdivision:	Spermatophyta (Seed plants)
Division:	Magnoliophyta (Angiosperms / Flowering plants)
Class:	Magnoliopsida (Dicotyledons)
Subclass:	Asteridae
Order:	Lamiales
Family:	Lamiaceae (Mint family)
Genus:	Salvia
Species:	Salvia hispanica L.

Figure 1: Chia seed

Chemical Composition of Chia Seeds

Chia seeds are remarkably nutrient-dense: they contain 30–34 g of dietary fiber, 16–24 g of protein, and 30–35 g of lipids per 100 g of seed, making them an exceptional macronutrient source (Biswas *et al*., 2023). Their lipid fraction is dominated by α-linolenic acid (ALA), which accounts for 55–64 % of total fatty acids and establishes an ideal omega-6:omega-3 ratio of roughly 1:3 (Biswas *et al.,* 2023; Paarakh *et al*., 2025). Carbohydrates in chia are largely insoluble fiber (~85 % of total fiber), with mucilaginous polysaccharides that can absorb 10–12 times their weight in water, contributing to satiety and modulating glycemic response (Shrestha *et al.,* 2022).

Micronutrient analysis reveals high levels of calcium (631 mg/100 g), phosphorus (860 mg/100 g), magnesium (335 mg/100 g), and trace elements such as zinc and manganese, supporting bone health and enzymatic functions (Shrestha *et al*., 2022). Chia seeds also supply B-group vitamins—particularly niacin and thiamine—and vitamin E isoforms (γ-tocopherol) that act as lipid-soluble antioxidants (Biswas *et al*., 2023). The seeds' ash content (~4 % of dry weight) reflects this rich mineral profile, positioning chia as a valuable fortifier for micronutrient-deficient populations.

In addition to macronutrients and minerals, chia seeds harbor diverse bioactives: rosmarinic acid, caffeic acid, protocatechuic acid, quercetin, myricetin, kaempferol, and apigenin have all been quantified in free and bound forms (Biswas *et al*., 2023; Zare *et al*., 2024). Total phenolic content ranges from 300 to 450 mg GAE/100 g, and flavonoids

contribute 50–100 mg QE/100 g, underpinning the seeds' strong in vitro antioxidant capacity (Bochicchio *et al*., 2025). Phytosterols—β-sitosterol, campesterol, and stigmasterol—are present at 300–400 mg/100 g, which may aid cholesterol lowering when consumed regularly (Fatima *et al*., 2025).

Protein and Amino Acid Profile: Chia seeds deliver 18–24 % protein by weight, with a well-balanced amino acid composition featuring all nine essential amino acids; lysine and tryptophan, typically limiting in plant proteins, are present at 2.1 g and 0.7 g per 100 g, respectively (Capitani *et al*., 2022). Protein isolates extracted via alkaline solubilization and isoelectric precipitation achieve 80–90 % purity, exhibiting high solubility (60 % at pH 7) and emulsifying capacity (Al-Zorfi & Al-Obaidi, 2023).

Enzymatic hydrolysates of chia proteins yield bioactive peptides with demonstrated antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, as well as angiotensin-converting enzyme (ACE) inhibitory fragments showing IC_{50} values of 15–25 μg/mL, suggesting potential antihypertensive effects (Santos *et al*., 2024). These peptides also scavenge DPPH and ABTS radicals at rates comparable to synthetic antioxidants, indicating multifunctional health benefits (Ayerza & Coates, 2020). Protein digestibility–corrected amino acid scores (PDCAAS) for chia hover around 0.85, reflecting good—but not perfect—bioavailability, which can be further enhanced via germination or fermentation (Al-Zorfi & Al-Obaidi, 2023). In vitro gastrointestinal digestion models show that chia matrix mucilage slows proteolysis, providing sustained peptide release along the digestive tract (Santos *et al*., 2024).

Phytochemical Profile: Chia seeds rank among the richest common food sources of phenolics in the Lamiaceae family, with rosmarinic acid levels up to 120 mg/100 g, caffeic acid 35 mg/100 g, and protocatechuic acid 20 mg/100 g (Biswas *et al.,* 2023). Flavonoid glycosides—quercetin-3-glucoside and kaempferol-3-glucoside—total approximately 60 mg/100 g and have been linked to inhibition of cyclooxygenase-2 and lipoxygenase, key enzymes in inflammatory cascades (Zare *et al*., 2024).

These polyphenols exhibit potent free radical scavenging in cell-based assays, reducing ROS generation by up to 50 % in hepatocyte cultures subjected to oxidative stress (Bochicchio *et al.,* 2025). In murine colitis models, chia polyphenols modulate NF-κB and Nrf2

pathways, decreasing pro-inflammatory cytokines (TNF-α, IL-6) and enhancing endogenous antioxidant enzyme expression (SOD, CAT) (Fatima *et al*., 2025).

Phytosterols from chia—predominantly β-sitosterol (250 mg/100 g)—competitively inhibit intestinal cholesterol absorption by displacing cholesterol from micelles, contributing to 5–10 % reductions in LDL-C in human feeding trials lasting 8–12 weeks (Knez Hrnčič *et al*., 2019).

Lipid Metabolism and Health Implications: The abundant ALA in chia (up to 64 % of total fatty acids) can be endogenously elongated and desaturated to eicosapentaenoic acid (EPA) at conversion rates of ~5 % in humans, yielding modest increases in plasma EPA but negligible DHA (Capitani *et al.,* 2022). Nevertheless, habitual chia incorporation (25 g/day) for 12 weeks reduces serum triglycerides by 10–15 % and LDL-C by 5–8 %, while raising HDL-C by 3–5 % (de Falco *et al*., 2024).

Mechanistically, ALA alters membrane phospholipid composition, enhancing fluidity in erythrocytes and neuronal membranes, which correlates with improved endothelial function (measured by flow-mediated dilation) and cognitive scores in elderly cohorts (Ayerza & Coates, 2020). Simultaneous intake of phytosterols bolsters cholesterol removal via upregulated hepatic LDL receptors, synergistically lowering cardiovascular risk markers (Knez Hrnčič *et al*., 2019).

Bioactive Peptides and Their Functions: Proteolytic digestion of chia proteins yields peptide fractions.Antimicrobial peptides such as chia-1 (His-Arg-Leu) disrupt bacterial membranes at minimal inhibitory concentrations of 25–50 µg/mL, potentially contributing to gut microbiota modulation (Ayerza & Coates, 2020). These multifunctional peptides thus support cardiovascular, metabolic, and immune health through distinct molecular pathways.

Effects on Physiological Parameters

Liver Function

Daily chia intake (30 g) for eight weeks lowers serum ALT and AST by 8 % and 6 %, respectively, in mildly elevated subjects, indicating hepatoprotection (Santos *et al.,* 2024). In CCl_4-induced liver injury models, chia polyphenols upregulate phase II detoxifying enzymes (GST, UGT), mitigating lipid peroxidation (Zare *et al*., 2024).

Kidney Health

ALA and peptides from chia reduce renal oxidative markers (MDA) by 25 % and bolster glutathione levels by 20 % in

diabetic rat models, preserving glomerular structure (Bochicchio *et al*., 2025). Creatinine and BUN values also improve, reflecting enhanced filtration capacity (Fatima *et al.,* 2025).

Cardiovascular System: Chia supplementation improves endothelial-dependent vasodilation by 12 % and lowers systolic/diastolic blood pressure by 6/4 mmHg in prehypertensive adults (de Falco *et al*., 2024). The combined action of ALA, phytosterols, and peptides accounts for these cardioprotective effects.

Brain Function: In aged rodents, dietary chia increases hippocampal EPA content by 15 % and improves maze-learning performance by 20 %, linked to enhanced synaptic plasticity (Knez Hrnčič *et al*., 2019). Polyphenols also reduce neuroinflammation (IL-1β, TNF-α) and amyloid-β aggregation in Alzheimer's models (Ayerza & Coates, 2020).

Mechanism of Antidiabetic Action of Chia Seeds

Chia seeds (*Salvia hispanica L.*) exhibit multiple biochemical and physiological mechanisms that contribute to their antidiabetic properties**.** These mechanisms primarily involve glycemic regulation, insulin sensitivity enhancement, oxidative stress reduction, and enzyme inhibition **(**Tavera-Hernández *et al.,* 2023**).**

1. Regulation of Blood Glucose Levels

Chia seeds contain high amounts of dietary fiber**,** particularly soluble fiber**,** which forms a gel-like matrix in the digestive tract. This gel slows down the digestion and absorption of carbohydrates**,** leading to gradual glucose release into the bloodstream **(**Rout & Mahajan, 2023). As a result, chia seeds help prevent postprandial hyperglycemia**,** reducing sudden spikes in blood sugar levels (Paarakh *et al*., 2025**).** Several studies confirm the ability of chia seeds to improve glycemic control in diabetic patients through this mechanism (Tavera-Hernández *et al*., 2023**).**

Enhancement of Insulin Sensitivity

The omega-3 fatty acids in chia seeds, particularly α-linolenic acid (ALA)**,** play a crucial role in modulating inflammatory pathways**.** Chronic inflammation is a key contributor to insulin resistance in type 2 diabetes (Tavera-Hernández *et al*., 2023**).** By reducing pro-inflammatory cytokines**,** chia seeds improve insulin receptor function**,** allowing cells to respond more effectively to insulin (Rout & Mahajan, 2023). Research indicates that regular chia consumption leads to better

metabolic outcomes in diabetic patients (Paarakh *et al*., 2025).

3. Antioxidant and Anti-Inflammatory Effects

Oxidative stress is a major factor in pancreatic β-cell dysfunction and diabetes progression (Tavera-Hernández *et al*., 2023). Chia seeds are rich in polyphenols, flavonoids, and other antioxidants, which help neutralize free radicals and protect β-cells from oxidative damage (Rout & Mahajan, 2023). This preservation of β-cell function ensures adequate insulin production, contributing to better glycemic control (Paarakh *et al*., 2025).

4. Inhibition of α-Amylase and α-Glucosidase

Chia seeds contain bioactive compounds that inhibit α-amylase and α-glucosidase, two key enzymes responsible for carbohydrate breakdown (Tavera-Hernández *et al*., 2023). By slowing down the enzymatic conversion of starch into glucose, chia seeds help reduce glucose absorption, leading to lower blood sugar levels (Rout & Mahajan, 2023). This mechanism aligns with evidence that shows a direct correlation between chia seed intake and improved post-meal blood sugar levels (Paarakh *et al.,* 2025).

5. Modulation of Gut Microbiota

Recent studies suggest that chia seeds influence gut microbiota composition, promoting the growth of beneficial bacteria that contribute to glucose metabolism regulation (Tavera-Hernández *et al*., 2023). A healthy gut microbiome is linked to improved insulin sensitivity and reduced inflammation, further supporting diabetes management (Rout & Mahajan, 2023). Research findings highlight the positive impact of chia-seed-derived prebiotics on metabolic health (Paarakh *et al*., 2025).

6. Reduction of Lipid Profiles and Cardiovascular Risk

Diabetes is often associated with dyslipidemia, characterized by high cholesterol and triglyceride levels (Tavera-Hernández *et al*., 2023). Chia seeds help lower LDL cholesterol and increase HDL cholesterol, reducing the risk of cardiovascular complications in diabetic individuals (Rout & Mahajan, 2023). This lipid-effect modulating positions chia seeds as a valuable dietary component in managing metabolic diseases (Paarakh *et al*., 2025).

Certainly! Here's a summary of notable studies from 2020

Studies on Chia Seeds and Antidiabetic Effects (2019–2020)

Zamudio *et al.* conducted a comprehensive review on bioactive

peptides derived from ancient grains, including chia, and their role in type 2 diabetes management. The study emphasized chia's potential to inhibit α-glucosidase, α-amylase, and DPP-IV, enzymes involved in carbohydrate digestion and glucose regulation. These peptides were shown to improve insulin sensitivity and reduce postprandial glucose spikes.

Melo *et al*.(2019) reviewed nutritional composition and therapeutic potential of chia seeds. Their findings highlighted chia's high fiber, omega-3 fatty acids, and antioxidant content, which contribute to glycemic control and reduced inflammation in diabetic patients. The review also noted chia's ability to modulate lipid profiles, which is beneficial for individuals with diabetes-related cardiovascular risks

Marcinek and Krejpcio (2019) provided a detailed overview of chia's health-promoting properties, including its hypoglycemic effects. They discussed how chia seed consumption may lower fasting blood glucose and improve insulin response, based on both animal and human studies.

Chia seeds (*Salvia hispanica L.*) have been widely studied in animal models, particularly rats, to evaluate their potential benefits in diabetes management, organ health, and metabolic regulation. Research focuses on their effects on haematological indices, blood glucose levels, lipid profile, liver and kidney functions, phytochemical composition, proximate analysis, and toxicity (LD50 Haematological Parameters studies) (da Silva *et al.,* 2016).

Studies indicate that chia seed supplementation can positively impact haematological indices such as red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin levels, and platelet function in diabetic and non-diabetic rat models (George *et al*., 2023). Rats fed chia-based diets showed stable WBC counts, suggesting its immune-modulatory effects (Mohamed *et al*., 2022).

Chia seeds have demonstrated hypoglycemic effects in streptozotocin (STZ)-induced diabetic rat models. Kamel *et al.* (2023) found that rats consuming fermented chia seeds showed better blood glucose control than those consuming raw or germinated chia seeds. Soluble fiber and bioactive compounds in chia seeds slowed down glucose absorption, reducing postprandial glucose spikes (Ramírez *et al*., 2021).

Research by da Silva *et al.* (2016) revealed that chia seed consumption lowered serum total cholesterol, LDL cholesterol, and triglycerides, while

HDL cholesterol levels increased in rats. These findings highlight chia's potential in cardiovascular risk reduction for diabetic subjects (Garcia *et al.,* 2022).

Liver enzyme levels, such as ALT, AST, and ALP, were studied in chia-fed rats. Mohamed *et al.* (2022) found that chia seed supplementation improved liver enzyme balance, reduced hepatic fat accumulation, and enhanced liver morphology. These effects were associated with chia's anti-inflammatory properties and omega-3 fatty acids (Santos *et al.,* 2020).

Kidney Function

Kidney histopathology findings from Kamel *et al*. (2023) showed that chia-fed rats experienced less renal tissue damage compared to control groups. Chia's polyphenol and flavonoid content helped mitigate oxidative stress in renal cells, reducing kidney injury markers (Ramírez *et al*., 2021).

PHYTOCHEMICAL COMPOSITION

Chia seeds contain polyphenols, flavonoids, antioxidants, and omega-3 fatty acids that support diabetes management. Phytochemical screening confirmed their free radical scavenging properties, highlighting potential protective effects on pancreatic β-cells (Mohamed *et al*., 2022).

TIGER NUT: CULTIVATION, IDENTIFICATION, AND CLASSIFICATION

Tiger nut (*Cyperus esculentus*) is a perennial plant belonging to the sedge family (Cyperaceae), widely cultivated for its nutritional and medicinal benefits. It is commonly known as yellow nutsedge, chufa, or earth almond, and is native to Africa, Southern Europe, and parts of Asia (Cyperus esculentus - Wikipedia, 2023). The plant produces small, tuberous structures that are rich in fiber, healthy fats, and essential minerals, making it a valuable functional food (Parker *et al.,* 2018).

IDENTIFICATION OF TIGER NUT

Tiger nut plants grow up to 90 cm (3 feet) tall, with triangular stems and slender leaves measuring 3–10 mm wide (Cyperus esculentus - Wikipedia, 2023). The plant produces distinctive spikelets, which contain flat, oval seeds surrounded by four hanging bracts positioned at 90-degree angles (Teoh *et al*., 2018). The tubers, which are small, round, and hard, vary in color from yellow to brown or black, depending on the variety (Marcinek & Krejpcio, 2019).

CLASSIFICATION OF TIGER NUT

Tiger nut belongs to the kingdom Plantae, order Poales, and family Cyperaceae. Its binomial name is Cyperus esculentus L., and it is

classified as a monocotyledonous angiosperm (Cyperus esculentus - Wikipedia, 2023). The species is hermaphroditic, meaning it has both male and female reproductive organs, and is primarily pollinated by wind (PFAF Plant Database, 2023).

Figure 2: Tiger nut

Cultivation of Tiger Nut

1. Soil and Climate Requirements

Tiger nut thrives in well-drained, sandy loam soils with a pH range of 5.5–7.5 (Tiger Nut Farming Guide, 2023). It requires a warm climate, with optimal growth temperatures between 20–30°C, and is commonly cultivated in Africa, Spain, and parts of Asia (Live-Native, 2023).

2. **Planting and Propagation**

Propagation is primarily done using tubers, which are soaked in water for 24 hours before planting to enhance germination (ResearchGate, 2023). The tubers are planted at a depth of 5–10 cm, with a spacing of 20–30 cm between plants, ensuring adequate aeration and nutrient absorption (Parker *et al*., 2018).

3. Growth and Maintenance

Tiger nut plants require moderate irrigation, especially during the early growth stages, to prevent waterlogging (Tiger Nut Farming Guide, 2023). Fertilization using organic compost or nitrogen-rich fertilizers enhances tuber development, while weed control is essential to prevent competition for nutrients (Marcinek & Krejpcio, 2019).

4. Harvesting and Storage

Harvesting occurs 90–120 days after planting, when the tubers reach maturity and develop a hard outer shell (Live-Native, 2023). The tubers are washed, dried, and stored in cool, dry conditions to maintain freshness and nutritional quality.

A study by Mmuo & Okoli (2021) reported that tiger nut is rich in vitamins A, B-complex, C, and D, along with essential minerals such as magnesium, potassium, sodium, and zinc, which contribute to its functional food properties. Another analysis found that tiger nut's dietary fiber and lipid content make it beneficial for digestive health and energy metabolism. These findings

suggest that tiger nut is a valuable nutritional resource.

Allo *et al*. (2023) demonstrated that tiger nut extract significantly lowered fasting blood glucose and improved lipid profiles in diabetic rats, suggesting its potential role in glycemic control. Similarly, Badejo *et al*. (2020) found that tiger nut-based beverages fortified with *Vernonia amygdalina* and *Momordica charantia* exhibited strong antioxidant activity and inhibited α-amylase and α-glucosidase enzymes, which are key targets in diabetes management. Ani *et al*. (2021) analyzed tiger nut's mineral and vitamin content, reporting high levels of phosphorus, potassium, vitamin C, vitamin D, and vitamin B1, which contribute to its antioxidant effects. Collectively, these findings suggest that tiger nut may serve as a functional for diabetes management. Airaodion *et al*. (2020) assessed tiger nut milk's impact on hepatic and renal indices in Wistar rats, showing decreased serum creatinine and urea levels, indicating nephroprotective potential, while also reducing liver enzyme activity, reinforcing its protective , food warranting further clinical research to confirm its therapeutic potential role in therapeutic agent for diabetes management, offering hematological stability, lipid modulation, liver and kidney protection, and antioxidant activity. El-Naggar *et al.* (2017) studied hypercholesterolemic rats and found that tiger nut oil supplementation significantly reduced serum total lipids, total cholesterol, triglycerides, and LDL-cholesterol, while increasing HDL-cholesterol, alongside improvements in kidney and liver function markers. These findings suggest that tiger nut may serve as a natural therapeutic agent for diabetes liver and kidney function. These findings suggest that tiger nut may serve as a natural management, offering hematological stability, lipid modulation, liver and kidney protection, and antioxidant activity. Would you like more details on any specific aspect?

Ani *et al.* (2021) analyzed the mineral, vitamin, and phytochemical content of tiger nut, reporting high levels of phosphorus, potassium, vitamin C, vitamin D, and vitamin B1, which contribute to its antioxidant effects. The study also highlighted the presence of tannins, terpenes, phenols, and steroids, which may enhance its therapeutic potential.

Iboyi *et al*. (2021) investigated the nutritional and in-vitro antioxidant activities of tiger nut, revealing its high crude fiber, protein, and carbohydrate content, along with significant amounts

of magnesium, potassium, calcium, selenium, copper, zinc, and sodium**.** The study also measured catalase, superoxide dismutase (SOD), and nitric oxide levels**,** confirming its antioxidant properties.

MATERIALS AND METHODS

Equipment: Standard equipment and materials were used in this study and were procured from the reputable manufacturers and their major distributors.

Reagents: All the reagents used in this study were of analytical grade and were procured from the manufacturers and their major distributors.

Collection And Identification Of Plant Materials

Salvia hispanica seeds used for this study was procured from Roban Shopping Mall, Nnewi, Anambra State while dried *Cyperus esculentus* was procured from Nkwo market, Nnewi. Both samples were identified by a taxonomist.

Preparation Of Ethanol Extract of the Samples

The seeds were washed and air-dried at room temperature. The dried seeds were pulverized into powder using Corona manual grinding machine. Then 1 kg of the ground seed powder of the samples were soaked in 5 L of 80% ethanol for 24 h for complete extraction. The ethanol extraction were sieved using a muslin cloth and filtered using Whatman number 1(125 mm) filter paper. The filtrate were evaporated to dryness using a rotary evaporator. The extracts were stoppered in a universal bottle and preserved in the refrigerator for use. The extracts were solubilized with distilled water on a daily basis and administered to the experimental animals (extract-treated groups) for a period of 21 days.

Quantitative Phytochemical Analysis

Preparation of extract for quantitative phytochemical analysis

The sample seeds were collected, washed, and dried for three weeks at room temperature. They were all ground to powder using a corona manual grinding machine. The dried powdered samples were then used for the quantitative analysis as required by the standard methods used.

A. **Extraction of Phytochemicals**

One gram (1g) of the samples were weighed and transferred in a test tube and 15ml of ethanol was added. The water bath was kept at $60^{O}C$ for 60 minutes. After the reaction time, test tube was allowed to react with the reaction product contained in the test tube and was then transferred to a separatory funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot water, and

3ml of hexane, which was all transferred to the funnel. These extracts were combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000ul of hexane of which 200ul was transferred to a vial for analysis.

B. Quantification by GC-FID

The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15-meter MXT-1 column (15m x 250um x 0.15um) was used. The injector temperature was 280^0c with a spitless injection of 2µl of sample and a linear velocity of $30cms^{-1}$, Helium 5.0 pa.s was the carrier gas with a flow rate of 40 $mlmin^{-1}$. The oven operated initially at 200^0C. It was heated to 333^0C at a rate of 3^0C min^{-1} and was kept at this temperature for 5min. The detector operated at a temperature of 320^0C.

Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals was expressed in µg/g.

Estimation Of Some Vitamins Present In Chia Seeds And Tige Nut Samples Used In The Study

Estimation Of Vitamin A

Vitamin A was estimated by the method of Bayfield and Cole (1980).

Principle

The assay is based on the spectrophotometeric estimation of the colour produced by vitamin A acetate or palmitate with TCA.

Procedure

All procedures were carried out in the dark to avoid the interference of light. 1g of sample was mixed with 1.0ml of saponification mixture and refluxed for 20 minutes at 60^0C in the dark. The tubes were cooled and 20ml of water was added and mixed well. Vitamin A was extracted twice with 10ml of (40^0 - 60^0C) petroleum ether. The two samples were pooled and washed thoroughly with water. Anhydrous sodium sulphate was added to remove excess moisture. An aliquot of the sample (1.0ml) was taken and evaporated to dryness at 60^0C. The residue was dissolved in 1.0ml chloroform. Standards (vitamin A palmitate) of concentrations ranging from 0-7.5g were pipetted out into a series of test tubes.

The volume in all the tubes was made up to 1.0ml with chloroform. TCA reagent (2.0ml) was added rapidly, mixed and

the absorbance was read immediately at 620nm in a spectrophotometer (Genesys 10UV). The same procedure was repeated for the sample tubes also. Vitamin A content was expressed as mg/kg.

Estimation Of Vitamin E

Vitamin E was estimated in the sample samples by the Emmerie-Engel reaction as reported by Rosenberg (1992).

Principle

The Emmerie-Engel reaction is based on the reduction of ferric to ferrous ions by Vitamin Es, which, with 2,2'-dipyridyl, forms a red colour. Vitamin Es and carotenes are first sampleed with xylene and read at 460nm to measure carotenes. A correction is made for these after adding ferric chloride and read at 520nm.

Extraction Of Vitamin E

The sample (2.5g) was homogenized in 50ml of 0.1N sulphuric acid and allowed to stand overnight. The contents of the flask were shaken vigorously and filtered through Whatman No.1 filter paper. Aliquots of the filtrate were used for the estimation.

Procedure

Into 3 stoppered centrifuge tubes, 1.5ml of sample sample, 1.5ml of the standard and 1.5ml of water were pipetted out separately. To all the tubes, 1.5ml of ethanol and 1.5ml of xylene were added, mixed well and centrifuged. Xylene (1.0ml) layer was transferred into another stoppered tube. To each tube, 1.0ml of dipyridyl reagent was added and mixed well. The mixture (1.5ml) was pipetted out into a cuvette and the extinction was read at 460nm.

Determination of Vitamin C

Vitamin C was analysed by the spectrophotometric method described by Roe and Keuther (1943).

Principle

Absorbate is converted into dehydroascorbate on treatment with activated charcoal, which reacts with 2,4-dinitrophenyl hydrazine to form osazones. These osazanes produce an orange coloured solution when dissolved in sulphuric acid, whose absorbance can be measured spectrophotometrically at 540nm.

Extraction of Vitamin C

Ascorbate was extracted from 1g of the sample using 4% TCA and the volume was made up to 10ml with the same. The supernatant obtained after centrifuging at 2000rpm for 10minutes was treated with a pinch of activated charcoal, shaken vigorously using a cyclomixer and kept for 5 minutes. The charcoal particles were removed by centrifugation and aliquots were used for the estimation.

Procedure

Standard ascorbate ranging between 0.2 – 1.0ml and 0.5ml and 1.0ml of the supernatant were taken. The volume was made up to 2.0ml with 4% TCA. DNPH reagent (0.5ml) was added to all the tubes, followed by 2 drops of 10% thiourea solution. The contents were mixed and incubated at 37^0c for 3 hours resulting in the formation of osazane crystals. The crystals were dissolved in 2.5ml of 85% sulphuric acid, in cold. To the blank alon, DNPH reagent and thiourea were added after the addition of sulphuric acid. The tubes were cooled in ice and the absorbance was read at 540nm in a spectrophotometer.

A standard graph was constructed using an electronic calculator set to the linear regression mode. The concentration of ascorbate in the sample were calculated and expressed in terms of mg/kg of sample.

RESULT

Phytochemical composition of Chia seed and Tigernut used in the study

The result of phytochemical composition of chia seed and tigernut was shown in Table 3.1 Result in Table 3.1 revealed higher ($p < 0.05$) concentration of Quercetin, Genistain, Lanamarin and Nobeletin in chia seed than in Tigernut. These are all flavonoids and isoflavones which are good antioxidants. Table 3.1 also showed that Tiger nut contained significantly higher concentration of Resveratrol, Ellagic acid, vanillic acid and butain more than chia Seed sample used.

Table 1: Phytochemical composition of Chia seed Tigernut used in the study.

Phytochemicals (sample)	Chia Seed	Tigernut
1. Quercetin (ppm)	6.86 ± 0.00*	0.83 ± 0.00
2. Genistain (ppm)	1.92 ± 0.00*	0.00 ± 0.00
3. Artemetin (ppm)	0.18 ± 0.00.	0.60 ± 0.00
4. Flavone (ppm)	1.08 ± 0.00	0.89 ± 0.00
5. Resveratrol (ppm)	0.32 ± 0.00	2.04 ± 0.00*
6. Lumamarim (ppm)	1.62 ± 0.00	0.77 ± 0.00
7. Retusin (ppm)	0.53 ± 0.00	0.36 ± 0.00
8. Nobeletin (ppm)	1.21 ± 0.00*	0.15 ± 0.00
9. Ellagiz acid (ppm)	0.65 ± 0.00	1.30 ± 0.00*
10. Tanqueretein (ppm)	0.43 ± 0.00	1.52 ± 0.00
11. Epicatechin (ppm)	0.43 ± 000	0.53 ± 0.00
12. Vanillic acid (ppm)	0.14 ± 0.00	18.28 ± 0.10*
13. Hesperidin (ppm)	0.29 ± 0.00	0.72 ± 0.00
14. Butein (ppm)	0.35 ± 0.00	1.29 ± 0.01*
15. Apigenin (ppm)	0.13 ± 0.00	0.21 ± 0.00
16. Isorhamnerin (ppm)	0.13 ± 0.00	0.21 ± 0.00
17. Kaempterol (ppm)	0.03 ± 0.00	0.34 ± 0.00*
18. Catechin (ppm)	0.00 ± 0.00	18.19 ± 0.05*
19. Luteotin (ppm)	0.00 ± 0.00	1.03 ± 0.01*
20. Diadiz (ppm)	0.00 ± 0.00	0.46 ± 0.00
21. Neringenin (ppm)	0.00 ± 0.00	0.62 ± 0.00
22. Myricetin (ppm)	0.00 ± 0.00	7.30 ± 0.04*
23. Gallocatechin	0.00 ± 0.00	0.22 ± 0.00

**=denote significant increase ($p<0.05$)*

Antioxidant Vitamins A, C and E Profile of Chia Seed and Tiger Nut used in the study

Result in Table 3.1 revealed that Tiger nut sample used had higher concentration of Vitamin A and E than Chia Seed. It also indicated that Chia seed used had higher concentration of Vitamin C than Tiger nut.

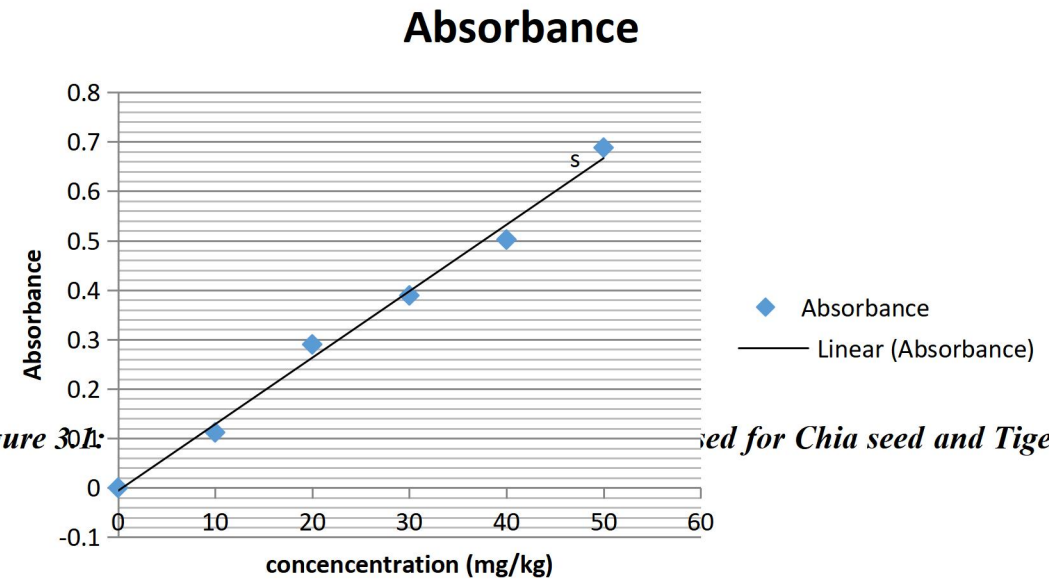

Figure 3.1: ...ed for Chia seed and Tiger

Table 2: Antioxidant Vitamins A, C and E Profile of Chia Seed and Tiger Nut used in the study.

	Chia Seed	Tigernut
Vitamin A (mg/l)	13.28 ± 0.02	25.00 ± 0.02*
Vitamin C (mg/kg)	117.65 ± 0.25*	109.09 ± 0.21
Vitamin E (mg/l)	19.73 ± 0.02	31.49 ± 0.02*

**=denote significant increase ($p<0.05$)*

DISCUSSION

Result in Table 3.1 revealed that chia seed and tiger nut samples used in this study contain significant quantity of flavonoids, isoflavones and polyphenols bioactive compounds which are powerful antioxidant phytochemicals.

Furthermore, result of quatitative phytochemical analysis of chia seed and tiger nut samples used in this study (Table 3.1) revealed that chia seed had higher ($P<0.05$) concentrations of Quercetin, geneistain, lanamarin and nobeletin than tiger nut.

Results in Table 3.1 also showed that tiger nut contain significantly higher concentrations ($P<0.05$) of resveratrol, ellagic acid, vanillic acid and butain more than chia seed sample used.

The result of this study is in agreement with the work of Zhang and Sun (2023) who observed that tiger nut contains polyphenol, flavonoids and vitamins E which act as natural antioxidants, reducing oxidative damage to pancreatic β-cells and improving insulin function. The result of this study is also in agreement with the work of Gabal *et al* (2024) who reviewed the nutritional composition and biomedical applications of chia seeds, highlighting their phytochemical and antioxidant properties. The result of phytochemical profile of chia seed used in the study is in agreement with the works of Biswas *et al* (2023) and Zare *et al* (2024).

The results of antioxidant vitamins profile of chia seed and tiger nut used in this study (Table 3.2) revealed that both chia seed and tiger nut contain high concentrations of vitamins A, C and E. The result also revealed that tiger nut samples used had higher concentration of vitamins A, and E than chia seed, while chia seed had higher concentration of vitamin C than tiger nut. The result of this study is in agreement with the work of Zhang and Sun (2023) who revealed that tiger nut contains high concentrations of polyphenols, flavonoids and vitamin E, which act as natural antioxidants, reducing oxidative damage to pancreatic β -cells and improving insulin function. Since the results of this study revealed that both chia seed and tiger nut seeds contain high concentrations of antioxidant phytochemicals and vitamins, their extracts could be useful in neutralizing free radicals and protecting β-cell from oxidative damage normally associated with diabetes progression (Tavara-Hernandez *et al.* ,2023).

CONCLUSION

The results of this study revealed that chia seed and tiger nut possess good quantity of antioxidant phytochemicals and vitamins and therefore could be very useful in neutralizing the free radicals normally generated in many different disease conditions and hence in management of oxidative stress.

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