PHYTOCHEMICAL SCREENING, ANTIMICROBIAL ASSESSMENT, AND ISOLATION OF A NOVEL BIOACTIVE FRIEDELANE-TYPE TRITERPENOID FROM THE STEMBARK EXTRACTS OF *Uapaca ambanjensis* Leandri.

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ABSTRACT

The hexane, dichloromethane, ethyl acetate and methanol extracts of the stem bark of *Uapaca ambanjensis* were investigated for their phytochemical constituents and activity against selected microorganisms. Whereas all the phytochemicals, except anthraquinones, are indicated in various extracts, the most impressive antimicrobial potencies of extracts compared to standard drugs were observed for methanol (against *Salmonella typhi* and *Candida albicans*), n-hexane (against *Staphylococcus aureus*, *C. albicans*, *S. typhi* and *Klebisiela pneumoniae*) and ethyl acetate (against *Pseudomonas aeruginosa*). Chromatographic separation and purification of the methanol extract led to the isolation of compound 1 (labelled G24) which upon characterization using 1D and 2D NMR was elucidated to be a novel friedelane–type pentacyclic triterpenoid, 2β-propanoyloxy-friedelan-3-one. *In vitro* antimicrobial screening of the new compound showed that it has both gram-positive and gram-negative antibacterial, and antifungal potencies with the strongest activities against *P. aeruginosa*, *C. albicans*, *S. aureus* and *Streptococcus pyogenes*. It is most likely a medicinal principle or antibiotic with activity against ailments for which the stated microbes are implicated, and may also account for the ethnomedicinal uses of the crude plant extract to treat typhoid fever, other fevers, skin diseases and stroke.

Key Words: *Uapaca ambanjensis*; 2β-propanoyloxyfriedelan-3-one; ethnomedicinal; antibacterial; antifungal; medicinal principle.

1. INTRODUCTION

Research inquests in modern drug development begin with the screening of medicinal plants for bioactive agents followed by their isolation, characterization, and antimicrobial investigation. Countless physiologically active compounds have been isolated spanning many medicinal classes, some following their traditional uses, others not. However, health challenges (such as diseases with no cure, recurrence of resistant strains, inadequate quantity of isolated bioactive compounds, and the threat of extinction of plants themselves) still point to the need to break new grounds [1]. Of particular concern are the incidence of multi-resistant *Salmonella typhi* strains and liver damage by *cirrhosis* [2,3]. The cure for today's incurable diseases, solutions to microbial resistance to hitherto effective drugs, and answers to other medical questions are believed to still rest with un-researched plants [4]. They can avail novel phytochemicals with the

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complexity, highly informative chemical structures, and new types of activities related to new drugs, drug leads, or drug precursors to *Uapaca ambanjensis* **Leandri** (Phyllanthaceae) is a flowering plant of the genus Uapaca. The genus is native to Africa and Madagascar [5-7]. It has been sighted in the Middle Belt areas of Northern Nigeria and some parts of South Eastern Nigeria. Ethno-medicinally infusions of *U. ambanjensis* leaf and stem back in water are said to cure typhoid fever, other fevers, and skin diseases in Middle belt areas. Partly dried leaves are boiled with water and drunken warm while fresh stem bark is crushed and squeezed with little water and drunken. For treatment of skin diseases like smallpox an infusion of the bark and leaf is drunk and used to bathe and wet the quarantined patient. In addition to these, in the South East, the fruit is crushed, mixed with gin or palm wine, squeezed out and drunk for treatment of stroke, or robbed for measles and chicken pox. In this study, phytochemical and antimicrobial activity tests were carried out on different solvent extracts of the plant stembark, and the methanol extract subjected to chromatographic separation and purification for the purpose of isolation, characterization and antimicrobial assessment of isolated compounds.

2. MATERIALS AND METHODS

Collection and Identification of Plant Material
The stem barks of *U. ambanjensis* mature plant were harvested in May 2017 from different locations in the local forests of Aieje village in Edumoga District, Okpokwu Local Government Area of Benue State, Nigeria. The plant material was identified and authenticated by Mr. Namadi Sunusi and the voucher specimen (number 965) was stored at the Herbarium Unit, Department of Biological Sciences, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria.

Extraction of Plant
The stem bark was air-dried at room temperature and subsequently pulverized using mortar and pestle. About 866g of it was subjected to Microwave-Assisted Extraction (MAE) using the method described by Akacha *et al.* [8]. A fraction of plant material that has gone full cycle was exhaustively washed and filtered in sequence with n-hexane, dichloromethane, ethyl acetate, and methanol. The combined extracts for each solvent were concentrated with the aid of a Rotary Evaporator and left to dry in a fume hood to give the crude product.

Phytochemical Screening
The crude solvent extracts were subjected to phytochemical tests for flavonoids, alkaloids, saponins, tannins, glycosides, cardiac glycosides, anthraquinones, steroids, and triterpenes using the methods of Trease and Evans [9,10].

Chromatographic Fractionation and Isolation A fraction of the methanol extract that separated as crystals on concentration was harvested, dissolved in a minimum amount dichloromethane, and subjected to chromatographic separation on a glass column using silica gel (60:120 mesh) as stationary phase

and gradients of n-hexane-ethyl acetate mixtures as mobile phase [11]. Thin-layer chromatography (TLC) on aluminum plates pre-coated with silica gel (60, F254 Merck, KGaA) was both used to select the best solvent system for column chromatography and to analyze eluents. Developed chromatograms were monitored by color observation, exposure to ultraviolet light (UV GL-58 Mineralight Multiband UV-254/366 nm), and spraying with 10% H_2SO_4, followed by heating at 100 ^{o}C for 5 minutes. Eluents of similar Rf profiles were pulled together. In this way, a single compound was harvested from n-hexane-ethyl acetate (7:3) mixture and gave a single spot on TLC analysis (Rf = 0.659).

NMR Analysis

The NMR Spectra of the isolated compound were run in $CDCl_3$ solution on a Bruker Topspin 3.2 DDU 500 42 instrument. 1D and 2D techniques deployed include *Proton Night* (^{1}H-NMR), ^{1}H-^{1}H COSY, ^{13}C-DEPTQ, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC [12,13]. Chemical shifts were expressed in δ (ppm) against tetramethylsilane (TMS) as a reference, and coupling constants (*J*) in Hertz.

Antimicrobial Screening

The antimicrobial activities were checked against both gram-positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes*), and gram-negative bacteria (*Salmonella typhi, Klebisiela pneumoniae, Pseudomonas aeruginosa*), and fungi (*Candida albicans* and *Aspergillus niger*). The microbe strains were clinical isolates sourced from the Department of Medical Microbiology Ahmadu Bello University Teaching Hospital Zaria. They were sub-cultured from agar slants into sterile Nutrient Agar tubes for bacteria and Sabouraud Dextrose Agar for fungi. Manufacturer's advice was followed in all preparations. McFarland turbidity standard was used in 0.9% sterile normal saline, while incubation periods depended on organism.

Determination of Antimicrobial Zones of Inhibition

Antimicrobial sensitivity tests by zones of inhibition were done using the Agar Well Diffusion Methods [14-16] but for four different concentrations of suspected antimicrobial agent (100μL each). Ciprofloxacin (10μL) at 10μg/mL and Terbinafine (10μL) at 50μg/mL were used respectively as positive antibacterial and antifungal controls while 20% Dimethyl sulphoxide (DMSO) was used as a negative control.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration of the antimicrobial agent was determined using the Agar Dilution method [17], [16] on ten different concentrations.

Determination of Minimum Bactericidal/Minimum Fungicidal Concentrations (MBC/MFC)

The Petri dishes in the MIC determination where a microbial growth was inhibited were used to determine the MBC and MFC. The micro filter paper discs by which the organisms had been seeded before being incubated were aseptically

lifted and used to subculture the microbial strain (if any) on fresh 5mL sterile Nutrient Broth medium for bacteria or Sabourand Liquid medium for fungi, in bottles labeled according to the organism and inhibiting concentration. After repeating the incubation cycles the bottles were examined for any visible colony growth (turbidity). The lowest concentration for which no visible growth was seen was recorded as the MBC/MFC.

3. RESULTS AND DISCUSSION

Phytochemical and antibacterial assessment of solvent extracts.

The n-hexane extract revealed the presence of alkaloids and steroids/triterpenes only. The dichloromethane, ethyl acetate and methanol extracts tested positive for all the phytochemicals classes except anthraquinones. However, dichloromethane extract also did not indicate saponins. From Table 1, *U. ambanjensis* stem bark extracts at a concentration of between 12.5mg/mL and 100mg/mL showed antibacterial zones of inhibition (ZOI) that range from 10mm to18mm. The values for the reference drug at

50μg/mL lie between 29 mm and 37mm. The highest antibacterial ZOIs of extracts at 100mg/mL were shown by methanol (18mm against *S. typhi*), n-hexane (18mm against *S. aureus,* 17mm against *S. typhi, and K. pneumoniae*), and ethyl acetate (17mm against *P. aeruginosa)*. The lowest MICs of 12.5mg/mL (Table 2) were shown by methanol, dichloromethane and ethyl acetate extracts against *S. typhi*, and n-hexane extract against *S. aureus,* while the lowest MBCs of 25mg/mL were observed for n-hexane extract (against *S. aureus*) and ethyl acetate extracts against *P. aeruginosa* (Table 3).

The highest antifungal ZOIs (at 100mg/mL) of 18mm, 16 mm, and 15mm were observed for activity against *C. albicans* by the n-hexane extract, ethyl acetate extract, and methanol extract respectively while the values for the antifungal drug at 30μg/mL are respectively 15mm,16mm and 15mm. The lowest MICs of 25mg/mL against the fungi were observed for n-hexane and methanol extracts.

Table 1: Diameters of antimicrobial Zones of Inhibition (mm) of *U. ambanjensis solvent extracts*

EXTRACT	ORGANISM	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)	CIP (10μg/mL)	TBF (50μg/mL)
N-hexane	*Salmonella typhi*	17	15	12	00	37	-
	Staphylococcus aureus	18	16	13	00	30	-
	Streptococcus pyogenes	00	00	00	00	30	-
	Klebisiela Pneumoniae	17	15	12	00	31	-
	Pseudomonas aeruginosa	16	14	12	00	31	-
	Candida albicans	18	16	13	00	-	15
	Aspergillus niger	14	00	00	00	-	48
Dichloromethane	*Salmonella typhi*	15	12	11	00	36	-
	Staphylococcus aureus	16	13	10	00	30	-
	Streptococcus pyogenes	16	13	10	00	30	-
	Klebisiela Pneumoniae	15	12	10	00	31	-

	Organism						
	Pseudomonas aeruginosa	13	11	10	00	30	-
	Candida albicans	14	11	00	00	-	15
	Aspergillus niger	14	00	00	00	-	48
Ethyl acetate	*Salmonella typhi*	16	14	12	10	36	-
	Staphylococcus aureus	14	12	10	00	30	-
	Streptococcus pyogenes	13	10	00	00	29	
	Klebisiela Pneumoniae	13	10	00	00	31	
	Pseudomonas aeruginosa	17	15	13	00	31	
	Candida albicans	16	13	00	00	-	16
	Aspergillus niger	14	11	00	00	-	48
Methanol	*Salmonella typhi*	18	16	14	12	35	-
	Staphylococcus aureus	15	12	00	00	30	-
	Streptococcus pyogenes	15	12	00	00	35	-
	Klebisiela Pneumoniae	14	11	00	00	31	-
	Pseudomonas aeruginosa	15	12	11	00	30	-
	Candida albicans	15	13	11	00	-	15
	Aspergillus niger	14	00	00	00	-	48

Key: CIP = *Ciprofloxacin* (antibacterial) TBF=*Terbinafine* (antifungal)

Table 2: Minimum Inhibitory Concentration (mg/mL) of *U. ambanjensis* solvent extracts.

ORGANISM	MINIMUM INHIBITORY CONCENTRATION (mg/mL)			
	N-Hexane	Dichloromethane	Ethyl acetate	Methanol
Salmonella typhi	25.0	12.5	12.5	12.5
Staphylococcus aureus	12.5	25.0	25.0	50.0
Streptococcus pyogenes	100.0	25.0	50.0	50.0
Klebisiela Pneumoniae	25.0	25.0	50.0	50.0
Pseudomonas aeruginosa	50.0	25.0	25.0	25.0
Candida albicans	25.0	50.0	50.0	25.0
Aspergillus niger	25.0	50.0	50.0	50.0

Table 3: Minimum Bactericidal/Fungicidal Concentration (mg/mL) of *U. ambanjensis solvent extracts*

ORGANISM	MINIMUM BACTERICIDAL / FUNGICIDAL CONCENTRATION (mg/mL)			
	N-Hexane	Dichloromethane	Ethyl acetate	Methanol
Salmonella typhi	50.0	100.0	100.0	50.0
Staphylococcus aureus	25.0	100.0	100.0	100.0
Streptococcus pyogenes	-	50.0	100.0	100.0
Klebisiela Pneumoniae	50.0	100.0	100.0	100.0
Pseudomonas aeruginosa	100.0	100.0	25.0	50.0
Candida albicans	50.0	100.0	100.0	100.0
Aspergillus niger	-	100.0	100.0	100.0

The significant activities of the extracts against *S. typhi*, *K. pneumoniae*, and *P. aeruginosa* are noteworthy as gram-negative strains are usually deemed resistant to plant extracts [18]. Also of note is the more widespread activity of the n-hexane extract, a fraction that tested positive to only alkaloids and steroids/triterpenes. The equally impressive potency of the methanol and n-hexane extracts especially against *S. typhi*, *S. aureus* and *C. albicans* informed the further fractionation of the former which lead to the isolation of compound **1** since the crude extract was ethno-medicinally administered as infusions of polar solvents for the treatment of typhoid fever and skin diseases. This is with the hindsight of the spate of multi-resistant *Salmonella typhi* strains and the hope a new drug lead to typhoid fever might create [19].

Structural Elucidation of Compound 1

The structural characterization of the isolated compound was done using the results from 1D and 2D NMR [12,13] and by comparing with literature data of related compounds. Proton decoupled DEPTQ was utilized for ^{13}C assignment and multiplicity determination of carbons [20]. HSQC was used to establish [1]H-^{13}C correlation linking carbons and their attached protons while ^{1}H-detected HMBC, was used to complement ^{13}C assignment and 'piecing together' of the structure via long-range correlations between protons and carbons [21,13]. Assignments were verified and

supported by ^{1}H–NMR and ^{1}H-^{1}H COSY and as follows:

Compound 1 (G24) : white crystals (MeOH); mp 282-286°C; ^{1}H NMR (CDCl$_3$, 500 MHz) δ 4.24 (d, J = 3.3,1H), 2.36 – 2.28 (m, 1H), 2.22 (dp, J = 20.5, 6.7 Hz, 3H), 1.91 (dd, J = 13.1, 7.1 Hz, 1H), 1.72 – 1.58 (m, 2H), 1.43 (s, 1H), 1.33 (s, 1H), 1.28 (s, 1H), 1.20 (d, J = 6.1 Hz, 4H), 1.12 (s, 1H), 0.99 (s, 1H), 0.95 (s, 3H), 0.82 (s, 3H), 0.66 (s, 1H); ^{13}C NMR (CDCl$_3$, 126 MHz) δ 206.76 (CO, C-3), 173.18 (COO, C-1'), 78.8 (CH,C-2), (59.46 (CH, C-10), 58.19 (CH, C-4), 53.08(CH, C-8), 42.82(CH, C-18), 41.50 (CH$_2$, C-6), 41.28 (C,C-5), 39.69 (C,C-13), 39.23 (CH$_2$,C-22), 38.30 (C,C-14), 37.47 (C,C-9), 36.02 (CH$_2$,C-16), 35.62 (CH$_2$,C-11),35.33 (CH$_2$,C-19), 35.00 (CH$_3$,C-30), 32.80 (CH$_2$, C-21), 32.42 (CH$_2$,C-15), 32.09 (CH$_3$,C-28),31.76 (CH$_3$,C-29), 30.49 (CH$_2$,C-12), 29.98 (C,C-17), 29.66 (CH$_2$,C-1), 28.15 (C,C-20), 22.26 (CH$_2$,C-2'), 20.23 (CH$_3$,C-27), 18.63 (CH$_3$,C-26),18.22 (CH$_2$,C-7),17.92 (CH$_3$,C-25),14.64 (CH$_3$,C-24), 6.80 (CH$_3$,C-3') ,6.79 (CH$_3$,C-23).

Table 4 shows the ^{13}C chemical shifts (ppm) of compound 1 (G24), their DEPTQ multiplicity, chemical shifts of attached protons (ppm) from HSQC data, and HMBC correlation. A comparison of the spectral data of the isolated compound with that of friedelan-3-one (*friedelin*) clearly suggests that it is its derivative related to 2α-acetoxy friedelan-3-one (*cerine acetate*) and 2β-acetoxy friedelan-3-one (*epicerine acetate*). The spectral data of the three compounds is shown in Table 5 while their structures are shown

in Figures 1 to 4. As in *cerine acetate* and *epicerine acetate*, the oxy-carbon in compound **1** is in C-2 (or α-) to the keto-group of *friedelin* and is part of an ester group. This is backed by the presence of a peak at δ 173.22 ppm which suggests an ester carbonyl peak, another at δ 78.8ppm which represents an oxy-carbon peak, and the absence of a proton broad singlet peak in its ^{1}H-NMR spectrum (which rules out an alcoholic OH). The less shielded carbinyl carbon peak at δ78.8, together with the oxy-methyl hydrogen peak that is also relatively downfield at δ 4.3 ppm (proton NMR) confers on the 'oxy' group an equatorial (rather than an axial) stereochemistry [22,23]. Further comparison of other ^{13}C resonances of the isolated compound with those of *cerine* acetate and *epicerine* acetate (Table 5) indicates that it is, in effect, structurally closer to *epicerine* acetate than it is to *cerine* acetate.

Furthermore, two unaccounted peaks (a prominent methylene peak of 22.26 and an up-

field methyl peak of 6.80) suggest that the oxygen atom at C-2 may not just be part of an acetoxy group but rather a propionyloxy (propanoyloxy) group. This is substantiated by the downfield resonation of the acid ester carbonyl peak at 173.22 as against 169.9 in *epicerine* [24] and the slight downfield resonation of the C-1 methylene carbon at 29.66 ppm compared to 28.5ppm in *epicerine acetate*. It also explains the resonation of the C-3 keto carbon at 206.78 ppm compared to 205.1 ppm in *epicerine acetate*. The new compound is thus *epicerine* propionate. That is 2β-(propanoyloxy) friedelan-3-one (or 2β-(propionyloxy) friedelan-3-one). This structural deposition is supported very strongly by the information from selected HMBC correlations (Figure 5). Oxy-propionate substructures have been severally cited among phytochemicals including triterpenes and steroids of medicinal value [25].

Table 4: ^{13}C and ^{1}H-NMR data of G24 (Compound 1) and observed ^{1}H-^{13}C-HSQC-1JCH and ^{1}H-^{13}C-HMBC-nJCH.

Cn	DEPTQ[a] CHn	δC[b]	^{1}H-^{13}C-HSQC-1JCH[c]	Selected HMBC long-range [1]H-^{13}C couplings. (Carbons to which protons are coupled)	Selected HMBC long-range [1]H-^{13}C couplings. (Protons coupled to carbon)
1	CH$_2$	29.66	0.97,2.09	C10	C1
2	CH	78.43	4.3	-	-
3	C	206.78	-	-	3H-23
4	CH	58.19	1.96/1.97	C23, C24, C5/C6	3H-24
5	C	41.28	-	-	1H-4

6	CH$_2$	41. 50	1.47/1.45,2.08	C10	1H-4,3H-24
7	CH$_2$	18.22	1.20	C26	3H-24
8	CH	53.08	1.11		3H-25, 2H-11/2H-15, 3H-27(l)
9	C	37.45	-	-	-
10	CH	59.46	1.29	C25	3H-25, 1H-4, 2H-1, 2H-6
11	CH$_2$	35.62	1.0	C8	3H-25
12	CH$_2$	30.49	-	-	-
13	C	39.69	-	-	-
14	C	38.30		-	3H-27
15	CH$_2$	32.41	1.0	C8	-
16	CH$_2$	36.02	1.28	C28	-
17	C	29.98	-	-	2H-22
18	CH	42.82	2.01		-
19	CH$_2$	35.33	0.66	C17	-
20	C	28.15	-	-	-
21	CH$_2$	32.80	2.02	-	-
22	CH$_2$	39.23	0.66	C17	-
23	CH$_3$	6.79	0.59	C3,C2,C24	1H-4
24	CH$_3$	14.64	0.44	C4,C5/C6, C7	1H-4,3H-23
25	CH$_3$	17.92	0.59	C10,C8,C11	1H-10
26	CH$_3$	18.63	0.77	-	-
27	CH$_3$	20.23	0.72	C14,C8	-
28	CH$_3$	32.09	-	-	2H-16
29	CH$_3$	31.78	0.71	-	-
30	CH$_3$	35.00	0.66/0.67	-	-
O$\underline{C}$OCH$_2$CH$_3$	C	173.18	-	-	2H-2[1]
OCO$\underline{C}$H$_2$CH$_3$	CH$_2$	22.26	2.02	C1[1]	-
OCOCH$_2$$\underline{C}H_3$	CH$_3$	6.80	0.66	-	-

a. Multiplicity in ^{13}C obtained by DEPTQ. b. [13]C chemical shifts assigned from DEPTQ and HMBC.
    c. [1]H Proton chemical shift from HSQC

Table 5: ^{13}C NMR data comparison of 2α-acetoxy friedelan-3-one (*cerine acetate*), 2β-acetoxy friedelan-3-one (*epicerine acetate*), friedelan-3-one (*friedelin*) and Compound 1(G24)

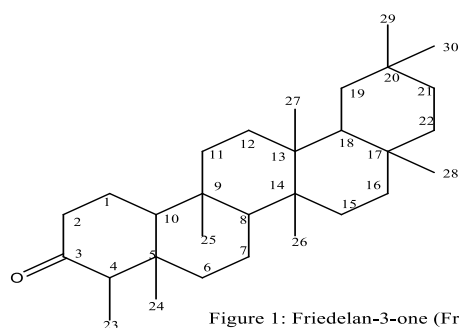| *Carbon Position*	[13]*CShift (ppm) of Cerine acetate* [24]	[13]*C Shift (ppm) of Epicerine acetate* [24]	[13]*C Shift (ppm) of Friedelin* [26]	[13]*C Shift (ppm) of Compound 1* (**Experimental**)	*CHn* (*DEPTQ*)
1	28.0	28.5	2.23(t)	29.66	CH$_2$
2	76.4	76.4	41.5(t)	78.43	CH
3	207.8	205.1	213.2(s)	206.78	C
4	53.2	56.1	58.2 (d)	58.19	CH
5	43.0	42.4	42.1(s)	41.28	C
6	40.9	41.0	41.3(t)	41.50	CH$_2$
7	18.1	18.1	18.2(t)	18.22	CH$_2$
8	53.1	53.1	53.1(d)	53.08	CH
9	36.7	37.4	37.4(s)	37.45	C
10	54.2	57.2	59.5(d)	59.46	CH
11	35.3	35.6	35.6(t)	35.62	CH$_2$
12	30.2	30.4	30.5(t)	30.49	CH$_2$
13	39.5	39.7	39.7(s)	39.69	C
14	38.2	38.3	38.3 (s)	38.3	C
15	32.2	32.4	32.4 (t)	32.42	CH$_2$
16	35.9	35.9	36.0 (t)	36.02	CH$_2$
17	29.8	29.9	30 (s)	29.98	C
18	42.7	42.8	42.8 (d)	42.82	CH
19	35.1	35.3	35.3 (t)	35.33	CH$_2$
20	28.0	28.1	28.2 (s)	28.15	C
21	32.7	32.7	32.8 (t)	32.80	CH$_2$
22	39.1	39.2	39.2 (t)	39.23	CH$_2$
23	6.3	6.5	7.0(q)	6.79	CH$_3$
24	13.9	14.5	14.6(q)	14.64	CH$_3$
25	17.7	17.9	17.9(q)	17.92	CH$_3$
26	18.5	18.5	20.2(q)	18.63	CH$_3$
27	19.9	20.1	18.6(q)	20.23	CH$_3$
28	32.0	32.0	32.1(q)	32.09	CH$_3$
29	31.7	31.7	35.0(q)	31.78	CH$_3$
30	34.8	34.9	31.8(q)	35.00	CH$_3$
OC**O**CH$_3$	169.5	169.9	-	-	C
OCO**C**H$_3$	20.9	20.7	-	-	CH$_3$
O**C**OCH$_2$CH$_3$	-	-	-	173.18	C
OCO**C**H$_2$CH$_3$	-	-	-	22.26	CH$_2$
OCOCH$_2$**C**H$_3$	-	-	-	6.80	CH$_3$

Figure 1: Friedelan-3-one (Friedelin)
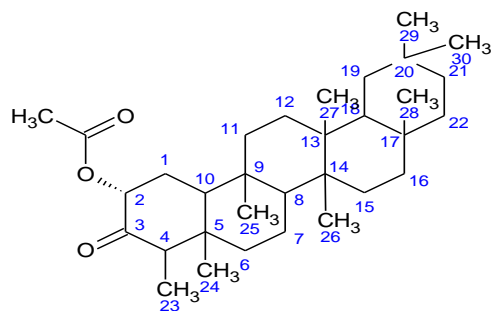
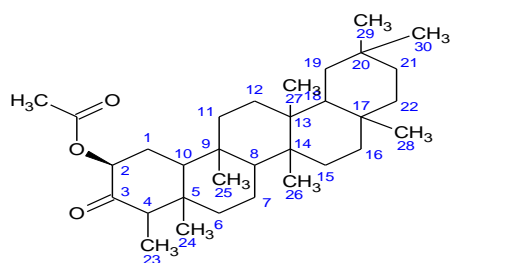Figure 2: 2α-(acetoxy)friedelan-3-one (cerine acetate)

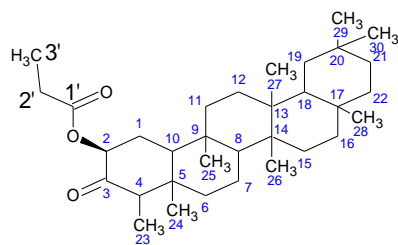Figure 3: 2β-(acetoxy)friedelan-3-one (*epicerine acetate*)

Figure 4: 2β-(propanoyloxy)friedelan-3-one (epicerine propanoate)
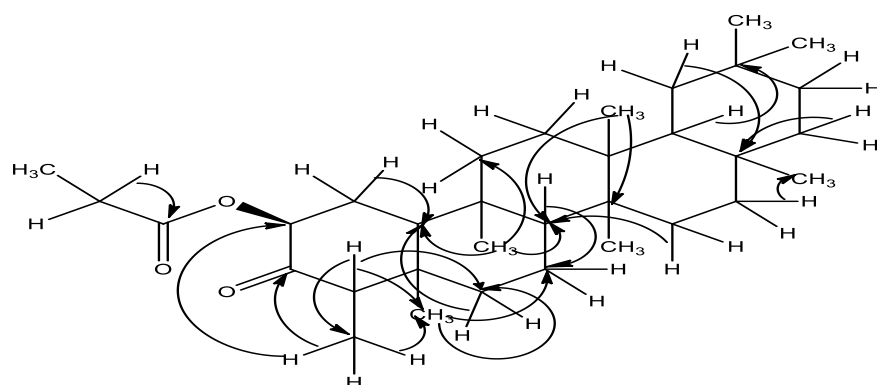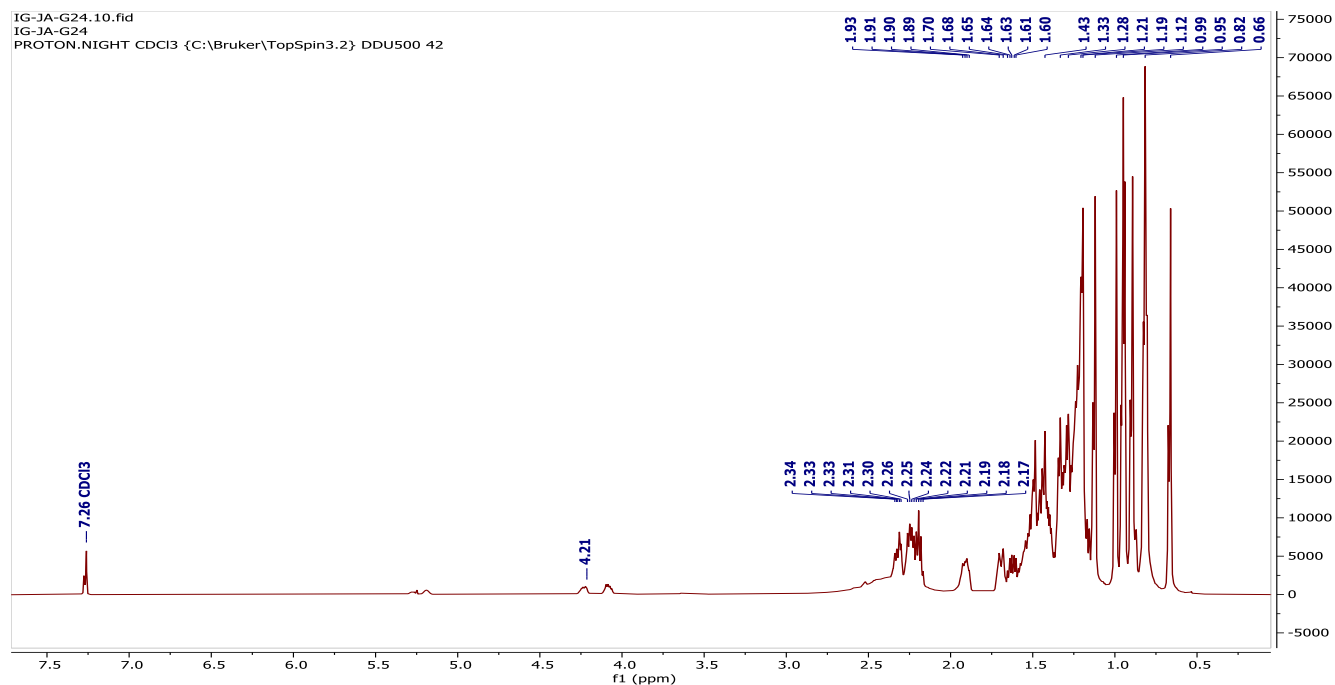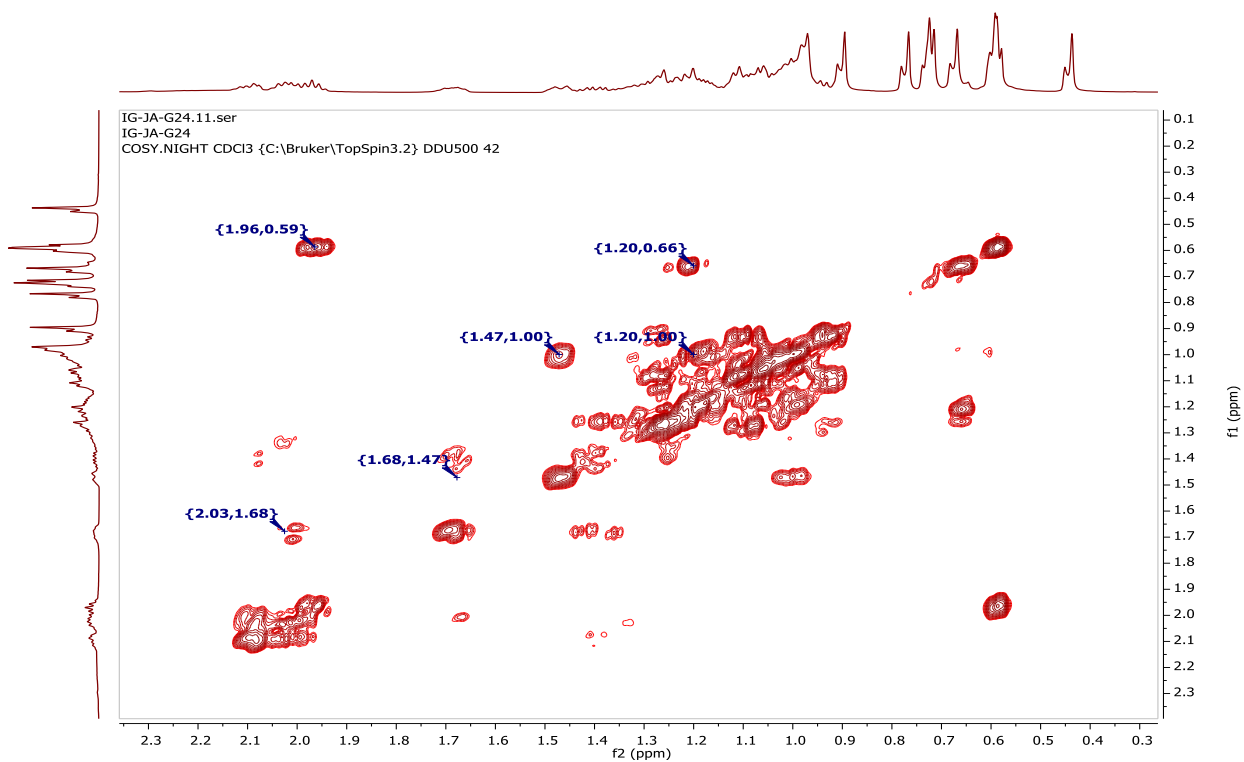

Figure 5: Structure and prominent HMBC correlations of compound (G24)

NMR spectra of G24 (Compound 1) are shown in Figures 6a-e.

Figure 6a: Proton. Night NMR of G24

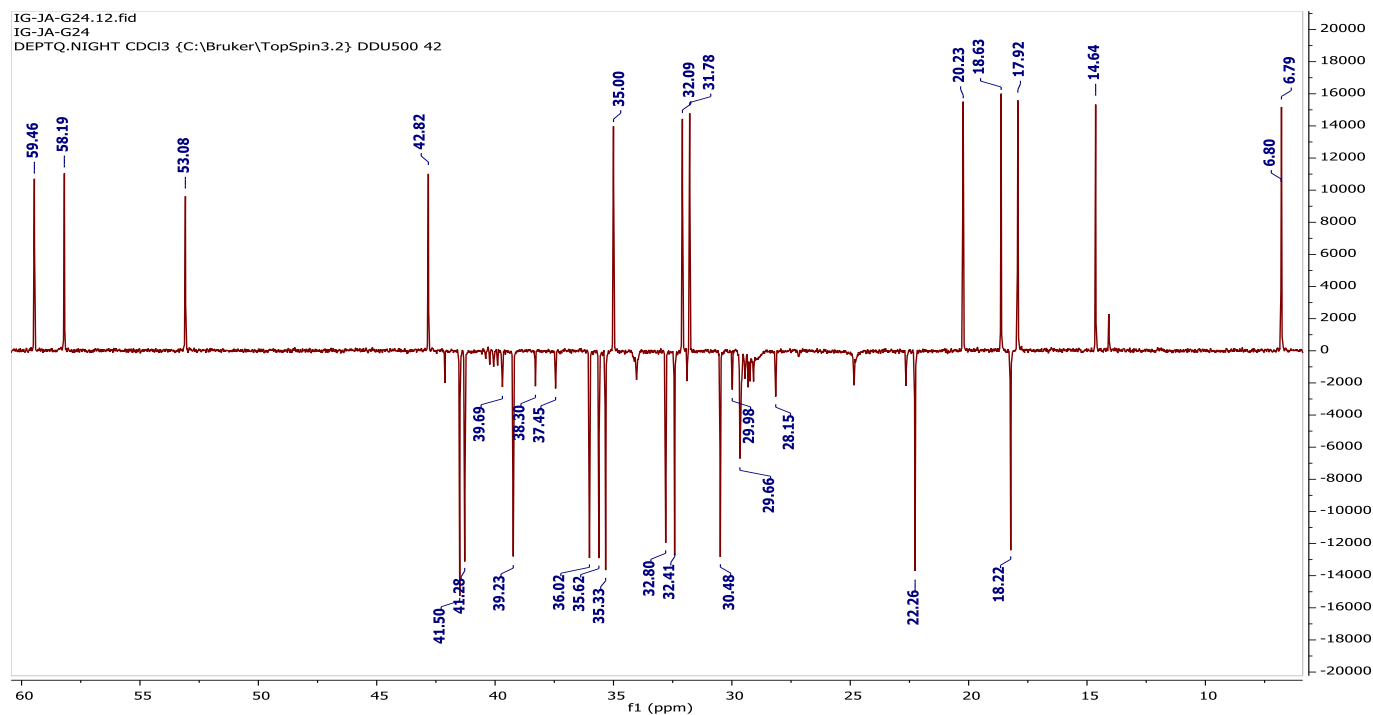**Figure 6b**: COSY Night NMR of G24

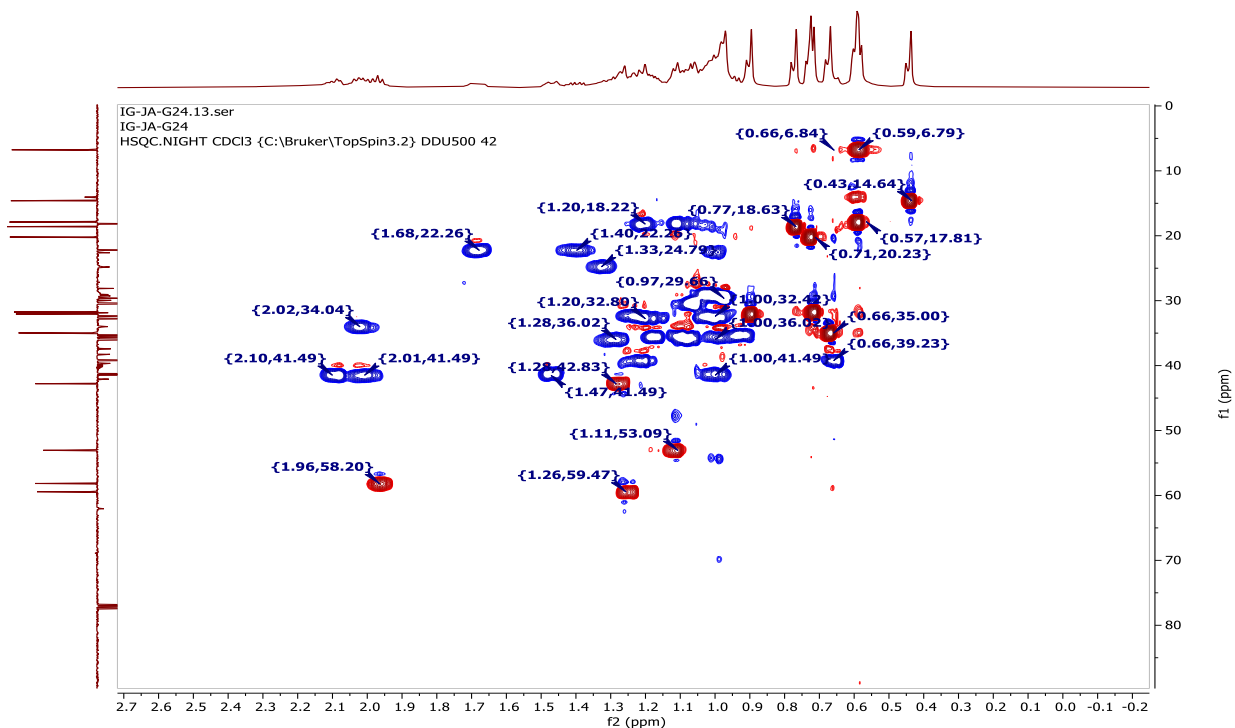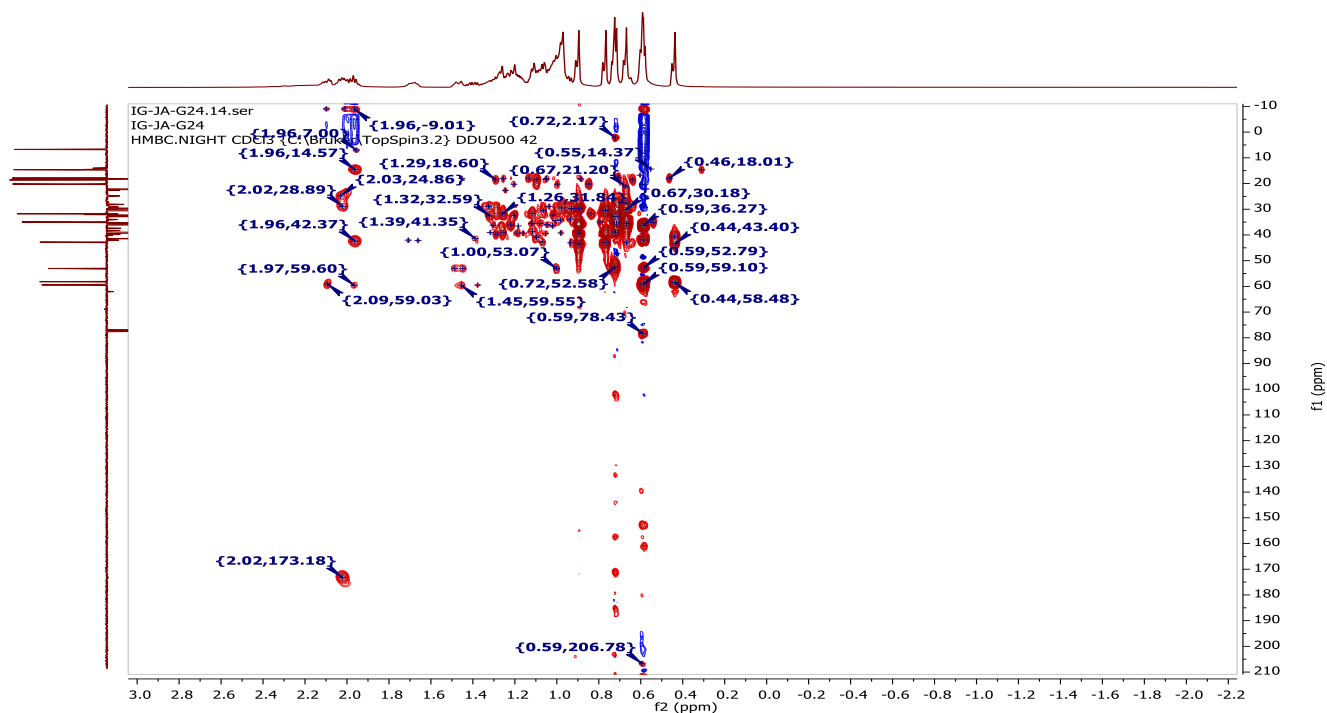**Figure 6c**: DEPTQ. Night (Zoomed in)

Figure 6d: HSQC. Night NMR spectra of G24

Figure 6e: HMBC. Night NMR spectrum of G24

2.3 Antimicrobial Potency of Compound 1 (G24)

From Table 6, the new compound at 10mg/mL had the most prominent antimicrobial ZOIs against *P. aeruginosa* (18mm), *C. albicans* (17mm), *S. aureus* (16mm), and *S. pyogenes* (16mm) with activity still observed at 1.25mg/mL except for *C. albicans*. The Standard drugs (*Ciprofloxacin* at 10μg/mL and *Terbinafine* at 50μg/mL) had ZOIs of 32mm,16mm,30mm, and 32mm respectively against the respective microbes.

Table 6: Diameter of Zone of Inhibition (mm) of compound **1** (G24) against microorganism

ORGANISM	10 (mg/mL)	5 (mg/mL)	2.5 (mg/mL)	1.25 (mg/mL)	CIP 10μg/mL	TBF 50μg/mL
Salmonella typhi	0	0	0	0	37	-
Staphylococcus aureus	16	14	12	11	30	-
Streptococcus pyogenes	16	14	12	11	32	-
Klebisiela Pneumoniae	13	11	0	0	31	-
Pseudomonas aeruginosa	18	16	14	12	32	-
Candida albicans	17	15	12	0	-	16
Aspergillus niger	0	0	0	0	0	48

Key: CIP=*Ciprofloxacin* (antibacterial), TBF=*Terbinafine* (antifungal

The isolated compound expectedly showed greater activity against the selected microbes than the crude extracts as evidenced in the ZOI values obtained at lower concentrations ranges of the compound. For instance, whereas the lowest concentration of a crude extract that inhibited microbial growth was 12.5mg/mL (Table 2), the activity of the isolated compound was still observed at as low as 1.25mg/mL for three microbes. There are great chances that minimum concentration of inhibition will even be lower since MIC is not an absolute value. The 'true' MIC is a point between the lowest test concentration that inhibited microbial growth and the next lower test concentration [27]. Further dissection of the concentration was limited by little quantity of isolated compound, but the concentration gradient applied to determining zones of inhibition provided sufficient result to confirm strong microbial activity most especially against *P. aeruginosa*, *C. albicans*, *S. aureus*, and *S. pyogenes*.

Thus looking at the ailments for which the respective microbes are implicated, compound **1** may have potency against *otitis, endophthalmitis, endocarditis, meningitis, pneumonia, and septicemia (P. aeruginosa)* [28]*;* urinary yeast infection, urinary tract infections, genital yeast infection, oral thrush and mucocutaneous candidiasis (*C. albicans*) [29]; abscesses, furuncles, cellulitis, bloodstream infections, pneumonia, or bone and joint infections (*S. aureus*) [30]; scarlet fever, bacteremia, pneumonia, necrotizing fasciitis, myonecrosis and Streptococcal Toxic Shock Syndrome (*S. pyogenes*) [31]. The new compound, 2β-(propanoyloxy) friedelan-3-one (or *epicerine propionate)* is most likely a medicinal principle or antibiotic or with antimicrobial potency against both gram-negative and gram-positive bacteria, as well as a fungus.

Conclusion

The N-hexane extract of *U. ambanjensis* indicated the presence of alkaloids and steroids/triterpenes only, but turned out to show the most prevalent antibacterial activity with potency against *S. aureus, S. typhi, K. pneumoniae, and C. albicans.* The methanol and ethyl acetate extracts indicated all the phytochemical classes except anthraquinones but the former had very impressive antimicrobial showing against *S. typhi*, *S aureus* and *C. albicans* while the later had the strongest activity against *P aeruginosa*. The novel compound isolated from methanol extract, 2β-propanoyloxy-friedelan-3-one, expectedly showed both gram-positive and gram-negative antibacterial, and antifungal potencies with the strongest activities against *P. aeruginosa*, *C. albicans*, *S. aureus* and *Streptococcus pyogenes*. It may have antibiotic activity against ailment for which the stated microbes are implicated, and may also be responsible ethnomedicinal uses of the plant to treat typhoid fever and skin diseases.

DECLARATIONS

Competing interests

There are no competing interests.

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