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Isolation, Characterization and Antitrypanosomal Evaluation of a Proposed Pentaglycosylated-7,4'-Dimethoxy Kaempferol Derivative from Ethanol Extract of *Vernonia Perrottettii* Promising Against *Trypanosoma* Parasite.

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Abstract

Human African Trypanosomiasis (HAT) remains a significant public health challenge despite considerable progress in disease control and management. The continued emergence of drug-resistant *Trypanosoma* strains, coupled with the severe adverse effects associated with many existing chemotherapeutic agents, has limited the long-term effectiveness of current treatment strategies. Resistance mechanisms have been linked to mutations in P2 and AQP2 transporters, deletion or modification of genes encoding these transport proteins, and, in some cases, enhanced drug efflux mechanisms. Consequently, the search for novel, safe, and effective antitrypanosomal agents from natural sources remains an important area of research. This study investigated the phytochemical composition and trypanocidal potential of *Vernonia perrottettii* leaf extract, a medicinal plant traditionally employed in Northern Nigeria for the treatment of HAT. Fresh leaves were collected, authenticated, air-dried, pulverized, and extracted by ethanol maceration. Qualitative phytochemical screening of the crude extract was conducted using standard analytical procedures. The results revealed the presence of alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, and cardiac glycosides. Bioactive constituents were isolated through silica gel (200 mesh) column chromatography, leading to the purification of a compound structurally characterized and predicted as a pentaglycosylated-7,4'-dimethoxy kaempferol derivative. The isolated compound was subsequently evaluated for in vitro trypanocidal activity using the Nigerian Institute for Trypanosomiasis Research (NITR) assay protocol. The compound exhibited significant antitrypanosomal activity with an IC_{50} value of 0.335 µM, demonstrating potency comparable to the reference drug, Diminazene diaceturate (IC_{50} = 0.24 µM). These findings highlight the therapeutic potential of the isolated flavonoid derivative as a promising lead compound for HAT drug development and warrant further investigation through in vivo efficacy, toxicity, and cytotoxicity studies.

Keywords

Human African trypanomiasis, Phytochemical screening, Chromatographed, Structurally elucidated, Biological activity.

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INTRODUCTION

The emergence of unique phytochemicals on global therapeutic stage can never be over emphasized because; herbal remedies in health management are as old as ethno medicines in various regions of the world (Yadav, 2025). A Neglected Tropical Disease (NTD) of interest is the Human African Trypanosomiasis (HAT), this disease constitutes a menace which several countries have fought to a standstill and are as a result recognized as HAT free by the World Health Organization (Barrett *et al.*, 2024). *Trypanosoma brucei* protozoan parasites are responsible for the HAT disease also known as sleeping sickness, it is transmitted by the bite of an infected Tsetse fly *Glossima spp.*; the two forms of the disease in Africa are caused by two subspecies known as *Trypanosoma brucei rhodesiense and Trypanosoma brucei gambiense* (Busse and Muller, 2009).

The approved drugs for the treatment of this disease depends on the type of *Trypanosoma* species causing the infection and the stage of infection.These drugs include Melarsoprol, Suramin, Pentamidine, Fexinidazole, and Nifurtimox eflornithine combination therapy (Lindner *et al.*, 2020) and (Setzer *et al.*, 2012). Currently there is no approved vaccine for the disease and all the clinical available chemotherapeutic drugs have been noted to be either ineffective with time due to resistance or characterized by severe side effects (Lindner et al., 2020).

In the rural African settings where recommended drugs are inaccessible medicinal plants have been recommended by traditionalists for the treatment of such infections, and a few have experimentally produced a good protozoan activity result when tested for potency (Setzer *et al.*, 2012). The current search for the acclaimed trypanosomal remedy in *Vernonia perrottettii* considering its traditional use in northern Nigeria presents an additional pool of documented research added to the limited literatures with respect to the plant's trypanosomal activity and phytochemicals.

Presently, related *Vernonia* plant genus like *Vernonia lasiopus* with documented reports of isolated Sesquiterpene lactones including vernolepin, vernomenin, vernodalin, 8-desacylvernolide and 11, 13-dihydrovernodalin has shown an *in-vitro* anti-trypanocidal activity (Kimani *et al.*, 2017). In view of the presence of Sesquiterpene lactones also isolated from *Vernonia perrottettii* (Aliyu, 2017) this research is expected to be promising with good chances of having a trypanocidal active component.

MATERIALS AND METHODS

Analytical grade solvents and chemicals such as absolute Ethanol (98%), Methanol (99.5%), Chloroform (99.8), Diethyl ether (99%), Acetic acid (99%), Ethyl acetate (99.5), n-Hexane (99%), Acetone (99.8%), But-1-ol (99%), Acetonitrile (99.5), Ammonia, Sodium hydroxide (99.8%) and Hydrogen chloride were obtained from Sigma-Aldrich Chemicals and used without further purification.

Other instruments used include: pHep pH-tester (HANNA INSTRUMENT), C-Parmer RV-200 rotary evaporator, Analtech UV cabinet 254nm shortwave and 366nm long wave, E200-LED Trinocular compound microscope, optical absorption spectrum device Carry630 FT-IR spectrometer by Agilent Technologies and Bruker Avance III HD 500MHz NMR Spectrometer (Germany).

Plant's Authentication Preparation and Extraction

Vernonia perrottetii plant samples were collected at random from Unguwar Maigero in Chikun Local government area of Kaduna State Nigeria on latitude 10.39N and longitude 7.27E, the plant was authenticated at the Biological Science Department Kaduna State University herbarium with voucher number KASU/BSH/156 deposited. The plant leaves were collected and washed with distilled water then dried under shade. The dried leaves were pulverized into powder and thereafter a portion (500g) of the dried leaves was macerated in a maceration jar using 1.5dm^3 of ethanol at room temperature for seven days (Bitwell *et al.*, 2023). The macerated sample was thereafter filtered using Whatmann paper no.1 and the filtrate was concentrated *in vacuo* to a small volume and allowed to dry on water bath to afford the crude extract.

Preliminary Phytochemical Screening

The ethanol crude extract was phytochemically screened for the presence of alkaloids, flavonoids, terpenoids, phenols and tannins as well as Saponins and cardiac glycosides using standard procedures by Trease and Evans, 2009: Pharmacognosy methods.

Fractionation of Ethanol Extract

The residue (Marc) obtained from the ethanol extraction above was further re-macerated in 1dm^3 of 0.01mol/dm^3 H_2SO_4 for 2 days (48hrs) then filtered using Whatmann filter paper and thereafter concentrated in a rotary evaporator to a volume of 300ml. The acidic concentrated solution was neutralized to a pH of 7 using 15% ammonia solution and furthermore, adjusting the pH to alkaline by adding more of the 15% ammonia solution until the pH meter reads 9. The alkaline solution was partitioned with chloroform in a separatory funnel and the two fractions collected separately, thereafter each fraction was concentrated in a rotary evaporator and allowed to dry then collecting the crude solid crystal. The crude component collected from the alkaline fraction was designated as crude sample D and subsequently used for Thin Layer Chromatography (TLC) and Column Chromatography.

TLC and Column Chromatographic Isolation of Crude Sample D

Thin layer chromatography was carried out on crude sample D crystal using standard commercially available aluminum sheet coated with silica gel adsorbent mesh 60F254 (Merck) in line with TLC analysis of plant material by Zych and Pyka-paja, 2025. An isocratic solvent system combination consisting of ethanol, acetone, n-hexane, methanol and ethyl acetate in the ratio 3:1.5:1.5:1:1 respectively was used for developing the resulting chromatogram which was examined under UV light at 366nm.

The TLC experiment formed the basis for the column chromatography the silica gel mesh size 70-230 was activated in an oven at 100°C then using the TLC solvent mixture combination of ethanol, acetone, n-hexane, methanol and ethylacetate in the ratio 3:1.5:1.5:1:1 respectively, a slurry of the cool activated silica gel was poured into a 2Ltr capacity column as stationary phase. The column chromatographic procedure was done according to Abubakar and Muhammad, 2017 in 5,6-dehydrokawain from Rhizome of *Alpinia mutica Roxb*. The loading was 3% by mass of crude separating sample (Analyte) to total mass of silica gel used as stationary phase; while the flow rate was adjusted to 0.5ml/min.

The resulting compound after the column chromatographic process was labeled isolated sample D, which was subjected to spectroscopic characterization and *in vitro* trypanocidal test.

***In vitro* Anti-trypanosomal Activity Procedure.**

This experiment was conducted at the Nigerian Institute for Trypanosomiasis Research (NITR) Kaduna with ethical approval reference number S/3714/TR/2025/0006 Trypanosoma brucei brucei was obtained from NITR and inoculated intraperitoneally into a mouse serving as parasite donor animal, the mice were properly monitored for 7 days to ascertain the presence of trypanosomes. The animal was sacrificed (according to the animal management guidelines of the Nigerian Institute for Trypanosomiasis Research) to get the infected blood sample which was collected in an EDTA bottle.

The blood was diluted with RPMI-1640 cell culture medium (HEPES, L-Gulatmine, D-glucose and $NaHCO_3$) having 2% DMSO as described by Olanrewaju *et al.*, 2019: Anti-trypanosomal evaluation of *Ximenia americana* root bark; this was slightly modified to have a minimum of approximately 3.1×10^7 parasites/ml (averagely 8 trypanosomes per field). The parasite count was estimated using the rapid matching method according to Herbert and Lumsden, 1976: A rapid "matching" method for estimating the host trypanosoma brucei parasitaemia.

50mg of the isolated compound was dissolved in 100µL (0,1ml) of dimethyl-sulfoxide (DMSO) and was made up with 4900µL (4.9ml) RPMI-1640 cell culture medium; the resulting 10mg/mL concentration of prepared compound was diluted serially to obtain 5mg/mL, 2.5mg/mL, 1.25mg/mL and 0.625mg/mL. Fifty microliters (50µL) of each prepared concentration were measured separately in duplicate and transferred into a 96-well plate, then out of the earlier prepared infected blood sample having Trypanosoma brucei brucei 35µL was added into each of the 96-well plate already having 50µL of prepared compound hence making a total volume of 85µL in each well; which was incubated in 5% CO_2 at 37°C.

Diminazene diaceturate (Trade name: Seminazene; Hebei Kexing Pharmaceutical Co. Ltd. China) was used as a positive standard drug control while 2% DMSO in RPMI-1640 medium was used as negative control since 2% DMSO was used all through for other sample preparations above. Microscopic examination was carried out after 1hr and 4hrs of incubation using a microscope magnification of ×40. The experiment was conducted in duplicate pattern to reduce error; and percentage (%) inhibition calculated as:

$$\frac{Total\ No.\ of\ parasite\ count\ in\ negative\ control - Total\ No.\ of\ parasite\ count\ in\ tested\ compound}{Total\ No.\ of\ parasite\ count\ in\ negative\ control} \times 100$$

RESULTS AND DISCUSSION

Table 3.0: Physical properties of the extracts and isolated compound

Sample type	Yield (%)	Colour	Nature (Texture)
Ethanol extract	4%	Dark green	Sticky-solid
Isolated sample	2.9%	Chocolaty-orange	Pasty-solid

About 500g *Vernonia perrottettii* yielded 20g of dark green sticky-solid ethanolic crude extract as shown in table 3.0, while the column chromatographic separation process of crude sample D crystal that was fractionated and modified to a pH of 9 from the ethanol extract as described above gave 0.58g (2.9%) of chocolaty-orange colored pasty-solid sample.

The preliminary phytochemical screening of the ethanol crude extract revealed the presence of alkaloids, flavonoids, terpernoids, phenols, tannins, saponin and cardiac glycosides. This qualitative phytochemical investigation revealed a rich broad spectrum of phyto classes consistent with reports from other *Vernonia* species especially *Vernonia amygdalina* and *Vernonia lasiopus* which are been reported as rich source of sesquiterpene lactones and flavonoids (Erasto *et al.*, 2011).

The confirmation of these secondary metabolites could support the ethno medicinal relevance of *Vernonia perrottettii* used by local herbalist in the management of uncomplicated fever. The column chromatographic separation of crude sample D crystal as described above produced a dark chocolaty-orange colored sample that is a pasty-solid compound in nature as shown in Table 3.0 above. The TLC of this isolated compound showed an R_f value of 0.48; the sample was designated as isolated compound (sample) D for spectroscopic characterization and in vitro trypanocidal activity investigation.

FT-IR Analysis of Isolated Compound (Sample) D

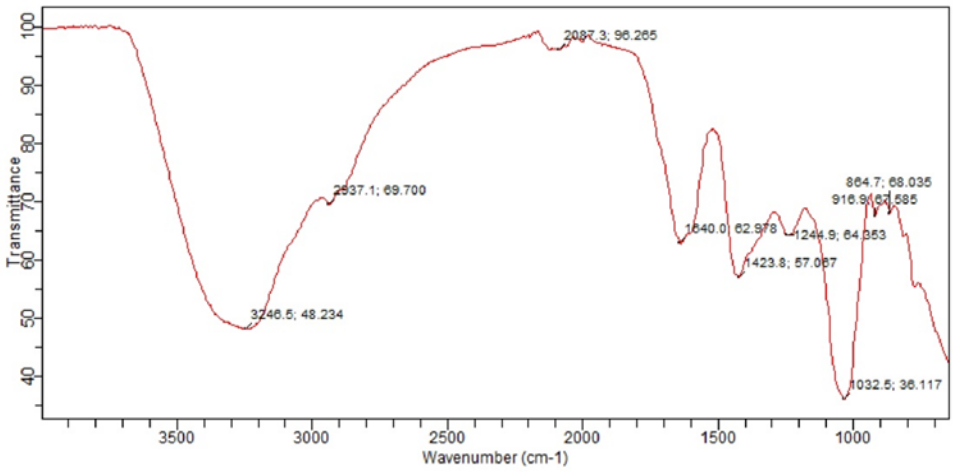


Figure 1: FT-IR spectra for isolated compound (sample) D

The Fourier Transform Infrared (FT-IR) spectra in figure 1 showed the significant characteristic bond types showing various functional groups present in the molecule as inferred and compared with standard reference values by Dudley and Ian, 2006 (Spectroscopic methods in organic chemistry). All major characteristic bands observed were inferred and tabulated as shown in Table 3.1 below.

Table 3.1: FT-IR characteristic bands for isolated compound (Sample D)

Bands (cm^{-1})	**Bonds**	**Characteristics Inference**
3246.5	O-H (broad)	Hydroxyl absorption
2937.1	C-H	Alkane stretch absorption
1640.0	C=C-C=C	Conjugated double bond absorption
1244.9	C-O	Carbon-oxygen stretch absorption

Inference: Dudley and Ian (2006)

The major characteristic bands observed in the FT-IR spectrum (Fig.1.0) includes, an alkane $(C\text{-}H)_{str}$ absorption stretch at 2937.1cm^{-1}, a broad hydroxyl $(O\text{-}H)_{str}$ absorption stretch at 3246.59cm^{-1}, and a conjugated carbon double bond $(C=C\text{-}C=C)_{str}$ absorption stretch at 1640.09cm^{-1} also prominent within the fingerprint region is a carbon–oxygen bond $(C\text{-}O)_{str}$ absorption stretch at 1244.9cm^{-1}. The observed bond types were reaffirmed by literature reference in Field *et al.*, 2007 (Organic structures from spectra), these characteristic bond types are expected to define the molecular structure of the isolated compound when further elucidated on NMR spectroscopy.

NMR Analysis of Isolated Compound (Sample) D

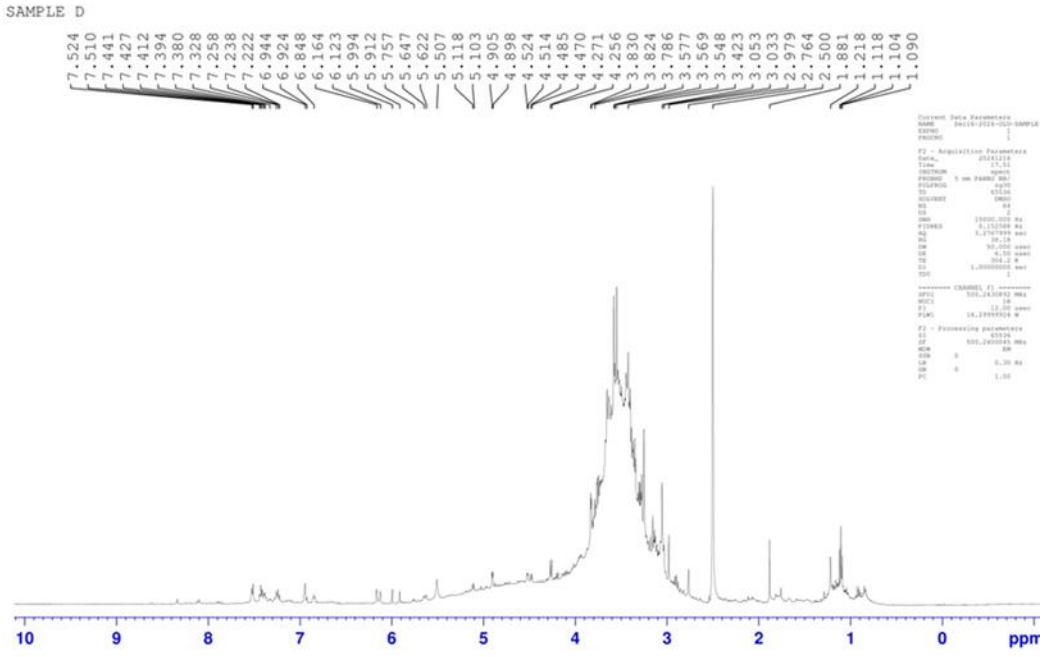


Figure 2: Proton NMR for isolated compound (sample) D

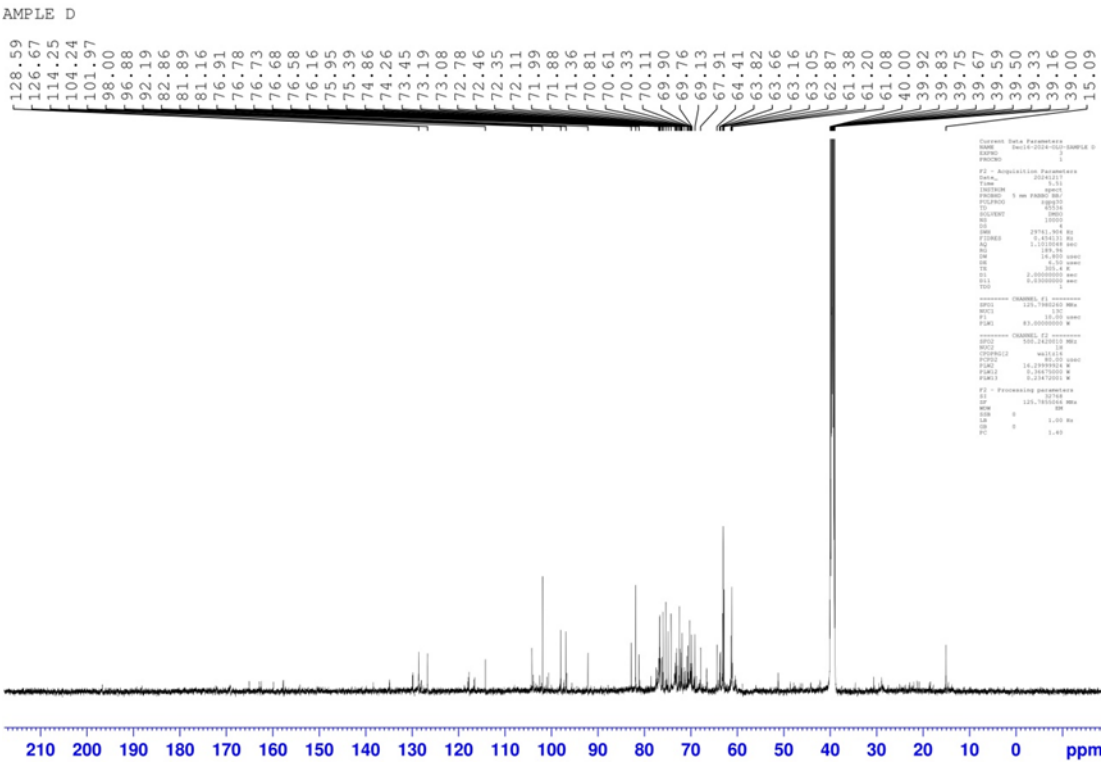


Figure 3: ^{13}C NMR Spectrum for isolated compound (sample D)

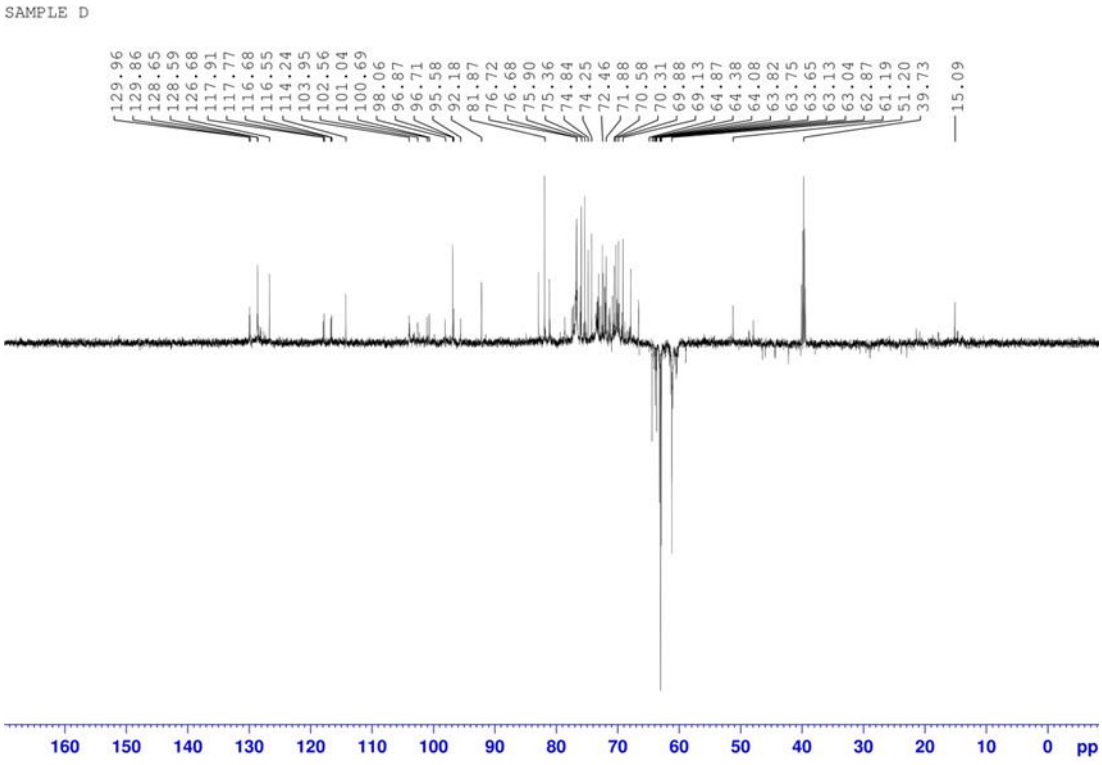


Figure 4: DEPT Spectrum for isolated compound (sample D)

The NMR spectroscopy of the isolated compound was carried out using Bruker Avance III HD 500MHz spectrometer (Germany). Discreet analysis of 1D ^{13}C-NMR spectrum in Figure 3 and DEPT 135 spectrum in Figure 4 revealed significant chemical shifts observed for the different categories of carbons in the molecular structure of the compound as follows: ^{13}C-NMR (DMSO - d_6, 126 MHz) δ, 173.0, 165.12, 162.92, 158.51, 157.85, 157.73, 134.91, 129.95, 129.85, 118.75, 116.55, 104.24, 100.7, 98.00, 96.88, 92.19, 81.16, 77.44, 76.91, 76.78, 76.68, 76.04, 74.86, 74.26, 72.78, 72.11, 71.99, 71.88, 71.50, 71.36, 70.81, 69.88, 69.76, 66.60, 63.82, 62.87, 61.20, 61.08, 55.52, 51.20, 42.20, 24.50 and 15.08, with all units in ppm.

Similarly, further analysis of the 1D proton spectrum in Figure 2 supported by corresponding correlations on 2D H-H COSY in Figure 5 revealed the chemical shifts for different categories of protons and their calculated coupling constants (J) as follows: ^{1}H-NMR (DMSO - d_6, 500MHz) δ, 7.412 d, J=7.5Hz, 6.944d, J=8.5Hz, 6.164 d, J=2Hz, 6.123 d, J=2Hz, 5.111 d, J=7.5Hz, 4.902d, J=3Hz, 4.517d, J=5Hz, 4.480d, J=7.5Hz, 4.280d, J=7.5Hz, 3.832m, 3.830m, 3.824, 3.786, 3.577, 3.500, 3.450, 3.423, 3.053, 3.033, 2.979, 1.218, 1.104t, J=7Hz, and 7Hz; all peak units in ppm.

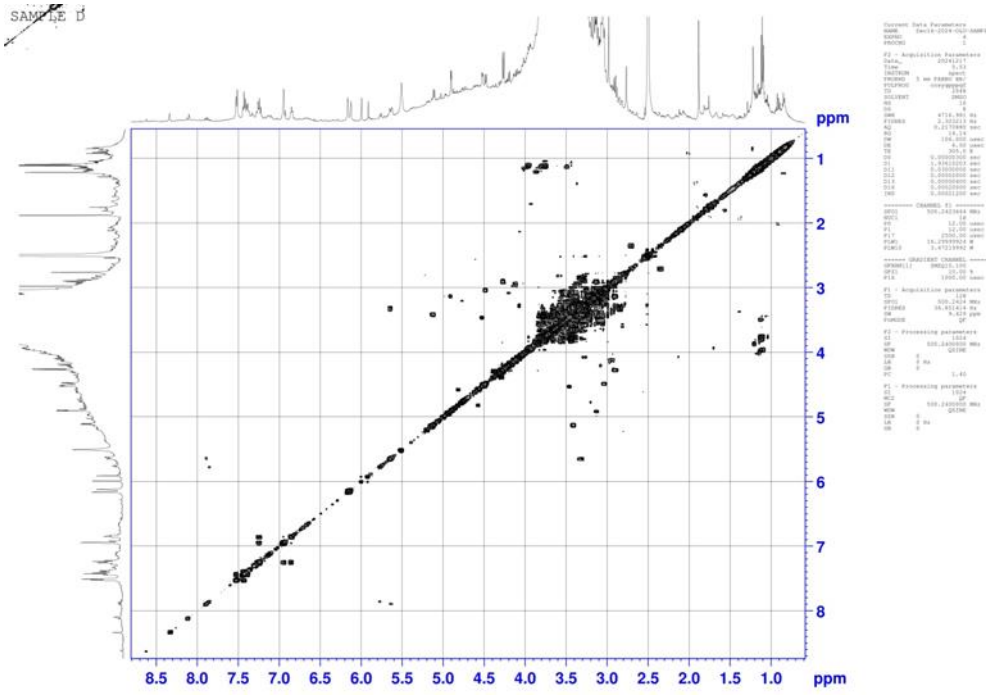


Fig 5: H-H COSY Spectrum for isolated compound (sample D)

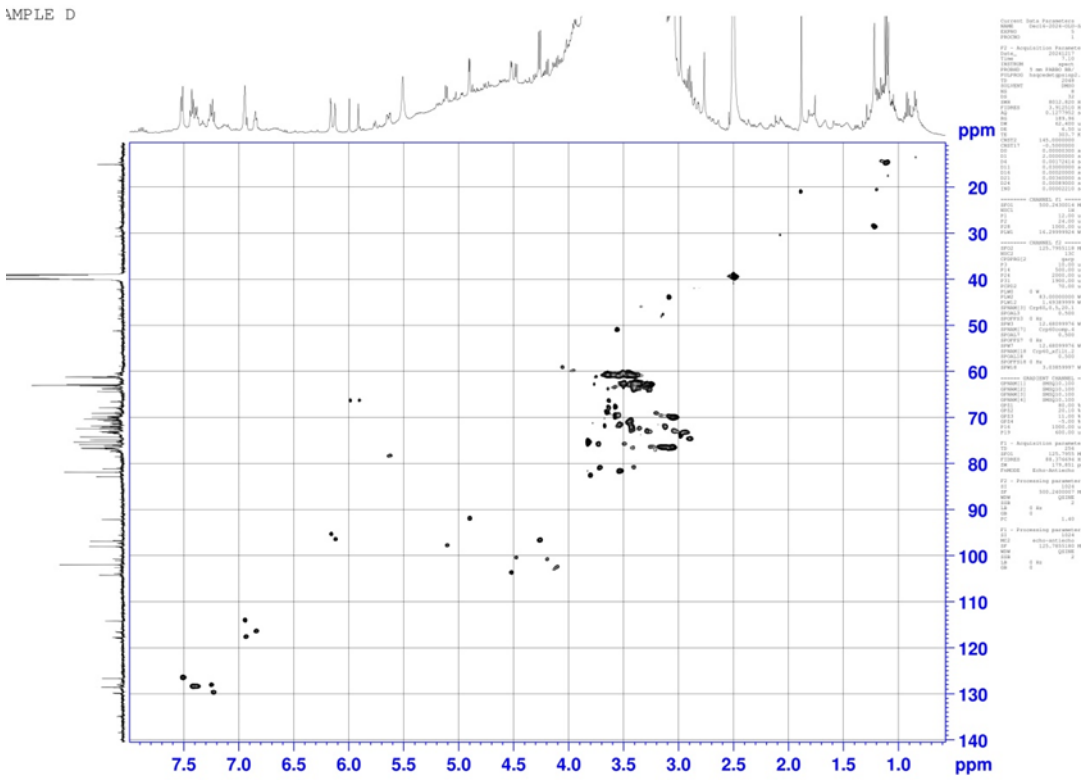


Fig 6: HSQC Spectrum for isolated compound (sample) D

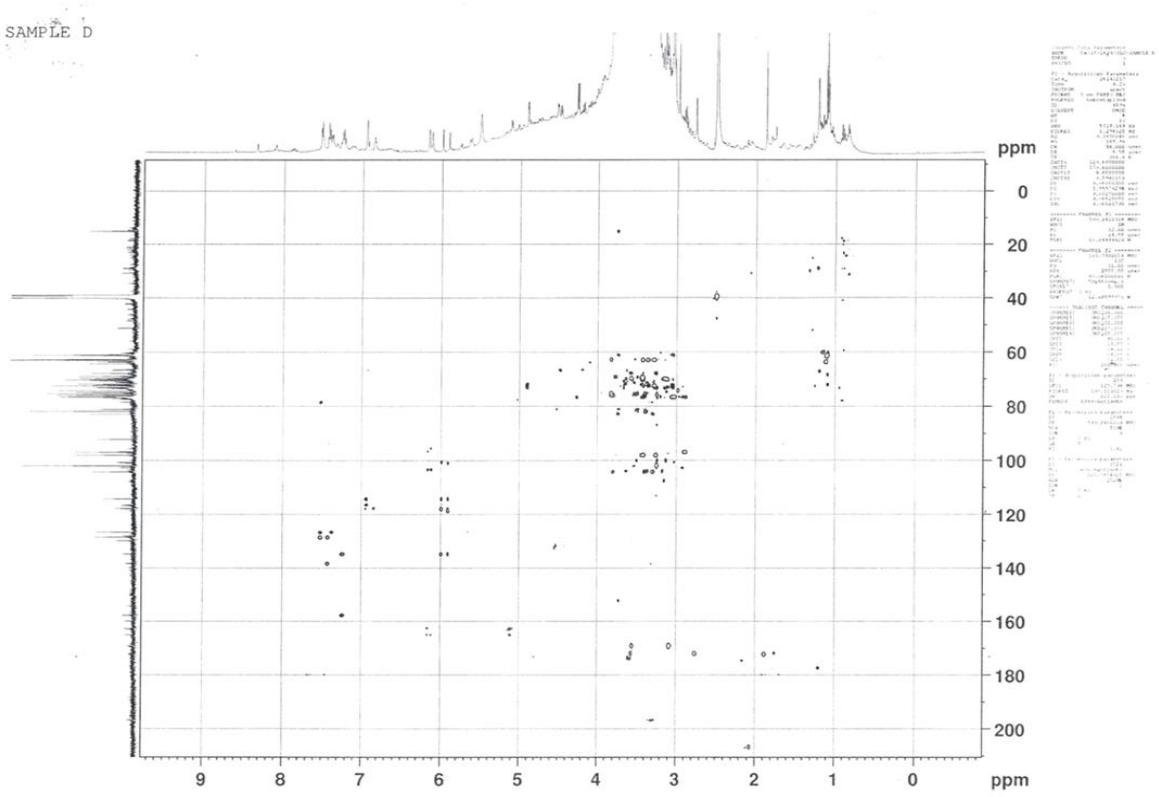


Fig 7: HMBC Spectrum for isolated compound (sample D)

Five prominent proton peaks at 5.111ppm, 4.902ppm, 4.517ppm, 4.480ppm and 4.280ppm, within the range of anomeric protons (Battistel *et al.*, 2015) were on HSQC in Figure 6 directly bonded to carbons at 98.00ppm, 92.19ppm, 104.23ppm, 101.97ppm and 96.71ppm respectively: these carbons being highly deshielded than expected for the normal sp^3 carbon peaks, when studied further with relevant spectra correlations on H-H COSY (Figure 5), HMBC (Figure 7) as well as their coupling constants (J), the anomeric properties of five observed glycone components as shown in Table 3.2 were revealed.

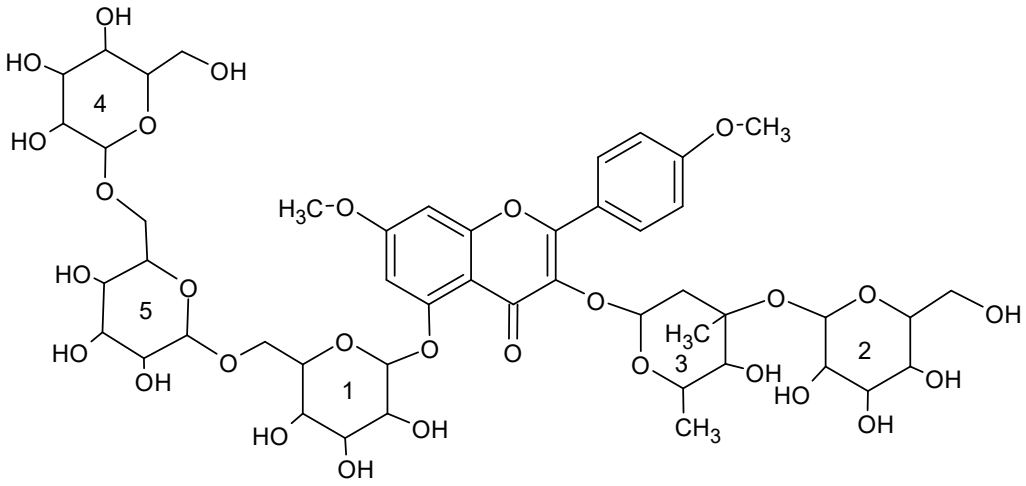
Table 3.2: Analysis of Anomeric Carbons and Protons in Compound (Sample) D

^{13}C-NMR$_{(ppm)}$	^{1}H-NMR$_{(ppm)}$	**Coupling Constant**
98.00	5.111	7.5H_z
92.19	4.902	3.5H_z
104.24	4.517	5.0H_z
101.97	4.480	7.5H_z
96.71	4.280	7.5H_z

A critical study of the high number of oxygenated carbon peaks between 61ppm to 82ppm in Figure 3 with their corresponding proton peaks between 3.02ppm to 3.80ppm in Figure 2 showed significant overlap of proton peaks within this range leading to obvious densely concentrated signal peaks especially in the H-H COSY (Figure 5) and HMBC (Figure 7) spectra hence supporting the presence of a number of cyclic glycone (sugar) moieties (Davis and Prestegard, 2023) and (Ohadoma *et al.*, 2016).

Two of these anomeric carbon peaks were further observed to be linked via a glycosidic bond to the aglycone core (98.00ppm and 104.23ppm) while others were bonded to a sister sugar ring moiety (92.19, 101.97and 96.71ppm) as shown on the HMBC spectrum in Figure 7. A careful analysis of the highly deshielded downfield peaks beyond the anomeric carbon and proton regions on the 1D and 2D spectra shows a good correlation with a documented literature on Isolation and characterization of flavonol Glycosides from leaves extract of *Lupinus arboreus* (Agrawal, 1992) consistent for a flavonone aglycone.

Further analysis of proton peaks showed prominent characteristic doublet (J=2Hz) peak at 6.164ppm and another similar doublet peak at 6.123ppm correlating on HSQC in Figure 6 with carbon peaks at 96.88ppm and 95.58ppm respectively: these peaks are characteristic features reflecting carbons at position C(6) and C(8) respectively on a normal flavonone moiety (Charisiadis *et al.*, 2014). The assignments of peaks for the flavonone aglycone and various glycone (sugar pyranosyls) with respect to 1D (^{1}H-NMR, ^{13}C-NMR and DEPT) and 2D (HMBC, HSQC and H-H COSY) spectra, corresponding with various studied literature references were carefully represented in Table 3.3. The analysis and assignment of different categories of carbons and protons led to the structural prediction of a compound seen as 3 - O - (α -mycarosy l - (1-3) - α - glucopyranosyl) -5 - O – { β - glucopyranosyl - (1-6) , (1-6) - β - diglucopyranosyl } - 7, 4' dimethoxy kaempferol with molecular structure as shown below.



3-*O*-(α-Mycarosyl-(1-3)-α-glucopyranosyl)-5-*O*-{β-glucopyranosyl-(1-6), (1-6)-β-diglucopyranosyl}7,4'dimethoxy kaempferol

Table 3.3: NMR spectra assignment analysis for 3-*O*-(α-Mycarosyl-(1-3)-α-glucopyranosyl)-5-*O*-{β-glucopyranosyl-(1-6),(1-6)-β-diglucopyranosyl}7,4'dimethoxy kaempferol.

Position	^{13}C- NMR$_{(ppm)}$	Literature Values (ppm)	^{1}H-NMR (ppm)	H-HCOSY	HMBC
***C*2**	157.73	157.0	-	-	-
***C*3**	134.9	134.5	-	-	-
***C*4**	173.0	178.0	-	-	-
***C*4a**	104.24	105.8	-	-	-
***C*5**	162.92	160.9	-	-	-
***C*6**	96.88	99.4	6·164 d, J=2H$_z$	-	*C*(5), *C*(7)
***C*7**	165.12	161.7	-	-	-
***C*8**	95.58	94.6	6·123 d, J=2H$_z$	-	*C*(7), *C*(5)
***C*8a**	157.85	156.1	-	-	-
***C*1'**	118.75	120.7	-	-	-
***C*2'**	129.95	130.7	7·412 d,J=7.5H$_z$	H-*C*(3')	*C*(6'), *C*(4')
***C*3'**	116.55	115.4	6·944 d, J=8H$_z$	H-*C*(2')	*C*(1')
***C*4'**	158.51	160.0	-	-	-
***C*5'**	116.55	115.4	6·848 d, J=7H$_z$	H-*C*(6')	*C*(1')
***C*6'**	129.85	130.7	7·412 d,J=7.5H$_z$	H-*C*(5')	*C*(2'), *C*(4')
4-*OC*H$_3$	51.20	-	-	-	*C*(4')
7-*OC*H$_3$	55.52	-	-	-	*C*(7)

β-Glucopyranosyl (1); Refeference: Pomin-Vitor, (2012)

Position	^{13}C- NMR	Literature Values (ppm)	^{1}H-NMR (ppm)
1 Cgp	98.00	96.0	5.111 d 7.5Hz
2 Cgp	74.86	74.1	3.423
3 Cgp	76.91	75.8	3.5 – 3.7*
4 Cgp	71.88	71.2	3.5 – 3.8*
5 Cgp	69.88	69.8	3.5 – 3.7*
6 Cgp	61.20	61.3	3.824m
α-Glucopyranosyl (2); Reference: Pomin-Vitor, (2012)			
1'Cgp	92.19	91.4	4.902 d 3.5Hz
2'Cgp	71.99	71.8	3.450
3'Cgp	72.78	72.4	3.6 – 3.85*
4'Cgp	71.36	71.2	3.6 – 3.85*
5'Cgp	69.76	69.2	3.577
6'Cgp	61.08	60.3	3.830m
α-Mycarosyl (3); Reference: Bruheim *et al.*, 2004			
1'Cm	**104.24**	**102.4**	**4.51 d, J=5Hz**
2'Cm	42.20	41.50	2.979/3.053
3'Cm	71.50	70.03	-
4'Cm	76.04	78.01	3.033
5'Cm	66.60	67.09	3.505
6'Cm	15.09	17.30	1.104 t, J=7 Hz, 7Hz
7'Cm	24.50	25.21	1.218s
β - Glucopyranosyl (4); Reference: Richter and Berger, 2013.			
1'Cgp	**100.70**	**98.5**	**4.480 d, J=7.5Hz**
2'Cgp	81.16	79.0	3.40
3'Cgp	77.44	78.1	3.50 – 3.7*
4'Cgp	76.68	76.7	3.50 – 3.7*
5'Cgp	72.11	71.8	3.50 – 3.7*
6'Cgp	63.82	63.2	3.832m
β - Glucopyranosyl (5) Reference: Pomin-Vitor, (2012)			
1'Cgp	**96.88**	**95.90**	**4.30 d, 7.5Hz**
2'Cgp	74.26	74.1	3.053
3'Cgp	76.78	75.8	3.3 – 3.5*
4'Cgp	70.81	71.2	3.4 – 3.7*
5'Cgp	69.76	69.8	3.4 – 3.6*
6'Cgp	62.87	61.3	3.824m

Keys: Overlapping peaks = (*); Glucopyranosyl = gp; Mycarosyl = m and Carbon = C

The absence of hydroxyl peaks for carbon at positions 3,5,7 and 4' as expected in a normal Kaempferol molecule within the range of 9ppm to 12ppm and sometimes beyond (Agrawal, 1992) confirms the substitution of all hydroxyl protons on the Kaempferol aglycone. The five anomeric proton peaks at 5.111ppm, 4.902ppm, 4.517ppm, 4.480ppm and 4.280ppm, were observed on HMBC in Figure 7 to have a long bond coupling relationship with carbon peaks at 162.10ppm, 72.4ppm, 134.10ppm, 63.82ppm and 62.87ppm respectively.

This informed the basis for the glycosidic linkages to the aglycone and other sugar pyranosyl moieties as represented by the predicted structure above. The configuration of the sugar glycones were confirmed in view of their coupling constants (Davis and Prestegard, 2023) hence identifying 5.111ppm, 4.480ppm and 4.280ppm as beta (β) while 4.902 and 4.517 were identified as alpha (α). Furthermore, it is important to state that the triplet methyl proton peak at 1.104, (J=7Hz, 7Hz) being adjacent to the proton at 3.505ppm on carbon 5Cm as shown on H H-COSY (Figure 5) is a characteristic identity of the mycarosyl sugar ring moiety (Hamano *et al.*, 1990). Further analysis of the mycarosyl moiety revealed the presence of methylene carbon peak at 42.20ppm (2Cm) with diastereotopic proton peaks (2.979/3.053) and a methyl carbon peak at 24.50 (7Cm) with a corresponding singlet proton peak at 1.218ppm (Bruheim *et al.*, 2004), the characterized mycarosyl corresponds with studied literature values in "Chemical diversity of polyene macrolides produced by strepmyces noursei" (Bruheim *et al.*, 2004),

In vitro Antitrypanosomal Activity of Compound (Sample) D

The research was conducted at the Nigerian Institute for Trypanosomiasis Research (NITR), in accordance with the institutes' animal management guidelines. The experiment was done in duplicate with Diminazene diaceturate as the reference standard drug (Hebei Kexing Pharmaceutical Co. Ltd. China). The results are as shown in Table 4.1 and 4.2 represents the average parasitamiae, percentage inhibition and percentage viability evaluated from the *in vitro* antitrypanocidal experiment conducted for various tested concentrations of compound and drug.

The half Inhibitory Concentration (IC_{50}) was calculated using Prism Graphpad software version 10, using the nonlinear regression the IC_{50} was calculated in mg/ml and subsequently converted to micro molar (µM) using the molecular mass of the isolated compound.

Table 4.1: Antitrypanosomal Activity Result of Compound (Sample) D

IC_{50} = 0.335μM

Results after 1hr			
Concentration(mg/ml)	Average Parasite Count	% Inhibition	% Viability
0.625	23.0	33.3	66.7
1.250	19.3	44.1	55.9
2.500	10.5	69.7	30.3
5.000	10.3	70.1	29.9
10.000	9.8	71.6	28.4
Results after 4hrs			
0.625	0.5	96.6	3.4
1.250	0.2	98.6	1.4
2.500	0.0	0.0	0.0
5.000	0.0	0.0	0.0

Table 4.2: Antitrypanosomal activity of Diminazene diaceturate (positive control)

IC_{50} = 0.24μM

Results after 1hr			
Concentration(mg/ml)	Average Parasite Count	% Inhibition	% Viability
0.625	3.9	88.5	11.5
1.250	1.1	96.9	3.1
2.500	0.5	98.5	1.5
5.000	0.0	100	0.0
10.000	0.0	100	0.0
Results after 4hrs			
0.625	1.6	95.3	4.7
1.250	0.5	100	0.0
2.500	0.0	100	0.0

Diminazene diaceturate the positive reference standard drug produced an active IC_{50} of 0.24µM killing more than 85% at the lowest tested concentration confirming its clinical use and standing as a benchmark for evaluating the trypanosomal efficacy of the tested compound.

The isolated compound (Sample) D revealed a potent antitrypanosomal activity with an IC_{50} of 0.335µM being more active at higher concentrations, this result was obtained simultaneously with both the positive control and tested compound under the same experimental conditions. Generally, both tables in Figure 4.1 and 4.2 were concentration dependent for the positive control and the evaluated compound, this evaluated compound proved active with a small IC_{50} difference of 0.085µM when compared with the reference standard drug.

The activity of this compound is not surprising in view of its flavonoid core because they have been proven to exhibit a very wide range of antytrypanosomal activity on the basis of structure activity relationship for individual compounds (Tasdemir *et al.*, 2006). The flavonoids wide spectrum of antitrypanosomal activity ranges from highly active nano molar inhibitors with $IC_{50} \cong 0.068\mu M$ to moderately potent low micro molar compounds with $IC_{50} \approx 1\text{-}10\mu M$ as well as weaker flavonoid compounds with IC_{50} values above 10µM. This variation has been proven in an extensive screening study of flavonoids libraries and related analogues (Tasdemir *et al.*, 2006) and (Schmidt *et al.*, 2009).

Although on the basis of the rule of structure activity relationship (SAR) the extensive glycosylation of the isolated compound it is expected to negatively affect the potency of the compound due to decreased membrane permeability but this was overcome by good aqueous solubility of the highly glycosylated compound (as a result of multiple hydroxylated sugar moieties) hence facilitating more drug uptake and suggesting a prodrug-like character (Tasdemir *et al.*, 2006) and (Costi *et al.*, 2016). Furthermore, it is important point out that the presence of methoxy substituents could enhance the lipophilicity and somehow compensate for the polarity introduced by glycosylation (Schmidt *et al.*, 2009).

CONCLUSION

The evaluation of *Vernonia perrottettii* traditionally used for the management of trypanosomiasis in this research has expanded the limited documented reports on this plant especially its trypanocidal activity. The preliminary qualitative phytochemical screening of the plant's ethanol extract confirmed positive for alkaloids, flavonoids, terpernoids, phenols, tannins, saponin and cardiac glycosides. This provided a broad spectrum of phytochemicals explored for the isolation of potentially active compound predicted as 3-*O*-(α-Mycarosyl-(1-3)-α-glucopyranosyl)-5-*O*-{β-glucopyranosyl-(1-6), (1-6)-β-diglucopyranosyl} 7,4'dimethoxykaempferol.

This compound showed a good half inhibitory concentration (IC_{50}) of 0.335µM in comparison with Diminazene diaceturate the reference standard drug with 0.24µM hence confirming the active profile of this compound and recommended as a promising compound for further *in vivo*, cytotoxicity and X-ray crystallographic experiments.

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Appendix 1: Sample of isolated chemical compound (sample) D