Original research article
UDC 635.71:543.9(669)
DOI: https://doi.org/10.46784/e-avm.v18i1.439

NUTRITIVE AND PHYTOCHEMICAL QUALITY AND PHYTOBIOTIC PROPERTY OF *PHYLLANTHUS AMARUS* (SCHUM. & THONN.) ORIGINATING FROM NIGERIA

Anthony Eduviere[1], Evelyn Ojugbeli[2], Christiana Akpo[3], Oghenesuvwe Okpara[4], Joy Acha[2], Innocent Onyesom[2*]

[1] Department of Pharmacology, Delta State University, Abraka, Nigeria
[2] Department of Medical Biochemistry, Delta State University, Abraka, Nigeria
[3] Department of Medical Laboratory Science, Delta State University, Abraka, Nigeria
[4] Department of Animal Science, Delta State University, Abraka, Nigeria

Abstract

Negative impact of antibiotics and synthetic antioxidants to livestock production including poultry are rising. Therefore, the current shift to phytobiotics as alternative that can improve the productivity and quality of commercial animals by enhancing feed property and performance. However, the use of phytobiotics in our environment is limited due to scarce research outputs. This study evaluates the nutrient quality, phytochemical constituents and phytobiotic (antimicrobial and antioxidant) properties of *Phyllanthus amarus*, a non-toxic medicinal herb, using standard procedures and documented methods. The results revealed that *Phyllanthus amarus* contains high quality nutrients and rich medicinal phytochemicals in both essential oil and methanol extract which demonstrated significant ($p < 0.05$) antibacterial activity with selectable characteristics against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* Enteritidis from poultry isolates. The plant's samples (both extracts) also inhibited the growth of *Eimeria tenella*, a protozoan that causes coccidiosis in chickens. Furthermore, two of the gas chromatography-mass spectrometry identified compounds 6-methoxy-4-methyl-5-((5-[3-(trifluoromethyl)phenol]plenty)oxy)-8-quinolinylamine and glyoxalbis (2,4-dinitrophenyl-hydrazone) were respectively identified to have high docking interactions with bacterial ATP synthetase and OxyR protein complex, involved in bacterial protein synthesis and oxidative stress

[1*] Corresponding Author: ionyesom@delsu.edu.ng

defense. In addition, these compounds inhibited Keap1 complex which induces oxidative stress in chickens. Thus, 6-methoxy-4-methyl-5-((5-[3-(trifluoromethyl)phenol]plenty)oxy)-8-quinolinylamine and glyoxalbis (2,4-dinitrophenyl-hydrazone) could be optimized and studied as hits for discovery of phytobiotics with antibacterial and antioxidants properties for use in livestock, especially poultry.

Key words: Phytobiotics, Antibacterial, Antioxidant, *Phyllanthus amarus*, Phytochemical

NUTRITIVNI I FITOHEMIJSKI KVALITET I FITOBIOTSKE OSOBINE *PHYLLANTHUS AMARUS* (SCHUM. & THONN.) POREKLOM IZ NIGERIJE

Anthony Eduviere[1], Evelyn Ojugbeli[2], Christiana Akpo[3], Oghenesuvwe Okpara[4], Joy Acha[2], Innocent Onyesom[2*]

[1] Katerdra za farmakologiju, Delta Univerzitet, Abraka, Nigeria
[2] Katedra za medicinsku biohemiju, Delta Univerzitet, Abraka, Nigeria
[3] Katedra za medicinsko-laboratorijsku dijagnostiku, Delta Univerzitet, Abraka, Nigeria
[4] Katedra za stočarstvo, Delta Univerzitet, Abraka, Nigeria

Kratak sadržaj

Neželjeno dejstvo antibiotika i sintetičkih antioksidanasa na stočarsku proizvodnju, uključujući živinarstvo, sve je izraženije. Zbog toga dolazi do prelaska na fitobiotike koji predstavljaju alternativu koja može poboljšati produktivnost i kvalitet komercijalnih životinja putem unapređenja svojstava i iskorišćenja hrane. Međutim, upotreba fitobiotika u našem okruženju je ograničena zbog nedostatka rezultata relevantnih istraživanja. Ova studija procenjuje nutritivni kvalitet, fitohhemijska jedinjenja i fitobiotska svojstva (antimikrobna i antioksidativna) biljke pelin - *Phyllanthus amarus*, netoksične lekovite biljke, korišćenjem standardnih procedura i dokumentovanih metoda. Rezultati su pokazali da *Phyllanthus amarus* sadrži visokokvalitetne nutritivne materije i bogata lekovita fitohemijska jedinjenja u oba ekstrakta – etarskom ulju i metanolnom ekstraktu, koji su pokazali

značajnu ($p < 0,05$) antibakterijsku aktivnost sa selektivnim karakteristikama protiv *Escherichia coli*, *Staphylococcus aureus* i *Salmonella* Enteritidis izolovanih iz živine. Uzorci biljke (oba ekstrakta) takođe su inhibirali rast *Eimeria tenella*, protozoe koja izaziva kokcidiozu kod pilića. Pored toga, dva jedinjenja identifikovana gasnom hromatografijom sa masenom spektrometrijom — 6-metoksi-4-metil-5-((5-[3-(trifluorometil)fenol]plenti)oksi)-8-kinolinilamin i glikoksalbis (2,4-dinitrofenilhidrazon) — pokazala su visoke vrednosti molekulskog vezivanja sa ATP sintetazom bakterija i OxyR proteinskim kompleksom, koji su uključeni u sintezu proteina i odbranu od oksidativnog stresa kod bakterija. Pored toga, ova jedinjenja su inhibirala Keap1 kompleks, koji indukuje oksidativni stres kod živine. Shodno tome, 6-metoksi-4-metil-5-((5-[3-(trifluorometil)fenol]plenti)oksi)-8-kinolinilamin i glikoksalbis (2,4-dinitrofenilhidrazon) mogli bi biti optimizovani i dalje proučavani kao potencijalni kandidati za otkrivanje fitobiotika sa antibakterijskim i antioksidativnim svojstvima za upotrebu u stočarstvu, a posebno u živinarstvu.

Ključne reči: Fitobiotici, Antibakterijski, Antioksidant, *Phyllanthus amarus*, Fitohemijski

INTRODUCTION

Phyllanthus amarus (Schum. & Thonn.), a tropical herb popularly called *stonebreaker*, belongs to the Phyllanthaceae family (Onyesom et al., 2023). It is found in various regions of the world, especially in tropical Asia, Africa, and South America. *P. amarus* has been used in Nigeria traditional medicine for centuries to treat a variety of ailments, like jaundice, liver diseases, digestive disorders, malaria, and kidney stones (Okom et al., 2023). Its medicinal properties have recently drawn considerable scientific attention, with modern research validating many of its traditional applications, especially its antioxidant (Ghasemzadeh et al., 2012), anti-inflammatory (Ghosh et al., 2022), antimicrobial (Ghasemzadeh et al., 2012), and hepatoprotective (Ghosh et al., 2022) activities.

One of the main areas of focus in the current research is phytobiotic property of *P. amarus*. Phytobiotics are plant-based substances that boost animal health by enhancing gut function, increasing immunity and providing antimicrobial protection, and serving as natural alternatives to antibiotics. This is particularly relevant and important in modern livestock farming, where there is a growing demand for sustainable, antibiotic-free alternatives for disease

management, especially in poultry production (Chen et al. 2025). *P. amarus* has been reported to contain several bioactive compounds, including flavonoids, alkaloids, tannins, and saponins, all of which contribute to its medicinal properties (Rakhee and Regin, 2008), including phytobiotic (antioxidant, anti-inflammatory, and antimicrobial) potential.

Rising concerns over the use of synthetic antioxidants and antibiotics as growth promoters in livestock feed have driven the search for alternative solutions that can deliver comparable performance and benefits, without the associated negative effects. One promising alternative currently under investigation is the use of phytobiotics (Filazi and Yurdakok-Dikmen, 2019; Alagawany et al., 2021). Over 5,000 different phytobiotics have been discovered from various sources which include herbs, fruits, legumes, essential oils, vegetables and whole grains, and are being assessed for their capacity to improve overall health and growth performance of animals. It is important to note that phytobiotics have numerous desirable characteristics (minimal side effects, low residue levels, and absence of resistance development) (Dialoke et al., 2020; Li et al., 2021; Mandey and Sompie, 2021) which makes them a promising alternative for enhancing animal health and productivity (Kikusato, 2021).

Various sources of literature highlight numerous beneficial effects of phytobiotics—including antioxidative, antimicrobial, and anti-inflammatory properties, as well as the promotion of probiotic flora growth in the gut (Filazi and Yurdakok-Dikmen, 2019). These effects are largely attributed to the diverse bioactive compounds found in phytobiotic substances, such as terpenoids, flavonoids, alkaloids, phenols, glucosinolates, and glycosides (Ulrikh et al., 2018; Pashtetsky et al., 2020).

Interest in phytobiotics with natural antimicrobial and antioxidant activities in poultry industry has been increasing in recent years due to growing resistance to antibiotics and the ever more present risk of chronic diseases among consumers of poultry products from treated birds. Phytobiotics as natural antioxidants and antimicrobials are cheaper, safer, more available, easily accessible, and they significantly minimize oxidative reactions in products during storage and prevent metabolic diseases in animals including poultry (Pashtetsky et al., 2019). Ginger has gained increased attention due to its rich array of medicinal compounds, which have strong antioxidant and antimicrobial properties (Ghasemzadeh et al., 2012). Supplementation with ginger at 5.10 or 15 g/kg has been reported (Ademola et al., 2006) to enhance growth performance of broilers by stimulating appetite and improving digestive properties, thanks to the constituents gingerol and shogaol. Moreover, it has been demonstrated that the addition of 5 mg/kg of *Camellia sinensis* powder en-

hanced carcass characteristics, blood lipid profile and growth performance of broilers (Ndomou et al., 2018). It has also been found that *Annona muricata* leaves have antioxidant activity by scavenging free radicals and chelating metals (Baskar et al., 2007).

Currently there is not much information about the use of *P. amarus* as phytobiotic in Nigeria. Therefore, this study examined the nutrient quality, phytochemical constituents, antioxidant and antimicrobial properties of the methanol leaf extract and essential oil obtained from *P. amarus* grown in our environment (Abraka, Delta State, Nigeria). The data from this study should provide important baseline information for further research that may culminate in the use of the plant's compounds as phytobiotics in livestock, and poultry farming in particular.

MATERIAL AND METHODS

Plant Collection and Preparation of Dry Sample

Mature, fresh whole samples of *P. amarus* were collected in Abraka, Ethiope East LGA of Delta State, Nigeria. Abraka (Long 6.1023468 and Lat 5.7894321) is located at the tropical rain forest zone of Nigeria at 22.5 m above sea level with a monsoon climate. The relative humidity oscillates between 83 to 95% and the annual rainfall is about 252.67 mm with two seasons: wet and rainy (March – September) and dry (November – February), while the ambient yearly temperature varies between 25.65 °C and 33.46 °C. The soil is soft, clayey, containing a high percentage of fine particles with over 50% measuring less than 0.075 mm in size (Weather and Climate, 2024).

The plant samples were collected between June and August, 2023 and authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State (FHI 10978). After authentication, the leaves were plucked and washed with running tap water, and then rinsed with distilled water and dried at room temperature (26 - 31 °C) for 21 days. The dried leaf sample was grounded by a crusher (Moulinex, France) and sieved (pore diameter = 0.4 mm) to get fine powder which was stored in a desiccator for use.

Nutrient Analysis of the Dried Plant Sample

Proximate composition, mineral element and vitamin constituents of the plant (powder) were analyzed in order to assess the nutritive quality of the plant.

The proximate composition (protein, crude fibre, fat, carbohydrate, mois-

ture, ash and calorie) which constitute the various classes of nutrients present in samples were determined using the standard methods of the Association of Official Analytical Chemists, AOAC (1990). Moisture content was determined by drying the plant's powdered leaf sample to constant weight at 105 °C in a drying oven. Kjeldahl method was used to measure the crude protein content. The nitrogen content was multiplied by factor of 6.25 to estimate the protein content. Crude fat was quantified using the Soxhlet extraction method with petroleum ether as solvent. The acid-detergent fiber (ADF) method was used to access the crude fiber. The ash content was estimated by incinerating the plant powdered leaf sample in a muffle furnace at 550 °C. Then the carbohydrate content was calculated by difference, subtracting the sum of moisture, protein, fat, and ash content from 100 %.

Mineral elements (five macro: potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P) and sodium (Na), and five micro: copper (Cu), manganese (Mn), chromium (Cr), zinc (Zn) and iron (Fe)) were determined using the standard procedures already documented by AOAC (1990). The powdered leaf samples were digested using a mixture of concentrated nitric and perchloric acids and the resulting solution was analyzed for metal content by atomic absorption spectrophotometry (AAS).

The content of vitamins (A, B_1, B_2, B_3, B_6, B_9, C, D, and E) in the plant's powdered leaf sample was analyzed by high performance liquid chromatography (HPLC) as reported by Achikanu et al. (2022).

Preparation of Plant's Methanol Extract and Essential Oil

Twenty grams (20 g) of the fine powdered sample was soaked in 100 mL of 95% methanol at room temperature (25 - 31 °C) for 72 h with frequent agitation. The extract was first filtered using Whatman filter paper (No. 42, 125 mm), followed by filtration through cotton wool. The resulting filtrate was evaporated to dryness in a hot water bath for 72 h and then the obtained methanol extract was refrigerated for further use.

The essential oil was extracted by hydrodistillation method (Begum et al., 2018).

Analysis of phytochemical constituents in Methanol Extract and Essential Oil

Quantitative phytochemical profiling was conducted using the prepared crude methanol extract and the oil sample in order to determine the amounts

of the various phytochemical constituents in the crude methanol extract and oil sample of *P. amarus*. Standard methods whose procedures have been documented (Ameen et al., 2021) were adopted for screening.

Determination of Antimicrobial Activity of Methanol Extract and Essential Oil

Determination of minimum inhibition concentration (MIC) was conducted in a 96-well plate with methanol extract (ME) and essential oil (EO) (Li et al., 2019) ranging from 5 – 2,500 µg/mL, and 100 µL of working inoculum solution (prepared from the poultry bacterial isolates – *Escherichia coli, Staphylococcus aureus, Salmonella* Enteritidis and *Eimeria tenella*) per well. Gentamycin was the positive control and methanol, the solvent, negative control. Following incubation at 37 °C for 48 h, the plate wells were visually examined to assess the lowest concentration at which colour changed to pink from purple, and this was recorded as the value of MIC (Drummond and Waigh, 2000). In general, MIC values ≤ 100 µg/mL indicate good activity (Eloff, 2004), values up to 320 µg/mL are considered reasonable, while values greater than 640 µg/mL are regarded as poor activity (Adamu et al., 2013).

The cytotoxicity levels of ME and EO of *P. amarus* against Vero monkey kidney cells were assessed by the MTT (3-[4,5- dimethylthiazol-2-yl] -2,5- diphenyltetrazolium bromide) reduction assay as described previously (Mosmann, 1983) with slight modifications (Elisha et al., 2017). The cell growth inhibition (i.e. cytotoxicity in percentage) was calculated by comparison with untreated cells. Plant extracts with cytotoxicity activity at 50% (CC_{50}) ≤ 20 µg/mL are regarded as toxic (Zirihi et al., 2005).

The selectivity indices (SI) of *P. amarus* ME and EO was determined by the ratio of cytotoxicity (CC_{50}) and MIC values of the tested bacteria in the same units of measure (SI = CC_{50}/MIC). SI > 1 indicates that extracts are more toxic to the bacterial cells than the host cell (Makhafola et al., 2012).

Gas Chromatography – Mass Spectrometry Analysis of Phyllanthus amarus Methanol Extract and Essential Oil

Gas chromatography-mass spectrometry (GC-MS) analysis of the ME and EO samples of *P. amarus* was performed using 7890 A GC system, 5675 C Inert MSD with triple-Axis detector as previously described (Ameen et al., 2021). The column, with a length of 30 m, an internal diameter of 0.2 µm, and a thickness of 250 µm, was treated with phenyl methyl siloxane. Other conditions of

the GC-MS include the following: ion source temperature (EI), 250 °C, interface temperature; 300 °C, pressure; 16.2 psia, out time, 1.8 mm, 1 µL injector in split mode having split ratio 1:50 with injection temperature of 300 °C. The column temperature started at 35 °C for 5 min and then changed to 150 °C at the rate of 4 °C/ min. The temperature was, however, increased to 250 °C at the rate of 20 °C/ min and held for 5 min. The total elution was 47.5 min. Ms Solution software provided by supplier was used to control the system and acquire the data, while the identification of the compounds was carried out by comparing the mass spectra obtained with those of the standard mass spectra from National Institute of Standard and Technology (NIST) database. The identity of the spectra above 95% was used to ascertain the name, molecular weight and structure of the components in the ME and EO samples of *P. amarus*.

In Silico Study

The GC–MS identified compounds in the ME and EO samples of *P. amarus*, and the validated targets important in bacterial protein synthesis and oxidative stress defense were virtually studied using the computer-aided drug design (CADD) (Naik et al., 2022).

The GC–MS identified phytochemicals from the *P. amarus* and a standard drug structure were downloaded from the PubChem data base. The structures were optimized using the Spartan 14 software and saved in pdb file format. A three-dimensional X-ray crystal structure of the validated protein targets (penicillin-binding protein, RNA polymerase, DNA gyrase, ATP synthetases, dihydrofolate synthase, dihydrofolate reductase, 30s subunit, 50s subunit, peptidoglycan) involved in bacteria protein synthesis, and proteins (OxyR, MarR, HypS, cysteine glutathione peroxidase, peroxiredoxins, thioredoxin reductase, extra cellular thioredoxins, glutaredoxins, catalase and SOD) operational in bacterial oxidative stress defense were retrieved from the RCSB PDB (Research Collaboratory for Structural Bioinformatics, Protein Data Bank, https://www.rcsb.org/) website. Using the Molegro Virtual Docker, incorrect bonds and side chain anomalies were rebuilt and optimized. After that, all potential active binding sites of the targeted phytocompounds were identified using the Molegro Virtual Docker.

Using the Molegro Virtual Docker, a receptor grid box was generated once the target protein active binding site has been predicted. The created ready-to-dock phytochemicals library was docked against each target protein to explore the ligand–protein interactions. After docking, the phytochemicals with the lowest molecular docking scores were selected and visualized using the Dis-

covery Studio Visualizer tool to analyze the protein–ligand complex structures.

The evaluation of drug-likeness, pharmacokinetics, and toxicity profile of drug candidates are significant steps in drug discovery process (Wu et al., 2020). The pharmacokinetics (absorption, distribution, metabolism, and excretion), toxicity, and some physicochemical properties of the top-scored phytocompoundsfrom *P. amarus* GC-MS profile were assessed using Swiss-ADME and pkCSM-pharmacokinetic online web-based tools.

The molecular structures of the identified bioactive compounds were optimized by the DFT/Becke-3-Lee-Yang-Parr (B3LYP) method using a 6-31G* basis set (Haunschild et al., 2019). Then, the optimized structures were used to determine the frontier molecular orbital energies of the chosen bioactive compounds and the energy gap was calculated from the lowest unoccupied molecular orbitals (E-LUMO) and highest occupied molecular orbitals (E-HOMO). The molecular orbital energy diagrams were visualized in the Spartan 14 software.

Assessment of In Vitro Antioxidant Activities

The ability of *P. amarus* ME and EO to scavenge DPPH (2, 2- diphenyl-1-picrylhydrazyl) radical was evaluated in accordance with the Braca et al. (2002) method described by Ndomou et al. (2018). A total of 4.5 mL of 0.002% alcoholic DPPH solution was added separately to 0.5 mL of extract samples and standard solutions at varying concentrations (250, 500, 1,000, and 2,000 µg/mL), resulting in final product concentrations ranging from 25 to 200 µg/mL. The samples were kept at room temperature in the dark for 30 min and then the absorbance of the resulting solution was measured at 517nm using a spectrophotometer (BioMate, Germany). The absorbance of the samples, control, and blank was measured using distilled water as the reference. The antioxidant activity (AA) was calculated as follows:

$$AA\% = \left(\frac{((AbScontrol - AbSsample) X\ 100)/AbScontrol}{AbScontro} \right)$$

In addition, the efficient concentration 50 (EC_{50}) was determined according to the antioxidant activity.

The standard TBARS (Thiobarbituric acid reactive substances) method was adopted to determine the degree of lipid peroxidation inhibition in isolated rat liver homogenate by the ME and EO samples of *P. amarus* (Shabbir et al., 2013).

From freshly excised liver of rat, 10× homogenate was made in cold phosphate buffer saline (pH 7.4). Extract and each fraction were added to 100 µL of (15 mM) ferrous sulphate followed by addition of 3 mL of homogenate. After incubation for 30 min, 0.1 mL of this reaction mixture was combined with 1.5 mL of 10% TCA. After 10 minutes of incubation, the mixture was filtered, and the resulting supernatant was transferred into a tube containing 1.5 mL of 0.67% thiobarbituric acid (TBA) solution (in 50% acetic acid) and placed in boiling water bath for 30 min. Concentration of chromogen formed was measured at 535 nm. Anti-lipid peroxidation was assessed using the following formula:

$$\text{inhibition} = (\text{control} - \text{test}) / \text{control X } 100$$

Statistics

The obtained data were expressed as Mean ± SEM for triplicate determinations. Statistical significance was determined by one-way ANOVA test using GraphPad prism 5- software. $P < 0.05$ was accepted as significant.

RESULTS

In order to evaluate the functional food characteristics of *P. amarus* and its possible utilization as nutraceutic, the nutritive quality of the powdered sample and phytobiotic properties of ME and EO from the herbal plant were analyzed. Nutritive quality was determined by investigating the proximate composition, mineral elements contents and vitamin constituents of the plant's dried powdered leaf sample.

Nutritive Quality

Proximate Composition

The results obtained from the analysis of proximate composition of the medicinal herb are shown in Figure 1.

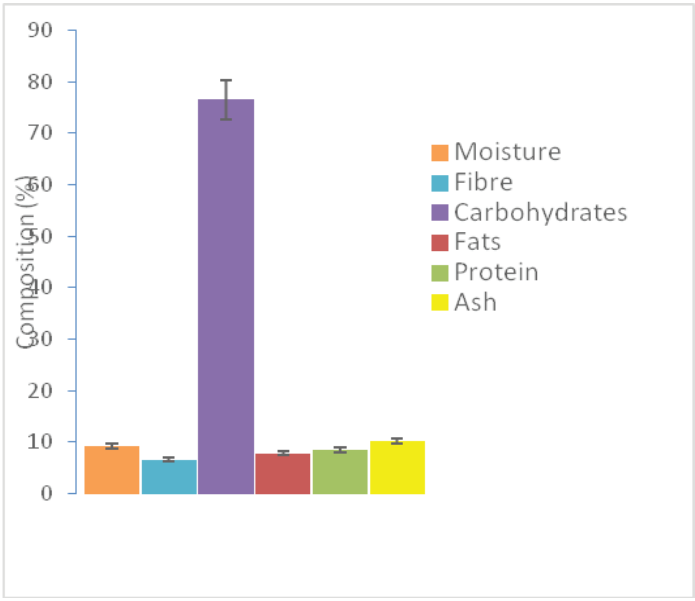


Figure 1. Proximate Composition of *P. amarus*
Values are expressed as Mean ± SEM for triplicate determinations, and calorie content = 456.150.04 kCal/100 g

The data (Figure 1) show high amounts of carbohydrates, calories (energy), and ash, but moderate contents of protein, fibre, fat, and low concentration of moisture.

Mineral elements

Table 1. Mineral Contents of *P. amarus*

Minerals	Content (mg/g)
Magnesium	0.331 ± 0.002
Zinc	0.062 ± 0.001
Calcium	3.476 ± 0.012
Sodium	0.079 ± 0.003
Potassium	2.210 ± 0.005
Phosphorous	0.095 ± 0.004

Minerals	Content (mg/g)
Iron	0.173 ± 0.007
Copper	0.007 ± 0.001
Manganese	0.036 ± 0.002
Chromium	0.015 ± 0.013

Values are expressed as Mean ± SEM for triplicate determinations

Ten mineral elements were analyzed and include the following: Mg, Zn, Ca, Na, K, P, Fe, Cu, Mn and Cr with considerable good amounts of Mg, Fe, Ca and K, moderate concentrations of Na, Mn, Zn and P, but trace quantities of Cu and Cr.

Vitamin constituents

The nine (9) vitamins identified and quantified in this study include vitamin A, B_1, B_2, B_3, B_6, B_9, C, D, E, and are shown in Table 2.

Table 2. Vitamin Constituents of *P. amarus*

Vitamins	Constituents (mg%)
A	0.768 ± 0.001
B_1	0.003 ± 0.0002
B_2	0.001 ± 0.0003
B_3	0.060 ± 0.002
B_6	0.019 ± 0.003
B_9	0.405 ± 0.001
C	7.05 ± 0.002
D	0.145 ± 0.001
E	1.615 ± 0.001

Data are represented as Mean ± SEM for triplicate determinations

The results (Table 2) show high proportion of vitamin C, moderate amounts of vitamins A, E, B_3, B_6, B_9, and D, and low levels of vitamin B_1 and B_2.

Phytochemical profile

Phytochemicals (plant-derived chemicals) play varied functions in plant defense and protection, human health, agriculture, industries, and environment. Several classes (flavonoids, alkaloids, saponins, phenols, triterpenoids, glycosides, tannins, carotenoids, etc.) of phytochemicals are recognized.

Figure 2 shows the quantitative phytochemical evaluation of the ME and EO of *P. amarus*

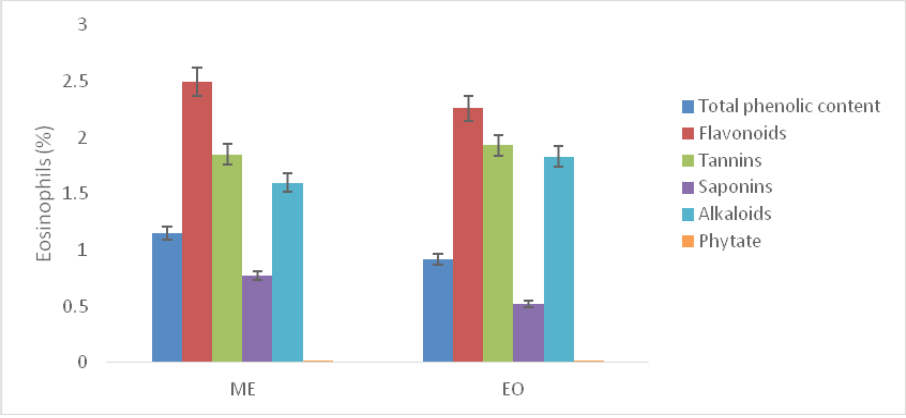


Figure 2. Quantitative Phytochemical Profile of *P. amarus*
Data represents mean values for n = 3 determinations

P. amarus leaf ME and EO contain good amounts of flavonoids, phenolics, tannins and alkaloids, but moderate concentration of saponins and trace level of phytate, an anti-nutrients.

Cytotoxicity, minimum inhibitory concentration and selectivity

The cytotoxicity, MIC and SI indices for the EO and ME samples of *P. amarus* against some poultry isolates (Bacteria: Gram-negative (*E. coli, S.* Enteritidis), Gram-positive (*S. aureus*) and protozoan (*E. tenella*)) were evaluated and results are presented in Table 3.

Table 3. Cytotoxicity MIC and SI of EO and ME of *P. amarus*

	ME	**EO**
CC_{50} (µg/mL)	**207**	**202**
S. aureus		
MIC (µg/mL)	100	150
SI	2.07	1.35
E. coli		
MIC_{50} **(µg/mL)**	50	50
SI	4.14	4.04
E. tenella		
IC_{50} **(µg/mL)**	20.00	15.00
SI	10.35	13.47
***S.* Enteritidis**		
MIC (µg/mL)	150	200
SI	1.38	1.01

CC_{50} = Cytotoxicity at 50% inhibition. SI= CC_{50} / MIC

Gentamycin MICs (µg/mL) (*S. aureus*: 12.5; *E. coli*: 6.25, *E. tenella*: 25.0; *S.* Enteriditis: 12.5).

Significant activity: MIC < 100 µg/mL; Moderate activity: 100 < MIC ≤ 625 µg/mL; Weak activity: MIC ≥ 625 µg/mL (Dao et al., 2020). SI > 1 indicates that extracts (ME and EO) are less toxic to the host cell than the bacteria (Makhafola et al., 2012; Elisha et al., 2017).

Results (Table 3) indicate that the ME and EO of *P. amarus* is safe and non-toxic since their CC_{50} values exceeded 20 µg/mL. Also, their MIC values against *E. coli* showed significant activity (very active), but moderate with respect to the other organisms. However, their selectivity indices against all organisms reveal that the EO and ME of *P. amarus* are more toxic to the bacteria than the host, and hence, can be selected for further study.

GC-MS analysis

In order to identify the active antibacterial compounds in the EO and ME

samples of *P. amarus*, the GC-MS profile was established and sixty-six (66) compounds (31 in ME and 35 in EO) were identified.

In silico study

The 66 GC-MS identified compounds were docked against targets involved in bacterial protein synthesis and oxidative stress defense. Subsequently, the virtual properties of the selected hit compounds were studied by CADD.

Following the ADME-Toxicity and Density Functional Theory (DFT) assessments, the most reactive compounds with drug-like properties against each bacterial class of targets were identified. Therefore, 6-Methoxy-4-methyl-5-((5-[3-(trifluoromethyl)phenol]plenty)oxy)-8-quinolinylamine, found in both ME and EO was observed to particularly react with ATP synthase, an enzyme involved in bacterial protein synthesis. Then, glyoxalbis (2,4-dinitrophenyl-hydrazone), present only in the ME reacted the most with oxyR complex protein, operational in bacterial oxidative stress defense system.

The GC-MS characteristics (Figure 3a & b), docking profile (Table 4), ADME-Toxicity data (Table 5) and DFT analysis of the two identified most active compounds are stated as indicated.

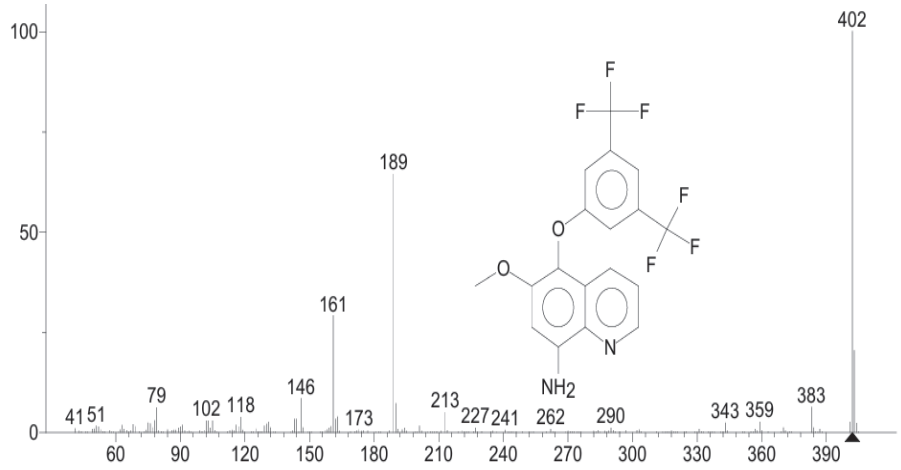


Figure 3a. GC–MS Chromatogram and structure of 6-Methoxy-4-methyl-5-(-[3-(trifluoromethyl)phenyl]pentyl)oxy)-8-quinolinylamine

Retention time (RT) = 12.215 min, Molecular weight (MW) = 418, Molecular formular (MF) = $C_{23}H_{25}F_3N_2O_2$, Peak area (PA) equivalent to concentration= 5.392%

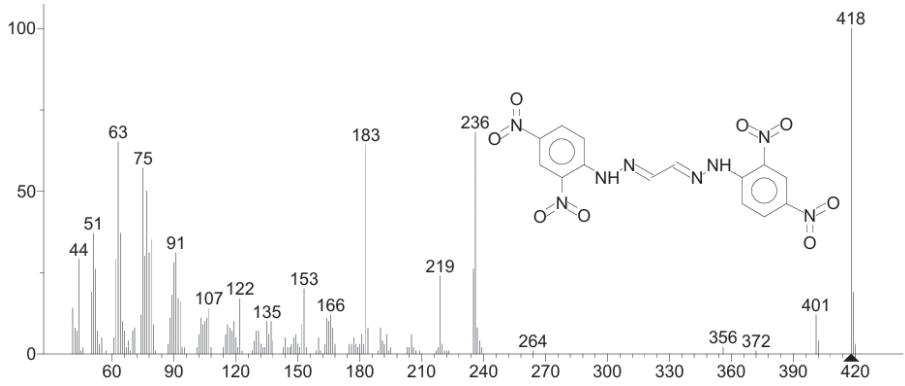


Figure 3b. GC – MS chromatogram and structure of glyoxalbis (2,4-dinitrophenyl-hydrazone)
RT = 12.310 min, MW = 418, MF = $C_{14}H_{10}N_8O_8$, PA equivalent to concentration = 15.518 %.

Table 4. The mole dock score of the two most active compounds

Compound	Compound ID	E-Total (kCal/mol)
6-Methoxy-4-methyl-5-(-[3-(trifluoromethyl) phenyl]pentyl)oxy)-8-quinolinylamine	609015	-198.064
Drug (Amoxicillin trihydrate)	21960594	-138.238
Glyoxalbis (2,4-dinitrophenyl-hydrazone)	3890919	-165.558
Drug (Allicin)	65036	-74.242

E-total are the mole dock scores for binding of compounds 1 and 2 to ATP synthetase, and compounds 2 and 4 to OxyR protein complex.

6-Methoxy-4-methyl-5-((5-[3-(trifluoromethyl)phenyl]pentyl)oxy)-8-quinolinylamine formed eight conventional hydrogen bonds, LYS80 (2.66 Kcal/mol), LYS80 (2.58 Kcal/mol), ARG99 (2.89 Kcal/mol), SER111 (1.81 Kcal/mol), ARG222 (2.68 Kcal/mol) ARG222 (2.54 Kcal/mol), SER111 (1.99 Kcal/mol), GLU101 (2.15 Kcal/mol). It also, formed three carbon hydrogen bonds, LYS80 (2.73 Kcal/mol), HIS110 (2.69 Kcal/mol), ALA261 (2.95 Kcal/mol). In addition, it formed seven hydrophobic bonds, PHE114 (3.66 Kcal/mol), PHE114 (3.78 Kcal/mol), ILE77 (4.23 Kcal/mol), ARH267 (3.55 Kcal/mol), PHE114 (4.77 Kcal/mol), ARG267 (5.01 Kcal/mol) with ATP syn-

thetases protein. The standard drug, Amoxicillin Trihydrate also interacted via fourteen hydrogen bonds, ARG99 (2.45 Kcal/mol), ARG99 (2.83 Kcal/mol), GLN116 (1.68 Kcal/mol), ARG267 (2.70 Kcal/mol), VAL216 (2.46 Kcal/mol), ASP47 (2.54 Kcal/mol), ASP195 (1.67 Kcal/mol), SER75 (2.67 Kcal/mol), VAL216 (2.99 Kcal/mol), ARG267 (2.29 Kcal/mol), GLU215 (2.22 Kcal/mol), ASP47 (2.37 Kcal/mol), GLN116 (2.33 Kcal/mol). It also, created two hydrophobic bonds, TYR194 (5.23 Kcal/mol), PHE114 (4.18 Kcal/mol) with ATP synthetases (PDB ID: 3reu) protein. Glyoxalbis (2,4-dinitrophenyl-hydrazone) (CID = 3890919) formed two GLU126, two GLU196, an ASP195, two GLY197, GLY223, two MET225, SER224, PRO99 at respective bond distance. A standard drug Allicin (CID = 65036) formed three hydrogen bonds with GLN217, THR218, THR218 at respective bond distances. It also formed three hydrophobic bond LYS114, and two TR192 at different distances as shown in Table 4 and Figures 4 a-d docking complexes.

Docking Complexes

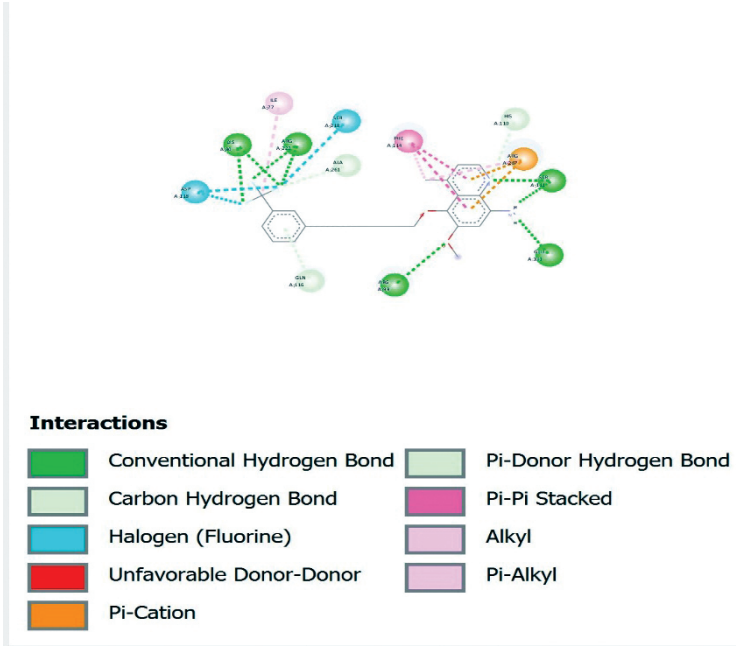


Figure 4a. 6-Methoxy-4-methyl-5-((5-[3-(trifluoromethyl) phenyl] pentyl) oxy)-8-quinolinylamine in complex with ATP synthetases

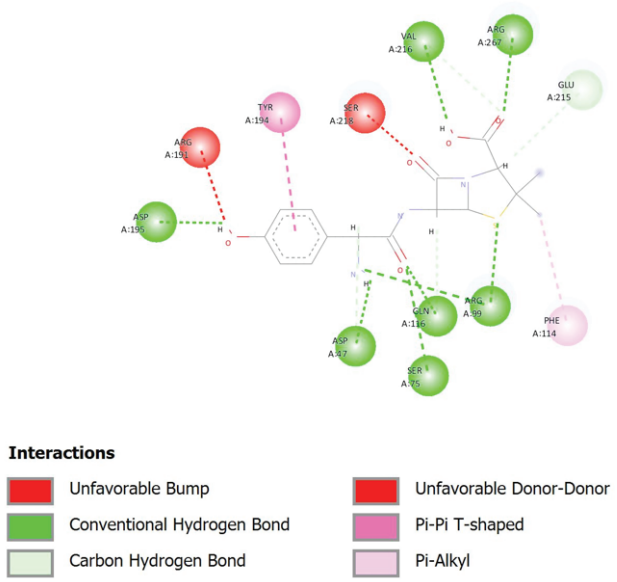


Figure 4b. 2D representation of Glyoxalbis (2,4-dinitrophenyl-hydrazone) in complex with OxyR protein.

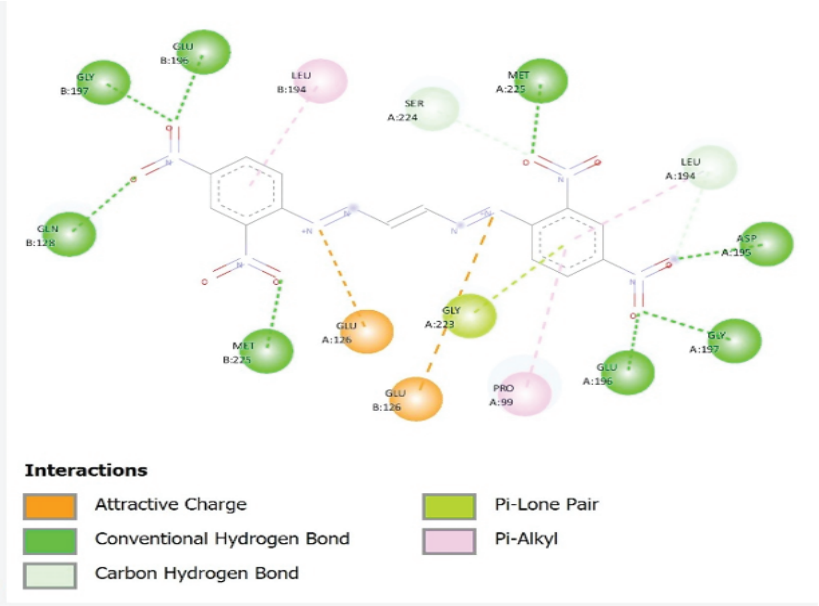


Figure 4c. Amoxicillin Trihydrate (Standard drug) in complex with ATP synthetases protein

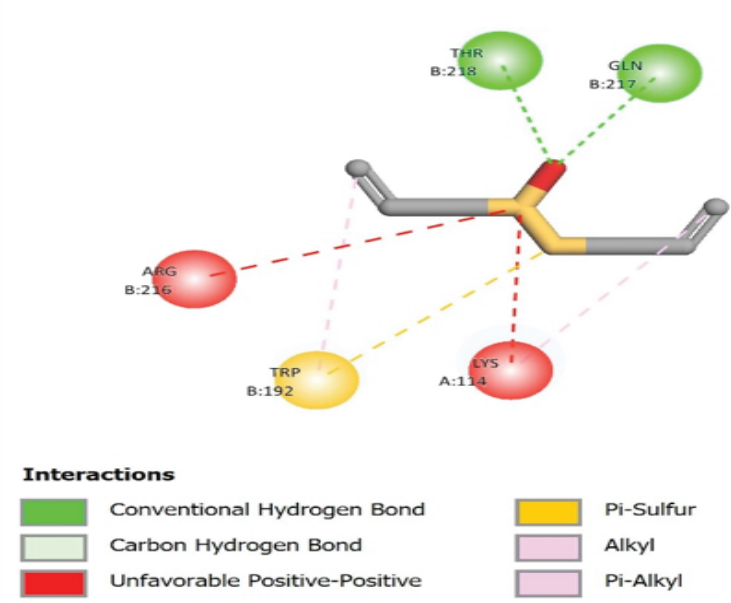


Figure 4d. 2D representation the standard drug (Allicin) in complex with OxyR protein

Analysis of Absorbtion Distribution Metabolism and Excretion (ADME) and Physicochemical Properties of the Active Compounds

Data obtained from the analyses are shown in Table 5.

Table 5. Physicochemical and pharmacokinetic (ADME) data for the selected most active compounds

Parameter	Compounds ID			
	609015	**21960594**	**3890919**	**65036**
Formula	$C_{23}H_{25}F_3N_2O_2$	$C_{16}H_{25}N_3O_8S$	$C_{10}H_8N_4O_4S$	$C_6H_{10}OS$
MW (g/mol)	418.45	419.45	280.26	162.27
Number of heavy atoms	30	28	19	9
Number of aromatic heavy atoms	16	6	11	0

Parameter	Compounds ID			
	609015	**21960594**	**3890919**	**65036**
Fraction C_SP_3	0.35	0.44	0.10	0.33
Number of rotatable bonds	9	5	3	5
Number of H-bond acceptors	6	9	5	1
Number of H-bond donors	1	7	1	0
Molar refractivity	112.81	103.73	71.40	45.88
TPSA (*Å*^{2})	57.37	185.95	141.73	61.58
Solubility class	Moderately soluble	Poorly soluble	Soluble	Very soluble
GI absorption	Low	No	High	High
BBB permeation	No	2	No	Yes
Violation of Lipinski's rule	0	1	0	0
Violation of Veber rule	0	0	1	0
Bioavailability score	0.55	0.17	0.55	0.55
Synthetic accessibility	3.03	4.50	3.23	3.60

Compound ID: 609015 (6-Methoxy-4-methyl-5-((5-[3-(trifluoromethyl)phenol] plenty)oxy)-8-quinolinylamine), 21960594 (Amoxicillin anhydrate – drug), 3890919 (Glyoxalbis (2,4-dinitrophenyl-hydrazone), and 65036 (Allicin – drug)

The observed results presented (Table 5) for the pharmacokinetic (ADME) and physicochemical parameters indicate that in line with the Lipinski's rule of five, no compound violated the rule. The compounds are soluble and have high gastrointestinal absorption with relative ease of synthesis, but varied BBB (brain-blood barrier) permeation. However, the data for each bioactive compound compared well with the respective standard drug.

Toxicity

The toxicity profile of the two active compounds and their respective standard drugs are shown in Table 6.

Table 6. Toxicity profile of the top-scored active compounds

	Compound ID			
Parameter	**609015**	**21960594**	**3890919**	**65036**
AMES toxicity	NO	NO	YES	YES
Max. tolerated dose	0.04	0.85	0.48	0.74
hERG 1 inhibition	NO	NO	NO	NO
LD_{50} (mol/kg)	2.61	1.57	2.86	2.37
Hepatotoxicity	YES	YES	YES	NO
Skin sensitivity	NO	NO	NO	YES
***T. pyriformis* toxicity**	0.42	0.29	0.95	0.90
Minnow toxicity	-3.85	5.62	3.08	1.24
Carcinogenicity	No	No	No	No

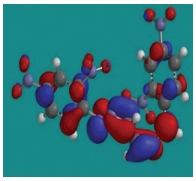
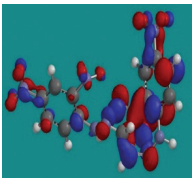
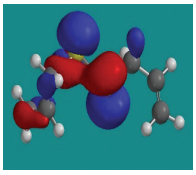
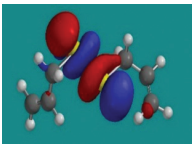
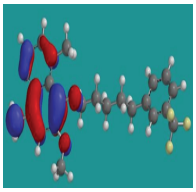
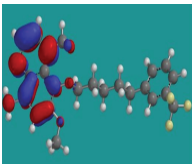
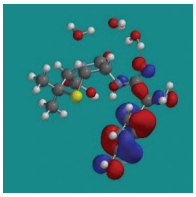
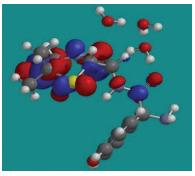
All the compounds are reported to be non-toxic with human tolerated dosage ranging from 0.04-0.85.

Compound ID: 609015 (6-Methoxy-4-methyl-5-((5-[3-(trifluoromethyl)phenol]plenty)oxy)-8-quinolinylamine), 21960594 (Amoxicillin anhydrate – drug), 3890919 (Glyoxalbis (2,4-dinitrophenyl-hydrazone), and 65036 (Allicin – drug)

Density Functional Theory

Molecular structure and reactivity are largely explained using the Frontier Molecular Orbital in organic chemistry (Rozeboom et al., 1981), while electronic properties of molecules can be described through the use of HOMO-LUMO bandgap energy (Feng et al., 2018). ENERGY gap between HOMO and LUMO helps to determine the stability of chemical bonds. Compounds with high energy gap have low chemical reactivity, while compounds with low energy gap have higher reactivity and lower stability (Miar et al., 2020). The two bioactive compounds are selected based on their energy gap, ΔE. The compounds' E-HOMO-E-LUMO diagrams and energy gap, ΔE values are shown in Table 7.

Table 7. HOMO-LUMO diagrams and energy gap, ΔE values of selected top-scored binding compounds and standard drugs.

COM-POUND ID	HOMO	EHO-MO	LUMO	ELU-MO	Energy Gap (ΔE).
3890919		-6.60		-3.2	3.4
65036		-6.40		-1.3	5.1
609015		-4.8		-1.0	3.8
21960594		-6.3		-0.7	5.6

Compound ID: 609015 (6-Methoxy-4-methyl-5-((5-[3-(trifluoromethyl)phenol] plenty)oxy)-8-quinolinylamine), 21960594 (Amoxicillin anhydrate – drug), 3890919 (Glyoxalbis (2,4-dinitrophenyl-hydrazone), and 65036 (Allicin – drug)

Computational Antioxidant Activity of the Most Active Compounds

Along with antimicrobial activity, phytobiotics are expected to exhibit antioxidant activity in order to counteract the bacterial and metabolic-induced oxidative stress in the host (poultry products). So, the two antibacterial-identified compounds and a natural antioxidant used in poultry (curcumin) were

docked with the Keap1 protein complex, which promotes oxidative stress in chickens (Kovesi et al., 2024), and the docking results are shown in Table 8 and Figure 5a-c.

Table 8. The mole-dock scores of the two most active compounds against Keap1 protein complex

Active Compound	COMPOUND ID	E-Total (kCal/mol)
6-Methoxy-4-methyl-5-((5-[3-(trifluoromethyl)phenyl]pentyl) oxy)-8-quinolinylamine	609015	-136.665
Glyoxalbis (2,4-dinitrophenyl-hydrazone)	3890919	-157.752
Curcumin	969516	-161.582

6-Methoxy-4-methyl-5-((5-[3-(trifluoromethyl) phenyl] pentyl) oxy)-8-quinolinylamine and glyoxalbis (2,4-dinitrophenyl-hydrazone) had mole-dock score of -136.665k Cal/mol and -157.752kCal/mol, respectively. They both showed a lower mole-dock score compared with the standard drug, curcumin, which had -161.582 kCal/mol. Glyoxalbis (2,4-dinitrophenyl-hydrazone) has a better potential as Keap1 inhibitor as evidenced by the dock score relative to that of the standard, curcumin. This could guide further research into optimizing these compounds or exploring new derivatives that may enhance their binding properties.

Figure 5 indicates the interaction of 6-methoxy-4-methyl-5-((5-[3-(trifluoromethyl)phenyl]pentyl)oxy)-8-quinolinylamine (5a), glyoxalbis (2,4-dinitrophenyl-hydrazone) (5b) and the standard, curcumin (5c) in complex with (5FZN) Keap1 protein.

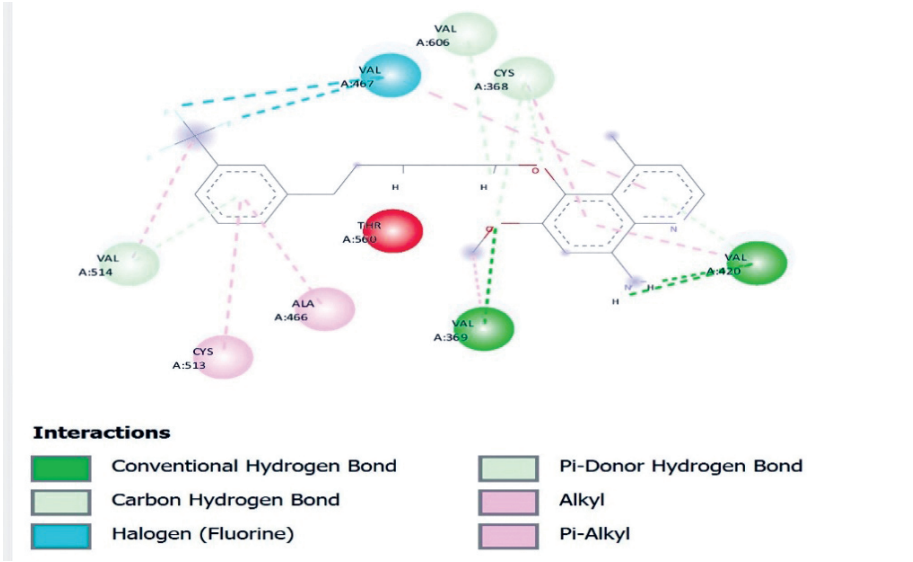


Figure 5a. Interaction of 6-methoxy-4-methyl-5-((5-[3-(trifluoromethyl)phenyl] pentyl)oxy)-8-quinolinylamine in complex with (5FZN) Keap1 protein

6-Methoxy-4-methyl-5-((5-[3-(trifluoromethyl) phenyl] pentyl) oxy)-8-quinolinylamine formed eight hydrogen bonds with VAL369 (1.84 Å^2), VAL420 (2.34 Å^2), VAL420 (2.68 Å^2), CYS368 (2.31 Å^2), CYS368 (2.99 Å^2), VAL606 (2.74 Å^2), VAL420 (2.95 Å^2), and VAL514 (2.87 Å^2). It also formed three halogen bonds, three VAL467 bonds at 3.28 Å^2, 3.30 Å^2, 3.60 Å^2 distance respectively. Lastly it formed eleven hydrophobic bonds, three VAL514 (at 4.78, 3.75, and 4.51 Å^2 distances), VAL369 (3.82 Å^2), ALA466 (4.44 Å^2), CYS513 (5.13 Å^2), VAL514 (4.67 Å^2), CYS368 (5.15 Å^2), VAL420 (4.23 Å^2), VAL420 (5.45 Å^2), AND VAL467 (5.09 Å^2) with Keap1 protein.

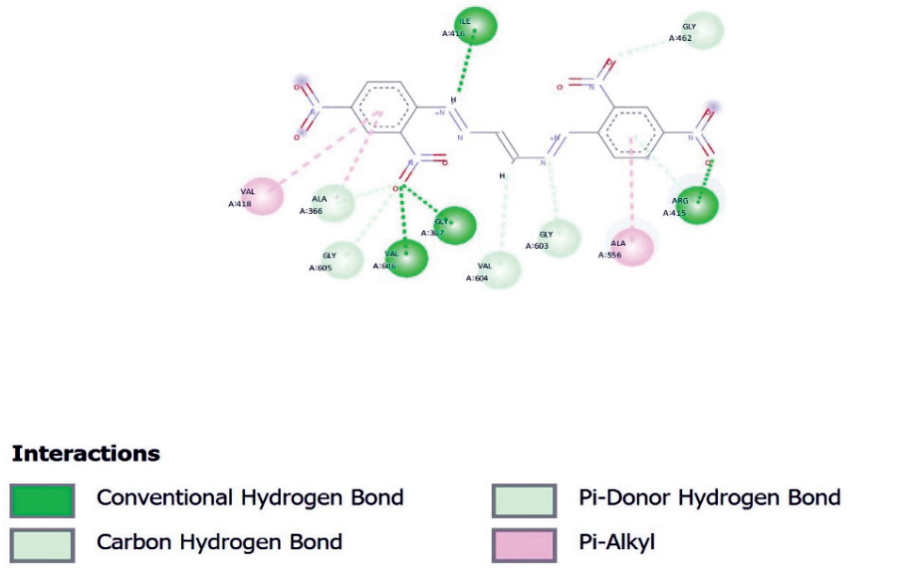


Figure 5b. Interaction of glyoxalbis (2,4-dinitrophenyl-hydrazone) in complex with (5FZN) Keap1 protein

Glyoxalbis (2,4-dinitrophenyl-hydrazone) formed ten hydrogen bonds, GLY367 (1.69 Å^2), ARG415 (1.35 Å^2), VAL606 (1.86 Å^2), ILE416 (2.78 Å^2), ILE416 (2.78 Å^2), ALA366 (2.45 Å^2), GLY462 (2.70 Å^2), GLY603 (2.38 Å^2), GLY605 (2.16 Å^2), VAL604 (2.42 Å^2) and ARG415 (2.83 Å^2). It also formed four hydrophobic bonds, ALA366 (4.33 Å^2), VAL418 (5.31 Å^2), ARG415 (4.10 Å^2) and ALA556 (3.79 Å^2) with Keap1 protein.

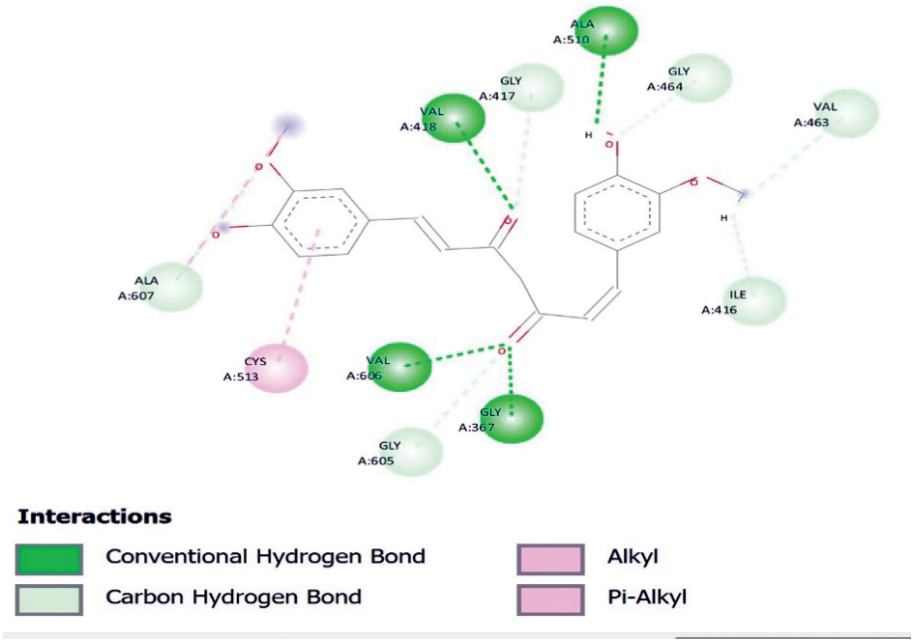


Figure 5C. Interaction of curcumin in complex with (5FZN) Keap1 protein

The standard drug, curcumin formed ten hydrogen bonds, GLY367 (1.58 Å^2), VAL418 (2.11 Å^2), VAL606 (1.87 Å^2), ALA510 (1.81 Å^2), GLY417 (2.68 Å^2), GLY464 (1.64 Å^2), GLY605 (2.51 Å^2), ALA607 (2.29 Å^2), ILE416 (2.52 Å^2) and VAL463 (2.84 Å^2). It also formed two hydrophobic bonds, ALA607 (3.59 Å^2) and CYS513 (5.41 Å^2) with Keap1 protein.

In Vitro Antioxidant Activity of ME and EO

In order to verify the virtual antioxidant property of the compounds in ME and EO samples of *P. amarus*, their *in vitro* 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging and lipid peroxidation inhibition were evaluated and results are shown in Table 9 and Figure 8 (a and b).

Table 9. *In vitro* antioxidant activity of ME and EO of *P. amarus*

	IC_{50} (µg/mL)	
Sample	**DPPH**	**LPI**
ME	11.10 ± 1.63^{a}	39.43 ± 3.42^{a}
EO	15.62 ± 1.56^{b}	60.60 ± 4.71^{b}
Vitamin C	10.68 ± 2.01^{a}	36.42 ± 3.61^{a}

Values are expressed as Mean ± SEM for triplicate determinations, and data bearing another superscript differ significantly ($p < 0.05$).

ME which had both active compounds, demonstrated the higher DPPH radical scavenging and lipid peroxidation inhibition activity which compared well with the standard drug, Vitamin C, but EO, containing only one of the bioactive compounds had lower DPPH scavenging and lipid peroxidation inhibition activity somewhat different from the standard drug. The values (Table 9) used to evaluate the samples' (ME and EO) antioxidant activity (DPPH and LPI) were obtained from the dose-response curve.

Low Mole dock scores indicate better binding affinity. So, from Table 8, glyoxalbis (2,4-dinitrophenyl-hydrazone) has stronger binding affinity to Keap 1, similar to the curcumin, the standard. However, the ME demonstrated more potent antioxidant activity (Table 9) than the EO. ME contains both compounds which may have acted in synergy to promote antioxidant activity demonstrated by the ME.

DISCUSSION

Photogenic feed additives (PFA) or phytobiotics are used in feed to promote health and growth performance of livestock, mainly because of their high quality nutrients and rich medicinal phytochemicals that have antimicrobial (Cheng et al., 2018) and antioxidant (Onyesom et al., 2015) properties.

This study shows that the leaves of *P. amarus* contain high quality nutrients (proximate, vitamins and mineral elements) and medicinal phytochemicals (high amounts of flavonoids, phenols, tannins and alkaloids, but low levels of phylate and anti-nutrients) necessary to enhance performance and maintain good health. These findings are in agreement with previous reports (Mazumder et al., 2006; Ameen et al., 2021; Achikanu et al., 2022). *P. amarus*, therefore, possesses adequate nutrients and medicinal secondary metabolites with the

potential for supporting growth, performance and good health.

P. amarus is globally distributed and it is a herb known for its therapeutic potential with long history of use in traditional system due to the significant medicinal attributes imparted by the phytochemical constituents. This study reveals the presence of such secondary metabolites (alkaloids, flavonoids, phenols, tannins and saponins) in the leaves of *P. amarus*. Ameen et al. (2021) reported that tannins content was the highest (1.85 ± 0.03%), followed by anthocyanins, alkaloids, phenolics, glycosides, saponins and triterpenoids (0.13 ± 0.10%) in the methanol leaf extract of *P. amarus*.

Moreover, this study shows that the leaf ME and EO of *P. amarus* exhibited notable antibacterial effects against certain poultry isolates (*E. coli, S. aureus, S.* Enteritidis) with selectable indices, SI > 1. The proposed antimicrobial mechanism of EOs is linked to the disruption of cytoplasmic membrane and cell wall which in turn increase cell permeability and reduce virulence. The MICs for the ME of *P. amarus* against *S. aureus* (100 µg/mL), and *E. coli* (50 µg/mL) have been reported (Dakpogan et al., 2019). In our study, the MICs of both ME and EO of *P. amarus* against *E. coli*, *S. aureus*, and *S.* Enteritidis, as well as the IC_{50} for *E. tenella*, are presented in Table 3. Based on the classification of activity by Dakpogan et al. (2019), both ME and EO of *P. amarus* demonstrated strong antibacterial activity against poultry-derived *E. coli*. Against the other organisms, ME and EO showed moderate activity. Importantly, cytotoxicity data (CC_{50} values) suggest no toxic effects on the host. The antimicrobial potential of *P. amarus* against multiple antibiotic-resistant bacteria has been studied (Dakpogan et al., 2019). Also, the anticoccidal activity of *P. amarus* has been evaluated (Akin-Osanaiye et al., 2011) and our results on the causative protozoan (*E. tenella*; Table 3) align with theirs. Ethyl oleate present in the ME of *P. amarus* has demonstrated pronounced antimicrobial activity and showed broad spectrum of MICs (Priya et al., 2013). It is important to note that quorum sensors play essential role in bacteria pathogenesis. So, several plant extracts have been screened to attenuate pathogenic bacteria (Mamza et al., 2012). These authors, therefore, postulated that the ME of *P. amarus* could potentially inhibit quorum sensing molecules in *P. aeruginosa* PA101 by interfering with the swimming motility, production of pyocyanin, and the lux: lecA expression. On this premise, the compounds of *P. amarus* can be used as antimicrobials for pathogenic bacteria.

To identify the antibacterial bioactive compounds in *P. amarus* and possibly speculate their likely mechanism of action, the GC-MS profiled compounds were studied virtually by the CADD method. Sixty-six (31 in ME and 35 in EO) compounds were identified with three overlapping. The GC-MS

analysis of compounds in the ethanol extract of *P. amarus* has been reported (Oshomoh and Uzama-Avenbuan, 2020). When compared with our GC-MS data for the ME, there were similarities in the forty-two (42) compounds identified by Oshomoh and Uzama-Avenbuan (2020). However, Mamza et al. (2012) identified nine (9) major compounds from the GC-MS profiling of the ethanol leaf extract of *P. amarus*, and based on Dr. Duke's phytochemical and ethnobotanical database, Mamza et al. (2012) speculated the activity of these nine compounds to include the following: antimicrobial, 8 (3,5-di-t-butyl phenol, hexadecanoic acid, 10-octadecanoate, 9-hexadecenal, glycerol-1,3-dipalmitate, 2,13-octadecadiene-1-ol, dioctyl ester and heptonoic acid), antioxidant; 1(3, 5-di-t-butyl phenol), antiinflammatory, 1(3, 5-di-t-butyl phenol), antifungal, 1(methyl 14-methylpentadecanoate). We also identified methyl 14-methylpentanoate, which has been suggested to possess antifungal properties (Oshomoh and Uzama-Avenbuan, 2020); however, its antifungal activity was not assessed in this study. Nonetheless, we identified two important compounds (Figures 3a & b) with different modes of antibacterial action (Table 4). 6-Methoxy-4-methyl-5-((5-[3-(trifluoromethyl)phenol]plenty)oxy)-8 quinolinyl- amine strongly bounded to bacterial ATP synthase (-198.063kCal/mol), but, that with the standard drug, amoxicillin trihydrate is -138.234kCal/mol. Bacteria ATP synthase has two (2) functional subunits; the F1F0-ATP synthase is the primary ATP synthase complex in bacteria that generates ATP from proton motive force (PMF), the FO subunit transports proton and generates PMF, while F1 forms ATP from ADP and Pi. Therefore, ATP synthase plays essential role in bacteria protein synthesis by provision of energy needed for translational events. Thus, it provides the energy (ATP) required for binding of ribosomes to mRNA, assembly of initiation complex, formation of peptide bond between amino acids during elongation phase of translation, assembly/disassembly of ribosomes and its translocation along mRNA.

Several researchers have reported the importance of ATP in bacteria ATP synthesis. Anisimova et al. (2018) demonstrated that the rates of ATP synthesis correlate with the rates of protein synthesis in *E. coli*. In addition, it has been shown that the initiation factors, IF 2 needs ATP to bind to ribosomes in order to initiate translation (Marshall et al., 2009). Marshall et al. (2009) have observed that the elongation factor EF-Tu utilizes ATP to hasten the formation of peptide bond during translation. Furthermore, the study on ribosome-associated protein RsfA by Pontes et al. (2016) indicated that ATP is essential and so, required for the assembly and disassembly of ribosomes from mRNA. Altogether, the inhibition of bacteria ATP synthase by 6-methoxy-4-methyl-5-((5-[3-(trifluoromethyl) phenol] plenty) oxy)-8-quinolinylamine could sig-

nificantly disrupt bacterial protein synthesis, thereby impairing their survival and leading to cell death.

In addition, we also observed that another *P. amarus* compound – glyoxalbis (2,4-dinitrophenyl-hydrazone) interacted strongly with oxyR protein complex (-165.558 kCal/mol) relative to the standard drug (inhibitor), allicin (-74.242 kCal/mol). OxyR, oxygen or oxidative stress regulator or repressor, is a transcriptional regulator protein which modulates the expression of genes engaged in protecting bacteria against free radicals, especially H_2O_2 and the consequent oxidative stress. OxyR plays vital role in bacterial response to oxidative stress. It responds to changes in bacterial cellular redox state, senses H_2O_2, and activates the production of antioxidant enzymes (catalase, kat G, alkyl hydroperoxide reductase, ahpCF, and glutathione reductase, gor). OxyR regulates biofilm formation in response to oxidative stress in *E. coli* (Maksimova et al., 2023) and its mediated antioxidant defenses has been noted to contribute to bacterial tolerance of antibiotics (Li et al., 2021). Altogether, the two compounds may exhibit synergistic antibacterial action, and glyoxalbis (2,4-dinitrophenyl-hydrazone) could enhance bacteria tolerance of itself and other compounds, an action that could prevent bacterial resistance to these promising poultry antimicrobials.

Having identified the antimicrobial compounds in *P. amarus*, it is yet important to determine their antioxidant activity in poultry chickens, and this was accomplished by investigating docking with Keap1 which revealed scores of -136.665 kCal/mol and -157.752 kCal/mol for 6-methoxy-4-methyl-5-((5-[3-(trifluoromethyl)phenol]plenty)oxy)-8-quinolinylamine and glyoxalbis (2,4-dinitrophenyl-hydrazone), respectively, but with the standard, curcumin, -161.582 kCal/mol (Table 6). Keap1 inhibitors have been studied in poultry chickens for their potential to improve performance and health. Keap1 inhibitors are known to activate Nrf2 pathway which enhance antioxidant defenses in chickens. Reports on curcumin's inhibition of Keap1 in poultry chickens are limited. However, curcumin (50mg/kg feed) inhibited Keap1 and activated Nrf2, reducing oxidative stress (Basiouni et al., 2023), lipid peroxidation and inflammation in broiler chickens (Wang et al., 2020). Curcumin is a natural compound found in turmeric.

The in vitro antioxidant activity of ME and EO of *P. amarus* was consistent with virtual screening results, which identified 6-methoxy-4-methyl-5-((5-[3-(trifluoromethyl)phenol]pentyl)oxy)-8-quinolinylamine and glyoxalbis (2,4-dinitrophenylhydrazone) as the most active compounds. Dakpogan et al. (2019) reported that *P.amarus* ethanol leaf extract demonstrated the most significant antioxidant activity (IC_{50}= 5.83±0.05 µg/mL for DPPH radical scav-

enging) of the twenty (20) plants studied. The lipid peroxidation inhibition activity of ME compared well with the standard, and was found to be better than the EO and that of the ME of *Mayteneusroyleanus* leaves (79.12±0.5µg/mL) (Shabbir et al., 2013). The identified compounds may have contributed synergistically to the antioxidant activity observed for the ME of *P. amarus*.

CONCLUSION

This study demonstrates that *P. amarus* from Nigeria possesses high nutriphytochemical value, exhibiting significant antimicrobial, antioxidant, and nutritional properties. The plant's high essential vitamins, protein content, and minerals, in addition to its potent antioxidant and antimicrobial properties, make it a promising source of phytobiotic in veterinary applications, particularly in poultry production**.**

The identification of the active compounds 6-methoxy-4-methyl-5-((5-[3-(trifluoromethyl)phenol]pentyl)oxy)-8-quinolinylamine and glyoxalbis (2,4-dinitrophenylhydrazone) supports further investigation into their antimicrobial and antioxidant properties for potential applications in both animal health and human medicine. These compounds hold promise for future drug development aimed at combating oxidative stress, inflammation, and microbial infections in animals.

Observations from this study have wider implications for sustainable agriculture, providing a natural alternative to synthetic antibiotics in animal husbandry. Future research should therefore concentrate on investigating the synergistic effects of *P. amarus* with other phytobiotics, assessing its long-term impacts on various animal species, and optimizing its use in different production systems, which could contribute to both animal health and food security.

ACKNOWLEDGEMENT

The research was supported by the Tetfund IBR grant processed by the TETFund IBR Grant Committee, Delta State University, Abraka, Nigeria.

Author's Contribution

OI designed the study, drafted the proposal and supervised the project. OET, ACO, OO and AJO conducted the different aspects of the laboratory investigation, overseen by EAT who managed the grant. The manuscript was prepared by OI and all authors approved its submission.

Competing interest

Authors declare that there is no conflict of interest.

REFERENCES

Achikanu, C., Ujah, I., Ezenwali, M.O. 2022. Vitamin content of *Phyllanthus amarus* leaf. IOSR Journal Of Pharmacy And Biological Sciences, 17(6): 61-65. https://doi.org/10.9790/3008-1706036165

Adamu, M., Naidoo, V., Eloff, J.N. 2013. Some southern African plant species used to treat helminth infections in ethnoveterinary medicine have excellent antifungal activities. BMC complementary and alternative medicine, 12: 213-219. https://doi.org/10.1186/1472-6882-12-213

Ademola, S.G., Farinu,G.O., Ajayi, A.O., Babatunde, G.M. 2004. Growth, haematological and biochemical studies on garlic and ginger-fed broiler chickens. Moor Journal of Agricultural Research, 5(2): 122–128. https://doi.org/10.4314/mjar.v5i2.31809

Akin-Osanaiye, C.B., Gabriel, A.F., Alebiosu, R.A. 2011. Characterization and antimicrobial screening of ethyl oleate isolated from *Phyllanthus amarus* (Schum and Thonn). Annals of Biological Research, 2(2): 298–305.

Alagawany, M., Elnesr, S.S., Farag, M.R., Abd El-Hack, M.E., Barkat, R.A., Gabr, A.A., Foda, M.A., Noreldin, A.E., Khafaga, A.F., El-Sabrout, K., Elwan, H.A.M., Tiwari, R., Yatoo, M.I., Michalak, I., Cerbo, A.D., Dhama, K. 2021. Potential role of important nutraceuticals in poultry performance and health-a comprehensive review. Research in veterinary science, 137:9–29. https://doi.org/10.1016/j.rvsc.2021.04.009.

Ameen, O.A., Hamid, A.A., Yusuf, Q., Njoku, O.G., Oseni, T.O. Jamiu, W. 2021. Quantitative and qualitative assessment of phytochemicals in methanolic extracts of hurricane weed (*Phyllanthus amarus* Schumach. & Thonn) Journal of Applied Sciences and Environmental Management, 25(2): 159-165. https://dx.doi.org/10.4314/jasem.v25i2.4

Anisimova A.S., Alexandrov A.I., Makarova N.E., Gladyshev V.N., Dmitriev S.E. 2018. Protein synthesis and quality control in aging. Aging (Albany NY), 10(12):4269-4288. https://doi.org/10.18632/aging.101721

Association of Official Analytical Chemists, A.O.A.C. 1990. 15th ed., Washington D.C., USA, 1178- 1205.

Basiouni, S., Tellez-Isaias, G., Latorre, J.D., Graham, B.D., Petrone-Garcia, V.M., El-Seedi, H.R., Yalçın, S., El-Wahab, A.A., Visscher, C., May-Simera, H.L., Huber, C., Eisenreich, W., Shehata, A.A. 2023. Anti-inflammatory and antioxidative phytogenic substances against secret killers in

poultry: current status and prospects. Veterinary sciences, 10(1): 55. https://doi.org/ 10.3390/vetsci10010055

Baskar, R., Rajeswari, V., Kumar, T.S. 2007. In vitro antioxidant studies in leaves of Annona species. Indian journal of experimental biology, 45(5): 480-485.

Begum, M., Lyngdoh, W., Sharma, H.K. 2018. Formulation and evaluation of mosquito repellent ointment prepared with the essential oil of *Zanthoxylum acanthopodium DC*. Indigenous to Northeast India. International Journal of Green Pharmacy, 12(3): S518-S527.

Braca, A., Sortino, C., Politi, M., Morelli, I., Mendez, J. 2002. Antioxidant activity of flavonoids from *Licania licaniae*flora. Journal of Ethnopharmacology, 79(3): 379–381, https://doi.org/10.1016/S0378-8741(01)00413-5

Chen, Y., Liu, Y., Zhao, C., Ma, J., Guo, J. 2025. Antibiotic resistance gene pollution in poultry farming environments and approaches for mitigation: A system review. Poultry Science, 104(3): 104858. https://doi.org/10.1016/j.psj.2025.104858

Cheng, C., Xia, M., Zhang, X., Wang, C., Jiang, S., Peng, J. 2018. Supplementing oregano essential oil in a reduced-protein diet improves growth performance and nutrient diigestibility by modulating intestinal bacteria, intestinal morphology, and antiioxidative capacity of growing-finishing pigs. Animals, 8(9): 159. https://doi.org/10.3390/ani8090159

Dakpogan, H.B., Houndonougbo, V.P., Pomalegni, C., Ahounou, J.E., Chrysostome, C. 2019. Evaluation of the effect of *Phyllanthus amarus*, *Jatropha curcas* and *Piliostigma thonningii* on experimental chicken coccidiosis. Journal of Animal and Plant Sciences (JAPS), 42(2): 7269–7278.

Dao, L., Tran, M.P., Caroline,D.,Joëlle, Q.L., Bui, T.B.H., Le Thi, B., Truong, Q.N., Bui, T.B.H., Do Thi, T.H., Nguyen, T.P., Patrick, K., Marie-Louise, S. 2020. Screening and comparative study of *in vitro* antioxidant and antimicrobial activities of ethanolic extracts of selected Vietnamese plants. International Journal of Food Properties, 23(1): 481–96. https://doi.org/10.1080/10942912.2020. 1737541

Dialoke, N., Onimisi, P., Afolayan, M. 2020. Performance, blood parameters and economic indices of broiler chickens fed graded levels of chestnut (*Castenea sativa*) phytobiotics as replacement for antibiotics growth promoters. Nigerian Journal of Animal Production. 47(2):161–70. https://doi.org/10.51791/njap.v47i2.123

Drummond, A.J., Waigh, R.D. 2000. The development of microbiological methods for phytochemical screening Recent Research Developments in Phytochemistry, 4: 143-152.

Elisha, I.L., Botha, F.S., McGaw, L.J., Eloff, J.N. 2017. The antibacterial activity of extracts of nine plant species with good activity against Esche-

richia coli against five other bacteria and cytotoxicity of extracts. BMC complementary and alternative medicine, 17(1): 133-142. https://doi.org/10.1186/s12906-017-1645-z

Eloff, J.N. 2004. Quantifying the bioactivity of plant extracts during screening and bioassay-guided fractionation. Phytomedicine: International Journal of Phytotherapy & Phytopharmacology, 11(4): 370–371.

Feng, J., Dong, H., Pang, B., Shao, F., Zhang, C., Yu, L., Dong, L. 2018. Theoretical study on the optical and electronic properties of graphene quantum dots doped with heteroatoms. Physical Chemistry Chemical Physics, 20(22): 15244–15252. https://doi.org/10.1039/C8CP01403E

Filazi, A., Yurdakok-Dikmen, B. 2019. Nutraceuticals in poultry health and disease. In *Nutraceuticals in veterinary medicine*. Eds. R. Gupta, A. Srivastava, R. Lall, Cham: Springer. 661–672.

Ghasemzadeh, A., Jaafar, Z.H., Karimi, E., Ibrahim, M.H. 2012. Combined effects of CO_2 enrichment and foliar application of salicylic acid on the production and antioxidant activities of anthocyanin, flavonoids and isoflavonoids from ginger. BMC complementary and alternative medicine, 12: 229-241. https://doi.org/10.1186/1472-6882-12-229

Ghosh, A.B.M., Banerjee, A., Chattopadhyay, S. 2022. An insight into the potent medicinal plant *Phyllanthus amarus* Schum. and Thonn. The Nucleus: an international journal of cytology and allied topics, 65(3): 437-472. https://doi.org/ 10.1007/s13237-022-00409-z.

Haunschild, R., Barth, A. French, B. 2019. A comprehensive analysis of the history of DFT based on the bibliometric method RPYS. Journal of Cheminformatics, 11(1): 72-81. https://doi.org/10.1186/s13321-019-0395-y

Kikusato, M. 2021. Phytobiotics to improve health and production of broiler chickens: functions beyond the antioxidant activity. Animal bioscience, 34(3):345-353. https://doi.org/10.5713/ab.20.0842.

Kovesi, B., Worlanyo, A.P., Kulcsar, S., Ancsin, S., Erdelyi, M., Zandoki, E., Mezes, M., Balogh, K. 2024. Curcumin mitigates ochratoxin A-induced oxidstive stress and alters gene expression in broiler chicken liver and kidney. Acta Veterinaria Hungarica, 72(1): 41-50. https://doi.org/10.1556/004.2024.01016

Li, J., Li, W., Luo, S., Ma, C., Liu, S. 2019. Alternate ultrasound/microwave digestion for deep eutectic hydrodistillation extraction of essential oil and polysaccharide from *Schisandrachinensis* (Turez) Baill. Molecules, 24 (7): 1288-1301. https://doi.org/10.3390/molecules24071288

Li, L., Sun, X., Zhao, D., Dai, H. 2021. Pharmacological applications and action mechanisms of phytochemicals as alternatives to antibiotics

in pig production. Frontiers in Immunology, 12:798553. https://doi.org/10.3389/fimmu.2021.798553

Makhafola, T.J., Samuel, B.B., Elgorashi, E.E., Eloff, J.N. 2012. Ochnaflavone and ochnaflavone 7-O-methyl ether two antibacterial Biflavonoids from *Ochna pretoriensis* (Ochnaceae). Natural product communications, 7(12): 1601–1604.

Maksimova, Y., Zorina, A., Nesterova, L. 2023. Oxidative stress response and E. coli biofilm formation under the effect of pristine and modified carbon nanotubes. Microorganisms, 11(5), 1221. https://doi.org/10.3390/microorganisms11051221

Mamza, U.T., Sodipo, O.A., Khan, I.Z. 2012. Gas chromatography-mass spectrometry (gc-ms) analysis of bioactive components of *Phyllanthus amarus* leaves. International Research Journal of Plant Science, 3(10): 208-215.

Mandey, J.S., Sompie, F.N. 2021.Phytogenic feed additives as an alternative to antibiotic growth promoters in poultry nutrition. In *Advanced studies in the 21st century animal nutrition*. Eds. L. Babinszky, J. Oliveira, E. Mauro Santos, London: IntechOpen Limited: 19. https://doi.org/10.5772/intechopen.99401

Marshall, R.A., Aitken, C.E., Puglisi, J.D. 2009. GTP hydrolysis by IF2 guides progression of the ribosome into elongation. Molecular cell, 35(1):37-47. https://doi.org/10.1016/j.molcel.2009.06.008

Mazumder, A., Mahato, A., Mazumder, R. 2006 . Antimicrobial potentiality of Phyllanthusamarus against drug resistant pathogens. atural product research, 20(4): 323-326. https://doi.org/10.1080/14786410600650404

Miar, M., Shiroudi, A., Pourshamsian, K., Oliaey, A.R., Hatamjafari, F. 2021. Theoretical investigations on the HOMO–LUMO gap and global reactivity descriptor studies, natural bond orbital, and nucleus-independent chemical shifts analyses of 3-phenylbenzo[*d*]thiazole-2(3 *H*)-imine and its *para* -substituted derivatives: Solvent and substituent effects. Journal of Chemical Research, 45(1–2): 147–158. https://doi.org/10.1177/1747519820932091

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods, 65(1-2): 55–63. https://doi.org/10.1016/0022-1759(83)90303-4

Naik, S.R., Bharadwaj, P., Dingelstad, N., Kalyaanamoorthy, S., Mandal, S.C., Ganesan, A., Chattopadhyay, D., Palit, P. 2022. Structure-based virtual screening, molecular dynamics and binding affinity calculations of some potential phytocompounds against SARS-CoV-2. Journal of Bio-

molecular Structure and Dynamics, 40(15): 6921–6938. https://doi.org/10.1080/07391102.2021.1891969

Ndomou, S.C.H., Tsapla, C.T., Djikeng, F.T., Teboukeu,G.B., Kohole, H.A.F., Ngoudjou, D.T., Mube, H.K., Womeni, H.M. 2018. Correlation between performance, carcass traits and blood lipid profile of broilers chicken fed with rations supplemented with plant powders. Journal of Applied Life Sciences International, 17(2): 1–12. https://doi.org/10.9734/JALSI/2018/40681

Okom, S.U., Ojugbeli, E.T., Okpoghono, J. and Onyesom, I., 2023. Safety and antimalarial therapeutic index of alkaloid-rich extract of Phyllanthus amarus Schumach. & Thonn. in mice. Heliyon, 9(12):e23078. http://doi.org/10.1016/j.heliyon.2023.e23078

Onyesom I., Ezedom T., Mordi J.C., Aganbi E., Elu C.O., Acha J.O. 2023. Assessment of Phytochemical Constituents and In Vivo Antimalarial Activity of Methanol Extract and Solvent Fractions of Phyllanthus amarus Schum. and Thonn. European Chemical Bulletin, 12(10): 4249-4270.

Onyesom, I., Onumaechi, I.F., Ehiwario, J., Dagana, R. 2015.Antiplasmodial activity of *Phyllanthusamarus* preserves renal function in *Plasmodium berghei* infected mice. European Journal of Medicinal Plants, 5 (1): 109 – 116. http://doi.org/10.9734/EJMP/2015/13230

Oshomoh, E.O., Uzama-Avenbuan, O. 2020. Quantitative phytochemical composition and bioactive constituents of ethanolic extracts of *Phyllanthus amarus* (Schum. et Thonn) leaves. European Journal of Agriculture and Food Sciences, 2(4): 59-62.

Pashtetsky, V., Ostapchuk, P., Il'yazov, R., Zubochenko, D., Kuevda, T. 2019. Use of antioxidants in poultry farming. IOP Conf. Series: Earth and Environmental Science 341:012042. http://doi.org/10.1088/1755-1315/341/1/012042

Pashtetsky, V., Ostapchuk, P., Kuevda, T., Zubochenko, D., Yemelianov, S., Uppe, V. 2020. Use of phytobiotics in animal husbandry and poultry. E3S Web of Conferences, 215:02002. https://doi.org/10.1051/e3sconf/202021502002

Pontes, M.H., Yeom, J., Groisman, E.A. 2016. Reducing Ribosome Biosynthesis Promotes Translation during Low Mg^{2+} Stress. Molecular cell, 64(3):480-492. http://doi.org/10.1016/j.molcel.2016.05.008

Priya, K., Yin, W.F., Chan, K.G. 2013. Anti-quorum sensing activity of the traditional Chinese herb, *Phyllanthus amarus*. Sensors, 13(11): 14558–14569. http://doi.org/10.3390/s131114558

Rakhee, R.N., Regin, S.A. 2008. Integrating the science of pharmacology and

bioinformatics. *Phyllanthus* "The wonder plant". Advanced Biotechnology. 10: 28-30.

Rozeboom, M.D., Tegmo-Larsson, I.M., Houk, K.N. 1981. Frontier molecular orbital theory of substitutent effects on regioselectivities of nucleophilic additions and cycloadditions to benzoquinones and naphthoquinones. The Journal of Organic Chemistry, 46(11): 2338–2345. https://doi.org/10.1021/jo00324a026

Shabbir, M., Khan, M.R., Saeed, N. 2013. Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of *Maytenusroyleanus* leaves. BMC complementary and alternative medicine. 13: 143-158. https://doi.org/10.1186/1472-6882-13-143

Ulrikh, E.V., Khaliullin, R.S., Ganieva, I.A., Izhmulkina, E., Arzjutov, M. 2018. The content of biologically active substances in phytobiotics used for agricultural animals and poultry. International Journal of Engineering & Technology 7(3):445-449. https://doi.org/10.14419/ijet.v7i3.14.17040

Wang, Y., Xia, L., Guo, T., Heng, C., Jiang, L., Wang, D., Wang J., Li K., Zhan, X. 2020. Research Note: Metabolic changes and physiological responses of broilers in the final stage of growth exposed to different environmental temperatures. Poultry Science, 99(4): 2017-2025. https://doi.org/10.1016/j.psj.2019.11.048

Weather and Climate. 2024. The Global Historical Weather and Climate Data. Retrieved from: http://weatherandclimate.com/nigeria. Accessed: 18.10.2024.

Wu, F., Zhou, Y., Li, L., Shen, X., Chen, G., Wang, X., Liang, X., Tan, M., Huang, Z. 2020. Computational Approaches in Preclinical Studies on Drug Discovery and Development. Frontiers in Chemistry, 8: 726. https://doi.org/10.3389/fchem.2020.00726

Zirihi, G.N., Mambu, L., Guédé-Guina, F., Bodo, B., Grellier, P. 2005. In vitro antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. Journal of ethnopharmacology, 98(3): 281–285. https://doi.org/10.1016/j.jep.2005.01.004

Received: 18.02.2025.
Accepted: 26.03.2025.