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Original Research Article

Varvain Glucoside from the Stem Bark of *Ceiba Pentandra* Controls Postharvest Loss in *Dioscorea alata* Tubers

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ABSRTACT

Ceiba pentandra is a plant renowned for its wound healing ability and antimicrobial characteristics. This work focused on isolating and characterizing antimicrobial compounds from the stem bark of *Ceiba pentandra*. Plant extracts were prepared by successive maceration of the ground stem bark in hexane, ethyl acetate, and methanol. The extracts were subjected to column chromatography on silica gel to isolate compounds that could be used as antimicrobial agents against tuber rot in *Dioscorea alata*. The compounds obtained were varvain-3-O-glucoside, ferulic acid, catechin-3-O-glucoside, and a mixture of sitosterol and stigmasterol. The compounds were identified using NMR and high-resolution mass spectrometry techniques, and their identities were verified by comparing their data with information found in literature reports. Antimicrobial assays of the extracts and isolated compounds against bacterial and fungal isolates from *Dioscorea alata* tubers revealed that the compounds are active against tuber rot bacteria and fungi with MICs between 50.0 and 12.5 µg/mL. The extracts were less active, with MICs between 50.0 and 25.0 µg/mL. In conclusion, the relatively low MICs indicate the ability of these compounds to inhibit the growth of pathogenic microorganisms commonly associated with postharvest losses of *Dioscorea alata* tubers.

Keywords: *Ceiba pentandra*, *Dioscorea alata*, Antimicrobial activity, Bacteria, Fungi, Spectroscopy

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Introduction

The search for new drugs and dietary supplements derived from plants and natural resources has increased in recent years due to drug resistance and the toxicity of synthetic drugs.[1] Ethnopharmacological practices and phytochemistry are revealing natural products that could be developed as drugs for the treatment of infectious diseases.[2] Orthodox medicine is now receptive to the use of natural antimicrobial agents and other drugs derived from plants.[3] *Ceiba pentandra* (L) Gaertn, commonly known as the Kapok tree, is a tropical tree of the order Malvales and the family Malvaceae.[4] The plant was selected based on ethnobotanical reports on the plant in the treatment of microbial diseases and conditions classified by traditional healers as "infection" or "inflammation". Leaves serve as bandages for ulcers, sprains, tumours, abscesses and vitiligo.[5] The bark macerates are employed as diuretics and aphrodisiacs and for the treatment of headache, stomach ailments, diarrhoea, gonorrhoea, oedema, fever, asthma, rickets, and type II diabetes. The plant's stem bark contains significant amounts of secondary metabolites, including alkaloids, tannins, saponins, anthraquinones, flavonoids, cardiac glycosides, steroids, and phenols.[6] Previous research revealed an isoflavone glucoside, vavain-3-0-beta-glucoside, and its aglycon vavain from the stem bark of plants.[7]

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Two sesquiterpene lactones with mild antimicrobial effects were also isolated from the root bark of the plant. Additionally, a compound called 2,7-dihydroxy-8-formyl-7-hydroxy-5-quinone was isolated from the heartwood of the plant.[8] These compounds were all obtained and characterized using chromatographic and spectral analysis. The combination of column chromatography with spectroscopic (NMR) and mass spectral, investigations has facilitated the separation and characterization of compounds derived from plant sources.[9] *Dioscorea alata*, or water yam, belongs to the genus *Dioscorea*, family Dioscoreaceae.[10] It contains mainly carbohydrates but also has several good nutrients.[11] With an annual yield of almost 13 million tons, it serves as a crucial food crop and a significant source of revenue for communities in West Africa.[12] During storage, tubers suffer microbial attack, deterioration or rot, and losses of up to 50% may result.[13] Some rot-causing microorganisms of water yam tubers include *Fusarium* species, *Aspergillus niger, Mucor circinelloides, Aspergillus flavus, Erwinia carotovora, Phyzopus stolonifera, Serretiamarcescens, Rhizoctonia solani and Armillariella mellea.*[14] Special attention has been given to plants due to their potential to provide safer alternatives to synthetic antimicrobial agents. Hence, this study aimed to investigate the efficacy of compounds isolated from *Ceiba petandra* stem bark against several pathogenic bacteria and fungi responsible for tuber rot in *Dioscorea alata*. This is an initial report of the activity of these isolated compounds against the tuber rot microorganism *Dioscorea alata*.

Materials and Methods

General

Column chromatography was performed using silica gel 60 with a particle size range of 0.040-0.063 mm and a mesh size of 230-400 according to the ASTM standard. Aluminium sheets that were precoated with silica gel F250 (Merck, Germany) were used for thin-

layer chromatography (TLC). A Bruker AVIII (400 MHz) spectrometer from Bruker, Germany, was used to acquire nuclear magnetic resonance (NMR) (^{1}H, ^{13}C, COSY, HSQC, and HMBC) spectra. The solvents used for the experiment were $CDCl_3$ and acetone-d6. The spectra were referenced against the residual solvent peaks ($CDCl_3$ = 7.26 ppm and CD_3COCD_3 = 2.05 ppm). An Agilent 6130B Single Quadrupole LC/MS System was used to acquire high-resolution mass spectra.

Collection of plant material

The stem bark of the plant was collected in November 2023 from the North-core area of Joseph Sarwuan Tarka University, Makurdi, Nigeria (latitude: 7.7322° N, longitude: 8.5391° E). The plant was identified at the Department of Forestry of the University, where it was submitted as a voucher specimen and assigned a voucher number (UAM/FH/0440).

Extraction and Isolation

The stem bark of the plant was pounded using a wooden mortar until a rough consistency was achieved. One kilogram of this powder was weighed into three glass bottles and macerated successively with 6.0 litres of hexane, ethyl acetate, and methanol for a duration of 48 hours each. Hexane, ethyl acetate, and methanol extracts were obtained by filtering the extracts and eliminating the solvents under vacuum. The ethyl acetate extract was subjected to column chromatography with 50 g of silica gel slurry in a glass column (40 cm × 3 cm id) packed in a mixture of 95% hexane and 5% ethyl acetate. The extract was separated using gradient elution with varying concentrations of ethyl acetate in hexane (5-50%). A total of 64 fractions (20 mL each) were collected. Similarly, the methanol extract was fractionated using 95:5-10:90 ethyl acetate-methanol mixtures, yielding 85 portions of 20 mL each. The fractions were dried and then analysed using TLC. Similar fractions were analysed using NMR and LC–MS.[15]

Antimicrobial assay

Antimicrobial sensitivity test

Approximately 0.5 grams of each extract was weighed and placed in sterile vials. To this solution, 2.5 mL of dimethyl sulfoxide (DMSO) was added, followed by the addition of 7.5 mL of sterile distilled water to form a total volume of 10 mL, resulting in a concentration of 50 mg/mL. This was the starting concentration of the extracts employed to assess the antimicrobial activity. The bacteria and fungi were cultured on Mueller Hinton agar (MHA) and Sabouraud dextrose agar (SDA), respectively, as the corresponding growth media. The media were prepared following the guidelines provided by the manufacturer, sterilized at a temperature of 121 °C for 15 minutes, poured into sterilized petri dishes, and then left to cool and harden. First, 0.1 mL of the standard inoculums of the test bacteria was added to Mueller Hinton agar, whereas 0.1 mL of the standard inoculums of the test fungus was added to Sabouraud dextrose agar. The inocula were equally distributed on the media using a sterile swab. The culture plates were left to air dry for approximately 5 minutes. An aseptic cork borer with a diameter of 6 mm was utilized to create evenly spaced wells in the central region of each inoculated medium. Each well received approximately 0.1 mL of the standardized solution at a concentration of 50 mg/mL. The culture plates were then left undisturbed for approximately 30 minutes to allow the extracts to spread before incubation. Controls were implemented by introducing the inoculums into media plates that contained ciprofloxacin (2 mg/mL) for bacteria and fulcin (2 mg/mL) for fungi rather than using the extracts. The Mueller Hinton agar plates were placed in an incubator set at 37 °C for 24 hours. Similarly, the Sabouraud dextrose agar plates were incubated at the same temperature for 7 days. Using a transparent ruler, the inhibition zone diameters were measured in mm to assess antimicrobial activity. The experiment was repeated three times, and the average of two separate measurements was used for each microorganism.[16]

Determination of the minimum inhibitory concentrations (MICs)

The broth dilution method was used to determine the lowest inhibitory concentrations of the extracts and compounds. The bacterial isolates were prepared using Mueller Hinton broth, while the fungal isolates were prepared using Sabouraud dextrose broth. Approximately 10 mL of the broth was distributed into test tubes, sterilized at a temperature of 121 °C for 15 minutes, and then allowed to cool. A turbid solution with a turbidity level of 0.5 on Mc-Farland's standard scale was prepared. A physiological saline solution was produced, and 10 mL was aliquoted into aseptic test tubes. The test microbes were introduced and kept in a controlled environment at 37 °C for 24 hours for the bacterial isolates and at 37 °C for 7 days for the fungi. The test microorganisms were diluted in normal saline until their turbidity equalled that of Mc-Farland's scale, as determined by visual inspection. The extract was diluted in sterilized broth to obtain concentrations of 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, and 3.13 mg/mL. Similarly, the compounds were diluted to concentrations of 50, 25, 12.5, 6.25, and 3.13 µg/mL. After determining the concentrations of the extract and compound in the sterile broth, approximately 0.1 mL of the test microbes in normal saline was introduced to the different concentrations: bacteria to Mueller Hinton broth and fungi to Sabouraud dextrose broth. Bacteria and fungi were incubated at 37 °C for 24 and 7 days, respectively. The broth test tubes were checked for turbidity, indicating growth. The minimal inhibitory concentration was the lowest extract or compound concentration in broth without turbidity.[17]

Results and discussion

Extracts

The weight and nature of the extracts are given in Table 1.

Table 1: Yield and appearance of the extracts from *Ceiba pentandra*

Extract	Yield	Physical appearance
Hexane	6.4 g	Oily red (gummy)
Ethyl acetate	1.5 g	Dark brown
Methanol	5.5 g	Deep red

Characterization of compounds

Characterization of CPM 24 as a Varvain-3′-O-beta-d-glucoside (Figure 1)

The compound was isolated as a white crystalline solid from the methanol extract. HRLC-MS detected a molecular ion peak at m/z 345.0970 [M+H]+ (calc. 345.0974), corresponding to the chemical formula $C_{18}H_{16}O_7$. The proton spectrum of the compound revealed an H-bonded hydroxyl proton signal at δ_H 12.90 ppm, consistent with that of a C-5-OH-substituted flavonoid. Two meta-coupled proton signals at δ_H 6.56 (d, J = 2.2) and 6.37 (d, J = 2.2) are typical of flavones' 5,7-disubstituted ring A. There was an oxymethylene proton singlet at 8.35 ppm, which is typical for the H-2 proton of isoflavones. A second set of meta-coupled aromatic protons were found at 8.11 (1H, d, J = 2.0) and 7.05 (1H, d, J = 1.9) for the tetra-substituted benzene ring, which must be the isoflavone's ring B. Three methoxy protons were detected at 3.92, 3.87, and 3.82 (O-CH_3); hence, the compound must be a trimethoxy-substituted isoflavone. In addition, a glycoside anomeric proton was found at 5.00 ppm along with other sugar protons between 3.62 and 4.13 ppm. This implies that the compound may contain a sugar moiety and thus a glycoside. The ^{13}C spectrum showed a total of 24 signals, including a carbonyl at δ_C 180.6, an anomeric carbon signal at 101.6 and five other sugar carbons between 61.8 and 77.5 ppm. Other signals were attributed to the methoxy carbons at 55.6, 55.7 and 60.2 cm-1, confirming the presence of a sugar molecule attached to the compound. This sugar must be glucose, as the -CH_2-OH (C-6) in glucose was present at 61.9 ppm. Based on these findings, the compound was determined to be a glucoside of a trimethoxy-substituted isoflavone, and its 2D (COSY, HSQC, and HMBC) spectra were studied to confirm the structure as follows: long-range (HMBC) couplings (^{3}J and ^{2}J) from the 5-OH proton revealed C-5, C-6, and C-10, whereas correlations from H-2 revealed C-1′, C-3, C-4, and C-9. The methoxy protons were found to be connected by ^{3}J correlations at C-4′, C-5′, and C-7. The long-range correlation between the anomeric proton and C-3′ confirmed that the glucose moiety was attached to C-3′. Other correlations and couplings were as expected; hence, the compound was identified as Varvain-3′-O-

beta-d-glucoside and validated by comparing its NMR chemical shift assignments (Table 2) to published literature reports.[18]

Table 2: ^{1}H (400 MHz) and ^{13}C (100 MHz) data for Varvain-3′-O-beta-d-glucoside in acetone-d_6

Position	Experimental		Literature*	
	^{1}H δ ppm	^{13}C δ ppm	^{1}H δ ppm	^{13}C δ ppm
1	-	-	-	-
2	8.35	154.8	8.50	155.2
3	-	122.8	-	122.2
4	-	180.6	-	180.0
5	-	162.4	-	161.7
6	6.37 (d, *J* = 2.0 Hz)	98.0	6.44	98.1
7	-	166.0		165.3
8	6.56 (d, *J* = 2.0 Hz)	92.1	6.67	92.5
9	-	158.0	-	157.3
10	-	105.9	-	105.3
1’	-	126.3	-	125.7
2’	7.11 (d, *J* = 2.2 Hz)	110.7	6.99	109.9
3’	-	150.9	-	150.6
4’	-	139.2	-	138.3
5’	-	153.4	-	152.6
6’	7.05	107.9	6.98	107.4
5-OH	12.90 (s)	-	12.92	-
7-OMe	3.92 (s)	55.6	3.88	55.9
5’-OMe	3.87 (s)	55.6	3.81	56.1
4’-OMe	3.82 (s)	60.2	3.75	60.2
Glu-1	5.00	101.5	4.91	100.8
Glu-2	3.54	73.8	3.25-3.32	73.2
Glu-3	3.53	77.2	3.25-3.32	76.8
Glu-4	3.44	70.6	3.13-3.18	69.8
Glu-5	3.53	77.2	3.25-3.32	77.0
Glu-6	3.91, 3.68	61.7	3.42-3.47	60.7
Glu-2-OH	5.35		5.32	
Glu-3-OH			5.09	
Glu-4-OH			5.01	
Glu-6-OH	4.46		4.55	

Characterization of CPM-50 as a catechin 3-O-glucoside (Figure 2)
The compound was isolated from the methanol extract and obtained as a white crystalline solid. High-resolution liquid chromatography–mass spectrometry (HRLCMS) analysis of the molecule detected only the aglycone moiety, which appeared as an [M+H]+ ion with a mass-to-charge ratio (m/z) of 291.0682 (calculated as 291.0689, $C_{15}H_{14}O_6$). The proton spectrum of the compound indicated that two methine protons were both deshielded and oxygenated. These protons were observed with chemical shifts of 4.17 ppm (H-3) and 4.86 ppm (H-2). Two aromatic proton signals that are meta-coupled were detected at δ_H 6.24 (d, J = 2.3) and 6.06 (d, J = 2.3). These signals are characteristic of 5,7-disubstitution on ring A of flavones. Three further aromatic proton signals corresponding to an ABX substitution on a benzene ring were detected at chemical shifts of 7.03 (H-2'), 6.82 (H-6'), and 6.78 ppm (H-5'). Two aliphatic methylene signals were observed, with chemical shifts of 3.37 ppm (1H, dd, J = 11.4, 9.7 Hz) and 2.85 ppm (1H, dd, J = 9.0, 3.8 Hz). Furthermore, a glycoside anomeric proton was detected at a chemical shift of 4.85 ppm, along with additional sugar protons ranging from 3.62 to 4.13 ppm. This suggests that the compound could have a sugar component, indicating the presence of a glycoside. The ^{13}C-NMR spectrum revealed signals for 15 carbons, without the presence of a carbonyl carbon. Therefore, it may be inferred that the compound is either a flavan or flavanol. The compound had two aromatic CH groups at 95.6 (C-6) and 97.1 (C-8), as well as two oxygenated carbons at 78.5 (C-2) and 66.0 (C-3). These carbons were determined to be part of a catechin moiety connected to a sugar at C-3 (130.1). The presence of an anomeric carbon at 101.0 ppm (Glu-1) and an oxymethylene glucose carbon at 65.3 ppm (Glu-6) confirms this. The remaining carbon signals were attributed to quaternary aromatic carbons. The compound was identified as catechin-3-O-glucoside through analysis of its 2D spectra. Its structure was confirmed by cross-referencing the chemical shift assignments with literature reports.[18] Table 3.

Characterization of CPE-47 as ferulic acid (Figure 3)
The compound was isolated as white solid crystals from the CPE-47 fraction, where it was present as a minor constituent. The molecular ion peak $[M+H]^+$ of the compound had a mass-to-charge ratio (m/z) of 195.0653 (calculated as 195.0657) in positive mode HR-LCMS. This corresponds to the molecular formula $C_{10}H_{10}O_4$. The compound has a double bond equivalence of six, potentially arising from the presence of one aromatic ring and two double bonds. The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) showed two doublets that were trans-coupled. The first doublet appeared at δ_H 6.29 (1H, d, J = 15.9, H-8), while the second doublet appeared at 7.60 ppm (1H, d, J = 15.9, H-7). Three aromatic proton signals, which were coupled in an ABX pattern, were detected at chemical shifts of 7.06 ppm (1H, dd, J = 8.2 Hz and 2.0 Hz, assigned as H-6), 6.90 ppm (1H, d, J = 8.5 Hz, assigned as H-5), and 7.03 ppm (1H, d, J = 2.0 Hz, assigned as H-2). Additionally, it exhibited a methoxy signal at 3.92 (3H, s, 5-OCH_3). The ^{13}C-NMR spectrum displayed a signal at δ_C 172.0 ppm, indicating that a carboxylic acid carbonyl group (C-9) was more susceptible to external influences. Additionally, there were two olefinic CHs at 115.7 and 144.7 ppm, three aromatic CHs at 113.2, 119.5, and 124.8 ppm, two oxygenated aromatic carbons at 143.7 and 152.7 ppm, a quaternary carbon at 126.3 ppm, and a methoxy carbon at 56.0 ppm. The compound was determined to be ferulic acid, and its NMR spectral data (Table 4) matched the information found in the literature.[19]

Characterization of CPE-59 as Stigmasterol (Figure 4) and β-Sitosterol (Figure 5)
The fraction was obtained as a white, crystalline substance from the plant's ethyl acetate extract. The ^{1}H NMR spectrum of $CDCl_3$ revealed the presence of a triterpene-type molecule with a specific number of methyl protons. Closer examination revealed a mixture of sitosterol and stigmasterol. Three olefinic protons were found at δ_H (ppm) 5.36 (d, 2H, J = 5.4 Hz, H-5 for both compounds), 5.17 (dd, 1H, J = 15.2, 8.6 Hz, H-22a for stigmasterol), and 5.03 (dd, 1H, J = 15.2, 8.7 Hz, H-22b for stigmasterol), with a 2:1:1 integration ratio. The spectra also revealed an oxymethine proton at 3.54 (1H, tdd, J = 11.1, 5.3, 4.1 Hz, and H-3 for both compounds). Between 0.69 and 1.58 ppm, a series of tertiary and secondary methyl groups were identified, including two singlets at 1.01 and 0.69 traceable to Me-19 and Me-18 and four doublets at 0.93 (Me-21), 0.85 (Me-26), 0.83 (Me-29), and 0.81 (Me-27). Their ^{13}C NMR spectra revealed the expected number of carbons, with some overlapping signals. There was an oxygen-bearing carbon at δ_C 71.8 ppm (C-3) and four olefinic carbon signals at 121.7 (C-6), 129.3 (C-22), 139.3 (C-23), and 140.8 (C-5). Their spectra were identical to those described in the literature for sitosterol and stigmasterol, as confirmed by the masses measured for their $[M+H]^+$ ions using HRLCMS. Sitosterol had a mass ion at m/z = 427.3946 (calc. 426.3861, $C_{30}H_{50}O$),

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whereas stigmasterol had a mass of 424.3775 (calc. 424.3705, $C_{30}H_{48}O$).[20]

Antimicrobial assay results

The zones of inhibition for the extracts and compounds are given in Table 5.

The minimum inhibitory concentrations for the extracts and compounds are given in Table 6.

Table 3: ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for catechin-3-O-glucoside in acetone-d_6.

Position	Experimental		(Hidenori et al., 2002)	
	^{1}H δ ppm (mult, *J* Hz)	**^{13}C δ ppm**	**^{1}H δ ppm**	**^{13}C δ ppm**
1	-	-	-	-
2	4.87	79.4	4.80 (1H, s)	82.4
3	4.17 (d, 11.2)	66.8	4.17 (1H, m	68.7
4	2.87, 2.08	28.4	2.86 (1H, dd, J) 4.8 and 16.5), 2.74 (1H, dd, J) 3.0 and 16.5)	28.4
5	-	156.9		157.6
6	6.07 (d, 2.1)	96.7	5.90 (1H, d, J) 2.1 Hz	96.3
7	157.9	162.6		157.9
8	6.24 (d, 2.1)	95.9	5.93 (1H, d, J) 2.1	95.5
9	-	157.3	-	157.3
10	-	101.7	-	100.0
1’	-	131.9	-	132.2
2’	7.42 (m)	115.4	6.96 (1H, d, J) 1.5	115.3
3’	7.33 (m)	145.7	-	145.9
4’	7.32 (m)	145.5	-	145.7
5’	7.33 (m)	115.5	6.75 (1H, d, J) 8.1 Hz	116.0
6’	7.42 (m)	119.5	6.80 (1H, dd, J) 1.5 and 8.1 Hz	119.4
Glu-1	4.85	101.5	4.77	103.4
Glu-2	3.49	73.3	3.49	75.5
Glu-3	3.47	77.2	3.47	77.4
Glu-4	3.59	69.7	3.41	71.2
Glu-5	3.47	77.1	3.41	77.3
Glu-6	3.89, 3.36	65.3	3.89, 3.72	62.8

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Table 4: Chemical shift assignments for ferulic acid in acetone-d6

Position	Experimental data (δ ppm)		Literature (Sajjadi et al. 2012)	
	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
1	-	126.3 (C)	-	126.68
2	7.03 (1H, d, *J* = 2.0 Hz).	113.2 (CH)	7.09 (d, *J* = 2 Hz)	109.48
3	-	143.7 (C)	-	147.05
4	-	152.7 (C)	-	148.37
5	6.90 (1H, d, *J* = 8.5 Hz)	119.5 (CH)	6.97 (d, *J* = 8 Hz)	114.39
6	7.06 (1H, dd, *J* = 8.2, 2.0 Hz)	124.8 (CH)	7.14 (dd, *J* = 8, 2 Hz	123.57
7	7.60 ppm (1H, d, *J* = 15.9 Hz)	144.7 (CH)	7.75 (d, *J* = 15 Hz)	146.81
8	6.29 (1H, d, *J* = 15.9 Hz)	115.7 (CH)	6.34 (d, *J* = 15 Hz)	114.78
9	-	172.0 (C)	-	171.36
3-OCH_3	3.92 (3H, s)	56.0 (CH_3)	3.98 (s)	55.98

Table 5: Zone of inhibition of the extracts and isolated compounds

Microorganism	Extracts			Compounds				Control*	
	Hexane	Ethyl acetate	Methanol	CPM-24	CPM-50	CPE-47	CPE-59	CFX	FN
		ZOI in mm		Bacteria		ZOI in mm			
Staphylococcus sp	22	23	-	-	26	28	-	-	
Bacillus subtilis	26	28	25	28	-	30	25	30	
Erwinia carotovora	22	24	24	26	30	-	24	24	
Klebsiella oxytoca	23	-	25	-	-	29	25	-	
Pseudomonas aeruginosa	-	-	-	28	32	-	-	-	
Enterococcus sp	25	27	26	24	29	28	26	-	
				Fungi					
Aspergillus niger	-	-	-	22	20	25	-		30
Aspergillus flavus	20	27	25	-	22	26	25		27
Fusarium oxysporum	24	26	24	24	20	28	24		31
Rhizopus stolonifera	25	24	26	26	-	29	26		28

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Table 6: Minimum inhibition concentrations of the extracts and isolated compounds

Microorganism	Extracts			Compounds			
	Hexane	Ethyl acetate	Methanol	CPM-24	CPM-50	CPE-47	CPE-59
			Bacteria				
	MIC in mg/mL			**MIC in µg/mL**			
Staphylococcus sp	25.0	25.0	-	-	25.0	12.5	-
Bacillus subtilis	25.0	12.5	25.0	12.5	-	12.5	25.0
Erwinia carotovora	25.0	25.0	25.0	25.0	12.5	-	25.0
Klebsiella oxytoca	25.0	-	25.0		-	12.5	25.0
Pseudomonas aeruginosa	-	-	-	12.5	12.5	-	-
Enterococcus sp	25.0	25.0	25.0	25.0	12.5	12.5	-
			Fungi				
Aspergillus niger	-	-	-	25.0	25.0	25.0	-
Aspergillus flavus	25.0	25.0	25.0	-	25.0	25.0	25.0
Fusarium oxysporum	25.0	25.0	25.0	25.0	25.0	12.5	25.0
Rhizopus stolonifera	25.0	12.5	25.0	25.0	-	12.5	25.0

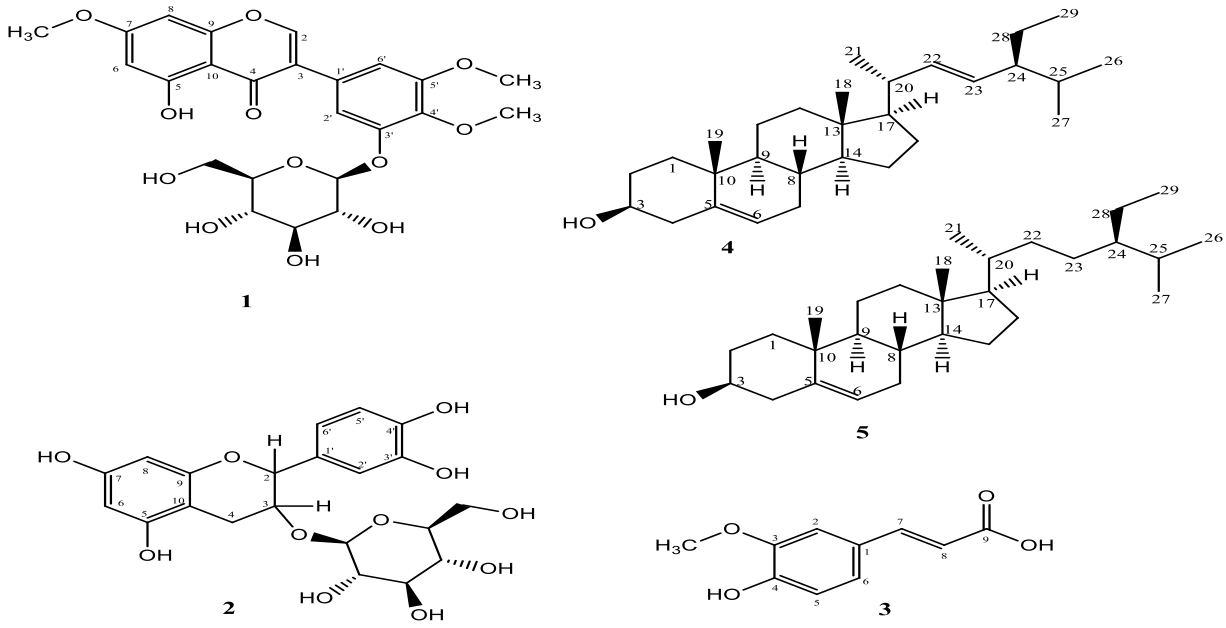

Figure 1: Isolated compounds

Discussion

All the isolated compounds were identified using NMR and mass spectral data, and the experimental results matched published reports. They were all obtained pure enough for unambiguous characterization. The aglycone vervain was not isolated in this study; hence, comparison of the glycoside and the aglycone was not carried out. This will be a good starting point for further studies. The antimicrobial activity results (Tables 5 and 6) show the efficacy of various extracts and compounds isolated from *Ceiba pentandra* in combating microbial strains that commonly lead to postharvest loss of *Dioscorea alata* tubers in Benue State, Nigeria. The largest zones of inhibition were recorded for the CPE-47 and CPM-50 compounds against *Bacillus subtilis* and *Erwinia carotovora*, respectively. The methanol extract exhibited significant antimicrobial activity against various bacteria, including *Staphylococcus* sp., *Erwinia carotovora*, and *Enterococcus* sp. The inhibitory impact ranged from moderate to strong, with minimum inhibitory concentrations (MICs) ranging from 25.0 mg/mL to 12.5 mg/mL. Fraction CPM-24 had significant efficacy against *Bacillus subtilis* and *Enterococcus* sp., demonstrating a minimum inhibitory concentration (MIC) of 12.5 µg/mL. CPE-47 (ferulic acid) exhibited a modest level of efficacy against *Bacillus subtilis* and *Erwinia carotovora*. CPE-59, a mixture of sitosterol and stigmasterol, demonstrated significant efficacy against most of the bacterial strains that were examined. The ethyl acetate extract exhibited moderate activity against *Aspergillus niger* and *Rhizopus stolonifera*, with a minimum inhibitory concentration (MIC) of 12.5 mg/mL. The compound CPM-24 demonstrated strong antifungal activity against *Aspergillus flavus* and *Fusarium oxysporum*, with a promising minimum inhibitory concentration (MIC) of 25.0 µg/mL. In addition, CPE-47 exhibited potent inhibitory activity against most fungal strains examined, with minimum inhibitory concentration (MIC) values ranging from 12.5 µg/mL to 25.0 µg/mL. The compounds CPM-24 (varvain-3'-O-beta-d-glucoside) and CPE-47 (ferulic acid) had notable antimicrobial activity against the bacteria and fungi that cause tuber rot in *Dioscorea alata*. These compounds had low minimum inhibitory concentrations (MICs), indicating strong inhibitory effects on the development of harmful microorganisms. The antimicrobial effect of these compounds is likely due to multiple processes, including the rupture of cell membranes, which causes the release of important cellular components, eventual cell lysis, and the inhibition of microbial growth. Disruption of metabolic processes vital for survival and replication can occur by interfering with key enzymes in bacteria. Ferulic acid hinders specific enzyme pathways that are crucial for the growth of microorganisms.[21] Varvain-3'-O-beta-d-glucoside has the potential to disrupt the synthesis of nucleic acids in microorganisms, which can impede the replication of DNA or the transcription of RNA, ultimately inhibiting the growth of microorganisms.[21] The results indicate that the extracts and compounds obtained from *Ceiba pentandra*, specifically CPM-24 (varvain-3'-O-beta-d-glucoside) and CPE-47 (ferulic acid), show promise as natural antimicrobial agents against pathogenic bacteria and fungi that cause tuber rot in *Dioscorea alata*. The comparatively low minimum inhibitory concentrations (MICs) demonstrate the effectiveness of these chemicals in suppressing the growth of harmful microorganisms that are frequently linked to postharvest losses. This could have significant implications for agricultural practices and food security in the region.

Conclusion

The extracts from *Ceiba pentandra* were subjected to phytochemical investigation, resulting in the identification of varvain-3'-O-β-d-glucoside, ferulic acid, catechin-3-O-glucoside, and a combination of sitosterol and stigmasterol. The compounds Varvain-3'-O-beta-d-glucoside and ferulic acid exhibited notable antibacterial effects on the bacteria and fungi that cause tuber rot in *Dioscorea alata*. These compounds had low minimum inhibitory concentrations (MICs), suggesting strong inhibitory effects on the proliferation of pathogenic microorganisms. The findings of this study suggest that both the extracts and compounds have potential use in the production of novel antimicrobial agents. These findings could have substantial implications for the use of more eco-friendly agents for disease control in crops if followed up.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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