PROXIMATE ANALYSIS AND INVITRO ANTIOXIDANTS CAPACITY ON MAX GLP-1

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A PROJECT SUBMITTED TO THE

DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCE, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THR REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCES (B.SC) IN MEDICAL BIOCHEMISTRY

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CERTIFICATION

This is to certify that EHIMWENMA OGHOSA HOLICE with matriculation number BMS2101395 of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin ,Benin City, in partial fulfillment of the requirements for the award of Bachelor of Science(B.sc) degree in Medical Biochemistry

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EXTERNAL EXAMINER **DATE**

DEDICATION

I dedicate my work to Almighty God, whose power, grace and discernment led me through every phase of this project work. May his light continue to guide and inspire me in whatever I do. All praise be unto Him.

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ABSTRACT

The proximate Analysis and In vitro Antioxidant Capacity of Max GLP-1, a nutraceutical supplement marketed for glucagon-like peptide-1 (GLP-1) activity enhancement, glucose control, and appetite regulation, were assessed in this study. Max GLP-1 is made with sorghum polyphenols, citrus flavonoids, and postbiotic compounds. There is little scientific evidence to support its nutritional and biochemical qualities, despite its growing consumer use. Moisture, ash, crude protein, crude fat, crude fiber, and carbohydrate contents were measured using standard AOAC techniques, and antioxidant activity was evaluated using 2,2-diphenyl-1-picryhydrazyl(DPPH)) radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays. Max GLP-1 is primarily carbohydrate-based (53.84%) with a high moisture content (42.74%) and very low levels of protein (1.42%), fiber (0.42%), fat (0.01%), and ash (1.57%), according to the proximate analysis. This indicates low nutrient density and suggests that phytochemicals, rather than macronutrients, are the primary source of its functional qualities. In contrast to the considerably higher activity of the reference standard, ascorbic acid (95.44% and 89.25%, respectively), antioxidant testing revealed moderate activity, with 44.61% 2,2-diphenyl-1-picryhydrazyl (DPPH)) radical inhibition and 41.56% Ferric Reducing Antioxidant Power (FRAP) lowering capacity. Although not in amounts equivalent to strong antioxidants, these results verify the existence of somewhat active antioxidant components such polyphenols and flavonoids. Overall, the study does not support claims about GLP-1 augmentation, weight regulation, or metabolic benefit, but it does offer some scientific evidence for the supplement's antioxidant capabilities. To support the more general health claims connected to Max GLP-1, more study incorporating phytochemical measurement, bioactivity profiling, and clinical trials is needed.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

In recent years, the focus on metabolic health and weight management has shifted from pharmaceutical remedies to natural and nutraceutical options. Max GLP-1 is a herbal-based dietary supplement that supports the body's natural synthesis of Glucagon-Like Peptide-1 (GLP-1). While prescription GLP-1 medications like semaglutide (Ozempic) and liraglutide (Saxenda) have been well-studied and approved for diabetes and obesity management, supplements like Max GLP-1 are marketed as natural, plant-derived support systems for appetite regulation and blood sugar balance (Cleveland Clinic, 2025; Harvard Health, 2025).

According to its manufacturer, Max GLP-1 is a dietary or nutraceutical supplement that boosts the body's natural synthesis of GLP-1, reduces cravings, supports pancreatic function, maintain a healthy metabolism and weight control. It is normally sold in capsule form, but patch versions are also available in specific product packages. However, unlike prescription pharmaceuticals, regulatory bodies such as the United States Food and Drug Administration (FDA) have not approved the supplement for illness treatment or prevention. The proglucagon gene encodes GLP-1, a peptide hormone released by intestinal L-cells in response to meal intake. It regulates glucose levels by increasing insulin production from pancreatic β-cells, inhibiting glucagon release, prolonging stomach emptying, and promoting satiety (Holst, 2022). These combined effects make GLP-1 a potent target for metabolic control and GLP-1 might be described as the body's ‘fullness and balance hormone’.

The United States Food and Drug Administration (FDA) and the Medicines and Health Care Products Regulatory Agency (MHRA) (MHRA2025) state that supplements are governed as

foods rather than medications. This implies that their manufacturers are not obliged to prove clinical efficacy prior to commercialization, even if they must be safe for ingestion. Therefore, even though Max GLP-1 might have beneficial nutritional effects, it shouldn't be used in place of prescription drugs. However, medical experts suggest being cautious. Before using the product, anyone who are pregnant, nursing, have diabetes, or are taking medication should speak with a doctor. Drug-supplement interactions are possible since it includes bioactive substances that could affect blood-sugar metabolism (Diatribe, 2025). Additionally, consumers should understand that phrases like "natural" or "herbal" on labels do not always imply efficacy or safety. Claims must be independently verified through laboratory examination, such as proximate composition, antioxidant activity. Studies like "Proximate Analysis and In-vitro Antioxidant Capacity of Max GLP-1," which assess the supplement's true nutritional and bioactive capabilities, are based on this scientific foundation.

1.2 Problem Statement:

Despite the fact that GLP-1 receptor agonist medications are useful for treating metabolic problems, many consumers are turning to herbal substitutes like Max GLP-1 due to its high cost and adverse effects. Nevertheless, there is little scientific data to support Max GLP-1, and the majority of claims lack peer-reviewed validation. Additionally, nothing is known about its antioxidant qualities and nutritional makeup. Therefore, to confirm the supplement's biochemical properties and any health effects, a thorough proximate analysis and in-vitro antioxidant evaluation are required.

1.3 Justification of Study

Although the Max GLP-1 supplement is marketed as herbal nutraceutical that increases natural GLP-1 secretion for appetite, metabolism, and blood-sugar regulation, there is little scientific evidence to support its cla ims, particularly with regard to its nutritional makeup

and antioxidant potential. For this reason, the study is crucial. While In-vitro antioxidant assays will assess its capacity to counteract free radicals associated with chronic diseases like diabetes, obesity, and cardiovascular disorders. Proximate analysis will identify its protein, fat, fiber, carbohydrate, moisture, and ash contents to determine its dietary value. This study will provide empirical validation, improve consumer and professional understanding, support regulatory transparency, and encourage the safe use of herbal nutraceuticals because Max GLP-1 contains bioactive substances such berberine, curcumin, and catechins with unknown effects.

1.4 Aim of Study

To evaluate the proximate composition and in-vitro antioxidants capacity of Max GLP-1 dietary supplement.

1.5 Objectives of the study

- To ascertain the Max GLP-1 supplement's proximate composition (moisture, ash, crude protein, crude fat, fiber, and carbohydrate content).
- To evaluate Max GLP-1's in vitro antioxidant capability using common laboratory tests E.g 2,2- Diphenyl-1picrylhydraxyl (DPPH), 2,2'- Azino-bis(3 ethylbenzothiazoline-6-sulfonic acid) (ABTS) and Ferric Reducing Antioxidants Power (FRAP).

CHAPTER TWO

LITERATURE REVIEW

2.1 Theoretical Framework

Any scientific investigation must include a review of the body of current literature since it offers the theoretical and empirical framework for the research project. Understanding the proximate composition and antioxidant capacity of food supplements is essential for assessing their nutritional quality and potential health benefits in the context of food science and nutritional biochemistry. As a result, this chapter provides a thorough overview of the ideas, theories, and research pertaining to in-vitro antioxidant capacity and proximate analysis, especially as they apply to dietary supplements like Max GLP-1.

The Dietary supplement Max GLP-1 targets pathways linked to the proglucagon gene (GCG), whose expression gives rise to glucagon-like peptide-1 (GLP-1), the proglucagon gene produces the 30- or 31-amino acid an incretin hormone that is mostly produced in the colon and distal small intestine by enteroendocrine L-cells. It increases glucose-dependent insulin secretion, inhibits glucagon secretion, slows stomach emptying, and decreases hunger via acting on the central nervous system. It is released in response to nutritional intake (Holst, 2007).

GLP-1, which was identified in the 1980s as a post-translational product of proglucagon, is essential for maintaining glucose homeostasis because it suppresses glucagon release, delays gastric emptying, increases satiety, and stimulates insulin secretion in a glucose-dependent manner (Alotaibi *et al*., 2025). Its short half-life of 1-2 minutes in circulation due to its quick breakdown by dipeptidyl peptidase-4 (DPP-4) has prompted the creation of synthetic analogs and receptor agonists for type 2 diabetes treatment and becoming overweight. GLP-1 receptor agonists (GLP-1RAs), including tirzepatide, liraglutide, semaglutide, and exenatide, have transformed the treatment of metabolic disorders over the last ten years by exhibiting

protective benefits on the kidneys and heart in addition to glycemic control. The processing of proglucagon (GCG) yields GLP-1. GLP-1(7-37) and GLP-1(7-36) amide are the two main physiologically active variants in humans. The enzyme dipeptidyl peptidase-4 (DPP-4) quickly inactivates native active GLP-1 de plasma, resulting in an active circulating half-life of less than two minutes. Drug development tactics were motivated by this quick deterioration (Ahren and Schmitz 2004).

The proglucagon gene, which produces glucagon-like peptide-1 (GLP-1), is linked to Max GLP-1 has been shown to have antioxidant qualities in addition to its traditional incretin actions, making it a versatile therapeutic treatment against oxidative stress-related diseases (Bakry and Hamada 2025). Beta-cell dysfunction, endothelial damage, and chronic inflammation in diabetes are all influenced by oxidative stress, which is defined as an imbalance between reactive oxygen species (ROS) production and antioxidant defenses. By increasing cellular antioxidant capacity through nuclear factor activation, GLP-1 and its agonists lessen this.

Proximate analysis is a basic technique for figuring out the basic chemical composition of foods and supplements, By measuring important macronutrients such moisture, ash, crude protein, crude fat, crude fiber, and carbohydrate, (AOAC International, 2019). It is typically used for food and plant materials, its adaptation to peptide hormones like GLP-1 entails evaluating formulation stability, compositional purity, and amino acid profiling in pharmaceutical contexts. For synthetic GLP-1 analogs, proximate-like analysis might include quantification of peptide content, impurities, and excipients employing methods like mass spectrometry (MS) and high-performance liquid chromatography (HPLC). Such studies are necessary to guarantee prolonged release and receptor activation in vivo, as demonstrated by recent advancements in MaxGLP-1-albumin conjugates (Olukorede *et al*., 2024).The

combination of Max GLP-1's metabolic actions and antioxidant capability has created opportunities for new treatments.

Due to a growing awareness of oxidative stress and its role in chronic diseases like diabetes, cardiovascular problems, and obesity, the antioxidant potential of dietary supplements has received increased attention worldwide in recent years. Compounds known as antioxidants shield biological systems from the harmful effects of reactive oxygen species (ROS) and free radicals. Therefore, a substance's potential to prevent or lessen oxidative damage can be determined by its in-vitro antioxidant capacity (Halliwell and Gutteridge, 2015; Re *et al*., 1999). Prior to in-vivo validation, assessing antioxidant activity in vitro with tests like 2,2-diphenyl-1-picryhydrazyl(DPPH), 2,2azinobis(3-ethylbenzothiazolene-6-sulphonic acid)(ABTS). , or Ferric Reducing Antioxidants Power (FRAP) offers initial proof of the sample's functional potential.

However, there is little scientific data on the nutritional makeup and antioxidant potential of Max GLP-1. This emphasizes the necessity of empirical research that integrates proximate analysis and in-vitro antioxidant assessment to offer scientific support for the purported advantages of such supplements.

2.1.1 Concept of Proximate Analysis

One of the most basic and popular techniques for assessing the fundamental chemical composition of food substances, feed materials, and dietary supplements is proximate analysis, which provides an estimate of the major nutrient fractions—moisture, ash, crude protein, crude fat, crude fiber, and carbohydrates(AOAC International, 2019)—that make up the bulk of the sample . This analytical process offers a quick, affordable, and standardized way to determine the nutritional content of biological materials (FAO, 2020).

The phrase proximate analysis refers to the purpose of providing a "proximate," or approximate, picture of a sample's nutritional content rather than its precise molecular makeup. Each metric found during proximate analysis provides unique information about the sample's chemical and nutritional properties, as outlined below.

- Moisture Content: The amount of water in a food or supplement is known as its moisture content, and it is commonly stated as a percentage of the sample's total weight. Typically, determination is accomplished by oven-drying at 105°C until a consistent weight is attained (AOAC International, 2019). Since low moisture improves storability and high moisture encourages microbiological growth and chemical spoiling, moisture content is a crucial determinant of stability, freshness, and shelf life (Oduse *et al*.,2020).. To preserve product integrity and avoid clumping of active component degradation, the moisture content of powdered nutritional supplements like Max GLP-1 must be regulated.
- Ash Content:

Ash is the total amount of inorganic residue left over after all of the organic stuff in a sample has burned completely at high temperatures(usually 550–600°C).It acts as a guage for the overall mineral content, which includes vital components like calcium, potassium, sodium, iron, and zinc (FAO, 2020). Total ash measures the overall presence of minerals, however spectroscopic techniques (such as atomic absorption spectroscopy) can be used to perform specific mineral analysis later on. A supplement's ash content can reveal information about its mineral composition or whether non-nutritive inorganic residues are present.

- Crude Protein:

The kjeldahl method, which analyses total nitrogen in a sample and multiplies it by a conversion factor (often 6.25) to estimate crude protein (AOAC International, 2019), is frequently used to quantify protein content, a crucial nutritional metric. Growth, repair, the production of enzymes, and metabolic control all depend on proteins. Because, certain

bioactive peptides may contribute to metabolic and antioxidant effects, protein content is especially important in supplements like Max GLP-1 (Drucker, 2018). The functional and nutritional value of the food may also be improved by a high protein content.

- Crude Fat:

Crude fat, which indicates the samples total lipid content, is measured using solvent extraction techniques (such as Soxhlet extraction). A significant source of energy, fat also makes it easier for fat-soluble vitamins (A, D, E, and K) to be absorbed. Additionally, it affects the calorie density, taste, and texture of supplements. In the context of antioxidant evaluation, lipid fractions may contain lipophilic antioxidants such as carotenoids or tocopherols that contribute to overall antioxidant potential (Huang *et a*l., 2005).

- Crude fibre:

The indigestible part of plant materials is called crude fiber, and it is mostly made up of cellulose, hemicellulose, and lignin. Successive acid and alkaline digestion is used to determine it (AOAC International, 2019). Fiber is known to improve glycemic management, lower cholesterol absorption, and support digestive health. This is especially important for supplements that target glucose metabolism, including Max GLP-1 (Anderson *et al*., 2009). Thus, a supplement's crude fiber content offers information about its possible physiological advantages, particularly with regard to controlling blood sugar and weight.

- Carbohydrates contents :

A common method for calculating carbohydrates is to deduct the total of moisture, ash, protein, fat, and fiber from 100. With 4kcal/g, carbohydrates are the main source of energy in most diets. The kind of carbohydrates (complex vs. simple) in nutritional supplements affects metabolic impact, energy release, and digestion. Moderate carbohydrate content in GLP-1-

based supplements may assist postprandial glucose management by stimulating endogenous GLP-1 production (Holst, 2007).

2.1.2 Standard Procedures and Guidelines:

The Food and Agriculture Organization (FAO), the International Organization for Standardization (ISO), or the Association of Official Analytical Chemists (AOAC) have produced defined techniques for proximate analysis. These techniques guarantee consistency and repeatability of findings between laboratories. For instance (AOAC International, 2019), describes certain techniques for figuring out each proximal parameter such as:

AOAC 925.10 for moisture content

AOAC 923.03 for Ash

AOAC 979.09 for crude protein (Kjeldahl method)

AOAC 920.39 for crude fat (Soxhlet extraction)

AOAC 962.09 for crude fibre

Researchers can collect data that is compatible with international analytical standards and comparable to earlier investigations by adhering to these established techniques.

2.1.3 Principles of Proximate Analysis

By dividing a food or biological substance into its key chemical constituents—moisture, ash, crude protein, crude fat, crude fiber, and carbohydrate—proximate analysis is a traditional analytical technique used to ascertain the fundamental nutritional makeup of the material. Together, these elements provide information on the sample's proximate chemical composition and its nutritional and functional qualities (AOAC International, 2019). The Association of Official Analytical Chemists' (AOAC) standardized gravimetric, titrimetric,

and spectrophotometric techniques serve as the basis for the analysis. Among the fundamental analytical concept are:

- Moisture Contents: A weighed sample is oven-dried at 105 °C until a consistent weight is achieved in order to determine the moisture content. The water content is correlated with the weight loss. Microbial deterioration and enzymatic degradation are accelerated by excessive moisture.
- Ash content is the total amount of mineral residue left over after organic matter is completely burned at 550 °C in a muffle furnace. It helps assess mineral purity and fortification and reflects the sample's inorganic composition (such as Ca, Mg, Fe, and K) (James, 2013).
- Crude Protein: The Kjeldahl method, which analyzes total nitrogen and multiplies it by a conversion factor (often 6.25), is used to calculate crude protein. This is predicated on the idea that proteins contain roughly 16% nitrogen. The outcome shows the sample's ability to sustain enzymatic processes and tissue growth (AOAC International, 2019).
- Crude fat is extracted in a Soxhlet system using non-polar solvents like hexane or petroleum ether. Energy density, palatability, and lipid-soluble nutrient transport are all influenced by the fat content of the residue left behind after solvent evaporation.
- Crude fiber is produced by sequentially breaking down soluble proteins, lipids, and carbohydrates using diluted acid and alkali. Cellulose and lignin, which signal digesting bulk and gastrointestinal health advantages, make up the majority of the residue that remains.
- Carbohydrate (by Difference): Determined indirectly as:

100- (Moisture + Ash +Protein +Fat+ Fibre)

Collectively, these determinations yield a proximate profile that characterizes the foods nutritional value, energy potential, and processing quality.

2.1.4 Importance of Proximate Analysis:

Proximate analysis, according to (Oyeyinka *et al*., 2019), assists in determining whether a supplement's nutrient content corresponds with its intended physiological function. For Max GLP-1 proximate analysis will establish its nutrient profile particularly protein and fat levels which may influence its antioxidant properties and metabolic effects.

1. Nutritional Evaluation: Proximate composition makes it possible to compare a product's nutritional density and caloric value with dietary requirements. For instance, a high crude fiber content enhances digestion, whereas a high protein level suggests a possible supplement for muscle synthesis (Olapade and Aworh, 2012).
2. Quality Control and Standardization: Proximate analysis is used in the food and supplement sector as a quality-control method to guarantee product consistency, adherence to labeling regulations, and adulteration detection (FAO, 2016).
3. Formulation of Functional Foods and Supplements: Optimizing the nutritional ratios of functional formulations, like Max GLP-1, for metabolic and antioxidant effects requires an understanding of macronutrient composition.
4. Shelf-Life Prediction: A product's vulnerability to microbial growth and rancidity is mostly determined by its moisture and fat concentrations. Stability and storage life are enhanced by reduced moisture.

2.2 Concept of Antioxidants

Antioxidants are bioactive compounds that neutralize free radicals and Reactive oxygen species (ROS) to prevent or postpone the oxidation of other molecules. Oxidative reactions are normal metabolic processes in biological systems, but too much Reactive oxygen species (ROS) can harm proteins, lipids, and DNA in cells, causing oxidative stress and the emergence of chronic illnesses (Halliwell and Gutteridge 2015).

An imbalance between the body's antioxidant defense systems and Reactive oxygen species (ROS) production leads to oxidative stress. By scavenging free radicals, chelating metal ions, and preventing oxidative chain reactions, antioxidants reverse this imbalance.

2.2.1 Classification of Antioxidants

Antioxidants are substances that mitigate the harmful effects of reactive nitrogen species (RNS) and reactive oxygen species (ROS). They can be classified based on:

Based on origin

- Endogeneous antioxidants:

These antioxidants are synthesized naturally within the body. Examples include Superoxide dismutase, Catalase, Glutathione-peroxidase, Uric acid and Coenzyme Q10.

- Exogeneous Antioxidants: These antioxidants are obtained from diet mainly fruits, vegetables and supplements such as Max GLP-1.These include Vitamin C and E, Carotenoids ,Polyphenols and Flavonoids.

Based on their sources and modes of action, these antioxidants are typically divided into two main categories: enzymatic antioxidants and non-enzymatic antioxidants (Pizzino *et al*., 2017;Halliwell and Gutteridge 2015).

- Enzymatic Antioxidants:

Catalytic proteins known as enzymatic antioxidants reduce reactive oxygen species (ROS) by transforming them into less reactive species through enzymatic processes. They are the body's main line of defense against oxidative damage.

1. Superoxide Dismutase (SOD): The first line of defense against reactive oxygen species (ROS) is Superoxide Dismutase (SOD). It catalyzes the dismutation of the superoxide anion

(O_2^{-}•) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). Humans have three primary isoforms of Superoxide Dismutase (SOD): extracellular (EC-SOD), mitochondrial (Mn-SOD), and cytosolic (Cu/Zn-SOD) (Halliwell and Gutteridge, 2015). SOD stops chain reactions that can harm DNA and cell membranes by eliminating superoxide radicals.

2. Catalase (CAT): Catalase breaks down hydrogen peroxide into oxygen and water. It is mostly present in peroxisomes. It is especially active in the kidney, liver, and red blood cells. Catalase is essential for preventing oxidative cell damage because H_2O_2 can produce extremely reactive hydroxyl radicals through the Fenton reaction (Pizzino *et al*., 2017).

3. Glutathione Peroxidase (GPx): Glutathione Peroxidase (GPx) uses glutathione (GSH) as a substrate to convert hydrogen peroxide and lipid hydroperoxides to water and related alcohols. Glutathione reductase (GR) then uses Nicotinamide Adenine Dinucleotide Phosphate (NADPH) to regenerate the oxidized form of glutathione (GSSG). Cellular membranes are shielded against lipid peroxidation by Glutathione Peroxidase (GPx), which is dependent on selenium (Lobo *et al*., 2010).

4. Glutathione Reductase (GR): By converting Glutathione di-sulphide (GSSG) back to Glutathione (GSH), Glutathione Reductase (GR) preserves the cellular store of reduced glutathione, a significant non-enzymatic antioxidant. According to (Halliwell and Gutteridge 2015), this regeneration process guarantees a steady supply of Glutathione (GSH) for detoxifying reactions. These enzymes cooperate to preserve redox homeostastis, which keeps harmful radicals from building up in biological systems.

- Non-Enzymatic:

Low-molecular-weight substances that scavenge radicals, bind metal ions, or stop oxidative chain reactions are examples of non-enzymatic antioxidants. They are either produced naturally or obtained through food.

1. Vitamins

- Vitamin E (α-tocopherol), a lipid-soluble antioxidant, shields cellular and subcellular membranes. By stopping lipid peroxidation, It creates a stable tocopheroxyl radical by giving lipid radicals a hydrogen atom (Lobo *et a*l., 2010).
- Ascorbic acid, also known as vitamin C, is a water-soluble antioxidant that directly scavenges free radicals and replenishes oxidized vitamin E. Additionally, it prevents radical production through Fenton chemistry by reducing metal ions like Fe^{3+} to Fe^{2+}.
- Vitamin A: Membranes and Low-Density Lipoprotein (LDL) cholesterol are shielded from oxidative damage by vitamin A (retinoids and carotenoids), which are lipid-soluble pigments that quench singlet oxygen and prevent lipid peroxidation (Tanumihardjo *et al*., 2011).

2. Flavonoids and phenolic compounds

They are common secondary metabolites found in plant-based meals and supplements. They have hydroxyl groups that have the ability to donate hydrogen atoms in order to counteract free radicals. It has been demonstrated that flavonoids, phenolic acids, tannins, and anthocyanins have potent metal-chelating and radical-scavenging properties (Nwachukwu *et al.,* 2021)

3. Glutathione (GSH):

The body produces this tripeptide (γ-glutamyl-cysteinyl-glycine), which is essential for detoxification since it immediately neutralizes free radicals and acts as a substrate for Glutathione Peroxidase (GPx). Its capacity to reduce is due to its thiol group (-SH) (Pizzino *et al*., 2017).

4. Minerals and Trace Elements:

Antioxidant enzymes require cofactors such as iron, manganese, copper, zinc, and selenium. For example, Cu, Zn, and Mn are cofactors for SOD, whereas selenium is essential for Glutathione Peroxidase.

5. Additional Dietary Antioxidants: Other substances with antioxidant action include melatonin, uric acid, and coenzyme Q10. They either renew additional antioxidants or scavenge reactive species (Halliwell and Gutteridge, 2015).

2.2.2 Enzymatic and Non-Enzymatic Interactions:

The network of enzymatic and non-enzymatic antioxidants is complimentary. For instance, oxidized vitamin E is regenerated by vitamin C, while superoxide radicals are transformed into innocuous water molecules by the sequential action of Glutathione Peroxidase and Superoxide dimutase. In both hydrophilic and lipophilic settings, this joint defense makes sure that oxidative stress is reduced (Pizzino *et al*., 2017).

2.2.3 Pertinence to the Current Research:

Both enzymatic and non-enzymatic antioxidants contribute to the biological effects of dietary supplements like Max GLP-1. The presence of phenolic compounds, peptides, or other bioactive molecules that function through the previously mentioned processes may determine the antioxidant activity of the supplement. Thus, determining the kind and potency of antioxidants in Max GLP-1 is essential to comprehending its in vitro antioxidant capacity and health consequences.

2.2.4 Mechanisms of Antioxidants Action:

Antioxidants shield biological systems from the harmful effects of reactive nitrogen species (RNS) and reactive oxygen species (ROS). Several metabolic pathways are involved in their

methods of action, which either stop radical chain reactions, stop the production of free radicals, or fix oxidative damage after it has already happened(Halliwell and Gutteridge,2015; Pizzino *et al*., 2017).

- Preventive Antioxidants Mechanisms:

Preventive antioxidants work by either decreasing the production of reactive species or preventing biological targets from accessing them. They stop oxidative chain reactions before they start. Among the main preventive measures are:

A. Metal ion Chelation

Transition metals such as iron (Fe^{2+}/Fe^{3+}) and copper (Cu^{+}/Cu^{2+}) catalyze the formation of reactive hydroxyl radicals through the Fenton and Haber-Weiss reactions:

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {\cdot}OH$

B. Reactive oxygen species are catalytically eliminated by enzymatic antioxidants such as glutathione peroxidase (GPx), Catalase (CAT) and Superoxide dismutase (SOD) before they interact with biomolecules.Superoxide radicals ($O_2^{-}\bullet$) are converted by SOD (Superoxide Dismutase) into oxygen and Hydrogen Peroxide(H_2O_2). H_2O_2 is broken down by CAT (Catalase) into oxygen and water. Gpx (Glutathione Peroxidase) uses glutathione (GSH) to convert H_2O_2 and lipid hydro-peroxides to water and nontoxic alcohols (Lobo *et al*., 2010; Pizzino *et al*., 2017).

C. Pro-oxidants compartmentalization

In order to prevent uncontrollable redox reactions, cells sequester metal ions and ROS in particular organelles (such as peroxisomes) or bind them to proteins (such as ferritin binds Fe^{3+}) (Halliwell and Gutteridge, 2015).

2 Repair and Regeneration Mechanisms:

Certain antioxidants maintain an ongoing protective network by regenerating or recycling other oxidized antioxidants

A. Antioxidants Synergy:

- Vitamin C regenerates vitamin E From its oxidized tocopheroxyl radical state,
- Glutathione (GSH) regenerates oxidized ascorbate (Pizzino *et al*., 2017) In addition to being a substrate for glutathione peroxidase (GPx),

By so doing, this interaction enhances the overall antioxidant capacity of cells and supplements such as Max GLP-1.

B. Reparing proteins and DNA

The consequences of oxidative stress are reversed by enzymes such as methionine sulfoxide reductase and DNA glycosylases, which repair oxidatively damaged proteins and DNA, respectively (Halliwell and Gutteridge, 2015).

3 Inhibition of lipid Peroxidation:

Membrane damage results from the oxidative breakdown of polyunsaturated fatty acids, a process known as lipid peroxidation. This process is inhibited by antioxidants at several stages:

- Inhibition of Initiation: Superoxide Dismutase (SOD) and Catalase (CAT) stop reactive oxygen species (ROS) from initiating lipid oxidation.
- Propagation Inhibition: By giving hydrogen to lipid radicals, vitamin E and flavonoids break the chain.
- Termination: The process is stopped by radical-radical recombination, which produces stable products (Lobo *et al*., 2010).

In vitro antioxidant tests of 2,2-diphenyl-1-picryhydrazyl(DPPH), 2,2 azinobis(3-ethylbenzothiazolene-6-sulphonic acid)(ABTS), and Ferric Reducing Antioxidants Power (FRAP) tests are based on these pathways (Re *et al*., 1999; Nwachukwu *et al.,* 2021). These tests are frequently used to evaluate the antioxidant potential of food samples, plant extracts, and dietary supplements.

2.2.5 Roles of Antioxidants in Health

Antioxidants have several physiological roles including:

i. Defence against oxidative damage:

Prior to attacking essential cellular components, they eliminate Reactive oxygen species (ROS). For example, vitamin E (α-tocopherol) prevents lipid peroxidation in membranes, while vitamin C (ascorbic acid) scavenges free radicals in aqueous environments and regenerates vitamin E from its oxidized form (Lobo *et al*., 2010).

ii. Preventing chronic illnesses:

By reducing oxidative stress, research has connected antioxidants to protection against cancer, neurological diseases like Alzheimer's, and cardiovascular diseases (Pizzino *et al*., 2017).

iii. Cellular longitivity and antiaging:

Antioxidants prolong cell life and enhance tissue function by slowing cellular senescence through the reduction of oxidative stress (Halliwell and Gutteridge, 2015).

iv. Foods functional and nutritional value:

Antioxidants stop oxidation-induced rancidity, color changes, and nutritional loss in food systems. Due to safety concerns, natural antioxidants derived from plants, such as polyphenols and flavonoids, are becoming more popular than synthetic ones, such as

butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Nwachukwu *et al.,* 2021).

2.2.6 Dietary sources of Antioxidants:

Fruits, vegetables, herbs, drinks, and dietary supplements are rich sources of antioxidants.

Typical sources of antioxidants includes:

- Citrus fruits, berries, guava, and broccoli are good sources of vitamin C.
- Nuts, seeds, vegetable oils, and spinach are good sources of vitamin E.
- Carotenoids include leafy greens, tomatoes, sweet potatoes, and carrots.
- Green tea, cocoa, grapes, and herbal supplements like Max GLP-1, which may contain plant-based antioxidants, are examples of polyphenols and flavonoids (Drucker, 2018; Nwachukwu *et al.,* 2021).

Together, these substances increase the body's antioxidant capacity. For example, vitamin C maintains its ability to scavenge radicals by regenerating oxidized vitamin E.

2.2.7 Antioxidants and Human Health

The pathophysiology of metabolic illnesses such as type 2 diabetes mellitus, where excess ROS decreases insulin sensitivity and pancreatic β-cell activity, is greatly influenced by oxidative stress. By improving glucose metabolism and lowering oxidative inflammation, glucagon-like peptide-1 (GLP-1) supplements, including Max GLP-1, may have indirect antioxidant effects (Drucker, 2018).

Additionally, in vitro research on antioxidant capacity sheds light on how supplements fight oxidative stress by lowering power, metal chelation, and free-radical scavenging—all of which enhance cellular and metabolic health overall (Nwachukwu *et al.,* 2021).

2.3 In-vitro Antioxidants Assays Method

2.3.1 Introduction

The ability of a substance, extract, or supplement to scavenge free radicals is assessed using laboratory-based analytical methods called in-vitro antioxidant assays (Nwachukwu *et al.,* 2021).

These asssays aid in understanding the mechanism of antioxidant activity, comparing the activities of various samples, and measuring antioxidant capacity. They are crucial instruments for evaluating the possible health advantages of dietary supplements as Max GLP-1, herbal items,and functional foods(Re *et al*., 1999).No single technique can fully capture all antioxidant pathways since oxidative damage in biological systems is caused by a variety of reactive species. For a more trustworthy assessment, several complementary in-vitro tests based on single electron transfer (SET) or hydrogen atom transfer (HAT) principles are frequently used (Nwachukwu *et al.,* 2021).

2.3.2 Principles of in-vitro Antioxidant Assays

The two main tenets of antioxidants assays are:

1. Assays for Hydrogen Atom Transfer(HAT):

Measures Antioxidants' capacity to donate hydrogen atoms to quench free radicals can be measured. Examples include Assays for linoleic acid peroxidation and oxygen radical absorbance capacity (ORAC) (Prior *et al*., 2005).

2. Single Electron Transfer (SET) Assays:

Determine an antioxidant's ability to decrease an oxidant by transferring one electron. 2,2-diphenyl-1-picryhydrazyl (DPPH)),2,2-azinobis(3-ethylbenzothiazolene-6-sulphonicacid)

(ABTS) and Ferric Reducing Antioxidants Power (FRAP) tests are a few examples (Nwachukwu *et al.,* 2021).

2.3.3 Common In-vitro Antioxidant Assay Methods

Below are the main assays widely used in antioxidant research and applicable for Max GLP-1

1. 2,2-diphenyl-1-picryhydrazyl(DPPH) Radical Scavenging Assay:

One of the most straightforward and trustworthy methods for assessing radical-scavenging activity is the 2,2-diphenyl-1-picryhydrazyl(DPPH)) assay.

The basic idea is that 2,2-diphenyl-1-picryhydrazyl(DPPH) is a stable free radical with a significant absorption at 517 nm and has a deep violet color. A spectrophotometer can be used to quantify the drop in absorbance that occurs when an antioxidant reduces 2,2-diphenyl-1-picryhydrazyl(DPPH) • to 2,2-diphenyl-1-picryhydrazyl(DPPH) -H by donating an electron or hydrogen atom (Brand-williams *et al*.,1995).

DPPH . + AH- → DPPH-H + A.

The antioxidant potency is shown by the percentage inhibition or IC_{50} value; stronger antioxidant activity is correlated with lower IC_{50}values.

Since the 2,2-diphenyl-1-picryhydrazyl(DPPH) assay is quick, repeatable, and doesn't require complicated reagents, it is frequently utilized for natural extracts and supplement (Nwachukwu *et al.,* 2021).

2. 2,2 azinobis(3-ethylbenzothiazolene-6-sulphonic acid)(ABTS) Radical Cation Decolorization Assay:

Another widely used electron transfer based technique is the 2,2 azinobis(3-ethylbenzothiazolene-6-sulphonic acid(ABTS),Which involves using potassium Per-sulfate, 2,2 azinobis(3-ethylbenzothiazolene-6-sulphonic acid)(ABTS) is oxidized to its radical cation 2,2 azinobis(3-ethylbenzothiazolene-6-sulphonic acid)(ABTS+.), resulting in a blue-green hue with a maximum absorbance at 734 nm. Antioxidants decrease absorbance proportionally to antioxidant content by reducing 2,2 azinobis(3-ethylbenzothiazolene-6-sulphonic acid)(ABTS) back to its colorless state(Re *et al*.,1999).

ABTS+. + AH → ABTS + A. + H+

Benefits

- Suitable for both hydrophilic and lipophilic antioxidants
- Fast and sensitive
- Trolox Equivalent Antioxidant Capacity (TEAC), which compares the sample's activity to that of Trolox, a vitamin E mimic, can be used to express the results.

3. Assay for Ferric Reducing Antioxidants Power (FRAP):

The Ferric Reducing Antioxidants Power (FRAP) assay assesses how well antioxidants reduce (Fe^{3+}) ions to ferrous ions (Fe^{2+}).

In an acidic environment, a Fe^{3+}–TPTZ (2,4,6-tripyridyl-s-triazine) complex is reduced to its blue-colored Fe^{2+}–TPTZ form. The potential to reduce antioxidants is indicated by the rise in absorbance at 593 nm (Benzie and Strain, 1996).

Fe^{3+}(TPTZ) + e- → Fe^{2+}(TPTZ).

Benefits

i. Easy and affordable
ii. Extracts from food and plant are highly reproducible
iii. Measure total reproducing power: representing electron donating potential

Limitation: Thiol-type antioxidants and substances acting through hydrogen atom transfer pathways are not well measured by Ferric Reducing Antioxidants Power (FRAP) (Nwachukwu *et al.,* 2021).

4. Assay for Hydrogen Peroxide(H_2O_2) Scavenging:

Despite not being a free radical, Hydrogen peroxide readily diffuses across membranes and when metal ions are presents produces hydroxyl radicals(•OH).

Antioxidants limit the generation of hydroxyl radicals by breaking down H_2O_2 into oxygen and water. Spectrophotometric measurements of the residual H_2O_2 are made at 230nm.

$H_2O_2 + 2AH \rightarrow 2H_2O + A_2$

Antioxidants' capacity to neutralize peroxide species and stop oxidative damage in biological systems is revealed by this assay.

5. Reducing Power Assay

This test evaluates antioxidants' capacity to donate electrons to convert Fe^{3+} to Fe^{2+}.

The samples antioxidants converts potassium ferric cyanide (Fe^{3+}) to ferrous (Fe^{2+}), which reacts with ferric chloride to generate a Perl's Prussian blue complex. Reduced strength is indicated by the absorbance at 700nm.

Greater electron-donating capacity and, thus, stronger antioxidant potential are indicated by higher absorbance.

2.3.4 Importance of In-vitro Assays in Supplement Evaluation

Understanding the antioxidant processes of dietary supplements like Max GLP-1, which are marketed for health advantages related to oxidative stress reduction, requires in-vitro experiments. These tests:

1 Verify health claims using scientific evidence.

2 Permit standardization and supplement quality control.

3 Assist in establishing a correlation between antioxidant activity and proximate composition, such as protein, lipid, and phenol concentration.

4 Function as a screening stage prior to biological tests conducted in vivo (Nwachukwu *et al.,* 2021: Lobo *et al*., 2010).

In conclusion, Reliable and affordable methods for determining antioxidant capability are in-vitro antioxidant tests. The most popular ones for food and supplement research include 2,2-diphenyl-1-picryhydrazyl(DPPH)), 2,2 azinobis(3-ethylbenzothiazolene-6-sulphonic acid) (ABTS), Ferric Reducing Antioxidants Power (FRAP), and Hydrogen Peroxide (H_2O_2) scavenging tests. Together, these techniques provide a comprehensive picture of Max GLP-1's antioxidant capacity. Each technique represents a different facet of antioxidant activity, such as radical scavenging, reducing power, or metal ion reduction.

2.4 Overview of Max GLP -1

MaxGLP-1 is touted as a "natural GLP-1 booster" for healthy weight control, with one capsule per day said to reduce cravings and appetite, improve blood sugar stability, and assist users "lose fat, keep muscle." According to the company's website (Max GLP-1, 2025), A proprietary "GLP-1 Weight Management Blend" (502 mg total) and chromium picolinate 200 µg (571% DV) are listed in the manufacturer's Supplement Facts (PDF). Redaxin (sorghum

polyphenol extract), Eriomin (lemon flavonoid/eriocitrin-rich powder), Pozibo (Heat treated/ postbiotic Lactobacillus Paracasei D3.5) and chromium picolinate are the names of the unique blend ingredients. Hypromellose, silica, magnesium stearate, and microcrystalline cellulose are additional excipients used in capsules (30 servings per container; 1 pill each serving).

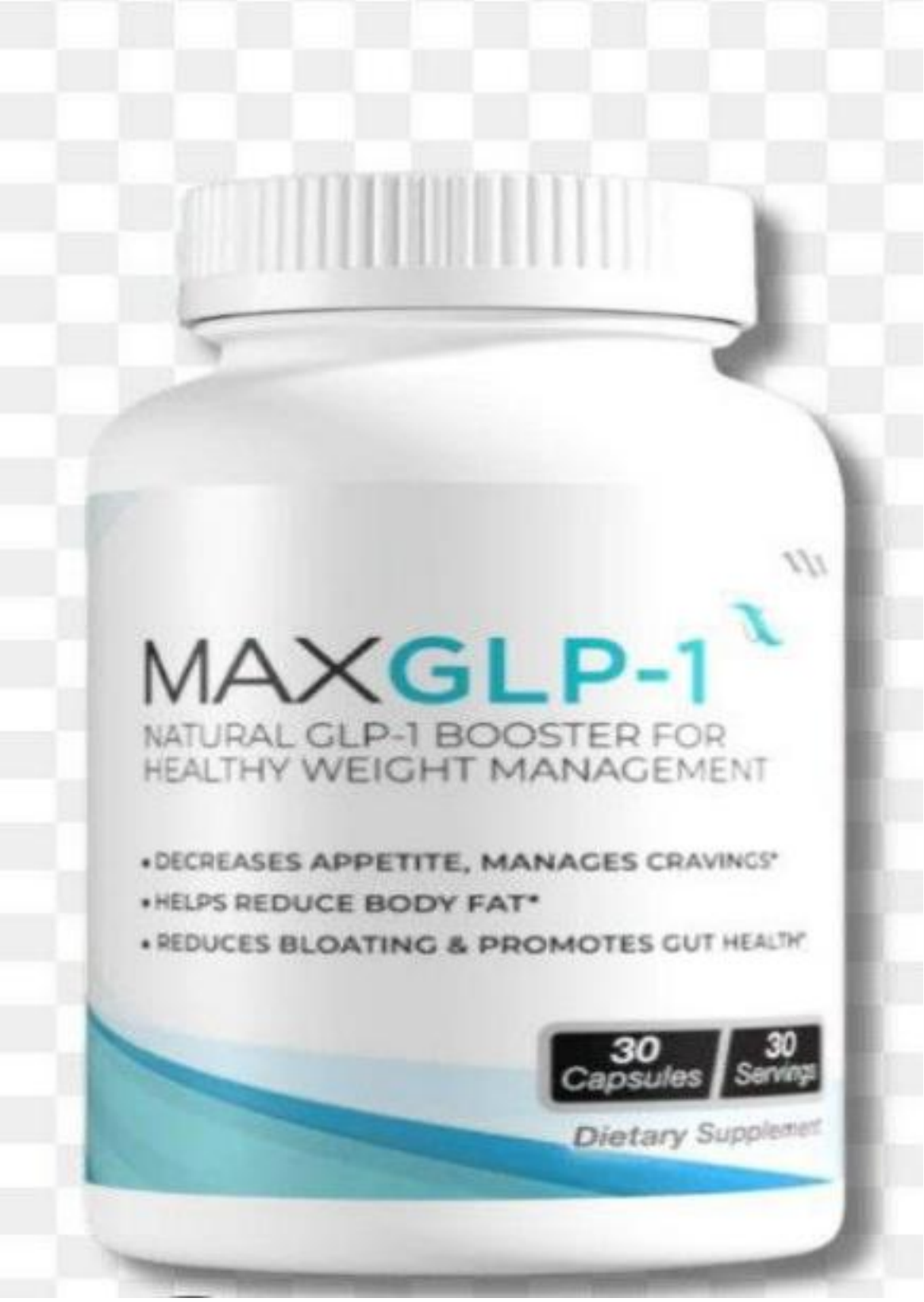

Figure 1: Image of Max GLP-1

The innovative mix of Max GLP-1 includes

- Redaxin (sorghum polyphenol extract):

A standardized polyphenol extract from sorghum leaf and sheath (Sorghum bicolor). Sorghum is high in phenolic compounds (flavonoids, 3-deoxyanthocyanidins, phenolic acids).

Evidence: Multiple reviews and primary research show that sorghum polyphenols have antioxidant, anti-inflammatory, and metabolic properties in vitro and in animal models; certain human dietary studies suggest that eating sorghum products improves antioxidant markers or glycemic response. However, actual clinical evidence that sorghum leaf extracts raise GLP-1 in humans is sparse; the greatest data points to antioxidant/anti-inflammatory potential rather than demonstrated incretin regulation (Wu *et al.,* 2023).

- Eriomin (lemon flavonoid/eriocitrin-rich powder):

A standardized lemon flavonoid extract (rich in eriocitrin) used as a nutraceutical ingredient.

Evidence: Human clinical trials (randomized, double-blind crossover) have shown that Eriomin supplementation improves some glycemic markers and metabolic biomarkers in people with high blood glucose; some trial reports and manufacturer-supported publications show small increases in GLP-1 and improvements in markers such as fasting glucose and inflammatory cytokines after weeks of supplementation. These studies are small in size yet peer-reviewed (Cesar *et al*., 2022). Thus, Eriomin has some clinical evidence of mild effects on glucose metabolism, which could be linked to incretin (GLP-1) pathways.

- PoZibio (heat-treated/postbiotic Lactobacillus paracasei D3.5)

A heat-inactivated (non-viable) preparation of L. paracasei strain D3.5 sold as a postbiotic (PoZibio). Postbiotics are microbial products or inactivated microorganisms that can affect gut health and host physiology (Lioyd,A.,*et al*., 2024).

Evidence: A growing body of preclinical and early clinical literature suggests that heat-inactivated Lactobacillus strains (postbiotics) can modulate gut barrier function, inflammation, and metabolism; small human pilot studies and sponsored trials have investigated the safety, gut health, and well-being outcomes for PoZibio in particular. Postbiotics may alter host metabolism via immune/gut signaling (and theoretically modulate GLP-1 via microbiota-gut hormone axes), but large independent trials have yet to provide direct, robust human evidence that PoZibio raises GLP-1 levels.

- Chromium picolinate(200µg per capsule):

A popular chromium salt supplement that has been researched for its effects on glucose metabolism and insulin sensitivity (Talab *et al.,* 2020).

Evidence: Systematic reviews and clinical research yield inconsistent results: some trials demonstrate moderate benefits in glycemic control or insulin sensitivity with chromium supplementation (particularly in specific subgroups), while others show no impact. Chromium's impact is mostly connected to insulin signaling and metabolism, rather than direct GLP-1 activation. Notably, chromium can interact with anti-diabetic drugs, therefore caution is suggested in medicated patients.

Other capsule excipients include hypromellose, microcrystalline cellulose, magnesium stearate, and silica. (One pill equals one serving; each container holds 30 servings).

2.4.1 Claimed Health Benefits:

Max GLP-1 is not a pharmacological GLP-1 agonist, despite being marketed as a natural or nutraceutical booster of GLP-1 action. Alternatively, it might include plant extracts or bioactive substances that are thought to

1. Boost natural GLP-1 levels to reduce appetite: allowing you to eat less and feel fuller for longer.
2. Reduce cravings and sugar-related snacking: promotes better dietary control.
3. Support metabolism and fat breakdown: promote fat loss with clinically-studied patented ingredients.
4. Support healthy blood sugar levels and have consistent energy throughout the day.
5. Support healthy aging, balances metabolism, energy and healthy aging part of a broader wellness claim.

2.4.2 How These Ingredients Could Potentially Influence Max GLP-1 or Antioxidant Status (mechanistic view):

- Eriocitrin (Eriomin): citrus flavonoids have been shown in small trials to slightly raise circulation GLP-1 and improve glycemic indicators, perhaps through DPP-4 inhibition or enteroendocrine modulation.
- Sorghum polyphenols have high antioxidant and anti-inflammatory properties in vitro and in animals (Wu *et al.,* 2023) they may reduce oxidative stress and inflammation, thereby improving metabolic health (although direct GLP-1 upregulation evidence is weak) .
- PoZibio (postbiotic): Postbiotics can alter gut immunity and microbial metabolites that influence enteroendocrine cells (perhaps modulating GLP-1), however human mechanistic studies for this strain are preliminary.

- Chromium picolinate: aids insulin signaling in some persons; its effect on GLP-1 is indirect at best.

2.4.3 Safety, Regulation and Important Cautions

- Dietary supplement status: MaxGLP-1 is marketed as a dietary supplement, not an FDA-approved medicine. Manufacturer pages incorporate the typical disclaimer that the FDA has not reviewed the claims (Max GLP-1, 2025).
- Regulatory context and caution: Regulators and independent reporting have issued warnings concerning unapproved GLP-1 medications and unregulated internet merchants marketing peptide versions of GLP-1 drugs (Semaglutide, Tirzepatide), which are distinct from botanical or nutraceutical "GLP-1 boosters." Consumers should distinguish between pharmaceutical GLP-1 receptor agonists (prescription medications) and dietary supplements promoted to "boost GLP-1." The FDA has taken action against companies that offer unlicensed GLP-1 medicines online (Diatribe, 2025).

Realistic safety Remarks, Talk to your doctor about using supplements if you take prescription medications for diabetes or weight loss because certain elements, such chromium, might alter blood sugar levels and interfere with medications. Although there is no long-term safety information on particular combos, postbiotics and high-dose flavonoids are generally well tolerated in trials (Talab *et al*., 2020).

2.5 Research Gaps

1. Max GLP-1's nutritional and antioxidant potential cannot be accurately evaluated due to a lack of published proximate composition data, including moisture, protein, fat, fiber, ash, and carbohydrates.

2. Dosage transparency: The proprietary blend conceals the actual amount of each component, hindering any significant dose-response or association with antioxidant results.

3. Lack of validated extraction and assay methods: There is no established technique for extracting or assessing antioxidants from Max GLP-1, which raises concerns concerning reproducibility and comparability of results.

4. In-vivo or clinical confirmation is lacking, as no studies have directly assessed antioxidant biomarkers or GLP-1 levels after taking Max GLP-1.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

MaxGLP-1 is a nutritional dietary supplement made from minerals, microbiological substances, and medicinal plants that are obtained from a licensed pharmacy situated in Lagos Nigeria. The product has intact manufacturer seals, validated manufacturing and expiration dates, and is registered with the National Agency for Food, Drug Administration and Control (NAFDAC).

3.1.1 Apparatus:

At the time of purchase, the Apparatus utilized in the research study was at experimental standard and was purchased from a certified vendor. Among them are

1. Beakers (50, 150 and 250ml) (Pyrex England)
2. Conical flasks (Pyrex England)
3. Pipettes (1,10 and 25ml)
4. Cardboard papers
5. Cotton wool[Fantastik, England]
6. Cover slip[Pyrex, England]
7. Detergent
8. Gloves[Fantastik, England]
9. Masking tape
10. Measuring cylinder[Pyrex, England]
11. Micro pipettes [Microlux, England]
12. Nose mask[Fantastik, England]

13. Plain tube [Fantastik, England]

14. Universal bottles[Fantastik, England]

15. Stop watch

16. Test tube racks and test tubes

3.1.2 Equipment

Major equipment used includes:

1. Refrigerator (Citizens PRC4246)
2. T70UV/VIS Spectrophotometer (PG Instruments Ltd., UK.)
3. HH-W Constant Temperature Water Bath (B. Bran Sc. Inst. Company, England)
4. Analytical weighing balance (Metter H-80 Germany).
5. Water distiller (B. Bran Sc. Inst. Company, England).
6. Simple Weighing Balance (Adventurer OHAUS AR1530).
7. Micro-plate reader (PG Instruments Ltd., UK).
8. 80-2 model Electric Centrifuge (B.Bran Scientific Instrument Company England).

3.1.3 Reagents

All the chemicals and reagents used in this study were of analytical grade at the time of purchase. They include;

1. The Proximate Determination Reagents (MyBioSource.com, USA).
2. Malondialdehyde (MDA) Reagent (MyBioSource.com, USA).
3. In-vitro Antioxidant Estimation Reagent (MyBioSource.com, USA).
4. Total Antioxidant Capacity (TAC) Colorimetric Assay Kit (MyBioSource.com, USA).
5. Chloroform, hydrochloric acid (HCl)[May and Bayer, England]
6. Distilled water[Trigas, UNIBEN]
7. Ethanol[BDH, England]

8. Petroleum ether[BDH, England]
9. Potassium hydroxide (KOH)[May and Bayer, England]
10. Methanol[BDH, England]
11. Ammonium and Ammonium Sulfate[BDH, England]

3.2 Methods

3.2.1 Proximate Analysis;

1. Determination of Moisture content:

Moisture content was evaluated using the oven-drying method, 2 ml of the sample was weighed using an analytical balance and recorded as the initial weight, and the dish containing the sample was placed in a preheated oven at 105°C. The sample was dried for 4 hours, then taken from the oven and placed in a desiccator to cool for 25 minutes. The sample was weighed again and recorded as the final weight.

2. Determination of Ash content:

The ash content was calculated using the AOAC (2000) technique.

Principle:

The total ash complete is estimated by removing all organic material using ignition.

Procedure

One gram of samples was placed in crucibles and fired in a furnace at 500-600oC for three hours, until it totally ashtened. It was then chilled in a dessicator before being promptly weighed at room temperature.

Calculation:

$$\text{Ash Content (\%)} = \frac{W2 - W0}{W1 - W0} \times 100$$

W_0 = Weight of the empty crucible (g)

W_1 = Weight of the crucible + sample before ashing (g)

W_2 = weight of crucible + ash after ashing (g)

3. Determination of crude fibre:

This was determined using the enzymatic-gravimetric approach established by A.O.A.C. (AOAC International 2019).

Procedure

A one-gram sample (w_0) was cooked in 200ml of sulfuric acid for 30 minutes. The sample was then filtered through a muslin cloth and washed with hot distilled water. The residue was mixed with 200ml of sodium hydroxide and boiled for 30 minutes before being washed with hot distilled water. It was also washed with hydrochloric acid. It was then rinsed three times with petroleum ether. The product was drained, oven-dried, cooled in a desiccator, and weighed (w_1). The sample was ashed at 500oC for 90 minutes in a muffle furnace, cooled in a desiccator, and weighed (w_2).

Calculation:

$$\text{Crude Fiber (\%)} = \frac{\mathbf{W2 - W1}}{\mathbf{W0}} \times \mathbf{100}$$

W_0 represents the weight of the dry sample (before digestion).

W_1 = weight of crucible + residue from acid/alkali digestion (before ashing)

W_2 = Weight of crucible + ash after ignition in furnace

4. Determination of carbohydrate content:

The carbohydrate content was calculated by subtracting one hundred from the sum of the protein, fiber, and fat percentage contents.

% carbohydrate = 100 - (%lipid + %protein + %ash + %fiber).

5. Determination of crude protein

Nitrogen was measured using the micro-Kjeldahl method (A.O.A.C 2000), and crude protein content was calculated by multiplying the nitrogen concentration by a factor of 6.25.

Principle:

In the presence of catalysts, proteins and other food components are digested with sulphuric acid to produce ammonium sulphate, which is then neutralized with alkali to produce ammonium, which is then steam distilled directly into a hydrochloric acid containing the indicator methylene red. The reaction between hydrochloric acid and ammonium produces ammonium chloride, which is then titrated with sodium hydroxide.

Procedure:

The micro kjeldahl flask held one milliliter of 4% CuSO4, H2SO4, and 0.8g of K2SO4. A particular amount of the test sample was put to the reagents in a micro Kjeldahl flask. It was digested at low temperature until the icing stopped, and then at high temperature until the solution was clear, pale yellow, or light blue. The flask was allowed to cool before gradually adding 4ml of distilled water and distilling the contents using a Kjeldahl distillation device. Ten milliliters of 30% NaOH were used to free ammonium during the distillation process. Ammonium was collected in 0.01 M HCl (a drop of methylene red was added to the solution).

The distillation was Titrated with 0.01 M NaOH. The nitrogen content of the sample was estimated using the volume of HCl neutralized by NaOH.

Calculations

$$\text{Nitrogen (\%)} = \frac{\textbf{Titre value(blank)} - \textbf{Titre value(distilate)} \times \textbf{0.14}}{\textbf{Weight of } \boldsymbol{sample}}$$

Protein = % nitrogen x 6.25

$$\text{\% protein} = \frac{\textbf{Protein} \times \textbf{100}}{\textbf{Initial Weight}}$$

6. Determination of Crude fat

Crude fat was evaluated by Soxhlet extraction (A.O.A.C. International 2019).

Principle

The free lipid content consists of neutral fats (triglycerides) and free fatty acid was evaluated by extracting the dried and pulverized material with diethyl ether in a continuous extraction equipment (Soxhlet extractor).

Procedure

The weight of an empty flask was calculated. One gram of sample was wrapped and placed within an extraction thimble. To prevent sample loss, the thimble was stuffed with cotton wool. The thimble was inserted inside the extractor. An already-weighed, clean, and dry soxhlet extractor flask was affixed to the extractor's bottom. Petroleum ether (500ml) was placed into a dry soxhlet flask, and the heating mantle was turned on, causing the petroleum ether to boil. Heating was maintained for eight hours, following which the solvent was entirely drained into a flask and dried by distillation. The flask was removed and dried to a consistent weight.

Calculation:

Weight of empty porous thimble=w_o

Weight of thimble +ground sample=w_1

Weight of ground sample= w_1- w_o

Weight of empty extraction flask=w_2

Weight of extraction flask + ether=w_3

$$\text{Lipid (\%)} = \frac{(w3-w2)\times 100}{w1-w0}$$

3.2.2 In-Vitro Antioxidant Capacity

1. 2,2-diphenyl-1-picryhydrazyl(DPPH) Radical Scavenging Assay

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH)) radical is a stable free radical whose color changes from purple to yellow when reduced by antioxidants. The degree of decolorization reflects the samples scavenging potential.

The 2,2-diphenyl-1-picryhydrazyl(DPPH) radical samples scavenging potential was performed using the method reported by Blois in 1958. A 0.1M solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was prepared in methanol.1mL of the solution was mixed with varying concentration (10-100 µg/mL) of the sample. The mixture was incubated in the dark room temperature for 30 minutes, and absorbance was measured at 517nm using a UV-Vis Spectrophotometer.

2. Ferric Reducing Antioxidant Power (FRAP) Assay

Principle: In an acidic environment, a Fe^{3+}–TPTZ (2,4,6-tripyridyl-s-triazine) complex is reduced to its blue-colored Fe^{2+}–TPTZ form. The potential to reduce antioxidants is indicated by the rise in absorbance at 593 nm (Benzie and Strain, 1996).

$Fe^{3+}(TPTZ) + e- \rightarrow Fe^{2+}(TPTZ)$.

Procedures: Incubate 1mL of Ferric Reducing Antioxidants Power (FRAP) reagent with 100 µL of sample at 37°C for 30 minutes, Measure absorbance at 593nm.

DATA ANALYSIS:

The result were expressed as percentages and Mean ± SEM (Standard error for the mean) for Proximate Analysis and Invitro antioxidant Capacity respectively.

CHAPTER FOUR

RESULT

4.1 Proximate Composition on MAX GLP-1

The nutritional makeup and physicochemical properties of MAXGLP-1 were ascertained by proximate analysis. The mean % values were calculated after three batches were examined. Table 4.1 displays the findings.

Table 4.1: Proximate Composition of MAXGLP-1 (per 100 g, n = 3)

According to the findings, MAXGLP-1 has a high percentage of carbohydrate (53.84% of the total composition), suggesting that the product is mostly composed of carbohydrates. Additionally, the formulation had a high moisture content of 42.74%, indicating significant water retention. 1.57% of the total mineral composition was made up of ash. Low amounts of crude protein, crude fiber, and crude fat were found at 1.42%, 0.42%, and 0.01%, respectively.

Sample	Moisture Content (%)	Crude Protein (%)	Crude Fibre (%)	Crude Fat (%)	Ash (%)	Carbohydrate (%)
MAXGLP-1	42.740	1.420	0.420	0.012	1.565	53.844

4.2 Invitro Antioxidants Potentials of Max GLP-1

Two complimentary in vitro tests were used to assess MAXGLP-1's antioxidant qualities: Ferric Reducing Antioxidants Power (FRAP) lowering activity and 2,2-diphenyl-1-picryhydrazyl(DPPH) radical scavenging activity. Table 4.2 displays the findings.

Table 4.2: Antioxidant Activity of MAXGLP-1 Compared with Ascorbic Acid.

The 2,2-diphenyl-1-picryhydrazyl(DPPH) radical scavenging assay measures a sample's capacity to eliminate free radicals by donating electrons or hydrogen atoms. With a 2,2-diphenyl-1-picryhydrazyl(DPPH) radical scavenging activity of 44.61 ± 2.91%, MAXGLP-1 demonstrated a moderate capacity to neutralize radicals under test circumstances. Ascorbic acid, the reference antioxidant, showed a greater inhibition of 95.44 ± 0.39%, in line with its well-established strong antioxidant profile. MAXGLP-1's comparatively reduced inhibition indicates a detectable but moderate ability to diminish 2,2-diphenyl-1-picryhydrazyl(DPPH) radicals.

MAXGLP-1 recorded has a reducing activity of 41.56 ± 1.04% in the Ferric Reducing Antioxidants Power (FRAP) assay, which measures an antioxidant's capacity to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions, while ascorbic acid obtained 89.25 ± 0.65%. The Ferric Reducing Antioxidants Power (FRAP) findings align with the 2,2-diphenyl-1-picryhydrazyl(DPPH) assay, revealing that MAXGLP-1 contains electron-donating characteristics capable of reducing oxidized intermediates, albeit at levels lower than the reference antioxidant.

Assay	**MAXGLP-1 (% Inhibition)**	**Reference Antioxidant**	**Standard (% Inhibition)**
2,2-diphenyl-1-picryhydrazyl(DPPH) radical scavenging	44.61 ± 2.91	Ascorbic acid	95.44 ± 0.39
2,4,6-tripyridyl-s-triazine (FRAP) reducing activity	41.56 ± 1.04	Ascorbic acid	89.25 ± 0.65

The values are given as mean ± SEM (n = 3).

All measurements were performed in triplicate, and results are expressed as mean ± SEM to reflect the reproducibility and precision of the assays. MAXGLP-1 showed consistent antioxidant activity in both tests, indicating the presence of bioactive components with the ability to reduce and scavenge radicals.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

Max GLP-1's proximate analysis revealed a composition dominated by carbohydrates (53.84%), with a high moisture content (42.74%) and minimal amounts of protein (1.42%), fat (0.01%), fiber (0.42%), and ash (1.57%). This suggests that the formulation is primarily made up of powders derived from plants and excipients based on carbohydrates rather than nutrient-rich raw materials. These results are consistent with earlier proximate assessments of herbal items and therapeutic plants. Herbal powders frequently show carbohydrate dominance, as demonstrated by (Quadri *et al*., 2021), who reported carbohydrate values ranging from 40.9–55.6% and similarly low macronutrient levels in many African plants. In line with patterns seen in powdered herbal supplements, (Fategbe *et al*.,2021) also discovered that the pericarp of Xylopia aethiopica contains more carbohydrates than protein or fat. These results demonstrate that the nutritional profiles commonly reported for botanical-based formulations are consistent with the proximate makeup of Max GLP-1.

However, the moisture content of Max GLP- 1 (42.7%) is substantially greater than typical values for dried herbal extracts, which commonly lie between 8–12% depending on drying conditions. Inadequate drying during processing or hygroscopic plant components could be the cause of elevated moisture levels. Over time, this high moisture content may weaken the efficacy of bioactive phytochemicals and jeopardize product stability.

The study found moderate antioxidant activity, with 44.61% 2,2-diphenyl-1-picryhydrazyl (DPPH) radical inhibition and 41.56% Ferric Reducing Antioxidants Power (FRAP) reduction power. These results are lower than those of traditional antioxidants such as ascorbic acid but similar with those predicted from mixed nutraceutical formulations.

According to (Wu *et al*.,2023), concentrated sorghum polyphenol extracts, which are comparable to the Redaxin component in Max GLP-1, have shown significantly greater antioxidant activity under controlled extraction conditions. The study's lower readings could be the result of excipients diluting the active ingredients and potential deterioration brought on by a high moisture content.

Furthermore, clinical investigations have shown that the supplement's recognized ingredient, eriomin (eriocitrin), has antioxidant and glycaemic regulating properties. Nevertheless, pure, defined dosages (about 200 mg/day) were used in these investigations. It is impossible to ascertain whether the product contains Eriomin in sufficient numbers to duplicate these clinical outcomes because Max GLP-1 is a proprietary mix and does not reveal exact constituent quantities. This probably explains why, in comparison to research employing pure extracts, the antioxidant capacity found in this study is moderate.

Moreover, gut-mediated metabolic effects, including possible GLP-1 regulation, have been connected to postbiotics like PoZibio. Robust human evidence is still scarce, though. Thus, while biologically plausible, GLP- 1 enhancing claims cannot be confirmed based solely on proximate composition or antioxidant assays.

Generally Max GLP-1 follows the nutritional and antioxidant patterns typical of many herbal nutraceuticals: low macronutrient density, moderate antioxidant activity, and reliance on phytochemical components for biological effects, according to a comparison of the study's findings with other research. In order to substantiate the metabolic and GLP-1-related claims, more study incorporating phytochemical measurement, HPLC profiling, stability testing, and controlled in-vivo studies is necessary due to the extremely high moisture content and moderate antioxidant capability.

5.2 Conclusion

The in vitro antioxidant capacity and proximate composition of Max GLP-1, a herbal nutraceutical sold for GLP-1 augmentation, hunger control, weight management, and glucose regulation, were assessed in this study. The product's low quantities of protein, fat, fiber, and ash, along with its high moisture content and dominance of carbohydrates, indicate a poor nutritional density. 2,2-diphenyl-1-picryhydrazyl(DPPH) (44.61%) and Ferric Reducing Antioxidants Power (FRAP) (41.56%) studies revealed moderate antioxidant activity, indicating the presence of bioactive chemicals originating from plants, albeit much weaker than that of conventional ascorbic acid. Although the supplement shows some antioxidant potential, it is not a potent antioxidant formulation, and its claimed metabolic advantages have not been clinically confirmed. The study emphasizes the necessity of more dose-response studies, in vivo investigations, and clinical trials to support the evidence-based application of health claims.

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APPENDIX I

USED EQUIPMENT

- UV-Visible Spectrophotometer
- Analytical equilibrium
- An electric centrifuge
- The Muffle Furnace
- The oven
- Soxhlet Extractor
- Water Bath
- Desiccator
- Typical glassware used in laboratories

APPENDIX II

USED REAGENTS

- 2,2-diphenyl-1-picryhydrazyl(DPPH)
- Ferric Reducing Antioxidants Power (FRAP)
- Total Antioxidant Capacity (TAC)reagents
- Methanol and ethanol
- HCl, H_2SO4, and NaOH
- Copper sulfate catalyst
- Petroleum ether
- Distilled water
- Chloroform

APPENDIX III

FORMULATION INFORMATION FROM THE MANUFACTURER

Excipients:

Microcrystalline cellulose

Hypromellose

Lemon extract

Flavor components

Active Ingredients:

Redaxin (Sorghum polyphenols)

Eriomin (Citrus flavonoids)

PoZibio (Postbiotics)