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Phenolic and Anti-Nutrients Compositions of Selected Underutilized Seeds: African Oil Bean Seed (*Pentaclethra macrophylla*), Cashew Nut Seed (*Anacardium occidentale*), Cucumeropsis Seed (*Cucurbita maxima*), Groundnut Seed (*Arachis hypogeal*) and Soya Bean Seed (*Glycine max*)

[1]Augustine Ogbonna Akpuru (Lead/Main Author)
Department of Biochemistry
University of Nigeria, Nsukka, Nigeria

[2]Livinus Ifeanyi Anyanwu (Co-Author)
University of Tulsa, USA

[3]Suzy Lewechi Ik-Ukennaya (Co-Author)
Lagos State University, Nigeria

[4]Chika Happiness Orji (Co-Author)
University of Nigeria, Nsukka

[5]Annastacia Gbemudu Vincent (Co-Author)
University of Uyo, Nigeria

Abstract:- The study aimed to assess the levels of anti-nutrients and phenolic compounds in under-utilized seeds such as African oil bean seed (Pentaclethra macrophylla), cashew nut seed (Anacardium occidentale), cucumeropsis seed (Cucurbita maxima), groundnut seed (Arachis hypogeal), and soya bean seed (Glycine max). The goal was to understand the impact of these compounds on the nutritional value of the seeds and to identify potential preventive measures. Neglected and under-utilized crops are plant species that have historically been used for food, fiber, fodder, oil, or medicinal purposes but have diminished in importance over time due to specific supply and utilization constraints. These constraints may include poor shelf life, unrecognized nutritional value, low consumer awareness, and reputational issues (such as being considered famine food or "poor people's food," often due to changes in agricultural practices). Anti-nutrients are substances commonly present in food that can be harmful to humans and can limit the availability of nutrients to the body. Sun-dried soya bean and groundnut seeds were ground, while African oil bean, cucumeropsis, and cashew nut seeds were mechanically dehulled and ground without heat. Oxalate and phytate levels were determined through titration, and carotenoid levels were determined spectrophotometrically. Various methods were used to determine the presence of tannins, trypsin inhibitors, cyanogenic glycosides, hemagglutinins, saponins, alkaloids, phenols, steroids, and flavonoids. It was found that African oil bean seed had higher concentrations of the analyzed anti-nutrients and phenolic compounds compared to the other seeds studied.

Keywords:- *Antinutrients, Phenolic Compounds, Underutilized Seeds, Nutritional Value, Oxalate and Phytate Levels, Titration and Spectrophotometry.*

I. INTRODUCTION

A. Overview of Under-Utilized Seeds

In today's industrial landscape, along with emerging health challenges and issues related to climate change, there is an increasing need for innovative solutions on a global scale. The patterns of industrial development and the health challenges that have emerged in the last five decades have made it essential to devise new strategies for addressing previously unforeseen difficulties. This has also emphasized the importance of biological diversity in development patterns. While the exact number of species on Earth is still unknown, approximately 1.75 million species have been identified (CBD, 2000). Despite some species being extensively developed and utilized, the majority of life forms remain underdeveloped, underexploited, and overlooked (Magbagbeola et al., 2010). Currently, only around 30 plant species are utilized to fulfill 95% of the world's food energy requirements, while a large portion of plant diversity remains untapped and at risk. Certain threatened species play a crucial role in pollution mitigation and ensuring the sustainability of human life, health, and well-being (Aju, 2010). This diverse range of biodiversity represents an invaluable natural resource that forms the basis of human well-being (Aju, 2010). Recent global events have demonstrated the potential for some underutilized species to become valuable crops.

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Neglected and underutilized crops are plant species that have been domesticated for food, fiber, fodder, oil, or medicinal purposes for centuries or even millennia, but have diminished in importance over time due to specific supply and utilization constraints. These constraints include, among other things, poor shelf life, unrecognized nutritional value, limited consumer awareness, and reputational issues (e.g., being considered famine food or "poor people's food," sometimes due to the modernization of agricultural practices). Some crops have been neglected to such an extent that the genetic erosion of their gene pools has become so severe that they are often deemed lost crops (Williams and Haq, 2002). Underutilized seeds encompass African oil bean seed, soybean seed, groundnut seed, cucumeropsis seed, cashew nut, bambara seed, neem seed, dacryodes, pumpkin seed, melon seed, sesame seed, and so on.

The demand for plant and crop characteristics is changing, leading neglected crops to address the barriers to wider production and utilization. Several previously overlooked crops, like Oil palm and Soya bean, are now globally significant. While it may seem that options for expanding neglected seeds for large-scale agriculture are becoming limited, many species have the potential to contribute to food security, nutrition, dietary diversity, health, and income generation, as well as provide environmental benefits. Defining "proper" or "correct" levels of use is challenging, but numerous neglected species are not fully utilized considering their nutritional value and productivity. Just three crops - maize, wheat, and rice - make up approximately 50% of the world's calorie and protein consumption (FAO, 1997). Despite there being at least 12,650 edible species names, about 95% of the world's food needs are met by only 30 plant species. In contrast, many neglected and underutilized plants have the potential to be used for food and other purposes on a larger scale, and are referred to as "minor", "orphan", "promising", or "little-used". Determining the characteristics of an "underutilized" crop is not straightforward, but they typically possess the following traits:

- Linked with the cultural heritage of their places of origin
- Poorly documented local and traditional crops regarding their distribution, biology, cultivation, and uses
- Adapted to specific agroecological niches and marginal land
- Lack or weak formal seed supply systems
- Solely or predominantly used in localized areas
- Grown in traditional production systems with minimal external inputs
- Receive limited attention from research, extension services, policy and decision-makers, donors, technology providers, and consumers
- Might be highly nutritious and/or possess medicinal properties or other multiple uses

Neglected crops are mainly cultivated by traditional farmers and although they may be distributed beyond their original centers, they tend to occupy specific niches in local production and consumption systems. These crops are crucial for the subsistence of local communities but are often overlooked by mainstream research and development efforts. Underutilized crops are marginalized by farmers and consumers due to agronomic, genetic, economic, environmental, and cultural factors and were historically significant in the community. They continue to play a vital role in the subsistence and economy of impoverished people in the developing world, particularly in the agro-biodiversity-rich tropics. Despite their potential for dietary diversity and the provision of micronutrients, such as vitamins and minerals, they receive minimal research and development attention. In addition to their commercial potential, many underused crops also offer significant environmental benefits due to their adaptation to marginal soil and climate conditions.

B. Anti-Nutrients and Phenolic Compounds Present in Most Under-Utilized Seeds

Anti-nutrients or anti-nutritional factors may be defined as those substances generated in natural feedstuffs by the normal metabolism of species and by different mechanisms (for example inactivation of some nutrients, diminution of the digestive process or metabolic utilization of feed) which exert effects contrary to optimum nutrition. Compounds or substances which act to reduce nutrient intake, digestion, absorption, and utilization and may produce other adverse effects are referred to as anti-nutrients or anti-nutritional factors (Akande *et al.*, 2010). Seeds of legumes and other plant sources contain in their raw state, wide varieties of anti-nutrients which are potentially toxic. Being an anti-nutritional factor is not an intrinsic characteristic of a compound but depends upon the digestive process of the ingesting animal. Trypsin inhibitors which are anti-nutritional factors for monogastric animals, do not exert adverse effects in ruminants because they are degraded in the rumen (Cheeke, and Shull, 1985). Many plant components have the potential to precipitate adverse effects on the productivity of farm livestock. These compounds are present in the foliage and seeds of virtually every plant that is used in practical feeding (D'Mello, 2000). The major anti-nutrients mostly found in plant protein sources are saponins, cyanogenic glycosides, tannins, phytic acids, aflatoxin, oxalates, goitrogens, lectins (haemagglutinins), protease inhibitors, trypsin inhibitor and amylase inhibitors.

The planet is home to around 300,000 known species of higher plants, which produce a wide variety of chemicals with diverse structures and classes (over 200,000 distinct chemical entities have been isolated and identified). The compounds can be classified into primary and secondary metabolites. Metabolic compounds can be categorized into primary and secondary types based on their structure and function in plant growth and development (Fiehn, 2002). While primary metabolites such as sugars, fatty acids, amino acids, and nucleic acids are essential for basic photosynthesis and respiratory metabolism, secondary metabolites are more diverse and are found in specialized cells, thought to be crucial for plants' survival in their environment. Plants have metabolic pathways leading to tens of thousands of secondary products that help them respond effectively to various stress factors from their surroundings. These pathways are developed from essential primary metabolic pathways

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through gene duplication, enabling the duplicated genes to perform new functions and play optimized roles in new pathways, thereby contributing to the developmental growth of plants. The accumulation of secondary metabolites often indicates the onset of different developmental stages, and their correct accumulation pattern is ensured through strict spatial and temporal control of gene expression. Additionally, the transport of metabolic intermediates and the regulation of gene expression by ontogeny and the circadian clock play integral roles in plant secondary metabolism (Nascimento and Fett-Neto, 2010).

Secondary metabolites also serve as defense mechanisms against herbivores, microbes, viruses, and competing plants, as well as signaling compounds to attract pollinating or seed-dispersing animals and protect the plant from ultraviolet radiation and oxidants. The pattern of secondary metabolites in plants is complex, varying between tissues and organs, among individuals, and across populations. These secondary metabolites are classified into different groups based on their biosynthetic pathways and structural characteristics (Lattanzio et al., 2008).

Phenolic compounds, one of the most widely distributed secondary metabolites, are present throughout the plant kingdom in various types, depending on the phylum under consideration. Although phenolics are uncommon in bacteria, fungi, and algae, vascular plants contain the full range of polyphenols, and it is estimated that around 2% of all carbon photosynthesized by plants is converted into flavonoids or related compounds (Roberds and Antolovich, 1997). Vascular plants synthesize thousands of different phenolic compounds, many of which are continually being characterized. Phenolic compounds can be found in various forms, such as esters, amides, glycosides of hydroxycinnamic acids, and glycosylated flavonoids. Additionally, some phenolic-containing polymers, like lignin, suberin, and pollen sporopollenin, are present in plants. The distribution of these compounds varies, and many are used as biomarkers for taxonomic studies. Phenolic compounds have been selected through evolution in different plant lineages to address specific needs, enabling plants to adapt to changing environmental challenges over time (Boudet, 2007). For instance, the successful adaptation of certain higher members of the Charophyceae to land was largely achieved through the massive formation of "phenolic UV light screens" (Graham et al., 2000). The phenylpropanoid pathway leading to lignin involves a set of biochemical reactions that were already present in vascular plants 400 million years ago with the emergence of erect vascular land plants. The metabolic backbones have evolved over time to provide specific adaptations to different plant families and the impressive biochemical diversity we observe (Boudet, 2007). These phenolic compounds, which make up about 40% of organic carbon in the biosphere, are formed through the shikimic acid pathway or the malonate/acetate pathway, also known as the polyketide pathway, and related biochemical pathways. The rate-limiting step in recycling biological carbon is the reassimilation of these compounds back to carbon dioxide during biodegradation (mineralization) (Croteau, 2000).

In terms of the definition of plant phenolics, "phenol" is a chemical term that describes a phenyl ring with one or more hydroxyl substituents. The term "polyphenol" could be used to describe natural products featuring at least two phenyl rings with one or more hydroxyl substituents and their functional derivatives, but within the context of plant phenolics, this definition is inadequate. This is because it would include compounds such as gossypol, the phenolic carotenoid 3-hydroxyisorenieratene (I), or the phenolic female sex hormone oestrone (II), which are primarily terpenoid in origin. Plant tissues synthesize a vast array of non-structural constituents that have various roles in plant growth and survival, in addition to playing cell wall structural roles. Therefore, the term "plant phenolics" encompasses a highly diverse group, with several thousand chemically known members with a wide range of identified structures, including monomeric, dimeric, and polymeric phenolics. Various classes of phenolics have been categorized based on their basic skeleton, including simple phenol, benzoquinones, phenolic acids, acetophenone, phenylacetic acid, hydroxycinnamic acid, coumarin, phenylpropanes, chromones, naphthoquinones, xanthones, stilbenes, anthraquinones, flavonoids, isoflavonoids, neoflavonoids, bitriflavonoids, lignans, neolignans, lignins, catechol melanins, and condensed tannins. Low molecular weight phenolics are universally present in higher plants, with some being common across various plant species and others being species-specific. Higher molecular weight proanthocyanidins (also called condensed tannins) are the most abundant polyphenols in woody plants but are typically absent in herbaceous plants. Hydrolysable tannins have a more restricted occurrence than proanthocyanidins, being found in only 15 of the 40 orders of dicotyledons (Veitch and Grayer, 2011).

C. Common Anti-Nutrients and Phenolic Compounds in Plants

It has been established that most plant seeds which serve as sources of protein and nutrients, contain in tandem, anti-nutrients and phenolics in varying degrees. These include:

- *Oxalates*

The name oxalate originates from its presence in wood sorrel, a type of plant known as oxalis. Early interest in oxalate toxicity stemmed from cases of severe or fatal human poisoning after consuming large amounts of leaves from plants such as rhubarb, which contain significant levels of oxalates (Osagie, 1998). Oxalates impact calcium and magnesium metabolism and form complexes with proteins that inhibit peptic digestion. Unlike monogastric animals, ruminants can consume substantial quantities of high-oxalate plants without adverse effects, largely due to microbial breakdown in the rumen. Most seeds' hulls contain oxalates, and meals must be completely processed to avoid toxicities (McDonald et al., 1995). Oxalic acid binds with calcium to create insoluble calcium oxalate, which hampers the absorption and utilization of calcium in animal bodies (Olomu, 1995).

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➢ *Saponins*

Saponins are a diverse group of naturally occurring triterpene or steroidal glycosides found in various plants, including legumes and oilseeds like kidney beans, peanuts, and chickpeas (Jenkins and Atwal, 1994). Research has shown that saponins can impact animal performance and metabolism in several ways: causing red blood cell breakdown, lowering blood and liver cholesterol levels, slowing growth, inducing bloat in ruminants, affecting smooth muscle activity, inhibiting enzymes, and reducing nutrient absorption. Saponins have also been found to change cell wall permeability, leading to toxic effects when consumed (Belmal et al., 1999). Studies have demonstrated that saponins bind to small intestinal cells, thus affecting the absorption of nutrients through the intestinal wall (Johnson et al., 1986). The effects of saponins on young chickens have been observed to impair growth, feed efficiency, and interfere with the absorption of dietary fats, cholesterol, bile acids, and vitamin A (Jenkins and Atwal, 1994).

➢ *Trypsin Inhibitor*

Protease inhibitors can be found throughout the plant kingdom, including in the seeds of most cultivated legumes. These inhibitors can block the activity of proteolytic enzymes in animals' gastrointestinal tracts. Raw legume seeds contain trypsin inhibitor, a type of protease inhibitor. Protease inhibitors are the most commonly encountered anti-nutrients of plant origin. When trypsin inhibitors are present in the diet, they lead to the formation of an irreversible condition called the enzyme-trypsin inhibitor complex. These inhibitors are partially responsible for the growth-retarding property of raw legumes. This retardation is linked to the inhibition of protein digestion, although there is evidence suggesting that pancreatic hyperactivity also plays a role. Trypsin inhibitors have been associated with reduced protein digestibility and pancreatic hypertrophy. They are polypeptides that form stable complexes with trypsin, obstructing its enzymatic action. Protease inhibitors can be activated by heat, especially moist heat, due to the even distribution of heat.

In soybeans, trypsin inhibitors result in inactivation and loss of trypsin in the small intestine, triggering the release of cholecystokinin and reducing pancreatic synthesis of excess trypsin, as well as the burden on the body's sulfur-containing amino acids. The potential beneficial effects of protease inhibitors are still not fully understood, although populations with high intake of soybean and its products have shown lower incidences of pancreatic cancer.

➢ *Phytate*

Phytic acid, also known as inositol hexaphosphoric acid, creates insoluble compounds with crucial minerals such as calcium, iron, magnesium, and zinc in food, making them unabsorbable into the bloodstream (Bingham, 1978). Man excretes approximately half of the phosphorus content of phytic acid unchanged, leaving the rest unavailable for use. Understanding the phytate content can help calculate the amount of available phosphorus out of the total phosphorus in a diet. Phytic acid and its breakdown products are linked to hindering calcification in rats (Robert and Yudkin, 1999).

➢ *Tannins*

Tannins, which are water-soluble phenolic compounds with a molecular weight greater than 500 Daltons, have the ability to precipitate proteins from an aqueous solution. There are two main groups of tannins: hydrolysable tannins and condensed tannins. Condensed tannins are commonly found in leguminous forages and seeds. Cattle and sheep are sensitive to condensed tannins, while goats show greater resistance (D'Mello, 2000). Tannins can form a complex with dietary proteins, affecting their digestibility, and can also bind and inhibit endogenous proteins, such as digestive enzymes (Kumar and Singh, 1984).

The formation of tannin-protein complexes involves both hydrogen bonding and hydrophobic interactions. Protein precipitation and the incorporation of tannin phenolics into the precipitate depend on the pH, ionic strength, and molecular size of the tannin. Both processes increase with an increase in the molecular size of the tannin (Kumar and Horigome, 1986). However, tannins become insoluble and lose their protein-precipitating capacity when their molecular weight exceeds 5,000 Daltons, highlighting the importance of the degree of polymerization in assessing the role of tannins in ruminant nutrition (Lowry, 1990).

Tannins have been found to interfere with digestion by exhibiting anti-trypsin and anti-amylase activity. Hesper et al. (1993) found that condensed tannins were responsible for the testa-bound trypsin inhibitor activity of faba beans. Furthermore, tannins interfere with dietary iron absorption (Rao and Desothale, 1998) and can form complexes with vitamin B12. Tannins cause significant growth depression in rats, potentially due to reduced digestibility of dietary protein and, to a lesser extent, available carbohydrates and lipids (Griffiths, 2000). Other reported adverse nutritional effects of tannins include intestinal damage and the potential for tannins to have a carcinogenic effect (Butler, 1989).

➢ *Cyanogenic Glycosides*

Cyanide, which is a type of cyanogenic glycoside, effectively blocks cytochrome oxidase and disrupts the aerobic respiratory system. When combined with sugar, hydrocyanic acid forms a harmless compound called cyanogenic glycoside (Bolhuis, 2003). The inhibition of the respiratory chain at the cytochrome oxidase level is eliminated through soaking and cooking, making the cyanogenic glycoside content in vegetables and fruits non-toxic.

➢ *Hemagglutinins*

Hemagglutinins, similar to antibodies, are proteins that can agglutinate red blood cells with a high level of specificity, acting strongly on one type of erythrocyte while having little or no effect on another type (Jaffe, 2003). Plant hemagglutinins are known as phytohemagglutinins (PHA), which are also referred to as lectins. Although seeds have the highest concentrations of lectins, their levels in leaves are low due to translocation. The biological effects of hemagglutinins are likely due to their attraction to sugars and their ability to bind to the carbohydrate components of intestinal cells, causing a non-specific disruption in nutrient absorption

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(Liener, 1985). In fodder trees, particularly robin and ricin are notable hemagglutinins. In cattle, the lectin from Robinia pseudoacacia, known as robin, has been identified as the cause of symptoms such as anorexia, lethargy, weakness, and hind limb paralysis (Cheeke and Shull, 1985). Ricin, which is found in castor beans (Ricinus communis), has been documented to induce poisoning in livestock of all classes. Due to ricin, de-oiled castor seed cake is rarely used as livestock feed.

➢ *Carotenoids*

More than 600 naturally occurring pigments called carotenoids are synthesized by plants, algae, and photosynthetic bacteria. These vibrant molecules are responsible for the yellow, orange, and red colors found in many plants (IARC, 1998). The majority of carotenoids in the human diet come from seeds and vegetables. The most common dietary carotenoids include A-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthin. A-carotene, β-carotene, and β-cryptoxanthin are provitamin A carotenoids, which means the body can convert them to retinol. On the other hand, lutein, lycopene, and zeaxanthin cannot be converted to retinol and therefore do not have vitamin A activity. Carotenoids are broadly divided into two categories: carotenes and xanthophylls.

For intestinally absorbed dietary carotenoids, they must first be released from the food matrix and then incorporated into mixed micelles (Yeum and Rusell, 2002). Therefore, the presence of fat in a meal is necessary for carotenoid absorption.

➢ *Alkaloids*

Alkaloids, which mostly consist of basic nitrogen atoms, are a category of naturally occurring chemical compounds (Andreas, 2009). Within this category are compounds with neutral or weakly acidic properties, and some synthetic compounds with a similar structure are also referred to as alkaloids. Alkaloids are present in a variety of seeds and have a wide range of pharmacological effects, such as anti-malarial (e.g. quinine), anti-asthma (e.g. ephedrine), and anti-cancer properties (e.g. homoharringtonine) (Kittakoop et al., 2014), as well as cholinomimetic effects (e.g. galantamine) (Russo et al., 2013), analgesic effects (e.g. morphine) (Raymond et al., 2010), antibacterial properties (e.g. chelerythrine) (Cushnie et al., 2014), and antihyperglycemic activities (e.g. piperine) (Qui et al., 2014).

It is important to note that some alkaloids, such as atropine and tubocurarine, can be toxic, and others have been utilized in entheogenic rituals or as recreational drugs. Additionally, when present in a seed or food substance, alkaloids are typically associated with a bitter taste.

➢ *Phenols*

Substances having a benzene ring, one or more hydroxyl substituents, and their functional derivatives are referred to as phenolic compounds (Waterman and Mole, 1994). There are several known sources of phenols, including sorghum, olive oil, grapes, beans, spices, and herbs (Moure et al., 2001). These substances reduce the oxidation of low-density lipoproteins (LDL), which lowers the risk of heart disease, among their many other beneficial impacts on human health (Bonilla et al., 1999). Numerous phenols with antioxidant qualities, both high and low molecular weight, have been investigated and are suggested for use as antioxidants to prevent lipid oxidation (Moure et al., 2001). It is known that phenolics containing many hydroxyl groups are very useful in stopping lipid oxidation.Furthermore, according to Boure et al. (2001), phenolic compounds are known to have antiviral, antibacterial, antimutagenic, and anticarcinogenic qualities. Based on their size, phenolic chemicals found in plants can be roughly categorized into three primary classes: phenolic acids, flavonoids, and tannins (Scalbert et al., 2002). Benzoic and cinnamic acids are the precursors of phenolic acids, which have methoxy and hydroxyl groups replaced at different aromatic ring positions (Marinova and Yanishlieva, 2003). Syringic acid, vanillic acid, ferulic acid, and caffeic acid are a few examples of phenolic acids (Pratt and Hudson, 1990).

Polyphenols have low bioavailability, meaning that most of what is consumed is extensively metabolized and excreted (Manach et al., 2005). Gallic acid and isoflavones may have an absorption rate of less than 5% (Manach et al., 2005), with even lower absorption rates for catechins (flavan-3-ols), flavanones, and quercetin glucosides. The phenols with the lowest absorption rates are the proanthocyanidins, galloylated tea catechins, and anthocyanins (Manach et al., 2005).

➢ *Flavonoids*

The majority of plant seeds and grains contain large concentrations of flavonoids, which are secondary plant metabolites. Flavonoids are secondary metabolites that frequently play crucial ecological roles in a plant's interactions with its surroundings. Examples of these roles include serving as pigments in leaves and flowers, acting as defensive substances against pathogens, herbivores, and predators, and supporting physiological processes like seed dormancy and maturation. Many significant chemicals are discovered under the sub-class of secondary metabolites, flavonoids. Flavone, flavonone, flavonol, and so on are among them. Multiple hydroxyl groups attached to phenolic rings are characteristics of flavonoids. They are hence referred to as polyphenolic. In plants, flavonoids are frequently found as glycosides. However, some flavonoid subclasses, including proanthocyanidins (condensed tannins), have a deleterious effect on the usage of grains and seeds in animal feed and can impart unwanted properties to human food products.

➢ *Steroids*

Compounds known as plant sterols and steroid hormones, or brassinosteroids, have a variety of biological effects. According to Williams (2011), they are necessary for plant growth, reproduction, and responses to a variety of biotic and abiotic stressors. Agronomically significant features like plant growth, photosynthesis, architecture, and flowering time are all regulated by steroids. According to Divi and Krishna (2009), steroids aid plants in fending off infections and disease attacks. A wide variety of grains,

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vegetables, fruits, legumes, nuts, and seeds naturally contain trace quantities of steroids. When consumed by people, they can lower the body's levels of low-density lipoprotein cholesterol, which lowers the risk of heart disease. Most steroids, such as sterols and stanols, resemble cholesterol chemically. The absorption of actual cholesterol into the bloodstream may be impeded by steroids.

II. MATERIALS AND METHODS

This study was conducted at the University of Nigeria, Nsukka's Department of Biochemistry from October 2015 to July 2016.

A. Sampling:

The five samples used in the analysis were soya bean (*Glycine max*), African oil bean (*Pentaclethra macrophylla*), groundnut seed (*Arachis hypogeal*), cucumeropsis seed (*Cucurbita maxima*), and cashew nut seed (*Anacardium occidentale*).

All chemicals and reagents used in this study were of analytical grade.

B. Sample Collection:

The samples were obtained from the Ogige market and subsequently taken to the Herbarium, Department of Plant Science and Biotechnology, University of Nigeria, for proper identification and labelling. Samples were obtained and dried. 1 litre of blood was obtained from a local breed of cow (Muturu) from an abattoir at Ikpa market, Nsukka. The blood sample was obtained from a freshly slaughtered cow.

C. Sample Preparation:

Soya bean and groundnut were ground with the grinder and stored in air-tight containers, separately. African oil bean seed, cucumeropsis, and cashew seeds were first dehulled and then ground to powder form and stored in separate containers, all labelled.

D. Analyses:

➢ *Flavonoid Determination:*

Each ground sample weighed 0.5 g, was macerated in 10 ml of ethyl acetate, and then steeped for three minutes in a boiling water bath. The Whatman No. 1 filter paper was used to filter it. In a test tube, 1 ml of diluted ammonia was added after pipetting 3 ml of the filtrate. The presence of flavonoids is indicated by the lowest layer's yellow coloring.

- *Analysis of Flavonoid:*

Each sample weighs 10 g, which is then put into a 250 ml beaker, dissolved in 70 ml of room temperature water, and allowed to stand for 15 minutes. After adding and thoroughly mixing 6.0 g of activated charcoal, the mixture was allowed to stand for 30 minutes before being filtered through a vacuum-powered, 60 ml flitted glass funnel that contained asbestos pad into a 400 ml beaker. The addition of two drops of HCl comes next. It was quantitatively transferred to a 50 ml volumetric flask and made up with water after being evaporated to around 40 ml in a steam bath. Water could be used to make more dilutions. At 233 nm, the absorbance was measured.Abs x 50/1000 = mg/100 g flavonoid is the concentration.

➢ *Saponins Determination:*

Each sample weighed 0.5 g, was macerated in 10 ml of petroleum ether, and then decanted into a beaker. Five milliliters of petroleum ether were used to rewash the sample mixture. After combining, the filtrates were dried out by evaporation. 6 ml of ethanol was then added to dissolve the residue. A test tube containing two milliliters was filled with two milliliters of chromogen solution. 30 minutes were given to the solution to stand. At 620 nm, absorbance was measured. The concentration of saponins was determined by building a calibration (standard) curve.

$$\text{Concentration} = \frac{\text{reading from the curve x D.F x 100 (mg/100 g)}}{1000}$$

D.F = dilution factor

Saponin Standard Curve: 0.2 g of each sample was weighed into 100 ml. A serial dilution of 0, 5, 10, 15, and 20 ml were pipetted into 5 different 50 ml volumetric flasks. Shaken and made up to volumes. The absorbance was read at 620 nm. The readings were plotted against the concentration of the various volumetric flask contents.

➢ *Alkaloids Determination*

The method Griffiths (2000) outlined was used to ascertain the samples' alkaloid contents. Each sample weighed 0.5 g, was macerated in 10 ml of ethanol (20% sulfuric acid):1:1, and then filtered. After pipetting 1 ml of the filtrate and mixing it with 5 ml of 60% H2SO4, it was left to stand for three hours. At 580 nm, absorbance was measured.

- *Analysis of Alkaloids:*

After pipetting 3.0 milliliters of each extract into two 250 milliliter beakers, 2 milliliters of phosphate buffer were added and the pH was adjusted to 7.0. After that, they were incubated at 370C in a water bath. 2.0 ml of casein solution that had been heated to 370C and left there for 20 minutes was added, along with 6.0 ml of 5% trichloroacetic acid. After letting the mixture remain at room temperature for an hour, it was filtered. At 580 nm, the absorbance and the blank were measured.Dilution factor (mg/100 g) x Reading from the curve equals concentration)

➢ *Tannin Determination*

The technique outlined by Jaffe (2003) was used to ascertain the tannin content of the seed samples. A 50 ml conical flask was filled with 0.5 g of each sample, macerated in 10 ml of distilled water, and filtered through Whatman No. 1 filter paper. After pipetting 5 milliliters of the filtrate into a test tube, 0.3 milliliters of 0.1 N ferric chloride (FeCl3 in 0.1 N HCl) were added. The addition of 0.3 ml of potassium ferricyanide (K3Fe(CN)6) at a concentration of 0.0008 M came next. At 760 nm, the absorbance was measured, and the

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results were plotted against the different standards' concentrations.

➢ *Phenol Determination:*

A 50 ml conical flask was filled with 0.5 g of each ground sample, macerated in 10 ml of 80% ethanol, and filtered through Whatman No. 1 filter paper. After pipetting 5 ml of the filtrate into a test tube, 0.5 ml of the folin ciocaiteus reagent was added. Two milliliters of 20% sodium carbonate were added after 30 minutes. At 650 nm, the absorbance was measured.

➢ *Cyanogenic Glycoside Determination:*

The alkaline picrate approach by Onwuka (2005) was the technique employed. Each ground sample weighed one gram, which was then macerated with fifty milliliters of distilled water in a fifty milliliter conical flask. The solutions of the samples were filtered using Whatman no1 filter paper and 1 ml of the filtrates were pipetted into various test tubes. After adding 4 milliliters of alkaline picrate solution to each solution, they were brought to a boil in a water bath for 5 minutes and then allowed to cool. At 490 nm, absorbance was measured.

➢ *Steroids Determination:*

Ten milliliters of ethyl acetate were added to each test tube containing 0.5 grams of each processed sample. After three minutes in a boiling water bath, the combinations were allowed to cool before being filtered through Whatman No. 1 filter paper. Two layers were created after the organic extracts and an equal volume of chloroform were combined. Each sample's 2 ml of the chloroform layer was pipetted into a separate test tube. Next, 5 ml of water was added, and 0.1 N NH4OH was used to bring the pH down to 7.0. It was eluted with Sephadex 100 and absorbance read at 240 nm.

$$\text{Concentration} = \frac{\text{Absorbance x D.F (mg/100 g)}}{2550}$$

2550 = extinction coefficient of steroids

D. F = Dilution factor

➢ *Oxalate Determination:*

Each sample's oxalate content was determined by applying the method described by Munro and Bassiro (2000). An electronic weighing balance was used to weigh 0.5 g of each sample into a beaker. Each was filled with 20 milliliters of water, a few drops of acetylacetic acid, and thoroughly shaken. Oxalate is present when a coloration turns greenish-black.Each sample was weighed again, yielding 2.0 g in a 50 ml conical flask. 20 ml of 30% HCl was then added, and the apparatus was left to stand for five minutes. Ammonium sulphate (NH4SO4), 4.0 g, was added, and the mixture was gently shaken to dissolve. It was let to calm down. After being decanted into a 25 ml volumetric flask, 30% HCl was added to make up the volume. After transferring each solution into a 50 ml volumetric flask, diethyl ether was added in the same volume. After adding ether and acetic acid (CH3COOH) to bring the pH down to 7.0, the mixture was centrifuged for ten minutes at 3000 rpm and then decanted into a 250 ml conical flask. Each was titrated with 0.1 M KMnO4, and at 490 nm, the absorbance of the titrated end products was measured.Concentration is calculated by taking the reading from the curve and multiplying it by D.F. to get 100 mg/100 g.

➢ *Phytate Determination*

Using the titration method outlined by Lucas and Markaka (1975), the phytate content of each seed sample was ascertained. 2.0 g of each sample was weighed into a test tube, mixed with 20 ml of distilled water and heated for 10 minutes. There were a few drops of picric acid added. A yellow-colored hue indicates the existence of phytic acid.Each sample weighted 0.5 g was extracted with 100 ml of 2.4% HCl for an hour at room temperature using a 500 ml round-bottom flask. The next steps were filtration and decantation. After pipetting 5 ml of the filtrate, 25 ml of water was added. To elute inorganic phosphates from the diluted samples, 10 ml was pipetted through amberlet resin grade 200–400 mesh into various test tubes. 0.7 M sodium chloride (NaCl) was added in 15 ml. At 520 nm, the absorbance was measured.

➢ *Trypsin Inhibitor Determination*

The samples' trypsin inhibitor contents were ascertained by applying the protocol outlined by Prokopet and Unlenbruck (2002). 50 milliliters of a 0.5 M NaCl solution were used to disseminate 1.0 g of each sample. The mixture was centrifuged for five minutes at 1500 rpm after being agitated for thirty minutes at room temperature. The filtrates from the supernatants were utilized in the experiment. Ten milliliters of each sample's substrate were mixed with two milliliters of standard trypsin solution. Using 10 milliliters of the same substrate as the blank, the mixture's absorbance was measured at 410 nm.

- *Standard Trypsin:*

N-alpha-benzoyl-DL arginine-P-nitroanilide (BAPA) was used to manufacture this. The substrate solution was treated with standard trypsin; that is, the test sample extract's trypsin inhibition activities were measured by standardizing the degree of inhibition of substrate trypsin hydrolysis.Expression of activity: The number of trypsin units inhibited (TIU) per unit weight (g) of the material under analysis represents the trypsin inhibitor activity. Under the conditions employed here, an increase of 0.01 absorbance units at 410 nm per 10 ml of the reaction mixture is arbitrarily defined as one trypsin unit (TU).

Consequently, TIU/mg = Sample Absorbance x 0.01F

Standard Absorbance of TIU/mg = b – ax F, where b is the test sample solution's absorbance.

a = the blank's absorbance

F is the experimental factor, denoted as F = 1/w x Vf/Va x D,

where w is the sample weight in grams and
Vf is the extract's total volume.
Va is the assay's volume of extract used.
D = Factor of dilution

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➢ *Hemagglutinin Determination*

Each sample's hemagglutinin content was ascertained by following Boyel's (2001) method. Twenty milliliters of 0.9% NaCl were mixed with two grams of each sample, and the mixture was shaken briskly for a minute. After letting the suspensions remain for an hour, the samples were centrifuged for ten minutes at 2000 rpm, and the suspension was filtered. Each was collected, and the supernatant was employed as a basic agglutinating extract.

- *Red Blood Cell Preparation:*

For this experiment, one liter of cow blood from the abattoir at Ikpa market, Nru, Nsukka, was used. On the same day, the preparation and collection were completed. Using a 5-milliliter syringe filled with ethylene-diamine tetraacetic acid (EDTA) anticoagulant, 3.5 milliliters of fresh whole blood were drawn. The whole blood was spun for 10 minutes at 2000 rpm in order to extract the plasma and red blood cells. Four volumes of 0.9% saline were mixed with one volume of red blood cells, centrifuged at 2000 rpm for ten minutes, and the supernatant was disposed of. Three saline washes were performed on the sediment cell until the supernatant had no color. After being cleaned, the red blood cells were suspended in phosphate buffer saline (4 milliliters of cells for every 100 milliliters of phosphate buffer). Ten parts of this suspension were then mixed with 2% trypsin solution and incubated for an hour at 370 degrees Celsius. To get rid of any remaining trypsin, the trypsinized red blood cells were subsequently rinsed four or five times with 0.9% saline. 100 ml of 0.9% saline was used to suspend 1.2–2 ml of parked cells. Each sample was placed in one of six microtiter plate wells. Agglutin extracts and a blank, consisting of 0.3 ml of 0.9% saline, were added to each well, with the exception of the wells that included all of the samples. Each well received 0.3 ml of a 2% solution of trypsinized red blood cells in saline following repeated dilution. The different wells' hemagglutinin patterns were interpreted.

- *Procedure:*

Each sample weighed 0.5 g and was then distributed across a 10 ml normal saline solution that had been pH 6.4-budded using a 0.01 M phosphate buffer solution. After 30 minutes of room temperature standing, the setup was centrifuged to extract the material. One milliliter of trypsinized blood was added to every 0.1 milliliter of extract diluents in a test tube. With just the blood cells in the test tube, this was mount-controlled. After the tubes were let to stand at room temperature for four hours, 1 milliliter of regular saline was added to each test tube, and it was left to stand for ten minutes before the absorbance at 620 nm was measured.The blank was a test tube with just blood cells and regular saline in it.

Hemagglutinin units per milligram of the sample are used to express the outcome.

Unit of hemoglobulin/mg = (b-a) x F,

Where b is the test sample solution's absorbance
a = the blank's absorbance (control)
F = (1/w x Vf/Va), which is the experimental factor. D.F.,
Where w is the sample weight
Vf = extract's overall volume
Va is the assay's volume of extract used.
D.F stands for dilution factor.

➢ *Carotenoid Determination:*

To extract the color material from each sample, 1.0 g was dissolved in 10 ml of acetone in a 50 ml conical flask, left to stand for 20 minutes, and then gently shaken every 4 minutes. They were stirred, then given time to calm down before being decanted into a test tube to produce a transparent solution. After adding and gently shaking 5 milliliters of hexane, two separate layers were visible. Using a separating funnel, the upper layer was separated, collected in a glass curvet, and the absorbance at 453 nm was measured.

$$\frac{\text{Abs x 10 x 1/5 x 104}}{2592}$$

Abs = absorbance

E. Statistical Analysis

The estimations of the phenolic compounds and anti-nutrient contents of the samples were carried out in triplicates (n = 3) and a one-way analysis of variance (ANOVA) was performed on the data using the SPSS 13 computer program (IBM Co., Chicago, IL., USA). Duncan's Multiple Range Test was used to compare means, and differences were deemed significant at $p < 0.05$.

III. RESULTS

A. Trypsin Inhibitor:

The findings in Table 1 demonstrate that each of the five underutilized seeds under investigation had the anti-nutrient trypsin inhibitor. African oil bean seed has a considerably ($p < 0.05$) higher concentration of trypsin inhibitor than cashew nut, groundnut seed, cucumeropsis, and soya bean.

Table 1: Trypsin Inhibitor Contents of Some Under-Utilized Seeds

Sample	Trypsin Inhibitor (TIU/mg)
Soya bean	3.619 ± 0.007
African oil bean seed	3.670 ± 0.006
Groundnut seed	2.247 ± 0.004
Cucumeropsis	2.385 ± 0.003
Cashew nut	2.584 ± 0.004

Results expressed as Means ± SD (n = 3)

A significance level of $p < 0.05$ is assigned to mean values with distinct letters serving as superscripts across rows and samples, whereas a non-significant level of $p > 0.05$ is assigned to mean values with the same letters serving as superscripts across rows and samples.

B. Tannins

All of the examined samples contained tannin, as indicated by the result in Table 2: soya bean, African oil bean, groundnut seed, cucumeropsis seed, and cashew nut seed. Statistical analysis of the result reviews that there is a significant difference at 0.05 confidence interval ($p < 0.05$).

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Soya bean tannin composition is significantly higher than found in the rest of the under-utilized seeds under examination.

Table 2: Tannin Contents of Some Under-Utilized Seeds

Sample	Tannin (mg/100g)
Soya bean	443.284 ± 0.003
African oil bean	388.013 ± 0.010
Groundnut seed	403.360 ± 0.002
Cucumeropsis	437.088 ± 0.623
Cashew nut	415.646 ± 0.008

Results Expressed as Means ± SD (n = 3)

A significance level of $p < 0.05$ is applied to mean values with distinct letters serving as superscripts across rows and samples, while a non-significant level of $p > 0.05$ is allocated to mean values with the same letters serving as superscripts across rows and samples.

C. Steroids

The result in Table 3 shows steroid composition of the examined under-utilized seeds. Steroid content is significantly ($p < 0.05$), higher in cucumeropsis seed than in the rest of the seeds under study.

Table 3: Steroids Contents of Some Under-Utilized Seeds

Sample	Steroids (mg/g)
Soya bean	4.474 ± 0.004
African oil bean	5.866 ± 0.006
Groundnut seed	6.675 ± 0.005
Cucumeropsis	9.147 ± 0.063
Cashew nut	5.642 ± 0.006

Results EXPRESSED as Means ± SD (n = 3)

Mean values with different letters acting as superscripts across rows and samples are assigned a significance level of $p < 0.05$, whereas mean values with the same letters acting as superscripts across rows and samples are assigned a non-significant level of $p > 0.05$.

D. Oxalate

Table 4 is the result of oxalate compositions in: soya bean, African oil bean, groundnut seed, cucumeropsis seed, and cashew nut. Oxalate content is significantly ($p < 0.05$), higher in soya bean than in the rest of the seeds.

Table 4: Oxalate Contents of Some Under-Utilized Seeds

Sample	Oxalate (mg/100 g)
Soya bean	0.254 ± 0.003
African oil bean	0.244 ± 0.004
Groundnut seed	0.147 ± 0.003
Cucumeropsis	0.125 ± 0.004
Cashew nut	0.148 ± 0.004

Results Expressed as Means ± SD (n = 3)

A significance level of $p < 0.05$ is assigned to mean values with distinct letters serving as superscripts across rows and samples, whereas a non-significant level of $p > 0.05$ is assigned to mean values with the same letters serving as superscripts across rows and samples.

E. Flavonoids

Table 5 displays the presence of flavonoids and phenolic compounds in each of the five underutilized seeds that were the subject of the investigation. African oil beans have a considerably ($p < 0.05$) higher flavonoid content than cashew nuts, groundnut seeds, cucumeropsis, and soybean.

Table 5: Flavonoids Contents of Some Under-Utilized Seeds

Sample	Flavonoids (mg/100 g)
Soya bean	400.985 ± 0.004
African oil bean	606.920 ± 0.006
Groundnut seed	63.217 ± 0.007
Cucumeropsis	10.386 ± 0.003
Cashew nut	564.945 ± 0.007

Results Expressed as Means ± SD (n = 3)

A significance level of $p < 0.05$ is assigned to mean values with distinct letters serving as superscripts across rows and samples, whereas a non-significant level of $p > 0.05$ is assigned to mean values with the same letters serving as superscripts across rows and samples.

F. Phytate

All five of the underutilized seeds under investigation included phytate, a phenolic component, as indicated by the results in Table 6. African oil beans have a considerably ($p < 0.05$) greater phytate concentration than groundnut seeds, cucumeropsis, soybean, and cashew nuts.

Table 6: Phytate Contents of Some Under-Utilized Seeds

Sample	Phytate (mg/100 g)
Soya bean	0.629 ± 0.005
African oil bean	1.884 ± 0.004
Groundnut seed	0.124 ± 0.003
Cucumeropsis	0.776 ± 0.004
Cashew nut	0.436 ± 0.010

Results Expressed as Means ± SD (n = 3)

Mean values with different letters acting as superscripts across rows and samples are assigned a significance level of $p < 0.05$, whereas mean values with the same letters acting as superscripts across rows and samples are assigned a non-significant level of $p > 0.05$.

G. Saponins:

Table 7 reports the presence of the anti-nutrient, saponin in all the 5 under-utilized seeds under study. The concentration of saponin in African oil bean is significantly ($p < 0.05$), higher than in groundnut seed, cucumeropsis, soya bean, and cashew nut.

Table 7: Saponins Contents of Some Under-Utilized Seeds

Sample	Saponins (mg/g)
Soya bean	3.543 ± 0.009
African oil bean	3.554 ± 0.007
Groundnut seed	3.338 ± 0.045
Cucumeropsis	3.365 ± 0.004
Cashew nut	3.129 ± 0.004

Results Expressed as Means ± SD (n = 3)

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A significance level of $p < 0.05$ is assigned to mean values with distinct letters serving as superscripts across rows and samples, whereas a non-significant level of $p > 0.05$ is assigned to mean values with the same letters serving as superscripts across rows and samples.

H. Phenol

Table 8 is the result of phenol compositions in: soya bean seed, African oil bean seed, groundnut seed, cucumeropsis seed, and cashew nut. Phenol content is significantly ($p < 0.05$), higher in cashew nut seed than in the rest of the seeds.

Table 8: Phenol Contents of Some Under-Utilized Seeds

Sample	Phenol (mg/100 g)
Soya bean	963.877 ± 0.006
African oil bean	854.193 ± 0.005
Groundnut seed	934.196 ± 0.003
Cucumeropsis	523.876 ± 0.006
Cashew nut	1183.234 ± 0.008

Results Expressed as Means ± SD (n = 3)

At $p < 0.05$, mean values with distinct superscripts across rows (across samples) are deemed significant, but mean values with the same letters as superscripts across the rows (across the samples) are considered non-significant at $p > 0.05$.

I. Cyanogenic Glycoside

Table 9 is the result of cyanogenic glycoside compositions in: soya bean seed, African oil bean seed, groundnut seed, cucumeropsis seed, and cashew nut. Soya bean seeds have a considerably ($p < 0.05$) higher amount of cyanogenic glycosides than the other seeds.

Table 9: Cyanogenic Glycoside Contents of Some Under-Utilized Seeds

Sample	Cyanogenic Glycoside (mg/g)
Soya bean	0.333 ± 0.004
African oil bean	0.136 ± 0.004
Groundnut seed	0.319 ± 0.004
Cucumeropsis	0.215 ± 0.004
Cashew nut	0.311 ± 0.006

Results Expressed as Means ± SD (n = 3)

A significance level of $p < 0.05$ is assigned to mean values with distinct letters serving as superscripts across rows and samples, whereas a non-significant level of $p > 0.05$ is assigned to mean values with the same letters serving as superscripts across rows and samples.

J. Carotenoid

Table 10 displays the presence and different compositions of carotenoids in all the samples that were tested, including cashew nut seed, groundnut seed, cucumeropsis seed, soybean seed, and African oil bean seed. The carotenoid composition of Cucumeropsis seeds is substantially ($p < 0.05$) higher than that of the other seeds.

Table 10: Carotenoid Contents of Some Under-Utilized Seeds

Sample	Carotenoid (mg/100 g)
Soya bean	0.221 ± 0.005
African oil bean	0.358 ± 0.005
Groundnut seed	0.574 ± 0.004
Cucumeropsis	0.962 ± 0.006
Cashew nut	0.614 ± 0.008

Results Expressed as Means ± SD (n = 3)

A significance level of $p < 0.05$ is assigned to mean values with distinct letters serving as superscripts across rows and samples, whereas a non-significant level of $p > 0.05$ is assigned to mean values with the same letters serving as superscripts across rows and samples.

K. Alkaloids

The alkaloid compositions of the following seeds are shown in Table 11: cashew nut, groundnut, cucumeropsis, soybean, and African oil bean. African oil bean has a considerably ($p < 0.05$) higher alkaloid content than the other seeds.

Table 11: Alkaloid Contents of Some Under-Utilized Seeds

Sample	Alkaloid (mg/100 g)
Soya bean	213.016 ± 0.009
African oil bean	850.416 ± 0.004
Groundnut seed	227.649 ± 0.008
Cucumeropsis	466.672 ± 0.006
Cashew nut	708.951 ± 0.007

Results expressed as Means ± SD (n = 3)

A significance level of $p < 0.05$ is assigned to mean values with distinct letters serving as superscripts across rows and samples, whereas a non-significant level of $p > 0.05$ is assigned to mean values with the same letters serving as superscripts across rows and samples.

L. Hemagglutinins

Table 12 displays the results of the analysis of the following samples: cashew nuts, groundnuts, cucumeropsis seeds, soybean seeds, and African oil bean seeds. All of these samples contained hemagglutinin. The hemagglutinin content of African oil beans is considerably ($p < 0.05$) higher than that of the other seeds.

Table 12: Hemagglutinin Contents of Some Under-Utilized Seeds

Sample	Hemagglutinin (HIU/mg)
Soya bean	0.322 ± 0.005
African oil bean	0.567 ± 0.005
Groundnut seed	0.556 ± 0.003
Cucumeropsis	0.538 ± 0.008
Cashew nut	0.416 ± 0.010

Results expressed as Means ± SD (n = 3)

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A significance level of $p < 0.05$ is assigned to mean values with distinct letters serving as superscripts across rows and samples, whereas a non-significant level of $p > 0.05$ is assigned to mean values with the same letters serving as superscripts across rows and samples.

IV. DISCUSSION

In this study, the composition of trypsin inhibitor in African oil bean seed, 3.670 ± 0.006 TIU/mg, is significantly ($p < 0.05$) higher when compared to the concentration in the rest of the seeds. Groundnut seed was found to have the least trypsin inhibitor concentration, 2.247 ± 0.004 TIU/mg. Groundnut seed, cucumeropsis seed, and cashew nut seed trypsin inhibitor contents, 2.247 ± 0.004 to 2.584 ± 0.004 TIU/mg, could be considered to be moderate. As a result of this, ingestion of high levels of African oil bean seed or soya bean seed, without proper processing to reduce the trypsin inhibitor content, may result in health challenges such as inactivation or loss of trypsin in the small intestine. The implication of this is that African oil bean seed would require intense preparation before it could serve as a protein source for man or livestock feed.

Table 2 result shows that the seeds' concentrations of tannins are on the low side, following the suggestion of Kessel (2002). The similarity between my result and that of Kessel could be a result of the use of seeds of similar species or the use of a similar method of analysis. Soya bean seed tannin content, 443.284 ± 0.003 mg/100 g is significantly higher ($p < 0.05$) compared to the other four seeds investigated. African oil bean seed has the least tannin content, 388.013 ± 0.010 mg/100 g. Tables 3 and 4 present steroid and oxalate compositions of the seed samples. The result of Table 3 shows that cucumeropsis seed steroid composition, 9.147 ± 0.005 mg/g is on the high side. The implication of this is that consumption of cucumeropsis seed may reduce the risk of heart disease. This is connected to the ability of steroids to reduce low-density lipoprotein cholesterol in the body as opined by Divi and Krishna (2009). However, care should be taken in the processing of cucumeropsis to minimize loss due to heating. Table 4 reviews a significant increase ($p < 0.05$) in oxalate composition in the 5 seeds. This result shows that the seeds' oxalate contents are well below the lethal dose, 2.5 g/kg suggested by Inuwa (2011). The difference in anti-nutrient and phenolic compound compositions of the seeds could be a result of differences in the geographical distribution of the seeds. In other words, the geographical location of plants affects the chemical composition of such plants and their products thereof.

The results in Tables 5 and 6 show the presence of flavonoids and phytate in all the seeds analyzed. This reviews that African oil bean seed flavonoid composition, 606.920 ± 0.006 mg/100 g is significantly ($p < 0.05$) higher than found in others. Cucumeropsis seed has the least flavonoid composition, 10.386 ± 0.003 mg/100 g, among the seeds. This suggests that African oil bean seed may have greater resistance to attacks by herbivores, predators, and pathogens than the rest of the seeds; and cucumeropsis low flavonoid content could be responsible for its vulnerability to attacks by disease pathogens and herbivores. African oil bean seed phytate content, 1.884 ± 0.004 mg/100 g, is significantly ($p < 0.05$) higher than found in others. African oil bean seeds should therefore be treated to lower their phytic acid level, which, if ingested in excessive amounts, may be hazardous to humans and livestock alike.

The mean concentration of saponin in soya bean seed, African oil bean seed, groundnut seed, cucumeropsis seed, and cashew nut seed were found to be: 3.543, 3.554, 3.338, 3.365, and 3.129 mg/g respectively. This agrees with the report of Uhegbu (2011), 0.14 ± 0.05 to 17 mg/g. This shows a moderate saponin concentration in the seeds. The seeds analyzed have high phenol contents. Cashew nut seed has the highest mean value, 1183.234 ± 0.008 mg/100 g of phenol content, followed by soya bean seed, 963.877 ± 0.006 mg/100 g. The high phenol content of cashew nut seed and soya bean seed suggests both could have some good antioxidant properties. Thus, consumption of cashew nut seed and soya bean seed could prevent lipid oxidation in living tissues (Medina, 1999). All the seeds contain cyanogenic glycoside in the range of 0.136 ± 0.004 to 0.333 ± 0.004 mg/g. This range is considered not to be harmful.

Results in Tables 10 and 12 show that all the seeds analyzed contain carotenoid and hemagglutinins in moderate levels, considered not harmful to the body. These seeds have concentrations of this anti-nutrient and phenolic substance that are within the safe range and not high enough to be harmful to health (Brown, 2007). Because the seeds have a low anti-nutrient concentration, they are safe to use. The seeds may be useful as an ethnomedical because anti-nutrients are needed at low concentrations to cause biochemical changes (Okaka and Okaka, 2001).

The result in Table 11 shows that African oil bean seed and cashew nut seed have higher alkaloid compositions than the rest of the seeds. This could mean that the seeds have some level of pharmacological activity as suggested by Cushnie (2014).

V. CONCLUSIONS

The full utilization of under-utilized seeds is hindered by the presence of anti-nutrients and phenolic compounds. Determining the nutritional potential of any plant protein source requires careful evaluation of the kind, amount, and concentration of these constituents as well as the nutrients' bioavailability to the consuming animal. Processing methods, supplementation of divalent minerals, and the addition of enzymes such as phytase can mitigate the effects of anti-nutrient and phenolic compounds. These seeds are positioned as a viable substitute for current high-cost protein and mineral sources that are in high competition with human consumption. The negative effects of these anti-nutritive components in plant protein sources can be reduced or eliminated, and their nutritional value can be increased, by using appropriate processing techniques or a combination of techniques. The detrimental effects of anti-nutrients in plant protein sources for animal nutrition can be lessened by supplementing with certain minerals, amino acids, and vitamins. Depending on

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the kind of plant, cultivar, and post-harvest procedures, these protein sources have different concentrations of anti-nutrient components.

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