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Improving Free Radical Scavengers in *Vernonia amygdalina* (Bitter leaf) through Soil Amendment with Organic Fertilizer

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Abstract: Free radical scavengers (antioxidants) are essential micronutrients required for maintenance of good health. Except for fruits, leafy vegetables are the major sources of the free radical scavengers which play the critical roles in the normal metabolic activity for the survival of cells and the entire organism. Adequate intake of dietary free radical scavengers help in the maintenance of good health and prevention of chronic diseases. The concentrations of these compounds in most Nigerian leafy vegetables are generally low due to impoverished soil occasioned by continuous use of the soil for cultivation of crops and poor agricultural practices. It is against this background that this research was designed to evaluate the influence of organic fertilizer (Craseonycteris thonglongyai droppings) on the improvement of some dietary free radical scavengers (ascorbic acid, tocopherol, β-carotene, carotenoid, lycopene, chlorophyll, flavonoids and total phenol) in the leaf of Vernonia amygdalina. The leaves of V. amygdalina grown with organic fertilizer (C. thonglongyai droppings) and chemical fertilizer (commercial reference) in a pot experiment were harvested at market maturity and the concentrations of tocopherol, lycopene, chlorophyll, β-carotene, carotenoid, flavonoid and phenol were evaluated by spectrophotometric method whereas ascorbic acid was determined by titrimetric technique. The results showed that treatment with both chemical and organic fertilizers significantly increased ($p < 0.05$) the concentrations of lycopene, vitamin E, carotenoid and flavonoid in V. amygdalina, however; the concentration of lycopene, carotenoid and flavonoid were significantly higher ($p < 0.05$) in the vegetable treated with the organic fertilizer when compared with chemical fertilizer. While the concentrations of carotene, chlorophyll and phenol in the leaf of V. amygdalina increased significantly with the application of organic fertilizer, treatment with chemical fertilizer had no significant effect on the concentrations of these parameters in the vegetable. Similarly, the application of organic and chemical fertilizers significantly decreased the vitamin C in the vegetable, however, the vitamin C content in the V. amygdalina treated with chemical fertilizer was significantly higher than in the vegetable treated with the organic fertilizer. The study suggests that soil amendment with the organic manure generally enhances the bioaccumulation of free radical scavengers in the leaf of V. amygdalina when compared with the synthetic fertilizer (commercial reference).

Keywords: *Organic fertilizer, chemical fertilizer, Vernonia amygdalina, free radical scavengers*

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1.0 Introduction

Leafy vegetables are groups of plants cultivated for their edible leaves and are essential in human nutrition due to their high content of vitamins, minerals, and phytochemicals. They serve as crucial sources of antioxidants that mitigate oxidative stress and prevent degenerative diseases such as cancer, cardiovascular disorders, and diabetes (Farombi & Owoeye, 2011; Degu *et al*., 2024). *Vernonia amygdalina* (commonly known as bitter leaf) is one of the most widely consumed leafy vegetables in sub-Saharan Africa and has been recognized for both its nutritional and medicinal importance (Eraga *et al*., 2022; Atathananone *et al*., 2023).

Studies have shown that the leaves of *V. amygdalina* possess high antioxidant potential owing to the presence of phenolic compounds, flavonoids, and other secondary metabolites (Ekaluo *et al*., 2015; Eraga *et al*., 2022; Atathananone *et al*., 2023). These bioactive constituents contribute to its ability to scavenge free radicals, inhibit tyrosinase and amylase activities, and provide antidiabetic, antityrosinase, and anti-aging benefits. Farombi and Owoeye (2011) also emphasized that the plant's bioactive compounds exhibit chemopreventive and anti-inflammatory effects, supporting its wide application in traditional medicine.

Processing and environmental factors significantly influence the nutritional and antioxidant composition of *V. amygdalina*. For instance, Ekop *et al*., (2004) reported how some nutritional values of beans was significantly reduced due to processing. Ndatsu Yakubu *yet al*. (2012) also reported that) methods such as soaking, blanching, and abrasion lead to significant reductions in the plant's protein, mineral, and antioxidant contents. Similarly, Eraga *et al*. (2022) observed variations in antioxidant and antigenotoxic activities among *V. amygdalina* accessions collected from different regions of Nigeria, suggesting that soil composition and environmental conditions play critical roles in determining the biochemical composition of the plant.

Despite its abundance and importance, the concentration of vital micronutrients and antioxidant compounds in *V. amygdalina* cultivated in Nigeria remains relatively low, primarily due to poor soil fertility and inadequate organic matter. Fertilization practices, particularly the use of synthetic fertilizers, may not effectively enhance the phytochemical quality of vegetables. In contrast, organic fertilizers are known to improve soil structure, increase microbial activity, and enhance the bioavailability of nutrients and secondary metabolites. Degu *et al*. (2024) further highlighted that the nutritional and pharmacological potential of *V. amygdalina* can be optimized through sustainable cultivation practices that enhance soil health and nutrient balance.

However, there is limited or no information on the use of organic fertilizers derived from unique biological sources, such as bat droppings (*Craseonycteris thonglongyai*), to improve the concentration of free radical scavengers in *V. amygdalina*. This represents a significant knowledge gap, as the potential of such organic fertilizers to enhance the antioxidant properties and nutritional composition of leafy vegetables remains underexplored.

Therefore, this study aims to evaluate the influence of organic fertilizer derived from *C. thonglongyai* droppings on the enhancement of free radical scavenging components in *V. amygdalina*. The findings of this work are expected to provide insights into the potential of biologically sourced organic fertilizers to improve the nutritional quality of leafy vegetables, support sustainable agriculture, and contribute to food security and public health.

This study is significant because it addresses the persistent problem of poor soil fertility, which limits the accumulation of essential antioxidants in *Vernonia amygdalina*, a widely consumed leafy vegetable in Nigeria. By evaluating the effect of an organic fertilizer derived from *Craseonycteris thonglongyai* droppings, the research provides evidence-based insight into sustainable soil amendment strategies capable of improving the nutritional and phytochemical quality of vegetables. The findings contribute to food security, public health, and environmentally friendly agricultural practices by demonstrating a viable alternative to synthetic fertilizers. This work also expands scientific knowledge on the use of biologically sourced organic manure to enhance free radical scavenging compounds in leafy vegetables.

2.0 Materials and Methods

2.1 Study Area

The experiment was conducted in a pot at the Department of Biochemistry, Faculty of Natural Sciences, Ibrahim Badamasi Babangida University (IBBU), Lapai, Niger State, Nigeria. Lapai is a Local Government Area in Niger State adjoining the Federal Capital Territory, Abuja. Its headquarters is located in Lapai town along the A124 highway between latitude 9°03′17.60″N and 9°05′07.22″N, and longitude 6°33′49.53″E and 6°35′38.47″E (NIPOST, 2009), with a total landmass of approximately 3,051 km².

2.1.1 Geology of the Study Area

The study area, Lapai, is characterized by two major geological formations: the sedimentary rocks to the south and the basement complex to the north. The sedimentary region comprises sandstones and alluvial deposits, especially along the Niger Valley and in parts of Gulu, Muye, and eastern Lapai town. These areas possess extensive flood plains of the River Niger, making them fertile zones suitable for agriculture.

To the north, the basement complex consists predominantly of granitic outcrops (inselbergs) that form a gently undulating terrain. These features dominate the landscape toward Paikoro Local Government Area. The university is situated within this basement complex zone, characterized by well-drained, sandy loam soils suitable for controlled pot experiments (Olowolafe & Dung, 2000).

2.1.2 Climate

The area experiences two distinct seasons — the dry and wet seasons. The annual rainfall ranges from 1,200 mm in the north to 1,600 mm in the south, with the rainy season lasting between 150 and 210 days. Mean maximum temperature remains high throughout the year, averaging 32 °C, especially from March to June. The lowest minimum temperatures (20–22 °C) occur between December and January under the influence of the tropical continental air mass. The dry season begins in October, while the wet season extends from April to October, with an average annual rainfall of 1,334 mm. August and September record the highest precipitation (300–330 mm). The monthly mean temperature peaks in March (30–40°C) and is lowest in August (22.3°C).

These climatic conditions are favorable for the cultivation of leafy vegetables such as Vernonia amygdalina, which thrives in tropical environments with moderate rainfall and high temperatures (Degu *et al*., 2024).

2.2 Source of Cuttings

Cuttings of *Vernonia amygdalina* (bitter leaf) were obtained from the National Horticultural Research Institute (NIHORT), Ibadan, Oyo State, Nigeria. The selection was based on the institute's certification of the variety for experimental use due to its known antioxidant potency and consistent growth rate (Eraga *et al*., 2022).

2.2.1 Source of Organic Fertilizer (Craseonycteris thonglongyai Droppings)

The dried droppings of *C. thonglongyai* were carefully collected into clean sacks from a natural cave in Faso, Edati Local Government Area, Niger State. The droppings were sun-dried, pulverized, and stored under hygienic conditions before use. This organic material was selected for its potential high nitrogen and phosphorus content typical of bat guano, which enhances soil fertility (Baldock, 2007; Musa *et al*., 2016b).

2.3 Soil Sampling and Analysis

Soil for the pot experiment was obtained from a site at the Main Campus of IBBU, Lapai, where the land had been left fallow for several years. Surface (0–20 cm) soil samples were collected, air-dried, and sieved through a 2 mm mesh. The chemical composition of the *C. thonglongyai* droppings and the physicochemical properties of the soil were determined following the procedure of Bieganowski *et al*. (2015). Parameters analyzed included pH, organic carbon, total nitrogen, available phosphorus, and exchangeable cations (Ca, Mg, K, Na). All analyses were carried out in triplicate to ensure reliability and accuracy.

2.3.1 Fertilizer Treatment and Application

The *C. thonglongyai* droppings were air-dried, pulverized, and homogenized into fine particles, after which 100 g was applied per pot containing 20.00 kg of soil. The synthetic fertilizer (NPK) was applied at the recommended rate of 30 mg N/kg soil, 30 mg P_2O_5/kg soil, and 22 mg K_2O/kg soil (NIHORT, 1983; Musa, 2010). The control group received no fertilizer application. All fertilizers were applied at the base of the plant at 2 weeks after planting (WAP) to ensure optimal nutrient uptake.

2.3.2 Agronomic Conditions

Two cuttings of *V. amygdalina* were planted per pot containing 20.00 kg of soil. After germination, the plants were thinned to one per pot to avoid competition. A Completely Randomized Design (CRD) was adopted with three soil treatments, namely, (i) Control (no fertilizer), (ii) synthetic fertilizer (NPK) and (iii) *C. thonglongyai* organic fertilizer (100 g/20 kg soil)

Each treatment had 10 pots replicated three times, giving a total of 90 experimental units. Plants were watered twice daily (morning and evening) using a watering can, except on rainy days. The experimental site was kept clean and pest-free, and the pots were occasionally raised to prevent root penetration into the ground (Musa, 2016a; FAO, 2019).

2.4 Determination of Free Radical Scavenger Contents

Freshly harvested leaves of *V. amygdalina* at the vegetative phase were analyzed using standard biochemical methods. Carotenoid and lycopene contents were determined following Zakaria *et al*. (1979), while tocopherol content was analyzed using the Emmerie–Engel reaction described by Rosenberg (1992). Chlorophyll a and b contents were quantified according to Whitney *et al*. (1990) and Branisa *et al*. (2014). Ascorbic acid (vitamin C) was evaluated using the 2,6-dichlorophenol-indophenol titration method of Pirdawd *et al*. (2025). β-carotene content was determined using ethanol and petroleum ether extraction as described by Musa *et al*. (2010). Flavonoid concentration was analyzed using the aluminum chloride colorimetric method of Chang *et al*. (2005), and total phenol content was determined following Singleton *et al*. (1999). All spectrophotometric readings were taken at their respective wavelengths, and results were expressed as milligrams per 100 grams of fresh weight (FW).

2.5 Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 16.0). Results were expressed as mean ± standard error of mean (SEM). Comparisons between treatments were performed using one-way Analysis of Variance (ANOVA), and Duncan's Multiple Range Test (DMRT) was used for post-hoc comparison of means. A p-value < 0.05 was considered statistically significant (Steel *et al*., 1997).

3.0 Results and Discussion

3.1 Results

3.1.1 Physical and Chemical Properties of Soil

The result of physical and chemical properties of the soil used for the pot experiment is presented in Table 1. The pH of the soil, which is 6.7, implies that the soil is neutral. The calcium, sodium and organic carbon of the soil are low, while potassium and nitrogen are very low. Similarly, whereas available phosphorus and magnesium contents are moderate, the base saturation is very high.

3.1.2 Chemical Properties of the Organic Fertilizer (C. thonglongyai Droppings)

The chemical characteristics of the organic fertilizer derived from *C. thonglongyai* droppings are presented in Table 2. The manure exhibited a strongly acidic pH value of 6.02, indicating its potential to influence soil acidity and nutrient solubility when applied to cultivated soil. The levels of organic carbon, phosphorus, total nitrogen, potassium, and sodium were remarkably high, suggesting that the droppings are a rich source of essential macronutrients required for vegetative growth and the biosynthesis of secondary metabolites.

In contrast, magnesium and calcium were present in moderate and low concentrations, respectively, implying that the manure contributes limited amounts of these nutrients compared with the major macronutrients. Notably, the concentrations of copper and zinc were considerably high. These micronutrients are important cofactors in several enzymatic processes and play critical roles in the synthesis of antioxidant compounds, chlorophyll formation, and overall plant metabolic function.

Overall, the nutrient profile of *C. thonglongyai* droppings indicates that it is a nutrient-dense organic amendment capable of enhancing soil fertility and supporting improved accumulation of bioactive compounds in leafy vegetables such as *Vernonia amygdalina*.

Table 1: Physical and chemical properties of the soil (0 – 20 cm depth) used for the experiment

Parameters	Values
Sand (%)	87.30 ±0.23
Silt (%)	7.70 ±0.05
Clay (%)	5.00 ± 0.04
Textural class	Sand
pH (H_2O)	6.70 ±0.02
pH ($CaCl_2$)	7.06 ± 0.13
Organic carbon (g kg^{-1})	4.07 ± 0.10
Total nitrogen (g kg^{-1})	0.22 ± 0.01
Available phosphorus (g kg^{-1})	12.66 ± 0.15
Na^+ (cmol kg^{-1})	0.26 ± 0.03
K^+ (cmol kg^{-1})	0.03 ± 0.01
Mg^{2+} (cmol kg^{-1})	3.30 ±0.12
Ca^{2+} (cmol kg^{-1})	4.00 ± 0.30
Acidity (cmol kg^{-1})	0.14 ± 0.02
CEC (cmol kg^{-1})	7.73 ± 0.21
EC (cmol kg^{-1})	7.59 ± 0.23
Base saturation (%)	99.19 ± 6.10

CEC = Cation exchange capacity, EC = Exchangeable cations. Values represent Mean ± SEM of triplicate determinations. SEM = Standard error mean.

Table 2: Chemical properties of the organic manure (*C. thonglongyai* droppings)

Parameters	Values
pH (H_2O)	6.02 ± 0.14
Organic carbon (g kg^{-1})	30.07 ± 1.03
Total nitrogen (g kg^{-1})	20.16 ± 0.12
Available phosphorus (mg kg^{-1})	32.45 ± 3.01
Na^+ (cmol kg^{-1})	6.21 ± 0.04
K^+ (cmol kg^{-1})	4.09 ± 0.50
Mg^{2+} (cmol kg^{-1})	3.02 ± 0.21
Ca^{2+} (cmol kg^{-1})	2.51 ± 0.12
Cu^{2+} (cmol kg^{-1})	1.86 ± 0.10
Zn^{2+} (cmol kg^{-1})	1.24 ± 0.04

Values represent Mean ± SEM of triplicate determinations. SEM = Standard error mean.

Values are expressed as mean ± standard error of the mean (SEM) of triplicate determinations. Application of both fertilizers produced significant ($p \leq 0.05$) increases in the concentrations of several antioxidant nutrients—particularly lycopene, vitamin E, carotenoid, and flavonoid—compared with the unfertilized control. Among these, V. amygdalina plants treated with the organic fertilizer recorded the highest concentrations of lycopene, carotenoid, and flavonoid, indicating a superior enhancement of antioxidant capacity relative to chemical fertilizer treatment.

In addition, the concentrations of β-carotene, chlorophyll, and phenol significantly ($p \leq 0.05$) increased in plants amended with the organic fertilizer, while chemical fertilizer treatment did not cause significant changes in these parameters when compared with the control. Interestingly, both fertilizer treatments resulted in a significant ($p \leq 0.05$) reduction in vitamin C concentration; however, the decline was less pronounced in the chemically fertilized plants, which retained relatively higher vitamin C content than the organically fertilized ones. Overall, these findings suggest that soil amendment with organic fertilizer (*C. thonglongyai droppings*) enhances the synthesis and accumulation of non-enzymatic antioxidant compounds such as carotenoids, flavonoids, and phenolics in *V. amygdalina* more effectively than chemical fertilizer. The results also confirm the existence of a trade-off between vitamin C content and the accumulation of other antioxidant phytochemicals under fertilized conditions.

Table 3: Effect of Organic and Chemical Fertilizers on the Concentrations of Free Radical Scavengers in *V. amygdalina*

Parameter	Control	Organic Fertilizer	Chemical Fertilizer	F-value	p-value	Significant Difference (α = 0.05)
β-carotene (μg/100 g)	1200 ± 32.13[a]	1600 ± 22.67[b]	1200 ± 33.47[a]	16.72	0.002	Yes
Vitamin C (mg/100 g)	287.8 ± 37.26[c]	253.0 ± 31.60[a]	270.4 ± 28.96[b]	9.31	0.008	Yes
Lycopene (mg/g)	50.6 ± 11.82[a]	135.9 ± 9.60[c]	97.3 ± 8.51[b]	21.44	0.001	Yes
Chlorophyll (mg/100 g)	40.0 ± 2.01[a]	70.0 ± 3.01[b]	50.2 ± 2.10[a]	18.23	0.002	Yes
Vitamin E (mg/100 g)	0.60 ± 0.05[a]	0.91 ± 0.02[b]	0.90 ± 0.01[b]	12.56	0.004	Yes
Carotenoid (mg/g)	297.4 ± 16.77[a]	362.3 ± 26.77[c]	320.8 ± 19.78[b]	10.72	0.006	Yes

Flavonoid (mg/100 g)	51.6 ± 9.73[a]	85.9 ± 5.73[c]	71.7 ± 7.65[b]	14.89	0.003	Yes
Phenol (mg/100 g)	81.3 ± 6.51[ab]	86.4 ± 11.79[b]	73.9 ± 5.68[a]	8.52	0.011	Yes

(Values are Mean ± SEM, n = 3. Means with different superscripts differ significantly at p ≤ 0.05 using Tukey's test.)

3.2 Discussion

The textural class of the soil used for the present study was sandy, indicating low water retention capacity and poor nutrient-holding potential. However, the soil's loose structure confers good aeration, permeability, and ease of tillage. The high base saturation observed agrees with the report of FAO (2006), suggesting that the exchange sites of the soil were largely occupied by basic cations such as Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}, even though the absolute nutrient concentrations remained low. The generally low levels of essential macronutrients (N, P, K) and organic carbon observed may be attributed to continuous cultivation and leaching losses, which deplete organic matter and diminish soil fertility over time (Lawal *et al*., 2022; Abu *et al*., 2023). This justifies the need for soil amendment using organic or inorganic fertilizers to restore fertility and enhance crop productivity.

The organic manure used in this study—droppings of *Craseonycteris thonglongyai* showed relatively high concentrations of organic carbon, nitrogen, phosphorus, and potassium, confirming its potential as a nutrient-rich organic amendment. The abundance of zinc and copper in the manure also provides trace elements essential for enzymatic activation and chlorophyll synthesis in plants. These findings support earlier reports that organic fertilizers improve soil microbial activity, structure, and nutrient cycling, thereby promoting sustainable plant growth and yield (Abu *et al*., 2020; Musa *et al*., 2022; Adegbite *et al*., 2024).

The significant increases in lycopene, vitamin E, carotenoid, and flavonoid contents in V. amygdalina leaves treated with both organic and chemical fertilizers relative to the control indicate that nutrient supplementation enhanced the synthesis of antioxidant metabolites. These phytochemicals are critical in protecting plant tissues against oxidative stress and are beneficial to human health due to their roles in scavenging reactive oxygen species (ROS) (Musa, 2021; Oyedeji *et al*., 2023). Notably, plants grown with organic fertilizer accumulated higher levels of β-carotene, chlorophyll, carotenoid, flavonoid, and phenolic compounds than those treated with chemical fertilizer. This observation reinforces the increasing preference for organically produced vegetables, which are often reported to contain higher concentrations of health-promoting antioxidants and secondary metabolites (Makinde *et al*., 2010; Musa, 2016b; Abu *et al*., 2023).

The superior performance of the organic fertilizer treatment can be explained by its slow nutrient release pattern and microbial-mediated mineralization. Unlike inorganic fertilizers, which dissolve rapidly and are prone to leaching, organic amendments decompose gradually, maintaining a more balanced supply of nutrients over time. Microbial activity associated with organic matter decomposition also enhances soil biological health, releasing nutrients such as carbon, nitrogen, and phosphorus in forms readily available for plant uptake (Musa *et al*., 2019; Adetunji *et al*., 2024). This sustained nutrient availability may account for the increased biosynthesis of carotenoids, phenols, and flavonoids in organically fertilized plants.

Interestingly, the observed decrease in vitamin C concentration following fertilization with both organic and inorganic fertilizers aligns with earlier findings (Musa *et al*., 2010; Musa, 2021). This inverse relationship between nitrogen availability and vitamin C synthesis may result from the nitrogen-induced reduction in carbohydrate reserves, as ascorbic acid biosynthesis depends on carbohydrate metabolism. Although vitamin C was highest in the unfertilized plants, the values recorded in fertilized treatments remained above the recommended dietary intake of 60 mg/100 g (George, 1999), suggesting that fertilization did not compromise the vegetable's nutritional adequacy.

The overall findings demonstrate that organic manure application (C. thonglongyai droppings) enhances the bioaccumulation of natural antioxidants in V. amygdalina more effectively than synthetic fertilizer. The enriched antioxidant profile of organically cultivated V. amygdalina supports its potential health benefits, particularly in mitigating oxidative stress–related and degenerative diseases. Therefore, the use of organic fertilizers not only promotes soil fertility and environmental sustainability but also improves the functional nutritional quality of this important leafy vegetable.

4.0 Conclusion

The findings of this study revealed that the soil used for the cultivation of *Vernonia amygdalina* was sandy with low nutrient and organic matter content, justifying the need for soil amendment to improve fertility. The application of both organic and chemical fertilizers significantly enhanced the concentrations of several antioxidant compounds in the leaves of *V. amygdalina* compared with the control, indicating that soil enrichment promotes the synthesis of bioactive nutrients. However, plants treated with organic fertilizer (*Craseonycteris thonglongyai* droppings) showed markedly higher levels of β-carotene, lycopene, carotenoid, chlorophyll, flavonoid, and phenolic compounds than those treated with chemical fertilizer, suggesting that the organic manure provides a more balanced and sustained nutrient supply that enhances antioxidant accumulation. Although vitamin C concentration decreased following fertilizer application, its level in the treated plants remained nutritionally sufficient.

In conclusion, the study demonstrates that the use of organic manure derived from *C. thonglongyai* droppings significantly improves soil fertility and enhances the accumulation of natural antioxidants in *V. amygdalina* compared to synthetic fertilizers. This implies that organic fertilization promotes the production of nutritionally superior and health-promoting vegetables while contributing to environmental sustainability and long-term soil health.

Based on the findings, it is recommended that farmers adopt the use of organic manure such as *C. thonglongyai* droppings in the cultivation of *V. amygdalina* and similar leafy vegetables to improve crop quality, enhance antioxidant content, and reduce dependence on chemical fertilizers. Further studies should explore the long-term effects of this organic fertilizer on soil microbial activity, nutrient cycling, and yield performance under field conditions.

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Data availability

The microcontroller source code and any other information can be obtained from the corresponding author via email.

Author contributions

Matthew T. Tsepav conceived and designed the study, coordinated the experimental setup, ensured methodological adherence, and contributed significantly to the interpretation of the data. Abduljelili Uthman performed the biochemical analyses, assessed antioxidant parameters with precision, and provided essential support in interpreting the results. Amanabo Musa supervised the entire research process, validated the analytical procedures, interpreted the findings, and took the lead in preparing, reviewing, and revising the manuscript for publication.