FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
TÜRKİYE

INVESTIGATION OF SOME BIOCHEMICAL PARAMETER IN FIVE DIFFERENT *SALONUM MELONGENA* L. CULTIVARS

Zulaiha Gidado MUKHTAR

Doctoral Thesis

DEPARTMENT OF CHEMISTRY
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This thesis, which was prepared according to the thesis writing rules of the Graduate School of Natural and Applied Sciences, Fırat University, was evaluated by the committee members who have signed the following signatures and was unanimously approved after the defense exam made open to the academic audience.

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DECLARATION

I hereby declare that I wrote this Doctoral Thesis titled "Investigation of Some biochemical parameter in Five different Salonum Melongena L. Cultivars " in consistent with the thesis writing guide of the Graduate School of Natural and Applied Sciences, Firat University. I also declare that all information in it is correct, that I acted according to scientific ethics in producing and presenting the findings, cited all the references I used, express all institutions or organizations or persons who supported the thesis financially. I have never used the data and information I provide here in order to get a degree in any way.

07 April 2021

Zulaiha Gidado MUKHTAR

PREFACE

The first paragraph should give information about the importance, difficulties, limits and motivation factors of the thesis subject. This information should be written by the thesis author and should not be quoted. If necessary, 2-3 short paragraphs can be created. In a new paragraph following this information, the contributions of the persons, institutions and organizations who first count as direct contributions to the thesis studies and then indirectly contribute should be expressly acknowledged.

With a deep sense of humility and gratitude to God, I would foremost like to express my most profound and heart felt appreciation to my hard working supervisor - Prof. Sinan Saydam, who was not only a teacher par excellence, but also a remarkable mentor and father figure. His faith in me, and his kindness, patience, empathy and generosity was endless during my PhD. training journey - I will forever be indebted to him. My sincere gratitude also goes to Prof. Fikret Karatas and Prof. Dursun Özer who further taught and showed me the value of hard work and due diligence in my fact-finding mission that was at the core of my training. My appreciation also goes to Meltem Çakmak who patiently guided me in the laboratory.

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May Allah SWT reward you all and give me the resilience to forever make you proud.

Zulaiha Gidado MUKHTAR
Elazıg, 2021

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ABSTRACT

Investigation of Some biochemical parameter in Five different *Salonum Melongena* L. Cultivars

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This study aimed to determine and compare varietal variations in five eggplant varieties with diverse phenotypes sampled from Turkey (dark and light eggplant) and Nigeria (white garden egg, bitter apple and bitter tomato). The concentrations of amino acids, vitamins, and stress biomarkers analysed using HPLC; and the total phenolic content (TPC), total flavonoid content (TFC) total antioxidant capacities (TAC) based on ABTS and DPPH assays using spectrophotometer were investigated and compared.

Our findings determined the concentration of 19 amino acids in all varieties ranging from 23.5 – 33625.4 µg/g DW, with the lowest and highest determined for leucine and lysine, respectively. Based on RDA, all of the varieties can contribute to dietary adequacy in terms of histidine, lysine, methionine, phenylalanine, threonine, tryptophan and white garden egg and bitter apple varieties had adequate amounts of valine. Likewise, all the varieties were nutritionally low in leucine.

Additionally, the concentration of 12 vitamins determined, ranging from 0.13 – 2543.02 µg/g DW with the lowest and highest for vitamin A and vitamin B_5, respectively. Relative to the RDA, all the varieties are excellent sources of the water-soluble vitamins; some are nutritionally significant in terms of vitamin A (white garden egg, bitter apple and bitter tomato) and vitamin E (dark eggplant and bitter tomato).

4-Hydroxynonenal ranging from 24.6 – 48.3 µg/g DW was determined, with the lowest and highest for bitter apple and white garden egg respectively. The concentration of MDA ranging from 1.50 -18.41µg/g DW. All the varieties of eggplant used in this study contained both reduced (GSH) and oxidized (GSSG) forms of glutathione. GSH levels were found to range from 364.85 – 5930.32 µg/g. The white garden egg had the highest amount of both GSH and GSSG while the bitter tomato and dark eggplant had the lowest GSH and GSSG levels, respectively.

The TPC (706.30 – 1260.32 µg GAE/g) was determined in the following order -light eggplant < bitter tomato< bitter apple< dark eggplant< white garden egg. While the TFC (167.50 – 356.51 µg QE/g) was determined in the following order- bitter apple < light eggplant< bitter tomato< dark eggplant< white garden egg. For the TAC, the scavenging activity for the ABTS radical (365.44 – 809.52 µg/g) was higher than for the DPPH radical (83.86 - 243.02 µg/g). The white garden egg exhibited the highest TAC in both assays while the bitter tomato and bitter apple had the lowest in the ABTS and DPPH assays, respectively.

Our findings highlighted a wide disparity in the levels of the nutrients, stress biomarkers, TPC, TFC and TAC within and between the eggplants sampled from the different countries attributed to varietal difference and growth conditions. This presents opportunities for dietary inclusion and other stakeholders to select and develop improved plant varieties

Keywords: Eggplant, Bitter apple, Bitter tomato, White garden egg, Amino acid, Vitamin, Antioxidant, HPLC

ÖZET

Beş Farklı *Salonum Melongena* L. Çeşidinde Bazı Biyokimyasal Parametrelerin İncelenmesi

Zulaiha Gidado MUKHTAR

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Bu çalışmada, Türkiye (koyu ve açık patlıcan) ve Nijerya'dan (beyaz bahçe yumurtası, acı elma ve acı domates) örneklenen çeşitli fenotiplere sahip beş patlıcan türündeki bazı biyokimyasal parametrelerin belirlenmesi ve karşılaştırılması amaçlanmıştır. Amino asit, vitamin ve stres biyomarker konsantrasyonları; ile ABTS ve DPPH testlerine dayalı toplam fenolik (TPC) ve flavonoid madde içerikleri (TFC) ile antioksidan kapasiteleri (TAC) HPLC ve spektrofotometrik olarak araştırılmıştır.

Elde edilen sonuçlardan, örneklerdeki amino asit miktarlarının 23.5 - 33625 µg/g dw arasında değiştiği; bunlar arasında en düşük ve en yüksek miktarın sırasıyla lösin ve lizin olduğu belirlenmiştir. RDA'ya göre, tüm çeşitler histidin, lizin, metiyonin, fenilalanin, treonin ve triptofan açısından günlük diyet yeterliliğine katkıda bulunabilir. Aksine, beyaz bahçe yumurtası ve acı elma çeşitlerinde yeterli miktarda valin bulunurken, koyu patlıcan, açık patlıcan ve acı domates çeşitleri besin açısından daha düşük değerlere sahiptir. Ayrıca, tüm çeşitlerde lösin miktarırın düşük olduğu gözlenmiştir.

Elde edilen sonuçlardan, bütün çeşitlerin suda çözünür vitaminler bakımından iyi birer vitamin kaynağı olduğu; bazıları A vitamini (beyaz bahçe yumurtası, acı elma ve acı domates) ve E vitamini (siyah patlıcan ve acı domates) bakımından önemli iken, likopen bakımından daha fakir olduğu bulunmuştur.

4-Hidroksinonenal miktarının, aşağıdaki artan sırada olduğu gözlenmiştir; acı elma <mor patlıcan <acı domates <siyah patlıcan <çeşitler arasında beyaz bahçe yumurtası. Bulgularımız, MDA konsantrasyonunun 1.50 -18.41µg/g DW arasında değiştiği tespit edilmiştir. Bu çalışmada kullanılan tüm patlıcan çeşitleri, glutatyonun indirgenmiş (GSH) ve okside (GSSG) formlarını içerdiğini göstermiştir. Beyaz bahçe yumurtası hem GSH hem de GSSG açısından en yüksek orana sahip iken, acı domates ve siyah patlıcan en düşük GSH ve GSSG miktarlarına sahip olduğu görülmüştür.

Toplam fenolik madde (TPC) miktarırın 706.30 - 1260.32 µg GAE/g arasında değiştiği belirlenmiştir. Toplam flavonoid (TFC) miktarının ise, 167.50 - 356.51 µg QE/g arasında değiştiği bulunmuştur. Toplam antioksidan kapasite (TAC) için, ABTS radikali giderme aktivitesinin 365.44 - 809.52 ug/g arasında değiştiği, DPPH radikal giderme aktivitesinin ise 83.86 - 243.02 ug/g arasında değiştiği, beyaz bahçe yumurtası, her iki testte de en yüksek TAC'yi sergilerken, acı domates ve acı elma sırasıyla ABTS ve DPPH testlerinde en düşük seviyeye sahip olduğu görülmüştür.

Bulgularımız, farklı ülkelerden örneklenen patlıcanlar arasındaki amino asit, vitamin, stres biyomarkır, TPC, TFC ve TAC seviyelerindeki, farklılığı iklim ve büyüme şartlarına bağlı olarak değişim gösterdiği sonucuna varılmıştır.

Anahtar Kelimeler: Patlıcan, Acı elma, Acı domates, Bahçe yumurtası, Amino asit, Vitamin, Antioxidan, Fenolik madde, Flavonoid,

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SYMBOLS AND ABBREVIATIONS

Abbreviations

4-HNE	: 4-Hydroxyneonal
ABTS	: 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid
DPPH	: 2, 2-diphenyl-1-picrylhydrazyl
EPA	: Environmental Protection Agency
EAA	: Essential Amino Acids
FAO	: Food and Agricultural Organization
GSH	: Reduced Glutathione
GSH-Px	: Glutathione Peroxidase
GSH-Red	: Glutathione Reductase
GSSG	: Oxidized Glutathione
GST	: Glutathione-s-transferase
HIV	: Human immunodeficiency virus
HPLC	: High performance liquid chromatography
MDA	: Malondialdehyde
MG	: Methyl Glyoxal
NADH	: Nicotinamide Adenine Dinucleotide
NADPH	: Nicotinamide Adenine Dinucleotide Phosphate
NEAA	: Non- Essential Amino Acids
OS	: Oxidative Stress
PG	: Peptidoglycan
PITC	: Phenylisothicyanide
PTC	: Phenylthiocarbomyl
ROS	: Reactive oxygen species
SEM	: Standard Error of the Mean
TEA	: Triethylamine
TPC	: Total Phenolic Contents
USEPA	: United States Environmental Protection Agency
WHO	: World Health Organisation

1. INTRODUCTION

Plants are vital resources with important nutritional, economic and ecological functions. They absorb sunlight and nutrients from the soil, which they use to synthesize and accumulate both primary and secondary metabolites, particularly in their leaves and fruits. The primary metabolites (e.g. amino acids, fatty acids, and vitamins) are essential to metabolic processes and serve to sustain the existence of all life forms [1]. For instance, in addition to their primary function as the building blocks of proteins and peptides, amino acids have several other biochemical and physiological roles as hormones, neurotransmitters and metabolic intermediates [2,3]. On the other hand, vitamins are a diverse group of unrelated organic compounds with even more diverse functions in living organisms. Their major roles are as coenzymes, hormones and antioxidants, while some vitamins have more specialized functions, for instance, vitamin C with its role in collagen formation [4]. Glutathione is an important tripeptide, initially presumed to function only as an antioxidant in cells where it regulates redox changes, further studies have shown it to perform other biochemical roles such as detoxification, modulation of gene expression and biosynthesis [5,6]. In contrast, secondary metabolites (e.g. phenolic compounds, alkaloids) are not directly used in metabolic processes but provide vital protective, healing and defensive support against both biotic and abiotic stresses [1]**.** Examples include flavonoids that are excellent water-soluble free radical scavengers contributing to the total antioxidant capacity of fruits and vegetables [7].

The recent advances in science have shown the crucial role diet plays in safeguarding and maintaining our health. It has immensely increased our grasp of the biochemical basis of free radical activity leading to oxidative stress, its role in the manifestation of a host of chronic metabolic disorders and the importance of nutrition in their prevention and management [8]. It shows how the normal metabolic processes that occur in all living systems and external factors - such as radiation, smoke, drugs, and other pollutants; contribute to the formation of free radicals. An innate antioxidant cellular mechanism neutralizes these highly reactive free radicals using molecules biosynthesized or obtained from the diet; or else they will accumulate, and attack biomolecules such as DNA, proteins and lipids and disrupt their normal functions. The nature of lipids makes them highly vulnerable to these oxidative attacks [9]. Malondialdehyde and 4-hyroxynonenal are some products of lipid peroxidation and are used as biological markers of oxidative stress. They interact with biomolecules, disrupting important physiological processes and have been reported to be mutagenic and carcinogenic [10]. Changes in lifestyle and dietary patterns, with the exponential rise in pollution means our bodies generate more free radicals than it can handle, consequently more antioxidants are required to neutralize them. The establishment of a relationship between oxidative stress and the pathogenesis of metabolic disorders has further generated interest in plant-derived nutrients and secondary metabolites, particularly those with antioxidant activities, which include vitamin C, vitamin E, carotenoids, and phenolic compounds that work synergistically with endogenous biomolecules like glutathione [7]. The food industry is also continuously seeking natural sources of antioxidant compounds for preservation and fortification to replace their potentially carcinogenic synthetic counterparts. Foods rich in antioxidants are ideal for use as functional foods to counteract the deleterious effects of free radicals [11].

Since time immemorial, man has consumed indigenous fruits because they are available at hand. The consumption of fruits therefore predates agriculture [12], as wild fruits and vegetables, and eventually domesticated varieties have been used to supplement the diet and sometimes serve as meals. Throughout the year, a wide variety of plants produce them at different times thereby providing a cheap, an unending supply of nutrients for man. Surprisingly, as diverse and available

as fruits are, their intake is relatively low in the world, with ironically the least intake in sub-Saharan Africa (SSA). It has been established that the world's population acquire most of its nutritional and energy requirements from a narrow range of crops with 80% dominated by cereal grains, pulses and oil seeds [13] , thus, less attention has been given to preserving and propagating fruit trees [14]. The 2016 state of the world's plants report declared that approximately out of every five plants, at least one faces the risk of worldwide extinction due to urbanization, erosion and climate change [15,16]. Some areas of sub-Saharan Africa are reported to have already lost from 45 to 77% of their natural vegetation [17]. Until recently, no literature exists on them; therefore, these diverse and rich species are not fully exploited and marketed worldwide. The preservation and promotion of indigenous fruits is restricted by a lack of cognizance of their nutritional value, loss of agro-biodiversity, non-existing fruit tree nursery systems, lack of fruit processing facilities and inefficient promotional channels, suggesting that a large share of potential food sources are underutilized [18].

Furthermore, while the western world is battling with the long-term effects of processed foods in engendering obesity, dental caries and a host of metabolic disorders; ironically, the developing countries have more than a third of their population undernourished or malnourished. In addition, massive rural-urban migration meant foregoing homegrown crops and the gathering of wild fruits and vegetables for processed and fast foods, which are seen as a symbol of social status and convenience. Thus, the abundant wealth of nutrients that nature has provided are more and more laying to waste, while there is an exponential rise in nutritionally-related metabolic disorders [15]**.** As recently as 2017, the Food and Agricultural Organization (FAO) reported that the number of people facing food and nutritional insecurity in the world escalated to 815 million in 2016, up from 777 million in 2015 [19]. These figures clearly show that food and nutrition insecurity is a global challenge at pandemic levels. This has lead to proliferation of various diseases and conditions such as poor growth, poor mental development, low immunity, mental retardation, cancer and atherosclerosis linked to an upsurge in mortality rates in worst cases [20,21]. With the unprecedented population explosion, endemic fruits can be included in the diet to contribute to reducing malnutrition and its related concerns. They can also be exploited for various industrial applications to create a value chain that will provide jobs and boost the economy. These fruits are also vital in times of food shortage such as during droughts and wars [19].

The highlighted factors have increased interest in researches on indigenous fruits where they have been found to be a repository of significant amounts of both primary and secondary metabolites [13]. The *Solanum* species comprises some of the world's most economically valuable vegetables such as tomato, potatoes, pepper and eggplant [1]. Eggplant fruits are commonly sold and consumed in global markets while still a relatively wild and underutilized fruit in Africa and parts of Asia [22]. A highly adaptable, low maintenance plant that can grow in tropical and temperate climates; it is one of the most phenotypically diverse fruits in the world with varieties of varied colors, shapes, and sizes. It forms an essential part of the diet of many countries including Turkey. Several cultures also use it in the traditional treatment of a wide range of ailments ranging from diabetes, chest infections, to allergies. It has been reported to be rich in fiber, some vitamins, minerals and secondary metabolites [7]. Selection and breeding to maximally balance physical, sensory, nutritional and functional value of fruits are in high demand [23]. However, there is limited and fragmented literature on the nutritional composition and antioxidant capacities of a large percentage of eggplant varieties, particularly wild species. This data is essential in order to promote and expand the usage of these species for dietary inclusion, nutrition-related projects, the food industry, programs and policies in agricultural and environmental sectors, as well as for labelling and trade regulations. Information on nutrient contents may facilitate the selection of priority

varieties for domestication and breeding programs aimed at improving food, nutrition and financial security [18]. Therefore, continuous assessment of the composition of endemic and underutilized fruits with a bid to uncover their possible role in delivering a healthy diet and in improving livelihoods cannot be over-emphasized.

1.1. Amino Acids

Amino acids (AA) are organic compounds having both an amino and a carboxylic acid functional group (Figure 1.1). The discovery of amino acids spans over a period of 200 years with professionals from the allied life sciences globally contributing until date. Though the term 'amino acids' was coined in 1898, asparagine was the first amino acid to be discovered in asparagus in 1806 while the first isolated amino acid was glycine in 1820. However, it took another two decades and a half, in 1925 to be precise, for the complete set of the twenty-proteogenic amino acids to be unveiled. By mid-twentieth century, a plethora of about two hundred natural amino acids have been discovered and elucidated. The latest discoveries were in 1973 of the rare seleno-cysteine amino acid, while pyrrolysine was discovered in 2002 [24,25].

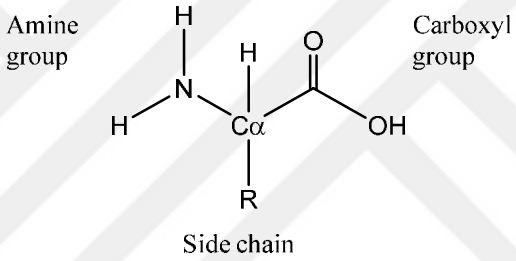

Figure 1.1 The basic structure of amino acids

Though over 300 amino acids have been identified in nature, only 20 are considered standard amino acids because they are the ones found in proteins. These proteogenic AAs are the substrates of peptides and proteins, and are responsible for their diverse biochemical roles. Each of the α-amino acids with exception of proline has a primary amino group and a carboxyl group bonded to the α-carbon atom or linked to the β-carbon atom e.g. taurine and β-alanine. They all possess an asymmetric carbon, are therefore chiral and optically active with the exception of glycine [26]. In addition to the amino and carboxylic acid functional group, each amino acid has a unique side group that accords them distinct biochemical properties and functions (Figure 1.1) [27]. The catabolism of dietary amino acids and glutamine occurs in the small intestine resulting in 30- 50% of them being inaccessible to other tissues as they provide fuel, intestinal anabolic precursors for biosynthesis and overall maintenance of our wellbeing [28].

Amino acid are best known as the building blocks of proteins. However, their biochemical functions are vast and varied, and can be broadly classified into four main categories: Messenger RNA translation; anabolic pathways (e.g. glutathione); anaplerotic homeostasis of biochemical pathways/cycles (e.g. Kreb's cycle); provision of substrates for non-nitrogen containing compounds (e.g. gluconeogenesis); and in neurotransmission/signal transduction. All of these are sensitive to, and dependent on the amino acid flow in the system, particularly from dietary intake [29].

1.1.1. Essential and Nonessential Amino acids

In 1912, Abderhalden investigated the effect of exclusion of different amino acids in the diet of adult dogs where he observed a negative nitrogen balance when they were fed a diet free of tryptophan and a positive nitrogen balance with a proline-free diet. Based on this, he reported a classification of amino acids based on the need for their dietary intake. Those that were biosynthesized were classified as nonessential (NEAAs or dispensable) and those that must be obtained from the diet as nutritionally essential (EAAs or indispensable) (Table 1.1) [30]. Rose and co-workers did further phenomenal studies on amino acid nutrition and metabolism initially in rats, then in adult human beings. The results from their work completely laid the groundwork for what we know about amino acids in the context of nutrition. Based on that, the benchmark dietary requirement of an amino acid for normal healthy growth, development and positive nitrogen balance was established [31]. Therefore, in broad terms, amino acids whose carbon skeletons our body cannot synthesis at all, or in amounts required to satisfy our physiological needs and thus must be provided by our diet are referred to as essential amino acids (EAA). On the other hand, amino acids which our body through its various anabolic pathways can biosynthesize in sufficient amounts to meet its needs are referred to as nonessential amino acids (NEAA).

In between these two extremes, are the semi-essential or conditionally essential amino acids. Sometimes, due to a variety of reasons such as in certain physiological states e.g. in pregnancy, our metabolic needs for certain nutrients may change and biosynthesis of certain nonessential amino acids may be insufficient. Therefore, some nonessential amino acids needs must be satisfied through the diet. Such amino acids are termed 'semi-essential' or 'conditionally essential' amino acids. However, it should be borne in mind that each amino acid whether classified as essential or not, has a crucial metabolic role it plays, which put together are responsible for normal cellular function and by extension overall good health and wellbeing [32]. Several controversies surround the amount of dietary protein requirement for optimal growth and development. Most of the controversy is associated with definition of protein adequacy based on nitrogen balance in relation to functions of each amino acid. In fact, recent emerging data may introduce a shift in protein dietary requirements of 'nutritionally non-essential amino acids' in both animals and humans for complete growth and development [33].

Table 2.1 Showing the classification of the 20 standard amino acids based on dietary requirement.

Nonessential Amino Acids	Essential Amino Acids
Alanine	Arginine[1]
Asparagine	Histidine
Aspartate	Isoleucine
Cysteine	Leucine
Glutamate	Lysine
Glutamine[2]	Methionine
Glycine	Phenylalanine
Proline	Threonine
Serine	Tryptophan
Taurine[3]	Valine
Tyrosine	

1. Classified as essential in young mammalians.
2. Classified as conditionally essential during stress and in neonates.
3. Classified as essential in neonates.

1.2.2. Biochemical roles of Amino Acids

Glycine

Glycine is a nonessential amino acid that was the first amino acid to be isolated, and purified from a protein (gelatin) sample in 1820 by Henri Braconnot using acid hydrolysis [34]. Though considered nonessential in humans, recent studies have established it to be conditionally essential due to some metabolic shifts (e.g. in pregnancy) and in some species such as birds. Its deficiency is characterized by immunity defects, and poor growth [35].

Glycine comprises twenty percent of the nitrogen of total protein and about eleven percent of total amino acids found in the human body [36]. It is an excellent one-carbon donor crucial to biochemical reactions. In hepatic tissues, its ability to donate methyl groups serves to balance the levels of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) in circulation [37].

Most of glycine (80%) is used for biosynthesis of proteins, especially in the young, to meet their developmental needs right from the womb. A number of key anabolic pathway require glycine, these include gluconeogenesis, methylation of DNA in gene expression and biosynthetic pathways for creatine, glutathione, bile salts, purine, and porphyrin, which translates to a reduction from the glycine recycling pool. It is therefore crucial that a fetus receives an adequate supply of glycine for proper growth and development. This is why it is classified as conditionally essential for fetal growth in some species [38].

Glycine is one of the amino acids found in structural proteins such as elastin and collagen, where it makes up about 33% of their total amino acid content. In fact, elucidation of the structural configuration of collagen shows that a glycine residue is placed after every two amino acid residues. This allows for the stability of its helical structure as it is conformed in such a way that the small glycine residue is inside the center of coil, where an otherwise larger amino acid cannot be placed [36]. A number of glycine-gated channels are found within some cells where glycine restricts the flow of calcium ions via hyperpolarization, thus producing an anti-inflammatory effect [39]; and an attenuation of skeletal muscle loss in cancer-induced mice models [40].

A study in 2018, evaluated the impact of oral glycine supplementation especially with age. Based on its metabolic roles, glycine will positively influence the elderly, serving to act as a hepato-protective, anti-inflammatory, cardio-protective, cyto-protective, and anti-atrophic agent. Since it plays a role in glutathione biosynthesis, oral glycine can increase glutathione levels that in turn will further prevent oxidative stress [41]. A recent review also pointed to a possible relationship between glycine plasma concentrations and some metabolic disorders. For instance, type two diabetes and obesity, where it was continuously observed that the plasma level of glycine remained low. The possible prophylactic and ameliorating effect of increased glycine levels against such metabolic disorders was suggested [34].

Alanine

L-Alanine (α-aminopropionic acid) is a nonessential amino acid, which was the first to be obtained through chemical synthesis before its presence as a part of protein was established. In 1850, A. Strecker initially synthesized alanine, using acetaldehyde, NH_4Cl and KCN, while in 1875, P.Schutzenberger and A. Bourgeois described a fractional hydrolysate of silk proteins as 70% other amino acids and remaining 30% as alanine [36].

L- Alanine is a gluconeogenic precursor, which is used during starvation via transamination to generate pyruvate and glutamate in the liver [42]. These intra- and intercellular reactions and transfers ensures the safe passage and elimination of nitrogen and sums up the glucose-alanine

cycle. It also makes alanine the second most abundant amino acid after glutamine to be found in circulation. The fact that these transfer reactions can be reversed acting as a two-way bridge between metabolism of two diverse and important macromolecules emphasizes the vital role of alanine that also serves to balance flux of carbon and nitrogen [43].

Though it is not a part of proteins, β-alanine plays many important biochemical roles in plants and is incorporated into pantothenate (vitamin B_5) which is involved in the carbon transporters-coenzyme A and acyl carrier proteins that are crucial to all forms of life. It also contributes to muscular health and function [44].

The Branched-Chain Family of Amino Acids – Leucine, Isoleucine and Valine

L-Leucine (α-aminoisocaproic acid), L- isoleucine (α-amino-β-methylvaleric acid) and L-valine (α-aminoisovaleric acid) constitute the three essential amino acids that have an aliphatic-branched side group and are thus, referred to as the branched-chain amino acids (BCAAs). In 1819, leucine was identified in cheese, making it the first BCAA to be discovered. It was isolated a year later by H. Braconnot in acid hydrolysates, who also named it as 'leucine'. E. Schulze determined its structure using chemical synthesis from isovaleraldehyde in 1891. Leucine is the most pervasive of all three BCAA in animal and plant proteins. On the other hand, valine was identified in 1856, in animal tissues. E. Fischer who elucidated its structure in 1906 coined the name 'valine'. In 1904, F. Ehrlich discovered isoleucine in molasses, three years later, in 1907, he reported the similarity in physical and chemical properties of isoleucine and leucine, and finally deduced its structure in 1908 [36].

The BCAAs are prevalent in proteins and serve as nitrogen stores making up approximately one-third of dietary needs of the essential amino acids [45]. By general standards, the BCAAs exhibit the greatest hydrophobicity, which forms the basis of their main biochemical roles. They are found along with methionine within the center of folded and coiled structures such as globular proteins like hemoglobin and amylase. They are vital to stabilizing such structures and enabling them to perform their biochemical functions. For instance, the hydrophobic setting they provide enables catalysis in such enzymes; the interactions of oxygen with hemoproteins and proteins in membranes to interface with lipids [46]. Hydrophobic amino acids are vital to the formation of alpha helical proteins like fibrinogen, myosin, keratin and some transcription factors. Coiled proteins such as leucine zippers, possess polypeptide chains with a repeat sequence of amino acid residues. The repeat sequences have a branched-chain amino acid placed at the unique reoccurring position, such that when the structure coils and the chains are packed, they interact and hold them together via hydrophobic interactions [47].

However, the differences in the length and nature of their branches, translates to variances in their biochemical roles and positions in proteins, and thus cannot be interchanged. For instance, while valine and isoleucine are found in beta-sheets, leucine is the predominant branched-chain amino acid in alpha helices and zippers [46]. Interchanging them has been reported to have implications on health, an example is the manifestation of a form of cardiomyopathy resulting from replacing isoleucine with valine in transthyretin [48,49] and an anomaly in plasma lipid profile simply by replacing valine with leucine in a peroxisome receptor [50].

The BCAAs have a modulatory effect on protein metabolism depending on the physiological needs, where they may stimulate biosynthesis or inhibit proteolysis, and therefore valuable in offsetting wasting. During certain physiological and pathological states, especially those synonymous with muscle wasting, the muscles have been reported to activate their biosynthesis from the corresponding branched-chain ketoacids. They are consequently useful during sports and

exercise, promote insulin activity, and improve the healing of wounds. Consequently, careful supplementation with them especially during therapy has been proposed to attenuate wasting, fatigue and to improve overall health [51,52]. The catabolism of the BCAAs has been reported to contribute to the aroma of fruits [53].

The Aromatic Family Amino Acids – Phenylalanine, Tyrosine and Tryptophan

Phenylalanine (α-amino-β-phenylpropionic acid), tyrosine (α-amino-β-p-hydroxyphenyl propionic acid), and tryptophan (α-amino-β-3-indolepropionic acid) make up the aromatic family of amino acids. Tyrosine was identified in a casein sample in 1846, then a year later in fibrin and serum albumin. Its isolation in a hydrolysate of casein in 1861 was a remarkable breakthrough, even though its structure was elucidated by chemical synthesis in 1883 by E. Erlenmeyer and A. Lipp. E. Schulze and J. Barbieri discovered phenylalanine in lupine sprouts in 1879 and isolated and its structure determined in 1883. In 1907, F.G. Hopkins and S.W. Cole discovered tryptophan from casein and Ellinger and C. Flamand elucidated its structure in 1907 [36].

Phenylalanine is an essential amino acid possessing a 4-hydroxy phenyl; tryptophan also an essential amino acid, has a heterocyclic indole ring, while tyrosine is a nonessential amino acid, and a product of the irreversible hydroxylation of phenylalanine in the liver or from the hydrolysis of dietary proteins. So far, in animals, this irreversible reaction represents the only identified anabolic pathway for aromatic amino acids. Thus, the availability of tyrosine relies heavily upon the adequate dietary intake of phenylalanine and its irreversible nature illuminates why if there is a deficiency of phenylalanine, tyrosine cannot replenish it [54]. Thus, the recommended daily intake of phenylalanine is set at 25 mg/kg of body weight [55]. Generally, the aromatic amino acids are the precursors of important biochemical compounds that mediate a wide variety of cellular processes. Their aromatic rings greatly contribute to the organizational conformation and physical stability of proteins. They also contribute to catalysis via acid-base exchanges and electron transfer reactions [56].

Phenylalanine is the precursor for tyrosine, which in turn is the precursor of a host of pigments, hormones, collagen and neurotransmitters such as epinephrine, norepinephrine, dopamine, thyroxine, and melanin. It may be broken down for energy via the Krebs's cycle and helps in catalysis and control of enzyme activity through its phosphorylation and de-phosphorylation via its hydroxyl group [57, 58]. The hydroxyl group of tyrosine also enables it to synchronize metals through oxidative and reductive reactions. Ferritin, a pervasive protein that stores iron, possesses a well-maintained residue of tyrosine in its iron center, which serves as a passive lone-electron biochemical energy store easing the oxidation of iron [59].

Tryptophan, an essential amino acid, is the most structurally complex of the amino acids. It is vital to the biosynthesis of the family of potent plant growth hormones called auxins, which influence and direct growth and development at strategic parts of the plant such as in the seeds, embryo, roots, stem, flowering, and during tropism [60]. Tryptophan is also the precursor of terpenoids, which are indole alkaloids and niacin [61].

Several disorders have been identified associated with a malfunction with the different enzymes associated with their metabolism. For example, insufficient or absence of phenylalanine hydroxylase in the liver leads to an accumulation of phenylalanine and manifests as phenylketonuria. It is characterized by seizures, motor, mental and learning defects [62]. A mutation in tyrosinase, the enzyme required for melanin biosynthesis results in albinism. It is a condition characterized by absence of coloration of the outermost covering including hair, skin, and eyes. Albinos tend to suffer more from poor vision, deaf-mutism, and are highly susceptible to

infection, sunburn and skin melanomas [63]. Disorders associated with tryptophan metabolism include certain types of cancers and Alzheimer's disease. Thus, key points in its metabolism have been targeted in the management and treatment of tumors and neuro-degenerative diseases [64].

The aromatic amino acids especially tryptophan, are responsible for the innate fluorescence exhibited by proteinous compounds. This fluorescence property is exploited in assessing variations in the configuration and functions of enzymes. Since the nature of the protein affects the strength of the fluorescence displayed by tryptophan, this unique property is the basis of techniques used to assess structure and arrangement of proteins. The technique involves real time identification of intrinsic protein with the aid of highly specific fluorescent probes [56].

The Sulfur-Containing Amino Acids – Methionine and Cysteine

There are four amino acids – methionine, cysteine, homocysteine and taurine, which contain sulphur, however only two - methionine and cysteine, are proteogenic. In 1922, methionine was identified in an acid hydrolysate of dairy protein and the presence of sulphur in it a year later. On the other hand, L-cysteine (α-amino-β-mercaptopropionic acid) was discovered in the bladder in 1810. In 1884, E. Baumann established it to give rise to cystine via its dimerization; however, the structures of both residues were not elucidated until 1903–1904 via chemical synthesis [26].

The presence of sulphur, which is electronegative accounts for their biochemical roles. For example, it makes all the difference between cysteine and serine that differ only by the presence of sulphur in cysteine, as cysteine easily forms disulphide bridges and dissociates with the same ease [65].

Methionine is an essential hydrophobic amino acid crucial to single carbon metabolism. It plays a role in biosynthesis of proteins, phospholipids; detoxification and combating oxidative stress [66], The hydrophobic nature of methionine places it in the center of globular proteins and enzymes, or in contact with the lipid bilayer of membranes. They serve a protective role against free radicals where they are oxidized to methionine sulphoxide, hence protecting membrane lipids and active sites of enzymes. The enzyme- methionine sulfoxide reductase; in a continuous sequence reverses the sulphoxide to methionine. Aging, atherosclerosis and several degenerative disorders have been linked to a buildup of methionine sulphoxide due to an impairment in the reductase enzyme activity. The use of methionine in the treatment of depression, inflammation, and liver diseases is also being explored [67].

Cysteine is a non-essential amino acid and its ability to form disulphide bridges is critical to protein folding, spatial conformation and stability that determines the different structures of proteins and peptides, and their subsequent biochemical function [68]. For example, a large amount of cysteine is found in structural proteins such as keratin; active sites of enzymes; and in immunoglobulins where disulphide linkages stabilizes their structure, vital to their function [69]. It is a component of glutathione [70], oxytocin and vasopressin and its use as a panacea to bronchitis, angina and respiratory distress is currently being investigated [71].

The Hydroxyl - Group Containing Amino Acids - Serine and Threonine

Serine (α-amino-β-hydroxypropionic acid) has a hydroxyl group in its side chain that is polar and uncharged and a nutritionally nonessential amino acid. It was first isolated from a type of silk protein hydrolysate called sericine in 1865. Later on, in 1901, it was isolated from acid hydrolysates of silk by E. Fischer and A. Skita. The structure of serine was determined in 1902 using chemical synthesis. In 1931, F.S. Daft and R.D. Coghill found out how highly unstable serine is, when heated in strong alkaline solution which served as a breakthrough in analyzing amino acids in protein. In

1932, F.A. Lipmann and P.A. Levene reported the discovery of phosphoserine in acid hydrolysates. This finding initiated and led to the discovery of protein phosphorylation in cells [36].

The main roles of serine are in metabolism and signaling where it serves as a source of one-carbon units. Serine plays a role in reductive biosynthesis and contributes to redox homeostasis. It interconnects metabolic flow from several important pathways where it links glycolysis to purine synthesis, folate-mediated one-carbon metabolism (FOCM), glutathione synthesis, and lipid metabolism [72]. It is therefore necessary for methylation of proteins, DNA and RNA, and anabolic pathways that generate biomolecules such as amino acids, nitrogenous bases, creatine, epinephrine and phospholipids. Its role in cell signaling and regulation is exemplified by its kinase- mediated phosphorylation [73]. Even though other sources of one-carbon donors exist, those derived from serine serve as precursors for *de novo* biosynthesis of adenosine, guanosine and thymidylate, and for the re-methylation of homocysteine in the methionine cycle. The biosynthesis of cysteine and glycine via the transsulfuration pathway also uses serine [74].

Though it was reported that tumor cells depend more on dietary serine than *de novo* synthesized serine, which points to how diet may be used in cancer management [74]. The antioxidant and anti-inflammatory effect of L-serine supplementation in age-related oxidative stress and obesity have been reported [75]. Serine deficiency shows symptoms associated with the central nervous system such as congenital microcephaly, seizures and severe psychomotor retardation [76].

L-Threonine (α-amino-β-hydroxybutyric acid) has a polar, uncharged side chain. It is a hydrophilic essential amino acid discovered in 1925 making it the last known protein amino acid to be discovered. A decade later, threonine was isolated from acid hydrolysates of casein by W. Rose and colleagues. This made it possible to prepare crystalline amino acids for research and dietary preparations.in protein. In 1952, for the first time, threonine as a phosphoester in proteins due to post-translation protein phosphorylation was reported [25]. Like lysine, threonine does not partake in transamination reactions and is therefore a strictly indispensable amino [77]. Consequently, an adult human requires a dietary intake of about 20 mg/kg body weight per day [78].

Threonine is essential to maintaining proper immune function, glycine requirements, gastrointestinal function, feather growth, optimal growth and development [79]. It exhibits exceptional metabolic plasticity that contributes to its unique role in metabolism [80]. A study investigated the effect of excess and deficiency of threonine in an animal model to assess its effect on protein synthesis. The tissue turnover rates in young growing animals is high particularly in organs like the intestine and skeletal muscle. Results showed that both excess and lack of threonine impaired protein synthesis as low growth was observed in the animals in both cases [81]. Embryonic stem cells (ESCs) are continuously going through self-renewal and differentiation while sustaining their pluripotency; threonine is strictly required during this process where it serves potent anabolic and signaling roles in the stem cells [82]. The intestinal epithelial lining protects the intestine from a host of diseases that include sepsis, inflammatory bowel disease, allergies, ulcer, and even type 1 diabetes**.** Thus, osmotically acting amino acids, such as glutamine and threonine with *in vitro* and *in vivo* intestinal cyto-protective activity are of interest. The cyto-protective effect of threonine following heat stress injury was also investigated, and it was observed to significantly elevate levels of heat shock protein (HSP 25 and 70) [83].

Amino acids with Acidic Side-Chains – Aspartate, Glutamate, Asparagine and Glutamine

It is a nonessential amino acid and was the first amino acid ever to be discovered in 1806 in asparagus juice. The lack of techniques for proper analysis delayed the recognition of asparagine until more than 100 years later. It was fully studied with the aid of enzyme hydrolysis of edestin (a type of globular protein found in hemp seeds) using pepsin, trypsin, and dipeptidase, and its role in proteins was finally reported [36]. Oxaloacetate serves as the precursor in *de novo* biosynthesis of asparagine in a series of transfer reactions [84]**.** Asparagine has vital roles in immunity, cell targeting, cell motility, and in protein structure and folding [85]. The antitumor activity of aspariginase has been reported, particularly for treatment of childhood acute lymphoblastic leukemia (ALL) where 40 to 60 % of patients went into remission [86].

L-Aspartate (α-aminosuccinic acid) has an acid polar side-chain that is negatively charged at neutral pH. It is a nonessential amino acid first identified in 1827 after alkaline hydrolysis of asparagine [36]. Aspartic acid is the precursor of the amino acids- methionine, threonine, isoleucine, and lysine and regulates the secretion of important hormones [87], pyrimidines, NAD and pantothenate - which donates nitrogen to the anabolic pathways of arginine and purine [88].

Aspartate is a vital metabolite in the urea cycle where it serves as a source of one of the amine groups on urea [89]. It is one of the amino acids that contribute to gluconeogenesis and serves as the precursor of asparagine via its transamidation. The malate-aspartate shuttle where aspartate and oxaloacetate are interchanged with ease also serves to transport reducing equivalents. It serves as the prevailing pathway in the heart with the degree of NADH transport into the mitochondria being 10 times higher than that of NADH transported by the glycerol-phosphate shuttle [90]. In addition, aspartic acid acts as a hydrogen acceptor in a chain of ATP synthase [91].

Hypertension is the number one identifiable risk factor for death worldwide and a leading risk factor for stroke, heart attack, heart failure, and chronic kidney disease. A study showed that aspartate or malate elevated L-arginine and NO levels, which in turn mitigates hypertension [92]. Recent studies have suggested the ameliorating role of oral aspartate supplementation in chronic fatigue state [93]. The normalization of enterohepatic circulation of bilirubin and bile acids linked with jaundice was observed with increased dietary intake of L-aspartic acid as it was observed to inhibit the activity of beta-glucuronidase that regulates their activities [94]**.** Since aspartate aminotransferase (AAT) synergizes with malate dehydrogenase to transfer electrons from NADH across the inner mitochondrial membrane, it has been the target of many studies to treat cancer. Its inhibition with oxamate was proposed, and may help arrest the growth and development of breast cancer cells [95,96].

L- Glutamate (α-aminoglutaric acid) has an acidic polar side-chain that is negatively-charged at neutral pH. It is a nonessential amino acid discovered in 1866 in wheat gluten. In 1907, K. Ikeda identified the monosodium salt of L-glutamic acid - monosodium glutamate while derivatives of glutamate (N -acetylglutamate and phosphoglutamate) are found as free amino acids in animal tissues [36]. All organisms are capable of biosynthesizing glutamate and is one of the most coded amino acids. Its abundance is attributed to the negative charge it bears, its stability and how it is metabolically generated and eliminated via its interconversion with the tricarboxylic acid cycle intermediate- α-ketoglutarate [97]. Apart from the provision of glutamate for cellular processes, the pathway for its biosynthesis serves as a route for the incorporation of nitrogen into several metabolic pathways. Purines and pyrimidines obtain about 50% of their nitrogen from glutamate biosynthetic pathway. While glutamate donates nitrogen groups to all amino acids [98], via the direct transfer of its amine to the 2-oxo precursors of various amino acids, e.g. serine, aspartate, leucine, isoleucine, phenylalanine, alanine, and tyrosine, while others acquire their amine

nitrogen indirectly. Likewise, apart from the amino group, the entire carbons in proline and most of those in arginine are obtained from glutamate [99].

The mammalian central nervous system has an abundance of glutamate where it serves as an excitatory neurotransmitter [100]. Glutamate is also the precursor of γ-aminobutyrate (GABA), the main inhibitory neurotransmitter in the mammalian central nervous system. Overall, glutamate contributes to shifts in synaptic strength that defines its function in cognitive tasks such as memory, perception and learning [101]. The biochemical functions of glutamate maybe summarized as being a building block of proteins, precursor of glutamine, facilitates shuttling of nitrogen, contributes to cell signaling, building block of glutathione, precursor of N-acetylglutamate, and found in active sites of enzymes [102]. It is also used as a food additive and flavor enhancer [103]. Glutamate has what is termed as 'umami taste' that increases gastro-intestinal secretions that enhance the digestive process [104]. A coordinated system between the metabolism of glutamate and ingestion of a meal rich in the branched-chain amino acids exist which elicits the release of insulin from the β-cells of the pancreas [105].

L-Glutamine (α-amino glutaramic acid) has a polar, uncharged side chain. It is a nonessential amino acid discovered in 1883 in beet juice. In 1904, E. Fischer reported it to produce ammonia when proteins undergo acid hydrolysis. However, it was recognized as a nutrient in 1965 due to work conducted by E.M. Neptune [36]. Glutamine is the most abundant amino acid and serves as an important donor of nitrogen for the anabolic pathways that produce nucleotides and nonessential amino acids [106]. Glutamine thus contributes to anaplerosis of the TCA cycle that is central to energy generation and biosynthesis [107]. The transamination of glutamine to generate α-ketoglutarate activates an atypical highly conserved serine\threonine kinase called mTOR (Mammalian target of rapamycin). TOR is a signaling molecule that assimilates a variety of stimuli to direct cell growth, autophagy, protein synthesis, cell motility, nutrient uptake, and ribosome biosynthesis [108]. Moreover, glutamine is also required for the maintenance of mitochondrial functions [109]. The role of glutamine in cell growth and development has made it the target of several studies on cancer therapy as even cancer cells rely upon glutamine, as such cancer patients experience glutamine depletion that ultimately leads to weakness and wasting of the body [108]. The inhibition of its metabolic pathway are some of the strategies that have been considered [110].

Basic Side Chain Amino Acids –Arginine Lysine, and Histidine

L-Arginine (2-amino-5-guanidinovaleric acid) has a basic and polar charged side chain at neutral pH. It is a semi-essential amino acid, first discovered in 1886 from extracts of lupine seedlings. With the elucidation of the urea cycle by H.A. Krebs and K. Henseleit in 1932, the metabolic role of arginine was unveiled [36]. Several vital metabolic processes require arginine; these include biosynthesis of creatine, polyamines, nitric oxide, urea cycle, cell cycle, and protein synthesis. There is an increased demand for arginine during rapid cellular growth, in such times *de novo* synthesis does not suffice and must be supplemented from the diet [111]. The urea cycle relies on arginine as an intermediate to transport nitrogenous waste and also contributes to acid-base balance [112]. Arginine is required for collagen biosynthesis that is necessary for wound healing, skin, joint, and bone health [113]. Based on the role of arginine in metabolism, it has been a potential target in cancer management including inhibition of its pathway and depleting arginine to inhibit several types of cancers [114].

L-Lysine (α, ε-diaminocaproic acid) has a basic polar side chain that is positively charged at neutral pH. It is an essential amino acid discovered by E. Drechsel in 1889 in casein hydrolysates. In 1891, he determined its structure while it was named 'lysine' by Ernst Fischer to depict how

urea was released upon its alkaline hydrolysis [36]. The main role of lysine is in protein synthesis and modulates biochemical processes ranging from signaling to stress response, apoptosis, proteolysis, and metabolism [115]. For instance, pathways for biosynthesis of carnitine, collagen and elastin involve a series of modifications of lysine [116]. Lysine is found in the domain of many enzymes where it participates in catalysis and makes their regulation possible via acetylation/deacetylation and methylation/demethylation, found widely in intermediary metabolism [117].

Lysine serves as a cleavage point for proteases made up of pairs of basic amino acid residues that include lysine i.e., Lys-Arg, Lys-Lys, or Arg-Lys [118]. A type of large muscle protein called titin, found in heart and skeletal muscle has a lysine-rich motif in its core, and contributes to their elasticity [119]. Lysine is part of the structural formation of collagen that is the framework for connective tissues [120]. It promotes the absorption of calcium by our body while reducing its loss in the urine, consequently, the potential role of lysine in prevention and management of osteoporosis has been investigated [121]. L-Lysine is reported to reduce anxiety-induced intestinal pathologies like diarrhea as it has an effect on intestinal tract receptors of the gut where it acts as a serotonin antagonist [122]. Deficiency of lysine has been observed to weaken the body's immune system and cell-mediated immune response [123].

L-Histidine (α-amino-β-imidazole propionic acid) has a basic polar side chain that is positively charged at neutral pH. It is classified as a nutritionally essential amino acid, predominantly for infants, and is vital throughout a plant's growth and development [124]. A. Kossel discovered it in acid hydrolysates of sturine protein in 1896 while its structure was described in 1904 [36]. Histidine is the most versatile of the 20 standard amino acids, which is from its imidazole side group that is aromatic, ionizable, a hydrogen bond donor and acceptor, and can coordinate metallic cations [125].

Although man obtains histidine solely from the diet, its metabolic pathway is quite broad and produces a variety of metabolites. For instance, histamine is a product of its decarboxylation [126]; while urocanic acid and ammonia, its deamination [127,128] Histidine is found in catalytic sites of enzymes whose mechanism of catalysis involves general acid-base catalysis, covalent modification, and proton/electron shuttling e.g. in serine esterases, such as trypsin, chymotrypsin, and acetylcholinesterase [128]. Histidine also serves as a precursor for some hormones such as thyrotropin-releasing hormone that induces secretion of thyroid stimulating hormone [129,130]. Galactosylceramide, which contributes to the compact nature of the myelin membrane, requires histidine residues for its maintenance [130]. The nitrogen atoms in the imidazole ring of histidine confer on it the ability to chelate metal ions such as zinc, manganese and copper. This plays a vital role where histidine-rich motifs in DNA transcription factors are involved in connecting proteins and nucleic acids via zinc-fingers [131]. Histidine is vital to globin biosynthesis and for erythropoiesis [132]. The presence of histidine decreases the rate at which free radicals that contribute to destruction of erythrocytes in circulation are produced, as such; histidine deficiency is characterized by anemia [133].

Several plant species have the ability to bioaccumulate nickel; this is due to the presence of free histidine that plays the role of a nickel-binding ligand, therefore enhancing the plant's tolerance to nickel. This affinity of nickel for histidine is used in metal-binding affinity chromatography, for example, an immobilized nickel column is used as a stationary phase to purify proteins with a histidine-tag [129]. Dietary intake of histidine-dipeptides such as carnosine and anserine may help in prevention and management of metabolic syndrome through their anti-inflammatory and antioxidant activities [134].

Imino Group-Containing Amino Acids - Proline

L-Proline (pyrrolidine-2-carboxylic acid) has a neutral, imino hydrophobic side chain that is conformationally restricted, and is a conditionally essential amino acid. Prior to its discovery as a part of proteins, it was the second amino acid to be chemical synthesized in 1900. A year later, E. Fischer who was not aware of its chemical synthesis, isolated proline from acid hydrolysates of casein and elucidated its structure**.** Proline and hydroxyproline are pervasive, forming about 33 percent of collagen [36]. Based on the amount (per-gram basis), necessary for total body protein synthesis, proline requirement is the highest amongst all amino acids [135]. The strength of proline non-covalent interactions, combined with its rigidity, plays a prominent role in protein stabilization and recognition processes [136].

Proline acts as a signaling molecule and acts a sensor of cellular energy levels [137]. It has been reported to be vital to cell differentiation, and fetal growth and development [138]. Proline levels were observed to significantly rise with cellular oxidative stress, this led to studies that confirmed its antioxidant activity [139]. In plants, it plays a defensive role against a variety of biotic and abiotic stresses and can accumulate in pollen, a unique property among the proteogenic amino acids [140]. A study observed that inhibition of proline metabolism significantly decreased melanoma cell growth by starving the cells of proline [141]. Thus, recent studies have been investigating ways to target key metabolites in the proline cycle in cancer therapy [142]**.**

An excess of proline in the blood, called hyperprolinemia, is characterized by neurological problems such as seizures, schizophrenia and intellectual disability [143]. Contrastingly, a study investigated the effect of acute proline deficiency on proline balance in rat. Their findings suggested that in proline deficiency, the small intestine could synthesize proline. This shows our body requires proline even if it is not nutritionally essential; and may produce what it requires for metabolic homeostasis [144]. That notwithstanding, in recent studies on infant nutrition it has been observed that even though most mammals including man, can synthesize proline, the rate may be inadequate for neonates [135].

1.2. Vitamins

Fruits are major contributors of vitamins to our diet [145]. Vitamins are a large group of chemically and functionally diverse organic compounds that are required in minute amounts for normal physiological function [146]. The concept of vitamins was coined by Casimir Funk, a polish biochemist in 1912 [145]. Vitamins are distinctly different from other nutrients both in structure, dietary requirement and metabolic roles. They are also distinct because they are not involved in structural formations and their breakdown does not generate significant amounts of energy for use by our cells. Rather, they each have specific metabolic and physiological roles that aid metabolism of other nutrients, and are required in trace amounts by our body. Most vitamins exist in foods in a provitamin inactive form that necessitate metabolic activation to perform their functions. That notwithstanding, their importance to general health and wellbeing cannot be over - emphasized as improper intake results in deficiency diseases – such as night blindness, beri-beri, pellagra; or toxicities - resulting in hypervitaminosis, as the case may be [147]. The functions of each vitamin forms the basis of its requirements and clinical manifestations of its deficiencies [4]. While our body can synthesize some vitamins, 13 vitamins must be acquired from the diet. These 13 essential vitamins are further categorised based on their relative solubility into fat-soluble and water-soluble vitamins [148].

Vitamins A. D. E. and K comprise the fat-soluble vitamins that are absorbed with dietary lipids in the intestine. Excess fat-soluble vitamins may be stored in the liver and fat tissues. The rest of vitamins B_1, B_2, B_3, B_7, B_9, B_{12} and vitamin C (ascorbic acid) are classified as the water-soluble vitamins and cannot be stored in our body. Since our bodies can store the fat-soluble vitamins, their toxicities may occur while their deficiency diseases are relatively less common while it is vice-versa for the water –soluble vitamins [149].

Although the vitamins are structurally diverse, each with its unique role, however, they work synergistically with each other to sustain our biochemical and physiological processes. Deficiency of one may affect the function of another, thus in assessing deficiency this must be borne in mind [150]. For instance, a deficiency of folate can manifest with thiamine deficiency symptoms since it contributes to thiamine activation. While thiamine deficiency in turn may affect pyridoxine function [151].

1.2.1. Vitamin C - Ascorbic acid

Vitamin C is a lactone made up of six-carbons synthesized from glucose [152] and exists as its ascorbate anion at physiological pH. While most plants and animals can synthesise vitamin C, primates cannot synthesize it as they lack L-gulonolactone oxidase required for the final step of its biosynthesis. Most citrus fruits such as lemons and oranges are rich sources of vitamin C [153].

Vitamin C is a potent water-soluble antioxidant and serves as an enzyme cofactor for several vital anabolic and gene regulatory enzymes [154]. Its role as an antioxidant is because it has the ability to donate electrons sequentially and serve as a reducing agent. Several classes of compounds are scavenged by vitamin C, examples include free radicals such as reactive oxygen (superoxide and peroxyl radicals); reactive nitrogen species; reactive compounds such as nitrosamines and ozone; products of the reactions of radicals or reactive compounds e.g. α-tocopherol scavenges free radicals and is radicalised in the process, vitamin C neutralises it back to α-tocopherol and; transition metals such as iron and copper [155].

Eight enzymes require vitamin C - three participate in collagen synthesis, two in carnitine biosynthesis while the rest are involved in biosynthesis of norepinephrine, amidation of peptide hormones and regulation of tyrosine metabolism [155]. Consequently, deficiency of vitamin C leads to scurvy because of the incomplete crosslinking of collagen during its synthesis. Scurvy is characterized by symptoms such as gingivitis, body pain, bone aches, and slow healing of wounds. Vitamin C is also required for DNA demethylation. Thus, deficiency of vitamin C may lead to phenotypic defects and disease such as cancer and neurodegeneration [156,157]. Vitamin C enhances the body's immunity in several ways. It complements and supports cellular reactions of the immune system and protects the epithelial linings. The motility and activity of neutrophils are enhanced by vitamin C as it protects them and aids oxidative destruction of bacteria within the neutrophils [158]. Vitamin C was recently reported to directly contribute to the growth and functions of osteoblasts [159].

Recent studies suggest vitamin C may have analgesic activity in specific clinical conditions and potentially ease suffering [154]. It is reported to protect the neurons from oxidative injury during re-oxygenation after hypoxia [160]. Vitamin C supplementation was investigated in critical care patients. It was reported to significantly reduce inflammation, pain, and cellular/organ injury [161]. It was also suggested to be useful in managing hypertension, sepsis, glycemic control, respiratory constriction, acute renal injury and generally reduce hospital stay [162]. Studies have investigated its anticancer potential based on its dehydroascorbate form being transported by

glucose transporters. Since cancer cells rely on glycolysis for energy, vitamin C could restrict glucose transport and limit their energy supplies [163].

Apart from inadequate dietary intake, certain life style choices and health conditions affect the bioavailability of ascorbate in the plasma. For instance, cigarette smoke [164], alcohol consumption, chronic kidney disease, dialysis, gastrointestinal disorders (e.g. ulcerative colitis, Crohn's disease) all lower its bioavailability and contribute to the manifestation of its deficiency symptoms [165]. People with particular genetic variants of its transporters are also more prone to ascorbate insufficiency [166].

1.2.2. Vitamin B_1 - Thiamine

Vitamin B_1 is an essential sulphur-containing water-soluble vitamin. Rich sources of vitamin B_1 include whole grains, nuts and meat. The recommended daily intake is 1.2 and 1.1 mg for adult males and females, respectively [167]. The highest concentrations are found in the liver, kidney, heart and skeletal muscle tissues [168].

Dietary vitamin B_1 generally exists as phosphate derivatives that are subsequently released by the action of phosphatases [169]. Being water-soluble, it is absorbed directly into blood circulation, can cross the blood-brain barrier and eventually excreted in the urine. While in circulation, it is activated to thiamin pyrophosphate (TPP) in the presence of magnesium. TPP is central to cellular energy generation. It serves as coenzyme for the pyruvate dehydrogenase complex that is important for carbohydrate metabolism and biosynthesis of myelin and acetylcholine. It partakes in the Krebs's cycle as a cofactor of α-ketoglutarate dehydrogenase that transforms α-ketoglutarate to succinate, a reaction that contributes to bioavailability of glutamate and gamma-aminobutyric acid (GABA) [170]. The transketolation of glucose-6-phosphate in the pentose phosphate pathway requires TPP that contributes substrates to vital anabolic pathways [171]. It is therefore vital to maintenance of cellular activity and neurological function [172,173].

Despite its availability in a varied range of foods, deficiency of vitamin B_1 is widespread in both developed and developing countries. It is seen in 'high-calorie malnutrition' where processed foods with high calories and low nutrient quality are consumed [174]. Processing techniques, contamination, poor dietary choices, alcoholism, eating disorders such as bulimia, disorders that affect its absorption and metabolism such as bariatric surgery and diabetes all contribute to thiamin deficiency [175,176]. Its deficiency is characterized by a decreased activity of relevant enzymes that negatively affects oxidative metabolism and results in decreased energy generation and subsequently several cellular processes are affected, particularly the neurons. This leads to Wernicke-Korsakoff syndrome (WKS) and beriberi with clinical symptoms that include brain lesions, encephalopathy, enlarged heart, psychosis, and muscle wasting [177].

1.2.3. Vitamin B_2 - Riboflavin

Vitamin B_2 is it a water-soluble vitamin that consists of a ribityl-side chain linked to an aromatic isoalloxazine ring structure [178]. Dairy products, dark green vegetables, offal and fatty fish are rich sources of vitamin B_2. The RDA of riboflavin for adults and children ranges from 0.9 to 1.3 mg/d. and 0.5 to 0.6 mg/d, respectively [179]. Most of vitamin B_2 in foods is in the form of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which are hydrolyzed to riboflavin in the intestine by phosphatases from where it is absorbed and actively transported into circulation and finally excreted in the urine. Intestinal bacteria in the colon also produces riboflavin,

which is also absorbed into circulation. Most of it is found enzyme-bound in tissues, or else it is hydrolyzed and excreted [180].

FMN and FAD participate as coenzymes in diverse reactions central to intermediary metabolism. They are part of reactions involving lipid metabolism, detoxification, signaling and protein folding [179]. Riboflavin contributes to redox homeostasis via an FAD – dependent enzyme essential to regulating physiological ratio of reduced to oxidized forms of glutathione [180].

Deficiency of riboflavin is usually seen in general malnutrition, alcoholism, thyroid malfunction and malabsorption from the GIT. Its deficiency manifests as neuro-generative and skin disorders [181]. The plasma levels of stroke patients were observed to be low in riboflavin, suggesting a possible role of its supplementation to reduce the risk of acute stroke [182]. Recently, it was reported that an enzyme that can further add a fourth ring to the three-ring structure of vitamin B_2 has been identified. A prenylated $FMNH_2$ is produced which may give more bioactive roles to vitamin B_2 [183].

1.2.4. Vitamin B_3 – Niacin

Niacin may be found in its acid (nicotinic acid) or amide form (nicotinamide). Rich dietary sources include meats, nuts, mushroom, yeast, beverages, and beans [184]. It is absorbed from the intestine into circulation from where it enters the liver for its metabolism, after which it is excreted in the urine. A dietary allowance of 15 mg/day of niacin is recommended [185]. It is also synthesized from tryptophan in the liver [186].

Niacin is the precursor of the cofactor's nicotinamide adenine dinucleotide (NAD^+), its reduced form (NAD(P)H) and its phosphorylated form (NAD(P)H) They are vital to all aspects of biochemical transformations that occur in cells [187]. For instance, NADH is produced in the Krebs's cycle from NAD^+, its ensuing oxidation results in synthesis of ATP. NADPH contributes to several biosynthetic pathways including that of purines, pyrimidines and in reduction of glutathione [188]. NAD^+ participates in diverse post-translational modification reactions that involve reversible introduction of one or more ADP-ribose moieties into a protein. This serves to regulate several biochemical processes including DNA repair, cell development, immune and inflammatory reactions [189].

Nicotinamide has been used for decades in the treatment of various skin disorders because of its anti-inflammatory activity; and ability to reduce sebum secretion and regulate fatty acid synthesis [190]. Significant improvement was observed when used to treat acne, atopic dermatitis, ageing, and skin pigmentation. Its inhibitory effect on cytokines and steroid-sparing effect has made it part of dermalogical preparations for the treatment of autoimmune blistering dermatoses [191].

Ultraviolet rays increase the activity of poly ADP ribose polymerase-1 (PARP-1) which consumes NAD^+ and contributes to cell death. This causes aging and increases the risk of cancer. Nicotinamide prevents cell senescence by providing energy and inhibiting PARP-1 via feedback inhibition [192]. The role of some NAD-dependent enzymes in gene regulation and key metabolic pathways have made niacin a feasible target in prevention and management of metabolic disorders including cancer and cardiovascular disease [193].

Deficiency of niacin leads to pellagra and is mainly seen in chronic insufficient dietary intake of niacin and tryptophan; disorders that prevent their absorption and in alcoholism [194]. Initial signs include poor appetite, lethargy, irritability, abdominal pain, and glossitis. The skin, nervous system, and gastrointestinal tract which have significant cellular turnover and demand for energy are affected, with manifestation of the four 'Ds': Dermatitis, Diarrhea, and Dementia leading to Death [195].

1.2.5. Vitamin B_5 – Pantothenic Acid

Vitamin B_5 is found in a diverse range of foods including off al nuts, dairy products, seafood and grains. A daily intake of 5 mg/d of pantothenic acid is recommended. It is the precursor of the 4′-phosphopantothine moiety of coenzyme A and the acyl carrier protein (ACP) and thus contributes to the tricarboxylic acid pathway and several anabolic pathways including those of cysteine and flavonoids [196]. A highly regulated process converts pantothenic acid to pantethine- its bioactive form, which possesses a sulfhydryl-group required by coenzyme A (CoA) [197]. Coenzyme A is a ubiquitous and vital cofactor to many cellular processes such as synthesis of carboxylic acids, lipids, and non-ribosomal peptides [198]. Therefore, vitamin B_5 contributes directly and indirectly to maintaining energy generation, the Krebs's cycle, total lipids, stress levels and detoxification. Consequently, its deficiency may limit energy balance and immunity [199].

Pantethine was reported to favorably alter total, LDL and non-HDL cholesterol in low to moderate cardiovascular risk subjects [200]. High levels of C-reactive protein (CRP) are observed in low-grade inflammation, which is a risk factor for atherosclerosis. Increased dietary intake of vitamin B_5 in human subjects over a five-year period was reported to lower CRP levels suggesting its possible cardio-protective role [201].

1.2.6. Vitamin B_6 – Pyridoxine

Vitamin B_6 can be sourced from a variety of foods such as meats, fruits, vegetables and our intestinal flora. The recommended daily allowance for adults is 1.7 and 1.5 mg/day for males and females respectively [202]. Its biochemical roles are either as a coenzyme or as an antioxidant [203]. About 140 reactions require pyridoxal phosphate (PLP) - dependent enzymes, for instance, the anabolic pathways of lipids, transamination of amino acids, and glycogenolysis [204,205]. Vitamin B_6 is essential to biosynthesis of haemoglobin [206] and some neurotransmitters (e.g. serotonin, dopamine, and γ-aminobutyric acid (GABA) [207]. The antioxidant activity of vitamin B_6 was recently investigated and reported by Ehrenshaft *et al.* [208].

Deficiency of vitamin B_6 is rare except with general malnutrition, disease (e.g. HIV, anaemia); alcoholism- as acetaldehydes compete with its binding sites; and drugs that interfere with its metabolism e.g. isoniazid and carbamazepine [209]. Decreased levels of erythrocyte glutamic oxaloacetic transaminase activity are observed in vitamin B_6 deficiency. Clinical symptoms include dermatitis, microcytic anaemia, confusion, neuropathy and seizures [210].

Vitamin B_6 supplementation was reported to reduce oxidative stress and the risk of metabolic complications in diabetic patients [211]. A study investigated the ameliorating effect of vitamin B_6 on inflammation and oxidative stress markers in rats subjected polymicrobial sepsis. It was reported to decrease neutrophil infiltration and levels of oxidative markers in the liver and thus reduces oxidative stress [212].

1.2.7. Vitamin B_9 – Folate

Vitamin B_9 is found in dates, nuts, leafy vegetables, liver, fruits and fortified grains [213]. The recommended daily allowance is 50 micrograms for an adult. The liver can store up to 10 to 20 mg, an adequate amount for about 90 days. In the body, it is found as folate derived from folic acid while its reduction to tetrahydrofolate renders it bioactive [214]. It serves as an acceptor and donor of one-carbon units in a variety of cellular processes and cofactor of methylation reactions [215]. It is required for the biosynthesis of nucleic acids and methylation of DNA [216]. It serves

as one-carbon unit shuttle in anabolic pathways of glycine, serine, purines, biotin, thymidylate, and pantothenate. In plants, it contributes to respiration and metabolism of phytochemicals such as alkaloids, carotenoids, chlorophyll and vitamins [215]. It also participates in the vitamin B_{12} - dependent reactions that produces methionine [214].

Though the liver can store it for a short –term, its deficiency can occur suddenly. Deficiency of folate has severe metabolic and clinical consequences as it results in DNA damage and mutation. Since folate is required for synthesis of thymidylate and methionine, in its absence, uracil is integrated into DNA in place of thymine, while lack of methionine hinders methylation of DNA causing point mutation, strand and chromosome breaks [217]. It manifests as megaloblastic anaemia, neural tube defects in the fetus, and neuropathy. It has further been linked to thrombocytopenia, cancer, mood swings and cognitive defects [218,219].

1.2.8. Vitamin B_{12} – (Cyanocobalamin)

Vitamin B_{12} is a family of cobalt-containing corrinoids and the most complex of the vitamins [220]. Sources include meats, dairy products and commensal intestinal flora. The daily allowance is 2.2 μg/day for adults. It is essential to development- from erythropoietic stem cells to mature red blood cell, biosynthesis of nucleoproteins and cell development. It also enhances the production of lecithin that is required for the biosynthesis of myelin sheaths [221].

The biochemical functions of vitamin B_{12} are classified into those that require either methylcobalamin or adenosyl cobalamin as a coenzyme. Methylcobalamin contributes to metabolism of propionate, methionine, and α-leucine to β-leucine [222]. Adenosyl cobalamin is a coenzyme for the transformation of L-methylmalonyl co-enzyme A to succinyl co-enzyme A in the Krebs's cycle. The maintenance of our nervous system requires vitamin B_{12} due to its role in synthesis of s-adenosyl methionine. In addition, the metabolism of all the macronutrients require vitamin B_{12}-dependent enzymes, particularly those with sulfhydryl groups [220].

Deficiency of vitamin B_{12} is caused by a variety of reasons that include lack of intrinsic factor, and other disorders. It presents as megaloblastic anaemia due to lack of active folate to drive DNA synthesis disrupting erythropoiesis. Other clinical manifestations include gastric injury, neuropsychiatric problems such as neuropathy and dementia due to disruption in myelin synthesis [223. Plasma homocysteine levels rise and predispose to stroke [224]. If detected early, most of the symptoms are reversible with supplementation and increased dietary intake of vitamin B_{12} [207].

1.2.9. Vitamin A

Vitamin A is derived from the diet from animal sources such as egg, milk, and cheese as pre-formed vitamin A (as retinyl esters) or from plant sources such as spinach and carrots in its inactive provitamin A form. In broad terms, 'Vitamin A' encompasses a group of similar compounds that share related biochemical roles. These include retinol, retinoic acid and retinal, while it is stored in the liver as retinyl palmitate [225]. Retinoic acid, which interchanges between its two isomeric forms, is the most bioactive [226]. Retinyl esters are lysed to retinol and free fatty acids in the intestine, whilst retinal reductase acts upon retinal to produce retinol [227].

The role of vitamin A in vision has been well studied and illuminated. It involves its transformation via a series of reactions, and the subsequent formation of a complex called rhodopsin that breaks down on exposure to light and sends signals to the brain [228]. The 'vitamin A' family play an important role in enhancing and regulating immunity. Its role in both adaptive and innate immune response has been the subject of several studies where it was established to

protect against multiple infections [226]. The epithelial membranes, which line the skin, eyes and organs, require vitamin A for their integrity. Deficiency of vitamin causes the epithelial linings to dry up and keratinise making it susceptible to infection. Its deficiency also affects the activity of neutrophils who protect the body against infection [229]. It is reported to contribute to regulating macronutrient metabolism [230]. Vitamin A is vital at each stage of our life cycle, right from embryonic stem cell development where it contributes to building vital organs such as the heart, brain and various organ systems. Therefore, its deficiency at this crucial stage may manifest as neural tube defects [225]. A 2006 study also reported its *in vitro* antitumor activity in cancer cells where it was able to induce cell death [231].

Consequently, communities with diets deficient in vitamin A are over time prone to reoccurring infections, night blindness, embryonic deaths and congenital defects [232]. The deficiency of vitamin A is still a public health concern in some developing countries particularly in children and pregnant women [233]. This prompted the World health organisation to mark it a 'public health hazard' and recommend supplementation and fortification of staple foods in communities where its deficiency diseases are prevalent [234]. However, since vitamin A is fat soluble, excessive intakes may lead to hypervitaminosis and eventually toxicity. Vitamin A toxicity is characterised by vertigo, nausea, headache and blurred vision [235]. The blood levels of retinol are tightly regulated and becomes lower only when hepatic stores of vitamin A are exhausted. Hence, serum or plasma levels of retinol are used as biochemical markers to assess vitamin A levels both for routine checks and for population studies [236].

1.2.10. Vitamin E

Vitamin E is a vital and potent antioxidant. It occurs naturally in plants as tocopherols and tocotrienols with both having four isomers (α, β, γ and δ). α- Tocopherol is considered the most bioactive and relevant form for man and other animals [228]. It is absorbed in the small intestine where its absorption is enhanced by presence of lipids, while its metabolism occurs in the liver [237]. Levels of α-tocopherol are used as a biochemical marker of vitamin E status. However, age, gender, and serum lipid levels affect its status [238]. Vitamin E is essential to the integrity of red blood cells. Since it protects the cells from free radical damage and destruction, it is believed to slow the progression of aging. Studies have also shown it to prevent the clumping of platelets and may thus have a cardio-protective role [239]. It may also protect against cancer, rheumatoid arthritis, cataracts, and neurological disorders [240,241]. Though the antioxidant activity of vitamin E is its most emphasized role and the basis of most of its investigated health benefits, recent studies have unveiled some of its non-antioxidant properties. For instance, its role in the regulation and immuno-pathogenesis of asthma have been reported [242].

Since most foods contain vitamin E, its deficiency is rare and seen mostly with genetic disorders, cystic fibroids, liver disease and gastrointestinal malabsorption. In developing countries where the deficiency diseases do occur, it is mainly in infectious diseases that are linked to oxidative stress such as HIV and malaria [240].

1.3. Plant Secondary Metabolites

Phytochemicals are bioactive secondary metabolite compounds found solely in plants. They have no metabolic role but play crucial synergistic and protective roles in plants. They protect plants from stresses like pollution, drought, microbial attack and radiation. Though they are not essential to the normal human metabolic processes, they work with other nutrients to enhance their efficient

use in the body. Phytochemicals act as antioxidants, antimicrobials, anticancer, modulators of enzymes, aid detoxification, immunity booster, decrease platelet aggregation, hormones modulators, and have antineoplastic activity. They generally protect human health and enhance our wellbeing [243]. Therefore, regular dietary intake is encouraged since our body cannot synthesize them. Most of the secondary metabolites that have proven to have prophylactic and therapeutic properties are used as functional foods, nutraceuticals and to produce pharmaceutical drugs [244].

1.3.1. Carotenoids

Carotenoids are plant secondary metabolites with 40 carbon atoms per molecule (tetraterpenoids). They absorb light in the 400-500 nm region of the visible spectrum thus act as natural pigments in plants and microorganisms and give yellow, orange and red colour to fruits and vegetables [245,246]. They are classified based on their functional groups – xanthophyll, which contain oxygen (e.g. lutein and zeaxanthin) and the carotenes that contain only the parent hydrocarbon chain without any functional group (e.g. α-carotene, β-carotene and lycopene) [247]. Carotenoids also provide most of our vitamin A needs in the form of provitamin A [248]. The main provitamin A carotenoids include β-carotene, β-cryptoxanthin, and α-carotene, which exhibit distinct biochemical activities [249]. In most of the Western countries, animal sources contribute approximately >70 % of their preformed vitamin A, while the rest of the <30 % are obtained from plants as provitamin A [250]. This is in contrast to developing countries where fruits and vegetables provide >70 % of daily vitamin A intakes in form of provitamin A carotenoids [248]. Carotenoids are potent antioxidants, which is the basis of their beneficial effects, however other mechanisms via which they may exert these effects have been proposed recently. For instance, they may exert their effects through regulation of cell growth, control of gene expression, and gap-junction communication [251]. Bioactive compounds like carotenoids that have anti-inflammatory and antioxidant activity have cardio-protective effects since the pathology of most cardiovascular diseases are linked to inflammation and oxidative stress [252]. An *in vitro* combination of β-carotene and lycopene was reported to decrease the inflammatory response tumour necrosis factors attributed to their ability to scavenge free radicals and maintain redox homeostasis in addition to regulating the flow of nitric oxide (NO) [253].

Beta-Carotene

β- Carotene is an important part of various fruits and vegetables particularly orange to deep yellow ones such as carrots, sweet potato and green leafy vegetables [254]. It possesses eleven double bonds [251] and is symmetrically broken down to produce two molecules of vitamin A [255].

Beta – carotene is not classified as a general antioxidant, however, it protects cells from oxidative damage by scavenging singlet oxygen [256]. It has been reported to promote gap-junction communication and prompts the production of detoxification enzymes in the liver [257]. β-Carotene has been reported to be beneficial in reducing the risk of cardiovascular disease, osteoporosis, Alzheimer's disease, psoriasis, macular degeneration, infertility, night blindness and in treating erythropoietic protoporphyria [258]. It also protects against sunburn and erythema as generally carotenoids help protect the skin from UV radiation. Subjects with Alzheimer's disease (AD) were reported to show lower serum β- carotene levels in comparison to healthy control subjects [259]. This prompted several studies that showed a correlation between increased dietary intakes of β-carotene and reduced risk of AD [260]. One of the promising biomarkers for novel

protective approaches in AD are telomeres [261]. β- Carotene was found to play a role in protecting telomeres as it was reported to significantly increase its activity [262].

Lycopene

Lycopene is a non-provitamin A unsaturated carotenoid. It has eleven conjugated bonds and two unconjugated double bonds that accounts for its bioactive properties. It is susceptible to photo- and thermal degradation and exists naturally in its more stable trans- isomeric form. Approximately 50% of lycopene found in the human body is stored in the liver, testis and adrenal glands [263].

Lycopene is an effective *in vivo* and *in vitro* antioxidant that scavenges singlet oxygen and can sequester peroxyl radicals [264]. The anti-inflammatory activity of lycopene has been compared to that of dexamethasone in inhibiting inflammatory responses and controlling oxidative damage in uveitis [265], an inflammation of the eye that causes swelling and destroys its tissues [228]. Lycopene has also shown an ameliorating and neuroprotective activity in Alzheimer disease (AD). Long-term intake of lycopene significantly enhanced cognitive functions seen in Alzheimer's by reducing inflammatory responses and oxidative stress in rat neurons [266]. Lycopene protects against lipid peroxidation, increases high density lipoproteins and prevents oxidation of low-density lipoprotein (LDL) which altogether have a cardio-protective effect. Thus, a correlation between an increased intake of lycopene and a reduction in risk of cardiovascular disease has been reported by several studies [256].

Epidemiological studies have also linked increased consumption of lycopene and a decreased cancer risk [267]. These findings are supported by *in vitro* and *in vivo* experiments showing reduced proliferation, induced apoptosis and a decrease in the metastatic capacity of prostate cancer cells because of lycopene treatment [258,268]. Lycopene supplementation was found to have an ameliorating effect on the liver, which may reduce the risk of non-alcoholic fatty liver and hepatocellular carcinoma [269]. Lycopene may also protect against osteoporosis as it was shown to significantly improve bone quality [251].

1.3.2. Phenolic Compounds

Any compound that contains the basic structure of phenol (hydroxybenzene) is termed a phenolic compound. Phenolic compounds are the major phytochemicals in plants and possess one or more aromatic ring with at least one hydroxyl group. The major classes of phenolic compounds (non-flavonoids, flavonoids and tannins) differ in structure, which varies from the relatively simple to complex (Figure 1.2) [270]. Their structural configuration makes them efficient antioxidants and metal chelates. They are pervasive in fruits, legumes, cereals and vegetables. They greatly contribute to total water-soluble antioxidant capacity; protect plants from UV radiation and aggression from predators and parasites [271]. Recent researches on secondary metabolites in eggplants have shown them to contain mainly phenolic compounds, particularly flavonoids and phenolic acids. They are reported to have the most diverse and highest concentrations of phenolic compounds within the domesticated *Solanaceae* family [1]

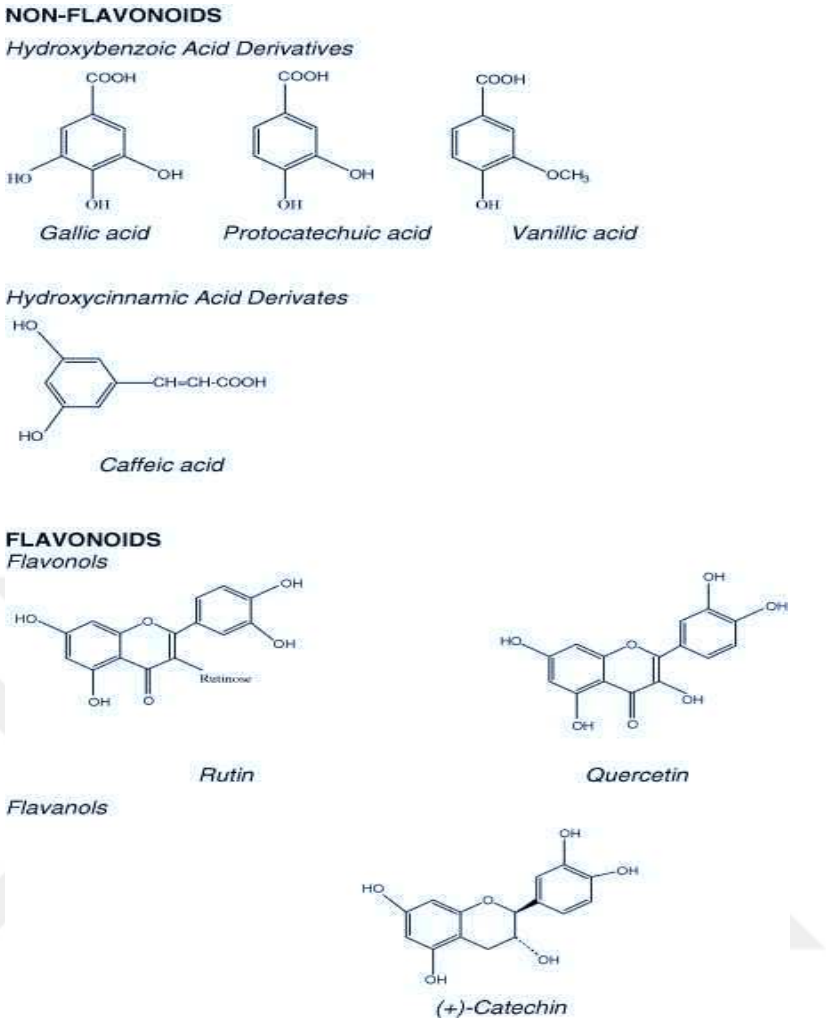

Figure 1.2 Chemical structures of phenolic compounds.

Flavonoids are largely more concentrated in the plant cell vacuoles. Their main structure constitutes three aromatic rings, which have been substituted at diverse points and varying degrees [272]. The main classes of flavonoids are classified based on the nature of these substitutions and oxidation level on the middle ring. Examples include flavonones (most citrus fruits), isoflavones (soybeans), flavonols (most fruits and vegetables), flavones (green leafy species), anthocyanidins (red, purple and blue fruits and vegetables) and flavanols (tea leaves, red grapes and wine). They are naturally present and stable as glycosylated esterified and polymerized derivatives. The sugar moieties increase polarity of the whole molecule making it easier to store in the plant cell vacuoles [273].

Several external and internal factors are reported to affect the concentrations of bioactive compounds found in plants. Any form of stress is a limiting factor, as the plant will make adaptive changes physically and biochemically to accommodate it. These adjustments may affect metabolic homeostasis, which in turn affects the metabolic profile of the plant [274]. For instance, it was observed that the concentration of phenolic compounds in eggplant fruits harvested during the spring was higher than that of fruits harvested during the summer months. This implies that environmental conditions affects the phenolic compound content [275]. A study compared the effect of farming methods (organic and conventional modes) on the polyphenolic content of two

eggplant cultivars (American (Blackbell) and Japanese (Millionaire). A significantly higher phenolic content, therefore higher total antioxidant activity was observed for the organically grown Japanese cultivar while it was vice versa for the American cultivar [276]. Another study indicated that the amount of phenolic compounds might depend on the varietal differences [277]. However, it is noteworthy that even though high concentrations of phenolic compounds are desirable for its antioxidant activity, it causes a faster browning of fruits. Polyphenol oxidase (PPO) oxidases phenolic compounds and in the process causes enzymic browning. This is a major challenge in the agricultural and food industry as it exacerbates post-harvest losses and increases processing costs [1].

1.4. Free radicals

The continuous metabolic activities occurring at the cellular level are what sustains all life forms. These metabolic activities are made-up of biochemical reactions that occur in the organelles such as mitochondria, chloroplasts, and membranes of all cells; and unfortunately, they leak electrons to O_2 and generate free radicals [278]. Exposure to external stresses like toxicity, irradiation, drugs, extreme temperatures, drought, infection, and pollutants, which unbalance homeostasis, also generates free radical species in the cell [279]. A few benefits of free radicals have been reported where they play signaling, anti-atherothrombotic, cytostatic, anti-microbial and cell growth regulatory roles [280]. However, free radicals are generally harmful, especially if allowed to accumulate, as they are highly reactive. As they accumulate, they cause oxidative stress, which in turn causes oxidative damage to our cells. They react indiscriminately with biomolecules triggering chaos as they cause lipid peroxidation, impair nucleic acids and DNA, inhibit enzymes, oxidize proteins, and initiate cell apostasies [281].

Membranes are particularly susceptible to lipid peroxidation and being pervasive, this has multilevel deleterious effects. These peroxidation products include conjugated dienes, aldehydes (malonyldialdehyde, acrolein and crotonaldehyde), 4-hydroxynonenal (4-HNE) and alcohols [281]. Lipid peroxidation compromises the integrity of the cell as it alters the assembly, configuration, organization, permeability and fluidity of lipid membranes, which are crucial to its physiological functions [282]. Permeability of the membrane is proportional to concentration of oxidized lipids, which results in leaks and shifts in water and ion gradients affecting biochemical and physiological properties of the cell [283]. A buildup of lipid peroxides has been implicated in ferroptosis, which is a type of iron-dependent controlled cell death [282]. All forms of DNA- nuclear, mitochondrial and chloroplastic; are subject to oxidative damage by free radicals, thus tampering with, or preventing the coding of proteins [281] and may manifest as mutation and carcinogenesis [284]. Likewise, a strong link between aging and debilitating metabolic disorders like diabetes, cancer, cataracts, dementia and atherosclerosis, and high levels of lipid peroxidation products have been established [285].

1.5. Stress Biomarkers

Biomarkers are metabolites or processes that can be measured as indicators of disorders, to monitor progression of disease, to develop drugs, and in maintenance of our wellbeing. Levels of biomolecules such as glutathione and peroxidation products like MDA and 4-HNE are used as markers of oxidative stress [286,287]. Our diet greatly influences the levels of these stress biomarkers in our system through dietary intake formed either in foods, or in their innate formation

and metabolism [288]. A disparity in amounts of pro-oxidant and antioxidant compounds in foods leads to both enzymatic and non-enzymatic oxidation of polyunsaturated fatty acids resulting in lipid peroxidation products. Food processing and storage conditions also contribute to their formation in foods [289]. Several studies have shown a possible relationship between the nutrient profile of foods and amounts of lipid peroxidation products. The amounts are attributable to processing methods, temperatures food is exposed to, storage methods and nature of the food sample [290,291]. The presence of certain pollutants and chemicals such as pesticides and additives may also predispose foods to increased rate of oxidation [292]. On the other hand, an increased intake of foods rich in antioxidant compounds such as garlic, green tea and fiber can help reduce lipid peroxidation products and other free radicals [293].

1.5.1. Glutathione

Glutathione (L--glutamyl-L-cysteinyl-glycine) is a ubiquitous intracellular water-soluble tripeptide antioxidant (Figure 1.3). It serves as a non-enzymatic defense against free radical species. Glutathione is a pervasive antioxidant that protects cells from oxidative stress and toxic compounds, thereby essential to maintaining redox balance and overall homeostasis [294]. The mutable oxidation of the thiol group in glutathione forms the basis of its antioxidant participation in redox reactions. It serves as an antioxidant in several ways- it may act directly on reactive species like $O^{2\bullet-}$, $^{\bullet}OH$ and neutralize them. It also forms covalent adducts with reactive electrophilic species via glutathiolation to protect lipids, DNA and proteins or it may donate protons. It participates in several defensive cellular reactions as a coenzyme and the ascorbate-glutathione cycle serves to regenerate ascorbic acid, another potent antioxidant [295]. It exists in its reduced form (GSH); or in its disulfide (GSSG) dimeric form which is its oxidized form. A steady equilibrium between these two forms- in which most of the glutathione is in the reduced form; is essential to conserving physiological redox homeostasis. Its excellent reducing capacity enable it participate in major metabolic processes of diverse biochemical, anabolic, physiological and toxicological nature. Examples include biosynthesis of nucleic acids, proteins, signaling, cellular growth, detoxification, regulation of enzymes, and in gene expression [296].

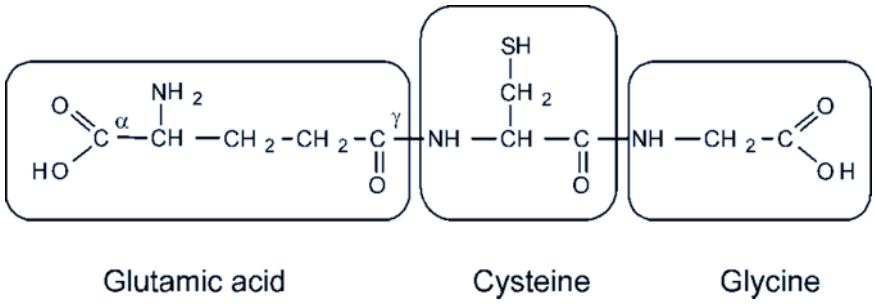

Figure 1.3 Structure of Glutathione

Since GSSG is cytotoxic, it must not be allowed to accumulate; and therefore ratio of GSH: GSSG is used as measure of oxidative stress [297]. Decreased levels of GSH are associated with increased proliferation of free radical species, inflammatory response, neurodegeneration and lower immunity, which predisposes to chronic metabolic disorders such as diabetes, cancer, HIV, Alzheimer's disease, and Parkinson's disease [298].

1.5.2. Malondialdehyde

Lipids are prone to free radical attack that initiates its chain-like decomposition. As they decompose, they release secondary products, which are mostly aldehydes. Their relative inter- and intra- cellular longevity and reactive nature are detrimental to molecular structures as they attack them and often cause irreversible damage [299]. One of the foremost and most studied lipid peroxidation products is malondialdehyde (MDA) [300]. It is largely a product of polyunsaturated fatty acids having over two methylene- interrupted double bonds [299]. MDA is a highly reactive electrophile due to its carbonyl functional group, thus it can react with a variety of biomolecules including DNA and amino acid residues [301], making it a prospective mutagenic, carcinogenic and atherogenic agent. This further underscores the importance of monitoring its levels and has become a widely accepted biomarker of lipid peroxidation and oxidative stress [302]. Lipid peroxidation products are formed in foods due to exposure to both biotic and abiotic stresses. MDA is one of the markers of chilling injury-induced damage in fruits stored at low temperatures [303]. Varieties of methods for its *in vivo* and *in vitro* assessment have been developed since the 1960s. From an analytical standpoint, the features of MDA exploited are its reactive nature with nucleophiles and its acidity [304].

1.5.3. 4-Hydroxynonenal

4- Hydroxynonenal (4-HNE) is an α-β-unsaturated electrophilic compound that was established as a toxic compound in the 1980s even though it was discovered much earlier. 4-HNE is amongst one of the most toxic, abundant and stable lipid peroxidation products. It functions as a signaling molecule where it coordinates aspects of cell development and some stress-sensitive transcription factors [305]. This is based on its capacity to interact with proteins that mediate gene expression and transduce cell signals such as receptors, kinases, phosphatases and transcription factors [306]. It is one of the breakdown products of the arachidonic acid and other polyunsaturated fatty acids. It is highly reactive, attributable to the presence of three different functional groups – a carbon-to-carbon double bond, a carbonyl group and a hydroxyl group [307]. One of the profound effects of 4-HNE is on the membranes where it interacts with lipids and proteins consequently distorting membrane structure and fluidity. This affects membrane integrity and function, for instance, it is reported to lower the activity of sodium–potassium pump and affect dopamine transportation [308]. Its role in increasing the risk and progression of aging, cancer, diabetes, Alzheimer's disease and other oxidative-stress induced disorders have been described. Thus, its concentration is used to assess oxidative stress and to monitor progression of related diseases [309].

Several studies have reported significant amounts of 4-HNE in a variety of food samples particularly fried foods and meats [310]. Processing methods such a freeze-drying have been shown to contribute to increased formation of 4-hydroxynonenal in foods [311]. The amounts of 4-hydroxynonenal in foods have been shown to vary significantly and were attributed to different factors including composition and antioxidant content. [312,313,314]. The oral toxicity of 4-HNE has been studied, and its long-term cytotoxic, hepatotoxic and nephrotoxic effects reported [315,316,317]. The use of amounts of 4-HNE as a marker of lipid peroxidation in foods and its correlation as an index of their consumption quality have also been proposed [318].

1.6. Antioxidants

Antioxidants are small, low molecular-weight molecules that are biosynthesized or obtained from the diet. They have the ability to permeate cells particularly those that are prone to free radical attack. They amass in parts of the cell that are most vulnerable and neutralize free radical species [319]. The delicate balance between the generation and neutralization of free radicals is tightly maintained by several elaborate cellular free radical scavenging antioxidant mechanisms [320]. These antioxidant mechanisms include both enzymatic and non-enzymatic free radical scavengers [321]. The enzymatic free-radical scavenging enzymes include superoxide dismutase (SOD), catalase and peroxidases [320]. Examples of the non-enzymatic free radical scavenging system include reduced glutathione (GSH), vitamin C (ascorbic acid), vitamin E (α-tocopherol), vitamin A (carotenoids), nicotinamide adenine dinucleotide (reduced form), carnosine, uric acid, bilirubin, flavonoids and phenolic compounds. These are obtained in different ways in the human body, for instance, glutathione, carnosine and nicotinamide adenine dinucleotide are anabolic products; uric acid and bilirubin are waste products of metabolism while vitamin C, vitamin E, phenolics and flavonoids are solely from dietary intake [319].

1.7. Total Antioxidant Capacity

After establishing the deleterious effects of free radicals and the roles of antioxidants and the diet in quenching them, it is essential to establish a standard method of assessing the antioxidant capacity of different foods and compounds. Though several methods have been developed to assess the free radical-scavenging capacity of antioxidants in living systems, and it is possible to distinctively profile the antioxidant in a specific plant, generally the total antioxidant capacity is assessed. This is more logical since the antioxidants work concertedly to scavenge free radicals-while one reduces the radical, the other regenerates the oxidized antioxidant. This is exemplified by the synergistic relationship between vitamin E and vitamin C [322] Therefore, measuring the total antioxidant capacity (TAC) is the most commonly used method [323]. Consequently, TAC is a measure of the cumulative activity of the antioxidant capacity of the antioxidants rather than their individual capacities based on an electron transfer reaction involving a colored oxidant [324].

Several methods have been described and developed over the years such as with 1,1-diphenyl-2-picryl-hydrazil (DPPH) radicals or the stable 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) [325].Though disparities have been observed in their reactions to different antioxidants, each has excellent stability and reproducibility under distinct experimental conditions [326]. The concept was first described by Miller and Co-workers in 1993 with the “Trolox equivalent antioxidant capacity” (TEAC) method which is based on the capacity of the antioxidants present in a given sample to reduce pre-formed ABTS radicals. Antioxidants in the sample, which suppress the color production, are measured as absorbance by a spectrophotometer [327]. Antioxidants such as Trolox (a water-soluble vitamin E analogue), which neutralizes the $ABTS^{\bullet+}$ radical, and decolorizes the solution is commonly used as control [301].

First developed in 1927 to measure tryptophan, the Folin-Ciocalteau (F-C) method of assay is the commonly used method for the total polyphenolic compounds in a sample. It is based on reduction of a metal ion by the antioxidant in the sample being oxidized as a measure of antioxidant capacity [328]. It is a relatively fast, cheap and easily reproduced method with low interference [329]. The Folin-Ciocalteau reagent is made up of a mixture of phosphomolybdic and phosphotungstic acids. The mixture forms a yellow color that changes to blue on being reduced by an antioxidant and occurs in alkaline conditions using sodium carbonate [330]. The yellow to blue

color transformation absorbs maximally at 760 nm and correlates to amount of total phenolic compounds in the sample [331]. The amount is generally stated as gallic acid or catechin equivalents [332]. On the other hand, total flavonoids using flavonoid aglycones are assessed to minimize variance [333]. The most commonly used aglycones in measuring total flavonoids are quercetin, myricetin, and kaempferol [334].

1.8. The Eggplant – Background

The *Solanum* genus belongs to the nightshade *Solanaceae* family and the scientific order *Polemoniaries* [335]. The genus has about 1500-2000 species and comprises some of the most populous, widespread and physically diverse species amongst the Angiosperms. Some of the world's most commercially cultivated crops are species under the *Solanum* genus (Figure 1.4), these include *S. lycopersicum L.*– tomato; *S. melongena L.*- eggplant; and *S. capsicum L.*-pepper [336].

1.8.1. Taxonomic Classification (http://plants.usda.gov/)

Kingdom: *Plantae*
Subkingdom: Tracheobionta
Superdivision: *Spermatophyta*
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: *Asteridae*
Order: Solanales
Family: Solanaceae
Genus: Solanum L.

Figure 1.4. Samples of varieties of fruit forms, colors, and proportions of *Solanum* species [337].

1.8.2. Historical Origins

The name '*Solanum*' is believed to be initially coined by Pliny the Elder, either from the Latin word '*solare*', which translates as 'to soothe'; or '*solamen*', which translates to 'a comfort', all describing the relaxing and soothing effect experienced after consuming the plant. Another postulation is the derivatisation of the name from the Latin word for 'sun' with reference to its nature as a plant of the sun [338]. On the other hand in 1706, Thomas Jefferson- a Botanist coined the name eggplant and was the first person to bring the plant to the United States. Other reports `claim the name eggplant was proposed in the eighteenth century based on the ovoid shape and white color of some varieties, however, depending on the country, it is also called aubergine or brinjal [339]. Even though most plants of the *Solanaceae* family are New World crops, the eggplant is a species of the Old World. As around the third century, an awareness of the eggplant started in India, followed by China about a hundred years later and then in Africa around the 9th century. Many theories regarding where eggplant was first domesticated have been postulated, however the most plausible is that it was initially cultivated in South East of the Asian continent in the 16th century [340]. *Solanum incanum* is presumed to be the initial fore bearer, which has evolved into the numerous varieties we have today [341]. The highest number of varieties of the eggplant are found in India where majority grow wild in the forest [339].

1.8.3. Description

The *Solanum* is a perennial, upright, yet spreading plant with herbaceous or woody stems. If it is woody, it matures to a small tree (Figure 1.5) of a height of up to 3 meters, while the herbaceous version does not exceed a height of 40-150 cm. It has prickles growing all over the branches and stem. The plant has leaves that are relatively large, rough and lobed. The leaves usually have a diameter of 5-10 cm, and a length of 10-20 cm [342]. Its ideal annual rainfall, pH and temperature requirements range from 700 - 1,000 mm, 6.5 - 7.5 and 20 – 26 °C, respectively. Though they thrive most in dark, rich and fertile soils, the *Solanum* species are highly adaptive and can grow in different types of soils and conditions. They mature within 3 to 4 months of planting and may continue for upto 12 months. The eggplant fruit matures dropping freely, either singly, or bunched with an average of 10 to 15 fruits (Figure 1.5). The fruit develops to weigh between 15 to 1500 grams depending on the variety [343]. The succulent fruit is hand-picked when it grows to a diameter of 4 to 6 cm and may weigh up to 100 g. Generally, harvested eggplant fruits are susceptible to extreme temperatures; ideally, if they are to be stored they are kept unwashed and refrigerated in perforated polythene bags [344].

Figure 1.5 The eggplant tree, showing the leaves and fruits.

The *Solanum* species fruits are categorized as berries, full of tiny edible seeds [345] and are profoundly physically different depending on the variety. In terms of size and proportions, some are ovoid; others are roundish while some are elongated. In 1976, Choudhury proposed a grouping of the different varieties of *S. melongena* which was centered on the phenotypical properties of the eggplant specifically the shape: '*S. melongena var. esculentum Dunal* (Nees) – round, oval or egg-shaped fruits; *S. melongena var. serpentinum L.*–long slender fruits; and *S. melongena var. depressum L.*–small, miniature fruits, dwarf, and early types" [346]. They are also of different colours and hues with some green, purple and white and may have stripes or dots (Figure 1.6) [347]. These properties and their origins are likewise, used as the basis of their identification. The white colored and egg-like shape of some cultivars gave it the name eggplant or garden egg, while the purple colored varieties are generally called aubergine [1]. They are further described as Chinese, Thai, Japanese, African, Indian and Oriental eggplants. Ninety-eight (98) accessions of eggplants are presently identified; however, they each share common parallel properties even as they have distinct physical significant differences to each other [339]. Twenty-five (25) have been identified in Nigeria [348], with some being endemic to Africa [1]. They are commonly called bitter apple (English) and yalo in Hausa, while some varieties are called bitter tomato (English) and da'ata or gauta (Hausa), afufa or anara (Igbo) and igba (Yoruba) [1].

The wild types usually produce smaller, bitter and even toxic fruits, while continuous selection has produced less bitter and non-toxic fruit types. The selected types are highly cultivated with all parts of the plants used as food or medicine [347]. A variety of the eggplant species have been domesticated and are cultivated around the world, however 'aubergine' is the most cultivated. It has smooth skin found in different hues of deep purple, green or black and has the largest fruit size compared to other species, especially the wild types [1]. The varieties share several traits and are closely linked to each other though they appear physically different attributed to genomic variances and ecological segregation [346]. They also share many traits that are of agronomical

value with tomato and potato. Some varieties are reported to be much better at withstanding both biological and physical stresses than others [349].

Figure 1.6 Showing the range of diversity in physical appearance of eggplant varieties.

1.8.4. Economic Importance

The eggplant is common to parts of Asia, Africa, and Europe. China with a production of 2.95 million tons is the world's largest producer of eggplant followed by India, Egypt, Iran and Turkey, which ranks fourth with 880,000 metric tons/year [1]. They are highly adaptive and thus spread over a wide range of climates from sub-Saharan Africa to the more temperate regions. It produces an appreciable amount of fruit which help keeps its prices reasonably low and therefore affordable [340].

The *Solanum* fruit is eaten fresh and raw or cooked, and has a spongy texture, but is also dried to eat when it is out of season. The fruit and leaves are used to flavor and enrich soups and stews. The fruit and the seed are used to curdle milk and in cheese making. The different varieties are sometimes interchanged for some uses [340]. It is a highly valued medicinal plant in Africa, particularly the sub-tropical and tropical regions spanning Western and Eastern Africa. Each part of the plant, from the foliage, to the roots, sap to the fruits are prescribed in local traditional treatment of a diverse range of maladies. The plant parts are used in various ways- sometimes as a decoction, chewed, or ground and applied as a poultice. Traditionally, it is used as an analgesic, and prescribed for abdominal pain, headache, menstrual cramps, joint pain, and inflammation. It is used for almost all skin conditions including burns, fungal infections, snakebites and sores. Decoctions made from the fruit are used to treat hypertension, cancer, diabetes and disorders

associated with the liver. The ameliorating effect of its extract is linked to the presence of strychnine and anthocyanin and has been demonstrated to effectively lower cholesterol levels in rats and man. Components of its anthocyanin have also shown antioxidant and antimutagenic properties [350]. A study identified seventy-seven (77) therapeutic uses of the *Solanum* species in parts of Fareast Asia. These included their use for topical problems, hemorrhoids, toothache, cough, diuretic, emetic, aphrodisiac, abortifacient, and hyperglycemic. It further shows its promise as a cheap source of nutrients and as a nutraceutical [340].

The fruits have a good moisture and fiber content with low calorific values, which makes them ideal functional foods for weight-loss, diabetics, and as laxatives. They are also repositories of antioxidants of different types particularly phytochemicals of phenolic compounds such as flavonoids, phenolic acids, and anthocyanins [276]. Antioxidants safeguard against peroxidation in the membranes including those of the brain, where it deters inflammation and encourages the flow of blood to the brain cells. This is the basis of its efficacy against brain-related maladies such as Alzheimer's disease [345,351]. They have also been reported to have a regenerative effect on rhodophsin, which in turn enhances the vision [352].

1.8.5. Nutrient Profile of Eggplant

In contrast to other members of the *Solanum* family like tomato, potato and pepper, it was only in recent years that the eggplant was the focus of researches. Though eggplant is not particularly considered as a rich repository of the main essential nutrients, it is currently in the spotlight as a functional food due to its antioxidant content [353]. However, the metabolite profile varies depending on several factors such as the variety, mode of farming, type of soil, storage condition, maturity and biotic/abiotic stress [354].

1.8.6. Therapeutic Benefits of Eggplant

Several health benefits have been attributed to all parts of the eggplant for both prophylaxis and therapy. Almost all cultures use it in folkloric medicine for the treatment of diverse ailments. An extensive study of traditional medicinal uses of different varieties of eggplant reported its various uses to include topical use to treat hemorrhoids, reduce swelling, as an antipyretic, as an emetic, laxative, aphrodisiac, hypoglycemic, for toothache, to relieve cough, stop bleeding, weight loss, appetite stimulant, for heart burn, hyperacidity, diuretic, induce sleep, skin compress for swelling, uterus stabilizer after a miscarriage, and to treat cancer, particularly skin cancer [340]. In Korean folklore medicine, it is used to treat gastric ulcers, stomatitis, warts, burns, acne, and frostbite [355]. Eggplant fruit contains a relatively high amount of fiber, which greatly reduces the risk of being constipated, hemorrhoids, stomach and colon cancer [339]. The high fiber and presence of complex carbohydrates also aids in dietary management of type II diabetes [356].

The possible role of eggplants in regulation of glucose and lipid metabolism have been investigated, which will have a positive impact on their related metabolic disorders such as diabetes, cardiovascular disease, obesity, cancer, hepatic steatosis and inflammation [357]. A study reported the hypoglycemic effect of an African variety of eggplant (*Solanum kumba*) in diabetic-induced rats [356]. A study conducted in Brazil on hypercholesterolemia human subjects was able to demonstrate a lowering effect of consuming an eggplant infusion, specifically *S.melongena* species, on total cholesterol and low-density lipoproteins. High serum levels of cholesterol and low-density lipoproteins predispose to cardiovascular disease and stroke. Although soluble fiber is believed to

aid in mopping up serum lipids, however, in this study the amount of fiber was not enough to account for the hypolipidemia and was attributed to the antioxidant effects of vitamin C and phenolic compounds [358]. A study assessed the cardio-protective properties of raw and grilled eggplant using an isolated perfusion heart model. Both samples exhibited cardio-protective function on the hearts with no significant difference in their capacities. The malondialdehyde and DPPH free radical scavenging activity of both groups were also the same [359]. Hans *et al* in 2003 reported the anti-inflammatory effect of eggplant extracts in mice where it significantly reduced edema and vascular permeability. This has positive implications in diseases such as heart disease and cancer since a rise in vascular permeability and tissue edema implies an increased infiltration of inflammatory mediators; decreases the efficiency of the heart in pumping blood and increases tumor-induced formation of new blood vessels, respectively [360]. Extracts of the eggplant fruit have been reported to be active against both gram-negative and gram-positive bacteria. A study tested the antibacterial activity of methanolic extract of eggplant fruit against twelve common human bacterial strains. The extract showed sensitivity against *B. subtilis, B. cereus, E. coli ATCC25922, Pseudomonas sp, S. aureus ATCC25923, V. cholera, B. subtilis, B. cereus, E. coli ATCC25922 and Pseudomonas sp strains* [361]. A study showed this lipid-lowering action was shown in normal and cholesterol-fed rats who were orally fed flavonoid from eggplant. Flavonoid extracts from eggplant were able to significantly lower levels of some oxidative stress biomarkers in rats [358].

1.9. Significance of the Study

The adage 'you are what you eat' elegantly captures the impact of the diet on our health and wellbeing. Recent studies have ascertained the relationship between contemporary dietary evolution and the proliferation of malnutrition, metabolic disorders and poverty. Globally, this vicious cycle has had a negative influence on all facets of development. The WHO, therefore, currently categorizes food and nutrition insecurity, which present one of the greatest current global challenge, as a pandemic. Consequently, the search for cheap, available and versatile sources of nutrients has captured the interest of all stakeholders. With less than 50% of the world's plants completely elucidated, there is a need to harness this unending and abundant resource. Eggplant is a resilient, low-maintenance crop that can grow almost anywhere around the world. Its relative availability, affordability, versatility and diversity makes it ideal for dietary inclusion and breeding programs especially for poor rural households, strife-ridden regions and with the looming exponential population explosion and threat of climate change. In comparison to other crops in the *Solanaceae* family, few studies have investigated eggplant's nutrient and secondary metabolite profile, with much lesser on the wild African varieties. In this study, the amounts of amino acids, vitamins, oxidants/antioxidants of different varieties of eggplant sampled from Turkey and Nigeria, are assessed and compared to each other. This provides essential information for consumers, academics, nutritionists, breeders and policy makers who are increasingly concerned about the nutritional, health, functional, industrial, ecological and economic benefits of crops.

1.10. Aim and Objectives

The aim of this study is to assess and compare the nutrient and antioxidant profile of fresh fruits of five (5) different varieties of eggplants - dark eggplant and light Eggplant sampled from Elazig, Turkey; and white Garden egg, bitter tomato and bitter apple sampled from Kano, Nigeria.

The specific objectives are to:

1) obtain fresh samples of the selected varieties of eggplants from Turkey and Nigeria,
2) determine the amino acid concentrations in the samples using HPLC,
3) determine the water-soluble and fat-soluble vitamin concentrations using HPLC,
4) determine the stress biomarkers – reduced and oxidized glutathione, malondialdehyde, and 4-hydroxynonenal concentrations using HPLC,
5) determine the total phenolic content, total flavonoid content and total antioxidant capacity using spectrophotometer,
6) compare the findings according to the different varieties of eggplant fruits collected, based on the contents of their amino acids, vitamins, stress biomarkers, total phenolic compounds, total flavonoids and total antioxidant capacity.

2. MATERIALS AND METHODS

2.1. Chemical Reagents and Equipment

All radical testing chemicals - vitamin A, β-carotene, lycopene, ascorbic acid, α-tocopherol, thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, folic acid, cyanocobalamin, quercetin (dehydrate), potassium persulphate, sodium nitrate, aluminum chloride, Gallic acid, flavone, Perchloric acid, butylated hydroxy toluene (BHT) and all other standard and chemical reagents were of analytical grade and purchased from Sigma Aldrich or Merck. Double distilled (dd H_2O) water was used throughout the work. All glassware was acid washed and rinsed with doubly distilled deionized water. Solvents used for liquid chromatography were of HPLC grade and solvents used as the mobile phase in HPLC were filtered using a membrane filter. HPLC analysis was performed with the Shimadzu Prominence-I LC-2030C 3D Model with PDA detector.

2.2. Samples

In this study, fresh eggplant fruits were purchased from local markets of Elazig city, Turkey and Kano, Nigeria in October 2019. Five types of varieties of eggplant (Table 2.1 and Figure 2.1) were used, namely, Dark Eggplant fruits, Light Eggplant fruits, Bitter Tomato, Bitter Apple and White Garden egg throughout the experiment. Each was washed thoroughly, and stored in the refrigerator at 4o C until required for further use.

Table 2.2. Showing the origins, varieties and descriptions of eggplant fruits sampled for this study

Origin	Description	Variety
Turkey	Dark, Oval	Dark Eggplant
Turkey	Light, Slim, long	Light Eggplant
Nigeria	Green, Rounded	Bitter Apple
Nigeria	Green / Reddish tones, Rounded	Bitter Tomato
Nigeria	White with green stripes, Ovoid	White Garden Egg

2.3. Sample Preparation

Several preparative steps, which include homogenization, extraction, concentration and drying of the samples were carried out prior to analysis for the purpose of this study**.** Each of the fruits was sampled, chopped and homogenized. The eggplant fruit samples were extracted with methanol using a Soxhlet extraction apparatus (Figure 2.2). Approximately 20.0 g of each homogenized sample was placed in a thimble made from filter paper and inserted into the extraction chamber. The extraction was done using 350 mL of methanol placed in a round bottomed flask mounted on a heating mantle, and maintained at 60 ^{o}C for about 24 hrs.

After the extraction, the flask, which contains the extract, is detached from the Soxhlet extraction setup and allowed to cool. The extract mixture was filtered through Whatman's No. 1 filter paper (Whatman International Limited, Maidstone, UK) and the flask rinsed with additional methanol. The residue was re-extracted again with methanol. The filtrate was concentrated in vacuum using a rotary evaporator under reduced pressure at 45 °C (Figure 2.3). It was detached

with the evaporation of the solvent and volume of the extract was made to a total of 50 mL with methanol. The extract was refrigerated at 4 °C until required for use.

Dark Eggplant

Light Eggplant

Bitter Apple

Bitter Tomato

White Garden Egg

Bitter Tomato

Figure 2.1 Shows the Eggplant varieties sampled for this study and their common names

Figure 2.2 Shows the soxhlet extraction setup for the solid-liquid extraction of the samples.

Figure 2.3 Shows the rotary evaporation setup for the extract.

2.4. Determination of Vitamins A, E, β-Carotene, Lycopene and 4-Hydroxynonenal content

The amounts of vitamins A, E, β-carotene, lycopene and 4-hydroxynonenal content in eggplant fruits were determined by High Performance Liquid Chromatography (HPLC) using methods described by [362,363,364] with minor modifications. The steps involved the handling, extraction and elimination of organic solvents in the sample done diligently to ensure complete abstraction from the sample.

The fresh sample of eggplant was homogenized using a homogenizer and approximately 2.0 g was weighed for extraction of vitamins A and E, β-carotene, lycopene and 4-hydroxynonenal. The weighed eggplant sample was placed in a polyethylene tube, and to each tube, 6.0 mL of ethyl alcohol was added then vortexed. After thorough mixing, the sample mixture was sonicated for 5 minutes with further vortex mixing after each minute during the sonication process. The tube containing the sample was placed in crushed ice during the sonication to keep it cool. After the ultrasonic break, the tubes were placed in a water bath for 5 minutes.

Then 1.0 mL of n-hexane was added to the mixture in the tube, vortexed, and then centrifuged at 4500 rpm for 5 minutes. After centrifugation, the topmost clear layer representing the n-hexane phase along with the first extract of retinol, β-carotene, lycopene, α-tocopherol and 4-hydroxynonenal was abstracted and placed in a glass test tube. The extraction with n-hexane is repeated for a second time, and n-hexane phase extracted again and then combined with the first extract. The glass tube was placed on a metal rack in a vacuum oven for evaporation to complete dryness. After drying completely, the residue in the tube was dissolved by addition of 1.0 mL of

methyl alcohol and mixing thoroughly using a vortex mixer. Subsequently, 1.0 mL of the dissolved residue was sampled and placed in HPLC vials for HPLC analysis. The described procedure was done for each of the samples in triplicate.

The equsil ODS-2 column (25.0 cm x 4.6 mm ID x 5.0 μm) was used in the determination of vitamin A, vitamin E, β-carotene, lycopene and 4-hydroxynonenal using a mixture of acetonitrile: methanol: water (63:33:4.0 v/v) as mobile phase. The mobile phase flow rate was set at 1.0 mL/min. The HPLC peaks were measured for vitamin A at 326 nm, vitamin E at 296 nm, β-carotene at 436 nm, lycopene at 460nm and 4-HNE at 232 nm.

2.5. Determination of vitamin C, B-vitamins (B_1, B_2, B_3, B_6, B_9, and B_{12}), Glutathione (GSH/GSSG) and Malondialdehyde content in Eggplant Fruits.

High Performance Liquid Chromatography (HPLC) was used to determine the amounts of vitamins C, B-vitamins (B_1, B_2, B_3, B_6, B_9, and B_{12}), glutathione (GSH/GSSG) and malondialdehyde content in the eggplant sampled.

The fresh sample of eggplant was homogenized using a homogenizer and approximately 2.00 g was weighed for extraction of vitamins C, B-vitamins, glutathione (GSH/GSSG) and malondialdehyde. The weighed sample was placed in a polyethylene tube, 1.0 mL of 0.5 M perchloric ($HClO_4$) acid was added to each homogenate and vortexed then each tube, 6.0 mL of distilled water was added then vortexed. After thorough mixing, the mixture was sonicated for 5 minutes, with further vortex mixing after each minute of sonication. Each tube is placed in crushed ice during sonication to keep the temperature down. After the ultrasonic break, the tubes were placed in a water bath for 5 minutes then centrifuged for 10 minutes at 6000 rpm. A precipitate is formed after centrifugation; 1.0 mL of the clear supernatant is sampled and placed in vials for HPLC analysis. The described procedure was done in triplicate for each of the samples.

The analysis was done using supelcosil LC-18 DB (100–5 ODS, 25 cm, 4.6 mm ID, 5 μm) column at a flow rate of 1.0 mL/min. For the mobile phase, 0.5 grams of potassium dihydrogen phosphate (KH_2PO_4) is dissolved in 1.0 liter of distilled water, then adjusted to pH of 3.4 by using O-phosphoric acid (H_3PO_4) and used for vitamin C. For the B-vitamins, a 1.0 liter volume of mobile phase made up of 250 mL methanol, 750 mL distilled water, 0.75grams heptanes-1- sulfonic acid sodium salt and 0.75mL tri-ethylamine (TEA) adjusted to pH of 2.8 by using o-phosphoric acid (H_3PO_4) was prepared and used.

As described by [365], the HPLC peak for vitamin C was measured at 244nm, while vitamin B_1 was measured at 247nm, B_2 at 269nm, B_3 at 262nm, B_5 at 212nm, B_6 at 291nm, B_9 at 273nm, B_{12} at 361nm as reported by [366,367]. The HPLC peaks for MDA was measured at 254nm [368] while GSH and GSSG were measured at 210nm [369].

2.6. Amino acid Analysis

2.6.1. Sample preparation

Approximately 4.00 g of each homogenized sample was weighed and then 6.0 mL of 6 M HCl was added to digest the samples for 24 hours. After digestion, it was filtered using Whatman No. 1 filter paper. Volume of each sample was made to 10 mL by adding distilled water, and then 1.5 mL was taken from each sample for amino acid analysis.

The amino acid standard solutions and hydrolyzed samples were neutralized by the addition of 50 µL of a 2:2:1 mixture of ethanol: water: triethylamine (TEA) (v/v), followed by thorough vortex mixing. They were then evaporated to complete dryness in a vacuum oven at ≤ 60 °C.

The amino acids in the samples were derivatised by the addition of 50 µL of a 7:1:1:1 mixture of ethanol: water: TEA: Phenylisothiocyanate (PITC -Edman's Reagent) (v/v) followed by thorough vortex mixing. The mixture was allowed to stand for 20 minutes at room temperature for the complete reaction to form phenylthiocarbamyl (PTC)-amino acids complex. The derivatised sample was evaporated to complete dryness again in a vacuum oven at 40 °C, and then stored at 4 °C until required for further use [370].

2.6.2. HPLC analysis

The amino acid analysis was done using HPLC with the column temperature maintained at 40 °C. Two mobile phases – eluent A and eluent B, were prepared and used for the analysis. Eluent A was made up of 150 mM $CH_3OONa \cdot 3H_2O$, 0.05% TEA, and 6% acetonitrile, pH 6.4; while eluent B consisted of 6:4 acetonitrile: water (v/v). The flow rate was maintained at 1 mL/minute throughout, and the PTC-amino acid eluted from the column were measured at 254 nm with the gradient program given in Table 2.2. The column was regenerated and equilibrated with eluent B for 10 mins before a new and freshly reconstituted sample was analyzed [370].

Table 2.2. Gradient program used for the separation of Phenylthiocarbamyl-amino acids

Time (Minute)	Flow Rate mL/min	% Eluent A	% Eluent B
0.01	0.8	90	10
12.00	0.8	70	30
16.00	0.8	65	35
16.01	0.8	50	50
25.00	0.8	100	0
30.00	0.8	50	50
30.01	0.8	10	90
35.00	0.8	10	90

2.7. Determination of Total Phenolic Content

Total phenolic content was determined using the Folin– Ciocalteu's phenol reagent colorimetric assay following slightly modified methods described by [371], and [372].

An aliquot (500 µL) of appropriately diluted sample extract was added to an aliquot (125 µL) of the Folin–Ciocalteu reagent and 500 µL of distilled water. The mixture was vortexed and allowed to stand for 6 min at room temperature, before the addition of 1250 µL of sodium carbonate (Na_2CO_3) (20 % w/v). An aliquot of distilled water added to make a total volume of 3.0 ml and vortexed thoroughly. The mixture was incubated in the dark at room temperature for 2 hours. After the incubation period, the absorbance was read at 760 nm using a UV-spectrophotometer versus prepared blank. The blank was prepared by replacing the sample extract with distilled water in the described procedure. This was done in triplicate and repeated for all extracts of the samples.

The total phenolic content was calculated from a calibration curve using gallic acid as standard. Total phenolic content of eggplant extracts were calculated from the standard calculation

curve and expressed as mean ± standard deviation in micrograms of Gallic acid equivalents per gram (µg GAE/g).

2.8. Determination of Total Flavonoid Content

Determination of the flavonoids content was achieved following the aluminum chloride ($AlCl_3$) method described by Huang *et al.* (2004) [373] with minor modifications. It is based on the spectrophotometric measurement of the reaction of flavonoid compounds with the colored NaNO2-Al (III)-NaOH complex. The presence of Al (III) produces a yellow colored complex that turns red upon the addition of sodium hydroxide (NaOH), the absorbance of which is read at 510.

Aliquots of the sample extract were mixed with 75 µL of sodium nitrate ($NaNO_3$) 5 % w/v) solution. After 6 minutes, 150 µL of aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) (10 % w/v) and 1.25 mL of distilled water were added. The mixture was allowed to incubate for 5 minute at room temperature. Finally, 0.5 mL of NaOH (10 % w/v) solution was added. The total volume was made to 4.75 mL with the addition of distilled water. For the blank, the extract was replaced with distilled water. The absorbance was read immediately at 510 nm against the prepared blank. Each assay was repeated to give three replicates for each sample extract.

A standard curve of quercetin dissolved in 80 % ethanol is prepared by substituting the sample extract. The total flavonoid contents was deduced from the standard curve and expressed as mean ± standard deviation µg quercetin equivalent (QE)/g.

2.9. Determination of ABTS free radical scavenging activity

The ABTS assay was carried out following methods described by [374] with minor modifications. It is based on the ability of the antioxidant compounds in the sample extract to scavenge the pre-formed blue-green ABTS radical, the disappearance of which is measured and calculated as the antioxidant capacity.

The blue-green ABTS radical cation working solution was prepared from mixing stock solutions of 7.4 mM ABTS solution and 2.6 mM potassium persulfate in equal quantities. The mixture was allowed to incubate at room temperature for 12 hours in darkness. After incubation, the solution was diluted by measuring 1.0 mL of the $ABTS^+$ solution and mixing it with 60 mL methanol. This diluted solution was adjusted with phosphate buffered solution to 7.4.The sample extract was mixed with 2850 µL of the ABTS solution and allowed to react in the dark for 2 hours. The absorbance was measured at 734nm using a spectrophotometer against a blank prepared without the extract. Each assay was repeated to give three replicates for each sample extract.

The results are deduced from the standard calibration curve using Trolox as a standard and are expressed in µg Trolox equivalents (TE)/g

$$\% \, Inhibition = \frac{(A_{C(0)} - A_{A(t)})}{A_{C(0)}} \cdot 100$$

2.10. Determination of DPPH free radical scavenging activity

The free radical scavenging activity of each of the sample extracts was assessed using 2, 2-diphenyl-β- picrylhydrazyl (DPPH) free radicals measured according to the methods descrıbed by [375] with minor modifications. It is based on the ability of antioxidants in the sample extract to scavenge the DPPH• which absorbs at 517 nm.

A solution of 0.135 mM DPPH in methanol was prepared and 1.0 mL of the solution was mixed with 1.0 mg of extract in methanol containing 0.2–1.0 mg/mL of the extract. The mixture was shaken vigorously, and incubated for 30 minutes in the dark at room temperature. The absorbance of the mixture was measured with a spectrophotometer at 517 nm, against a blank prepared without the extract, while BHT was used as standard. Each assay was repeated to give three replicates for each sample extract. A standard curve was prepared and used to calculate the DPPH scavenging activity.

$$DPPH\ Scavenging\ effects\ (\%)\ or\ Percent\ Inhibition = \frac{(A_0 - A_1)}{A_0} \cdot 100$$

3. RESULTS

A working graph was made for each of the parameters and used for the calculations. Data collected from HPLC chromatogram were calculated according to the peak area (Figure 4.1, 4.2 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 4.10, 4.11, 4.12, 4.13, 4.14 and 4.15) to obtain the concentration. For instance, based on the equation of a linear graph, the equation for beta-carotene was found to be y = 212.09x + 157.54. Chromatogram of beta-carotene in respect of peak area in the sample was then used to calculate concentration (x) by writing the peak area of beta-carotene instead of 'y' in the equation, where y is the values of peak area in the chromatogram. The result are multiplied by the dilution factor, where applicable.

The amounts of vitamin A, E, C, B_1, B_2, B_3, B_5, B6, B_9, B_{12}, MDA, beta-carotene, lycopene, glutathione reduced (GSH), glutathione oxidized (GSSG) and 4-hydroxynonenal in different eggplant fruits, were then calculated based on their respective working graphs and peak areas. Similarly, calculations of total phenolic and flavonoid content and free radical scavenging activity based on ABTS and DPPH assays were determined by their respective created graphs (Figure 4.17 and 4.18).

Table 3.1. Shows the amounts of amino acids in the five different varieties of eggplant fruits **(µg/g dw).**

Amino Acid	White Garden Egg	Bitter Apple	Dark Eggplant)	Light Eggplant	Bitter Tomato
Aspartic acid	1747.6±154.2	2277.9±196.4	3019.0±	972.4±71.2	4217.7±358.3
Glutamic acid	7712.7±520.4	3535.7±288.3	3206.0±	2784.3±237.4	7385.3±510.7
Asparagine	4215.9±356.4	3318.6±268.2	3269.0±	2985.8±240.4	5360.3±411.2
Serine	1665.1±149.,3	1317.1±119.3	1753.5±	1601.6±142.8	2181.6±359.8
Glycine	1822.2±161.1	3928.6±254.2	1526.7±	1394.5±114.0	4578.7±98.6
Glutamine	1315.9±102.3	927.9±77.3	782.8±	715.0±58.5	1219.1±1377
Histidine	13823.8±513.0	6903.6±468.2	20545.7±	18766.1±348.5	17398.5±6.5
Arginine	234.9±16.3	95.0±6.9	98.3±	89.8±6.4	89.7±238.3
Threonine	2381.0±198.0	1205.0±188	3014.7±	2753.5±230.0	2941.9±236.2
Alanine	2490.5±202.5	2286.4±191.4	1479.3±	1351.2±108.4	2978.7±15.2
Proline	442.9±32.3	163.6±13.3	256.9±	234.7±19.3	172.8±196.2
Tyrosine	1765.1±158.1	1472.1±112.8	1017.2±	929.1±75.3	2424.3±27.1
Valine	3246.0±250.0	1413.6±108.9	918.1±	612.6±49.8	388.2±376.1
Methionine	2628.6±224.2	3105.0±262	3710.4±	1886.6±163.3	5038.2±183.0
Cysteine	1861.9±159.9	1517.1±124.3	1876.7±	1096.9±79.4	2183.1±1.5
Leucine	255.6±18.4	90.0±6.5	175.9±	114.2±8.8	23.5±2407.4
Phenylalanine	465.1±34.6	2332.1±194.4	2438.8±	2829.1±221.7	2407.4±194.6
Tryptophan	1807.9±151.6	1355.0±106	1003.5±	1622.1±129.3	1691.2±138.4
Lysine	33625.4±571.3	13545.7±508.5	6800.9±	47018.9±327.3	26117.7±1816.0

Table 3.2. The percentage of RDA of the essential amino acids per 100g of each of the varieties of eggplant fruits

Essential amino acids	RDA[376]* FAO/WHO/UNU	White garden egg	Bitter apple	Dark eggplant	Light eggplant	Bitter tomato
Histidine	700	197.48	98.62	293.51	268.09	248.55
Leucine	2730	0.94	0.33	0.64	0.42	0.09
Lysine	2100	160.12	64.50	32.39	223.90	124.37
Methionine (with no cysteine)	1050	25.03	29.57	35.34	17.97	248.74
Phenylalanine (with no tyrosine)	1750	2.66	13.33	13.94	16.17	13.76
Threonine	1050	22.68	11.48	28.71	26.22	28.02
Tryptophan	280	64.57	48.39	35.84	57.93	60.40
Valine	1820	17.84	7.77	5.05	3.37	2.13

Table 3.3. Shows the amounts of vitamin A, E, C, β-carotene, lycopene, B_1, B_2, B_3, B_5, B_6, B_9, and B_{12} in five varieties of eggplant fruits (µg/g) DW.

Parameter	White garden egg	Bitter apple	Dark eggplant	Light Eggplant	Bitter tomato	Range difference
Vitamin A	2.33±0.03	3.63±0.10	0.63±0.04	0.13±0.01	2.65±0.45	27.08
Vitamin E	10.98±0.11	3.63±0.17	39.14±1.71	4.80±0.37	34.49±1.57	10.79
Beta carotene	4.29 ±0.04	5.13±0.05	30.47±0.38	1.87±0.05	21.40±0.37	16.33
Lycopene	6.79±0.11	4.33±0.05	1.78±0.08	1.52±0.01	3.20±0.36	4.47
Vitamin C	1136.2±4.2	579.2±3.4	476.1±3.1	421.8±0.3	357.4±1.7	3.18
Thiamine B1	95.56± 0.96	53.86±0.44	11.04±0.11	19.13±0.18	45.59±0.19	8.66
Riboflavin B2	5.44±0.03	3.80±0.13	4.74±0.04	5.18±0.01	1.90±0.01	2.87
Nicotinamide B3	265.9±0.3	96.2±0.7	83.28±0.23	104.0±0.5	119.1±4.3	3.19
Pantothenate B5	20.16±0.91	36.36±0.37	2543.0±27.0	1380.7±1.8	685.7±3.7	126.2
Pyridoxine B6	347.9±2.1	225.7±2.9	127.3±0.9	202.4±1.9	141.2±1.4	2.47
Folic Acid B9	55.87±0.49	26.86±0.31	-	78.58±0.89	102.1±1.1	3.80
Cyanocobalamin B12	9.84±0.03	3.88±0.03	1.10±0.09	2.02±0.01	3.79±0.01	8.99

Table 3.4. Shows the percentage of RDA [377] in µg/100 g dw of each of the varieties of eggplant fruits.

Vitamin	Average RDA (mg/day)	White garden egg	Bitter apple	Dark eggplant	Light eggplant	Bitter tomato
Vitamin A	0.8	29.13	45.36	7.86	1.68	33.09
Vitamin E	15	7.32	2.42	26.09	3.20	22.99
Lycopene	8	8.49	5.41	2.22	1.90	4.00
Vitamin C	46	246.10	128.71	105.81	93.74	79.41
Thiamine B_1	0.9	1061.78	598.41	122.61	212.60	506.53
Riboflavin B_2	1	54.40	38.00	47.41	51.81	18.97
Nicotinamide B_3	15	177.25	64.14	55.52	69.34	79.41
Pantothenate B_5	4	50.40	90.89	6357.54	3451.77	1714.34
Pyridoxine B_6	1.4	2485.26	1612.24	909.49	1446.01	1008.41
Folic Acid B_9	0.5	1117.46	537.14	-	1571.66	2042.64
Cyanocobalamin B_{12}	0.2	492.05	193.95	54.75	100.80	189.70

Table 3.5. Shows the amounts of stress biomarkers - GSH, GSSG, MDA and 4-HNE in the eggplant fruits (μg/g) DW.

Parameter	White Garden Egg	Bitter apple	Dark eggplant	Light eggplant	Bitter tomato
GSH.	5930.32±475.38	1511.93±23.74	408.45±2.13	812.44±4.91	364.85±14.84
GSSG.	1961.59±56.99	866.29±5.64	224.83±0.91	418.50±2.44	230.01±2.20
MDA	18.41±0.33	1.5±0.16	1.90±0.01	3.62±0.04	3.16±0.15
4-HNE	48.25±1.55	24.57±0.31	36.29±0.83	31.81±1.63	33.56±0.21

Table 3.6 Shows the amounts of total phenolic content, total flavonoid content, ABTS and DPPH scavenging activities of the different varieties of the eggplants (μg/g) DW.

Sample	ABTS	IC_{50} (DPPH)	Total Flavonoids	Total Phenolic
Dark Eggplant	692.24±5.1	95.09±1.03	263.79±6.93	1120.69±11.2
Light Eggplant	532.28±3.2	113.78±1.87	192.05±0.40	706.30±10.6
Bitter Tomato	365.44±2.8	99.27±0.83	214.71±0.90	732.35±6.7
Bitter Apple	464.29±4.6	83.86±0.96	167.5±5.12	868.57±12.8
White Garden	809.52±3.8	243.02±1.31	356.51±3.44	1260.32±5.5

4. DISCUSSION

4.1. Amino acids

4.1.1. Aspartic acid

All the eggplant samples used in this study had measurable amounts of aspartic acid ranging from 972.44 - 4217.65 µg/g (DW), depicting a 4.3 fold range difference. Our findings showed the amount of aspartic acid determined in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato to be (Table 4.1) 1747.62, 2277.86, 3018.97, 972.44 and 4217.65µg/g (DW), respectively. The Nigerian bitter tomato (4217.65 µg/g) had the highest concentration of aspartic acid, while the light eggplant (972.44 µg/g) variety sampled from Turkey had the lowest.

A wide variation was observed in amounts of aspartic acid amongst both the Nigerian varieties and the Turkish varieties. Between the two Turkish varieties sampled in this study, the dark eggplant (3018.97 µg/g) had higher amounts that were 3.1 folds the aspartic acid content in the light eggplant (972.44 µg/g). Comparatively, between the Nigerian varieties, the bitter tomato (4217.65 µg/g) variety had the highest concentration determined to be 1.9 and 2.4 folds the amount of aspartic acid determined in the bitter apple (2277.86 µg/g) and white garden egg (1747.62 µg/g) varieties, respectively. Similarly, the bitter apple (2277.86 µg/g) variety had a concentration that was 1.3 folds, the amounts determined in white garden egg (1747.62 µg/g).

These variations were also observed between the values in the Turkish (972.44 – 3018.97 µg/g) and Nigerian (1747.62 – 4217.65 µg/g) varieties. All the Nigerian varieties had a higher concentration than the light eggplant, whereas the dark eggplant had higher amounts than the white garden egg and bitter apple varieties. In contrast, the Nigerian bitter tomato varieties had higher aspartic concentrations than both Turkish varieties. Our findings are consistent with the values of aspartic acid in eggplant fruits reported by Flick *et al.* [378] in three varieties of eggplants as 3.274, 2.666 and 1.969 (mg/g) (3274, 2666 and 1969 µg/g) DW. Contrastingly, lower values of 10.45 mg/100g DW (104.5 µg/g) were reported by Imo *et al.* (2019) [379] while a range of 5.31 - 9.52 mg/100g (53.1 – 95.2 µg/g) was reported by Mori *et al.* (2013) [380]. The variations observed in the concentration of aspartic acid of the different varieties are attributable to differences in their genetic makeup and geographical origins.

4.1.2. Glutamic Acid

The results revealed the presence of glutamate in all the samples used in this study ranging from 2784.3 - 7712.7 µg/g DW with a 2.8 fold range difference. Specifically, a glutamic acid concentration of 7712.7, 3535.7, 3206.0, 2784.3, and 7385.3 µg/g DW was determined (Table 4.1) in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato varieties of eggplant, respectively. The highest and lowest concentrations of glutamic acid were detected in white garden egg and light eggplant varieties, respectively.

Comparing and contrasting within and between the varieties sampled from Turkey and Nigeria. Within the Turkish samples, the dark eggplant had a higher concentration of glutamic acid than the light eggplant. For the Nigerian samples, the white garden egg and bitter tomato had 2.1-folds the concentration of glutamic acid determined in the bitter apple variety.

All the Nigerian varieties had higher concentrations of glutamic acid than the Turkish varieties. However, the bitter tomato and white garden egg varieties had 2.3 and 2.4; then 2.8 and

2.7 folds the amounts determined in the dark and light eggplant, respectively. In comparison, the bitter apple variety concentration differed by a narrower margin to those of the dark and light eggplant varieties. Our findings are consistent with range of values of glutamic acid reported by Flick *et al*. [378] 3.582 2.992 and 2.405 mg/g (3582, 2992, and 2405 µg/g) DW. In comparison, Imo *et al.* (2019) [379] reported significantly lower values of 9.05 mg/100g (90 µg/g) DW. While Ayaz *et al*. (2015) [381] reported similar values of 148.4–298.75 mg/100g) (1484 – 2987.5 µg/g) in different varieties of fresh eggplant fruits. The variations observed between the varieties are due to genotype variations and geographical origins of the eggplant fruit samples.

4.1.3. Asparagine

Our findings showed the presence of asparagine ranging from 2985.8 - 5360.30 µg/g DW with a 1.8-fold range difference in all the varieties of eggplants sampled for this study. The concentrations of asparagine were determined in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato to be (Table 4.1) 4215.9, 3318.6, 3269.0, 2985.8 and 5360.3 µg/g DW, respectively. The Turkish light eggplant was determined to have the lowest concentration while the Nigerian bitter tomato had the highest.

Between the Turkish varieties, the dark eggplant had a higher concentration of asparagine than the light eggplant. Within the Nigerian varieties, the bitter tomato variety had a higher concentration of 2.8 and 1.6 folds the concentrations in white garden egg and bitter apple varieties respectively. Whilst the white garden egg had, 1.8 folds the amounts in the bitter apple (3318.57 µg/g) variety. Comparing the concentrations in the Turkish varieties to their Nigerian counterparts showed all the Nigerian varieties to have higher concentrations of asparagine.

Contrastingly, Flick *et al*. [378] and Imo *et al*. [379] reported asparagine to be below detection limit in eggplant fruits. A study on different varieties of tomato reported asparagine values to range from 21.55 – 42.03 mg/100g DW [382]. The differences in the concentration of asparagine determined between the varieties sampled for this study and the literature are attributable to varietal differences and growth conditions, which have been reported to affect its occurrence and concentration in crops [383].

4.1.4. Serine

Based on this study, all the eggplant samples had a measurable amount of serine ranging from 1601.6 - 2181.6 µg/g DW with a 1.4 fold range difference. Our findings revealed the concentration of serine determined (Table 4.1) to be 1665.1, 1317.1, 1753.5, 1601.6, and 2181.6 µg/g DW in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato, respectively. The highest and lowest concentrations of serine was determined in the Nigerian bitter tomato and Turkish light eggplant varieties, respectively.

Similar to values of asparagine determined, the serine concentration in Turkish varieties differed by a narrow margin. Between the Nigerian varieties, the bitter tomato variety had the highest concentration that was 1.3 and 1.7 folds amounts determined in white garden egg and bitter apple varieties, respectively. While the white garden egg had 1.3 folds the concentration determined in the bitter apple variety.

Comparing between the Turkish and Nigerian varieties showed no particular trend. The Nigerian bitter tomato had 1.2 and 1.4 folds the amounts of serine determined in the Turkish dark and light eggplant varieties, respectively. In contrast, the Turkish varieties had higher amounts of

serine than the white garden egg and bitter apple varieties respectively. Flick *et al.* [378] reported lower serine values of 0.815, 0.568 and 0.562 mg/g (815, 568 and 562 µg/g) DW in three varieties of eggplants. Imo *et al*. (2019) [379] also determined significantly lower concentrations of 3.63 mg\100g (36.3 µg /g) DW in eggplants. Equally lower values were reported by Mori *et al*., (2013) [380] and Ayaz *et al*. (2015) [381] in different varieties of eggplants ranging from 1.58 – 6.78 mg/100 (15.8 – 67.8 µg / g) and 21.97 – 38.04 mg/100g (219.7 - 380.4 µg / g) FW, respectively. The disparities observed are due to the variations in the genetic makeup and geographical origins of each variety used for this study.

4.1.5. Glycine

Each of the eggplant varieties sampled for this study had measurable amounts of glycine, which ranged from 1394.5 - 4578.7 µg/g DW, depicting a 3.3 fold range difference. Specifically, the concentration of glycine in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined (Table 4.1) to be 1822.2, 3928.6, 1526.7, 1394.5 and 4578.68 µg/g (DW) respectively. The highest and lowest amounts of glycine were detected in the Nigerian bitter tomato and the Turkish light eggplant, respectively.

Analogous to values determined for asparagine and serine, the Turkish varieties had concentrations of glycine that differed by a narrower margin in comparison to the Nigerian varieties that had a wider range difference. The bitter tomato and bitter apple varieties had a higher concentration that was 2.5 and 2.2 folds the amounts of glycine in the white garden egg variety, respectively. Contrastingly, a narrower difference was observed between the values of glycine in bitter tomato and bitter apple varieties.

Relative to the concentration of glycine in the Turkish varieties; the Nigerian varieties all had a higher concentration. The bitter apple and bitter tomato (4578.7 µg/g) varieties had a higher concentration of 2.8 and 2.6; then 3.3 and 3.0 folds the glycine determined in the light and dark eggplant, respectively. Flick *et al.* [378] determined the glycine in eggplants to range from 0.542-0.776 mg/g (542- 776 µg/g) DW. Imo *et al*. [379] reported a glycine concentration of 4.00 mg/100g (40 µg/g) DW in an eggplant variety. These are inconsistent with our findings. These dissimilarities are due to the varietal differences and the geographical origins of the samples used in this study.

4.1.6. Glutamine

Our findings revealed the presence of glutamine ranging from 715.0 – 1315.9 µg/g (DW) in the eggplant varieties sampled for this study depicting a 1.8 fold range difference. Glutamine concentrations of (Table 4.1) 1315.9, 927.9, 782.8, 715.0 and 1219.1 µg/g DW were determined in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato, correspondingly. The highest and lowest concentrations were determined in the Nigerian white garden egg and Turkish light eggplant varieties, respectively.

Within the Turkish varieties, concentration of glutamine (715.0 – 782.8 µg/g) varied by a comparatively narrow margin. Similarly, within the Nigerian varieties the white garden egg and bitter tomato varieties had similar concentrations of glutamine that were 1.4 and 1.3 folds the concentrations determined in the bitter apple variety, respectively. Correlating the concentration of glutamine in the Turkish varieties (715.0 – 782.8 µg/g) to the Nigerian varieties (927.9 - 1315.9 µg/g) showed all the Nigerian varieties to have a higher concentration of glutamine. These observations are ascribed to the differences in the genetic makeup and geographical origins of the varieties used in this study.

Our findings are at disparity with the literature values of glutamine as Flick *et al.* [378] and Imo *et al*. [379] who also conducted amino acid analysis on different varieties of eggplant fruits (DW) did not report glutamine values. The disparities between our findings and the literature values may be due to the difference in the varieties and methodologies used.

4.1.7. Histidine

Our findings revealed the presence of histidine in all the varieties of eggplant fruits sampled for this study ranging from 6903.57 -20545.69 µg/g DW with a 3.0 fold range difference. This shows a significant variability in the concentration of histidine in the varieties. Specifically, the concentration of histidine in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined (Table 4.1) to be 13823.8, 6903.6, 20545.7, 18766.1 and 17398.5 µg/g DW, respectively. The highest concentration was detected in the Turkish variety while the Nigerian bitter apple had the lowest.

The Turkish varieties showed less range difference in their concentrations of histidine in comparison to the Nigerian varieties. Whereas a comparison between the Nigerian varieties showed a wider range difference. The bitter tomato and white garden egg had 2.5 and 2.0 folds the amounts determined in the bitter apple variety, respectively. While a narrower difference was observed between the white garden egg and bitter apple varieties. Correlating between the varieties showed those from Turkey to have a higher concentration of histidine than all the Nigerian varieties. The dark and light eggplant had a higher concentration that was 3.0 and 2.7 folds the amounts in bitter apple variety, respectively. The differences observed are due to the genetic variations and growth conditions of the varieties used in this study.

Flick *et al*. [378] determined values of 0.475, 0.338 and 0.332 mg\g (475, 338 and 332 µg /g) DW while Imo *et al.* [379] reported it as 1.92 mg/100g (19.2 µg/g) DW. Amadi *et al*. [384] reported values of 10.97, 17.05, and 18.02 mg/100g (109.7, 170.5 and 180.2 µg/g) FW in some varieties of eggplant fruits. These are inconsistent with the values of histidine determined in this study, which may be due to varietal differences, or methodologies used.

Correlating the values of histidine determined across the varieties to the RDA for histidine (700 mg/day) [376]. It was determined (Table 4.2) that the concentrations of histidine in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato to be equivalent to 197.5, 98.6, 293.5, 268.1, and 248.6 % per 100g DW respectively. Thus, all the varieties selected for this study may serve as excellent sources of histidine.

4.1.8. Arginine

All the varieties used in this study had measurable amounts of arginine, which ranged from 89.7- 234.9 µg /g DW with a 2.6 fold range difference. Based on our findings, the concentration of arginine in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined (Table 4.1) to be 234.9, 95.0, 98.3, 89.8 and 89.7 µg /g DW. The highest and lowest concentrations were determined in the Nigerian white garden egg and bitter tomato varieties, respectively.

Comparing between the Turkish varieties, the dark eggplant had a higher concentration of arginine than the light eggplant. Between the Nigerian varieties, a range difference of 2.6 folds was observed. The white garden egg had the highest concentration, followed by bitter apple, while the bitter tomato variety had the lowest.

Relating the values of arginine determined based on geographical source, the Nigerian white garden egg had a higher concentration of arginine than the Turkish varieties. In contrast, the dark eggplant had a higher arginine concentration than the Nigerian bitter apple and bitter tomato varieties. Conversely, all the Nigerian varieties with the exception of the bitter tomato had a higher concentration of arginine than the Turkish light eggplant. Flick *et al*. [378] reported values of arginine in varieties of eggplant fruits to be 1.206, 0.724 and 1.033 mg\g (1206, 724 and 1033 µg /g) DW. Contrastingly, Imo *et al*. (2019) [379] reported arginine values as 5.32 mg\100g (53.2 µg/g) DW. Conversely, Mori *et al.* [380] determined the arginine values in different varieties of eggplants to range from 4.50 to 23.08 mg\100g (45.0 – 230.80 µg/g) FW. Our findings are inconsistent with literature values. These variations are attributable to the differences in the varieties and geographical origins of the samples used in this study.

4.1.9. Threonine

Based on this study, each of the sampled varieties had detectable amounts of threonine that ranged from 1205.0 - 3014.7 µg /g DW with a 2.5 fold range difference. Our findings determined the concentrations of threonine in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato to be (Table 4.1) 2381.0, 1205.0, 3014.7, 2753.5 and 2941.9 µg /g DW. The Turkish dark eggplant (3014.7 µg /g) and the Nigerian bitter apple (1205.0 µg/g) varieties were determined to have the highest and lowest concentrations, respectively.

The varieties sampled from Turkey had concentrations of threonine that differed by a narrow difference, with the dark eggplant having a higher threonine concentration. Amongst the Nigerian varieties, the bitter tomato had the highest concentration of threonine 1.2 and 2.4 folds the amounts determined in the white garden egg and bitter apple varieties, respectively. While the white garden egg had 2.0 folds the concentration of threonine determined in the bitter apple variety.

Correlating the values of threonine in the varieties from Turkey and Nigeria showed no particular trend based on origins. The dark eggplant had a concentration that was higher than all the Nigerian varieties. In contrast, while the Turkish light eggplant had a higher threonine concentration than the white garden egg and bitter apple varieties; its concentration was lower than that of the bitter tomato. Flick *et al*. [378] determined threonine values in different varieties of eggplant of 0.493, 0.527 and 0.776 mg/g (493, 527 and 776 µg /g) DW. Imo *et al.* (2019) [379] reported significantly lower values of threonine in eggplant fruits of 2.11 mg\100g (21.1 µg /g) DW. These are lower than the findings in this study. These variabilities in the concentration of threonine across the varieties used in this study and the literature values are ascribed to their varietal differences and origins.

In terms of RDA (1050 mg/day) [376], a comparison with values of threonine determined in this study (Table 4.2) for white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined to be 22.7, 11.5, 28.7 , 26.2, 28.0 % per 100g DW, respectively. All the varieties contribute more than 10% of the RDA, and are therefore good sources of threonine.

4.1.10. Alanine

Our findings revealed the presence of alanine in the selected varieties used in this study to range from 1351.2 - 2978.7 µg /g DW depicting a 2.2 fold range difference. Individually, the concentration of alanine in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined to be (Table 4.1) 2490.5, 2286.4, 1479.3, 1351.2 and 2978.7 µg /g DW,

respectively. The bitter tomato variety had the highest concentration while the light eggplant had the lowest.

The Turkish varieties were determined to have concentrations of alanine that varied by a narrow difference, with the dark eggplant having a higher concentration. A comparison between the Nigerian varieties showed a comparatively wider variability in their concentrations of alanine. The bitter tomato variety had the highest concentration followed by white garden egg while the bitter apple had the lowest.

Correlating the concentration of alanine in the Turkish varieties to those determined in the Nigerian varieties. All the Nigerian varieties were determined to have a higher concentration of alanine than the Turkish varieties. Flick *et al.* [378] reported lower concentrations of alanine of 0.995, 0.658 and 0.677 mg\g (995, 658 and 677 µg /g) DW in different varieties of eggplants. Imo *et al*. (2019) [379] also reported significantly lower concentrations of alanine in eggplant of 3.84 mg\100g (38.4 µg /g) DW. These variabilities in the values of alanine observed are due to differences in the varieties and origins of the samples used for this study.

4.1.11. Proline

Each of the varieties of eggplant fruits sampled for this study had a measurable amount of proline, which ranged from 163.6 - 442.9 µg/g DW showing a 2.7 fold range difference. Our findings revealed the concentration of proline in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato determined to be (Table 4.1) 442.9, 163.6, 256.9, 234.7, and 172.8 µg /g (DW), respectively. The highest and lowest concentrations were detected in the Nigerian white garden egg and bitter apple varieties, respectively.

An appraisal of the concentrations determined in varieties based on their origins showed the Turkish varieties had a relatively narrow variability with the dark eggplant having a higher proline concentration. In contrast, the Nigerian varieties showed a wider variance, with the highest concentration of proline determined in the white garden egg, which was 2.7 and 2.6 folds the amounts in bitter apple and bitter tomato varieties, respectively. While the concentrations in the bitter tomato variety was higher than that of the bitter apple, it varied by a narrower margin. Correlating the concentration of proline in the Turkish varieties to their Nigerian counterparts. The Turkish varieties had a higher concentration than all the Nigerian varieties with the exception of the white garden egg. Flick *et al*. [378] determined higher proline values of 0.784, 0.585 and 0.534 mg/g (784, 585, and 534 µg/g) DW in three varieties of eggplants. Contrastingly, lower proline values of 3.05 mg\100g (30.5 µg /g) DW in eggplant fruits were reported by Imo *et al*. (2019) [379]. Conversely, Amadi *et al*. [384] reported proline values of 9.56, 11.25 and 12.47 mg\100g (95.6, 112.5 and 124.7 µg /g) in three varieties of fresh eggplants. The disparities observed in the concentration of proline are attributable to the varietal differences and geographical origins of the samples used for this study.

4.1.12. Tyrosine

The varieties of eggplant sampled for this study each had detectable levels of tyrosine that ranged from 929.1- 2424.3 µg /g DW depicting a 2.6 fold range difference. Our findings determined the concentration of tyrosine in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato to be 1765.1, 1472.1, 1017.2, 929.1 and 2424.3 µg /g DW. The Nigerian bitter tomato and the Turkish light eggplant varieties had the highest and lowest concentrations, respectively.

Comparing and contrasting between the concentrations of tyrosine determined in the Turkish varieties. The dark eggplant had a higher concentration of tyrosine than the light eggplant (929.1µg/g). Conversely, between the Nigerian varieties, the bitter tomato variety had a higher concentration that was 1.4 and 1.7 times the amounts determined in the white garden egg and bitter apple varieties, respectively. While the white garden egg variety had, higher concentrations that was 1.2 folds the amounts detected in the bitter apple variety. A comparison between the values determined in the Turkish varieties to their Nigerian counterparts showed the Nigerian varieties to have higher concentrations of tyrosine. A study by Flick *et al.* [378] reported values of tyrosine in three varieties as 0.419, 0.287 and 0.313 mg/g (419, 287 and 313 µg /g) DW which falls within range of the findings in this study. In contrast, Imo *et al*. (2019) [379] reported a significantly lower tyrosine value of 3.57 mg\100g (35.7 µg/g) DW in eggplant. The variations in the concentrations of tyrosine observed are due to the differences in the genetic makeup of the varieties sampled.

4.1.13. Valine

Each of the varieties of eggplant fruit sampled for this study had measurable amounts of valine ranging from 388.2 – 3246.0 µg/g DW showing a wide range variation of 8.4 folds. Based on our findings, the concentrations of valine in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined to be (Table 4.1) 3246.0, 1413.6, 918.1, 612.60 and 388.2 µg/g DW respectively. The highest and lowest concentrations were determined in the Nigerian white garden egg and bitter tomato varieties, respectively.

A comparison between the Turkish varieties showed that the dark eggplant had a higher concentration of valine that was 1.5 times the amounts determined in the light eggplant.

Within the Nigerian varieties, the white garden egg had a concentration that 2.3 and 8.4 folds the amounts of valine in the bitter apple and bitter tomato varieties, respectively. Likewise, the bitter apple variety had 3.6 folds the amounts determined in the bitter tomato variety.

Correlating the concentration of valine in the Turkish varieties to their Nigerian counterparts. The Nigerian white garden egg and bitter apple had higher concentrations of valine than the Turkish dark and light eggplant varieties. Contrastingly, both Turkish varieties had a higher concentration of valine than the Nigerian bitter tomato variety. The findings in this study are consistent with values reported by Flick *et al*. [378] who determined valine concentrations of 1.212, 0.807 and 0.795 mg/g (1212, 807, and 795 µg /g) DW in three varieties of eggplants. Contrastingly, Imo *et al*. (2019) [379] determined the concentration of valine in eggplant fruit as 5.36 mg\100g (53.6 µg/g) DW, which is significantly lower than the findings in this study. The observed variations are due to the varietal differences from genotype and origins of the eggplants sampled.

A comparison of our findings of valine concentrations to the RDA values (1820 mg/day) [376] (Table 4.2) for white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined to be 17.8, 7.8, 5.1, 3.4 and 2.1 % per 100g DW, respectively. This shows that while the white garden egg variety is as a good dietary source of valine; the bitter apple and dark eggplant varieties may be considered useful during shortages, while the light eggplant and bitter tomato varieties are poor sources of valine.

Methionine

Our findings revealed the presence of methionine in all the varieties used in this study ranging from 1886.6 - 5038.2 µg/g DW depicting a 2.7 fold range difference. The specific concentrations of methionine in white garden egg, bitter apple, dark eggplant, light eggplant and

bitter tomato were determined to be (Table 4.1) 2628.6, 3105.0, 3710.4, 1886.6 and 5038.2 µg/g DW, respectively. The Nigerian bitter tomato and the Turkish light eggplant varieties were determined to have the highest and lowest concentrations, respectively.

Between the varieties sampled from Turkey, the dark eggplant had a higher concentration of 2.0 folds the amount of methionine determined in the light eggplant. Within the Nigerian varieties, the bitter tomato had the highest concentration that was 1.9 and 1.6 folds the concentrations in white garden egg and bitter apple, respectively. While the bitter apple variety had 1.2 times the concentration detected in the white garden egg. Comparing and contrasting between the values of methionine determined in the Turkish (1886.6– 3710.4 µg/g) varieties to that of their Nigerian (2628.57 - 5038.2 µg/g) counterparts. The bitter tomato and variety had the highest concentration followed by the bitter apple, while the white garden egg variety had the lowest. In contrast, all the Nigerian varieties had a higher methionine concentration than the light eggplant. While the bitter tomato had a higher concentration than the dark eggplant, it in turn had a higher concentration than the white garden egg and bitter apple varieties. Flick *et al*. [378] reported methionine in three varieties of eggplant fruits to be below detection levels while Imo *et al*. [379] reported a significantly lower methionine value of 0.88 mg/100g (8.8 µg/g) DW. The differences observed are due to the varietal individualities and geographical origins of the eggplant fruits sampled.

Comparing the values of methionine determined in the eggplant samples to the RDA (1050 mg/day) [376] (Table 4.2) the percentages were calculated for white garden egg - 25.0 %; bitter apple - 29.6 %; dark eggplant - 35.3 %; light eggplant -18.0 % and bitter tomato -243.7% per 100g DW. All the varieties used in this study had levels of methionine that are considerably significant in dietary terms.

4.1.14. Cysteine

All the varieties of eggplant fruits sampled for this study had detectable amounts of cysteine found to range from 1096.9 – 2183.1 µg/g DW showing a 2.0 fold range variation. Our findings determined the concentration of cysteine in each of white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato to be (Table 4.1) 1861.9, 1517.1, 1876.7, 1096.9 and 2183.1 µg/g DW, respectively. The highest and lowest concentrations were determined in the Nigerian bitter tomato and Turkish light eggplant varieties, respectively.

A comparison between the values of cysteine determined in the Turkish varieties showed the dark eggplant to have a higher concentration that is 1.7 folds the amounts in the light eggplant. Within the Nigerian varieties, the bitter tomato had a higher concentration that was 1.2 and 1.4 folds the amounts detected in white garden egg and bitter apple, respectively. The white garden egg also had a higher concentration that was 1.2 times the values of cysteine in the bitter apple variety.

Comparing the values of cysteine determined in the Turkish varieties to the Nigerian varieties showed no specific trend. Whilst the bitter tomato had a higher concentration than all the sampled varieties, the Turkish dark eggplant had a higher concentration than the Nigerian white garden egg and bitter apple varieties. On the contrary, the Turkish light eggplant (139.3 µg/g) had a lower cysteine concentration than all the Nigerian varieties. While Flick *et al*. [378] reported cysteine to be below detection, Imo *et al.* (2019) [379] reported a significantly lower cysteine value of 0.78 mg/100g (7.8 µg/g) DW. The observed disparities are due to differences in the genetic makeup and geographical origins of the varieties studied.

4.1.15. Leucine

All the varieties of eggplant fruits sampled for this study had a measure of leucine, which ranged from 23.5 - 255.6 µg/g DW with a 10.9 fold range variation. Based on our findings, the leucine concentration in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined to be (Table 4.1) 255.6, 90.0, 175.9, 114.2, and 23.5 µg/g DW respectively. The Nigerian white garden egg (255.6 µg/g) and bitter tomato (23.53 µg/g) varieties had the highest and lowest concentrations, respectively.

Comparing the values determined between the Turkish varieties showed the dark eggplant to have a higher concentration that was 1.5 folds the amounts in the light eggplant. A comparison within the Nigerian varieties showed the white garden egg to have a higher concentration, 2.8 and 10.9 folds the values determined in the bitter apple and bitter tomato varieties, respectively. Whilst the bitter apple had 3.8 folds the amounts in the bitter tomato variety.

Correlating the values of leucine determined in the Turkish (114.2 – 175.86 µg/g) varieties to the Nigerian (23.53 – 255.56 µg/g) varieties, which had a wider range difference. The white garden egg variety had a higher leucine concentration than all the Turkish varieties sampled for this study. While in contrast, the Turkish varieties had higher concentrations than the bitter apple and bitter tomato varieties. Our findings are consistent with those of Imo *et al*. (2019) [379] who reported the concentration of leucine to range from 1.86 to 8.92 mg/100g (18.6 – 89.2 µg/g) DW. In contrast, Flick *et al.* [378] reported leucine values of 1.266 0.950 0.944 mg/g (1266, 950 and 944 µg/g) DW which are higher than the findings in this study. The disparities observed in the values of leucine are due to the differences in the varieties selected.

In relation to the RDA for leucine (2730 mg/day) [376], the varieties sampled for this study - white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined (Table 4.2) to contribute 0.94, 0.33, 0.64, 0.42 and 0.09 % per 100g DW, respectively. The values are significantly low and thus none of the varieties can be regarded as a good dietary source of leucine.

4.1.16. Phenylalanine

Based on this study, each of the varieties of eggplants sampled had detectable levels of phenylalanine ranging from 465.1 -2829.1 µg/g with a wide range difference of 6.1 folds. Our findings determined the concentration of phenylalanine in white garden egg, bitter apple, and dark eggplant, light eggplant and bitter tomato to be (Table 4.1) 465.1, 2332.1, 2438.8, 2829.1 and 2407.4 µg/g DW, respectively. The highest concentration was determined in the Turkish light eggplant variety, while the Nigerian white garden egg had the lowest.

A comparison of the phenylalanine values determined between the Turkish varieties showed no wide variability with the light eggplant having a higher concentration. In contrast, a wider variance of 5.2 folds was observed between the Nigerian varieties. The bitter tomato had the highest concentration of phenylalanine followed by bitter apple while the white garden egg had the lowest.

Correlating the concentration of phenylalanine determined in the varieties from different origins, showed the Turkish varieties to have a higher concentration than all the Nigerian varieties. The disparities observed in the concentration of phenylalanine are due to the varietal differences of eggplant fruits sampled for this study. Our findings are consistent with some of the values of phenylalanine determined in a study by Flick *et al*. [378] who reported values of 0.869, 0.617 and 0.617 mg/g (869, 617 and 617 µg/g) DW in three (3) different varieties. Contrastingly, Imo *et al*. [379] reported a lower value of 9.34 mg/100g (93.4 µg/g) DW in eggplants.

The percentage of phenylalanine in relation to the RDA (1750 mg/day) [376] (Table 4.2) were calculated for white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato to be 2.7, 13.3, 13.9, 16.2 and 13.8 % per 100g DW, respectively. Based on this, with the exception of the white garden egg variety, all of the varieties had concentrations of phenylalanine that were nutritionally significant.

4.1.17. Tryptophan

Based on our findings, each of the varieties of eggplant fruits sampled for this study had measurable levels of tryptophan ranging from 1003.5 - 1807.9 µg/g DW showing a 1.8 fold range difference. The individual concentrations of tryptophan in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined to be (Table 4.1) 1807.9, 1355.0, 1003.5, 1622.1, and 1691.2 µg/g DW, respectively. The highest and lowest concentrations were determined in the Nigerian white garden egg and Turkish dark eggplant, respectively.

Comparing the values of tryptophan determined in the Turkish varieties showed the light eggplant had a higher concentration that was 1.6 folds the amounts in the dark eggplant. Within the Nigerian varieties, the white garden egg variety had the highest concentration followed by the amounts determined in the bitter apple while the bitter tomato had the lowest. Correlating the values of tryptophan determined in the Turkish varieties (1003.5 – 1622.1 µg/g) to values in the Nigerian (1691.2 -1807.9 µg/g) varieties. All the Nigerian varieties had higher tryptophan concentrations than the dark eggplant. In contrast, the light eggplant had a higher concentration than the bitter apple variety, and lower concentrations than the white garden egg and bitter tomato varieties.

Flick *et al*. [378] did not report tryptophan values while Imo *et al.* [379] reported a significantly lower concentration of tryptophan 4.02 mg/100g (40.2 µg/g) DW in eggplant fruits. The differences observed are due to the varietal dissimilarities and geographical origins of the eggplants sampled.

A correlation of the values of tryptophan determined across the varieties to the RDA (280 mg/day) [376] were determined (Table 4.2) for white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato to be 64.6, 48.4, 35.8, 57.9 and 60.4 % per 100g DW, respectively. Based on this, all the varieties of eggplant sampled for this study had nutritionally significant values of tryptophan.

4.1.18. Lysine

Each of the varieties sampled for this study had detectable levels of lysine that ranged from 6800.9 – 33625.4 µg/g DW showing a 4.9 fold range variation. Our findings revealed the concentration of lysine in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato determined (table 3.1) to be 33625.4, 13545.7, 6800.9, 47018.9 and 26117.7 µg/g DW, respectively. The highest and lowest concentrations were determined in the Turkish light eggplant (47018.9 µg/g) and dark eggplant (6800.9 µg/g) varieties, respectively.

Comparatively, between the Turkish varieties, the light eggplant had a higher concentration of lysine that was 6.9 folds the amounts in the dark eggplant. Within the Nigerian varieties, the white garden egg (2118.4 µg/g) had the highest concentration followed by the bitter tomato (26117.7 µg/g) varieties and both had a concentration that was 2.5 and 1.9 folds the values in the bitter apple (13545.7 µg/g), respectively.

Correlating the values in the Turkish (6800.9 - 47018.9 µg/g) varieties to the Nigerian (13545.7– 33625.4 µg/g) varieties showed a wider range difference between the former. All the

Nigerian varieties had a higher concentration of lysine than the dark eggplant. While in contrast, the light eggplant had a higher concentration of lysine than all the Nigerian varieties. The variabilities observed are attributable to the difference in the variety and geographical origins of eggplants sampled for this study. Our findings are higher than lysine values reported by Flick *et al*. [378] who reported values of 0.769 0.541 and 0.541 mg/g (769.0, 541.0 and 541.0 µg/g) DW in three varieties of eggplant fruits. Likewise, Imo *et al*. (2019) [379] also determined significantly lower lysine values of 3.64 mg/100g (36.4 µg/g) DW in eggplant.

Based on our findings, we compared amounts of lysine detected in this study to the RDA (2100 mg/day) [376] (Table 4.2) determined for white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato as 160.1, 64.5, 32.4, 223.9 and 124.4 % per 100 g dw, respectively. Thus, all the varieties of eggplants used in this study had high levels of lysine and could contribute to its dietary adequacy.

Table 4.1. Shows the increasing order of amino acid concentrations in the five varieties of eg

Amino Acid	Min-Max (μg/g)	Amounts in the different varieties from the lowest to the highes
Leucine	23.5 - 255.6	Bitter tomato < Bitter apple < Light eggplant < Dark eggplant < Whi
Arginine	89.7 - 234.9	Bitter tomato < Light eggplant < Bitter apple< Dark eggplant < White
Proline	163.6 - 442.9	Bitter apple < Bitter tomato < Light eggplant < Dark eggplant < Wh
Glutamine	715.0 - 1315.9	Light eggplant < Dark eggplant < Bitter apple < Bitter tomato < Whit
Valine	388.2 -3246.0	Bitter tomato < Light eggplant < Dark eggplant < Bitter apple < W
Tryptophan	1003.5 - 1807.9	Dark eggplant < Bitter apple < Light eggplant < Bitter tomato < Wh
Serine	1601.6 - 2181.6	Bitter apple < Light eggplant < White garden egg <Dark eggplant <
Cysteine	1096.9 - 2183.1	Light eggplant < Bitter apple < White garden egg < Dark eggplant <
Tyrosine	929.1 - 2424.3	Light eggplant < Dark eggplant < Bitter apple < White garden egg <
Phenylalanine	465.1 -2829.1	White garden egg < Bitter apple < Bitter tomato < Dark eggplant < Li
Threonine	1205.0 - 3014.7	Bitter apple < White garden egg < Light eggplant < Bitter tomato < D
Alanine	1351.2 - 2978.7	Light eggplant < Dark eggplant < Bitter apple < White garden egg <
Aspartic acid	972.4 - 4217.7	Light eggplant < White garden egg < Bitter apple < Dark eggplant <
Glycine	1394.5- 4578.7	Light eggplant < Dark eggplant < White garden egg < Bitter apple <
Methionine	1886.6 - 5038.2	Light eggplant < White garden egg < Bitter apple < Dark eggplant <
Asparagine	2985.8 - 5360.3	Light eggplant < Dark eggplant < Bitter apple < White garden egg <
Glutamic acid	2784.3 - 7712.7	Light eggplant < Dark eggplant < Bitter apple < Bitter tomato < Whit
Histidine	6903.6 -20545.7	Bitter apple < White garden egg < Bitter tomato < Light eggplant < D
Lysine	6800.9 - 33625.4	Dark eggplant < Bitter apple < Bitter tomato < White garden egg < Li

In summary, based on the findings in this study (Table 4.1) the bitter tomato variety (Figure 4.1) sampled from Nigeria appears to be the richest in terms of the concentrations of the individual amino acids. It had the highest concentration of eight (8) – serine, cysteine, tyrosine, alanine, aspartic acid, glycine, methionine and asparagine, out of the 19 amino acids. Likewise, it had the second highest concentration of glutamine, tryptophan, threonine and glutamic acid. It was also determined to have average concentrations of phenylalanine, histidine and lysine; while in contrast, it had low concentrations of proline and the lowest concentrations of valine, leucine and arginine.

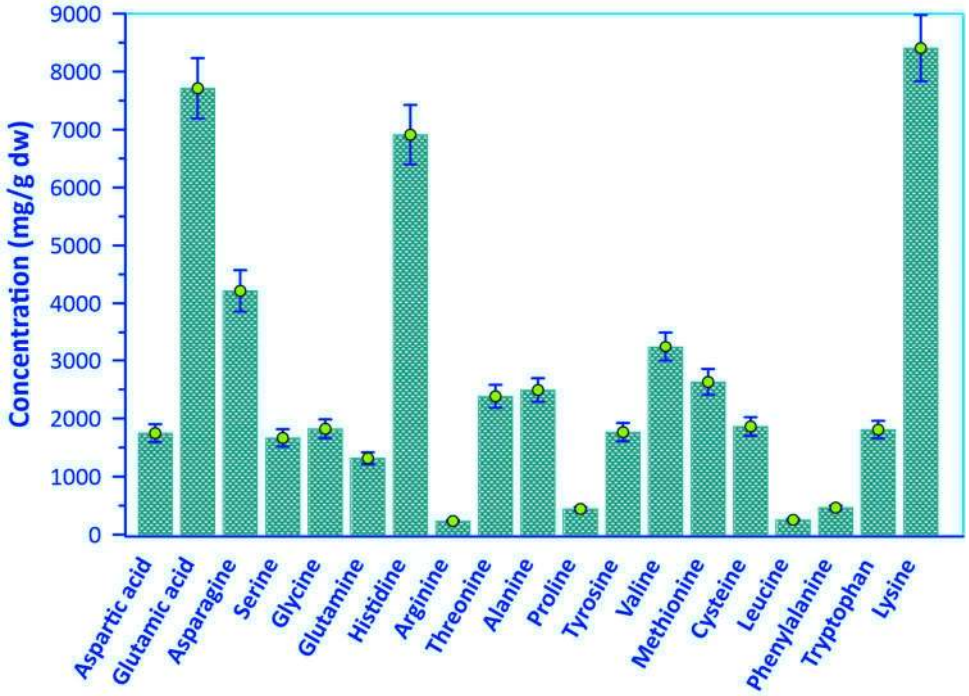

Figure 4.1. Shows the amino acid content of Bitter Tomato.

The white garden egg variety sampled (Figure 4.2) from Nigeria was determined to have the second highest concentrations of the amino acids determined in this study as it had highest concentrations of seven (7) - leucine, arginine, proline, glutamine, valine, tryptophan, and glutamic acid, out of the 19 amino acids assessed. It had the second highest concentration of tyrosine, alanine, asparagine, and lysine. The white garden egg also had average values of serine, cysteine and glycine. Comparatively though, it had low concentrations of threonine, aspartic acid, methionine and histidine and the lowest concentration of phenylalanine based on this study.

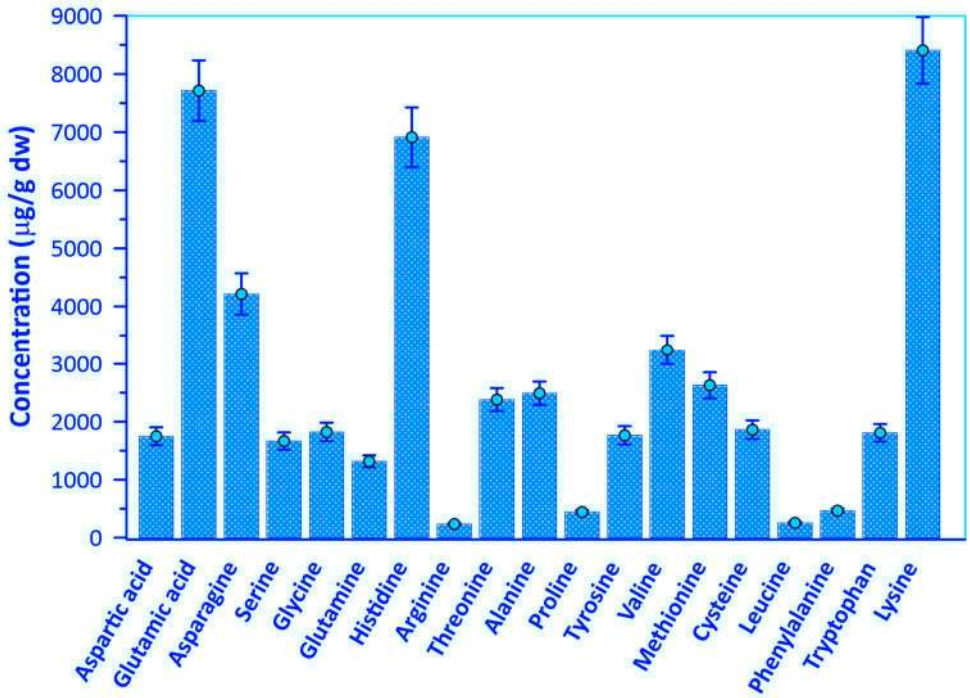

Figure 4.2. Shows the amino acid content of White Garden Egg.

Our findings showed the dark eggplant (Figure 4.3) variety sampled from Turkey to have the third highest concentration in terms of amino acids. It was determined to have the highest values of two (2) - threonine and histidine, amino acids. It had the second highest of seven - leucine, arginine, proline, serine, cysteine, phenylalanine, aspartic acid, and methionine, amino acids. While it had average values of valine, low values of glutamine, tyrosine, alanine, glycine, asparagine, and glutamic acid and the lowest of tryptophan, and lysine.

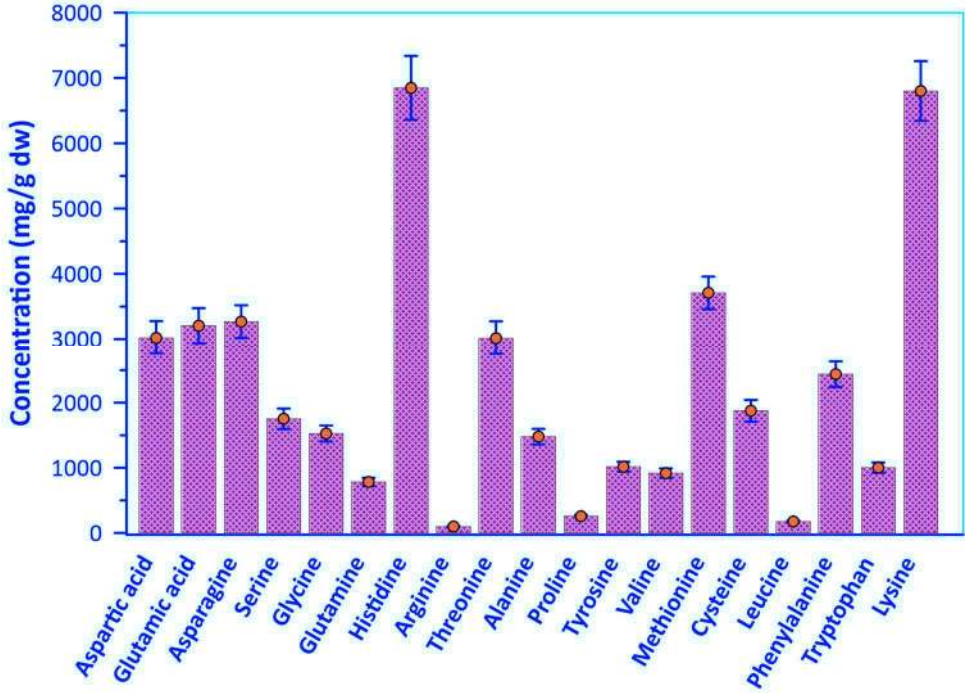

Figure 4.3. Shows the amino acid content of Dark Eggplant.

The bitter apple variety (Figure 4.4) sampled from Nigeria ranked fourth out of the five varieties in terms of concentration of the total amino acids assessed in this study. It had the second highest concentration of valine, glycine, and average values of arginine, glutamine, tyrosine, alanine, methionine, aspartic acid, asparagine, and glutamic acid. It was also determined to have low values of leucine, tryptophan, cysteine, phenylalanine, and lysine. Comparatively though, it had the lowest concentrations of proline, serine, threonine, and histidine determined in this study.

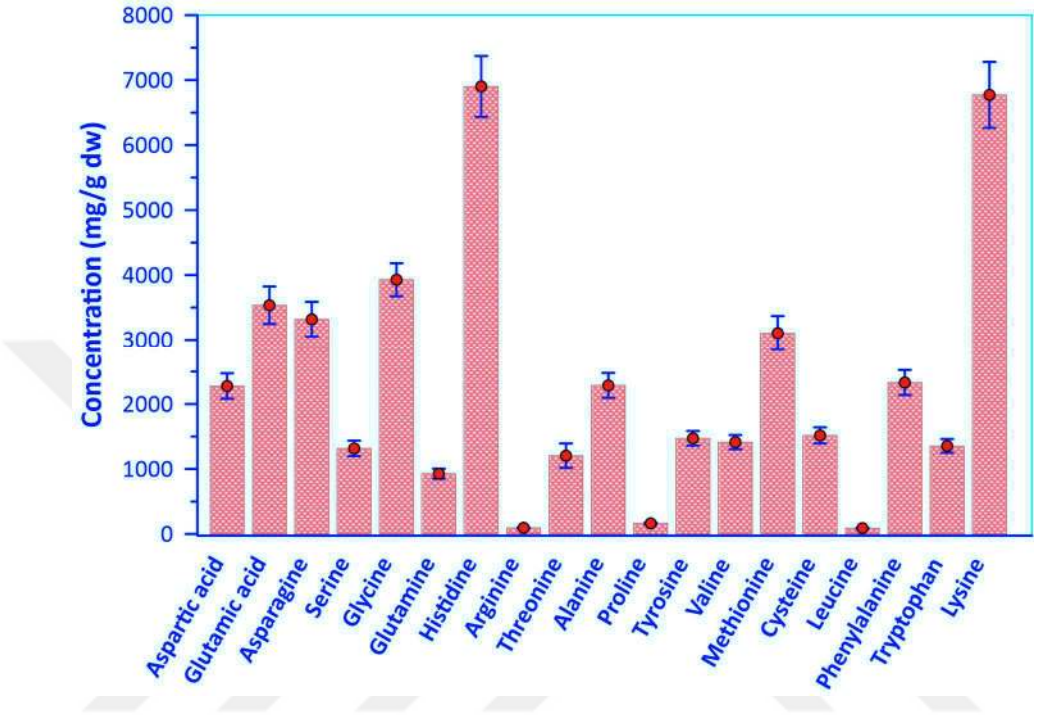

Figure 4.4. Shows the amino acid content of Bitter Apple.

Comparatively, the light eggplant variety (Figure 4.5) sampled from Turkey was determined to have the least concentrations of the amino acids determined in this study. While it was determined to have the highest concentrations of two (2) – phenylalanine and lysine, out of the 19 amino acids determined. Conversely, it had the second highest concentrations of histidine and average amounts of four (4) amino acids – leucine, proline, tryptophan, and threonine. However, it had low concentrations of arginine, valine, and serine, and the lowest of nine (9) - glutamine, cysteine, tyrosine, alanine, aspartic acid, glycine, methionine, asparagine, and glutamic acid, out of the nineteen amino acids assessed in this study.

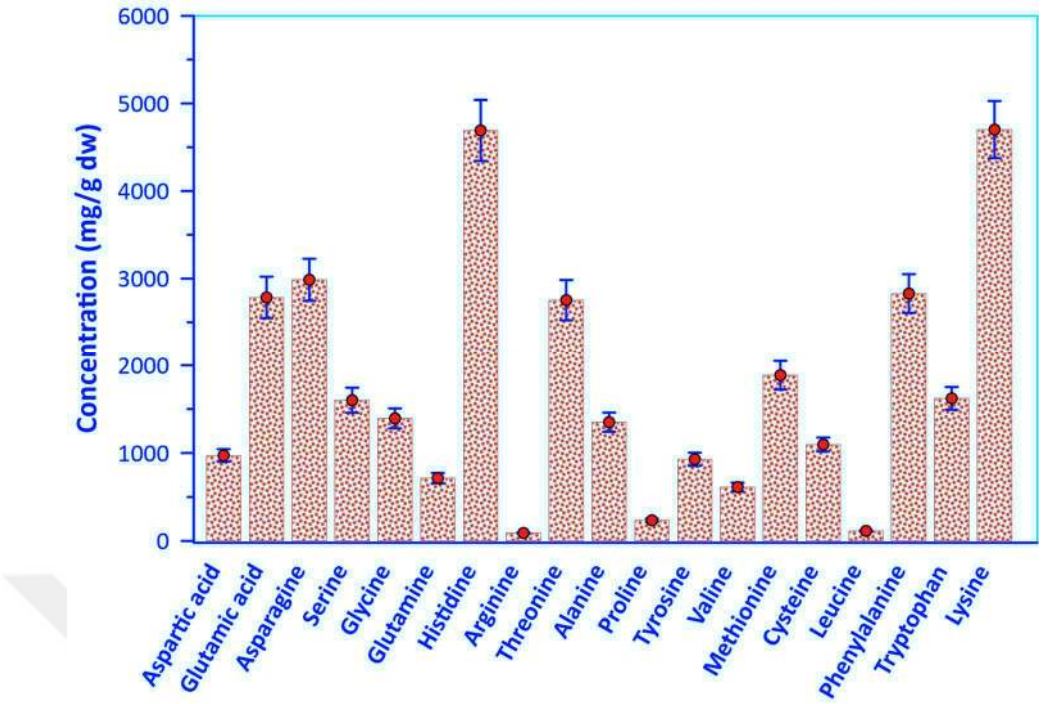

Figure 4.5. Shows the amino acid content of Light Eggplant.

Furthermore, our findings determined the concentration of the 19 amino acids in all the varieties to range from 23.5 – 33625.4 µg\g DW, with the lowest concentration determined for leucine while the highest was for lysine showing a significantly wide variability. The variations in the concentrations of the individual amino acids were in the following increasing order (Table 4.1) – Serıne < Asparagine and Tryptophan < Glutamine < Cysteine < Alanine < Threonine < Tyrosine and Arginine < Methionine and Proline < Glutamic acid < Histidine < Glycine < Aspartic acid < Lysine < Phenylalanine < Valine < Leucine. The narrowest range variation was in the values of serine determined across the varieties with a 1.4 fold difference. In contrast, the widest varietal variation in the concentrations was observed in the values of leucine with a 10.9 fold difference. This shows a significant varietal disparity in the values of amino acids detected in the different varieties of eggplant fruits sampled for this study.

Based on the findings in this study, the most abundant amino acid determined was lysine with values of up to 33625.4 µg\g DW in the white garden egg. The second most abundant is histidine at 20545.69 µg\g DW in dark eggplant. These are both nutritionally essential amino acids. Appreciable levels of amino acids such as glutamic acid, methionine, asparagine, glycine and aspartic acid were also determined across the varieties. Generally, the profile of the amino acids determined across the varieties are atypical of plant proteins where concentrations of methionine and lysine are characteristically low in comparison to animal proteins [378]. This is consistent with previous studies by Ogunka-Nnoka and Ekrika (2018) [385]; and Imo *et al.* (2019) [379] who observed a similar pattern of a predominance of glutamic acid, lysine and histidine in eggplants. In contrast, the least abundant concentrations were determined for arginine, proline, and leucine with a significantly low value of 23.5 µg\g for leucine. This further reiterates the broad varietal divergence in the amino acid profiles of the different varieties of eggplants sampled from Turkey and Nigeria for this study.

However, protein quality of any food is directly proportional to its nutritionally essential amino acid profile [386]. Foods are graded in terms of concentrations of a nutrient to be a 'good source' if they contribute a minimum of 10% and up to 19% of its recommended daily value (DV) per serving, while to be considered an 'excellent source' requires a provision of ≥ 20% of the DV per serving [387]. All the nutritionally essential amino acids (leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, histidine and arginine) were detected in all the varieties sampled for this study. The contribution of each of the varieties in terms of daily dietary adequacy (Table 4.2) of the essential amino acids were calculated based on the recommended daily allowance (RDA) reported by FAO/WHO/UNU [376] for a 70 kg adult per 100g (DW). Based on this, it was determined that all of the varieties can contribute to dietary adequacy in terms of histidine, lysine, methionine, phenylalanine, threonine and tryptophan, and may even provide an excess. Contrarily, while the white garden egg and bitter apple varieties had adequate amounts of valine, the dark eggplant, light eggplant and bitter tomato varieties had nutritionally insignificant values. Likewise, all the varieties are nutritionally low in leucine and, consequently cannot contribute to its dietary adequacy.

4.2. Vitamins

4.2.1. Vitamin A

The results of our study showed all the varieties of eggplant fruits contain different amounts of vitamin A ranging from 0.13 – 3.63 µg/g DW with a 27.1 fold difference. The vitamin A contents for white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined to be (Table 4.3) 2.33±0.03, 3.63±0.10, 0.63±0.04, 0.13±0.01 and 2.65±0.45 µg/g DW, respectively.

Comparing and contrasting between the five varieties, the amounts of vitamin A were determined in the following increasing order- light eggplant < dark eggplant < white garden egg < bitter tomato < bitter apple. Between the Turkish samples, the dark eggplant had 4.9 folds the amount of vitamin A in the light eggplant variety. While amongst the Nigerian varieties, the bitter apple had the highest concentration, 1.4 and 1.6 folds the amounts determined in bitter tomato and white garden egg varieties, respectively. While the bitter tomato variety had a higher concentration of 1.1 folds, the amounts in the white garden egg variety. Comparing the vitamin A values determined in the Turkish varieties to their Nigerian counterparts showed a vast difference. All the Nigerian varieties had a higher concentration of at least four folds the amounts determined in the Turkish varieties. Our results are consistent with the range of values of vitamin A in eggplant fruits reported in the literature by the USDA [377] for eggplant fruits as 1 µg/100g while Singh (2006) [388] reported a value of 0.2 µg /g and a value of 0.7µg/g was mentioned by Horna *et al*., (2007) [389]. Conversely, lower vitamin A contents of dried eggplants ranging from 1.88 -4.73 µg/100g (0.0188 – 0.0473 µg/g) DW were also reported by Çağlarırmak and Hepçimen [390]. The differences are due to the varieties selected for the study.

Our findings for vitamin A in each of the five varieties were also compared to average RDI of vitamin A for adults (0.8 mg/day) [377] (Table 4.4). The percentage of the RDI in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined to be 29.1, 45.4, 7.9, 1.7, and 33.1 % per 100g DW, respectively. Based on this, the white garden egg, bitter apple and bitter tomato varieties may be regarded as rich sources of vitamin A.

4.2.2. Vitamin E

All the eggplant fruit varieties used in this study had detectable levels of vitamin E ranging from 3.63 – 39.14 µg/g DW, depicting a 10.8-fold range difference. Our findings showed the vitamin E content of white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato to be (Table 3.3) 10.98±0.11, 3.63±0.17, 39.14±1.71, 4.80±0.37 and 34.49±1.57 µg/g DW, respectively.

Comparing between the concentrations of vitamin E in the five varieties were determined in the following increasing order- bitter apple < light eggplant < white garden egg < bitter apple < dark eggplant. A wide variation was observed in amounts of vitamin E amongst the Turkish varieties. The dark eggplant had a concentration that was 8.2 folds the amounts in the light eggplant. Comparatively, between the Nigerian varieties, a narrower difference was observed between vitamin E values in white garden egg and bitter apple varieties. Contrastingly, the bitter tomato had values 3.1 and 9.5 folds the values determined in the white garden egg and bitter apple varieties, respectively. These variations were also observed between the values in Nigerian (3.63 – 34.49 µg/g) and Turkish (4.80 – 39.14 µg/g) varieties. The Turkish dark eggplant had a higher concentration than all the Nigerian varieties, respectively. Contrastingly, with the exception of the bitter apple variety, the Nigerian varieties had a higher concentration than the light eggplant. The differences observed are due to varietal variations and origins of the samples. These have been previously reported to affect its concentrations in fruits [391,392]. Our findings corroborate with that of Fraikue *et al*. (2016) [339] who mentioned the amount of vitamin E in eggplant fruits to range from 0.2 - 0.3 mg/100g (2.0 - 3.0 µg /g). In contrast, Lagunovschi-Luchian *et al*. (2019) [393] reported a lower vitamin E content of 0.3µg /g in eggplant fruits, while Imo *et al.* [379]. reported higher values of 22.415 mg/100g DW.

Correlating the average RDI (15 mg/day) [377] to the values of vitamin E determined in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato to be 7.32, 2.42, 26.09, 3.20 and 22.99% per µg/100g DW, respectively, and may be considered for dietary adequacy accordingly.

4.2.3. Beta Carotene

All the eggplant fruit varieties sampled had detectable levels of beta-carotene ranging from 1.87 – 30.47 µg/g DW with a 16.3 fold difference. The white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato each contained beta-carotene determined to be (Table 3.3) 4.29 ±0.04, 5.13±0.05, 30.47±0.38, 1.87±0.05 and 21.40±0.37 µg/g DW, respectively.

Based on the results, the amounts of vitamin A in the five varieties were determined in the following increasing order- light eggplant < white garden egg < bitter tomato < bitter apple < dark eggplant. Comparing the amounts of beta-carotene determined, between the Turkish varieties, the dark eggplant variety had 16.3 folds the amounts in the light eggplant variety. Between the Nigerian varieties, correlating amounts in bitter tomato to those in white garden egg and bitter apple, it had 5.0 and 4.1 folds the amounts, respectively. Correlating amounts in the Turkish varieties to the Nigerian varieties, a wide disparity was observed. The amounts of beta-carotene in dark eggplant (3.534 µg/g) was higher than amounts detected in all Nigerian white garden egg, bitter apple and bitter tomato varieties, by 7.1, 6.0, and 1.4 folds, respectively. Contrastingly, amounts in the Turkish light eggplant was lower than amounts detected in all the Nigerian varieties. Our findings are consistent with a previous study by Msogoya *et al*. (2014) [394] who reported beta-carotene values of eggplant ranging from 7.09 – 8.14 µg/g. EL-Qudah (2009) [395] reported the

concentration of β-carotene to be 0.18 µ g g^{-1} in eggplant fruits, which is lower than values observed in this study. These variations are attributable to varietal differences and geographical origins of the samples used in this study that have previously been correlated to levels of beta-carotene in food samples [396].

4.2.4. Lycopene

All varieties of eggplant fruit used in this study had a measure of lycopene that ranged from 1.52 – 6.79 µ g/g DW showing a 4.5 fold range disparity. The white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato varieties each contained (Table 3.3) 6.79±0.11, 4.33±0.05, 1.78±0.08, 1.52±0.01 and 3.20±0.36 µ g/g DW of lycopene, respectively.

The amounts of lycopene were determined in the following increasing order- light eggplant < dark eggplant < bitter tomato < bitter apple<white garden egg. Comparing within the Turkish varieties, the dark eggplant variety had a higher concentration of lycopene than the light eggplant. Whilst between the Nigerian varieties, the white garden egg had the highest followed by the bitter apple variety and both had 2.1 and 1.4 folds the amounts in the bitter tomato variety, respectively. Comparing and contrasting between the Turkish and Nigerian varieties, the Nigerian varieties were all determined to have a higher concentration of lycopene. Lycopene was below detection in a study conducted on eggplant fruits by Kim *et al.* (2007) [397] and Mbondo *et al.,* (2018) [398]. While Arkoub-Djermoune *et al* (2016) [399] reported higher lycopene values of 12.84 ± 1.66 mg/100 g DW in eggplant fruit. The literature points to significant differences in lycopene content of eggplant and similar fruits [400]. The differences observed are thus, attributed to the varietal differences of eggplants sampled for this study.

Relating the RDI values of lycopene (8 mg/day) to the values determined in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato varieties each contained (Table 3.4) 8.5, 5.4, 2.2, 1.9 and 4.0 % of RDI per 100g DW, respectively. Based on this, the varieties selected in this study are not rich dietary sources of lycopene.

4.2.5. Vitamin C

Based on our findings each of the varieties had detectable levels of vitamin C ranging from 357.4 – 1136.2 µ g/g DW with a 3.2 fold range variation. The individual amounts of vitamin C in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined to be (Table 3.3) 1136.19±4.24, 579.21±3.39, 476.121±3.07, 421.81±0.34 and 357.35±1.73 µ g/g DW, respectively.

Comparing between values of vitamin C it was determined to be in following increasing order – bitter tomato < light eggplant < dark eggplant < bitter apple < white garden egg. Within the Turkish varieties, the dark eggplant had a higher amount of vitamin C than the light eggplant variety. Comparing and contrasting between the Nigerian varieties, the white garden egg variety had the highest amount of vitamin C that was 2.0 and 3.2 folds the amounts in bitter apple and bitter tomato varieties, respectively.

Correlating amounts of vitamin C in the Turkish varieties to those in the Nigerian varieties. The white garden egg and bitter apple from Nigeria were determined to be richer in vitamin C than both Turkish varieties. However, both Turkish varieties had higher amounts of vitamin C than the Nigerian bitter tomato variety. The variations observed in this study are attributable to varietal differences of the eggplants sampled. Msogoya *et al*. [394] and Imo *et al*. [379] reported vitamin C values in eggplant to be 7.67 – 8.98 mg/100g (76.7 – 89.8 µ g/g) and 21.460mg/100g (214.6 µ g/g)

DW, respectively. Ayaz *et al.* (2015) reported the vitamin C concentration in different eggplant cultivars to have a range of 7.69–11.74 mg/100 g (76.9 - 117.4 µg /g) which is consistent with our findings [381]. Our findings appear to be higher than the range of literature values of vitamin C in different eggplant fruits.

Comparing the RDA to the amounts of vitamin C determined (Table 3.4) in the five varieties (µg/100g): white garden egg – 246.1 %, bitter apple – 128.7 %, dark eggplant - 105.8 %, light eggplant – 93.7 % and bitter tomato – 79.4 % used in this study. This shows that the eggplant varieties used in this study can serve as excellent sources of vitamin C.

4.2.6. Vitamin B_1

The findings show each of the five varieties of eggplant fruit used in this study to contain varying levels of vitamin B_1 (Thiamine) ranging from 11.04 – 95.56 µg/g DW depicting an 8.7 fold difference. The amounts of vitamin B1 in the different varieties of eggplant fruit were determined to be (Table 4.3) 95.56± 0.96, 53.86±0.44, 11.04±0.11, 19.13±0.18, and 45.59±0.19 µg/g DW in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato, respectively.

Comparing and contrasting between the amounts of vitamin B_1 in the varieties of eggplant from Turkey and Nigeria showed them to be in the following increasing order- dark eggplant < light eggplant < bitter tomato < bitter apple< white garden egg. Within the Turkish varieties, the light eggplant variety had 1.7 folds the amount of vitamin B_1 determined in the dark eggplant. While between the Nigerian varieties, the white garden egg had the highest values, 1.8 and 2.1 folds the amounts in bitter apple and bitter tomato who had a narrower difference, respectively. Comparatively, the Nigerian varieties appear to be richer vitamin B_1 content than the Turkish varieties, as both Turkish varieties had significantly lower concentrations than all the Nigerian varieties. The white garden egg variety had 8.7 and 5.0 folds the amounts of vitamin B_1 in the Turkish dark and light eggplant varieties, respectively. Conversely, a 4.9 and 2.8 fold difference was observed between bitter apple variety sampled from Nigeria and the dark and light eggplant fruits, respectively. While the bitter tomato variety contrasted in values of vitamin B_1 to the dark and light eggplant varieties by 4.1 and 2.4 folds, respectively. A study by Imo *et al.* [379] reported lower vitamin B_1 values of 0.092 mg/100g (0.92 µg/g) DW in eggplant. Another study reported thiamine to range from 0.16-0.29 mg/100g (1.6 – 2.9 µg/g) DW in eggplants [390] which are also lower than our findings. The disparities observed in vitamin B_1 concentrations between the different varieties of eggplant fruits are due to varietal variations and origins.

Correlating the RDI (0.9 mg/day) (Table 4.4) to the amounts of vitamin B_1 determined in white garden egg, bitter apple, dark eggplant, light eggplant an bitter tomato to be 1061.8, 598.4, 122.6, 212.6 and 506.5 % per µg /100g DW. Based on our findings all the varieties of eggplant fruits used in this study could serve as rich sources of thiamine B_1.

4.2.7. Vitamin B_2

Vitamin B_2 (Riboflavin) ranging from 1.9 – 5.4 µg/g DW with a 2.9 fold range difference was determined in all the varieties sampled for this study. Our findings revealed the individual amounts of vitamin B_2 in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato determined to be (Table 3.3) 5.44±0.03, 3.80±0.13, 4.74±0.04, 5.18±0.01 and 1.90±0.01 µg/g DW, respectively.

The concentration of vitamin B_2 determined in all the varieties were found to be in the following increasing order – bitter tomato < bitter apple < dark eggplant < light eggplant < white

garden egg. Comparing values determined in the Turkish varieties; the light eggplant variety had higher amounts than the dark eggplant variety. While within the Nigerian varieties, the white garden egg had 1.4 and 2.9 folds the amounts in the bitter apple and bitter tomato varieties, respectively. While the values detected in the bitter apple variety were 2.0 folds the amounts in the bitter tomato variety. Correlating values of vitamin B_2 in the varieties sourced from the two different countries, both Turkish varieties had higher amounts than the Nigerian bitter apple and bitter tomato varieties. Contrastingly, the white garden egg had a higher concentration than both Turkish varieties. Our findings corroborates with the range of literature values of riboflavin determined by Imo *et al.* [379] who reported values of 0.215 mg/100g (2.15) DW in eggplants while Çağlarırmak and Hepçimen (2013) reported values of 0.39 mg/100g (3.9 µg/g) DW [390]. These discrepancies may be due to variations in geographical origins and variety of the eggplant fruits sampled for this study.

In relation to RDI (1 mg/day) values (Table 3.4) the percentage of vitamin B_2 the white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato contributed per µg/100g DW were determined to be 54.4, 38.0, 47.4, 51.8 and 19.0 %, respectively. Based on this, each of the varieties of eggplant sampled for this study appear to be rich sources of vitamin B_2.

4.2.8. Vitamin B_3

The results of this study showed all the varieties had detectable levels of vitamin B3 (Nicotinamide) ranging from 83.28-265.87µg/g DW with a 3.2 fold range difference. The individual amounts of vitamin B_3 in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined to be (Table 4.3) 265.87±0.34, 96.21±0.73, 83.28±0.23, 104.02±0.52 and 119.12±4.34 µg/g DW, respectively.

The concentrations of vitamin B_3 determined in all the varieties were found to be in the following increasing order – dark eggplant < bitter apple < light eggplant < bitter tomato < white garden egg. Between the Turkish varieties, the light eggplant had higher amounts of vitamin B_3 than the dark eggplant variety. Whilst for the Nigerian varieties, the white garden egg had the highest amounts of vitamin B_3, 2.2 and 2.8 folds the amounts determined in the bitter tomato and bitter apple varieties, respectively. Correlating the values in the Turkish varieties to their Nigerian counterparts, the latter appeared to be richer in vitamin B_3. All the Nigerian varieties had higher amounts of vitamin B_3 than the dark eggplant and light eggplant, except the light eggplant, which had higher amounts than the Nigerian bitter apple variety. Souci *et al.,* [401] and Imo *et al.,* (2019) [379] reported lower values of 0.60 mg / 100 g (6.0 µg/g) and 0.460 mg/100g (4.6 µg/g) DW in eggplant fruits, respectively. The observed variations are due to the varieties and the geographical origins of eggplant fruits used in the study.

In terms of RDI (15 mg/day), a comparison of values of vitamin B_3 determined in the varieties sampled in this study (Table 3.4) revealed the white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato varieties to contain 177.3, 64.1, 55.5, 69.3, and 79.4% per µg /100g DW. Consequently, all the varieties may serve as rich sources of vitamin B3.

4.2.9. Vitamin B_5

Based on our findings, all the varieties used in this study contained vitamin B_5 (Pantothenate) that ranged from 20.2 – 2543.0 µg/g DW with a 126.2 fold range difference. The amounts of vitamin B_5 were determined (Table 3.3) to be 20.16±0.91, 36.36±0.37, 2543.02±27.01, 1380.71±1.79, and 685.74±3.66 µg/g DW in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato varieties, respectively.

The concentrations of vitamin B_5 determined in all the varieties were found to be in the following increasing order – white garden egg < bitter apple < bitter tomato < light eggplant < dark eggplant. Between the Turkish varieties, the dark eggplant had 1.8 times the amount of vitamin B_5 determined in the light eggplant variety. While between the Nigerian varieties, the bitter tomato variety appeared to have the highest amounts of vitamin B_5, 34.0 and 18.9 folds the amounts determined in white garden egg and bitter apple varieties, respectively. In contrast, a narrower difference of 1.8-folds was determined between bitter apple and white garden egg varieties. Comparatively, the Turkish varieties had significantly higher amounts of vitamin B_5 than the Nigerian varieties. The light eggplant had 68.5, 38.0 and 2.0 folds the amounts determined in white garden egg, bitter apple and bitter tomato varieties from Nigeria, respectively. Wider variations of 126.2, 70.0 and 3.7 folds were observed between the amounts in the Turkish dark eggplant variety and white garden egg, bitter apple and bitter tomato varieties, respectively. Our findings corroborates with values reported by Nachar *et al.,* (2019) [400,402] who determined a pantothenic acid value of 7.3 mg/100g (73 µg/g) in eggplant. Contrastingly, Souci *et al.,*[401] and Imo *et al.* [379] reported lower values of 0.23 mg/100g (2.3 µg/g) and 0.035mg/100g (0.35 µg/g) DW in eggplants, respectively. The disparities observed are attributable to varietal differences of the eggplants sampled for this study.

Based on our findings, the percentages of RDI (4 mg/day) of vitamin B_5 in the different varieties of eggplant fruits were calculated (Table 3.4) per µg/100g DW – white garden egg (50.4 %), bitter apple (90.89 %), dark eggplant (6357.5), light eggplant (3451.8%) and bitter tomato (1714.3%). Based on this, all the varieties sampled are excellent dietary sources of vitamin B_5.

4.2.10. Vitamin B_6

Each of the eggplant varieties used in this study had measurable amounts of vitamin B_6 (Pyridoxine) ranging from 141.18- 347.94 µg/g DW depicting a 2.5 fold range difference. The amounts of vitamin B_6 in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined to be (Table 3.3) 347.94±2.06, 225.71±2.91, 127.33±0.86, 202.44±1.92, and 141.18±1.38 µg/g DW, respectively.

The varieties were determined to have concentrations of vitamin B_6 in the following increasing order –dark eggplant< bitter tomato < light eggplant < bitter apple< white garden egg. Conversely, between the Turkish varieties, the light eggplant had higher amounts of vitamin B_6 than the dark eggplant. Between the Nigerian varieties, the white garden egg had the highest amount that was 1.5 and 2.5 folds the amounts in the bitter apple and bitter tomato varieties, respectively. Correlating the values detected in the Turkish varieties to those in the Nigerian varieties, no particular trend was observed. The dark eggplant had lower amounts of vitamin B_6 than all the Nigerian varieties. In contrast, the light eggplant variety had higher amounts than the bitter tomato variety, and lower amounts than white garden egg and bitter apple varieties. A study by Imo *et al.* [379] determined vitamin B_6 concentrations to be 0.200 mg/100g (2.0 µg/g) DW in eggplants. Çağlarırmak and Hepçimen (2013) reported values of 0.39 mg/100g (3.9 µg/g) DW [390]. Our findings are higher than the values reported in the literature. The variations observed may be due to varietal differences of the samples selected for this study.

The amounts of vitamin B_6 were correlated to RDI (1.4 mg/day) values (Table 3.4) and the percentages of the daily value they contained determined. The white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato each contained 2485.3, 1612.2, 909.5, 1446.0 and 1008.4% (µg/100g) DW. Based on this, all the varieties used in this study appear to be excellent sources of vitamin B_6 and can contribute to its dietary adequacy.

4.2.11. Vitamin B_9

With the exceptıon of the dark eggplant variety, all the varieties had detectable levels of vitamin B_9 that ranged from 26.857 -102.132 µg/g DW showing a 3.8 fold range difference. Our findings revealed the individual amounts of vitamin B_9 in white garden egg, bitter apple, light eggplant and bitter tomato determined to be (Table 3.3) 55.87±0.49, 26.86±0.31, 78.58±0.89 and 102.13±1.13 µg/g DW, respectively.

The varieties were determined to have concentrations of vitamin B_9 in the following increasing order –bitter apple < white garden egg <light eggplant< bitter tomato. Conversely, between the Turkish varieties, while the light eggplant had appreciable amounts of vitamin B_9, it was below detection limit in the dark eggplant. Comparing between the Nigerian varieties, the bitter tomato had the highest amount 1.8 and 3.8 folds the amounts detected in the white garden egg and bitter apple varieties, respectively. Comparing values detected in the Turkish light eggplant variety to the Nigerian varieties. While the light eggplant had lower values than the bitter tomato, it however had 1.4 and 2.9 folds the values detected in white garden egg and bitter apple varieties, respectively. Çağlarırmak and Hepçimen (2013) [390] reported values of folate to be 30.04 and 29.28 µg/100g (0.3 and 0.29 µg/g) in dried eggplants while Imo *et al*. [379] reported it as 0.061 mg/100g (0.61 µg/g). Our values are higher than the reported literature values. The variations in values observed are due to differences in varieties of eggplants used in this study.

Correlating the values of vitamin B_9 determined in the eggplant samples to the RDI (0.5 mg/day). It was determined (Table 3.4) that white garden egg, bitter apple, light eggplant and bitter tomato contained 1117.5, 537.1, 1571.7 and 2042.6 % (µg/100g) DW. Therefore, all the varieties used in this study with the exception of the dark eggplant variety, are rich dietary sources of vitamin B_9.

4.2.12. Vitamin B_{12}

Our findings revealed detectable levels of vitamin B_{12} in all the varieties ranging from 1.1 – 9.8 µg/g DW with a 9.0 fold range variation. The amounts of vitamin B_{12} in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined to be (Table 3.3) 9.84±0.03, 3.88±0.03, 1.10±0.09, 2.02±0.01 and 3.79±0.01 µg/g DW, respectively.

The varieties were determined to have concentrations of vitamin B_{12} in the following increasing order –dark eggplant< light eggplant < bitter tomato < bitter apple< white garden egg. Comparing the values of vitamin B_{12} detected between the varieties, the Turkish light eggplant variety had double the amounts in the dark eggplant. Values determined in the Nigerian varieties, showed the white garden egg to have the highest amounts that was 2.5 and 2.6 folds those determined in bitter apple and bitter tomato varieties, which had similar values. Correlating amounts determined in the Turkish (1.10 – 2.02 µg/g) varieties to the Nigerian (3.79 – 3.88 µg/g) varieties; the Nigerian varieties had higher amounts of more than two- folds the amounts detected in light and dark eggplant varieties, respectively. Our findings are consistent with the values of vitamin B_{12} reported by Imo *et al.* (2019) [379] of 0.365 mg/100g (3.65 µg/g) in eggplants. The observed differences are attributable to varietal differences of eggplants sampled for this study.

Based on our findings, the percentages of vitamin B_{12} in relation to the RDA (0.2 mg/day) (Table 4.4) were calculated for white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato determined to be 492.1, 194.0, 54.8, 100.8 and 189.7 % (µg/100g) DW. Consequently, they can be included in the diet as excellent sources of vitamin B_{12}.

In summary, based on the findings in this study (Table 4.2) the white garden egg variety (Figure 4.6) sampled from Nigeria appears to be the richest in terms of the concentrations of the individual vitamins. It had the highest concentration of seven (7) – lycopene, vitamin C, vitamin B_1, vitamin B_2, vitamin B_3, vitamin B_6 and vitamin B_{12} out of the 12 vitamins assessed. Likewise, it had the third highest concentrations of vitamins A, E and vitamin B_9 while in contrast; it had low concentrations of beta-carotene and the lowest concentration of vitamin B_5.

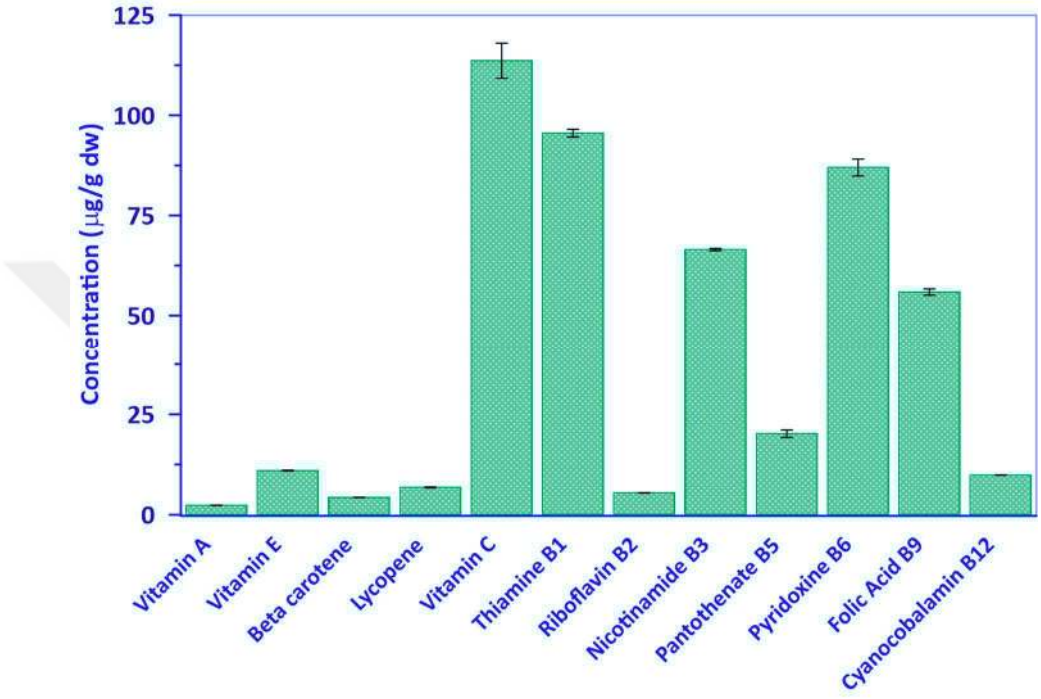

Figure 4.6. Shows the vitamin content of White Garden Egg.

Our findings revealed the bitter tomato variety (Figure 4.7.) sampled from Nigeria to be the second richest in terms of vitamins. It was determined to have the highest amounts of vitamin B_9 and the second highest of vitamin A, E, Beta-carotene, and vitamin B_3. Furthermore, it had average concentrations of lycopene vitamin B_1, vitamin B_5 and vitamin B_{12}. Conversely, it had low levels of vitamin B_6 and the lowest levels of vitamin C and vitamin B_2.

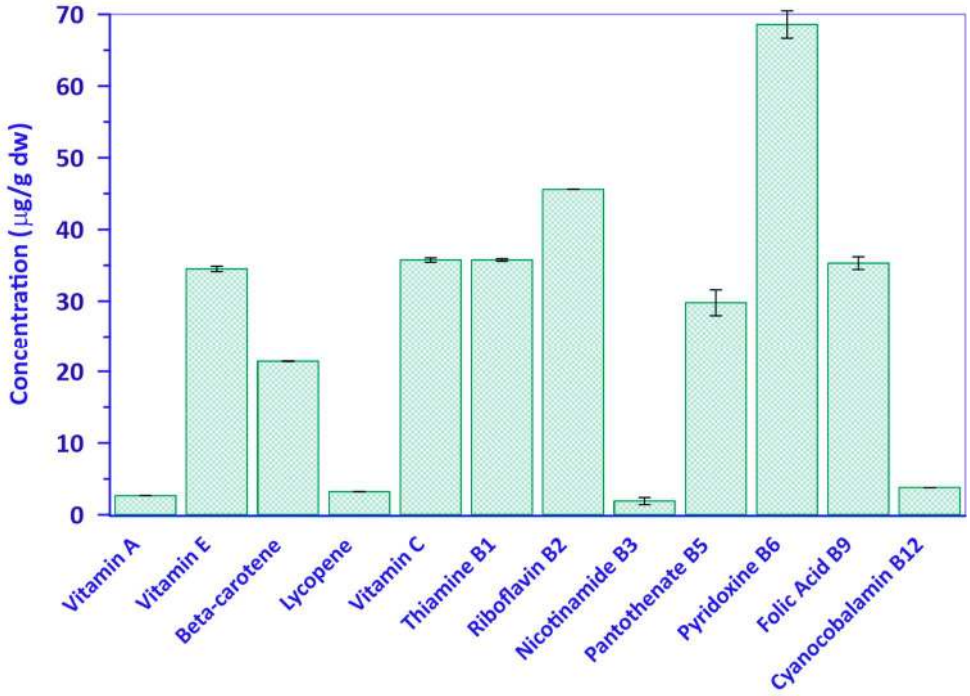

Figure 4.7. Shows the vitamin content of Bitter Tomato.

The third variety in terms of vitamin levels was determined to be the dark eggplant (Figure 4.8.) sampled from Turkey. It was revealed to have the highest concentration of vitamin E, Beta-carotene, and vitamin B_5. It also had average amounts vitamin C and vitamin B_2, and low amounts of vitamin A, lycopene and the lowest of vitamins B_1, B_3, B_6 and B_{12} while vitamin B_9 was below detection limits.

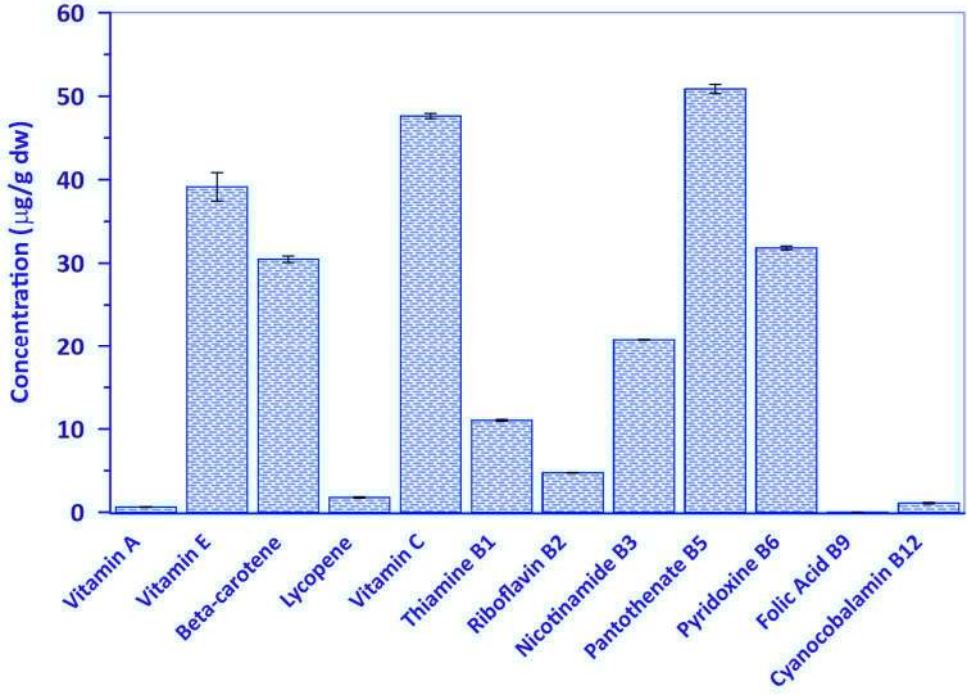

Figure 4.8. Shows the vitamin content of Dark Eggplant.

The bitter apple variety (Figure 4.9) sampled from Nigeria ranked fourth in terms of vitamins content. It had the highest concentrations of vitamin A, and second highest concentration of lycopene vitamin C, vitamin B_1 vitamin B_6 and vitamin B_{12}. It also had average values of beta-carotene, comparatively though, and low concentrations of vitamin B_2, vitamin B_3, vitamin B_5, and vitamin B_9 and the lowest amounts vitamin E.

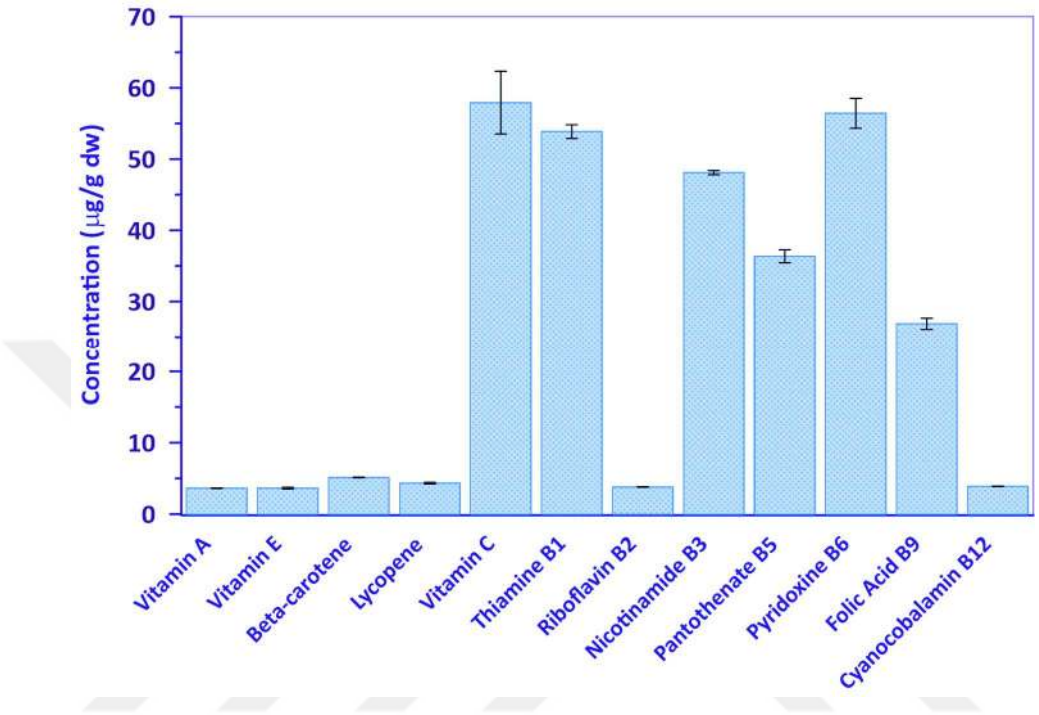

Figure 4.9. Shows the vitamin content of Bitter Apple.

Based on the findings in this study, the Turkish light eggplant variety (Figure 4.10) was determined to have the least concentrations of the vitamins determined in this study. It had the second highest levels of vitamin B_2, vitamin B_5 and vitamin B_9 and average amounts of vitamins B_3 and B_6. Contrastingly, it had low amounts of vitamin E, vitamin C, vitamin B_1 and vitamin B_{12} and the lowest amounts of vitamin A, beta-carotene, and lycopene.

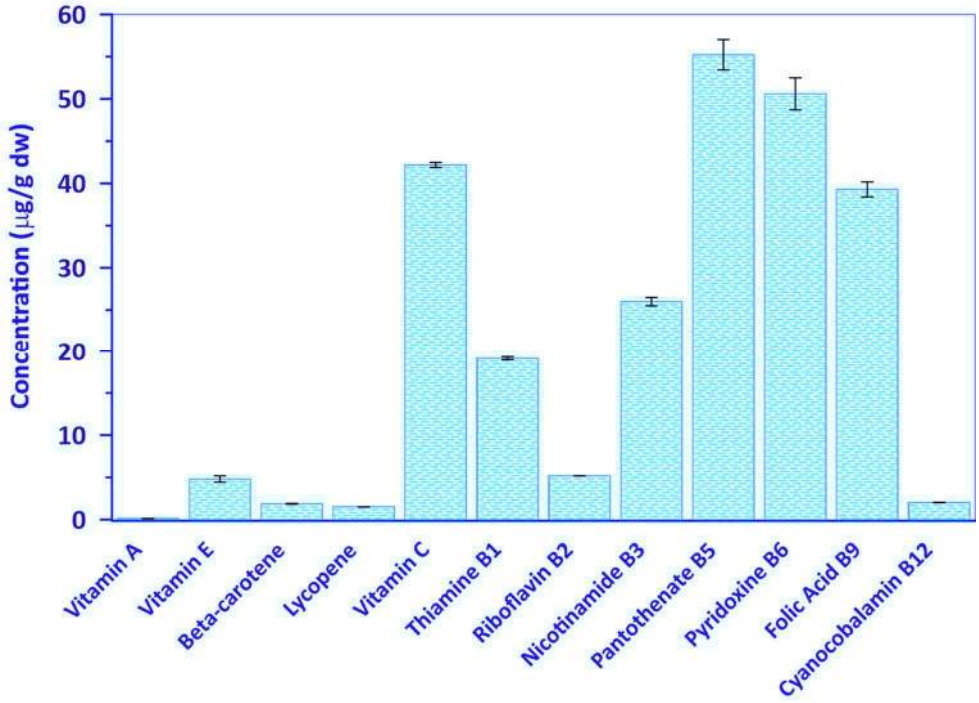

Figure 4.10. Shows the vitamin content of Light Eggplant.

Additionally, our findings determined the concentration of the all the vitamins in all the varieties to range from 0.13 – 2543.02 µg/g DW showing a significantly wide difference. The variations in the concentrations of the individual vitamins across the varieties were in the following increasing order (Table 5.2) – vitamin B_6 < vitamin B_2< vitamin C and B_3< vitamin B_9 < lycopene < vitamin B_1< vitamin B_{12}< vitamin E < Beta-carotene < vitamin A< vitamin B_5.

Across the varieties, the lowest vitamin concentration was determined for vitamin A in the Turkish light (0.13 µg/g DW) eggplant variety. Ironically, the most abundant values were determined for vitamin B_5, a water-soluble vitamin, in the dark eggplant, also a Turkish variety with values as high as 2543.02 µg\g DW while the white garden egg, sampled from Nigerian had as low as 20.16 µg/g DW for the same vitamin B_5. The narrowest varietal variation were in the values of vitamin B_6 determined across the varieties with a 2.5 fold difference. In contrast, the widest varietal variation in the concentrations was observed in the values of vitamin B_5 with a 126.2 fold difference. This illustrates the significant varietal difference in the values of vitamins determined in the different varieties of eggplants sampled for this study.

Table 4.2. Shows the increasing order of vitamin concentrations in the five varieties of eggplants.

Vitamin	Min-Max (µg/g)	Amounts in the different varieties from the lowest to the highest
A	0.13 – 3.63	light eggplant < Dark eggplant < White garden egg < Bitter tomato < Bitter apple
E	3.63– 39.14	Bitter apple < Light eggplant < White garden egg < Bitter Tomato < Dark eggplant
Beta carotene	1.87 – 30.47	Light eggplant < White garden egg < Bitter apple < Bitter tomato < Dark eggplant
Lycopene	1.52 – 6.79	Light eggplant < Dark eggplant < Bitter tomato < Bitter apple < White garden egg
C	357.35– 1136.19	Bitter tomato < Light eggplant < Dark eggplant < Bitter apple < White garden egg
B_1	11.04 – 95.56	Dark eggplant < Light eggplant < Bitter tomato < Bitter apple < White garden egg
B_2	1.90 – 5.44	Bitter tomato < Bitter apple < Dark eggplant < light eggplant < White garden egg
B_3	83.28 -265.87	Dark eggplant < Bitter apple < Light eggplant < Bitter tomato < White garden egg
B_5	20.16 – 2543.02	White garden egg < Bitter apple < Bitter Tomato < Light eggplant < Dark eggplant
B_6	141.18- 347.94	Dark eggplant < Bitter tomato < Light eggplant < Bitter apple < White garden egg
B_9	26.86 -102.13	Dark eggplant < Bitter apple < White garden egg < Light eggplant < Bitter tomato
B_{12}	1.10 – 9.84	Dark eggplant < Light eggplant < Bitter tomato < Bitter apple < White garden egg

According to the FDA [403] universal guidelines, if a particular food contains ≤ 5% of the recommended daily intake (RDI) of a nutrient per serving, usually estimated at 80 – 100 g for fruits, it is considered "low", while ≥ 20% of the RDI is considered "high". However, for vitamin A and C, the benchmark is ≥ 10% of RDI to be deemed relevant and 'healthy' or 'high' [404]. Based on these criteria, vitamin A levels for white garden egg, bitter apple and bitter tomato are high while those of dark and light eggplant are low. The vitamin E levels are high for dark eggplant and bitter tomato and low for the other varieties. Similarly, for the lycopene values, only the white garden egg variety had up to 8.5% whilst the rest were all low. Thus, based on the findings, the varieties sampled are generally "low" in the fat-soluble vitamins assessed in this study. Contrastingly, when compared to the RDI, all the varieties had levels of vitamin C, vitamin B_1, vitamin B_2, vitamin B_3, vitamin B_5, vitamin B_6, vitamin B_9 and vitamin B_{12} that were above the benchmark. Remarkably, the values determined for vitamin B_1, vitamin B_5, vitamin B_6 and vitamin B_9 were significantly high. This further reiterates the varietal difference in the vitamin profiles and nutritional value of the different varieties of eggplants sampled for this study.

4.3. Stress Biomarkers

4.3.1. 4-Hydroxynonenal

Each of the varieties used in this study had detectable levels of 4-hydroxynonenal which ranged from 3.035 - 4.564 µg/g with a 1.5 fold range variation. Our findings showed the 4-hydroxynonenal content investigated for white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato to be (Table 3.5 and Figure 4.11) 3.035±1.54, 3.435±0.31, 4.205±0.83, 4.038±1.63 and 4.564±0.21 µg/g (DW), respectively. The highest and lowest values were determined in the Nigerian white garden egg (3.035 µg/g) and bitter tomato (4.564 µg/g) varieties.

However, the amounts of 4-hydroxynonenal in the samples were in the following decreasing order - bitter tomato (4.564 µg/g) > dark eggplant (4.205 µg/g) > light eggplant (4.038 µg/g) >bitter apple (3.435 µg/g) > white garden egg (3.035 µg/g).

Between the eggplant fruit samples from Turkey, the dark eggplant had higher amounts of 4-hydroxynonenal than the light eggplant variety. While within the Nigerian samples, the white garden egg variety had 2.0 and 1.4 folds the amounts determined in the bitter apple and bitter tomato varieties. Comparing and contrasting between Turkish and Nigerian varieties, the white garden egg from Nigeria had higher amounts of 4-HNE than all the Turkish varieties. While in contrast, both Turkish varieties had higher or similar levels than the bitter apple and bitter tomato varieties. The variations observed are due to varietal differences and origins of the eggplants sampled for this study. Since 4-Hydroxynonenal is, a lipid peroxidation product formed due to oxidative stress [405], the levels also suggests that the varieties with lower 4-HNE values are better at withstanding stress.

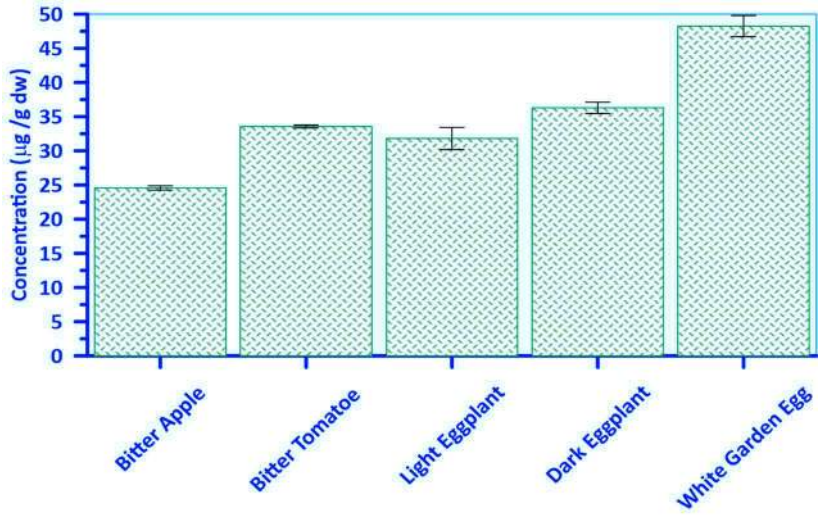

Figure 4.11. Shows a comparison of 4-HNE levels in the different varieties.

4.3.2. Malondialdehyde

Our findings revealed the presence of MDA in all the samples ranging from 1.50 -18.41µg/g DW with a 12.3 fold difference. The amounts of malondialdehyde in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined (Table 3.5 and Figure 4.12) to be 18.41±0.33, 1.5±0.16, 1.90±0.01, 3.62±0.04 and 3.16±0.15 µg/g DW, respectively. The highest

amount of MDA was detected in the white garden egg (18.41 µg/g) variety while the bitter apple (1.50 µg/g) variety had the lowest.

Based on the results, amongst the Turkish varieties, the light eggplant had 1.9 folds the amounts of MDA determined in the dark eggplant. Between the Nigerian varieties, the highest amount was detected in the white garden egg, which had 12.3 and 5.8 folds the amounts determined in bitter apple and bitter tomato varieties, respectively. Comparatively, the amounts in the bitter tomato variety were 2.1 folds the amounts determined in the bitter apple variety.

A comparison between the Turkish and Nigerian varieties showed no particular trend. The white garden egg had higher amounts, 9.7 and 5.1 folds the levels in the dark eggplant and light eggplant varieties, respectively. While the reverse was observed between the Turkish varieties and the bitter apple variety, as they both had higher amounts than the latter. In contrast, the Nigerian bitter tomato variety had lower amounts of MDA than the dark eggplant varieties while the Turkish light eggplant had a higher amount. The variations observed in amounts of MDA in all the varieties are attributable to the varietal differences which may contribute to its ability to resist stress or otherwise. Eggplant is generally a resilient plant, particularly to oxidative stress [406]. However, a study by Plazas *et al.,* (2019) reported a significant increase in amounts of malondialdehyde in stressed eggplants [357]. Based on this study, in terms of MDA content, all the varieties had low MDA levels and the bitter apple and dark eggplant varieties, which had the lowest, may be the most resilient to oxidative stress.

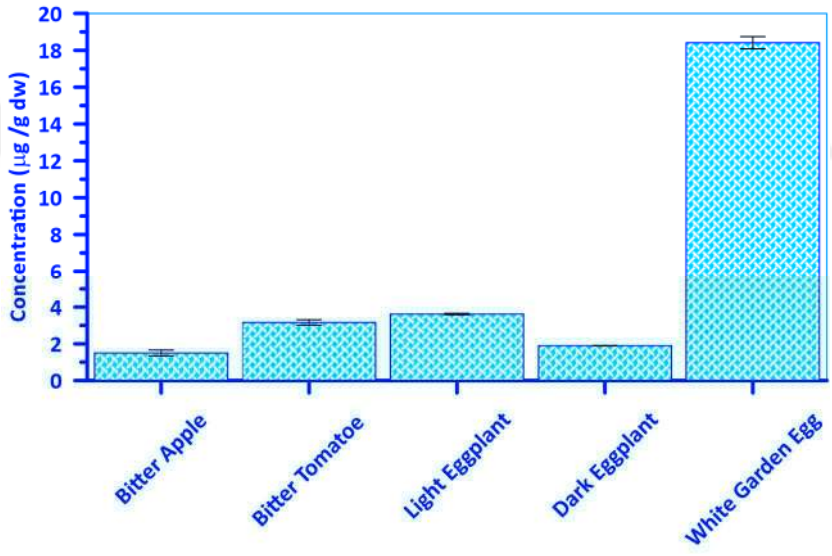

Figure 4.12. Showing a comparison in MDA levels between the eggplant varieties.

4.3.3. GSH and GSSG

All the varieties of eggplant used in this study contained both reduced (GSH) and oxidized (GSSG) forms of glutathione. While the reduced form was found to range from 364.85 – 5930.32 µg/g DW with a 16.3 fold variation, the oxidized form ranged from 224.83 – 1961.59 µg/g DW with an 8.8 fold difference. The findings of GSH levels in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato were determined (Table 3.6 and Figure 4.13) to be 5930.32±475.38, 1511.93±23.74, 408.45±2.13, 812.44±4.91, and 364.85±14.84 µg/g, respectively. While the amounts of GSSG in white garden egg, bitter apple, dark eggplant, light eggplant and

bitter tomato were determined (Table 4.6 and Figure 4.14) to be 1961.59±56.99, 866.29±5.64, 224.83±0.91, 418.50±2.44 and 230.01±2.20 µg/g DW, respectively. The white garden egg had the highest amount of both GSH and GSSG while the bitter tomato had the lowest GSH levels; the dark eggplant had the lowest GSSG levels.

Comparing between and amongst varieties, within the Turkish varieties, the light eggplant had double the amounts of both GSH and GSSG compared to the amounts of in the dark eggplant variety. On the other hand, amongst the Nigerian varieties, the white garden egg had 3.9/2.3 and 16.3/8.5 folds the amount of GSH/GSSG determined in bitter apple and bitter tomato, respectively. Whereas, the bitter apple had 4.1 and 3.8 folds the amounts of GSH and GSSG determined in the bitter tomato variety, respectively.

Correlating values of GSH and GSSG in the Turkish varieties to those in the Nigerian varieties, our findings revealed that the dark eggplant variety had lower values of GSH and GSSG than those detected in all the Nigerian varieties with the exception of GSH levels in bitter tomato. However, while the values of both GSH and GSSG in dark eggplant varied significantly with those detected in white garden egg and bitter apple, it varied by a relatively narrower margin with values determined in bitter tomato variety. Conversely, the white garden egg and bitter apple varieties had more than double the amounts of GSH and GSSG determined in the light eggplant variety, respectively. In contrast, the light eggplant had higher levels than the bitter tomato variety. The ratio of GSH: GSSG is used as a measure of oxidative stress, where higher levels of GSH are associated with increased antioxidant activity while GSSG is considered cytotoxic if allowed to accumulate [297]. Based on our findings in terms of GSH: GSSG, the white garden egg appears to have the highest antioxidant activity. The disparities observed in amounts and ratios of GSH to GSSG may be due to differences in the varieties of the eggplant fruits used in this study.

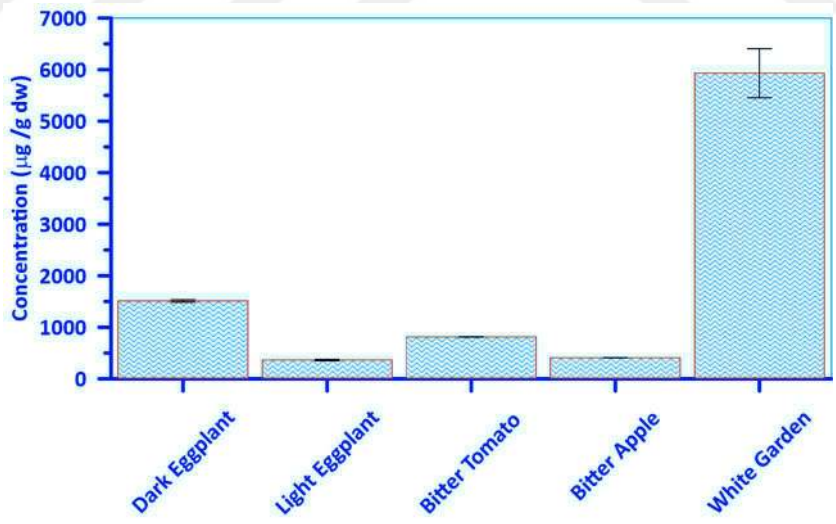

Figure 4.13. Shows a comparison of GSH levels between the different varieties of eggplants.

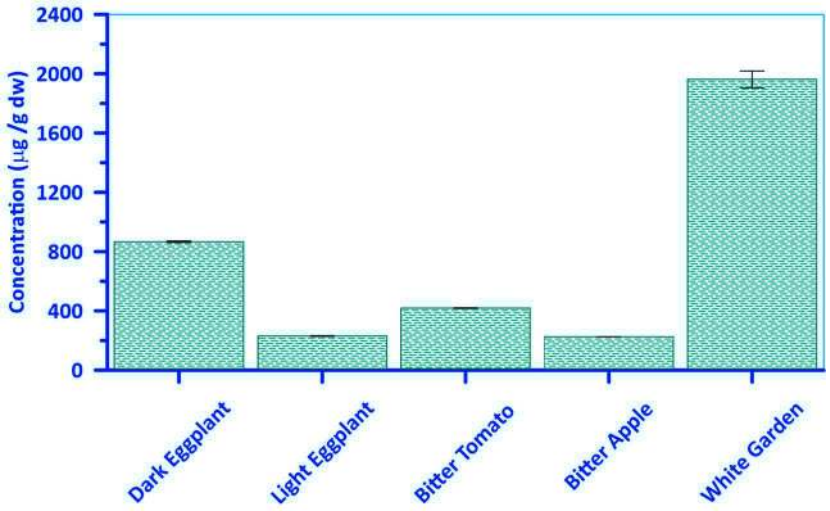

Figure 4.14. Shows a comparison of GSSG levels between the different varieties of eggplants.

Total Phenolic Content

The total phenolic content (TPC) in extracts of the eggplant fruit varieties sampled for this study were evaluated using Folin– Ciocalteu's method. All the varieties sampled had detectable levels that ranged from 706.30 - 1260.32 -µg Gallic acid equivalent (GAE)/g DW of extract value, depicting a 1.8 fold difference in content. Individually, the concentrations of total phenolic compounds in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato varieties were determined (Table 4.6) to be 1260.32±5.5, 868.57±12.8, 1120.69±11.2, 706.30±10.6 and 732.35±6.7 µg GAE/g DW, respectively. The Nigerian white garden egg had the highest concentration while the Turkish light eggplant variety had the lowest.

A comparison between the varieties sampled from Turkey showed the dark eggplant to have a higher concentration of 1.6 folds the amounts detected in the light eggplant. Comparing within the Nigerian varieties showed a range difference of 1.7 folds. The white garden egg had the highest concentration, 1.5 and 1.7 folds the TPC determined in bitter apple and bitter tomato varieties, respectively. While the bitter apple had 1.2 folds the amounts determined in the bitter apple variety. Correlating the TPC determined in the Turkish varieties to their Nigerian counterparts showed no specific trend though they had a similar range difference. The Turkish dark eggplant had a higher concentration than the Nigerian bitter apple and tomato varieties, while it had lower than the white garden egg. Contrastingly, the Turkish light eggplant had a lower TPC than all the Nigerian varieties.

Overall, the total phenolic content (Figure 4.15) in the varieties were found to be in the following decreasing order: white garden egg > dark eggplant > bitter apple > bitter tomato>light eggplant. The TPC in eggplants was observed to significantly vary widely- with low and comparably very high values also reported in the literature, with ranges of 23- 1,168 mg/100g DW, depending on the variety and external factors [1]. In 2006, Hanson *et al.* investigated the TPC in the methanolic extracts of 33 different eggplant cultivars sampled from diverse countries and found it to range from 74 – 1400 mg/100g DW [1]. Akanitapichat *et al.,* (2010) also investigated the variation in concentrations of TPC in five varieties of eggplant fruits as 739.36 –1116.13 mg GAE/100 g extract (DW) [321]. Nwanna *et al.* (2019) likewise observed a significant variation in TPC (253.1 – 499.2 mg GAE/100g DW) of eggplants sampled from diverse locations in Nigeria [356]. Over all, our findings are consistent with the range of values reported in the literature. The

variations observed in the TPC are therefore attributable to varietal differences and origins of the eggplants sampled for the study.

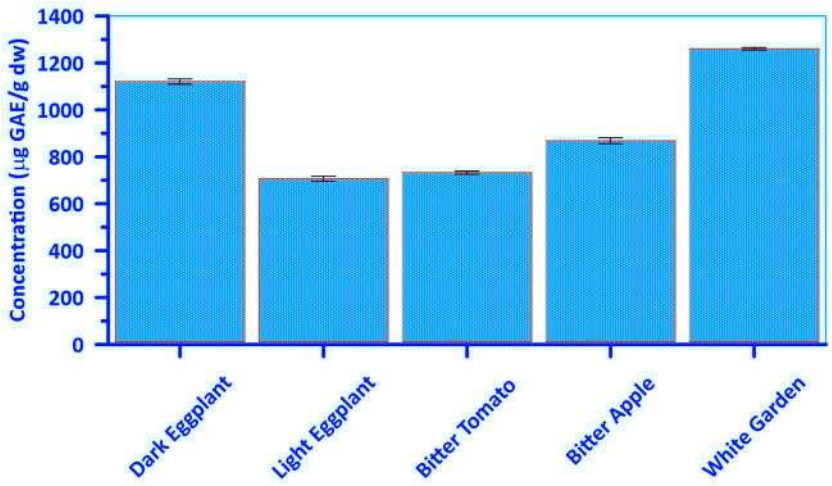

Figure 5.15 Shows a comparison of the total phenolic content of the different varieties.

4.4. Total Flavonoid content

The TFC were determined to range from 167.50 – 356.51 µg Quercetin equivalent (QE)/g DW of extract value, showing a 2.1 fold range difference. Individually, the concentrations of total flavonoid compounds in white garden egg, bitter apple, dark eggplant, light eggplant and bitter tomato varieties were determined (Table 3.6.) to be 356.51±3.44, 167.5±5.12, 263.79±6.93, 192.05±0.40 and 214.71±0.90 µg QE/g DW, respectively. The highest flavonoid content was determined in the white garden while the lowest was determined in the bitter apple variety.

Comparing between the TFC (Figure 4.16) determined in the Turkish varieties showed the dark eggplant to have 1.4 folds the TFC contents determined in the light eggplant. Between the Nigerian varieties, the white garden egg had the highest concentration, 2.1 and 1.7 folds the TFC determined in the bitter apple and bitter tomato varieties, respectively. While the bitter tomato variety had 1.3 folds the amounts determined in the bitter apple variety.

Correlating the TFC determined in the Turkish varieties to their Nigerian counterparts. The white garden egg had a higher TFC that was 1.4 and 1.9 folds the amounts determined in the dark and light eggplant varieties, respectively. In contrast, the dark eggplant had a higher TFC than the bitter apple and bitter tomato varieties. Conversely, the light eggplant had a lower and higher TFC than the bitter tomato and bitter apple varieties, respectively, though both by a narrow margin. The variations in the total flavonoids determined in this study are attributable to the differences in the genetic makeup of varieties and origins of the eggplants sampled for this study.

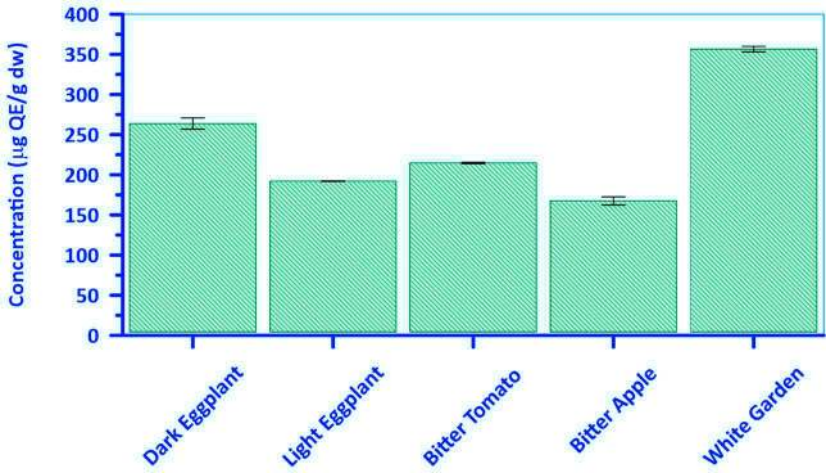

Figure 4.16. Shows a comparison of the total flavonoid content of the different varieties.

Overall, the total flavonoid contents in the varieties were found to be in the following decreasing order: white garden egg > dark eggplant > bitter tomato > light eggplant> bitter apple. The total flavonoids, which are a large class of phenolic compounds, have also been reported to vary significantly in eggplants, with values as low and high as 0.25 and 3954 mg/ 100g of extract value respectively, reported in the literature [407]. The TFC in eggplants have been reported to range from 16.13 - 18.52 mg QE/100 g (161.3 - 180.52 µg QE/g)DE by Boulekbache-Makhlouf *et al*. [408]. Kaur *et al*., (2014) [324] investigated TFC in a variety of eggplant fruits classified based on color and wild types and found it to range from 3 - 26 mg QE / 100g (30 to 260 µg QE/g). Another study by Akanichapitat *et al* reported values of TFC in varieties of eggplants to range from 1991.29–3954.20 mg CE/100 g DW [321]. A comparison between TFC in varieties sampled from Nigeria showed it to range from 152.4 - 392.0 mg QE/100 g DW as reported by Nwanna *et al*. [356]. Our findings are consistent with the range of literature values.

This study showed the total phenolic content (706.30 – 1260.32 µg (GAE)/g) across the different varieties from Turkey and Nigeria determined, expectedly, to be of a higher concentration and though a wider variation was observed in the total flavonoid content (167.50 – 356.51 µg QE/g extract). The mean values of total phenolic and flavonoid contents for all the varieties were calculated to be 937.65 µg GAE/g and 238.91 µg QE/g respectively showing a 3.9 fold content difference. The most and least significant deviation in TPC within the varieties were determined in the Nigerian bitter apple and white garden egg varieties, respectively, in contrast, for the TFC it was in the values of the Turkish dark and light eggplant varieties, respectively. Our findings revealed that the white garden egg and dark eggplant overall had the highest content of both total phenolic and flavonoid compounds. This is consistent with previous studies by Stratil *et al.,* (2006) [409] and Sharma *et al*. (2019) [410] that have reported a similar correlation in total phenolic contents of different varieties of eggplants.

4.4.1. ABTS

The ABTS free radical scavenging activities of the methanolic extracts of the different varieties of eggplants were determined to range from 365.44 – 809.52 µ g trolox equivalent/g DW with a 2.2 fold range difference. Individually, the white garden egg, bitter apple, dark eggplant, light eggplant, and bitter tomato showed (Table 4.6) - 809.52±3.8, 464.29±4.6, 692.24±5.1, 532.28±3.2 and 365.44±2.8 µ g/g ABTS radical scavenging activity, respectively. The white garden

egg (809.52 µg/g) extract had the highest ABTS free radical scavenging activity, while the bitter tomato (365.44 µg/g) extract had the lowest.

Comparing the free radical scavenging activities between and within the varieties from Turkey and Nigeria (Figure 4.17). Amongst the varieties from Turkey, the dark eggplant had a higher activity that was 1.3 folds that of the light eggplant. On the contrary, a wider difference in ABTS scavenging activity was observed between the Nigerian varieties. The white garden egg had the highest activity-1.7 and 2.2 folds that of the bitter apple and bitter tomato varieties, respectively. Relating the antioxidant capacities of the Turkish varieties to their Nigerian counterparts, whilst the ABTS scavenging activity of the Turkish varieties were higher than those of bitter apple and bitter tomato were, it was still lower than that of the white garden egg variety. Akanitapichat *et al.* [321] reports the ABTS radical scavenging activity of five varieties of eggplants to range from 53.18 – 105.63 µg/mL DW. While Nwanna *et al.* [356] reported a free radical scavenging activity of varieties of eggplants to range from 1.24 - 3.50 mmol TEAC/g). The observed dissimilarities are attributed to the differences in the varieties sampled for the study.

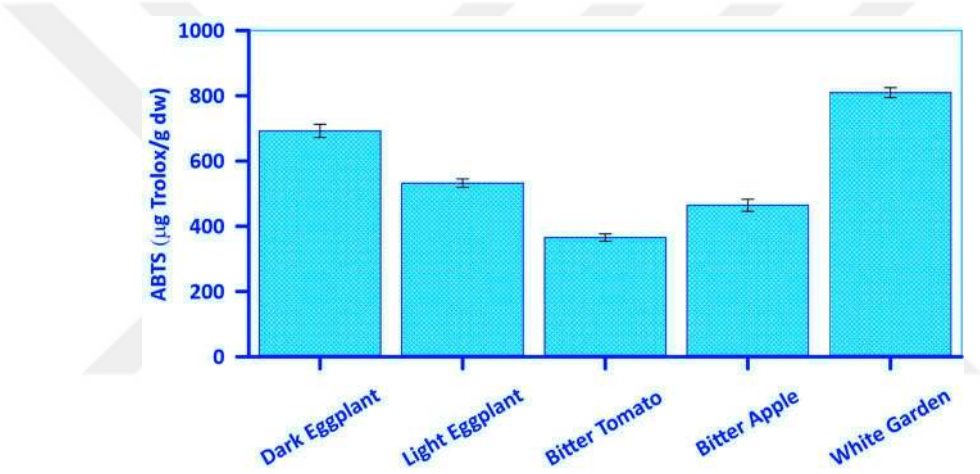

Figure 4.17. Shows a comparison of the antioxidant capacities of the different varieties based on ABTS assay.

4.4.2. DPPH

On the other hand, the DPPH free radical scavenging activities for the eggplant varieties sampled were found to range from 83.86 - 243.02 µg/g with a 1.4-fold range difference. Each of the methanolic extracts of white garden egg, bitter apple, dark eggplant, light eggplant, and bitter tomato were determined to have (Table 3.6) 243.02±1.31, 83.86±0.96, 95.09±1.03, 113.78±1.87 and 99.27±0.83 µg/g DPPH radical scavenging activity, respectively. Similar to TAC determined by ABTS assay, the white garden egg had the highest DPPH free radical scavenging activity while the bitter apple variety had the lowest.

Comparing and contrasting between the Turkish varieties showed the light eggplant to have a higher DPPH scavenging activity than the dark eggplant variety. Between the Nigerian varieties, the white garden egg variety had the highest scavenging activity followed by the bitter tomato variety, while the bitter apple variety had the lowest.

Correlating the TAC of the Turkish varieties to the Nigerian varieties (Figure 4.18) based on their DPPH free radical scavenging activities showed no particular trend. The Turkish dark eggplant variety had a lower activity than the Nigerian white garden egg and bitter tomato varieties. Contrastingly, the Turkish light eggplant had a higher free radical scavenging activity than the

Nigerian bitter tomato and bitter apple varieties. However, the Nigerian white garden egg had a higher activity than all the Turkish varieties. The findings in this study corroborates with a study by Prohens *et al*. (2013) [411] who observed a similar pattern.

The different varieties and assay methods used in this study to measure antioxidant activity in the methanolic extracts showed disparities in scavenging activity. The free radical scavenging activity as determined was found to vary for both ABTS and DPPH assays. The ABTS assay had higher and wider ranging values than the DPPH assay. The extracts from the different varieties also had an average free radical scavenging ability for ABTS radicals of 572.75 µg/g while it was 127.00 µg/g for DPPH radicals. Individually, the white garden egg exhibited the highest antioxidant capacity for both assays. In contrast, the lowest antioxidant activity was detected for bitter tomato for the ABTS assay, whereas the bitter apple showed the lowest for the DPPH assay. These variations are attributable to the varietal differences in the profile of phenolic content of the eggplant fruit varieties sampled for this study.

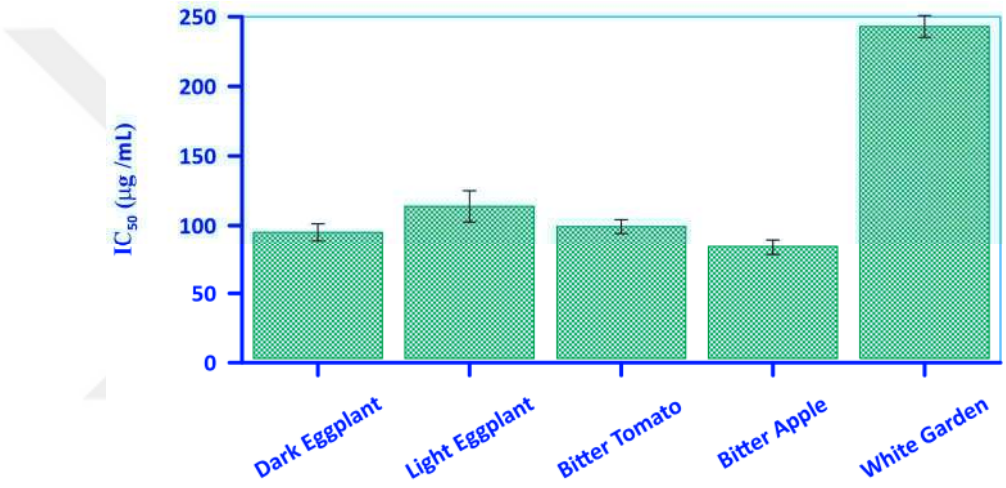

Figure 4.18. Shows a comparison of the antioxidant capacities of the different varieties based on DPPH assay.

Overall, the white garden egg variety had the highest concentration of TPC, TFC, and TAC for both assays. Rationally, the variety with the highest total phenolic content would have the highest antioxidant capacities, as observed, since eggplants are reported to be repositories of phenolic compounds that greatly contribute to their hydrophilic antioxidant value [354]. Previous studies by Thaipong *et al.,* (2006) [374] and Yang *et al*. (2004) [412] have also reported similar correlations.

That notwithstanding, each variety has its own unique metabolic profile, and by extension total phenolic content profile, which differs qualitatively and quantitatively. Thus, the antioxidant capacities of the extract from each variety is reflective of their total phenolic content profile and their individual capacities and synergies with the two distinct ABTS and DPPH radicals. Stommel and Whitaker (2003) [354] and Jacobo-Velazquez (2009) [413] adequately demonstrated these correlations between total phenolic content and their contributions to scavenging activities of various food samples. The total phenolic content determined in all the varieties were therefore correlated to the TAC assays to assess and compare their relationship using linear regression. Both assays showed a strong correlation to the total phenolic content, however, a stronger correlation was observed between the total phenolic content and the ABTS radical than with the DPPH radical.

This is indicative of the higher affinity of the phenolic content in the extracts for the DPPH radical than it had for the ABTS radical and their contribution to hydrophilic antioxidant capacity of the extracts. Likewise, the total flavonoid content was correlated and a strong correlation was observed with both radicals. Similar to observations with the total phenolic compounds, there was a stronger correlation between total flavonoid content and ABTS radical than with the DPPH assay radical. The strong affinities observed between the total flavonoid content and assay radicals suggests that their presence in the extracts significantly contributed to the total antioxidant activities of the extracts. Our findings corroborate with previously reported studies [414]. Hanson *et al*. (2006) [415], Kaur *et al*. (2014)[324] and Koley *et al*. (2019) [407] also reported a similar correlation between total phenolic content and free radical scavenging activities of eggplants, which have been observed to be affected by factors like genetics and the environment [416].

5. CONCLUSION

Our findings revealed all the varieties selected to have a measure of nineteen (19) of the proteogenic amino acids assessed in this study, ranging from 23.5 – 33625.4 μg\g DW depicting a wide variability. In relation to the RDA, all of the varieties can contribute to dietary adequacy in terms of histidine, lysine, methionine, phenylalanine, threonine and tryptophan. However, while the white garden egg and bitter apple varieties had adequate amounts of valine, the dark eggplant, light eggplant and bitter tomato varieties had nutritionally insignificant values. Likewise, all the varieties are nutritionally low in leucine and, consequently cannot contribute to its dietary adequacy.

With the exception of vitamin B_9 (folate) that was below detection limit in the dark eggplant variety sampled from Turkey, all the vitamins assessed were detected in the selected varieties. The concentrations were determined to range from 0.13 – 2543.02 μg\g DW showing a significantly wide difference. Relative to the RDA, all the varieties can serve as excellent sources of the water-soluble vitamins. However, whilst the white garden egg, bitter apple and bitter tomato varieties can be regarded as excellent sources of vitamin A, the dark and light eggplant were below the benchmark. Similarly, the dark eggplant and bitter tomato are nutritionally rich in vitamin E, while the other varieties were not. Likewise, in terms of lycopene all the selected varieties had low values, with only the white garden egg variety having up to 8.5% of the RDA. Thus, based on the findings, the varieties sampled have comparably lower concentrations of the fat-soluble vitamins assessed in this study.

Each of the varieties used in this study had detectable levels of the stress biomarkers (4-hydroxynonenal, malondialdehyde, and GSH/GSSG) assessed in this study. 4-Hydroxynonenal was determined in the following decreasing order – white garden egg > dark eggplant >bitter tomato >light eggplant > bitter apple. Our findings revealed the presence of MDA in all the samples with a 12.3 fold difference variation in the following decreasing order – white garden egg >light eggplant >bitter tomato > dark eggplant >bitter apple. Based on this, the bitter apple variety, which had the lowest, is the most resilient to lipid peroxidation-related oxidative stress. All the varieties of eggplant used in this study contained both reduced (GSH) and oxidized (GSSG) forms of glutathione.

This study showed the varieties sampled to have a significant difference in total phenolic (TPC) and flavonoid (TFC) content. Both the TPC and TFC determined showed a strong correlation with both assays used to assess TAC, however, a stronger correlation was observed with the ABTS radical scavenging activity in comparison to the DPPH radical scavenging activity.

Our findings highlighted a wide disparity in the levels of the nutrients, stress biomarkers, TPC, TFC and TAC within and between the varieties sampled from the different countries attributed to their varietal differences and origins. Consequently, such comparisons have unveiled differences that present further opportunities for dietary inclusion and other stakeholders to select and develop improved plant varieties with a preferred characteristic.

RECOMMENDATIONS

Based on the results of this study, below are summary of some recommendations for future research:

- A wider range of varieties of eggplants could be sampled to compare the variations in the parameters investigated in this study.
- The quantification of other components including mineral elements, enzymes and hormones in the eggplant varieties is also recommended.
- Elucidation and characterization of the profiles of phenolic compounds and other secondary metabolites in the varieties may be explored.
- The effect of different pre-treatments on the parameters studied and compared to assess the impact on the nutrient and secondary metabolite profiles of the different varieties.
- Assessment of health benefits suggested in the literature on experimental animals will also be desirable to confirm such claims and promote its usage.
- Selection and breeding to maximally balance physical, sensory, nutritional and functional value of fruits are in high demand. The results suggest a genetic variation that could be exploited in breeding eggplant varieties with a desired ideotype.

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APPENDICES

APPENDIX- 1: CALIBRATION GRAPHICS OF VITAMINS

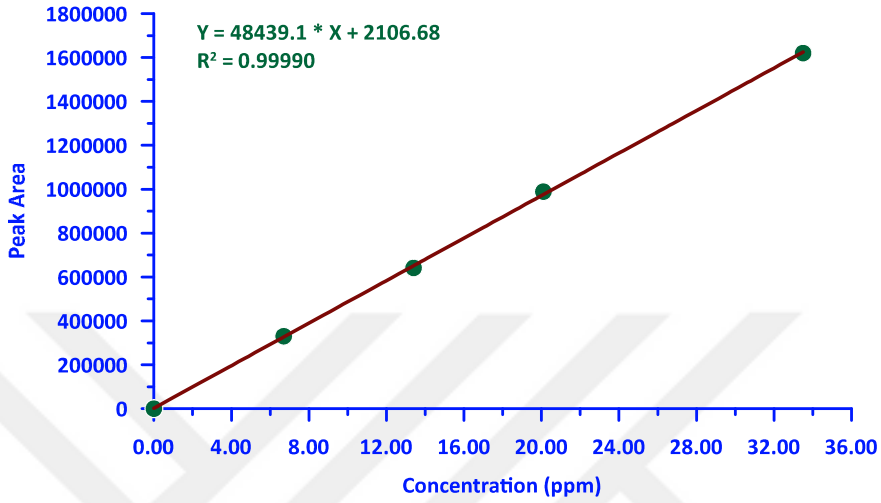


Figure A1.1 Ascorbic acid Calibration Graphic

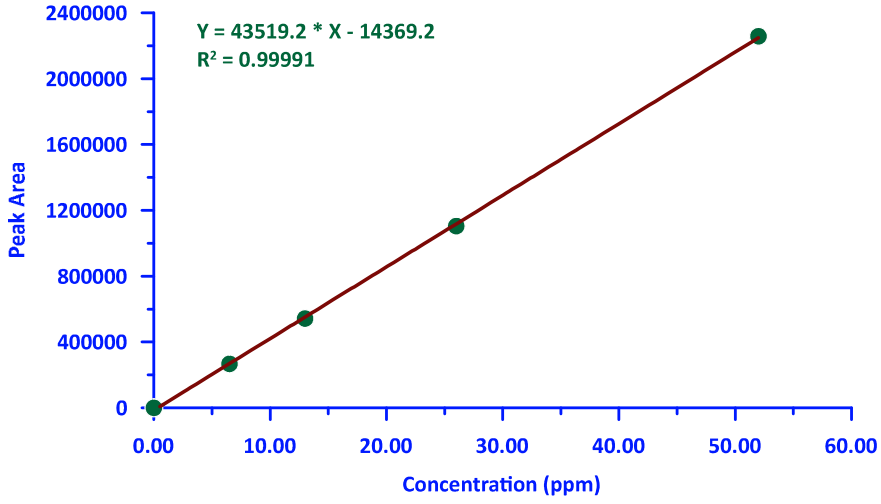
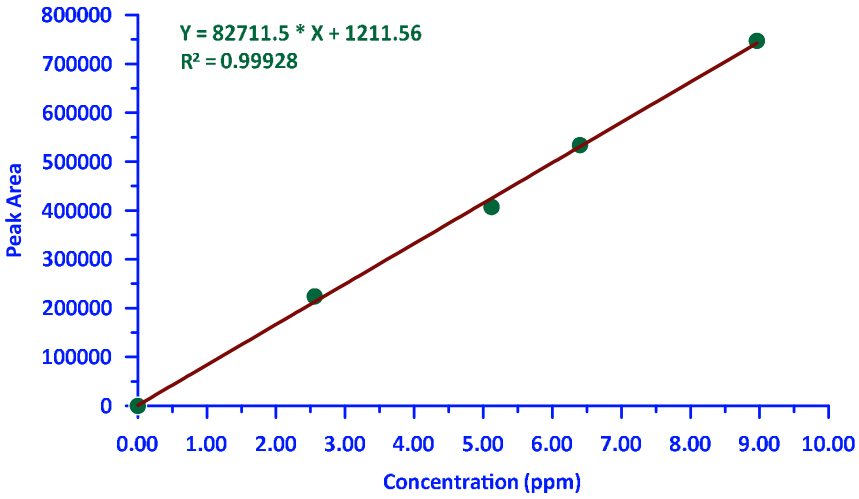


Figure A1.2 B_1 Vitamine Calibration Graphic



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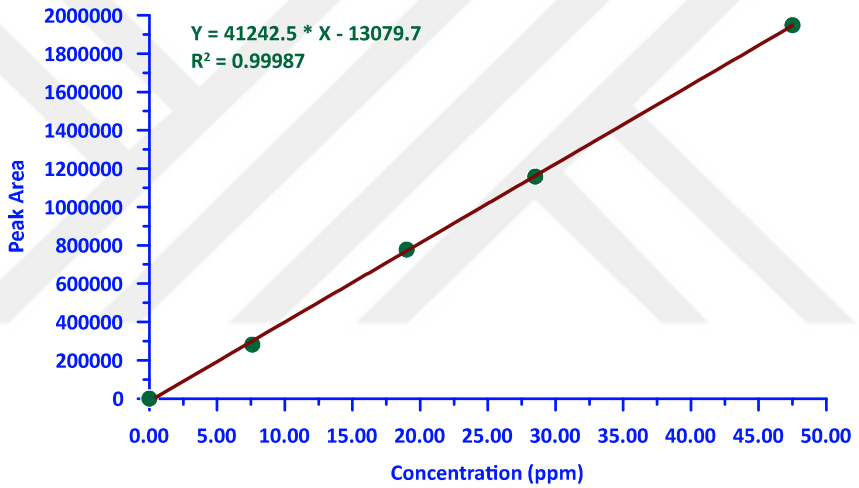

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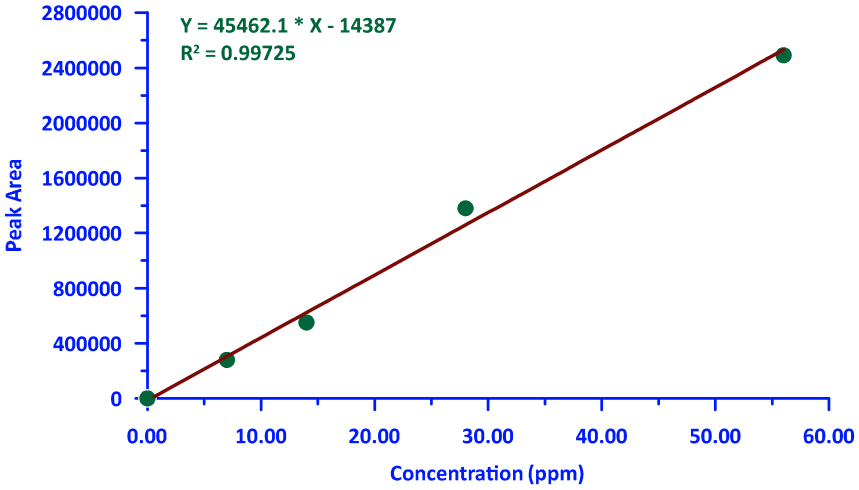

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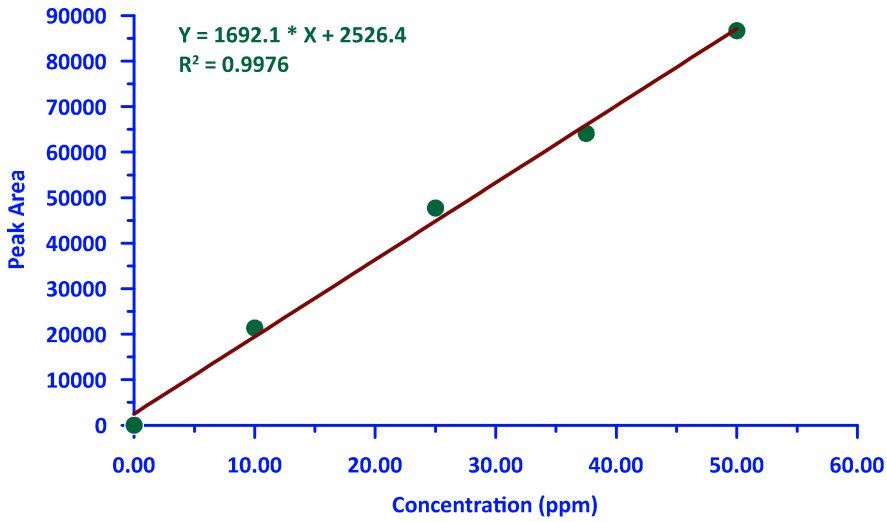

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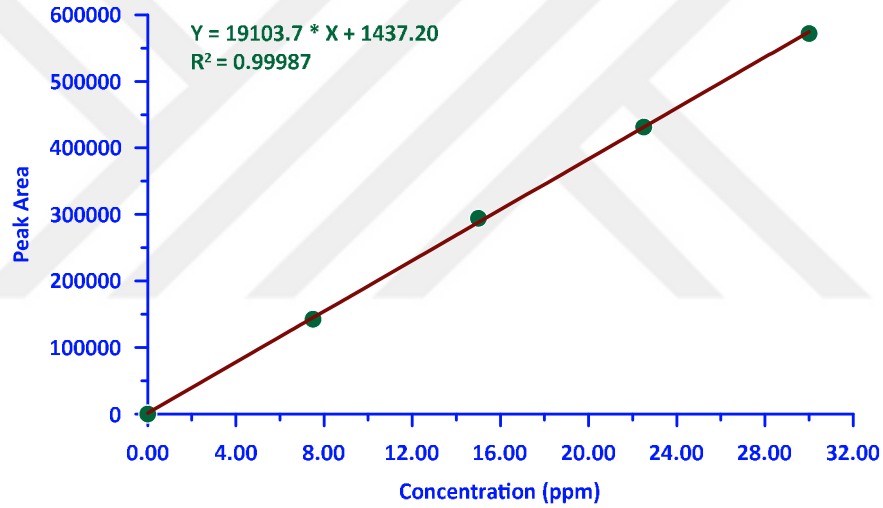


Figure A1.7 B_{12} Vitamine Calibration Graphic

APPENDIX- 2: CALIBRATION GRAPHICS OF STRESS BIOMARKERS

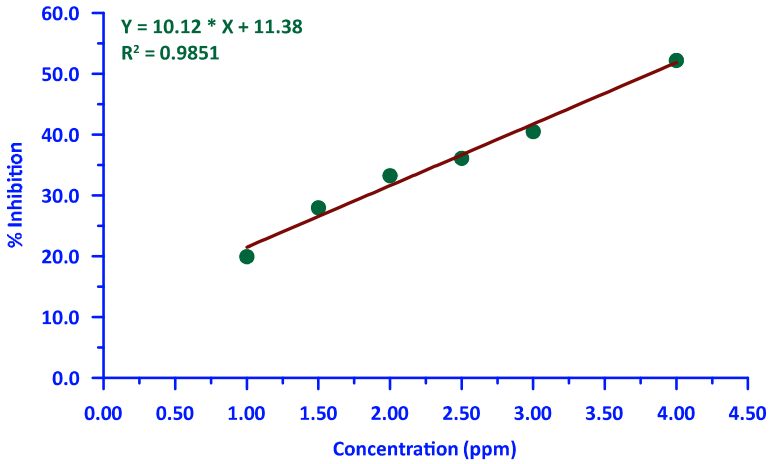


Figure A2.1 Trolox Calibration Graphic

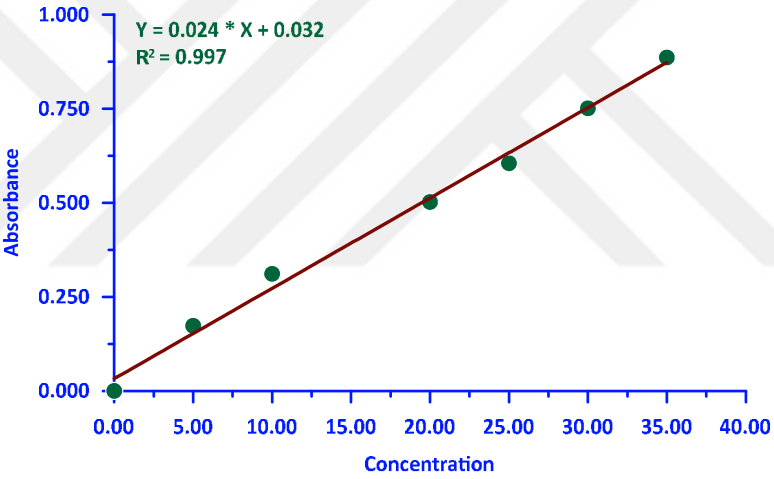


Figure A2.2 Quercetin Calibration Graphic

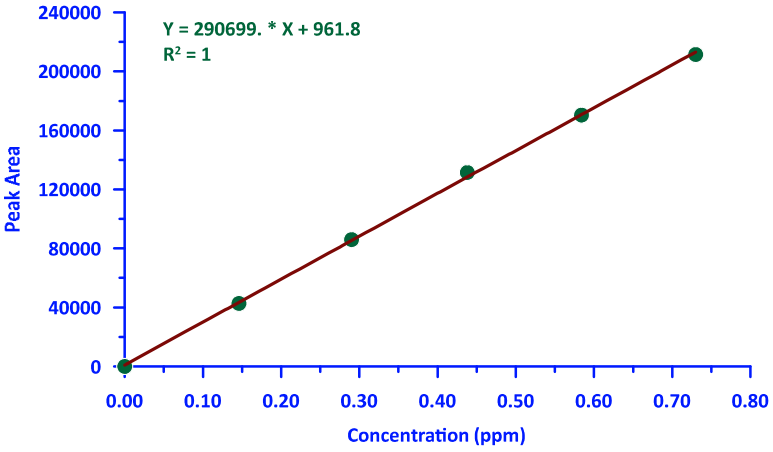


Figure A2.3 MDA Calibration Graphic

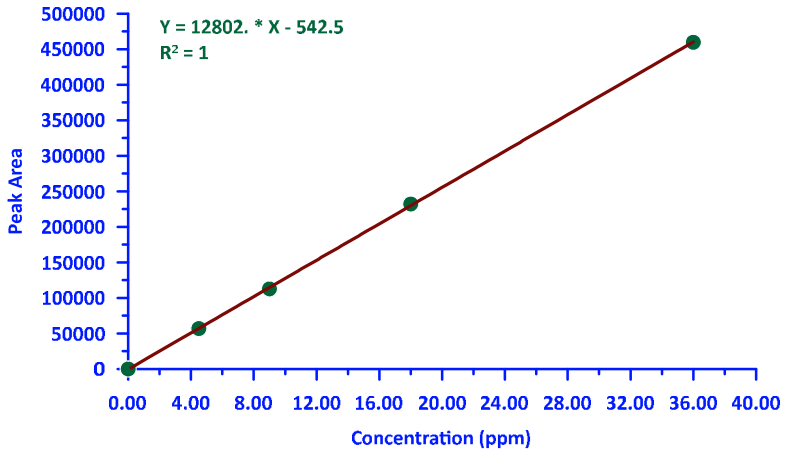
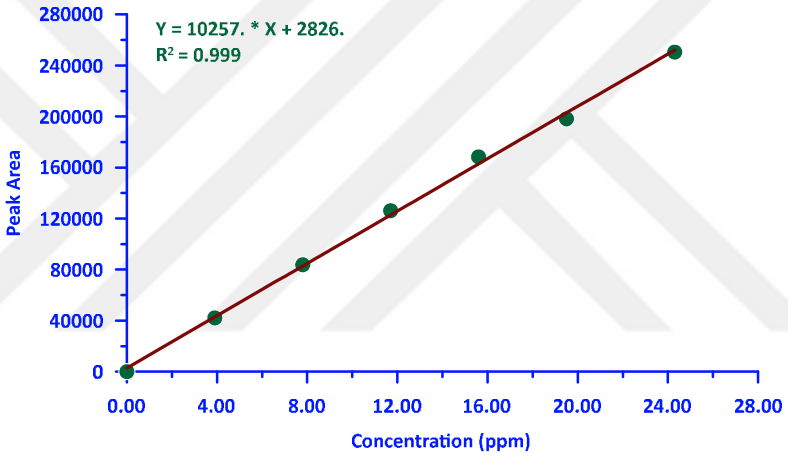


Figure A2.4 GSSG Calibration Graphic



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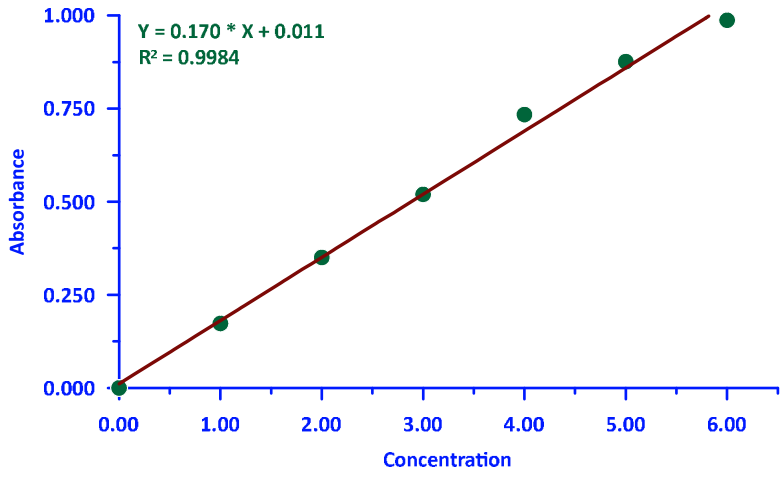


Figure A2.6 Galic Acid Calibration Graphic

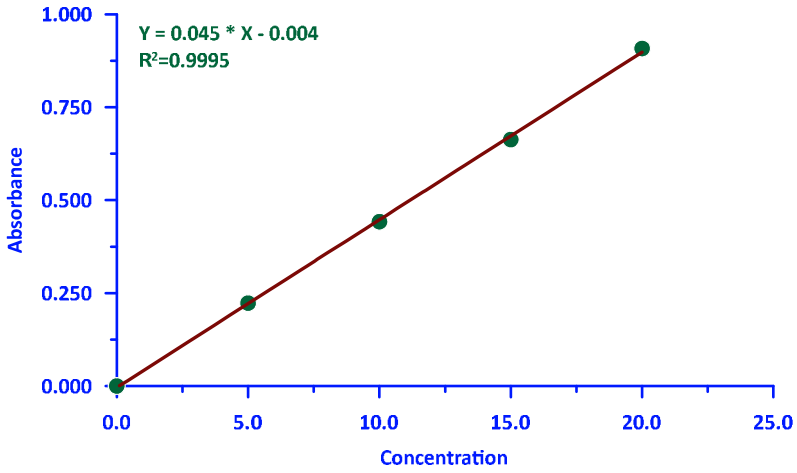
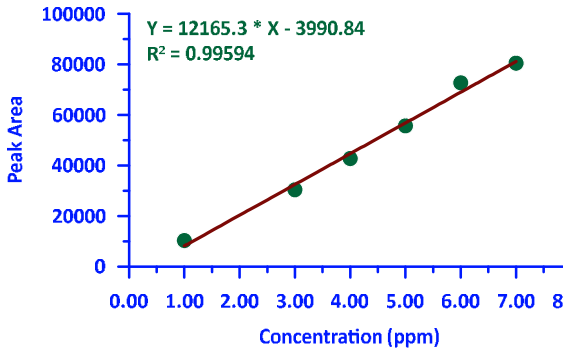
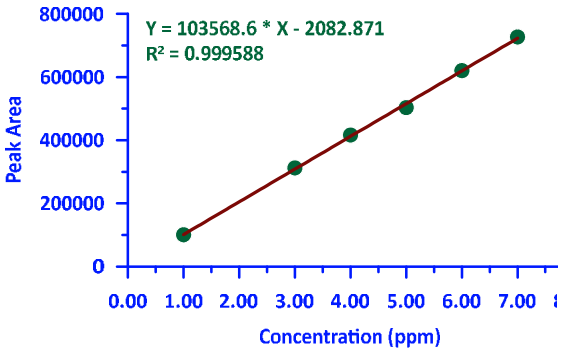


Figure A2.7 DPPH Calibration Graphic

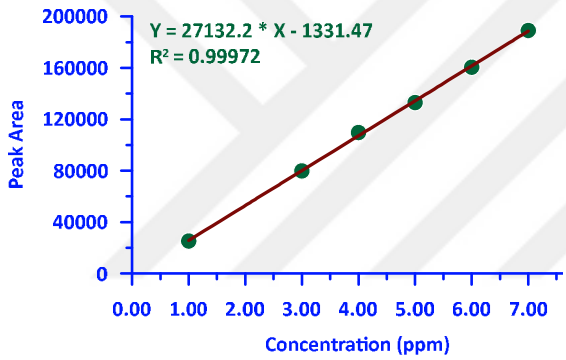
APPENDIX- 3: CALIBRATION GRAPHICS OF AMINO ACIDS



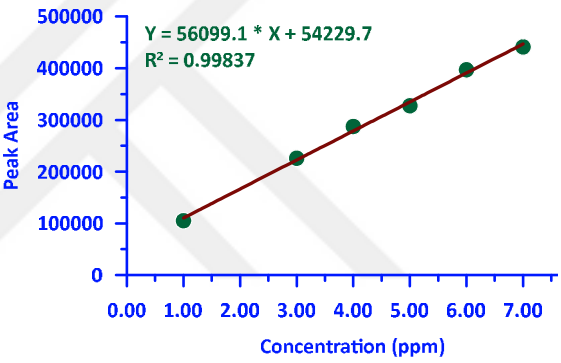
a) Alanine Calibration Graphics



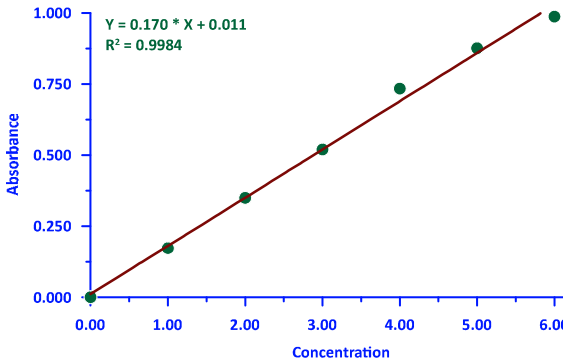
b) Arginine Calibration Graphics



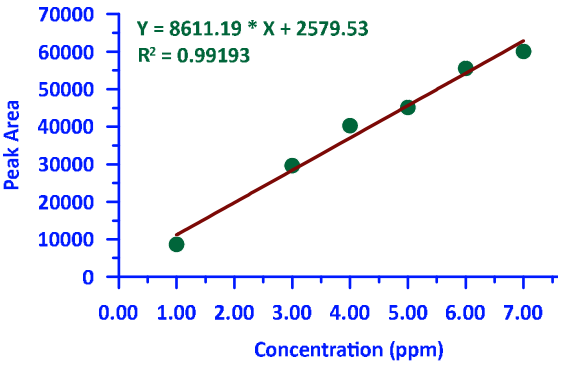
c) Asparagine Calibration Graphics



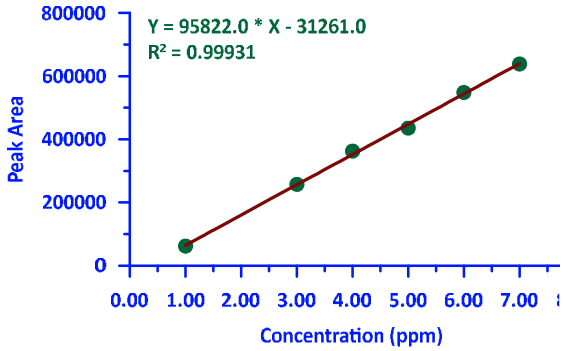
d) Aspartic acid Calibration Graphics



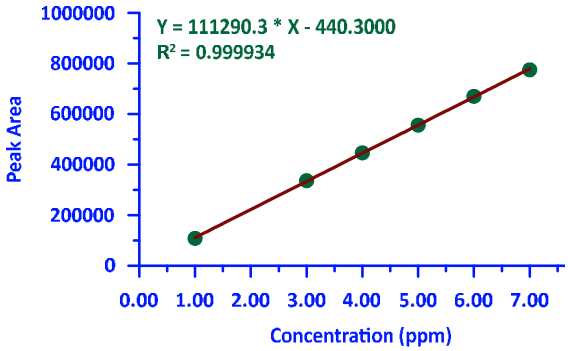
e) Gallic acid Calibration Graphics



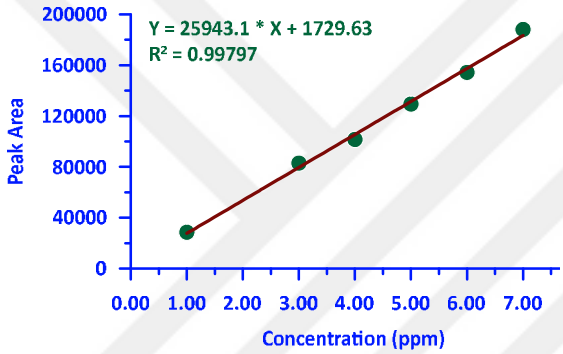
f) Glutamic acid Calibration Graphics



g) Glutamine Calibration Graphics



h) Glycine Calibration Graphics



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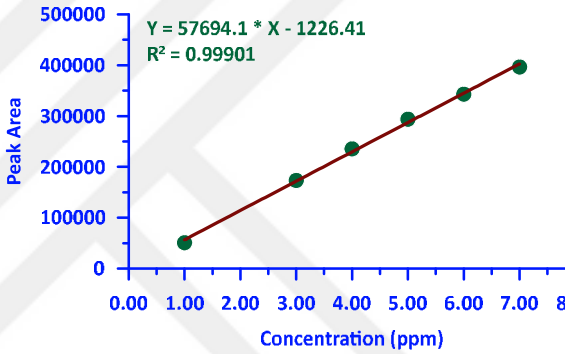
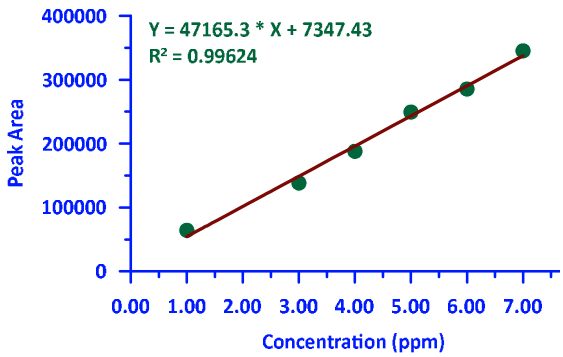
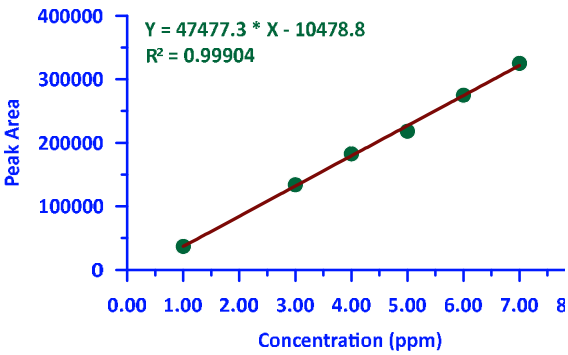


j) Leucine isoleucine Calibration Graphics



k) Lysine Calibration Graphics



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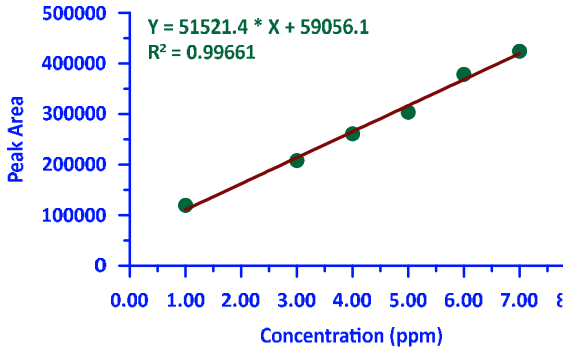

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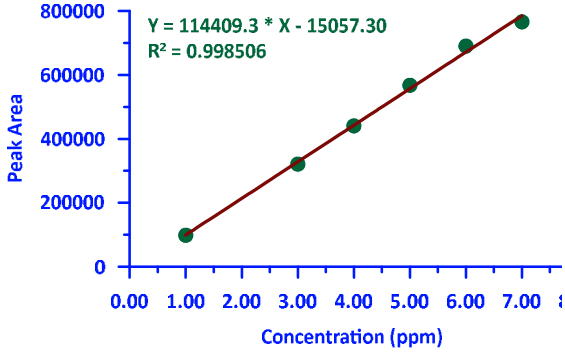

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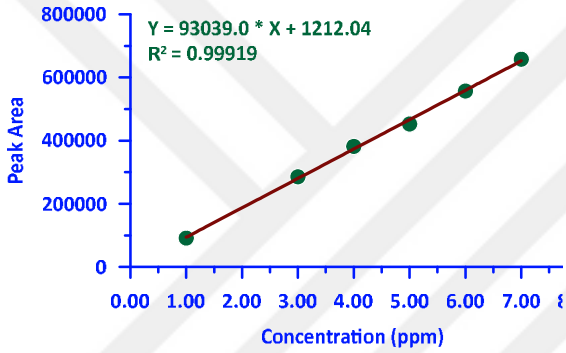

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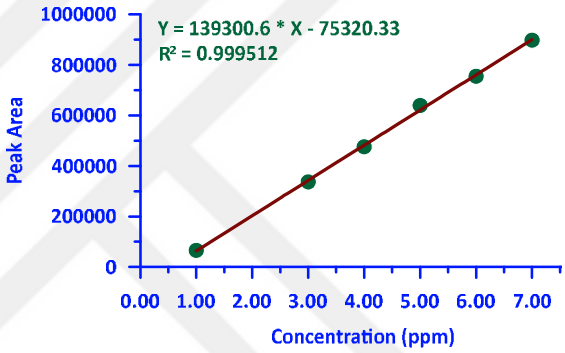

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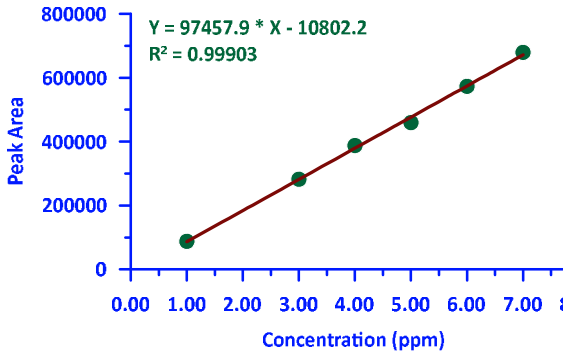

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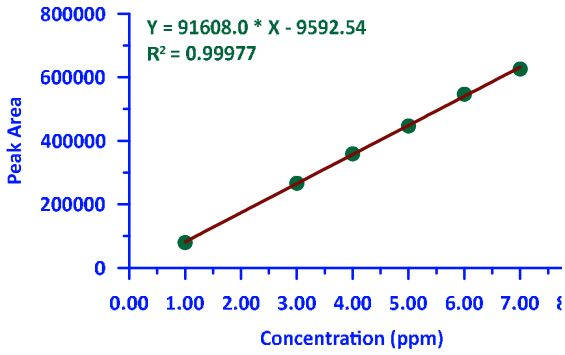
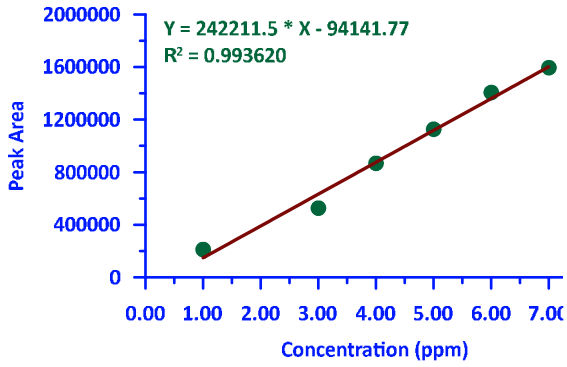
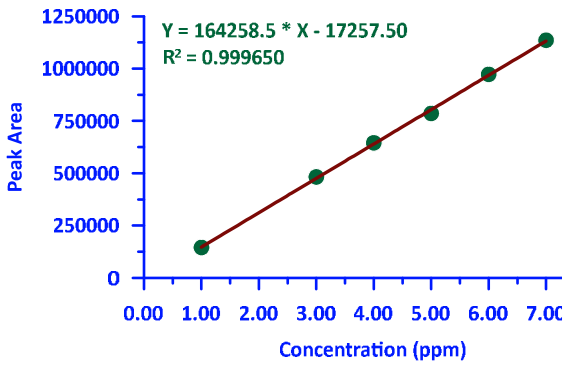


s) Thorazine Calibration Graphics



u) Tryptophane Calibration Graphics



y) Valine Calibration Graphics

CURRICULUM VITAE

Zulaiha Gidado MUKHTAR

PERSONAL INFORMATION

[redacted]

RESEARCH EXPERIENCES

- ✓ Laboratory Instruments – Spectrophotometer, HPLC
- ✓ Data analysis in nutrition/ food research and toxicology studies.
- ✓ Basic Computer skills – MSWord, PowerPoint, Excel.

WORK EXPERIENCE

Jan. 2018 – Date	**:** Kano State Polytechnic (School of Technology) Lecturer I
Jan. 2015 – Dec. 2017	**:** Kano State Polytechnic (School of Technology) Lecturer II
June 2011- Dec., 2014	**:** Kano State Polytechnic (School of Technology) Lecturer III
Aug. 2008 - July 2011	**:** Kano State Polytechnic (School of technology) Assistant Lecturer

ACADEMIC ACTIVITIES

Paper: Paper:

1. Kiyawa, S. A., **Mukhtar, Z. G,** Bayero, A. S. and Ibrahim, Y. I. (2019). Antioxidant and Antipyretic activities of Adasonia digitata (African Baobab) fruit, leaves and bark extract. *Chemistry Journal* Vol 2 (2019) ISSN: 2581-7507
2. **Zulaiha Gidado Mukhtar,** Yusuf Ibrahim Ibrahim, Hassan T. Kabara, Adamu J. Alhassan, Isa Yunusa, Zainab Rabiu, and Ahmad M. Isah (2019). *Leptadenia hastata Leaves Elicit Medicinal Properties*. *Chemr**j***.org/archive/volume-4-issue-4-2019, P.53-57. ISSN:2455-8990.
3. **Zulaiha Gidado Mukhtar**, Muhammad Salihu Ibrahim, Yusuf Ibrahim Ibrahim, Fikret Karatas (2019). *Amounts of Vitamin A, Vitamin E, Vitamin C, B-Carotene, Lycopene, Ghrelin, Glutathione, And MDA in Fruits of Diospyros Kaki L. Gida, The Journal of Food*, (2019) 44(4) 585-592, E-ISSN 1309-6273, ISSN 1300-3070. doi: 10.15237/gida.GD19033.

4. Zainab Rabiu, Fatima U. Maigari, Umma Lawan, and **Zulaiha Gidado Mukhtar** (2018). *Pineapple Waste Utilization as a Sustainable Means of Waste Management in:* Sustainable Technologies for the Management of Agricultural Wastes, eds. Zakaria, Zainul Akmar. *Springer pub***.** eISBN (pdf): 9789811050626; Printed ISBN: 978-981-10-5061-9.
5. Ibrahım M.S., Ibrahım Y. I., **Mukhtar Z. G.**, Karatas F. (2017). *Amount of Vitamin A, Vitamin E, Vitamin C, Malondialdehyde, Glutathione, Ghrelin, Beta-Carotene, Lycopene in Fruits of Hawthorn, Midland (Crataegus laevigata). J Hum Nutr Food Sci*. 5(3): 1112.
6. Ahmad Isah Muhammad, Isa Yunusa, Mohammed T. Bolori, Lawrence Ezeanyika, Hamisu Abdullahi Walla, **Zulaiha Mukhtar Gidado** (2017). *Malnutrition among Children under 5 Does Not Correlate with Higher Socio economic Status of Parents in Rural Communities. Open Access Library Journal* 04(09):1-15DOI: 10.4236/oalib.110390, ISSN Online: 2333-9721.
7. **Zulaiha Gidado Mukhtar,** Ibrahim, Y. I., and Muhammad, I.Y. (2016). *Practical and Experimental Biochemistry*, *ABU Press Ltd,* 1st edition. ISBN 978-978-54653-8-9.
8. Ahmad I. M., Yunusa I., Badayi M. Sani, Abba B., Abdullai S., Ameen Y., Illo I.I., Hassan H. Suleiman., Abubakar A.S., Abubakar Y.K., Ali N.R., Umar M.S., Haladu S. and **Zulaiha Gidado Mukhtar** (2016). *Impact and Cost Effectiveness Study of Hardo Intervention in Polio Eradication*. *EJPMR*, 2016, 3(8), 105-110. ISSN 2394 – 3211.
9. **Zulaiha Gidado Mukhtar,** Ciroma, B. A., Rabiu, Z., Ahamad, I.M., Ibrahim, Y. and Yunusa, I. (2016). *Cinnamomun cassia*, *Ficus carica* and *Fumaria officinallis* possesses aphrodisiac activity in male Wistar rats. *Annals of Experimental Biochemistry***.** 2015, 3(3):14-17.
10. Yunusa I., Ibrahim M. A., Yakasai H. A., Ahmad I. M., Odo C. E., **Gidado Z. M.,** Rabiu Z., Kabir N., Ezeanyika L. U. S. (2015). Heavy Metals in Female Adolescents. *International Journal of Public Health Research*. Vol. 3, No. 1, 2015, pp. 37-43.
11. Ahmad I. M., Yunusa I., Wudil A. M., **Gidado Z. M.,** Sharif A. A., Kabara H. T. (2015). Knowledge, Attitude, Perception and Beliefs of Parents/Care givers About Polio Immunization. *International Journal of Public Health Research.* Vol. 3, No. 5, 2015, pp. 192-199 (2015).
12. Ahmad, I.M., Wudil, A. M., Yunusa, I., **Gidado, Z. M**., Sharif, A.A. and Kabara, H.T (2015). Oral administration of aqueous bark extract of *Vitex doniana* affects serum electrolytes levels in Albino rats. *Point J. Med. Med. Res.* 1(1): 001-005.
13. Yunusa, I., Ibrahim, M.A., Ahmad I.M., Kabir N., **Gidado Z.M.**, Kabara H.T., and Ezeanyika L.U.S. (2014). Vitamin A status for sustainable development. *Scholars Academic Journal of Biosciences.* 3(1).
14. Yunusa I., Ahmad I.M., **Gidado Z.M.**, Rabiu Z., Kabir, N., Kabara, H.T., Ezeanyika L.U.S. (2014). Antioxidant vitamins and body mass index in adolescents. *American Journal of Food Science and Nutrition Research***.** 6(1): 60 – 65.

Proceedings:

1. **Zulaiha Gidado Mukhtar,** Yusuf Ibrahim Ibrahim, Muhammad Salihu, Fikret Karatas, Dursun Ozer and Sinan Saydam (2020). Antioxidant Capacity of Different Varieties of Eggplant Fruits. *Fourth International Students Science Congress book of abstracts*, İzmir, Turkey, 18th - 19th September 2020.
2. Muhammad Salihu, Sani Ado, Maryam Habeeb, **Zulaiha Gidado Mukhtar,** and Yusuf Ibrahim Ibrahim (2020).Indices of Oxidative Stress in Experimental Rats exposed to Smoke from Meperfluthrin based Mosquito Coil Insecticide. *Fourth International Students Science Congress book of abstracts*, İzmir, Turkey, 18th - 19th September, 2020.
3. **Zulaiha Gidado Mukhtar,** Yusuf Ibrahim Ibrahim, and Sinan Saydam (2020). Carotenoid Content of Eggplant Fruits. *International Conference Book of Abstracts* .Third International Eurasian Conference on Biological and Chemical Science. 'EurasianBioChem 2020' in Ankara, Turkey, 19th – 20th March 2020.
4. Yusuf Ibrahim Ibrahim, **Zulaiha Gidado Mukhtar** and Fikret Karatas (2020). Cloves (*Syzygium aromaticum*) and Black pepper (*Piper nigrum*) contain high amounts of Resveratrol. *International Conference Book of Abstracts* .Third International Eurasian Conference on Biological and Chemical Science. 'EurasianBioChem 2020' in Ankara, Turkey, 19th – 20th March 2020.
5. Umar Muazu Yunusa, Sulaiman Danjuma Dausayi, **Zulaiha Gidado Mukhtar,** Wasiu Ayodele Abibu and Isa Yunusa (2019). Sero-prevalence of HIV and HBV infections among pregnant women attending some antenatal care services in Kano State, Nigeria. *International Student Symposium book of abstracts,* Fifth International Student Symposium*,* Trakya University, Edirne, Turkey, 6th - 8th December 2019.
6. Yusuf Ibrahim Ibrahim, **Zulaiha Gidado Mukhtar** and Fikret Karatas (2019). Assessment of Resveratrol in some selected foods. *National academic conference book of abstracts.* 37th Annual National Conference of

the Nigerian Society of Biochemistry and Molecular Biology (NSBMB), Umaru Musa Yar'adua University, Katsina, November 4th-8th, 2019.

7. **Zulaiha Gidado Mukhtar**, Yusuf Ibrahim Ibrahim, Hassan T. Kabara, Adamu J. Alhassan, Isa Yunusa, Zainab Rabiu, and Ahmad M. Isah (2019). *Leptadenia hastata* leaves elicit medicinal properties. *1st National conference and Exhibition in Science and Technology (SLT 2019) book of abstracts.* Kano State Polytechnic, Dept of Science Lab.Tech. 24th – 27th March 2019.

8. Yusuf Ibrahim Ibrahim and **Zulaiha Gidado Mukhtar** (2019). Polytechnics and Biochemistry as the driving force for self- reliance. *1st National conference and Exhibition in Science and Technology (SLT 2019) book of abstracts*. Kano State Polytechnic, Dept of Science Lab.Tech. 24th – 27th March 2019.

9. **Zulaiha Gidado Mukhtar**, Yusuf Ibrahim Ibrahim, and Fikret Karatas (2019). Amounts of Vitamin A, Vitamin E, Vitamin C, B-Carotene, Lycopene, Ghrelin, Glutathione, and MDA in Fruits of *Diospyros Kaki L. National academic conference book of abstracts. Nigerian Institute of Food Science and Technology.* 42nd Annual Conference and Annual General Meeting, Abeokuta Nigeria, 15th - 18th October, 2018

10. **Gidado Z. M**., Yunusa I., Ibrahim. Y. Ahmad I. M., Alhassan, A.J. and Badamasi, B., (2016). Antioxidant and Antipyretic activity of bark, leaves and fruit extract of Baobab in albino rats. *National academic conference book of abstracts. Nigerian Society of Biochemistry and Molecular Biology (NSBMB) 34TH annual conference,* Ogun, November 2016.

Projects:

1. Vitamin A status of primary school aged children in Kano state, Nigeria sponsored by the Tertiary Education Trust Fund Institution based research grant (Tetfund IBR grant) – 2015.
2. The Phytochemical screening and the effect of the aqueous extract of *Leptadenia hastata* leaves on some liver and kidney enzymes in rats- 2011.
3. The Quality assessment of Promethazine hydrochloride tablets sold within Kano metropolis. -2004.