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Research Article

Antimicrobial activity of compounds isolated from the leaves of *Aspilia africana* (Pers.) C. D. Adams (Asteraceae)

Ekarika C. Johnson [a,*], Mohammad Ilyas [b], Mohammed I. Sule [b], Olorunfemi A. Eseyin [a], Hammed O. Oladimeji [a], Emmanuel I. Etim [a], and Aniefiok S. Udobre [a]

[a] *Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Nigeria*
[b] *Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Nigeria*

* **Corresponding author:** Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo P. M. B. 1017, Uyo, Nigeria; **Tel**.: +234-70-37476173; **Email**: rikaisable@gmail.com

Background: Incidences of serious failures in the treatment of infectious disease by antibiotics caused by the emergence and spread of drug resistant strains of the microorganisms/multiple drug resistant bacteria have led to new global search for more effective anti-infective microbial agents from natural sources. This study intends to examine the anti-microbial potentials of the leaves of *Aspilia africana,* which is employed in the treatment of wounds and sores by traditional medical practitioners in Nigeria.

Objective: To evaluate the anti-microbial potentials of the isolates from leaves of *Aspilia africana* (Pers.) C. D. Adams (Aristeraceae), using isolated clinical strains of pathogens such as *Staphylococcus aureus, Methicillin Resistant Staphylococcus aureus (MRSA), Streptococcus pyogenes, Bacillus substilis, Proteus vulgaris, Salmonella typhi, Shigella dysenteriae, Escherichia coli, Klebsiella pneumonia, Candida albicans* and *Candida stellafoidea*.

Methodology: Three compounds isolated from butanol fraction of the methanol extract of the dried powdered leaves of *Aspilia africana* through repeated silica gel column-chromatography and sephadex gel filtration, were evaluated for anti-microbial potentials using Agar-well diffusion method.

Results: The isolated compounds identified as oleanolic acid, ursolic acid, and corosolic acid by 1D, 2D-NMR and FITR spectroscopic analyses inhibited the growth of all the pathogens with inhibition diameters ranging between 25 – 33 mm compared with the standard drugs used. The MIC, MBC/MFC of the plant extracts ranged from between 5.00 and 10.00 mg/mL while that of the isolated compounds ranged between 0.0125 and 0.0500mg/mL

Discussion: From the results, we conclude that isolated compounds namely oleanolic acid, ursolic acid and corosolic acid are the bioactive constituents responsible for the anti-microbial activity of *Aspilia africana*.

Key words: Aspilia africana, Anti-microbial, Oleanolic acid, Ursolic acid, Corosolic acid

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1. Introduction

Microbial infection is the invasion of the body by microorganisms that can cause disease (American Heritage Dictionary, 2011). Microorganisms that can cause infection include bacteria, fungi, protozoans, and viruses. One type of microbial infection that poses serious health risk is wound infection. Studies have shown that 70 percent of the deaths of patients who have undergone surgery are caused by surgical site infections (Plowman, 2000). Infected wounds are wounds in which bacteria or other microorganisms have colonized, causing a delay in wound healing and deterioration of the wound. The most common causative organisms associated with wound infections include *Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus*

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(MRSA), Streptococcus pyogenes Enterococcus faecalis and Pseudomonas aeruginosa (Calvin, 1998). However, infected wounds result when the body's immune defenses are overwhelmed or cannot cope with normal bacterial growth. The skin contains bacteria (normal flora) which are normally harmless if the skin is intact. However, the protective barrier formed by the skin is disrupted when there is a wound, and these normal floras are able to colonize the injured area. This results in further tissue damage and may prolong wound healing by promoting more inflammation. Infected wounds can have serious local and systemic complications. The infection can also affect the surrounding tissues and may cause a bacterial skin infection (cellulitis) or an acute or chronic bacterial bone infection (osteomyelitis) (Wound Infection, 2016). If the infection spreads to the blood vessels, the bacteria may spread and cause infection in other areas of the body. Wound infection could be treated by the use of antibiotics that may be applied directly to the wound (topical) or taken orally (systemic) (Ngan, 2005)). Wound microbial infection has become a very serious problem and health risk due to emergence and spread of drug resistant strains of the microorganisms (Kapil, 2005; Todar, 2007). This is further compounded by the emergence of multiple drug resistant bacteria (MDR) which is now a major cause of failure in the treatment of infectious disease by antibiotics (Adeola et al, 2013). The search for alternative natural antibiotics has become the most feasible way of remedying the problem of failing drugs. That is why plants and other natural sources have become targets of research now.

One plant that is known to have many biological activities and is widely used in African traditional folkloric medicine is *Aspilia africana*. The leaves of *Aspilia africana* possess haemostatic, abortifacient, ecbolics, lactation stimulating, analgesic and sedative properties and is employed in the treatment of cutaneous, subcutaneous parasitic infection (including veterinary), naso-pharyngeal, skin and mucosal infections as well as venereal diseases (Burkill, 1995). *Aspilia africana* is an herb, which can grow up to 2 m high and occurs throughout the savannah and forested zones of tropical Africa. It is found in all parts of Nigeria (Dalziel, 1955). In this research work the anti-microbial potentials of the methanol extract, butanol fraction and isolates from leaves of *Aspilia africana* were studied using selected pathogens usually implicated in wound infection namely: *Staphylococcus aureus, Methicillin Resistant Staphylococcus aureus (MRSA), Streptococcus pyogenes, Bacillus substilis, Proteus vulgaris, Salmonella typhi, Shigella dysenteriae, Escherichia coli, Klebsiella pneumonia Candida albicans,* and *Candida stellafoidea*.

2. Materials and Methods

2.1 Reagents and Analytical Equipment

Melting points of the isolates were determined using Electrothermal Melting point Apparatus. The UV-spectrum was recorded on Pye Unicam. The silica gel for column chromatography (60 – 120 mesh), sephadex LH-20 and the analytical grade reagents used were obtained from Sigma Aldrich. Preparative TLC plates: silica gel 60 PF254 (Merck, Germany); visualized under $UV_{(\lambda max)}$ 254 and 364 nm and detection with I_2 and 10% ethanol H_2SO_4 spray. ^1H and ^{13}C NMR, COSY, HMQC and HMBC spectra: JEOL AS 400,400/100 MHz, 500, 500/100 MHz) spectrometer (Japan), DMSO-d_6 and $CDCl_3$ as solvents and TMS as internal standard. Solvent peaks were at 7.26 ppm for $CDCl_3$ and 2.50 ppm for DMSO-d_6. The chemical shifts were reported as δ ppm relative to TMS, Chemical shifs in ppm, coupling constants (J) in Hz. The samples were run at temperature of 25°C. Mass spectra were obtained using HR-EI-MS: JEOL-JMS-HX-110 mass spectrometer; EI, source at 250° and 70 eV. FTIR (KBr) was recorded using 8400S Shimadzu, Japan. The pathogenic microorganisms obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH), Zaria. Mueller-Hinton agar medium (Sigma-Aldrich, USA) was used for the anti-bacterial study while Sabouraud dextrose agar medium (Sigma-Aldrich, USA) was used for antifungal study and nutrient agar medium for storing and preserving the bacterial organisms. Ciprofloxacin (obtained from Trivenic Chemicals - India) and Fluconazole (from MDC Pharmaceuticals – India) were used as positive control drugs for bacterial and fungal strains respectively.

2.2 Collection and Identification of Plant Materials

The fresh leaves of *Aspilia africana* collected in Zaria, Northern Nigeria was identified by the taxonomist in the Herbarium unit of the Department of Biological Science, Ahmadu Bello University, Zaria. A voucher (Specimen No. **1146**) was deposited at the Herbarium. The plant material was washed to remove dust and other dirt and dried under shade for several weeks. This dried material was pulverized and sieved. The weighed, powdered sample was stored in a labeled airtight container until when needed for use.

2.3 Extraction

The powdered leaf material (1kg) was cold-macerated in H_2O-MEOH (30:70) for 72 h, filtered and concentrated *in-vacuo* to give dark-brown gummy methanol extract (150.8 g, 15.08 %). The extract was dissolved in water and the water soluble portion was sequentially and exhaustively extracted with n-hexane, dichloromthane, ethyl acetate and n-butanol to give (after removing the solvent *in-vacuo*), n-hexane (15.0 g, 1.50%), dicloromethane (18.50 g, 1.85 %), ethyl acetate (10.70g, 1.07 %) and n-butanol (35.80 g, 3.58 %) fractions respectively.

2.4 Hydrolysis of the n-butanol fraction

Acid hydrolysis (Prawat, et al, 1989) was performed on the n-butanol fraction, which was noted to froth copiously and the only fraction that showed substantial anti-microbial activity against the pathogens. 6 g of n-butanol fraction was dissolved in 50 mL methanol in a 250 mL beaker and diethyl ether was added slowly by the side of the beaker until the precipitation was complete. The precipitate was centrifuged and the solid residue dried to strip of the solvent completely. This was weighed and placed in a round-bottom flask to which 50 mL 4 M HCl (dioxane, 1:1 H_2O) was added. The set-up was refluxed on a water bath for 5 h at 90 °C. The resulting mixture was cooled and extracted with $CHCl_3$. The $CHCl_3$ (organic) extract was evaporated to dryness *in-vacuo*. The aqueous part was tested for the presence

of sugars using comparative TLC with simple monosaccharide sugars such as glucose, rhamnose, galactose, mannose and xylose.

2.5 Isolation

2 g of the BUOH fraction was subjected to chromatographic separation on silica gel (60-120 mesh) column (45 x 3.5cm) and eluted with $CHCl_3$/MEOH (99:1) in increasing order of polarity. This afforded 12 fractions (HYD1-12) based on thin layer chromatography (TLC) analyses. Fraction HYD4 on gel filtration with sephadex LH_{-20} eluted with $CHCl_3$ and MEOH afforded oleanolic acid **1** (225 mg). Fraction HYD6 afforded ursolic acid **2** (14 mg) on recrystallization with methanol. Fraction HYD7 was subjected to repeated gel filtration using sephadex LH_{-20} eluted with $CHCl_3$ and MEOH and finally purified with preparative thin layer chromatography to afford corosolic acid **3** (25mg)

2.6 *In-vitro* Antimicrobial Activity Assay

The antimicrobial activity of the isolates were determined using Agar-well diffusion technique (Adeniyi et al, 2013). 0.4 g each of the extracts and fractions was dissolved in 10 mL of the DMSO and further two-fold dilution was made to obtain concentrations of 40 mg/mL, 20 mg/mL, 10 mg/mL, 5 mg/mL and 2.5 mg/mL. For the isolated compounds, 0.002 g was dissolved in 10 mL DMSO to obtain initial stock concentration of 200 µg/mL, which was further diluted to 100 µg/mL, 50 µg/mL, 25 µg/mL and 12.5 µg/mL. 0.05 mg of the standard drugs was dissolved in 10 mL of DMSO to obtain a concentration of 50 µg/mL. Nutrient agar plates were each seeded with 0.1 ml of an overnight culture of each bacterial strain (equivalent to 10^7 CFU/mL), while the Sabourand dextrose agar plates were similarly seeded with each fungal strain. The seeded plates were allowed to set and then dried in the incubator at 37 °C for 20 min. A standard cork borer of 6 mm diameter was used to cut uniform wells on the surface of the agar, into which was added 0.1 mL test solutions suspended in DSMO (40 mg/mL for the extract and fractions and 200 µg/ml for the isolates). Nutrient agar plates with bacterial isolates were incubated at 37 °C for 24 h and Sabourand dextrose agar plates seeded with fungal strains were incubated at 25 °C for 72 h after which diameters of zones of inhibition were measured. This procedure was repeated for the standard chemotherapeutic agents used as positive controls namely Ciproflaxacin (50 ug/mL, for bacteria) and Fluconazole (50 ug/mL, for fungi). All the assays were performed in triplicate. The minimum inhibitory concentration (MIC) and minimum bacterialidal concentration (MBC)/minimum fungicidal concentration (MFC) tests were performed according procedures reported by Adeniyi et al, (2013).

3. Results and Discussion

The aqueous portion of the butanol fraction indicated the presence of only glucose when compared with a reference glucose sample. Structural characterizations were done using a combination of 1D, ^1H ^{13}C and 2D experiments. Hydrogen attachments to carbon were identified by DEPT; ^1H. H COSY and HMQC spectra. Long range correlations were found in the HMBC spectrum that also helped in assigning chemical shift to quaternary carbons. ^1H and ^{13}C resonance arrangements for **1**, **2** and **3** (**Figure 1**) are in perfect agreement with literature data (Seebacher et al, (2003).

Oleanolic acid (OA, **1**) was isolated as a white amorphous powder, mp 299-302 ºC. UV(λ_{max}): 221(c = 0.348, $CHCl_3$). The molecular formula $C_{30}H_{48}O_3$ was determined on the basis of EI-MS. (calcd. 456 for $C_{30}H_{48}O_3$). FTIR (KBr) cm^{-1}: 3436 (OH); (2931) C-H str, 1728 (C=C), 1389 (gem-dimethyl at C-4), 1218 (two methyls on qua-ternary carbon atom at C-20) and 1039 (C-O). ^1H NMR (400 MHz, DSMO-d_6) ppm: δ_H 5.35(1H, t, J=4 Hz, H-12, C=C), 3.15 (1H, dd, J = 3.5 Hz, H-3, OH), 2.27 (1H, t, J = 14 Hz), 1.15(3H, s, H-30), 0.91(3H, s, H-23), 0.93 (3H, s H-24) 0.93 (3H, s, H-27), 0.91(3H, s, H-29), 0.79 (3H, s, H-25) and 0.76 (3H, s, H-26). The ^{13}C NMR, HMBC, HMQC and DEPT NMR showed thirty carbon atoms, consisted of eight quaternary, five tertiary, ten secondary carbons and seven methyl groups: ^{13}C NMR (100 MHz, DSMO-d_6) ppm: δ_C 178.02(C-28), 144.12(C-13), 122.24 (C-12), 77.78 (C-3) 55.39 (C-5), 47.70 (C-9), 46.05 (C-19), 45.99 (C-17), 41.70 (C-8), 41.70 (C-14) 41.43 (C-18), 39.35(C-1), 38.69 (C-21), 38.51 (C-4), 37.01 (C-10), 33.64 (C-7), 32.84 (C-20), 32.55 (C-29), 30.51 (C-15), 27.89 (C-22), 27.63 (C-2), 27.29 (C-23), 25.45 (C-30), 23.32 (C-11), 23.07 (C-16), 22.95 (C-17), 18.33 (C-6), 16.80 (C-25), 15.50 (C-26) and 14.98 (C-24).

Ursolic Acid (UA, **2**) was obtained as a white amorphous powder (14 mg), mp 284-287°C, R_f : 0.70 ($CHCl_3$–MeOH, 9:1). UV(λ_{max}): 222 (c = 0.647, $CHCl_3$). The molecular formula $C_{30}H_{48}O_3$ was determined on the basis of EI-MS. (calcd. 456 for $C_{30}H_{48}O_3$). FTIR (KBr) cm^{-1}: 3450 (OH), 2931(CH stretch), 1644.37 (C=C), 1373 (gem-dimethyl at C-4) and 1045 (C-O). The NMR resonances are similar to those of oleanolic acid. ^1H NMR (400 MHz, DSMO-d_6) ppm: δ_H 5.58 (1H, brs, H-12, C=C) 4.08 (1H, dd, J=6.2, 11.0 Hz, H-3), 1.45(1H, H-18), 1.28 (3H, s, H-27), 1.03 (3H, d, J= 4Hz, H-29), 0.99 (3H, s, H-23), 0.99 (3H, s, H-24), 0.98 (3H, d, J=6.2 Hz, H-30), 0.96 (3H, s, H-25) and 0.93 (3H, s, H-26). ^{13}C NMR spectrum showed 30 signals, consisted of seven quaternary carbons, seven tertiary, nine secondary and seven methyl groups. ^{13}C NMR (100 MHz, CD_3OD) ppm: δ_C 173.69 (C-28), 145.19 (C-13), 121.65 (C-12), 80.58 (C-3), 55.26 (C-5), 47.55 (C-18), 47.55 (C-9), 47.24 (C-17), 41.70 (C-14), 39.81 (C-8), 39.62 (C-20), 39.62(C-19), 39.38 (C-1), 38.25(C-4), 37.83 (C-10), 37.15 (C-22), 33.34 (C-7), 30.06 (C-21), 29.18 (C-15), 28.75 (C-23), 28.40 (C-2), 25.96 (C-27), 24.28 (C-16), 23.69 (C-11), 21.28 (C-30), 18.27 (C-6), 17.51 (C-26), 16.78 (C-29), 16.58 (C-24) and 16.36 (C-25).

Corosolic Acid (CA, **3**) crystallized in MEOH as a white amorphous powder (25 mg), mp 238-242 °C; R_f: 0.79 ($CHCl_3$–MeOH, 9:1). UV (λ_{max}): 262 (c = 2.310, $CHCl_3$). The molecular formula $C_{30}H_{48}O_4$ was determined on the basis of EI-MS. (calcd. 472 for $C_{30}H_{48}O_4$). FTIR (KBr) cm^{-1}: 3472 (OH), 2930 (CH stretch), 1725 (C=C), 1389 (gem-dimethyl at C-4), 1167 and (tert. alc. C-O str.). ^1H-NMR (500 MHz, $CDCl_3$) ppm: δ_H 5.43 (1H, t, J = 4.0 Hz, H-12, C=C), 4.45 (1H, dd, J = 4.0, 12.0 Hz, H-2), 4.02 (1H, d, J = 10.1 Hz, H-3) and 3.09 (dd, J = 14.2, 4.0 Hz, H-18), five singlet methyls at δ_H 1.01 (H-23), 1.005, H-24), 1.021 (H-25), 1.031, (H-26), 0.965, (H-27), and two doublet methyls at δ_H 0.996 (J = 6.7 Hz, C-29) and 0.97, J = 6.8 Hz, H-30). ^{13}C NMR (100 MHz, $CDCl_3$) ppm: δ_C 173.749 (C-28), 145.00 (C-13), 121.658 (C-12), 80.622 (C-3), 64.418 (C-2), 64.418 (C-18), 55.260 (C-5), 47.556 (C-9), 47.555

(C-17), 47.242 (C-20), 46.795 (C-1), 41.725 (C-14), 40.849 (C-8), 39.818 (C-4), 39.374 (C-19), 38.258 (C-10), 34.873 (C-7), 34.434 (C-22), 29.696 (C-21), 29.175 (C-15), 28.394 (C-23), 24.792 (C-30), 23.792 (C-27), 23.607 (C-11), 22.687 (C-16), 19.026 (C-26), 18.268 (C-6), 16.992 (C-25), 16.811 (C-24), 16.772 (C-29).

OA and UA are isomeric triterpenic acids and always simultaneously exist in the same plant (Liu, 1995; Xu et al, 2012). Their spectroscopic data are similar depicting their structural similarities with the only difference being the positions of the methyl groups of C-29 and C-30. In oleanolic acid, the two groups are "geminally" attached to C-20 and this is a feature of β-amyrins to which oleanolic acid belong, thus making C-19 to have two protons attached to it. This is clearly established by appearance of a triplet observed at 2.27 ppm with a J value of 14.0 Hz in NMR spectra indicating coupling between a single proton at C-18 and two protons at C-19 as deduced from ^{1}H ^{1}H COSY and HMQC spectra. This triplet only appears if the compound is of theβ-type triterpene -oleanane ; where only protons (two protons) are attached to C-19 (Bhatt, 2011). This is confirmed by FTIR absorption at 1218 cm^{-1} (two methyls on qua-ternary carbon atom- indicating the two methyl groups on C-20 which distinguishes it from the isomer (ursolic acid, Fig. 1), which has one methyl group at this position (Mann et al, 2012). The α –type orientation in compound 2 was established by appearance of a doublet at δH 2.35 (br-d, J = 10.2 Hz); assigned to H-18, (a doublet appears here because the two groups attached to C-19 are hydrogen and methyl and the coupling between a single proton at C-18 and C-19 would produce a doublet (Bhatt, 2011). The FTIR spectrum of compound **2** shows that the peak at 1218 cm^{-1} is absent; showing that two methyls on qua-ternary carbon (C-20) atom- is absent confirming that **2** is an ursane triterpene. Compound 3 (Corosolic acid) is a derivative of ursolic acid having additional –OH group at C-2. The NMR data for compound 3 are similar to those of compound **2** (ursolic acid) except the chemical shift at δC 64.418 ppm which is assigned to C-2 because of the second OH in the molecule. In comparison, the ^{13}C NMR data of compound **3** showed a good agreement with reported data of corosolic acid (Park et al, 2002; Lee and Juang, 2005).

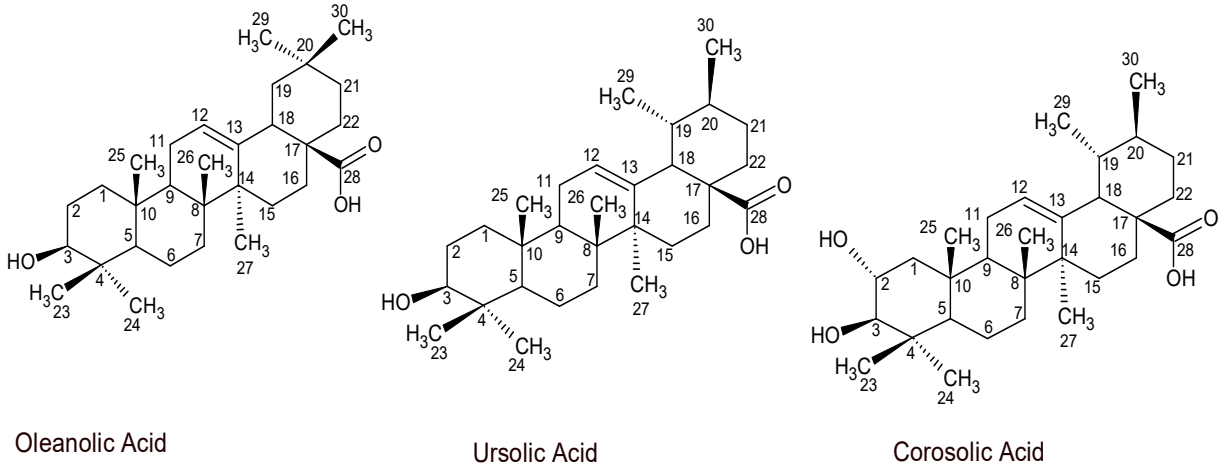

Figure 1: Structures of Oleanolic acid (OA, **1**), Ursolic Acid (UA, **2**) and Corosolic Acid (CA, **3**)

Table 1: Susceptibility of bacterial and fungal strains to the isolates, extracts and control drugs

Test Organism	Zone of inhibition (mm)						
	OA	UA	CA	ME	BE	CP	FL
Staphylococcus aureus	31.21±0.20	32.60±0.33	30.21±0.50	20.00±.0.3	24.10±0.1	37.00±0.0	0
MRSA	30.7±0.210	32.30±1.55	28.02±0.10	21.50±0.3	22.00±0.0	32.00±0.1	0
Streptococcus pyogenes	29.02±0.15	26.11±0.31	25.02±0.10	0.00	0.00	38.00±0.2	0
Bacillus cereus	30.25±0.20	27.10±0.70	26.25±0.20	20.10±0.1	27.11±0.5	40.30±0.3	0
Proteus vulgaris	25.22±0.80	30.55±0.67	29.15±0.80	0.00	0.00	0.00	0
Salmonella typhi	28.30±0.40	31.01±2.11	25.30±0.30	21.00±0.0	24.24±0.2	32.20±0.5	0
Shingella dysenteriae	26.10±0.10	27.06±0.89	27.10±2.50	0.00	0.00	39.30±0.4	0
Klesiella pneumonae	25.20±0.10	26.32±0.10	27.20±0.80	22.32±0.1	25.20±0.2	30.32±0.8	0
Escherchia coli	31.50±0.40	28.06±1.40	26.20±3.40	22.06±1.4	25.33±0.5	42.36±0.7	0
Candida albicans	26.02±0.20	25.30±0.50	25.08±0.70	20.30±0.5	22.10±0.1	0.00	40.2±0.2
Candida stellatoidea	27.22±0.20	25.40±2.21	27.25±0.90	10.0±0.2	12.0±0.2	0.00	34.1±0.2

Values are mean ± standard error of mean, diameter of cork borer = 6 mm

OA: Oleanolic acid; UA: Ursolic acid; CA: Corosolic acid; ME: Methanol extract; BE n-Butanol fraction; CP: Ciproflaxacin; FL: Fluconazole.

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Table 2: MIC and MBC of the isolates, extracts, and the control drugs on the susceptible bacteria and fungi strains

Organism		OA mg/mL	UA mg/mL	CA mg/mL	ME mg/mL	BF mg/mL	Control* mg/mL
Staphylococcus aureus	MIC	0.0250	0.0125	0.0250	5.00	5.00	0.003125
	MBC	0.0500	0.0125	0.0500	5.00	5.00	0.003125
MRSA	MIC	0.0250	0.0125	0.0500	5.00	5.00	0.003125
	MBC	0.0250	0.0125	0.0500	5.00	5.00	0.003125
Bacillus cereus	MIC	0.0250	0.0125	0.0250	5.00	5.00	0.003125
	MBC	0.0250	0.0125	0.0250	5.00	5.00	0.003125
Proteus vulgaris	MIC	0.0500	0.0125	0.0250	5.00	5.00	0.003125
	MBC	0.0500	0.0125	0.0250	5.00	5.00	0.003125
Pseudomenas fluorescence	MIC	0.0250	0.0500	0.0250	10.00	5.00	0.003125
	MBC	0.0250	0.0500	0.0250	10.00	5.00	0.003125
Salmonella typhi	MIC	0.0250	0.0125	0.0250	10.00	5.00	0.003125
	MBC	0.0250	0.0125	0.0250	10.00	5.00	0.003125
Shingella dysenteriae	MIC	0.0250	0.0125	0.0250	5.00	5.00	0.003125
	MBC	0.0250	0.0125	0.0250	5.00	5.00	0.003125
Klebsiella Pneumonae	MIC	0.0500	0.0125	0.0250	5.00	5.00	0.003125
	MBC	0.0500	0.0125	0.0500	5.00	5.00	0.003125
Escherchia coli	MIC	0.0500	0.0125	0.0250	10.00	10.00	0.003125
	MBC	0.0500	0.0125	0.0500	10.00	10.00	0.003125
Candida albicans	MIC	0.0500	0.0125	0.0500	0.00	10.00	0.003125
	MFC	0.0500	0.0125	0.0500	0.00	10.00	0.003125
Candida stellatoidea	MIC	0.0250	0.0125	0.0250	0.00	0.00	0.003125
	MFC	0.0250	0.0125	0.0250	0.00	0.00	0.003125

OA: Oleanolic acid, UA: Ursolic acid, CA: Corosolic acid, ME: Methanol extract, BF: Butanol fraction,
**Ciproflaxcin for Bacteria, Fluconazole for fungi. (0.001mg/mL =1.000 µg/mL)*

The isolates obtained from the leaves *Aspilia africana* demonstrated broad-spectrum anti-microbial activities as shown in **Table 1**. These microorganisms were selected based on their established identity as wound pathogens and are always implicated in wound sepsis and sores (Hugo and Russel, 1983). The results show that all the pathogens including highly resistant bacterial strain like MRSA, *C. albicans* and *C. stellatoidea* were highly susceptible, with diameter of zones of inhibition ranging between 25.00 and 32.00 mm (Table 1) (Ibeh and Uraih 2003). The anti-bacterial and anti-fungal activities of the plant extracts and isolated compounds compared favourably with that of the standard drugs (ciprofloxacin and fluconazole). The MIC of the plant extracts ranged between 5.00 and 10 mg/mL, while that of the isolated compounds ranged between 0.0125 and 0.0500 mg/mL. The MIC and MBC/MFC of the plant extracts and isolates were obtained as shown in **Table 2**. The LD_{50} of the methanol extract of the leaves was obtained from acute toxicity test as 4472.14 mg/kg. There are several reports of significant antimicrobial activity by OA, UA and CA isolated from diverse plants. Studies have shown that UA and OA are the specific agents responsible for the anti-mycobacterial activity of *Lantana hispida* and *Chamaedora tepejilote* (tropical plants from Mexico used in folklore medicine to treat tuberculosis and respiratory diseases).

The study shows, UA and OA isolated from these plants to have the *in vitro* anti-mycobacterial activity against the reference drug sensitive *Mycobacterium tuberculosis* strain H37Rv, mono-resistant H37Rv strains, several MDR clinical isolates and a group of non-tuberculous mycobacteria with MIC ranging from 12.5 to 100 µg/mL. The report also shows that UA and OA which are isomers, when combined produced *in vitro* intracellular and *in vivo* synergistic effects with lower MIC values (Jiménez-Arellanes et al, 2013).

In another study by Kim et al (2012), the minimum bacterial concentrations (MBC) values of UA and OA against 19 MRSA strains showed broad ranges; 4 to 32 µg/ml and 16 to >256 µg/mL, and minimum inhibitory concentrations (MIC) values of 4 – 8 µg/mL and 16 to 32 µg/mL respectively. This compares favourably with results obtained in this present study which range between 12.5 and 25 µg/ml for both MBC and MIC for MRSA. Rivero et al (2009) determined MIC for OA which ranged from 212 to 848 µg/mL for, *Klebsiella pneumoniae, Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Streptococcus pneumonia*; these values are far above the doses observed in this study. This indicates that the microbial activity of these triterpenic acids is not much higher than traditionally used antimicrobials, but the study on them is worthy because they are natural source products, and no resistance is found yet (Wolska et al, 2010). They are therefore potentially excellent agents for eradicating the growth of micro-organisms.

4. Conclusion

The findings of this study indicate that the bioactive isolates are very effective and can tackle the problem of resistance of the virulent microorganisms. It can be concluded that, these bioactive constituents are responsible for anti-microbial activity of the leaves of this plant. The isolation of oleanolic acid, ursolic acid and corosolic acid (pentacyclic triterpenic compounds) with well-defined therapeutic uses such anti-microbial, hepatoprotective anti-hyperglycaemic and anti-inflamatory from *A. aficana* lends credence to the traditional use of the plant in the treatment of these disease conditions especially those of microbial origins.

Conflict of Interest Declaration

The authors declare no conflict of interest.

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