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RESEARCH ARTICLE

INVESTIGATING THE BIOCHEMICAL PROFILE AND NUTRITIONAL COMPOSITION OF *VERNONIA AMYGDALINA* LEAVES: A STUDY ON ANTIOXIDANT PROPERTIES, PHYTOCHEMICAL COMPOSITION, ENZYME INHIBITION, AND PROTEIN ACTIVITY

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ABSTRACT

In this comprehensive study, the biochemical profile and nutritional composition of *Vernonia amygdalina* leaves were examined, focusing on their antioxidant properties, phytochemical composition, enzyme inhibition, and protein activity. A meticulous extraction process using 70% ethanol yielded an ethanolic extract of *Vernonia amygdalina* leaves powder (VLP), which exhibited substantial Vitamin C content (12.95 mg/g) and impressive antioxidant activity, as evidenced by a 2,2-diphenyl-1-picrylhydrazyl hydrate value of 60.24% and a ferric ion reducing antioxidant power value of 49.22 mg/g. The extract also demonstrated a significant 49.01% inhibition of lipid peroxidation, highlighting its potential in mitigating oxidative stress. Phytochemical analysis revealed the presence of flavonoids, alkaloids, phenols, saponins, and tannins, further emphasizing the therapeutic potential of *Vernonia amygdalina*. Moreover, the study unveiled the extract's inhibitory effects on key enzymes and proteins involved in metabolic processes, with notable rates of 29.34% for anti-glucosidase activity, 43.28% for alpha-amylase inhibition, 34.44% for albumin denaturation inhibition, and 57.04% for anti-proteinase activity. Additionally, the proximate analysis showcased the leaf's composition, with 14.13% crude protein, 23.53% crude fiber, 0.81% crude fat, 7.10% ash content, and 41.16% nitrogen-free extracts, underscoring its potential as a valuable source of dietary protein and fiber. These findings collectively emphasize the multifaceted health-promoting properties and nutritional value of *Vernonia amygdalina* leaves, advocating their utilization in various dietary and therapeutic applications.

KEYWORDS

Bitter leaf, Botanicals, Supplements, Nutraceuticals

1. INTRODUCTION

In recent years, there has been a growing interest in exploring the bioactive compounds present in various natural sources, particularly in botanicals, due to their potential health-promoting effects and therapeutic applications (Oloruntola, 2022; Oloruntola and Ayodele, 2022). *Vernonia amygdalina*, commonly known as bitter leaf, is a plant native to tropical Africa and is renowned for its extensive use in traditional medicine and dietary practices (Oladele et al., 2021). It has long been recognized for its bitter taste, which is attributed to the presence of various phytochemicals with diverse pharmacological activities (Okunlola et al., 2018).

Vernonia amygdalina is known to be a rich source of bioactive compounds, including polyphenols, flavonoids, alkaloids, and terpenoids, among others (Farombi and Owoeye, 2011). These phytochemicals are believed to contribute to the plant's numerous reported health benefits, which range from its potential antioxidant properties to its role in the management of certain metabolic disorders (Farombi and Owoeye, 2011). An in-depth analysis of the phytochemical composition of *Vernonia amygdalina* leaves will provide insights into the specific bioactive compounds present. Understanding the types and concentrations of these compounds can inform the development of dietary supplements and feed additives tailored to the nutritional needs of different animal species.

The antioxidant properties of *Vernonia amygdalina* have drawn particular attention, given the established link between oxidative stress and a myriad of chronic diseases, including diabetes, cardiovascular diseases, and cancer (Farombi and Owoeye, 2011; Wang et al., 2020). Antioxidants play a crucial role in neutralizing harmful free radicals, thereby mitigating cellular damage and reducing the risk of oxidative stress-related diseases (Engwa et al., 2022). By assessing the plant's antioxidant properties, its ability to safeguard animals against oxidative damage is uncovered.

Furthermore, the inhibition of key enzymes such as alpha-amylase and alpha-glucosidase holds promise in managing blood glucose levels (Gong et al., 2020), making *Vernonia amygdalina* a potential candidate for addressing conditions like diabetes (Tundis *et al*., 2010). This inhibition can have significant implications for the regulation of glucose metabolism in animals, which is vital for those prone to metabolic disorders. Additionally, its ability to inhibit protein denaturation and exert antiproteinase activity could have implications in preventing certain pathological processes (Farombi and Owoeye, 2011; Alara and Abdurahman, 2021). This modulation of protein function may have implications for protein digestion and utilization in animal diets.

Despite the growing interest in *Vernonia amygdalina*, comprehensive studies encompassing its biochemical profile, nutritional composition, and

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multifaceted bioactivities are still relatively limited. This study aims to bridge this gap by presenting a comprehensive investigation into the biochemical profile and nutritional composition of *Vernonia amygdalina* leaves. This study will focus on elucidating its antioxidant properties, phytochemical composition, enzyme inhibition potential, and protein activity modulation.

2. Materials and Methods

2.1 Processing and laboratory analysis of *Vernonia amygdalina* leaf

The leaves of *Vernonia amygdalina* were harvested from their parent plants, left to wilt, and then finely chopped using a stainless steel knife. Subsequently, they were spread thinly on clean polythene sheets and placed under a sheltered area for a period of 14 days to undergo the drying process. The dried and finely chopped *Vernonia amygdalina* leaves were subsequently ground into a powdered form, which we will refer to as *Vernonia amygdalina* leaf powder (VLP). This VLP was then carefully stored for further laboratory analysis. A total of 400 grams (400 g) of *Vernonia amygdalina* leaf powder (VLP) was combined with 2000 ml of 70% ethanol. This mixture was vigorously shaken for a duration of 6 hours and subsequently left undisturbed for an additional 48 hours. Afterward, the mixture was filtered through Whatman No. 1 filter paper to separate the solid from the liquid. The resulting ethanolic extract of VLP was then condensed under vacuum conditions using a rotary evaporator at a temperature range of 35–40°C. We obtained the analytical reagent-grade chemicals necessary for chemical analysis from Sigma-Aldrich. The evaluation of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity (Otles and Yalcin, 2012), the Ferric-reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996), and the assessment of lipid peroxidation inhibition (Bajpai et al., 2015) Tests were conducted for *Vernonia amygdalina* leaf powder (VLP) following the outlined procedures (Oloruntola, 2022).

The specific procedures for determining the phytochemical components were detailed in a prior report (Oloruntola and Ayodele, 2022). For the phytochemical analysis of *Vernonia amygdalina* leaf powder (VLP), Assays conducted included the assessment of total flavonoids, following a specific method (Surana et al., 2016), alkaloids were quantified using the gravimetric method (Adeniyi et al., 2009), according to the procedure outlined, total phenolic content was determined (Otles and Yalcin, 2012), total saponins were measured using the vanillin and concentrated sulfuric acid colorimetric technique (He et al., 2014), Following the method that has been presented, total tannins were quantified (Biswas et al., 2020), Steroids has been analyzed in accordance with the protocol (Madhu et al., 2016).

The comprehensive procedures for assessing the inhibition of alpha-amylase and alpha-glucosidase, as well as albumin denaturation inhibition and antiproteinase activity, have been previously documented (Oloruntola, 2022). Specifically; α-glucosidase inhibitory activity was determined using the method that has been described (Dej-adisai and Pitakbut, 2015). In accordance with the outlined protocol; the α-amylase inhibition study was conducted using the 3,5-Dinitrosalicylic acid (DNSA) method (Wickramaratne et al., 2016). Albumin denaturation inhibition and antiproteinase activity assays were carried out in accordance with the procedures specified (Osman et al., 2016; Rajesh et al., 2019).

Furthermore, *Vernonia amygdalina* leaf powder (VLP) underwent analysis using the Association of Official Analytical Chemists method (AOAC, 2010) to determine its content of crude fat, crude protein, crude fibre, ash, and nitrogen-free extract. The assessment of Vitamin Cin VLP was conducted based on the procedure that has been outlined by Benderitter with detailed methods reported earlier by Oloruntola, 2021 (Benderitter et al., 1998; Oloruntola, 2021)

2.2 Statistical Analysis

The assays were conducted in triplicate, and the results were averaged. Descriptive statistics were employed to analyze the collected data. Bar graphs were generated in Excel to enhance the visualization of the averaged data.

3. Results and Discussion

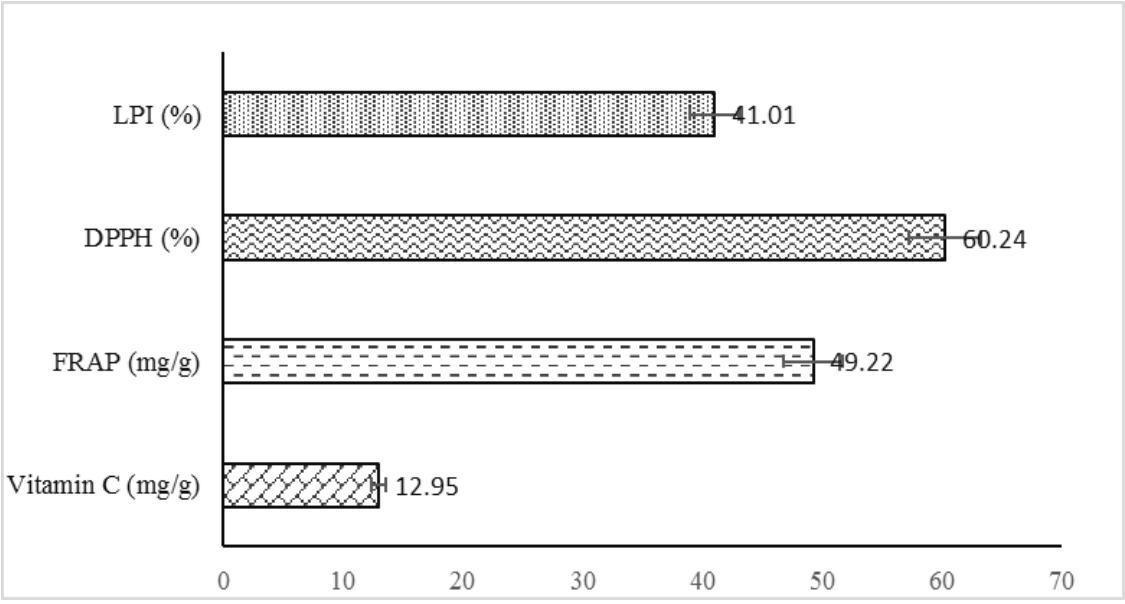

Figure 1: Vitamin C concentration and antioxidant activities of *Vernonia amygdalina* leaf

LPI: Lipid peroxidation inhibition; DPPH: 2,2-diphenyl-1-picrylhydrazyl hydrate;

FRAP: Ferric ion reducing antioxidant power.

Figure 1 presents data on the levels of Vitamin C and the antioxidant properties exhibited by *Vernonia amygdalina* leaf powder (VLP). Specifically, VLP contained a Vitamin C concentration of 12.95 mg/g. Furthermore, it displayed a 60.24% DPPH activity and a FRAP value of 49.22 mg/g. VLP also demonstrated a notable 49.01% inhibition of lipid peroxidation. The presence of Vitamin C (ascorbic acid) in plant materials like *Vernonia amygdalina* is significant for its potential health benefits. Vitamin C is a well-known antioxidant that plays a vital role in neutralizing harmful free radicals in the body. It also contributes to the biosynthesis of collagen, helps in wound healing, and enhances the absorption of non-heme iron from plant-based foods (Padaytty et al., 2003; Kaźmierczak-Barańska et al., 2020). *Vernonia amygdalina*, commonly known as bitter leaf, is recognized for its medicinal properties and nutritional content (Oladele et al., 2021). The reported concentration of 12.95 mg/g of Vitamin C in VLP suggests that it could be a valuable source of this essential nutrient. This finding underscores the potential dietary and therapeutic significance of *Vernonia amygdalina* as a natural source of Vitamin C. The antioxidant activities of VLP were assessed using two common methods: 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and Ferric ion reducing antioxidant power (FRAP). These assays are commonly employed to evaluate the ability of a substance to scavenge free radicals and reduce oxidative stress (Munteanu and Apetrei, 2021). The DPPH assay measures the ability of a substance to donate electrons and neutralize DPPH radicals (Baliyan et al., 2022). A higher percentage inhibition, in this case, 60.24%,

indicates a stronger ability of VLP to scavenge free radicals. This suggests that *Vernonia amygdalina* leaf powder possesses potent radical scavenging properties, which are attributed to its antioxidant compounds. The FRAP assay quantifies the reducing power of a substance. A higher FRAP value indicates a greater capacity to reduce ferric ions (Wojtunik-Kulesza, 2020). The result of 49.22 mg/g indicates that VLP has a substantial reducing ability, further supporting its antioxidant potential. The high DPPH inhibition and FRAP value suggest that *Vernonia amygdalina* leaf powder may be effective in combating oxidative stress and preventing cellular damage associated with free radicals. Lipid peroxidation is a process that damages cell membranes and biomolecules due to the action of free radicals on lipids. Inhibition of lipid peroxidation is a crucial aspect of antioxidant activity (Ayala et al., 2014). The result of 49.01% inhibition of lipid peroxidation by VLP indicates its ability to protect lipids from oxidative damage, which is important for maintaining cell membrane integrity and overall cellular health.

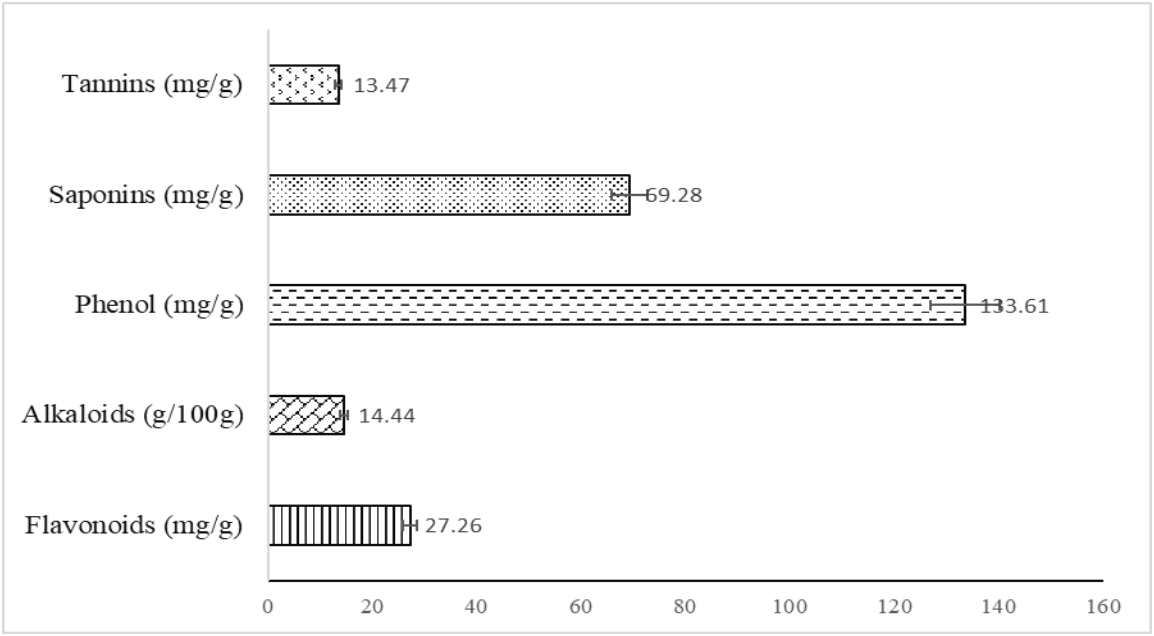

Figure 2: Phytochemical composition of *Vernonia amygldalina*

Figure 2 illustrates the phytochemical content of *Vernonia amygdalina* leaf powder. VLP contains flavonoids at a concentration of 27.26 mg/g, alkaloids at 14.44 g per 100 grams, phenol at 133.61 mg/g, saponins at 69.28 mg/g, and tannins at 13.47 mg/g. Phytochemicals are bioactive compounds found in plants that are known to have various health-promoting properties (Altemimi et al., 2017). The presence of significant concentrations of phytochemicals such as flavonoids, phenols, and tannins in *Vernonia amygdalina* leaf powder is of paramount importance for its use as a nutraceutical (Yu et al., 2021). These compounds are well-documented for their potent antioxidant properties. Antioxidants play a crucial role in neutralizing harmful free radicals in the body, thereby protecting cells and tissues from oxidative damage (Lobo et al., 2010). This antioxidant potential is particularly valuable in reducing the risk of chronic diseases such as cardiovascular disease, cancer, and neurodegenerative disorders (Pham-Huy et al., 2008). Saponins have been studied for their ability to lower cholesterol levels. High saponin content in *Vernonia amygdalina* leaf powder suggests a potential role in managing cholesterol levels, which is crucial for heart health (Nnanga et al., 2022).

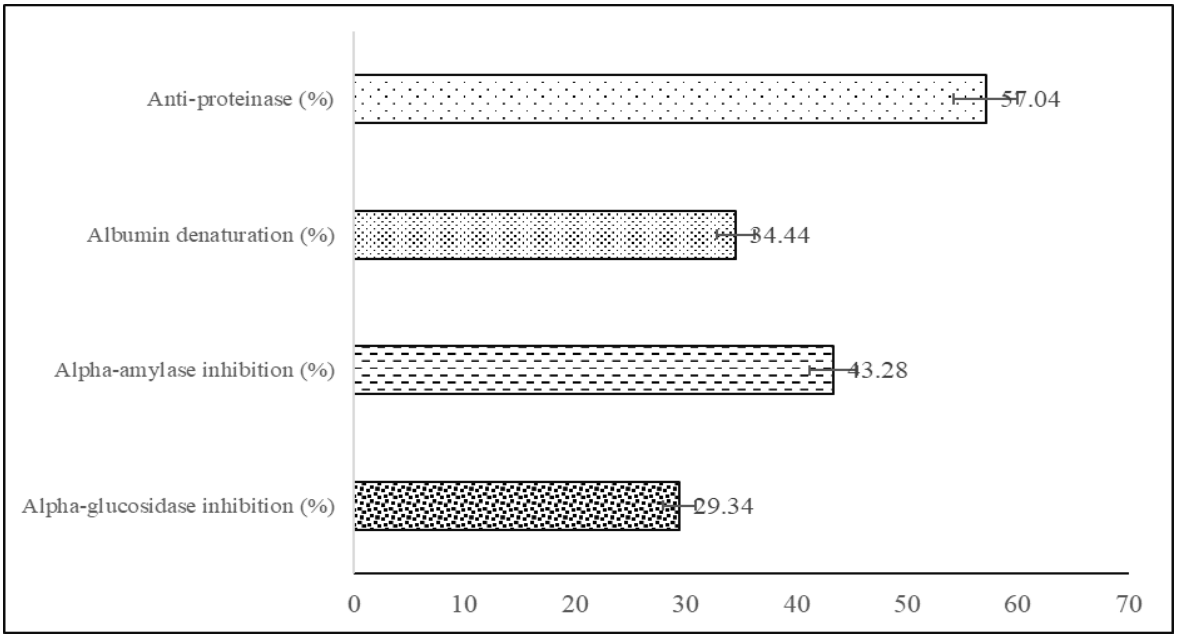

Figure 3: The alpha-amylase inhibition, alpha-glycosidase inhibition, albumin denaturation inhibition and antiproteinase activity of *Vernonia amygdalina* leaf.

Figure 3 illustrates the inhibitory effects of *Vernonia amygdalina* leaf on alpha-amylase, alpha-glycosidase, albumin denaturation, and antiproteinase activities. Specifically, the leaf displayed inhibitory rates of 29.34% for anti-glucosidase activity, 43.28% for alpha-amylase inhibition, 34.44% for albumin denaturation inhibition, and 57.04% for anti-proteinase activity. The results reveal the potential of *Vernonia amygdalina* leaf as a natural source of bioactive compounds with various inhibitory activities related to enzymes and proteins involved in metabolic processes. Alpha-amylase is an enzyme responsible for breaking down starch into simpler sugars. The 43.28% inhibition of alpha-amylase by

Vernonia amygdalina leaf suggests that it contains bioactive compounds that may help regulate post-meal blood sugar levels (Oboh et al., 2012). This could be valuable for individuals with diabetes or those looking to manage their blood sugar levels. Alpha-glycosidase is another enzyme involved in carbohydrate digestion, specifically in the breakdown of complex carbohydrates into glucose (Kashtoh and Baek, 2022). The 29.34% inhibition of alpha-glycosidase is indicative of the leaf's potential to slow down the absorption of glucose from dietary carbohydrates, which can be beneficial in controlling blood sugar spikes. Albumin is a major protein found in the blood and plays a vital role in maintaining osmotic pressure and transporting various substances (Belinskaia et al., 2021). The inhibition of albumin denaturation by 34.44% suggests that *Vernonia amygdalina* leaf may contain compounds that can protect proteins from undergoing structural changes when exposed to heat or other denaturing factors (Adedapo et al., 2014). This could have implications in various biomedical and food science applications. Proteinases, or proteases, are enzymes responsible for breaking down proteins. Inhibiting their activity can be beneficial in preserving protein-based foods and potentially have therapeutic applications in diseases associated with excessive protein degradation (Craik et al., 2011). The significant anti-proteinase activity of 57.04% indicates the potential of *Vernonia amygdalina* leaf to modulate proteinase function (Farombi and Owoeye, 2011).

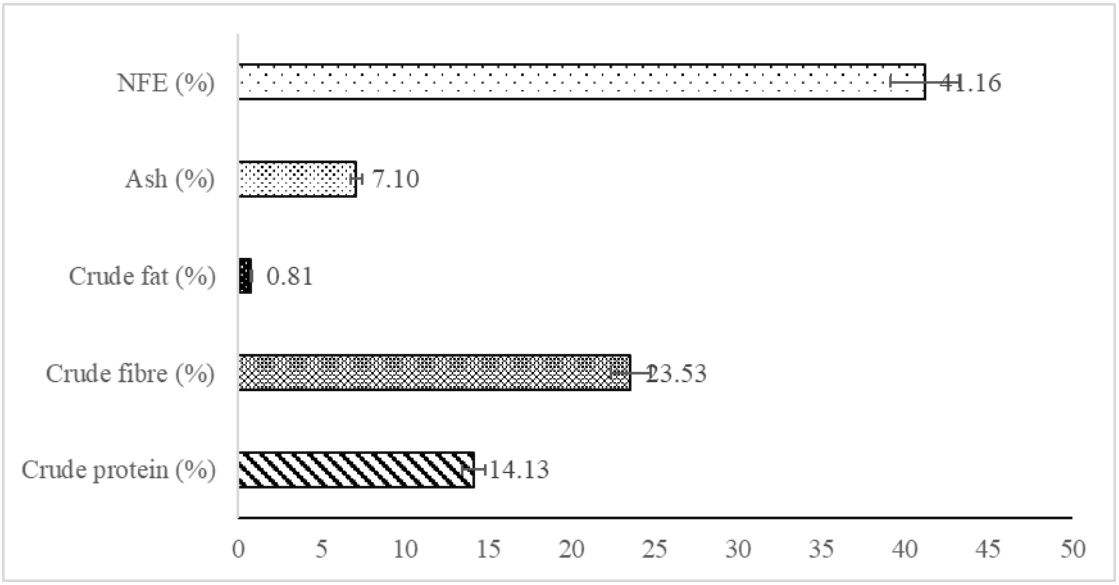

Figure 4: The proximate composition of *Vernonia amygdalina* leaf.

NFE: Nitrogen free extract

Figure 4 displays the proximate analysis of *Vernonia amygdalina* leaves, which reveals the following composition: crude protein at 14.13%, crude fiber at 23.53%, crude fat at 0.81%, ash content at 7.10%, and nitrogen-free extracts at 41.16%. The proximate composition analysis of *Vernonia amygdalina* leaf provides valuable insights into the nutritional and chemical makeup of this botanical resource. The relatively high crude protein content in *Vernonia amygdalina* leaf suggests its potential as a source of dietary protein. Proteins are essential macronutrients vital for various physiological functions in the human body. Incorporating *Vernonia amygdalina* leaf into the diet can contribute to protein intake, particularly in regions where protein sources are limited (Oboh, 2006). The substantial crude fiber content in *Vernonia amygdalina* leaf is indicative of its high dietary fiber content. Dietary fiber is known for its role in promoting digestive health and potentially aiding in weight management. Foods rich in fiber can help regulate bowel movements and reduce the risk of constipation, making *Vernonia amygdalina* leaf a potential dietary choice for promoting gastrointestinal well-being (Anderson et al., 2009). The low crude fat content indicates that *Vernonia amygdalina* leaf is a low-fat food source. This can be of interest to individuals seeking low-fat dietary options to manage their calorie and fat intake. However, the specific types of fats present in the leaf, such as saturated or unsaturated fats, can influence its nutritional profile and health benefits (Astrup et al., 2020). The presence of ash represents the inorganic mineral content in *Vernonia amygdalina* leaf. Minerals are essential for various physiological processes, including bone health, nerve function, and fluid balance. Understanding the ash content is crucial for assessing the leaf's potential contribution to mineral intake in the diet (Ali et al., 2020). Nitrogen-free extracts are a category of carbohydrates that do not contain nitrogen. The high content of nitrogen-free extracts suggests a significant carbohydrate component in *Vernonia amygdalina* leaf (Daramola et al., 2018). These carbohydrates can serve as a source of energy and may include various sugars, starches, and other non-nitrogenous compounds (Navarro et al., 2019).

4. Conclusions and Recommendations

In conclusion, the scientific findings highlight the potential health benefits of *Vernonia amygdalina* leaf powder (VLP). It contains a notable concentration of Vitamin C and exhibits strong antioxidant properties, as evidenced by high DPPH inhibition, FRAP values, and lipid peroxidation inhibition. These characteristics make VLP a promising candidate for further research and utilization in both nutritional and medicinal applications.The high concentration of phytochemicals in VLP is of significant importance for its potential use as nutraceuticals. These compounds contribute to VLP's antioxidant, anti-inflammatory, and potential disease-preventive properties. The leaf's nutritional composition, with its notable protein and fiber content, could contribute to the overall nutritional quality of these diets. Further research and development efforts are warranted to harness the health-promoting benefits of VLP and to explore its applications in functional foods and dietary supplements for improved health and well-being.

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