

Phytochemical Profiling, Mineral Analysis, Nutritional Composition, and Bioactivity Assessment of a Commercial Honey-Fortified Polyherbal Formulation

Eniayewu O.I.^{1*}, Emenem E.^{1,2}, Rotimi M.¹, Shittu A.¹, Sanusi A.¹, Afosi A.B.³, Abdullahi S.T.¹, Njinga N.S.¹, Aiyelero O.M.⁴, Akinpelu L.A.⁴, Giwa H.B.⁵, Williams F.E.⁵, Bakare-Odunola M.T.¹

¹Department of Pharmaceutical and Medicinal Chemistry, University of Ilorin

²Central Research and Diagnostic Laboratory, Ilorin, Nigeria

³Department of Pharmaceutics and Industrial Pharmacy, University of Ilorin, Nigeria

⁴Department of Pharmacology and Toxicology, University of Ilorin, Nigeria

⁵Department of Clinical Pharmacy and Pharmacy Practice, University of Ilorin, Nigeria.

Corresponding Author's Email & Phone No.: eniayewu.oi@unilorin.edu.ng, eniayewuoluwasegun@gmail.com; +2347033823109

Received on: October, 2025

Revised and Accepted on: November, 2025

Published on: December, 2025

ABSTRACT

HM-4 is a commercial polyherbal product used for the treatment of various illnesses, including hypertension and diabetes. It consists of honey and four different medicinal plants; *Curcuma longa*, *Aframomum melegueta*, *Zingiber officinale* and *Piper nigrum* in various unrevealed proportions. This study aims to determine the phytochemical, proximate and mineral composition, as well as in-vitro antidiabetic and antioxidant potential of HM-4. Phytochemical screening for the presence of bioactive compounds in the HM-4 was performed using standard methods. Proximate and mineral composition were determined using AOAC guidelines and atomic absorption spectrophotometry after wet digestion, respectively. Antioxidant potential was evaluated using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), nitric oxide and hydroxyl ion scavenging assays. In-vitro antidiabetic potential was determined using 3,5-dinitrosalicylic acid (DNSA) method. Phytochemical analysis identified several bioactive compounds including flavonoids, phenolics, alkaloids, tannins and terpenoids. Flavonoids content was 509.6 mg/QE, alkaloid 12.5 mg/100g, total phenolics 0.343 mg/GAE, and tannins 0.378 mg/100g. HM-4 possess good antioxidant property, with IC₅₀ values of 148 and 112 µg/ml for DPPH and ABTS assays, respectively. The HM-4 is rich in essential micronutrients, predominantly potassium. Proximate analysis revealed that HM-4 is rich in carbohydrate (89%w/v) and calories (1554%w/v). The HM-4 exhibits in-vitro antidiabetic property, with 57,1% alpha amylase inhibition at extract concentration of 500 µg/ml, indicating its potential as an adjunct in the management of diabetes.

The HM-4 is a valuable source of bioactive compounds and micronutrients, offering significant antioxidants and antidiabetic properties that could be further explored for the development of novel nutraceuticals

Keywords: Honey, polyherbal formulation, phytoconstituents, antioxidant, antidiabetic, proximate analysis.

1.0 INTRODUCTION

Medicinal plants remain a viable source of bioactive compounds. These compounds which may include, but not limited to alkaloids, flavonoids, terpenoids, and saponins, are known to possess various therapeutic properties which are continuously explored in the development of new drugs (Bergonzi *et al.*, 2022; Nasim *et al.*, 2022). The combination of multiple medicinal plants to produce a polyherbal formulation for therapeutic purposes has gained significant interest due to its synergistic effect which enhances its efficacy and minimizes untoward effects (Veenadevi *et al.*, 2024). Studies have shown that polyherbal medicines possess expanded spectrum of activity including antimicrobial, antidiabetic, anti-inflammatory, anti-cancer, antioxidant, anti-hypertensive and wound healing properties, neuroprotective (Patel *et al.*, 2025). Additionally,

antimicrobial resistance is limited with the use of polyherbal formulations further making them a more acceptable alternative to conventional medicines, particularly those used in the management of chronic health conditions (Patel *et al.*, 2025).

One major drawback with the use of polyherbal formulations is the absence of scientific evidence to ascertain its phytochemical compositions, nutritional value, quality and efficacy. Often times, their utility is largely based on ethnomedicinal claims which limit their optimal usage and their overall quality (Matthews, 2024). This drawback highlights the need to conduct scientific studies to validate the safety and efficacy of polyherbal formulations to optimize their usage. The present study aims to evaluate the phytochemical, mineral, and nutritional composition, as well as in-vitro

antioxidant and anti-diabetic potential of a commercial polyherbal formulation consisting of honey and four different medicinal plants; *Curcuma longa*, *Aframomum melegueta*, *Zingiber officinale* and *Piper nigrum*.

Honey is a popular and widely acceptable food source known to be very rich in bioactive compounds, including polyphenols and flavonoids (Rani & Verma, 2025). Numerous studies have established its nutritious values and therapeutic benefits including antioxidants, anti-inflammatory, antimicrobial and anticancer properties. Combination of honey with herbal extracts not only enhances the therapeutic effect of polyherbal formula through synergistic effect but also provides additional antimicrobial protection, enhances its taste as well as its absorption (Gośliński *et al.*, 2020; Ranneh *et al.*, 2021; Cilla *et al.*, 2022).

Curcuma longa, popularly known as turmeric is known to possess a wide range of pharmacological actions, including antibacterial and anti-fungal activities owing to the presence of essential elements, proteins, carbohydrates, vitamins, volatile oils, curcumin, and curcuminoids (Fuloria *et al.*, 2022). *Piper nigrum* is also rich in essential oils and possess notable biological activities such as antioxidant, antimicrobial and anti-inflammatory effects (Tekade *et al.*, 2024). Ginger (*Zingiber officinale*) is rich in bioactive compounds such as polyphenols, flavonoids in addition to 6-gingerol, gingerdiol, gingerdione, paradols, shogaols, sesquiterpenes, and zingerone, It is used as a food spice and exhibit anti-inflammatory, hypoglycemic and antioxidant actions (Paudel *et al.*, 2025). The seed of *Aframomum melegueta* was recently reported to possess neuromodulatory effect and potentially useful in anxiety and stress management (Pérez-Machín *et al.*, 2025).

The primary objective of this research was to assess the phytochemical, mineral and nutritional composition of HM-4, a commercial polyherbal product comprising of honey, *Curcuma longa* (rhizomes), *Aframomum melegueta* (seed), *Zingiber officinale* (rhizomes) and *Piper nigrum* (seed) in various unrevealed proportions. In addition, this study seeks to evaluate the antioxidant and antidiabetic effect of this formulation using standard in-vitro methods

2.0 MATERIALS AND METHODS

2.1 Sample Purchase and Extraction

HM-4 was purchased in Ile-Ife, Osun State, Nigeria. The brown-coloured granules of HM-4 were powdered using a mechanical grinder. About 10g of the powder was weighed and extracted with 200 ml of methanol for 48 h. The extract was filtered using a Whatmann No 1 filter and the filtrate was concentrated to dryness on a water bath at

a temperature of 50°C. All analysis performed on the extract were carried out at the Central Research and Diagnostic Laboratory, Ilorin, Nigeria.

2.2 Qualitative Phytochemical Screening

The presence of alkaloids, flavonoids, polyphenols, saponins, tannins, Phlobatannins, terpenoids, steroids, glycosides, starch, coumarin, amino acids, anthraquinones in methanolic extract of HM-4 were determined using standard methods

2.3 Quantitative Phytochemical Screening

The determination of the phytoconstituents of methanolic extract of HM-4 was performed using previously reported methods (Singh *et al.*, 2004; Ayodeji *et al.*, 2016; Ademoye *et al.*, 2018; Zaïri *et al.*, 2020)

2.3.1 Determination of Alkaloids

The total alkaloid content of the HM-4 extract was determined using the 1,10-phenanthroline method as previously described (Obadoni & Ochuko, 2002), with slight modification. A 0.01 g/mL solution of the extract was prepared in methanol and aliquot of 1 mL of this solution was transferred into a 10 mL volumetric flask, followed by the addition of 1 mL of 0.025 M ferric chloride prepared in 0.5 M hydrochloric acid and 1 mL of 0.05 M 1,10-phenanthroline prepared in ethanol. The mixture was diluted to volume with distilled water and incubated in a water bath maintained at 70 ± 2 °C for 30 min. The absorbance of the resulting, red-colored solution was measured at 510 nm against a reagent blank. Standard solutions of quinine were prepared and treated in the same manner as the samples. The total alkaloid content of the extract was estimated from the quinine calibration curve and expressed as milligrams of quinine equivalent per gram of extract (mg QE/g).

2.3.2 Determination of Total Phenolics

The phenol content in the methanol extract of HM-4 was determined using the Folin-Ciocalteu reagent method. A 2 mL solution of 10% Folin-Ciocalteu reagent and 1.6 mL of 2% sodium bicarbonate solution was added to 2 mL of HM-4 extract. The resultant solution was incubated at room temperature for 30 minutes and the absorbance measure at a wavelength of 765 nm. The concentration of phenol in the extract was extrapolated from standard absorbance versus concentration curve obtained from pure gallic acid over a concentration range of 0.1-0.7 mg/ml. The results were expressed as gallic acid equivalent (mg/g of extracted compound).

2.3.3 Determination of Total Flavonoid

To determine the total flavonoid content in HM-4 extract, about 1 mL of the extract and 4 mL of distilled were

mixed and 0.3 ml of 5% NaNO_2 . After 5 minutes, 0.3 ml of 10% AlCl_3 was added and allowed to stand for 6 minutes, after which 2 ml of 1 M NaOH was added and the resultant solution was made up to 10 ml with distilled water. The solution was mixed well, and the absorbance of the test solution recorded at 510 nm was measured against prepared reagent blank at 510 nm. Absorbance values of varying concentration of Quercetin were used to plot standard absorbance versus concentration curve. Flavonoid content in NM-4 was estimated from the standard curve and results expressed as mg of Quercetin equivalent per 100 g of fresh mass.

2.3.4 Determination of Steroids

This was done following the previously described method (Ademoye *et al.*, 2018). Briefly, 1 ml of the methanolic extract of HM-4 was reacted with 1 ml of a 4N H_2SO_4 after which 1 ml of 0.5% FeCl_3 solution and 0.25 ml of 0.5% (w/v) potassium hexacyanoferrate (III) solution were added. The resulting solution was boiled for 30 minutes at a temperature of 70°C , allowed to cool and diluted with 250 ml of distilled water. The absorbance of the resultant solution was recorded at 780 nm and the steroid content was computed from standard calibration curve (Madhu *et al.*, 2016).

2.3.5 Determination of Triterpenes

A 0.5 g of the NH-4 extract was placed in a 100 mL conical flask, after which 20 mL of a chloroform-methanol mixture (2:1, v/v) was added. The contents were thoroughly agitated and allowed to stand for approximately 15 minutes. The resulting supernatant was decanted, and the remaining residue was rinsed with another 20 mL portion of the same solvent mixture. The washed residue was centrifuged and subsequently reconstituted in 40 mL of 10% sodium dodecyl sulfate (SDS) solution. Thereafter, 1 mL of 0.01 M ferric chloride solution was added, and the flask was gently mixed before allowing it to stand for 30 minutes. A reagent blank was prepared by substituting the extract with distilled water. A calibration curve was generated using standard triterpene concentrations ranging from 1–5 mg/mL, prepared from a 100 mg/L stock solution. Absorbance readings for both the standards and the test samples were recorded at 510 nm using a spectrophotometer. The triterpene content of each sample was estimated from the calibration plot.

2.3.6 Determination of Coumarin

A stock solution of the HM-4 extract (0.01 g/mL) was first prepared. To 1 mL of this solution, 0.5 mL of 5 N sodium hydroxide was added, and the mixture was heated at 80°C for about 5 minutes before being allowed to cool. Subsequently, 0.75 mL of 5 N sulfuric acid (H_2SO_4) was added, followed by thorough mixing. Thereafter, 0.25 g

of anhydrous sodium carbonate was incorporated and mixed until completely dispersed. The resulting mixture was transferred into a separating funnel, and 50 mL of petroleum ether was added for extraction. The upper petroleum ether layer was collected into a clean flask and washed with 20 mL of distilled water. The solvent layer was evaporated on a water bath maintained at $50\text{--}55^\circ\text{C}$ to dryness. The residue obtained was dissolved in water and made up to 50 mL in a volumetric flask with continuous agitation. From this solution, 25 mL was taken into another flask, and 5 mL of 1% sodium carbonate solution was added. The mixture was then heated in a water bath at 85°C for 15 minutes with continuous stirring. After cooling, 5 mL of freshly prepared diazonium reagent was added and left to stand for one hour. The absorbance of the colored solution was read at 540 nm against a reagent blank. Quantification of coumarin was carried out using a calibration curve generated from standard coumarin concentrations ranging between 1 and 10 mg/L.

2.3.7 Determination of Tannin

To 1.0 g of the HM-4 extract was added 10 ml of distilled water and vortex mixed. This was incubated for 30 minutes at room temperature, centrifuged at 1500rpm and 500 μl of the supernatant was transferred into a clean test tube. To this was added 1.0 ml of Folin-C reagent and 0.5 ml of 17% sodium carbonate. 10.5 ml of distilled water was added to the reaction mixture and was incubated for 1hr at room temperature. The absorbance was read at 760 nm. Blank Solution was prepared as well but distilled water was used in place of sample. For standard, a 1-10ppm solution of tannic acid was prepared and concentration varied to obtain a calibration curve. The absorbance of the tannic acid standard solution was taken at 760nm as the sample. The amount of tannic acid in the samples were derived from the calibration curve.

2.3.8 Determination of Terpenoids

About 1.0 g of the HM-4 extract was weighed and soaked in 25 ml of 95 % ethanol for 24 h. The extract was filtered, and the filtrate was extracted with petroleum ether and concentrated to dryness. The dried ether extract was treated as terpenoids per 100g sample.

2.3.9 Determination of Glycosides

Approximately 0.5 g of the HM-4 extract was placed in a 250 mL conical flask, and 25 mL of chloroform was introduced. The mixture was agitated on a vortex mixer for about one hour to ensure thorough extraction. Afterward, the contents were filtered into a clean flask. To the filtrate, 10 mL of pyridine and 2 mL of a 2% sodium nitroprusside solution were added, and the mixture was vigorously shaken for 10 minutes. Subsequently, 3 mL of 20% sodium hydroxide solution

was added to develop a brownish-yellow coloration. Standard glycoside solutions (1–5 mg/mL) were prepared from a 100 mg/mL stock solution and processed under the same conditions as the test sample. Absorbance values for both the standards and the extract were recorded at 510 nm using a UV–Visible spectrophotometer. The concentration of glycosides in the sample was then estimated from the calibration curve obtained from the standard series.

2.3.10 Determination of Saponins

About 1ml of methanolic HM-4 extract was transferred into a 50ml volumetric flask and 2ml of 5% FeCl₃ solution was added and made up to the mark with distilled water. This was then allowed to stand for 30 minutes for a blood red colour to develop. Standard saponins solution (2-10 ppm) was prepared from saponins stock solution. The standard solutions were treated similarly with 2ml of 5% FeCl₃ solution as earlier described. The absorbance of the samples as well as standard saponin solutions were read using UV/Visible spectrophotometer at a wavelength of 380nm. The saponin content in the samples were derived from the standard curve plotted.

2.4 In-vitro Antioxidant Assay

2.4.1 ABTS Radical Scavenging Assay

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) assay performed as described by Re and coauthors (Re *et al.*, 1999). About 7 mM ABTS and 2.45 mM potassium persulfate were mixed in a 1:1 ratio and allowed to stand at room temperature (protected from light) for 24 hours to generate radicals. Radical stock solution was diluted with distilled water (1:89) to obtain an absorbance value of 0.70 ± 0.02 at 734 nm. The antioxidant activity of the extract was determined by adding 500 μ L of the prepared ABTS solution to 1.0 ml of the HM-4 extract or standard, incubated at 30°C in the dark and absorbance measured at 734 nm using distilled water as a blank. Vitamin C was used as a standard.

2.4.2 DPPH Free Radical Scavenging Assay

The method reported by Ademoye and co-authors was employed to assess the ability of HM-4 extract to neutralize DPPH free radicals (Ademoye *et al.*, 2018). This method involved an initial preparation of five different concentrations of HM-4 in methanol. To a 1 mL solution of each of these concentrations was added 1 mL of freshly prepared DPPH solution. The resultant solution was vortex-mixed and thereafter incubated in the dark at ambient temperature for 30 minutes. The absorbances of these solutions were measured using spectrophotometric method at a wavelength of 518 nm using ascorbic acid as a standard. Methanol in DPPH solution was used as the control. The percentage radical

scavenging activity of the HM-4 extract was calculated using the formula below

$$\% \text{ DPPH Scavenging Activity} = \frac{ABS_{Control} - ABS_{Sample}}{ABS_{Control}} \times 100$$

2.4.3 Ferric Reducing Antioxidant Property (FRAP) Assay

The assay was performed according to the method described by Gülçin and co-authors (Gülçin *et al.*, 2010). A 1mg/ml solution of the HM-4 extract and standard were prepared and diluted to various concentrations (100-500 μ g/ml). To 1ml of the extract or standard was added to 2.5 ml of sodium phosphate buffer and 2.5 ml of potassium ferrocyanide solution. The mixture was incubated at 50°C for 20 mins, allowed to cool, and 2.5 ml of trichloroacetic acid was added, centrifuge at 3000rpm for 5min. A 2.5 ml portion of the supernatant was transferred into another test tube and mixed with 2.5 ml of distilled water. To this was added 0.5 ml of Ferric chloride solution and absorbance measured at 700 nm. Gallic acid was used as a standard.

2.4.4 Nitric oxide Radical Scavenging Assay

The ability of nitric oxide radical scavenging ability of HM-4 was determined using a previously reported method (Awah & Wirnkor, 2010). This method involves the reaction of 500 μ L of different HM-4 extract concentrations (100-500 μ g/ml) with 2 ml solution of 10mM sodium nitroprusside in phosphate buffer solution. The resulting solution was thereafter incubated at 25°C for 1hr, following by reacting equal volume of resulting sample solution with Griess reagent. The final solution was allowed to stand for 30 minutes at room temperature (in the dark) before absorbance was measured at 546 nm. Ascorbic acid serves as the standard while phosphate buffer solution was used as the blank reagent. The experiment was performed in triplicate.

2.4.5 Hydroxyl Radical Scavenging Assay

To determine the hydroxyl radical scavenging potential of HM-4, a 0.1 mL volume of different concentration (100-500 μ g/mL) of the HM-4 extract was reacted with 500 μ L of 1,10 phenanthroline, 1000 μ L of Sodium phosphate buffer, 500 μ L of ferrous sulfate and 500 μ L of hydrogen peroxide. The resultant solution was vortex-mixed and allowed to stand at room temperature for 30 minutes after which absorbance was recorded at 536 nm using distilled water and ascorbic acid as blank and positive control respectively.

2.5 Alpha-amylase Inhibition Assay

The α -Amylase inhibitory activity was performed using a previously described method (Quazi *et al.*, 2022). The

inhibitory activity of the HM-4 extract and acarbose (standard) was calculated using the formula below

$$\text{Inhibitory activity (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_s is the absorbance in the presence of HM-4 extract or acarbose (standard) and A_c is the absorbance of control (all reagent without sample)

2.6 Proximate Analysis

The proximate composition (moisture content, ash content, lipid content, protein content, carbohydrate content, crude fiber and caloric value) of the methanolic extract of HM-4 was analyzed using standard methods (AOAC, 2005)

2.7 Mineral Analysis

Determination of the mineral content in HM-4 followed the procedure outlined by AOAC (2019) with slight modifications. An accurately weighed portion (5.0 g) of each sample was incinerated in a muffle furnace at 550 °C until white ash was obtained. The ash residue was digested in 10 mL of 10% hydrochloric acid, transferred through Whatman filter paper into a 100 mL volumetric flask, and diluted to volume with deionized water. The concentrations of mineral elements were quantified using an Atomic Absorption Spectrophotometer (PG Instruments, Model AA990). The final concentrations were expressed in mg/L (ppm) after correction for the applied dilution factor.

2.8 Data Analysis

Results were computed using Microsoft Excel and expressed in mean \pm SD. Graphs were plotted using GraphPad Prism version 8.4.0 software.

3.0 RESULTS

3.1 Phytochemical Screening

The results of qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolics, tannins, steroids, coumarins, terpenoids, glycosides, and starch in the methanolic extract of HM-4 (Table 1).

Table 1: Qualitative phytochemical screening of HM-4

Phytochemical	Results
Alkaloids	+
Amino acids	-
Phlobatannins	-
Tannins	+
Phenolics	+
Flavonoids	+
Steroids	+
Coumarins	+
Terpenoids	+
Glycosides	+
Anthocyanin	-
Saponin	+
Starch	+

The content of these phytochemicals in the extract varied significantly ($p < 0.05$), with flavonoids (509.67 ± 0.33 mg QE) and alkaloids (12.5 ± 0.30 mg/100 g) being the most predominant in the HM-4 (Table 2).

Table 2: Quantitative phytochemical screening of HM-4.

Phytochemical Constituents	Concentration (mg/100g)
Alkaloids	12.5 ± 0.30
Saponins	0.0075 ± 0.0001
Phenolics (mg/GAE)	0.343 ± 0.081
Flavonoids (mg/QE)	509.67 ± 0.33
Steroids	0.76 ± 0.075
Coumarins	0.039 ± 0.002
Terpenoids	1.68 ± 0.00
Glycosides	0.713 ± 0.0004
Triterpenes	0.168 ± 0.05
Tannins	0.378 ± 0.011

Values presented as Mean \pm SD ($n=3$)

3.2 In-vitro Antioxidant Evaluation

In this study, we determined the antioxidant potential of HM-4 using various in-vitro tests. The tests were performed at various extract concentration ranging between 100 and 500 $\mu\text{g/mL}$ and compared with standard antioxidant compounds, including ascorbic acid (ABTS and hydroxy radical scavenging activity), rutin (nitric oxide assay and gallic acid (DPPH and FRAP)). The graph of percentage scavenging activity versus extract concentrations for all the in-vitro assay tests is presented in Fig 1. Results of the DPPH assay indicate that the HM-4 possesses a concentration-dependent DPPH scavenging potential. The radical scavenging activity for DPPH at 500 $\mu\text{g/ml}$ is 72.5 ± 1.48 compared to gallic acid 77.8 ± 0.87 (Table 3). The 50% inhibitory concentration (IC_{50}) of the HM-4 (148 $\mu\text{g/ml}$) was noted to be significantly higher than that of standard (98 $\mu\text{g/ml}$), indicating moderate DPPH antioxidant capacity compared to the standards (Table 3). In the ABTS assay, a concentration-dependent radical scavenging HM-4 activity was observed. The percentage ABTS scavenging inhibition ranged between 13.2 ± 6.8 at 100 $\mu\text{g/ml}$ to 69.7 ± 0.25 at 500 $\mu\text{g/ml}$. The IC_{50} value of the HM-4 was lower than that of ascorbic acid (Table 3), indicating stronger antioxidant activity. The HM-4 had a ferric reducing antioxidant power of 1605 ± 63.6 $\mu\text{M Fe}^{2+}$ equivalents at 500 $\mu\text{g/ml}$ compared to standard gallic acid of 4750 ± 14.1 $\mu\text{M Fe}^{2+}$ equivalents, suggesting a moderate ferric reducing antioxidant capacity of the extract relative to standard. Nitric oxide scavenging activity of the HM-4 was noted to be comparable to rutin, with extract-rutin IC_{50} ratio of 0.92. Hydroxyl radical scavenging potential of the HM-4 appears to be concentration dependent with maximum percentage scavenging activity of 55.4 ± 0.84 recorded at

500 µg/ml compared to standard 78.5±0.62, at the same concentration.

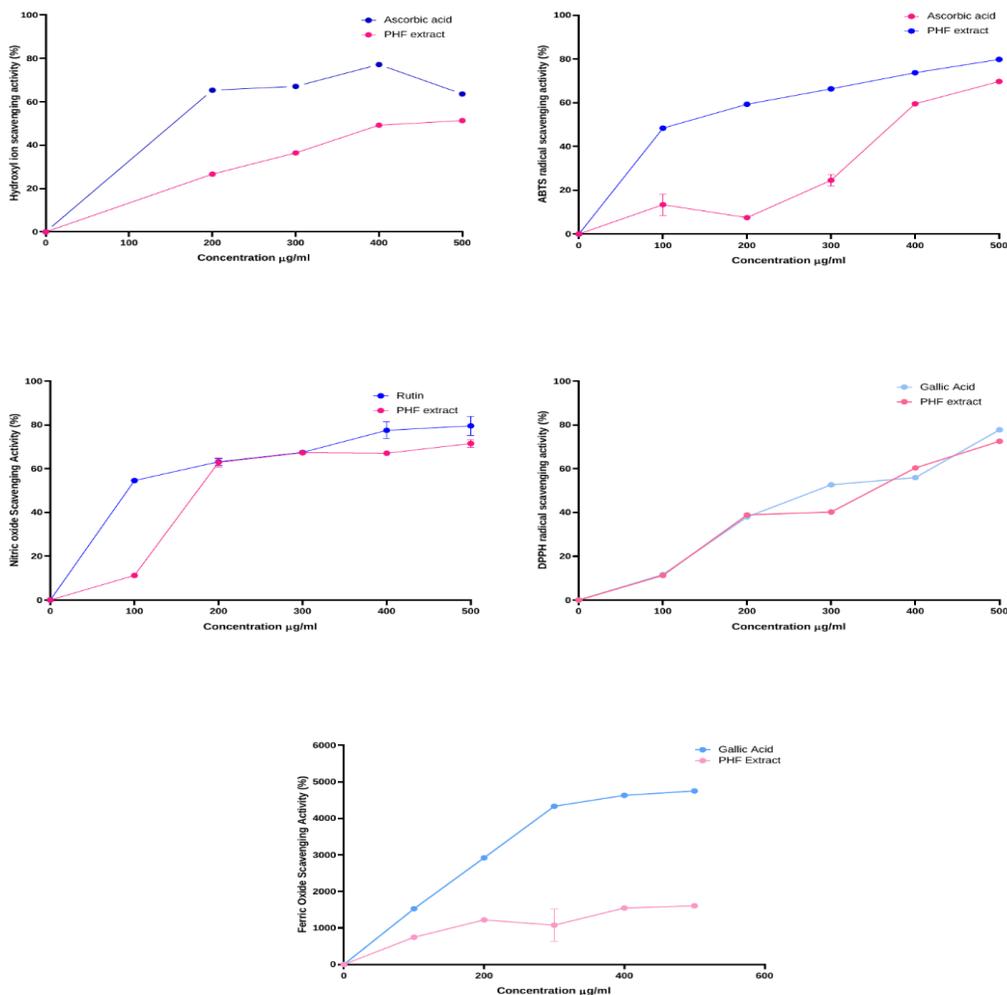


Figure 1: Graph of Percentage scavenging activity versus HM-4 concentrations

Table 3: Percentage Scavenging Activity and IC₅₀ of HM-4 and standard antioxidant compounds

In-vitro Antioxidant Assay	Concentration (µg/mL)	HM-4	Standard
% Scavenging Activity		Mean±SD	
ABTS	100	13.4±6.8	48.4±0.45
	200	7.52±0.51	59.3±0.45
	300	24.6±3.80	66.3±0.91
	400	59.5±1.47	73.7±0.91
	500	69.7±0.25	79.8±0.91
DPPH	100	11.26±0.64	11.6±0.29
	200	38.9±0.21	37.9±0.58
	300	40.2±0.42	52.7±0.58
	400	60.4±1.27	55.9±0.58
FRAP	500	72.5±1.48	77.8±0.87
	100	745±21.2	1525±35.4



	200	1220±42.4	2920±28.3
	300	1075±629	4330±28.3
	400	1545±21.2	4630±14.1
	500	1605±63.6	4750±14.1
Nitric Oxide	100	11.2±1.32	54.5±0.71
	200	62.9±2.97	63.1±2.14
	300	67.3±1.32	67.4±1.07
	400	67.1±1.00	77.5±5.35
	500	71.5±2.62	79.5±6.07
Hydroxyl	100	26.7±0.21	65.4±0.62
	200	36.4±1.70	67.1±0.62
	300	49.2±1.07	77.2±1.20
	400	51.4±0.64	63.6±0.62
	500	55.4±0.84	78.5±0.62
50% Inhibitory Concentrations (IC₅₀)		HM-4	Standards
ABTS		112	161
DPPH		148	98
FRAP		129	92
Nitric Oxide		190	206
Hydroxyl		211	200

3.3 Alpha Amylase Activity of HM-4

The antidiabetic potential of the HM-4 was evaluated by comparing the ability of HM-4 to inhibit alpha amylase enzyme to that of standard acarbose. The methanolic extract of HM-4 exhibited a concentration dependent inhibitory activity against the alpha amylase enzyme. The mean(SD) percentage of amylase inhibition was 13.0(0.57), 32.4(0.64), 47.9(1.13), 48.4(0.64) and 57.1(1.13), for 100, 200, 300, 400 and 500 µg/ml, respectively (Table 4). Although the inhibitory activity of the HM-4 increased with concentration, it was lower than that of acarbose.

Table 4: Alpha amylase inhibition assay

Concentration	HM-4 Extracts	Acarbose (Standard)
100	13.0±0.57	65.6±1.20
200	32.4±0.64	66.0±1.77
300	47.9±1.13	69.8±2.33
400	48.4±0.64	73.1±1.13
500	57.1±1.13	73.5±0.57

3.4 Proximate Composition

The proximate composition of the HM-4 are shown in Table 5. The results obtained indicate that the HM-4 is rich in crude fiber, carbohydrate and calories, with mean(SD) percentage values of 5.10(0.06), 89.6(0.14) and 1554(3.54), respectively. Lipid, protein and ash content of the formulation were minimal. A low moisture content of 2.29% was observed in this formulation.

Table 5: Proximate Composition of HM-4.

Proximate Composition	
Proximate Composition	%w/v (Mean±SD)
Moisture Content	2.29±0.26

Ash Content	1.29±0.13
Lipid Content	0.88±0.00
Crude Fibre	5.10±0.06
Protein Content	0.84±0.01
Carbohydrate Content	89.6±0.14
Caloric Value KJ/100g	1554±3.54

3.5 Mineral Composition of HM-4

The result of the mineral analysis of the HM-4 extract is presented in Table 6. It contains essential micro and macro elements, including iron, potassium, sodium, calcium, zinc, cobalt, phosphorus, nickel, manganese, chromium, and magnesium, in various amounts. Notably, the formulation is highly rich in iron (27.0±3.1), potassium (186.3±68.4), manganese (67.3±88.9), cobalt (184.4±0.00), phosphorus (14.3±5.51), chromium (3.35±4.7) and magnesium (150.8±35.8), measured in mean (SD) part per million(ppm).

Table 6: Mineral composition of HM-4.

Mineral Composition (ppm)	
Mineral	Mean±SD
Iron	27.04±3.06
Potassium	186.3±68.4
Sodium	31.4±30.3
Calcium	26.5±1.23
Zinc	0.16±0.18
Cobalt	184.4±0.00
Phosphorus	14.3±5.51
Nickel	2.74±2.17
Manganese	67.33±88.85
Chromium	3.35±4.69
Magnesium	150.8±35.8

4.0 DISCUSSION

Polyherbal formulation remains a viable therapeutic option in modern medicine due to the presence of diverse bioactive phytoconstituents which play a crucial role in optimizing human health (Aladejana, 2023). However, scientific evidence to validate the therapeutic utility of these formulations is often not established, a situation that can negatively impact their safety and efficacy (Eniayewu et al., 2020). In the present study, we confirm the phytochemicals present in HM-4 and established their relationship to its nutritional and therapeutic activities. It was noted that HM-4 is particularly rich in flavonoids and alkaloids, both of which are essential bioactive compounds for treating metabolic and oxidative stress-related disorders. The abundance of flavonoids in HM-4 can be linked to some of its claimed biological activities. For instance, literature reports have established that this phytochemical is abundantly present in honey, zingiber officinale and *curcuma longa* (Iweala et al., 2023; Haroen et al., 2024). Flavonoids are water soluble polyphenolic compounds with recognized ability to scavenge free radicals thus preventing lipid peroxidation and tissue damage (Mutha et al., 2021). Alkaloids possesses neuroprotective and anti-inflammatory properties and can also help regulate glucose metabolism (de Lima et al., 2025). Xu and co-authors reported the presence of a bioactive amide alkaloids from the stem of *Piper nigrum*, one of the components of HM-4 polyherbal formulation (Bergonzi et al., 2022). Tannins, steroids and terpenoids are essential bioactive compounds with various biological properties, including antimicrobial, anti-inflammatory and antioxidant properties (Yang et al., 2020; Maugeri et al., 2022). The coexistence of these phytochemicals with flavonoids and alkaloids further enriches the pharmacological profile of HM-4.

The antioxidant potential of HM-4 was demonstrated in this study. A concentration-dependent DPPH, ABTS, nitric oxide, and hydroxyl radical scavenging ability of HM-4 noted in this study provide a scientific justification for its use in conditions associated with oxidative stress. The HM-4 exhibited concentration-dependent radical scavenging activity in DPPH, ABTS, nitric oxide, and hydroxyl radical assays, alongside moderate ferric reducing antioxidant power. Of particular importance is the superior ABTS scavenging capacity of HM-4 relative to ascorbic acid, suggesting that HM-4 possesses broad-spectrum radical neutralization abilities. Also, the demonstrated nitric oxide scavenging potential comparable to rutin is also noteworthy, since excessive nitric oxide production is associated with inflammatory cascades, diabetic vascular complications, and neurodegeneration (Dash et al., 2025). The observed antioxidant properties of HM-4 revealed in this study may

be due to its phytochemical compositions. Results from this study revealed the presence of polyphenolic compounds in HM-4. These compounds, particularly flavonoids and alkaloids, are natural polyphenols with inherent capacity to effectively scavenge free radicals due to their redox properties.

The inhibition of alpha amylase, an enzyme essential in carbohydrate digestion and glucose absorption in human, is essential in controlling postprandial hyperglycemia (Gong et al., 2020). The ability of HM-4 to inhibit this enzyme suggests its potential in controlling elevated blood glucose level and its potential utility in the management of diabetes mellitus. Importantly, unlike synthetic inhibitors that often cause gastrointestinal discomfort, moderate inhibition of alpha amylase enzyme as seen with HM-4 may offer a more tolerable alternative in diabetes management (Ogunyemi et al., 2022). The proximate composition reported in the current research highlights the nutritional significance of HM-4. The formulation is rich in crude fibers, carbohydrates and calories, an indication of its potential to serve as a dietary source of energy. Fiber modulates gut microbiota thus aiding bowel mobility, glycemic control and reduce oxidative stress (Snauwaert et al., 2023). The low lipid and protein contents suggest limited risk of contributing to dyslipidemia, while the minimal moisture content indicates enhanced stability and shelf life, a critical factor in herbal medicine preservation. Mineral analysis of HM-4 revealed the presence of essential elements notably iron, potassium, magnesium, manganese, and chromium. Iron is important in formation of blood particularly in anemic and convalescent patients. On the other hand, Magnesium and potassium regulate cardiovascular and neuromuscular functions, while chromium is highly relevant to diabetes management, as this trace element enhances insulin action and glucose tolerance (Vincent, 2017). Summarily, findings from this study clearly indicate that HM-4 provides a promising option in the search for natural antioxidant and nutraceuticals

This study has a few drawbacks. Firstly, in-vitro methods were used to evaluate the nutritional and pharmacological benefits of HM-4. While these methods are very reliable, they may not completely account for in-vivo complexities, including bioavailability. Secondly, the phytochemical, nutritional and therapeutic potential of each of the components of HM-4 was not assessed individually in this study, limiting understanding of potential synergistic or additive interactions. Lastly, in-vivo safety and toxicity of HM-4 was not evaluated in this study.

5.0 CONCLUSION

The findings from our study established that HM-4 offers a promising option for the development of new natural nutraceutical and adjunct therapy for the management of chronic disease. More studies to further explore options to manufacture standardized formulation of the HM-4 as well as evaluating its in-vivo safety and efficacy is recommended

Authors' contributions

EOI conceptualized and performed the experiment, EE, RM, SA, AAB performed the experiment. STA, NNS, AOM, ALA, GHB, WFE, BMT contributed to the supervision of the research. All authors contributed to the writing, review and approval of the final version of the manuscript for submission

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