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Isolation, Characterization, and Assessment of the *In Vitro* Antibacterial and Antifungal Properties of Methanol Extracts and Friedelan-3-one from *Uapaca ambanjensis* (Leandri)

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ABSTRACT: *Uapaca ambanjensis Leandri* (Phyllanthaceae) is a largely un-researched plant whose crushed stem bark, leaf, and fruit infusions are ethno-medicinally claimed to cure typhoid fever, other fevers, skin diseases, and stroke. This work aimed at the isolation, characterization, and determination of antimicrobial potency of the methanol stem-bark extracts of *Uapaca ambanjensis*. Phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, tannins, saponins, steroids and triterpenes in the methanol extract. The antimicrobial assessment of the extract *against Salmonella typhi*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebisiela pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger* showed the diameter of zones of inhibition (ZOI) ranging from 11 to 18 mm at a concentration of 12.5mg/mL to 100mg/mL, with most prominent activities against *S. aureus*, *S. typhi and P. aeruginosa*. Chromatographic fractionation and purification led to the isolation of a pentacyclic triterpenoid (friedelan-3-one) labeled G23f-9. The compound showed antimicrobial potency evidenced by zones of inhibition against *S. aureus* (20 mm), *S. typhi* (16 mm), *S. pyogenes* (14 mm), *K. pneumoniae* (13 mm), and *P. aeruginosa* (13 mm) at a concentration of 100 µg/mL while the values for the standard drug, *Ciprofloxacin*, at 10 µg/mL are respectively 24 mm, 20 mm, 27 mm`1,20mm and 18mm. The Minimum Inhibitory Concentrations (MIC) were 12.5 µg/mL for *S. aureus*, 25 µg/mL for *S. typhi* and 100 µg/mL for the rest while the Minimum Bactericidal Concentrations (MBC) were 50 µg/mL for *S. aureus* and 100 µg/mL for *S. typhi*. The isolated compound showed some antifungal activity against *C. albicans* with ZOI of 11 mm at 100µg/mL while the antifungal drug, *Terbinafine*, gave 30 mm at 30 µg/mL. Phytochemical and antimicrobial results lay credence to some ethnomedicinal claims on the plant including its use to treat typhoid fever and some skin diseases. The biological assessment of the methanol extract and isolated compound reveals that the plant could have antibacterial and antifungal potentials. The isolation of Friedelan-3-one is reported for the first time from the plant *U. ambanjensis Leandri*.

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Medicinal plants have remained the major drivers of both traditional ethnomedicine and modern drug development, being hosts to diverse and complex bioactive compounds. The isolation, characterization, and determination of the antimicrobial activity of compounds from such plants are the very primary steps in modern natural product-based drug discovery. So, despite the onslaught of modern orthodox medicine and initial setbacks caused by its blooming, herbs-driven traditional medical system of treatment continued to grow in relevance, with synergy being the most recent advocacy in many countries. A recent WHO declaration insists that successful primary health care will come faster by marrying scientific and traditional information (WHO, 2019). Modern drug formulation using isolated active principles or their precursors not only dissipates the challenge of too little concentration of the isolates in plants but also

opens new doors for structure-activity manipulations leading to either more effective or entirely new drugs. On the other hand, ethnomedicinal uses of remedies in their crude or semi crude forms with advantages such as affordability, easy accessibility, fewer side effects, and natural (ecofriendly) healing, are also fast blossoming into orderly regulated systems of medicine (Alves & Rosa, 2007; Bandaranayake, 2006). Thus, in both ways plants increasingly remain the bedrock of human medication and medicinal solutions. A lot of diseases had no cure until drugs were eventually developed using inputs from plants: malaria before extraction of *quinine* from the Cinchona tree, cancer until the anticancer drug *Paclitaxel* (*Taxol*) was derived from the bark of *Taxus brevifolia*, etc. Effective drugs that, with time, developed resistance to microbes had equally been often replaced with better alternatives still from plants. From formerly most effective antimalarial, *quinine*, highly successful anti-bacterial, *penicillin*, to anti-schistosomiasis, *praziquantel*, the list is endless and perpetually calls for fresh discoveries and new drug options (Dankaddai and Okafor, 2019). Potentials to provide answers to today's unanswered medical questions may still rest with un-researched plants! The other way round, phytochemicals from such plants, in their skeletal diversity and structural complexity, could also be trusted to provide useful information about chemical structures and new types of action required for the standardization of traditional remedies. *Uapaca ambanjensis* Leandri. (Phyllanthaceae) is a largely un-researched flowering plant of the genus Uapaca. The genus is native to Africa and Madagascar (Mcpherson, 2011; Breteler, 2013). *U. ambanjensis* has been sighted in the North Central and South-Eastern states of Nigeria. Ethno-medicinally infusions of *U. ambanjensis* leaf and stem back are said to cure typhoid fever, other fevers, and skin diseases. 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, chickenpox, and measles an infusion of the bark and leaf is drunk and used to bathe and wet the quarantined patient. In addition to these, in South-Eastern Nigeria the fruit is crushed, mixed with gin or palm wine, squeezed out, and drunk for treatment of stroke (Ochibo, 2017; Ubochi, 2021). Thus, the objective of this work is to isolate, characterize and assess the *in vitro* antibacterial and antifungal properties of methanol extracts and antimicrobial activity of friedelan-3-one from *Uapaca ambanjensis* (Leandri).

MATERIALS AND METHODS

Collection and Identification of Plant Material; The stem barks of mature *U. ambanjensis* plants were harvested in May 2017 from different locations in the local forests of Aiede village in Edumoga District Okpokwu Local Government Area (7.04^{o} N, 7.91^{o} E), Benue State, North Central Nigeria. The plant material was identified and authenticated by the plant taxonomist, Mallam Namadi Sunusi, and the voucher specimen (Voucher number 965) was deposited 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. The powdered plant material (866 g) was exhaustively extracted sequentially with n-hexane, dichloromethane, ethyl acetate and methanol respectively using Microwave-Assisted Extraction (MAE) method as recommended by Chan *et al.* (2013) and adopted by Akacha *et al.* (2016). The methanol extract was concentrated using Rotary Evaporator at 40^{o}C and left to dry in a fume hood to give the crude extract.

Phytochemical Screening: The crude methanol extract was subjected to phytochemical tests for alkaloids, glycosides, cardiac glycosides, flavonoids, tannins, saponins, anthraquinones steroids, and triterpenes using standard qualitative methods of Trease and Evans (Evans, 2009; Abdisa & Kenea, 2021)

Fractionation and Isolation: The concentrated methanol extract deposited heterogeneous crystals on standing which were collected by filtration and the fraction prepared for chromatographic analysis (Harborne *et al.*, 1998). Thin-layer chromatography (TLC) on aluminum plates pre-coated with silica gel (60 F254, Merck, KGaA,64271 Darmstadt Germany) was used to estimate the most suitable solvent system for column chromatography. 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. The fraction was dissolved minimum amount of dichloromethane and about an equal mass of silica gel was added and both crushed to a fine dry powder before being loaded onto a slurry-packed silica gel (60:120 mesh) chromatographic glass column of dimension 70 cm by 2.5 cm. The column was eluted continuously with gradients of n-hexane-ethyl acetate mixture (100:0, 90:10, 80:20, etc.). About 114 eluents were harvested. The chromatographic fractions were analyzed with TLC and fractions with similar Rf profiles were combined in groups. A key fraction, G23, obtained from n-hexane-ethyl acetate 8:2 mixtures was re-chromatographed using a micro-scale

(pipette) column and eluted with narrower solvent mixture ratios. This led to the isolation of G23f-9 as white crystals which gave a single spot on TLC analysis. The isolate was subjected to proton and carbon-13 NMR and antimicrobial assessment.

NMR Analysis: NMR spectra were recorded on a Bruker Topspin 4.1.0 Avance version instrument at SF (MHz) 400.1300093(^{1}H) and SF(MHz) 100.6127690(^{13}C) in $CDCl_3$ solution and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constants (J) in Hertz.

Antimicrobial Susceptibility Testing (AST): Antibacterial activities were checked against *Salmonella typhi*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebisiela pneumoniae*, and *Pseudomonas aeruginosa*. while antifungal activities were checked against *Candida albicans* and *Aspergillus niger*. All microbes are clinical isolates obtained from the Department of Pharmaceutical Microbiology at Ahmadu Bello University, Zaria, Nigeria. The pathogens were checked for purity and maintained in slant agar. Loops of the test organisms were taken from the agar slants and sub-cultured in tubes containing the Sterile Nutrient Agar for bacteria and the Sabouraud Dextrose Agar for fungi. The tubes were incubated for 48 hours for bacteria and 72 hours for fungi until sporulation.

Determination of Zones of Inhibition (ZOI): The antibacterial and antifungal sensitivity tests were done using the Agar Well Diffusion Method (Kebede *et al*, 2021; Balouiri *et al*.., 2015; OIE Terrestrial Manual, 2012) run with a slight concentration gradient of the suspected antimicrobial agent. In this way, the diameters of zones of inhibition were measured for four different concentrations. Each of sterile media (20 mL) prepared following the manufacturer's instruction were dispensed into sterile Petri dishes and allowed to solidify. Each standardized culture (2 mL) was flooded over each dish and the excess was discarded to give an even spread. Equidistant wells (for four concentrations, standard drug, and negative reference) were bored aseptically into the seeded agar using a sterilized 8 mm diameter cork-borer and labeled. The wells were filled according to labeling with 100 µL of the extract at 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL as well as *Ciprofloxacin* (10 µL at a concentration of 10 µg/mL) as a positive control for bacteria, *Terbinafine* (10 µL at 50 µg/mL) as a positive control for fungi, and 20 % Dimethyl sulphoxide as a negative control for plant extract. The plates were loaded in duplicate as backup and left for one hour to allow for proper diffusion before incubating upright in the dark. After a maximum of 48 hours (at 37ºC) for bacteria and a minimum of 96 hours (at 30ºC) for fungi, depending on the organism (Benkova *et al*.., 2020), the dishes were observed for growth, and the diameters of any resulting zones of inhibition formed around wells measured in millimeter-scale using a transparent ruler.

Minimum Inhibitory Concentration (MIC): The Agar Dilution Method (Benkova *et al*.., 2020; Kebede *et al*, 2021) was used to determine the minimum inhibitory concentration of extract and isolated compound. Ten serial concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.390625, and 0.1953) in mg/mL of the test material were prepared and incorporated into the molten Muller Hinton agar (for bacteria) or Sabourand Dextrose agar (for fungi) in Petri dishes, using serial two-fold dilutions. Sterile micro filter paper discs (6mm in diameter) for each of the five bacterial strains (or two fungi) were placed on the solidified agar surface in a petri dish bearing a particular concentration. 10 µL of each standardized inoculum was then inoculated on the agar surface by impregnating the paper discs, and left for an hour to diffuse. The Petri dishes were loaded in duplicate and incubated under suitable conditions depending on the test microorganism. By visually comparing different strains in the series in a plate and observing for microbial growth, the MIC was determined as the lowest concentration at which no visible bacterial or fungal growth was observed.

Minimum Bactericidal/Fungicidal Concentration (MBC/MFC): The minimum bactericidal/fungicidal concentrations of the extracts and isolated compound were determined from the Petri dishes where a microbial strain showed no visible growths (i.e. where the growth of the microbial strain was inhibited) in the MIC determination. The micro-filter paper discs by which the organisms had been seeded before incubation were lifted and used to subculture the organism (if any) on fresh 5 ml sterile Nutrient Broth media for bacteria and Sabourand Liquid media for fungi. These were done in bottles labeled according to the organism and inhibiting concentrations. The incubation cycles were then repeated. The lowest concentration of each extract or isolated compound, in each case, for which there was no visible colony growth (turbidity) on the medium was recorded as minimum bactericidal/fungicidal concentration (Benkova *et al*.., 2020; Kebede *et al*, 2021).

RESULTS AND DISCUSSION

Phytochemical Constituents and Antimicrobial Activity: The methanol extract tested positive for flavonoids, alkaloids, saponins, tannins, glycosides, steroids, and triterpenes. Polar compounds cut across

most classes of phytochemicals given their structural diversity thus explaining their detection in methanol, and justified the interest in further subjecting its extract to fractionation and isolation. Truong *et al.* (2019) had reported that methanol extracted the highest content of phenolics, flavonoids, alkaloids, and terpenoids from *Severinia buxifolia* (Rutaceae) when compared to dichloromethane, chloroform, acetone, and even distilled water. The extract showed antimicrobial zones of inhibition (ZOI) in the range of 11 mm-18 mm at a concentration of between 12.5mg/mL and 100.0mg/mL (Table 1). The highest antibacterial ZOIs of extracts at 100mg/mL were obtained for activity against *S. typhi* (18 mm), *S. aureus* (15 mm), *S. pyogenes* (15 mm), and *P. aeruginosa* (15 mm). These are promising compared to the values for the reference drug, *Ciprofloxacin*, at 10 µg/mL. The lowest concentration of the extract that inhibited bacterial growth (MIC) is 12.5 mg/mL (Table 2) observed for activity against *S. typhi* and *S. aureus* followed by 25 mg/mL observed for activity against *P. aeruginosa*. The minimum bactericidal concentration (MBC) which represents the lowest concentration that caused the death of bacteria stood at 50mg/mL and was observed for activity against *S. typhi* and *P. aeruginosa*. The extract also showed some antifungal activity against *C. albicans* and *A. niger* with ZOIs of 15 mm and 14 mm respectively, at a concentration of 100 mg/mL, while the antifungal drug, *Terbinafine*, at 50 µg/mL gave 15mm and 48mm of respectively (Table 1). The antifungal MICs of the extract stood at 25mg/mL and 50mg/mL against *C. albicans* and *A. niger* respectively but the MFCs were 100mg/mL for activity against the two fungi (Table 2).

Table 1. Diameters of Zones of Inhibition (mm) of Methanol Extract of *U. ambanjensis* Stembark

Extract Concentration Organism	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)	CIP 10 µg/mL	TBF 50µg/mL
Salmonella typhi	18	16	14	12	35	-
Staphylococcus aureus	15	12	0	0	30	-
Streptococcus pyogenes	15	12	0	0	35	-
Klebisiela Pneumoniae	14	11	0	0	31	-
Pseudomonas aeruginosa	15	12	11	0	30	-
Candida albicans	15	13	11	0	-	15
Aspergillus niger	14	0	0	0	-	48

Key: - not determined

Table 2. Minimum Inhibitory Concentration and Minimum Bactericidal/Fungicidal Concentration (mg/mL) of Methanol Extract of *U. ambanjensis* Stembark

ORGANISM	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.13 mg/mL	1.56 mg/mL	0.78 mg/mL	0.39 mg/mL	0.195 mg/mL
Salmonella typhi	-	-ℂ	-	-ℂ	+	+	+	+	+	+
Staphylococcus aureus	-ℂ	-ℂ	+	+	+	+	+	+	+	+
Streptococcus pyogenes	-ℂ	-ℂ	+	+	+	+	+	+	+	+
Klebisiela Pneumoniae	-ℂ	-ℂ	+	+	+	+	+	+	+	+
Pseudomonas aeruginosa	-	-ℂ	-ℂ	+	+	+	+	+	+	+
Candida albicans	-ℂ	-	-ℂ	+	+	+	+	+	+	+
Aspergillus niger	-ℂ	-ℂ	+	+	+	+	+	+	+	+

Key: + = Growth, - = No Growth, -ℂ = Minimum inhibitory concentration, -ℂ = Minimum bactericidal/fungicidal concentration.

It is noteworthy that highest antibacterial ZOIs, lowest MICs, and MBCs of the extract were observed for activity against *S. typhi*, *S. aureus*, *P. aeruginosa*, and *C. albicans*. Activity against *S. typhi* underscores the reported ethnomedicinal use of *U. ambanjensis* to treat typhoid fever of which the crude drug was administered as infusions of water or ethanol. The equally impressive activity of the extract against *P. aeruginosa*, *S. aureus* and *C. albicans* have concurrence in the reported ethnomedicinal use of the crude plant extracts to treat skin diseases. *C. albicans* is liable for candidiasis, skin, genital yeast and urinary infections. *P. aeruginosa* is implicated in dermatitis, gastrointestinal, urinary tract, respiratory, bone, and joint infections while *S. aureus* is the causative agent for abscesses, meningitis, endocarditis, osteomyelitis, pneumonia and food poisoning (Bush & Charles, 2021). The lather strains are gram-negative, adjudged to be usually resistant to (crude) plant extracts (Odeh, *et al.*, 2016). The stem bark of U ambanjensis could be host to bioactive constituents that have potency against many of the ailments for which the highlighted microbes are implicated.

Structural Elucidation of Isolated Compound (G23f-9): G23f-9 was isolated as a white crystal of melting point, 261-265^{o}C (unverified), Molecular Formula: $C_{30}H_{50}O$ (Mr 426.7244) that showed a positive Liebermann–Burchard test for steroids and/or triterpenes. Information from the ^{1}H NMR spectrum of G23f-9 showed the absence of unsaturated proton, the presence of a proton quartet at δH 2.26 (H-4), a methyl

doublet at δH 0.89 (H-23), and methyl singlets at δH 0.72 (H-24), δH 0.87 (H-25), δH 1.01(H-26), δH 1.05 (H-27), δH 1.18 (H-28), δH 1.00 (H-29) and δH 0.95 (H-30). Its ^{13}C NMR spectra showed 30 carbons atoms, confirmed the absence of carbon-carbon multiple bonds, but has a carbonyl signal at 213.26 ppm typical of a saturated ring ketone. These data reflected the structural features of friedlan-3-one and matched closely with existing literature (Sangsuwon, *et al.*., 2013; Igoli & Gray, 2008; Hatem, *et al.*, 2015) to conclude that G23f-9 is the pentacyclic triterpenoid known popularly as friedelin (Table 3). This is the first time friedelan-3-one is reported isolated from *U. ambanjensis*. Figure 1 shows the structure and NMR spectral assignment of friedelin. The proton and carbon-13 NMR spectrum of the isolated compounds are shown in figures 2 and 3 respectively.

Table 3 Comparative Proton and Carbon-13 NMR Data of G23f-9 and Literature

Cn	*^{13}C Shift (ppm) ($CDCl_3$ 75MHz) (Sangsuwon, et al,, 2013)*	*^{13}C Shift (ppm) ($CDCl_3$ 400 MHz) (Hatem, et .al, 2015)*	*$_1H$ Shift/ δ(ppm) ($CDCl_3$, 300 MHz) (Abdullahi, et al., 2011)*	*^{13}C Shift (ppm) ($CDCl_3$) (Abdullahi, et al., 2011)*	*^1H Shift (ppm) ($CDCl_3$) (Isolate G23f-9)*	*^{13}C Shift (ppm) ($CDCl_3$) (Isolate G23f-9)*	*(CH)n*
1	22.20	22.29	1.95,1.71,(2H, ddd)	2.23(t)	1.95, 1.71(2H ddd, H-1a, H-1b)	22.286	CH_2
2	41.50	41.54	2.37,2.27(2H,ddd)	41.5(t)	2.37, 2.27(2H ddd, H-2a, H-2b)	41.573	CH_2
3	213.10	213.25	-	213.2(s)	-	213.26	C
4	58.20	58.24	2.25 (1H, q)	58.2 (d)	2.26 (1H,m,H-4)	58.229	CH
5	42.00	42.16	-	42.1(s)	-	42.153	C
6	41.50	41.30	1.74,1.28 (2H, d)	41.3(t)	1.77,1.28*(2H,m)	41.291	CH_2
7	18.20	18.25	1.49,1.36 (2H,m)	18.2(t)	1.48,1.34(2H,m)	18.239	CH_2
8	53.10	53.11	1.38 (1H dd)	53.1(d)	1.39 (1H, dd)	53.107	CH
9	37.40	37.45	-	37.4(s)	-	37.446	C
10	59.4	59.48	1.53 (1H,m)	59.5(d)	1.55 (1H, dd)	59.480	CH
11	35.60	35.63	1.45,1.26 (2H,m)	35.6(t)	1.48, 1.25 (2H,m)	35.625	CH_2
12	30.50	30.51	1.33,1.32(2H,m)	30.5(t)	1.33, 1.32 (2H m)	30.508	CH_2
13	39.70	39.71	-	39.7(s)	-	39.700	C
14	38.30	38.31	-	38.3 (s)	-	38.299	C
15	32.40	32.43	1.47,1.27 (2H,m)	32.4 (t)	1.48,1.25 (2H,m)	32.093	CH_2
16	36.00	36.02	1.58,1.35 (2H, m)	36.0 (t)	1.58,1.36 (2H,m)	36.010	CH_2
17	30.00	30.03	-	30 (s)	-	30.000	C
18	42.80	42.91	1.56 (1H,m)	42.8 (d)	1.55,(1H,m)	42.790	CH
19	35.30	35.04	1.37,1.22 (2H,m)	35.3 (t)	1.39,1.25(2H,m)	35.346	CH_2
20	28.10	28.18	-	28.2 (s)	-	28.174	C
21	32.80	32.78	1.50,1.31(2H,m)	32.8 (t)	1.48,1.33 (2H,m)	32.770	CH_2
22	39.20	39.29	1.51,0.95 (2H,m)	39.2 (t)	1.48,0.95 (2H,m)	39.255	CH_2
23	6.80	6.83	0.88 (3H,d)	7.0(q)	0.89 (3H,d,H-23)	6.829	CH_3
24	14.60	14.67	0.73 (3H,s)	14.6(q)	0.72 (3H,s,H-24)	14.660	CH_3
25	17.90	17.95	0.87 (3H,s)	17.9(q)	0.87 (3H,s,H-25)	17.949	CH_3
26	20.20	20.27	1.01 (3H,s)	20.2(q)	1.01 (3H,s,H-26)	20.262	CH_3
27	18.60	18.66	1.05 (3H, s)	18.6(q)	1.05 (3H,s,H-26)	18.670	CH_3
28	32.10	32.09	1.18 (3H,s)	32.1(q)	1.18 (3H,s,H-28)	31.781	CH_3
29	35.00	31.80	1.00 (3H,s)	35.0(q)	1.00 (3H,s,H-29)	35.028	CH_3
30	31.80	33.40	0.94 (3 H s)	31.8(q)	0.95 (3H,s,H-30)	32.422	CH_3

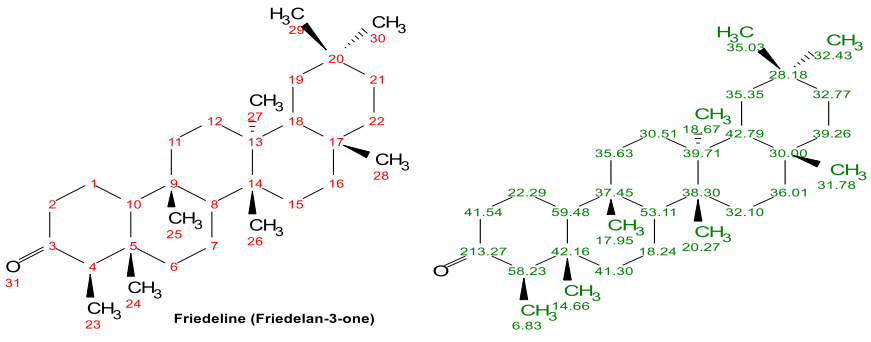

Fig 1: Structural and spectral assignment of isolated compound.

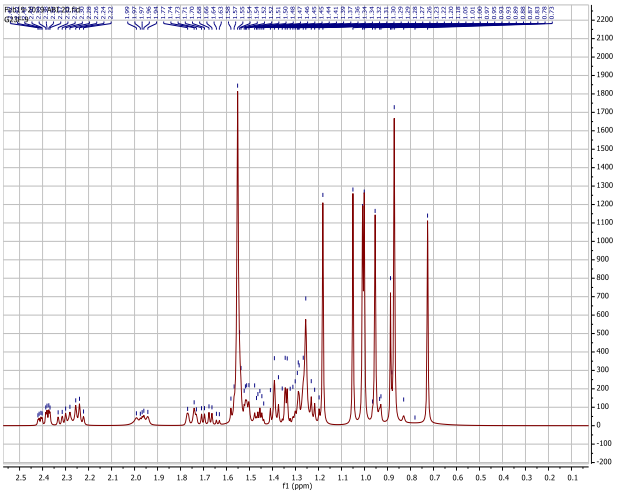

Fig 2: Proton NMR of G23f-9 (zoomed in)

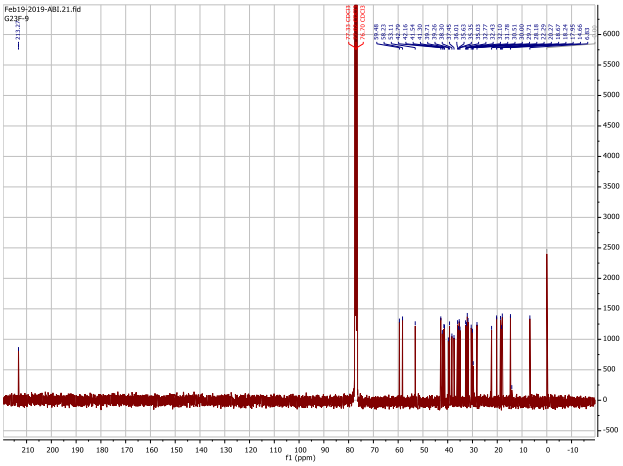

Fig 3: Carbon-13 NMR of G23f-9

Antimicrobial Activity of Isolated Compound: From the ZOI values in Table 4, the isolated compound (G23f-9) showed potency against *S. aureus* (20mm), *S. typhi* (16 mm), *S. pyogenes* (14 mm), *K. pneumoniae* (13 mm), and *P. aeruginosa* (13mm) at 100 µg/mL whereas the respective values for *Ciprofloxacin* (at 10µg/mL) are 24 mm, 20 mm, and 27 mm, 20mm, and 18mm. The antibacterial activity was, interestingly, still observable at 12.5 µg/mL (MIC) for S. aureus, and 25 µg/mL for *S. typhi* while the MBC stood at 50 µg/mL and 100µg/mL respectively (Table 5), indicating bactericidal activities. G23f-9 at 100 µg/mL also showed antifungal activity with ZOI of 11mm against *C. albicans* while *Terbinafine* at 30 µg/mL had antifungal ZOI of 30 mm against the fungus. Friedelan-3-one (Friedelin), the isolated compound, was earlier reported to have potency against *S. aureus*, Methicillin-resistant *S. aureus*, *P. aeruginosa*, and *C. albicans* (Odeh *et al*.., 2016). Also reported by the same authors are activity against *Streptococcus pneumoniae*, *Helicobacter pylori*, *Escherichia coli*, *Enterococcus faecalis*, *Candida tropicalis*, *Candida krusei*, and *Candida glabrata*. Its antimicrobial activity was linked to anti-inflammatory, anti-feedant, hepato-protective and growth inhibitory activities. Others also reported include cytotoxicity against human tumor cell lines (Mossi *et al*.., 2004), gastric antiulceragenic and leishmanicidal activity (Surendra and Corey, 2009) etc. Thus, it is an important medicinal compound with broad-spectrum antimicrobial potency.

Table 4 Diameters of Zones of Inhibition (mm) of isolated compound (G23f-9)

ORGANISM	100 (µg/mL)	50 (µg/mL)	25 (µg/mL)	12.5 (µg/mL)	CIP (10µg/mL)	TBF (30µg/mL)
Salmonella typhi	16	14	12	0	20	-
Staphylococcus aureus	20	16	14	12	24	-
Streptococcus pyogenes	14	0	0	0	27	-
Klebisiela Pneumoniae	13	0	0	0	20	-
Pseudomonas aeruginosa	13	0	0	0	18	-
Candida albicans	11	0	0	0	-	30
Aspergillus niger	**0**	**0**	**0**	**0**	**-**	**35**

Key: - not determined, CIP = Ciprofloxacin, TBF = Terbinafine

Table 5 Minimum inhibitory concentration and minimum bactericidal/fungicidal concentrations (µg/mL) of isolated compound (G23f-9)

ORGANISM	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.13 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL
Salmonella typhi	-ℂ	-	-ℂ	+	+	+	+	+	+	+
Staphylococcus aureus	-	-ℂ	-	-ℂ	+	+	+	+	+	+
Streptococcus pyogenes	-ℂ	+	+	+	+	+	+	+	+	+
Klebisiela Pneumoniae	-ℂ	+	+	+	+	+	+	+	+	+
Pseudomonas aeruginosa	-ℂ	+	+	+	+	+	+	+	+	+
Candida albicans	-ℂ	+	+	+	+	+	+	+	+	+
Aspergillus niger	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**

KEY: + = Growth, - = No Growth; -ℂ = minimum inhibitory concentration, -ℂ= minimum bactericidal /fungicidal concentration

Conclusion: Phytochemical and antimicrobial assays indicated that methanol stembark extract of *Uapaca ambanjensis* could be host to bioactive compounds with activity against gram-positive and gram-negative

bacteria. The stretching of antibacterial activity to low bactericidal concentrations against *S. typhi*, *P. aeruginosa and S. aureus*, and added antifungal activity against *C. albicans* seemed to lay credence to the reported ethnomedicinal uses of the crude plant extracts to treat typhoid fever and skin diseases. The isolation of Friedelan-3-one is, as far as we know, reported for the first time from *U. ambanjensis*.

List of Abbreviations
MAE-Microwave Assisted Extraction
TLC-Thin Layer Chromatography
AST-Antimicrobial Susceptibility Testing
ZOI-Zone of Inhibition
MIC-Minimum Inhibitory Concentration
MBC-Minimum Bactericidal Concentration
MFC-Minimum Fungicidal Concentration
NMR-Nuclear Magnetic Resonance
^{1}H NMR –Proton Nuclear Magnetic Resonance
^{13}C NMR – Carbon -13 Nuclear Magnetic Resonance
Mr- Relative molecular mass

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REFERENCES

Abdullahi, M; Kolo, I; Oyewale, OA; Amupitan, JO; Fatope, MO; Okogun, JI (2011). Antimycobacterial friedelane-terpenoid from the root bark of *Terminalia avicennioides*. *Am J. Chem* 2011;1(2):52-55.

Abdisa, Z; Kenea, F (2021). Phytochemical screening, antibacterial and antioxidant activity studies on the crude root extracts of *Clematis hirsuta*, *Cog. Chem.* 6 (1) pp 2-4

Akacha, LU; Dikko, JY; Khan, ME; Anyam, JV; Igoli, JO(2016). Phytochemical Screening and Antimicrobial Activity of Bryophyllum pinnatum Extracts, *Bri Biotech J*. 16(2): 1-8

Alves, RR; Rosa, IM (2007). Biodiversity traditional medicine and public health: Where do they meet? *J. Ethnobio*. Ethnomed., p.3

Balouiri, M; Sadiki, M; Ibnsouda, SK (2016). Methods for *in vitro* evaluating antimicrobial activity: A Rev, *J. Pharm. Anal.* 6(2016) pp71–79

Bandaranayake, WM (2006). "Quality control, screening, toxicity, and regulations of herbal drugs," In: Igbal, A; Farrukh, A; Muhammad, O (Eds) Modern Phytomedicine, Turning Medical Plants into Drugs, 10, p.25.

Benkova, M; Soukup, O; Marek, J (2020). Antimicrobial susceptibility testing: currently used methods and devices and the near future in clinical practice, *J. App. Microbio. (ISSN* 1364-5072) pp 808-811

Beutler, JA (2009). Natural products as a foundation for drug discovery. *Curr. Proto.Pharm.* 46(1), pp.9-11.

Breteler, FJ (2013), Uapaca (Phyllanthaceae) in the Guinea-Congolian Forest Region: a Synoptic Revision. *Plant Eco. and Evo.*, 146: 75-94.

Bush, LM; Charles, E(2021). Staphylococcal Infections, MSD Manual (Professional edition), Merck and Co. Inc, Kenilworth, NJ, USA, pp1-12

Chan, CH; Yusoff, R; Ngoh, GC (2013). Modeling and prediction of extraction profile for microwave-assisted extraction based on absorbed microwave energy. *Food Chem.*, 140(1–2), 147-153.

Dankaddai, H; Okafor, GCO (2019). Medicinal Potentials of Virgin Plants: The Case of *Beloperone asclepiadea* Nees (Acanthaceae), *Intl. J. Engr. Res. Tech*. (IJERT), Vol. 9 Issue 03, March 2020, pp 204.

Evans, WC (2009). Pharmacognosy, In: Evans, WC (ed), Trease and Evans Pharmacognosy 16th Edition, Saunders Ltd. London p191-293.

Hatem SA.; Ehab, AR; Abd-El-Salam I; Mohammed, A; El-Hela, A (2015). Phytochemical and Biological Investigation of *Ficus mysorensis* Cultivated in Egypt, *J. Pharm. Chem. Biol. Sci.* Volume 3(3): pp.396-407

Harborne, JB (1998). Phytochemical Methods (A Guide to Modern Techniques of Plant Analysis), Third Edition, Chapman and Hall, London, pp 6, 11-29.

Kebede, T; Gadisa, E; Tufa, A (2021). Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment multi-resistant microbes, In: Iddya, K (ed) Antimicrobial Role of Phytochemicals Extracted from Traditional Medicinal Plants

(https://doi.org/10.1371/journal.pone.0249253, March 26, 2021, pp5-6

McPherson, G (2011). A Review of Madagascan Uapaca (Euphorbiaceae s.L). *Adansoma*, I, II, (33), 221-231.

Mossi, AJ; Cansian, R; Carvalho, ZA; Dariva, C; Oliveira, VJ; Mazutti, M; Filho, IN; Echeverrigaray, S(2004). Extraction and characterization of volatile compounds in *Maytenus ilicifolia*, using high-pressure CO_2. *Fitoterapia*, 75: 168-178.

Ochigbo, C (68). Personal Communication, 12th October 2017-31st October 2017.

Odeh, IC; Tor-Anyiin, TA; Igoli, JO; Anyam, JV (2016). *In vitro* antimicrobial properties of friedelan-3-one from *Pterocarpus santalinoides* L'Herit, ex Dc, *Afr. J. Biotech.* Vol. 5(14), pp531-538

OIE *Terrestrial Manual* (2012). Laboratory Methodologies for Bacterial Antimicrobial Susceptibility Testing, *Guideline* 2.1. (http://www.oie.int/en/our-scientific-xpertise/reference-laboratories/list-of-laboratories/) pp 4-6.

Sangsuwon, C; Jiratchariyakul, W; U-pratya, Y; Kummalue, T (2013). Antiproliferative Effect and the Isolated Compounds of *Pouzolzia indica* Hindawi, Publishing Corporation, *Evid.-Based Compl. Alt. Med. Paper* ID 342352, pages 3-4.

Surendra, K; Corey, EJ (2009). A Short Enantioselective Total Synthesis of the Fundamental Pentacyclic Triterpene Lupeol. *J. Am. Chem. Soc.* 131: 13928-13929.

Truong, D; Nguyen, DH; Ta, NTA; Biu, AV; Do, TH; Nguyen, HC (2019). "Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidant, and In Vitro Anti-Inflammatory Activities of *Severinia buxufolia*", *J. Food Qual.* vol. 2019 (Article ID 8178294) pp 1-6

Ubochi, M (68), Personal Communication (Agbaje, Nwangele L.G.A. Imo State), 6th November,2021.

WHO Global Report on Traditional and Complementary Medicine (2019). Geneva: World Health Organization, License: CC BY-NC-SA IGO p.10-13, 2019.