

Phytochemical and Metabolomic Profiles of Ethanolic Extract of *Curculigo pilosa* Rhizomes for Animal Health

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Abstract. *Curculigo pilosa* is traditionally used in herbal medicine. This study aims to provide information on the secondary metabolites present in *C. pilosa* rhizomes powder, considering the growing interest in ethnomedicines in recent times. The rhizomes of *C. pilosa* were purchased from markets around Ogere, Ogun State, Nigeria. They were authenticated by a botanist, after which the rhizomes were washed and extracted using ethanol. The phytochemical composition and metabolomic profiles of the ethanolic extracts of *C. pilosa* were determined using gas chromatographic-mass spectrometric (GC-MS) methods. The results showed that the ethanolic extracts of *C. pilosa* had higher concentrations of phenols (9.27 ± 0.55 mg GAE/g), alkaloids (6.22 ± 1.95 g/100g), flavonoids (0.66 ± 0.05 g/100g), and saponins (0.51 ± 0.02 g/100g), but lower concentrations of glycosides (0.06 ± 0.01 g/100g), steroids (0.20 ± 0.02 g/100g), and tannins (0.09 ± 0.01 g/100g). The twenty-one bioactive compounds found in the extract include tetraethyl silicate ($C_8H_{20}O_4Si$), benzene, 1,2-dimethoxy- ($C_8H_{10}O_2$), 5-dodecene, (Z)- ($C_{12}H_{24}$), 7-tetradecene, (E)- ($C_{14}H_{28}$), spirohexane-1-carboxylic acid, ethyl ester ($C_9H_{14}O_2$), 1-dodecanol, 2-octyl- ($C_{20}H_{42}O$), formaldehyde, methyl (2-propynyl) hydrazone ($C_5H_8N_2$), D-allose ($C_6H_{12}O_6$), cyclohexane, 1R-acetamido-2cis,4trans-bis(acetoxy)-3trans-azido- ($C_{12}H_{22}N_4O_5$), 5-octadecene, (E)- ($C_{18}H_{36}$), acetamide, 2-(4-hydroxy-3-methoxyphenyl)- ($C_{11}H_{15}NO_3$), cyclopentanol, 1-(1-methylene-2-propenyl)- ($C_9H_{14}O$), 1,9-tetradecadiene ($C_{14}H_{26}$), 9-eicosine, (E) ($C_{20}H_{40}$), hexadecyl propyl ether ($C_{19}H_{40}O$), 9-octadecene, (E)- ($C_{18}H_{36}$), linoleic acid ethyl ester ($C_{20}H_{34}O$), 2-methyl-Z,Z-3,13-octadecadienol ($C_{19}H_{36}O$), 3-octadecene, (E)- ($C_{18}H_{36}$), 3-heptadecenal ($C_{17}H_{32}O$), tricyclo[5.4.3.0(1,8)] tetradecan-6-one, and 4-ethenyl-3-hydroxy-2,4,7,14-tetramethyl ($C_{20}H_{32}O_2$). Some of the phytocompounds identified in this study are biologically important and exhibit antimicrobial, antioxidant, and anti-inflammatory properties, which may hold therapeutic potential for both animal and human health.

Keywords: *Curculigo pilosa*, Metabolomics, Phytochemicals, Phytomedicine

Introduction

Despite the widespread use of modern medicine, herbal products are still used to treat illnesses in the majority of developing countries in Asia and Africa. Medicinal plants are plants that have therapeutic qualities (Okaiyeto and Oguntibeju, 2021; Akintunde et al., 2021a; Olumide et al., 2023). Numerous studies on the ethnomedicinal potentials of various phytonics in Africa, such as the seeds of *Moringa oleifera* (Akintunde et al., 2021a, 2024), *Carica papaya* (Olumide et al., 2022), *Phyllanthus niruri* (Tayo et al., 2022), and *Parquetina nigrescens* (Akintunde et al., 2023a), have been conducted. *Curculigo pilosa*, on the other hand, is one of those

medicinal plants with enormous potential for use in ethnomedicine (Titilayo and Adeyemi, 2010). *Curculigo pilosa* belongs to the family of Hypoxidaceae (Common name: Golden eye grass, African crocus; Local Nigerian names: Nupe - Echidungi; Hausa - Baka, ekuaku, Dooyar kureege; Yoruba - Epakun) (Sofidiya et al., 2011). Medicinal plants have been used since ancient times as food additives and to treat certain illnesses.

Another well-known species of *Curculigo*, *Curculigo pilosa* (Africa), has traditionally been utilized as an anti-epileptic agent, to enhance fertility, treat meteorism, act as a styptic, and manage drepanocytosis (Mad Nasir et al., 2021).

Additionally, it exhibits notable antidiabetic, antioxidant, and antibacterial properties (Palazzino et al., 2000; Shaba et al., 2014; Karigidi and Olaiya, 2019a, b). The crude extract of *C. pilosa* demonstrates the highest overall phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC), indicating a robust DPPH scavenging effect (Karigidi and Olaiya, 2019b).

The phytochemical and metabolomic profiles of ethanolic extracts from plant rhizomes have been explored in several studies. Titilayo and Adeyemi (2010) and Wang et al. (2021) identified various phytochemicals in the rhizomes, including alkaloids, saponins, and tannins, with Wang et al. also demonstrating the extract's anticandidal activity. Mad Nasir et al. (2021) expanded on the metabolomic analysis of *Curculigo latifolia*, a closely related species, and reported a high antioxidant capacity in the rhizome crude extract. Additionally, Shaba et al. (2014) reported on the antimicrobial and cytotoxic activities of the methanolic extract of *Curculigo pilosa* rhizomes, indicating the potential for these extracts in various applications.

Dipa et al. (2022) conducted a study on the phytochemical profiling and antioxidant activities of *Curculigo orchioides*. Phytochemical screening and estimation were employed to identify the secondary metabolites present in the methanolic extract of the plant's rhizomes. The plant demonstrated significant antioxidant properties, as evidenced by its ability to scavenge DPPH radicals. The presence of flavonoids, alkaloids, phenols, tannins, and saponins was verified by phytochemical screening. Upon phytochemical estimation, the following values were obtained: 103.36 mg of GAE/gm of phenol, 118.86 mg of GAE/gm of tannins, 21.20 mg/gm of alkaloids, 13.00 mg/gm of saponins, and 96.80 mg of QE/gm of flavonoids. GC-MS was used for additional

analysis. Furthermore, an antioxidant test revealed that *Curculigo orchioides* had IC₅₀ values of 49.529 µg/ml, with 39.91 µg/ml of garlic acid used as the standard. They however concluded that *Curculigo orchioides* contain promising phytochemicals and antioxidant activities.

Titilayo and Adeyemi (2010) also reported that *Curculigo pilosa* contained traces of anthraquinones, alkaloids, saponins, tannins, and cardenolides, among other phytochemicals. Furthermore, they observed that while the water extract (1000 mg/ml) was ineffective against the isolates, the ethanol extracts (500 mg/ml) and undiluted essential oil exhibited significant anticandidal activity. The minimum inhibitory concentration (MIC) of the ethanol extract for the tested isolates ranged from 0.020 to 1.500 mg/ml. The authors concluded that the identification and isolation of the active compounds in *C. pilosa* could potentially lead to the development of effective anticandidal phytomedicines.

In addition, Mad Nasir et al. (2021) observed that *Curculigo latifolia* leaf extract exhibited the highest level of antibacterial activity against *Staphylococcus aureus* (MIC = ±0.25 mg/mL, MBC = ±0.25 mg/mL) and *Salmonella choleraesuis* (MIC = ±0.25 mg/mL, MBC = ±0.25 mg/mL). Both the rhizome and leaf extracts of *Curculigo latifolia* were found to contain major metabolites, phenolic glycosides, and norlignans.

Based on these findings, the rhizomes of *Curculigo pilosa* offer numerous benefits for both human health and the agricultural and livestock sectors. In livestock development, these rhizomes have the potential to improve animal health due to their antimicrobial and antioxidant properties, which can help enhance the immune system of livestock. Additionally, they can be utilized as feed additives to improve livestock feed quality. However, the phytochemical composition of these

rhizomes remains largely unknown and warrants further investigation.

This study aimed to investigate the phytochemical and metabolomic profiles of *Curculigo pilosa* rhizomes. An understanding of the overall phytochemical and metabolomics of the rhizomes is necessary to optimize its potential as a source of food and medicine, both for human and animal health.

Materials and Methods

Source and Preparation of Experimental Material

Curculigo pilosa rhizomes were locally purchased from the Tollgate market in Ogere Remo, Ogun State, Southwest Nigeria, in September 2023. The rhizomes were peeled, cut into thin slices, and air-dried. After drying, the material was pulverized into a fine powder using a blender and stored in a dry, clean container with a tight lid. The powder was then dried at 105°C in a hot air oven as per the experimental design, and then cooled. The leaves were ground to a fine powder using a USHA MG 3473 laboratory grinder. The extraction process was carried out through maceration of the leaves in ethanol, using varying weight-to-volume ratios based on the experimental design. The mixtures were shaken vigorously and left to stand for 48 hours at room temperature.

The mixture was filtered through Whatman No. 1 filter paper, and the residue was subsequently macerated in an equal volume of ethanol for an additional 24 hours to extract more compounds. The resulting mixtures were then combined and evaporated to dryness under reduced pressure at approximately 40°C using an Eyela N-1001 vacuum rotary evaporator.

Phytochemical analysis.

Saponin quantification was performed using the afrosimetric method (Koziol, 1991), while the

gravimetric method (Harborne, 1973) was used to determine contents of alkaloids and flavonoids. All analyses done using triplicate samples.

Metabolomic Profiling of *Curculigo pilosa* Rhizomes

The ethanolic extracts of *Curculigo pilosa* rhizomes were subjected to GC/MS analysis at the Department of Chemistry, University of Lagos, Akoka. The GC/MS system used was an Agilent Technologies 7890A GC/MS, MSD 5975C detector, with a 7683B series injector. The analysis conditions were as follows: initial temperature of 100°C held for 2 minutes, ramped to a final temperature of 270°C at a rate of 10°C/min. A 1 µL injection of the 0.2 g/mL extract was used. The heater temperature was set at 250°C with a pressure of 3.2652 psi. The column used was a splitless HP5MS (30 m×320 µm×0.25 µm), with helium as the carrier gas at a purity of 99.9999% and an average velocity of 45.618 cm/s. The identification of constituent compounds was done by comparing the retention periods and mass spectra of the genuine samples acquired by GC with the mass spectra from the NIST Version 2.0 database library, Washington, DC, USA.

Statistical Analysis.

All data are presented as mean ± standard deviation, based on three independent measurements. Data analysis was performed using IBM SPSS Statistics version 20.

Results and Discussion

Table 1 shows the phytochemical analysis of *Curculigo pilosa* rhizomes. It was observed that the rhizomes contained alkaloids (6.22 g/100g), flavonoids (0.66 g/100g), glycosides (0.06 g/100g), saponin (0.51 g/100g), steroids (0.20 g/100g), phenols (9.27 mg GAE/g), and tannin (0.09 g/100g).

Table 1. Phytochemical Analysis of *Curculigo pilosa* Rhizomes

Parameter	Concentration
Alkaloids (g/100g)	6.22 ± 1.95
Flavonoids (g/100g)	0.66 ± 0.06
Glycosides (g/100g)	0.06 ± 0.02
Saponin (g/100g)	0.51 ± 0.02
Steroids (g/100g)	0.20 ± 0.02
Phenols (mgGAE/g)	9.27 ± 0.55
Tannin (g/100g)	0.09 ± 0.01

*Data are mean values ± standard deviation (SD) of duplicate results

The ethanol extract of *C. pilosa* rhizomes was analyzed by GC-MS (Figure 1), revealing twenty-one peaks based on their retention times. The active compounds identified, along with their retention time (RT), molecular formula (MF), molecular weight (MW), peak area (PA), structure, and pharmacological activities, are given in Table 2. The twenty-one compounds found in the extract are tetraethyl silicate (1.10), Benzene, 1,2-dimethoxy- (1.76), 5-Dodecene, (Z)- (3.73), 7-Tetradecene, (E)- (7.50), Spirohexane-1-carboxylic acid, ethyl ester (2.37), 1-Dodecanol, 2-octyl- (1.41), Formaldehyde, methyl (2-propynyl)hydrazine (1.79), D-allose (35.13), Cyclohexane, 1R-acetamido-2cis,4trans-bis(acetoxy)-3trans-azido- (1.86), 5-Octadecene, (E)- (8.67), Acetamide, 2-(4-hydroxy-3-methoxyphenyl)- (8.35), Cyclopentanol, 1-(1-methylene-2-propenyl)- (2.67), 1,9-Tetradecadiene (1.40), 9-icosine, (E) (4.47), Hexadecyl propyl ether (1.58), 9-Octadecene, (E)- (5.55), Linoleic acid ethyl ester (2.69), 2-Methyl-Z, Z-3,13-octadecadienoic (2.74), 3-Octadecene, (E)- (2.58), 3-Heptadecenal, Tricyclo[5.4.3.0(1,8)] tetradecane 6-one (1.44), and 4-ethenyl-3-hydroxy-2,4,7,14-tetramethyl (1.20).

The phytochemical composition of *C. pilosa* rhizomes is detailed in Table 1. Phytochemicals are plant-derived compounds that can exhibit both beneficial and toxic effects. While some phytochemicals offer significant health benefits, others may pose risks to the body (Nyirenda and

Kumwenda, 2023; Kumar et al., 2023). It was observed that *C. pilosa* rhizomes contained relatively high number of alkaloids.

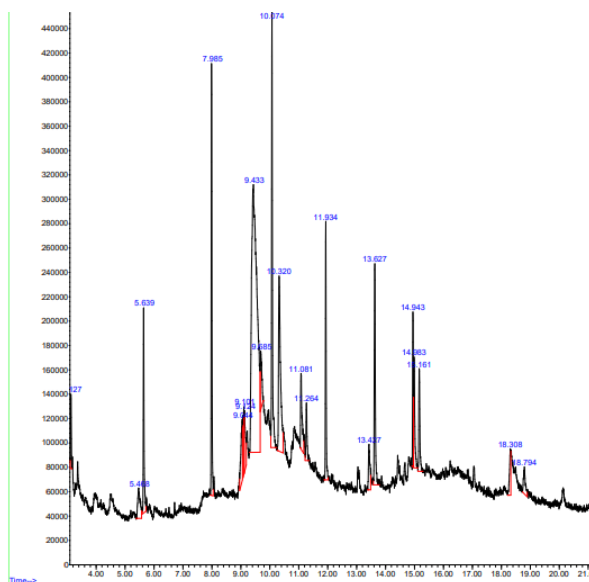
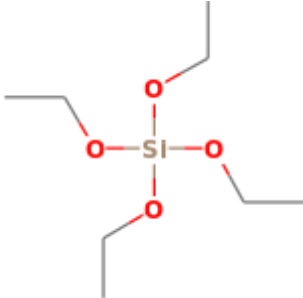
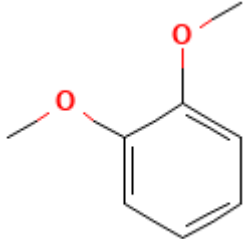
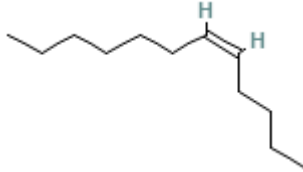
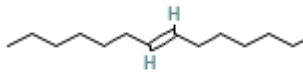
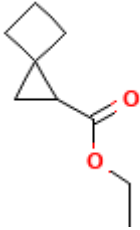
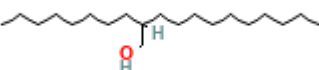
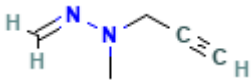
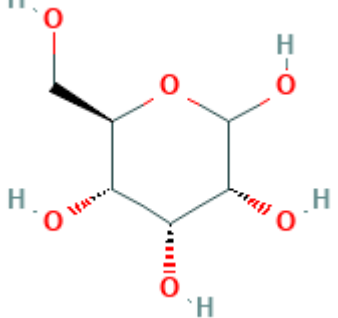
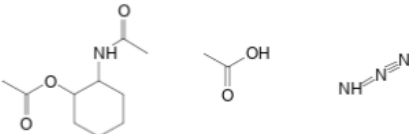



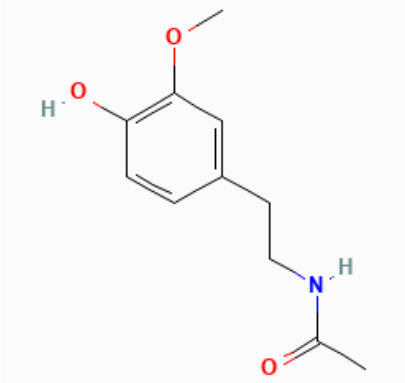
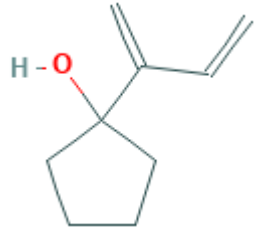
Figure 1: GC-MS chromatograms of ethanolic extract of *Curculigo pilosa*

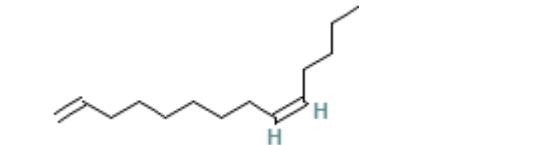


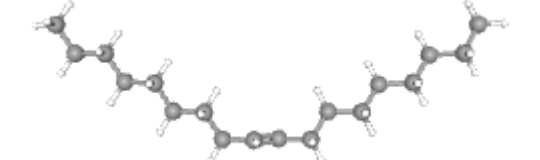
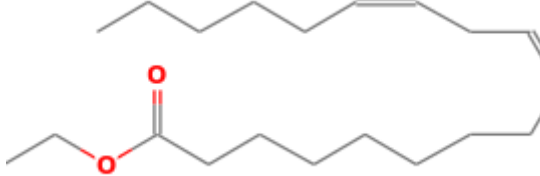
The values of alkaloids obtained were lower (12.80 mg/100g) than those obtained by Shaba et al. (2014). The variation might be due to the method of extraction, as crude methanolic extraction was used by Shaba et al. (2014). The value (6.22 g/100g) obtained for *C. pilosa* rhizomes in this study was, however, higher than the values reported for *Phyllanthus niruri* leaf (3.67 g/100g) by Olufayo et al. (2021). *Chromolaena odorata* leaf meal (1.66%) by Akintunde et al. (2021b).

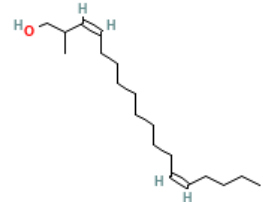

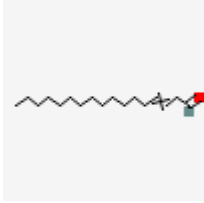
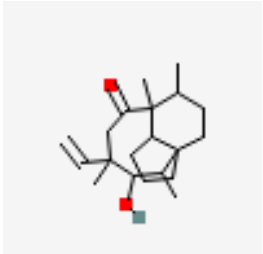
Table 2: GC-MS analysis of ethanolic extract of *Curculigo pilosa*

Peak No	RT	Compound name	MF	MW	PA (%)	Class of compound	Pharmacological Activity	Molecular structure
1	3.127	Tetraethyl silicate	$C_8H_{20}O_4Si$	208.33	1.10	Silicates	-	
2	5.468	Benzene, 1,2-dimethoxy-	$C_8H_{10}O_2$	138.16	1.76	Aromatic	Antioxidant Activity, Anti-Inflammatory Activity, Antimicrobial Activity, Neuroprotective Activity, and Antiplatelet Activity	
3	5.639	5-Dodecene, (Z)-	$C_{12}H_{24}$	168.31	3.73	Alkene	-	
4	7.985	7-Tetradecene, (E)-	$C_{14}H_{28}$	196.37	7.50	Alkene	-	

5	9.044	Spirohexane-1-carboxylic acid, ethyl ester	$C_9H_{14}O_2$	154.2 1	2.37	Carboxylic acid ester	Antioxidant, antimicrobial, neuroactive, anti-inflammatory, and analgesic effect, and receptor modulation.	
6	9.101	1-Dodecanol, 2-octyl-	$C_{20}H_{42}O$	298.5 5	1.41	Alcohols		
7	9.124	Formaldehyde, methyl(2-propynyl) hydrazone	$C_5H_8N_2$	96.13	1.79	Hydrazone		
8	9.433	D-Allose	$C_6H_{12}O_6$	180.1 6	35.1 3	Aldohexoses	Antidiabetic potential, antioxidant activity, and cell signaling.	
9	9.685	Cyclohexane, 1R-acetamido-2cis,4trans-bis(acetoxy)-3trans-azido-	$C_{12}H_{22}N_4O$	302.3 3	1.86	Acetamino-acetoxy-azido group	-	

10	10.074	5-Octadecene, (E)-	$C_{18}H_{36}$	252.5	8.67	Alkene	-	
11	10.320	Acetamide, 2-(4-hydroxy-3-methoxyphenyl)-	$C_{11}H_{15}NO$ 3	209.24	8.35	Acetamides	Antioxidant Activity, Anti-Inflammatory Effects, analgesic, antimicrobial, neuroprotective activity, and cardiovascular benefit	
12	11.081	Cyclopentanol, 1-(1-methylene-2-propenyl)-	$C_9H_{14}O$	138	2.67	Alcohols	Antioxidant activity, cell signaling modulatory, anti-inflammatory, enzyme	

13	11.26 4	1,9-Tetradecadiene	$C_{14}H_{26}$	194	1.40	Alkenes	inhibitory effect, metabolic, cytotoxic or anticancer effect	-	
14	11.93 4	9-Eicosene, (E)-	$C_{20}H_{40}$	280	4.47	Alkenes	-	-	
15	13.42 7	Hexadecyl propyl ether	$C_{19}H_{40}O$	284.5	1.58	Ethers	-	-	
16	13.62 7	9-Octadecene, (E)-	$C_{18}H_{36}$	252.5	5.55	Alkene	-	-	
17	14.94 3	Linoleic acid ethyl ester	$C_{20}H_{34}O$	306.5	2.69	Fatty acid esters	Cardiovascular health, skin health, inflammation regulation, and metabolic processes	-	

18	14.98 3	2-Methyl-Z,Z-3,13- octadecadienol	$C_{19}H_{36}O$	280.5	2.74	Alcohols	-	
19	15.16 1	3-Octadecene, (E)- 1	$C_{18}H_{36}$	252.5	2.58	Alkene	-	
20	18.30 8	3-Heptadecenal	$C_{17}H_{32}O$	252.4	1.44	Aldehydes	Antioxidant, antimicrobial, anti- inflammatory, enzyme inhibitory effect, metabolic, cytotoxic or anticancer effect	
21	18.79 4	Tricyclo[5.4.3.0(1,8)]tetradec an-6-one, 4-ethenyl-3- hydroxy-2,4,7,14-tetramethyl	$C_{20}H_{32}O_2$	304.5	1.20	Ketone, alkene, alkanol	-	

Almost on par with 6.64% reported for *Curcuma longa* rhizomes (Adebisi et al., 2021) and 5.94 mg/100g for seeds of unripe *Carica papaya* fruits (Akintunde et al., 2021c) and peels of ripe (6.44 mg/100g) and unripe (6.90 mg/100g) *Carica papaya* (Akintunde et al., 2022).

Natural compounds with heterocyclic nitrogen atoms are called alkaloids (Kurek, 2019). Because of their capacity to act as metal chelators, electron or hydrogen donors, or as scavengers of free radicals, alkaloids also have antioxidant and anticancer properties. Additionally, it has been reported that these alkaloids have a chemopreventive effect on tumor cells by depolymerizing or terminating the protein microtubules that form the mitotic spindle during cell division. This lowers the incidence of cancer by impeding the process of tumor cell division and separation. This bolsters the findings of research by Dibwe et al. (2023), who documented the antimutagenic, antigenotoxic, and ROS-scavenging properties of beta-carboline alkaloids, which are present in a range of foods and medicinal plants. Thus, the rhizomes of *C. pilosa* may have chemopreventive properties.

It was found that *Curculigo pilosa* rhizomes had a comparatively low concentration of flavonoids. The flavonoid level obtained in this trial (0.66 g/100g) was lower than that reported by Shaba et al. (2014) (44.88 mg/100g) and the 23.17 mg/g observed by Karigidi et al. (2020). The concentration of flavonoids in this study was also lower than the 2.30 mg/100g reported for the egg-lime-molasses mixture by Akintunde et al. (2023b) and 1.40 g/100g for *Phyllanthus niruri* by Olufayo et al. (2021), but higher than the values observed for the leaves of *Carica papaya* (Olumide et al., 2023). Being naturally occurring antioxidants, flavonoids are crucial in scavenging free radicals and preventing degenerative illnesses such as cardiovascular disease (Ullah et al., 2020; Bondonno et al., 2020; Patel, 2023). They also play a role in

regulating the cell cycle, inhibiting the growth of cancerous cells, and initiating apoptosis (Khan et al., 2021; Mir et al., 2023). To inhibit tumor formation, flavonoids can function as mild agonists or antagonists of estrogen, thereby modulating the activity of endogenous hormones, and as inhibitors of free-radical-mediated cytotoxicity and lipid peroxidation (Patel, 2023). In this way, they may help manage menopausal symptoms and provide protection against long-term conditions such as cancer and atherosclerosis. Their conjugated ring structures and hydroxyl groups enable them to act as antioxidants in vitro or in cell-free systems by scavenging superoxide anions, singlet oxygen, and lipid peroxy radicals, while stabilizing free radicals involved in oxidative processes through hydrogenation or complexation with oxidizing species (Abhinav et al., 2024).

There is very little glycoside concentration (0.06 g/100 g) in the rhizomes of *Curculigo pilosa*. The flavonoids, glycosides, and cardiac glycosides present in the rhizomes of *Curculigo pilosa* are known for their antioxidant properties. Flavonoids and glycosides reportedly treat capillary fragility by acting as anti-inflammatories and antioxidants (Maury et al., 2020). Their presence in the rhizomes of *Curculigo pilosa* suggests the plant's strong antioxidant and membrane-stabilizing abilities.

The study found that the saponin concentration in *Curculigo pilosa* rhizomes was moderate, at 0.51 g/100g. However, a higher level of saponins was reported by Shaba et al. (2014) after extracting the rhizome using methanol. Saponins are naturally occurring surface-active glycosides with characteristic foaming behavior. According to Paarvanova et al. (2023), saponins have an impact on hemolysis. The aglycone moiety of saponins is thought to have an affinity for the phospholipids found in the cell membrane, forming insoluble complexes, which is why saponins exhibit hemolytic activity. Rhodophil membranes are

lytically affected by saponins, with potential positive or negative effects. Erythrocytes may undergo suicidal cell death, or apoptosis, before hemolysis, which results in the removal of faulty erythrocytes before hemoglobin is released (Föller and Lang, 2020). Bhuyana et al. (2022) stated that when human erythrocytes are exposed to saponin, it stimulates Ca^{2+} entry, which causes cell membrane scrambling and ultimately leads to the suicidal death of the erythrocytes. Hemolysis is a parallel effect, followed by anemia and thrombosis. Both plants tend to produce more erythrocytes due to their significant saponin content (Airaodion et al., 2019). Additionally, saponins have been shown to reduce serum cholesterol levels, which may impact cholesterol metabolism. Their higher excretion can be explained by the formation of large mixed micelles resulting from the interaction between saponins and bile acids. As a result, the liver's accelerated metabolism of cholesterol lowers serum levels of the substance (Cao et al., 2024). Consequently, *C. pilosa* may be an effective natural treatment for conditions such as obesity, heart disease, and other disorders linked to cholesterol (Airaodion et al., 2019). There have also been reports of hypolipidemic properties for saponins. The high fiber content of saponins is the mechanism underlying their hypolipidemic action. Due to the fiber's strong binding to cholesterol, it helps the body eliminate it (Islam et al., 2021). Saponins can also reduce inflammation. Their noteworthy ameliorative effect may stem from the inhibition of inflammatory mediators, including prostaglandin, histamine, and serotonin, as well as their antioxidant properties, which prevent the production of reactive oxygen species (ROS), a major contributor to inflammation (Khan et al., 2022).

The rhizomes of *C. pilosa* contain steroids at low levels. The most well-known effect of steroids is on lipid metabolism (Atik et al., 2020). One class of steroids with anti-inflammatory

qualities is phytosterols, which also prevent the intestinal absorption of cholesterol (Li et al., 2022). Medications and various sex hormones are also derived from steroids. Additionally, steroids exhibit antibacterial properties, which are linked to membrane lipids and result in liposome leakage (Sari et al., 2024).

The phenolic content obtained in this trial was lower (9.72 mg GAE/1g) than that reported by Shaba et al. (2014) and Karigidi et al. (2020). Phenols are present in low amounts in *C. pilosa* rhizomes. The pharmacological and functional characteristics of the plant may be partially explained by the presence of these phenolic compounds. In aqueous solutions, these compounds donate a hydrogen atom or an electron-chelating metal ion to neutralize reactive oxygen species or free radicals (Kruk et al., 2022). Due to the functional groups present in each phenolic compound, the phenolic compounds extracted from plants exhibit a variety of biological properties, including antimicrobial, antioxidant, anti-inflammatory, anticancer, antidiabetic, and anti-mutagenic qualities (Kruk et al., 2022). However, according to this study, *C. pilosa* rhizomes contain some phenolic content, suggesting they may have potential uses as ethnomedicinal plants.

The rhizomes of *C. pilosa*, as observed in this study, contain low levels of tannin (0.09 mg/100g). These values are lower than those of 69.49 mg/100g for the crude methanolic extract of *C. pilosa* as reported by Shaba et al. (2014) and also lower than the value obtained for unripe *Carica papaya* seed reported by Akintunde et al. (2021c). This variation might be due to differences in the method of extraction. Tannins are recognized as antibacterial substances. These plant polyphenols, which are soluble in water, can precipitate proteins (Abdullahi et al., 2022). It has been reported that tannins inhibit the growth of microorganisms by precipitating microbial proteins, rendering them incapable of obtaining nutritional proteins.

Tannins inhibit the growth of numerous yeasts, bacteria, viruses, and fungi. Numerous physiological effects, including anti-irritant, antisecretolytic, antiphlogistic, antimicrobial, and antiparasitic effects, have been linked to tannins. Antiviral, antibacterial, antiparasitic, anti-inflammatory, anti-ulcer, and antioxidant properties have also been attributed to tannins (Abdullahi et al., 2022). The rhizomes contain phenols and flavonoids, which can help prevent oxidative stress by scavenging free radicals and bio-activating carcinogens for the liver to excrete (Chaudhary et al., 2023). This suggests that the rhizomes may be very helpful in treating conditions like neuro-inflammation, which can cause cell damage due to the presence of free radicals, as well as in slowing down the aging process.

Gas chromatography-mass spectrometry (GC-MS) serves as a reliable tool for identifying phytochemicals. GC-MS is undeniably a robust and powerful analytical method for delineating and characterizing phytochemicals present in diverse plant specimens (Ralte et al., 2022). The most abundant phytochemical revealed in this study is D-allose (Karigidi and Olaiya, 2019b; Maury et al., 2020), which is known to have some antioxidant activity and cell signaling effects (Ganesan and Xu, 2019; Yamazaki et al., 2022). D-allose, due to its limited capacity to provide energy, is considered a less vital nutrient for the majority of animals. Fatty acid derivatives, such as linoleic acid methyl ester, which were also found, are reported to be biologically significant in energy storage and utilization, cardiovascular health, and skin health (Sherratt et al., 2023). Secondary metabolites, notably alcohols, esters, carboxylic acids, aldehydes, and ketones, which are found in significant abundance within *Curculigo pilosa*, have the potential to serve diverse roles. These compounds may function as alternative energy sources, contribute to pheromone production, facilitate cell signaling, and act as

neurotransmitters. Consequently, they could make valuable contributions to animal nutrition and overall health.

Conclusion

In this study, some of the phytochemicals identified from the rhizomes of *Curculigo pilosa* are biologically important and possess antimicrobial, antioxidant, and anti-inflammatory potential. Therefore, they may have therapeutic significance for both animal and human health. These bioactive substances may contribute to the preventive or therapeutic applications of *Curculigo pilosa* rhizomes in the treatment of various chronic inflammatory and/or oxidative stress-related illnesses. *Curculigo pilosa* demonstrates significant potential in advancing the livestock sector, both in improving animal health and enhancing production, thereby contributing to sustainable and environmentally friendly livestock practices.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

- Abdullahi, A., Tijjani, A., Abubakar, A.I., Khairulmazmi, A., Ismail, M.R. 2022. Plant biomolecule antimicrobials: An alternative control measures for food security and safety. In Herbal Biomolecules in Healthcare Applications (pp. 381-406). Academic Press.
- Abhinav, S., Pentu, N., Rao, T.R. 2024. An updated review on flavonoids. The Pharma Innovation Journal, 13(2), 01-08. <https://doi.org/10.22271/tpi.2024.v13.i2a.25347>
- Adebisi, A.A., Olumide, M.D., Akintunde, A.O. 2021. Nutritive value and phytochemical screening of turmeric and clove as a potential photo-additive in livestock production. Nigerian Journal of Animal Science, 23(2), 142-152. <https://www.ajol.info/index.php/tjas/article/view/219045>.
- Airaodion, A.I., Olatoyinbo, P.O., Ogbuagu, U., Emmanuel, O.O., Akinmolayan, J.D., Adekale,

- O.A., Awosanya, O.O., Agunbiade, A.P., Oloruntoba, A.P., Obajimi, O.O., Adeniji, A.R., Airaodion, E.O. 2019. Comparative Assessment of Phytochemical Content and Antioxidant Potential of *Azadirachta indica* and *Parquetina nigrescens* Leaves. *Asian Plant Research Journal*, 2(3), 1-14.
- Akintunde, A.O., Toyé, A.A., Ademola, A.A. 2021a. Effects of dietary *Moringa Oleifera* seed meal on obesity, liver and kidney functional parameters of local and exotic chickens. *Aceh Journal of Animal Science*, 6(3), 97 – 103. DOI: 10.13170/ajas.6.3.20641.
- Akintunde, A.O., Ndubuisi-Ogbona, L.C., Ajayi, O.A., Chioma, C., Jimoh, W.A., Afodu, O.J. 2021b. Utilization of *Chromolaena odorata* leaf meal as a supplement in broiler chickens' diet. *Nigerian Journal of Animal Science*, 23(1), 189-198. <https://www.ajol.info/index.php/tjas/article/view/212029>.
- Akintunde, A.O., Kolu, P., Ndubuisi-Ogbona, L.C., Akinboye, O.E., Akintunde, I.A., Adewole, S.A. 2021c. Nutritive and Phytochemical Values of Unripe Seeds of *Carica papaya* and Prospects in Animal Nutrition. *Nigerian Research Journal of Chemical Sciences*, 9(2), 278-287.
- Akintunde, A.O., Kolu, P., Akintunde, I.A., Adewole, S.A., Akinboye, O.E., Afodu, O.J., Ndubuisi-Ogbona, L.C., Shobo, B.A. 2022. Evaluation of the Nutritive Values of *Carica papaya* Fruit Peels as A Potential Ingredient in Livestock Nutrition. *Animal Production*, 24(2), 104-113. DOI: <https://doi.org/10.20884/1.jap.2022.24.2.129>.
- Akintunde, A.O., Ndubuisi-Ogbona, L.C., Sobowale, A., Irevebo, H.E., Ojo, O.A., Oyewumi, S.O., Shobo, B.A., Akinboye, O.E., Ngozi, E.O. 2023a. Antioxidant Potentials of *Parquetina nigrescens* Leaf Extract Administration in Broiler Chicken Production. *International Journal of Pharmaceutical and Phytopharmacological Research*, 13(5), 19-26. <https://doi.org/10.51847/jHhpavJCEo>
- Akintunde, A.O., Ndubuisi-Ogbona, L.C., Olorunfemi, O.A., Ladele, M.M., Shobo, B.A., Adewole, S.A., Akinboye, O.E. 2023b. Nutritional and Ethno-Medicinal Potentials of Egg-Lime-Molasses Mixture in Livestock Production. *Asian Journal of Dairy and Food Research*, 42(3), 298-306. doi: 10.18805/ajdfr.DRF-285.
- Akintunde, A.O., Ndubuisi-Ogbona, L.C., Shobo, B.A., Akinboye, O.E., Animashaun, R.O., Oyekale, O.O. 2024. Value and Prospects of *Moringa oleifera* as Non-Conventional Feedstuff in Livestock Production: A Review. *Research Biotica*, 6(1), 17-27. <https://doi.org/10.54083/ResBio/6.1.2024/17-27>.
- Atik, N., Hayati, R.U., Hamijoyo, L. 2020. Correlation between Steroid Therapy and Lipid Profile in Systemic Lupus Erythematosus Patients. *Open Access Rheumatology*, 12, 41-46. <https://doi.org/10.2147/OARRR.S245662>.
- Bhuyana, A.A.M., Alzoubib, K., Fazioc, A., Brigliad, M., Faggioc, C., Langb, F. 2022. Suicidal Death of Human Erythrocytes Following Exposure to Pentostatin. *Paracelsus Proceedings of Experimental Medicine*, 1, 15-23. <https://doi.org/10.33594/000000576>.
- Bondonno, N.P., Lewis, J.R., Blekkenhorst, L.C., Bondonno, C.P., Shin, J.H., Croft, K.D., Woodman, R.J., Wong, G., Lim, W.H., Gopinath, B., Flood, V.M. 2020. Association of flavonoids and flavonoid-rich foods with all-cause mortality: The Blue Mountains Eye Study. *Clinical Nutrition*, 39(1), 141-150. <https://doi.org/10.1016/j.clnu.2019.01.004>.
- Chaudhary, P., Janmeda, P., Docea, A.O., Yeskaliyeva, B., Abdull Razis, A.F., Modu, B., Calina, D., Sharifi-Rad, J. 2023. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. *Frontiers in Chemistry*, 11, 1158198. <https://doi.org/10.3389/fchem.2023.1158198>.
- Dibwe, D.F., Takeishi, N., Oba, S., Sakurai, A., Sakurai, T., Tsukui, T., Chiba, H., Hui, S.P. 2023. Identification of a β -Carboline Alkaloid from Chemosselectively Derived Vanilla Bean Extract and Its Prevention of Lipid Droplet Accumulation in Human Hepatocytes (HepG2). *Molecules (Basel, Switzerland)*, 28(24), 8024. <https://doi.org/10.3390/molecules28248024>.
- Cao, S., Liu, M., Han, Y., Li, S., Zhu, X., Li, D., Shi, Y., Liu, B. 2024. Effects of Saponins on Lipid Metabolism: The Gut–Liver Axis Plays a Key Role. *Nutrients*, 16(10):1514. <https://doi.org/10.3390/nu16101514>.
- Dipa, M., Harishankar, M., Sharma, H.P. 2022. Phytochemical, antioxidant, and GC-MS analysis of a crude extract of *Curculigo orchoides Gaertn* rhizomes grown in hilly areas of Jharkhand. *Research Journal of Chemistry and Environment*, 26(5), 178-183. <https://doi.org/10.25303/2605rjce177183>.
- Föllner, M., Lang, F. 2020. Ion Transport in Eryptosis, the Suicidal Death of Erythrocytes. *Frontiers in Cell and Developmental Biology*, 8: 597. <https://doi.org/10.3389/fcell.2020.00597>.
- Ganesan, K., Xu, B. 2019. Anti-Diabetic Effects and Mechanisms of Dietary Polysaccharides. *Molecules*, 24(14), 2556. <https://doi.org/10.3390/molecules24142556>.

- Harborne, J. 1973. Phytochemical methods. Chapman and Hall, Ltd London. 49- 88.
- Islam, S.U., Ahmed, M.B., Ahsan, H., Lee, Y.S. 2021. Recent Molecular Mechanisms and Beneficial Effects of Phytochemicals and Plant-Based Whole Foods in Reducing LDL-C and Preventing Cardiovascular Disease. *Antioxidants* (Basel, Switzerland), 10(5): 784. <https://doi.org/10.3390/antiox10050784>.
- Karigidi, K.O., Akintimehin, E.S., Omoboyowa, D.A., Adetuyi, F.O., Olaiya, C.O. 2020. Effect of *Curculigo pilosa* supplemented diet on blood sugar, lipid metabolism, hepatic oxidative stress and carbohydrate metabolism enzymes in streptozotocin-induced diabetic rats. *Journal of Diabetes and Metabolic Disorders*, 19(2), 1173–1184. <https://doi.org/10.1007/s40200-020-00618-w>.
- Karigidi, K.O., Olaiya, C.O. 2019a. Antidiabetic activity of corn steep liquor extract of *Curculigo pilosa* and its solvent fractions in streptozotocin-induced diabetic rats. *Journal of Traditional and Complementary Medicine*, 10(6): 555–564. <https://doi.org/10.1016/j.jtcme.2019.06.005>.
- Karigidi, K.O., Olaiya, C.O. 2019b. In vitro antidiabetic, antioxidant, and anti-lipid peroxidative activities of corn steep liquor extracts of *Curculigo pilosa* and its solvent fractions. *Journal of Herbs, Spices & Medicinal Plants*, 25(4), 377-388.
- Khan, A.U., Dagur, H.S., Khan, M., Malik, N., Alam, M., Mushtaque, M.D. 2021. Therapeutic role of flavonoids and flavones in cancer prevention: Current trends and future perspectives. *European Journal of Medicinal Chemistry Reports*, 3, 100010. <https://doi.org/10.1016/j.ejmcr.2021.100010>.
- Khan, M.I., Karima, G., Khan, M.Z., Shin, J.H., Kim, J.D. 2022. Therapeutic Effects of Saponins for the Prevention and Treatment of Cancer by Ameliorating Inflammation and Angiogenesis and Inducing Antioxidant and Apoptotic Effects in Human Cells. *International Journal of Molecular Sciences*, 23(18), 10665. <https://doi.org/10.3390/ijms231810665>.
- Koziol, M.J. 1991. Afrosimetric estimation of threshold saponin concentration for bitterness in quinoa (*Chenopodium quinoa Willd*). *J. Sci. Food Agric*. 54(2), 211-219.
- Kruk, J., Aboul-Enein, B.H., Duchnik, E., Marchlewicz, M. 2022. Antioxidative properties of phenolic compounds and their effect on oxidative stress induced by severe physical exercise. *The Journal of Physiological Sciences*, 72(1), 19. <https://doi.org/10.1186/s12576-022-00845-1>.
- Kumar, A., Nirmal, P., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Eloheid, T., Sneha, K., Oz, F. 2023. Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. *Molecules* (Basel, Switzerland), 28(2), 887. <https://doi.org/10.3390/molecules28020887>.
- Kurek, J. 2019. Alkaloids - Their Importance in Nature and for Human Life. IntechOpen. doi: 10.5772/intechopen.85400.
- Li, X., Xin, Y., Mo, Y., Marozik, P., He, T., Guo, H. 2022. The Bioavailability and Biological Activities of Phytosterols as Modulators of Cholesterol Metabolism. *Molecules* (Basel, Switzerland), 27(2), 523. <https://doi.org/10.3390/molecules27020523>.
- Mad Nasir, N., Ezam Shah, N.S., Zainal, N.Z., Kassim, N.K., Faudzi, S.M.M., Hasan, H. 2021. Combination of molecular networking and LC-MS/MS profiling in investigating the interrelationships between the antioxidant and antimicrobial properties of *Curculigo latifolia*. *Plants*, 10(8), 1488. <https://doi.org/10.3390/plants10081488>.
- Maurry, G.L., Rodríguez, D.M., Hendrix, S., Arranz, J.C.E., Boix, Y.F., Pacheco, A.O., Díaz, J.G., Morris-Quevedo, H.J., Dubois, A.F., Aleman, E.I., Beenaerts, N., Méndez-Santos, I.E., Ratón, T.O., Cos, P. Cuyper, A. 2020. Antioxidants in Plants: A Valorization Potential Emphasizing the Need for the Conservation of Plant Biodiversity in Cuba. *Antioxidants* (Basel, Switzerland), 9(11), 1048. <https://doi.org/10.3390/antiox9111048>.
- Mir, S.A., Dar, A., Hamid, L., Nisar, N., Malik, J.A., Ali, T., Bader, G.N. 2023. Flavonoids as promising molecules in the cancer therapy: An insight. *Current Research in Pharmacology and Drug Discovery*, 6, 100167. <https://doi.org/10.1016/j.crphar.2023.100167>.
- Nyirenda, K.K., Kumwenda, F.D. 2023. Health Benefits and Toxicity Potential of Phytochemical Food Additives. IntechOpen. <https://doi.org/10.5772/intechopen.109251>.
- Okaiyeto, K., Oguntibeju, O.O. 2021. African herbal medicines: Adverse effects and cytotoxic potentials with different therapeutic applications. *International Journal of Environmental Research and Public Health*, 18(11), 5988. <https://doi.org/10.3390/ijerph18115988>.
- Olufayo, O.O., Tayo, G.O., Olumide, M.D., Akintunde AO. 2021. Assessment of the nutritive value of *Phyllanthus niruri* Linn. (Stonebreaker) leaves. *Nigerian Journal of Animal Science*, 23(3), 108-115. <https://www.ajol.info/index.php/tias/article/view/220739>
- Olumide, M.D., Akintunde, A.O., Kolu, P. 2022. Response of broiler chickens to substitution of

- vitamin-mineral premix with *Carica papaya* seed meal. *Journal of the Indonesian Tropical Animal Agriculture*, 47(3), 215-234. DOI: 10.14710/jitaa.47.3.215-234.
- Olumide, M.D., Akintunde, A.O., Shobo, B.A., Akinboye, O.E. 2023. Nutrient Evaluation and Phytochemical Analysis of Fresh and Dry Leaves of *Carica papaya*. *Indian Journal of Agricultural Research*, 57(2), 230-234. doi: 10.18805/IJARe.AF-726.
- Paarvanova, B., Tacheva, B., Savova, G., Karabaliyev, M., Georgieva, R. 2023. Hemolysis by saponin is accelerated at hypertonic conditions. *Molecules*, 28(20), 7096. <https://doi.org/10.3390/molecules28207096>.
- Palazzino, G., Galeffi, C., Federici, E., Delle Monache, F., Cometa, M.F., Palmery, M. 2000. Benzyl benzoate and norlignan glucosides from *Curculigo pilosa*: structural analysis and in vitro vascular activity. *Phytochemistry*, 55(5), 411-417.
- Patel, D.K. 2023. Therapeutic Potential of a Bioactive Flavonoids Glycitin from *Glycine max*: A review on medicinal importance, pharmacological activities and analytical aspects. *Current Traditional Medicine*, 9(2), 33-42. <http://dx.doi.org/10.2174/2215083808666220513143957>.
- Ralte, L., Khiangte, L., Thangjam, N.M., Kumar, A., Singh, Y.T. 2022. GC-MS and molecular docking analyses of phytochemicals from the underutilized plant, *Parkia timoriana* revealed candidate anti-cancerous and anti-inflammatory agents. *Scientific Reports*, 12(1), 3395. <https://doi.org/10.1038/s41598-022-07320-2>.
- Sari, S.R., Wardhani, R., Umar, F., Husain, D.R., Iwansyah, A.C. 2024. Antibacterial activity of Shallots (*Allium xwakegi* Araki.) cultivars in Palu Valley against Salmonella Typhi ATCC 27870 through in vitro and in silico evaluation. *Iranian Journal of Microbiology*, 16(2), 208-218. <https://doi.org/10.18502/ijm.v16i2.15354>.
- Shaba, E.Y., Mann, A., Yisa, J. 2014. Antimicrobial and cytotoxic activities and GC-MS analysis of phytocomponents of methanolic extract of *Curculigo pilosa* (Schum and Thonn) Engl. (Hypoxidaceae) rhizomes. *British Journal of Pharmaceutical Research*, 4(12), 1552.
- Sherratt, S.C.R., Libby, P., Budoff, M.J., Bhatt, D.L., Mason, R.P. 2023. Role of Omega-3 Fatty Acids in Cardiovascular Disease: The Debate Continues. *Current Atherosclerosis Reports*, 25(1), 1-17. <https://doi.org/10.1007/s11883-022-01075-x>.
- Sofidiya, M.O., Oduwole, B., Bamgbade, E., Odukoya, O. and Adenekan, S. (2011). Nutritional composition and antioxidant activities of *Curculigo pilosa* (Hypoxidaceae) rhizome. *African Journal of Biotechnology*, 10(75):17275-17281. <https://doi.org/10.5897/AJB11.1335>
- Tayo, G.O., Olufayo, O.O., Olumide, M.D., Akintunde, A.O. 2022. Growth and hematological parameters of Isa-brown pullets fed *Phyllanthus niruri* leaf meal as an additive at the chick phase. *Nigerian Journal of Animal Production*, 49(2), 130-139. <https://doi.org/10.51791/njap.v49i2.3470>
- Titilayo, G.I., Adeyemi, E. 2010. Phytochemical screening and in vitro anticandidal activity of extracts and essential oil of *Curculigo pilosa* (Schum and Thonn) Engl. Hypoxidaceae. *African Journal of Biotechnology*, 9(8), 1236.
- Ullah, A., Munir, S., Badshah, S.L., Khan, N., Ghani, L., Poulson, B.G., Emwas, A.H., Jaremko, M. 2020. Important flavonoids and their role as a therapeutic agent. *Molecules* (Basel, Switzerland), 25(22), 5243. <https://doi.org/10.3390/molecules25225243>.
- Wang, Y., Li, J., Li, N. 2021. Phytochemistry and Pharmacological Activity of Plants of Genus *Curculigo*: An Updated Review Since 2013. *Molecules*, 26(11), 3396. <https://doi.org/10.3390/molecules26113396>.
- Yamazaki, K., Hoshi, M., Tezuka, H., Morita, N., Hirayama, M., Sato, F., Yoshida, S., Saito, K. 2022. D-allose enhances the efficacy of hydroxychloroquine against Lewis lung carcinoma cell growth by inducing autophagy. *Oncology Reports*, 47(6), 1-10. <https://doi.org/10.3892/or.2022.8328>