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Full Length Research Paper

Triterpenoids from *Vernonia auriculifera* Hiern exhibit antimicrobial activity

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Phytochemical investigation of *Vernonia auriculifera* afforded farnesylamine, a sesquiterpene amine that has not been found previously in plant species, together with lupenyl acetate, oleanolic acid, β-amyrin acetate, β-amyrin, friedelanone, friedelin acetate, α-amyrin and β-sitosterol. The compounds were characterized using nuclear magnetic resonace (NMR) spectroscopy and comparison with literature values. The isolated triterpenoids exhibited moderate antibacterial activity; α and β-amyrin had minimum inhibitory concentration (MIC) of 0.25 mg/ml against *Staphylococcus aureus, Bacillus subtilis*, *Enterococcus faecium* and *Staphylococcus saprophyticus* while lupenyl acetate and oleanolic acid exhibited MIC of 0.25 mg/ml against *Stenotrophomonas maltophilia*. Sub-MIC exposure of *β*-amyrin acetate was effective in decreasing adhesion of *S. aureus, Klebsiella pneumonia* and *E. faecium* while oleanolic acid decreased adhesion of *K. pneumonia* and *Pseudomonas aeruginosa* significantly at sub-MIC. These compounds show potential for synergistic coupling with antimicrobial agents to improve therapeutic efficiency, in the face of rising bacterial resistance.

Key words: *Vernonia auriculifera*, triterpenoids, farnesylamine, antibacterial activity.

INTRODUCTION

The genus *Vernonia* (Asteraceae family) has more than 1000 species growing all over the world with more than 30 species growing in Kenya (Beentje, 1994; Oketch-Rabah et al., 1997). *V. auriculifera* is a small tree or woody herb that grows between 1 and 7.5 m high and is easily recognizable by its deep purple flowers. *V. auriculifera* has a wide variety of applications in traditional medicine. A drop of the juice squeezed from the crushed stem bark, inserted into the nostrils, is known to relieve headache (Kusamba, 2001). The Kikuyu people of central Kenya use the leaves of this plant as a wrap for pounded material used as a poultice (Kokwaro, 1976). Heated crushed leaves of *Aspilia mossambicensis,* are tied in the leaf of *V. auriculifera,* and then applied over the eyes to treat conjunctivitis (Muthaura et al., 2007). Cold water infusion of *V. auriculifera* is administered orally in Uganda and Kenya to treat fever associated with viral and bacterial infections (Muthaura et al., 2007; Freiburghaus et al., 1996). In Ethiopia, the roots are used to treat toothache (Mirutse et al., 2009) and snake poison (Mesfin et al., 2009).

Hydroperoxides of unsaturated fatty acid methyl esters previously isolated from *V. auriculifera* were found to have lethal toxicity (Keriko et al., 1995a). Plant growth stimulators have also been identified from this plant (Keriko et al., 1995b). Other *Vernonia* species that have received extensive phytochemical and pharmacological research include: *V. galamensis* (Miserez et al., 1996), *V. brachycalyx* (Oketch-Rabah et al., 1997), *V. colorata* (Rabe et al., 2002), *V. amagdalina* (Erasto et al., 2006), *V. cinerea* (Chen et al., 2006), *V. mapirensis* (Morales-Escobar et al., 2007), *V. cumingiana* (Mao et al., 2008), *V. ferruginea* (Malafronte et al., 2009) and *V. scorpioides* (Buskuhl et al., 2010). Members of the genus *Vernonia* are an excellent source of sesquiterpene lactones which

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Abbreviations: TMS, Tetramethylsilane**; MIC,** minimum inhibitory concentration; **TSB,** tryptone soy broth; **DMSO,** dimethyl sulfoxide; **LB,** Luria Bertani broth; **OD,** optical density; **IR,** Infrared; NMR, nuclear magnetic resonace; **FT-IR,** Fourier transform infrared spectroscopy; **ATR,** attenuated total reflection; **GC-MS,** gas chromatography- mass spectrometry.

include vernolide, vernolepin, vernodalin and hydroxyvernolide (Kupchan et al., 1969; Jisaka et al., 1993; Koshimizu et al., 1994). Other compounds have also been isolated from this genus such as triterpenoid glycosides, flavonoids, coumarins and benzofuranones (Miserez et al., 1996; Oketch-Rabah et al., 1997; Mao et al., 2008).

The current study was undertaken primarily to investigate the phytochemistry of *V. auriculifera* from which only fatty acids were previously isolated and to test the isolated compounds for antimicrobial activity since extracts of *Vernonia* species have been cited as antimicrobials in traditional medicine (Kokwaro, 1976).

MATERIALS AND METHODS

General experimental procedure

NMR spectra were recorded using a Bruker Avance^{III} 400 MHz spectrometer. All the spectra were recorded at room temperature with all chemical shifts (δ) recorded against the internal standard, tetramethylsilane (TMS). Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum 100 Fourier transform infrared spectroscopy (FT-IR) spectrometer with universal attenuated total reflection (ATR) sampling accessory. For gas chromatography-mass spectrometry (GC-MS) analyses, the samples were analysed on an Agilent GC–MSD apparatus equipped with DB-5SIL MS (30 m × 0.25 mm i.d., 0.25 µm film thickness) fused-silica capillary column. Helium (at 2 ml/min) was used as a carrier gas. The mass spectrometry (MS) was operated in the EI mode at 70 eV. Optical rotation was recorded using a PerkinElmerTM, Model 341 Polarimeter. Melting points were recorded on an Ernst Leitz Wetziar micro-hot stage melting point apparatus.

Plant material

The leaves, stem bark and root bark of *V. auriculifera* were collected in August, 2009 from Egerton University Botanical Garden, Rift Valley Province in Kenya. The plant was identified by taxonomist, Dr S. T. Kariuki, of the Botany Department, Egerton University, Kenya and a voucher specimen (Kiplimo, 02) was deposited in the Ward Herbarium, University of KwaZulu-Natal Westville, Durban, South Africa.

Extraction and isolation

The air-dried and ground plant material of *V. auriculifera* (823 g leaves, 710 g roots, 600 g stems) was sequentially extracted with organic solvents in order of increasing polarity including; hexane, dichloromethane, ethyl acetate and methanol, using a soxhlet apparatus for 24 h in each case. The yields obtained for each solvent were, hexane 66.68 g (leaves), 9.10 g (roots), 16.73 g (stems); dichloromethane 16.77 g (leaves), 5.13 g (roots), 9.25 g (stems); ethyl acetate 8.28 g (leaves), 0.93 g (roots), 5.02 g (stems) and methanol, 27.01 g (leaves), 20.28 g (roots), 15.09 g (stems).

Isolation and purification of Compounds 1, 3, 4, 5, 8 and 9

The hexane extract from the leaves (30 g) was separated by column chromatography using a step gradient of hexane: dichloromethane: ethyl acetate gradient, starting with 100% hexane stepped to 10, 20, 30, 50, 80 and 100% dichloromethane, followed by 20 and 30% ethyl acetate in dichloromethane. Twenty fractions of 100 ml each were collected in each step. Fractions 5 to 12 were combined and purified using 100% hexane, to produce farnesylamine (9) (12 mg). Fractions 21 to 25 were recrystallised in methanol to yield sitosterol (8) (78 mg). Fractions 41 to 67 were combined and separated with 20 and 30% dichloromethane in hexane. Lupenyl acetate (1) (52 mg) was obtained in fractions 8 to 12 while fraction 18 to 35 was further purified using 20% ethyl acetate in hexane where fractions 5 to 9 afforded β-amyrin acetate (4) (150 mg) and fractions 11 to 18 afforded a mixture of α-amyrin (5) and β-amyrin (3) (89 mg).

Isolation and purification of Compound 2

The ethyl acetate extract (0.93 g) from the roots was dissolved in dichloromethane and separated with a mobile phase consisting of a hexane: ethyl acetate step gradient 1:0 (fractions 1 to 10), 9:1 (fractions 11 to 20), 7:3 (fractions 21 to 38), 6:4 (fractions 52 to 64) and 3:7 (fractions 65 to 70). Fractions 22 to 27 were further purified with 20% ethyl acetate in hexane. Oleanolic acid (2) (25 mg) was obtained in fractions 9 to 13.

Isolation and purification of Compounds 6 and 7

The hexane extract from the stems (16.73 g) was subjected to column chromatography. The mobile phase consisted of a hexane: dichloromethane step gradient; 1:0 (fractions 1 to 45), 9:1 (fractions 46 to 66), 8:2 (fractions 67 to 80), 7:3 (fractions 81 to 98) and 1:1 (fractions 99 to 121). Friedelin acetate (7) (31 mg) was eluted in fraction 24 to 32 and the pure compound was obtained by recrystallisation in methanol. Friedelanone (6) (120 mg) was obtained by purification of fractions 55 to 80 using 10% dichloromethane in hexane as the mobile phase where the compound was eluted in fractions 7 to 15, followed by recrystallisation in methanol.

Farnesylamine (9)

White crystals, *m/z* (rel%): 221 $[M]^+$, 206 (1), 189 (3), 179 (3), 161 (3); IR spectra (V_{max} cm^{-1}): 3412, 2975, 1623, 968; ^{1}H NMR spectral data (400 MHz, $CDCl_3$) δ_H 5.11(H-2, 6, 10), 2.07 (H-1), 2.05 (H-8), 2.03 (H-9), 1.69 (H-12), 1.62 (H-13, 14, 15), 1.28 (H-4); ^{13}C NMR spectral data (400 MHz) 134.82 (C-3), 134.16 (C-7), 130.97 (C-11), 124.12 (C-6), 124.02 (C-10), 123.98 (C-2), 39.45 (C-1), 29.42 (C-4, 8), 27.99 (C-5), 26.48 (C-9), 25.41 (C-12), 15.71 (C-15), 17.39 (C-13), 15.75 (C-14).

BIOLOGICAL STUDIES

Minimum inhibitory concentration (MIC)

Four strains of gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 35032, *Klebsiella pneumoniae* ATCC 700603 and *Stenotrophomonas maltophilia* ATCC 13637) and five Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6051, *Enterococcus faecium* ATCC 19434, *Staphylococcus epidermidis* ATCC 14990 and *Staphylococcus saprophyticus* ATCC 35552) were selected for the determination of antimicrobial activity.

The antibacterial activities of the compounds were determined using the broth microdilution method as described by Andrews (2001). Bacterial strains were cultured for 18 h at 37°C in Tryptone Soy Broth (TSB) and standardized to a final cell density of 1.5×10^8 cfu/mL equivalent to 0.5 McFarland Standard. The 96-well plates

Table 1. Minimum inhibitory concentrations (MIC in mg/ml) of compounds isolated from *V. auriculifera.*

Microoganism	MIC ($mgml^{-1}$) of the test organisms					
	1	2	3 and 5	4	6	7
S. aureus	1.0	0.5	0.25	1.0	1.0	1.0
B. subtilis	1.0	0.5	0.25	1.0	0.25	1.0
E. faecium	0.5	1.0	0.25	0.5	1.0	1.0
S. epidermidis	1.0	0.25	0.5	1.0	0.5	0.5
S. saprophyticus	0.25	1.0	0.25	1.0	1.0	1.0
E. coli	0.12	1.0	0.12	0.5	1.0	0.5
K. neumoniae	1.0	0.5	0.5	1.0	1.0	1.0
P. aeruginosa	1.0	1.0	1.0	1.0	1.0	1.0
St. maltophilia	0.25	0.25	0.5	0.5	0.5	0.5

were prepared by dispensing into each well, 90 µl Muller-Hinton (MH) broth and 10 µl of the bacterial inoculum. Test compounds were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 10 mg/ml while tetracycline (a broad-spectrum antimicrobial agent) the positive control was dissolved in ethanol. Serial two-fold dilutions were made in a concentration range of 0.002 to 2 mg/ml. Wells containing MH broth only were used as a medium control and wells containing medium and cultures without the test compound were used as the growth control. Plates were covered to avoid contamination and evaporation and incubated for 24 h at 37°C. The MIC was described as the lowest concentration of the test compounds completely inhibiting the growth of microorganisms. The tests were done in triplicate on two separate occasions and the results are as shown in Table 1.

Anti-biofilm activity evaluation

To determine the anti-biofilm activity of β-amyrin acetate and oleanolic acid, three strains of Gram-negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 35032 and *K. pneumoniae* ATCC 700603) and four Gram-positive bacteria (*S. aureus* ATCC 25923, *S. aureus* ATCC 43300, *E. faecium* ATCC 19434, and *S. saprophyticus* ATCC 35552) were used. Bacterial isolates were cultured overnight in TSB to determine the effect of MIC, sub-MIC (0.5×MIC) and supra-MIC (2×MIC) exposures on biofilm formation. Cells were washed and resuspended in distilled water to a turbidity equivalent to a 0.5 McFarland standard. Wells of sterile, 96-well U-bottomed microtiter plates were each filled with 90 µl Luria Bertani broth (LB) and 10 µl of cell suspension, in triplicate. Based on individual MICs for each isolate the effect of MIC, sub-MIC and supra-MIC of β-amyrin acetate and oleanolic acid on bacterial adhesion was investigated. Plates were incubated aerobically at 37˚C for 24 h with shaking on an Orbit P4 microtitre plate shaker (Labnet).

The contents of each well were aspirated and then washed three times with 250 µl of sterile distilled water. To remove all the non-adherent bacteria, the plates were vigorously shaken and the remaining attached cells were fixed with 200 µl of 99% methanol per well. After 15 min, plates were left to dry and then stained for 5 min with 150 µl of 2% Hucker crystal violet. Excess stain was washed with running tap water and plates were left to air dry (Basson et al., 2008). The bound stain was resolubilised with 150 µl of 33% (v/v) glacial acetic acid per well. The optical density (OD) of the contents of each well was obtained at 595 nm using the Fluoroskan Ascent F1 (Thermolabsystems).

Tests were done in triplicate on two separate occasions and the results were averaged (Stepanović et al., 2000). The negative control for both assays was un-inoculated LB, while the positive control was tetracycline, with respective cell suspensions without β-amyrin acetate or oleanolic acid. OD_{595nm} values of treated cells were compared with untreated cells to investigate the increase/decrease of biofilm formation as a result of antimicrobial agent exposure. Treated and untreated samples were compared statistically using paired t-tests and Wilcoxon signed rank tests if normality failed (SigmaStat V3.5, Systat Software).

RESULTS AND DISCUSSION

The phytochemical investigation of *V. auriculifera* led to the isolation of eight triterpenoids 1 to 8 and a sesquiterpene amine (9). Extracts from the leaves were found to contain the sesquiterpene amine along with one lupane-type triterpenoid (lupenyl acetate 1), one ursane-type triterpenoid (α-amyrin 5), two oleanane-type triterpenoids (β-amyrin 3 and β-amyrin acetate 4) and a common steroid (sitosterol 8) (Figure 1). The stem bark afforded friedelanone (6) and friedelin acetate (7) belonging to the friedelane class. From the roots, oleanolic acid (2), the parent oleanane type triterpene, was isolated. Compounds 1 to 8 were identified using 2D NMR spectral data and by comparison with literature values, which supported the structures as lupenyl acetate (Jamal et al., 2008), oleanolic acid (Seebacher et al., 2003), β-amyrin, β-amyrin acetate, friedelin acetate and α-amyrin (Mahato and Kundu, 1994), friedelanone (Igoli and Gray, 2008) and sitosterol (Kamboj and Saluja, 2011). Although farnesylamine (9) was previously reported (Jones et al., 2003), here we are reporting the complete data for the first time.

Compound 9 was isolated as a colourless oily liquid; its molecular formula was assigned as $C_{15}H_{27}N$. The IR spectrum showed the presence of primary amine (3412 cm^{-1}) and (1623 cm^{-1}). The ^{13}C NMR spectrum showed the presence of six olefinic carbon resonances, 3-protonated carbons at δ_C 124.2 and 3-non-protonated carbons at δ_C 131-134. The olefinic methine resonances could also be seen at δ_H 5.11 in the ^1H NMR spectrum. The methylene carbon resonances were observed between δ_C 26.48 and δ_C 29.42 except for the methylene bonded to the amine group which was observed

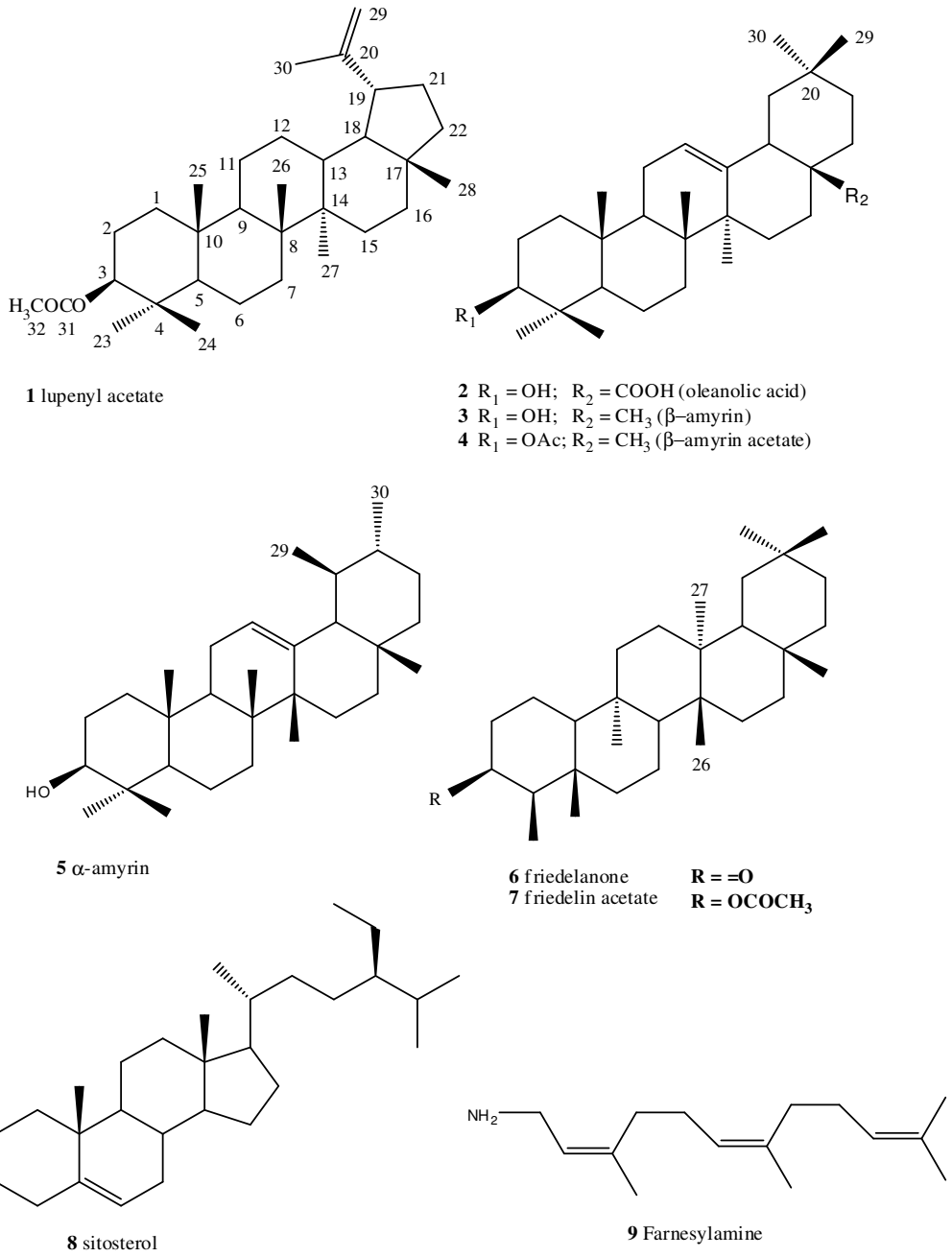

Figure 1. Structures of compounds (1-9) isolated from *Vernonia auriculifera.*

downfield at δ_C 39.47. All the methylene proton resonances, including 2H-1 were present at δ_H 2.05 except for one methylene resonance which appeared upfield at δ_H 1.27. Three of the four methyl proton resonances overlap at δ_H 1.62 (3H-13, 14, 15) and one is in a different chemical environment at δ_H 1.69 (3H-12). This same overlapping of the methyl carbon resonances can be seen in the ^{13}C NMR spectrum with methyl carbon resonances at δ_C 25.41, confirmed by the DEPT spectrum. This compound has been detected in an extract of the ant *Monomorium fieldi* Forel from Australia (Jones et al., 2003) and has only now been found in a plant species.

The triterpenic family of compounds to which all the isolated compounds belong are reported to possess antibacterial activity (Collins and Charles, 1987). The sesquiterpene, farnesylamine, could not be screened for antibacterial activity due to sample decomposition. MIC values recorded for all tested compounds (Table 1) suggested moderate antibacterial activity. The most

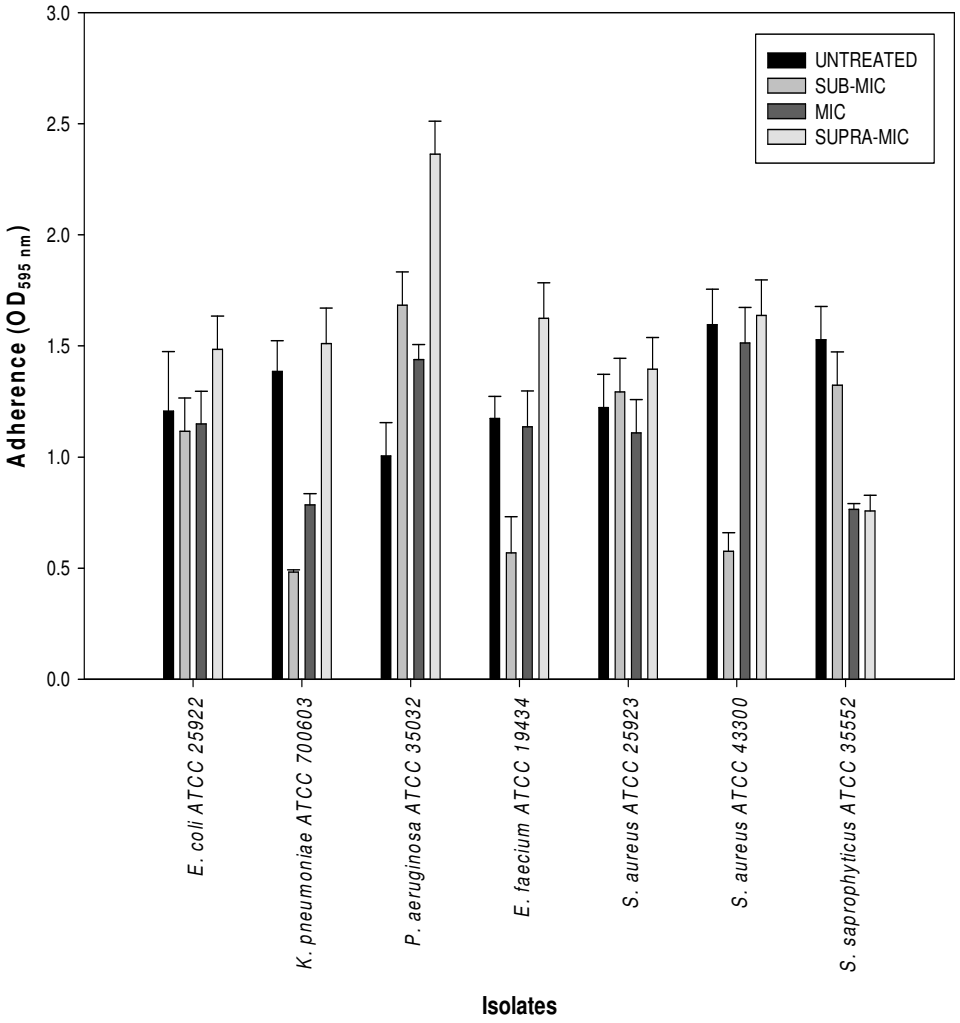

Figure 2. Antibiofilm results for β-amyrin acetate (4).

active compound was amyrin (mixture of α-and β-), with MICs of 0.12 mg/ml against *E. coli*, 0.25 mg/ml against *S. aureus, B. subtillis, E. feacalis, S. saprophyticus,* and 0.5mg/mL against *S. epidermis*, *K. pneumonia*, and *S. maltophilia.* The other compounds 3-7 had MIC of 0.5 mg/mL against *S. maltophilia.* The least active compounds were 6 and 7 with MIC of 1.0 mg/mL against six microorganisms. All tested compounds had MICs of 1.0 mg/mL for *P. aeruginosae* and 0.5 mg/mL for *S. maltophilia.* The oleanane triterpernoids (2-4) displayed better antibacterial activity than the friedelane triterpenoids (6-7). It is reported that the 28-COOH and ester functionality at C-3 contributes to pharmacological activities of pentacyclic triterpenes (Mallavadhi et al., 2004) like lupenyl which has greater antimutagenic activity than lupenyl acetate (Guevara et al., 1996). These effects are observed for friedelanone and friedelin acetate where the ketone has higher activity against *B. subtilis* than the ester.

Biofilm is a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other and are embedded in a matrix of extracellular polymeric substances they have produced. When planktonic bacteria adhere to surfaces, they initiate biofilm formation. The nature of biofilm structure and physiological attributes of biofilm organisms confer an inherent resistance to antimicrobial agents such as antibiotics, disinfectants or germicides (Donlan and Costerton, 2002).

β-amyrin acetate and oleanolic acid were tested for antibiofilm activity against seven strains of bacteria. *β*-amyrin acetate decreased adhesion of S. aureus (ATCC 43300), *K. pneumonia* and *E. faecium* significantly at sub-MIC concentrations (Figure 2). For K. pneumonia, this decreased adhesion was also seen at MIC concentrations and in S. saphrophyticus a marked decrease in adhesion was seen at MIC and supra MIC

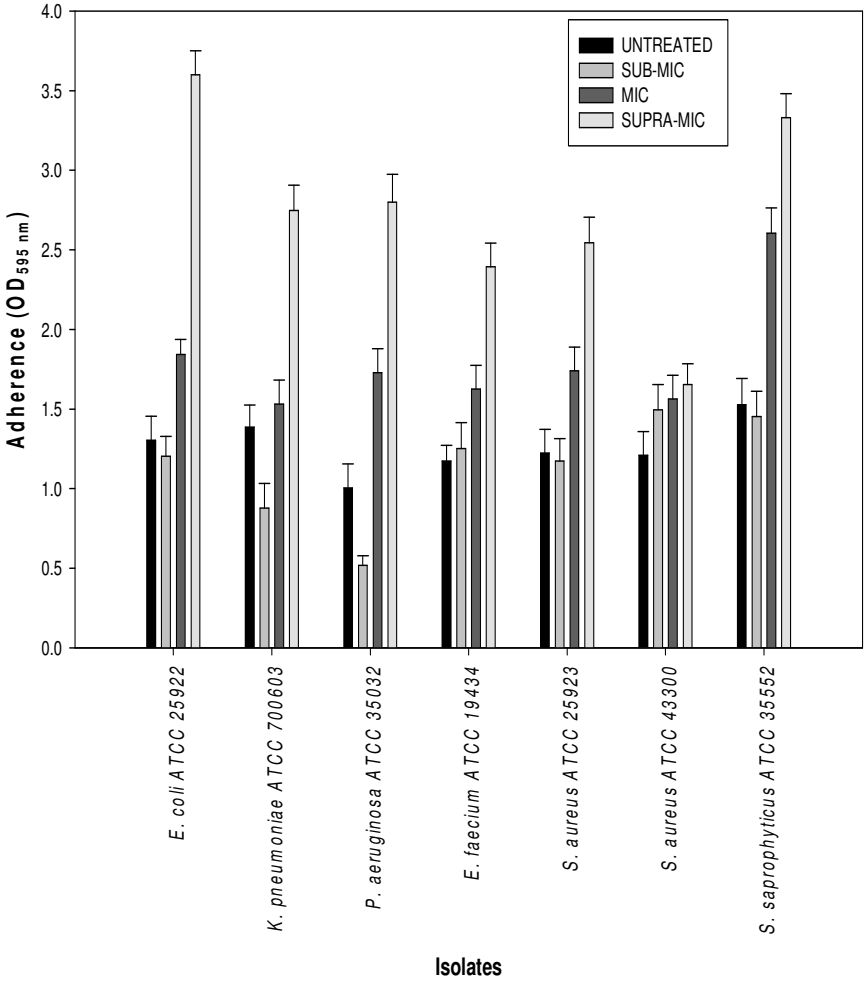

Figure 3. Antibiofilm results for oleanolic acid (2).

concentrations. Sub-MIC oleanolic acid exposure also decreased adhesion of *K. pneumonia* and *P. aeruginosa* significantly (Figure 3), but MIC and supra-MIC exposures of oleanolic acid increased adhesion of all tested bacterial strains. These results suggest that oleanolic acid and *β*-amyrin acetate that are relatively abundant, can be used at low concentrations to decrease adhesion of certain bacterial strains to abiotic surfaces. Since bacterial resistance to antibiotics and their survival are associated with their ability to form biofilms (Donlan and Costerton, 2002), compounds which decrease biofilm formation would be useful in being used in conjunction with other antibiotics to decrease bacterial resistance. Agents that decrease adhesion of bacteria may also be useful in improving the efficacy of antibiotics and hygiene in hospitals that have devices such as incubation tubes, catheters, artificial heart valves, water lines and cleaning instruments on which bacterial biofilm have been found (Donlan and Costerton, 2002).

Conclusion

This is the first report of a phytochemical investigation of *V. auriculifera*. The finding of a sesquiterpene amine in *V. auriculifera* is unique as it has not been isolated from a plant species before. Although the genus *Vernonia* is known to be a rich source of sesquiterpene lactones, none were isolated from *V. auriculifera*. However, eight pentacyclic compounds with moderate antibacterial activity were isolated. Oleanolic acid and *β*-amyrin acetate exhibited moderate anti-adhesion properties. These compounds show potential for synergistic coupling with antimicrobial agents to improve therapeutic efficiency, in the face of rising bacterial resistance, however this needs further investigation.

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REFERENCES

Andrews JM (2001). Determination of inhibitory concentrations. J. Antimicrob. Chemother., 48: 5-16.

Beentje H (1994). Kenya Trees, Shrubs and Lianas. National Museums of Kenya, Nairobi, pp. 564-570.

Buskuhl H, Oliveira LF, Blind ZL, Freitas RA, Barison A, Campos FR, Corilo YE, Eberlin NM, Caramori GF, Biavatti MW (2010). Sesquiterpene lactones from *Vernonia scorpioides* and their *in vitro* cytotoxicity. Phytochemistry, 71: 1539-1544.

Chen X, Zhan ZJ, Yue JM (2006). Sesquiterpenoids from *Vernonia cinerea*. Nat. Prod. Res. Part B: Bioact. Nat. Prod., 23(12): 1160.

Collins MA, Charles HP (1987). Antimicrobial activity of carnosol and ursolic acid: Two anti-oxidant constituents of *Rosmarinus officinalis* L. Food Microbiol., 4: 311-315.

Donlan RM, Costerton JW (2002). Biofilms: survival mechanisms of clinically Relevant Micro-organisms. Clin. Microbiol. Rev., 15 (2): 167-193.

Erasto P, Grierson DS, Afolayan AJ (2006). Bioactive sesquiterpene lactones from the leaves of *Vernonia amygdalina*. J. Ethnopharmacol., 106: 117-120.

Freiburghaus F, Ogwa NE, Nkunya MHH, Kaminsky R, Reto B (1996). *In vitro* antitrypanosomal activity of African plants used in traditional medicine in Uganda to treat sleeping sickness. Trop. Med. Int. Health., 6: 765-777.

Guevara AP, Amor E, Russell GR (1996). Antimutagens from *Plumeria acunimata* Ait. Mutation Research/Environmental mutagenesis and related subjects. 36(1): 67-72.

Igoli OJ, Gray IA (2008). Friedelanone and other triterpenoids from *Hymenocardia acida*. Int. J. Phys. Sci., 3(6): 156-158.

Jamal AK, Yaacob WA, Din LBA (2008). Chemical Study on *Phyllanthus reticulatus*. J. Phys. Sci., 19(2): 45-50.

Jisaka M, Ohigashi H, Takegawa K, Huffman MA, Koshimizu K (1993). Antitumoral and antimicrobial activities of bitter sesquiterpene lactones of *Vernonia amygdalina*, a possible medicinal plant used by wild chimpanzees. Biochemistry, 57: 833-834.

Jones TH, Clark DA, Heterick BE, Snelling RR (2003). Farnesylamine from the ant *Monomorium fieldi* Forel. J. Nat. Prod., 66(3): 325-326.

Kamboj A, Saluja AK (2011). Isolation of stigmasterol and β-sitosterol from petroleum ether extract of aerial parts of *Ageratum conyzoides* Asteraceae). Int. J. Pharm. Pharm. Sci., 3: 94-96.

Keriko JM, Nakajima S, Baba N, Isozaki Y, Ikeda K, Iwasa J, Alam MK (1995a). Hydroperoxides of unsaturated fatty acid methyl esters from Kenyan plant *Vernonia auriculifera* (Asteraceae). Yukagaku. 44(4): 338-340.

Keriko JM, Nakajima S, Baba N, Isozaki Y, Ikeda K, Iwasa J, Karanja PN (1995b). A plant growth regulator from *Vernonia auriculifera* (Asteraceae). Z. Naturforsch., 50(5/6): 455-458.

Kokwaro JO (1976). Medicinal Plants of East Africa. East Africa Education Publishers, pp. 56-70.

Koshimizu K, Ohigashi H, Huffman MA (1994). Use of *Vernonia amagdalina* by wild chimpanzee: Possible roles of its bitter and related constituents. Physiol. Behav., 56: 1209-1216.

Kupchan SM, Hemingway RJ, Karim A, Werner D (1969). Tumor inhibitors. XLVII. vernodalin and vernomygdalin, two new cytotoxic sesquiterpene lactones from *Vernonia amygdalina* Del. J. Org. Chem., 34: 3908-3911.

Kusamba C (2001). Contribution to the inventory of medicinal plants from the Bushi area, South Kivu Province, Democratic Republic of Congo. Fitoterapia, 72: 351-368.

Mahato SB, Kundu AP (1994). ^{13}C NMR spectra of pentacyclic triterpenoids. A Compilation and some salient features. Phytochemistry, 37: 1517-1575.

Malafronte N, Pesca M, Sabina B, Angela E, Luis MTN (2009). New flavonoid glycosides from *Vernonia ferruginea*. Nat. Prod. Comm., 4(12): 1639-1642.

Mallavadhi UV, Mahapatra A, Jamil K, Reddy PD (2004). Antimicrobial activity of some pentacyclic triterpenes and their synthesized 3-O-lipophilic chains. Biol. Pharm. Bull., 27: 1576-1579.

Mao RS, Jun SY, Ze Sheng Z (2008). Two new compounds from the stem of *Vernonia cumingiana*. Chin. Chem. Lett., 19: 180-182.

Mesfin F, Demissew S, Teklehaymanot T (2009). An Ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. J. Ethnobiol. Ethnomed., 5: 28.

Mirutse G, Zemede A, Zerihun W (2009). Medicinal plants of the Meinit ethnic group of Ethiopia: An ethnobotanical study. J. Ethnopharmacol., 124: 513–521.

Miserez F, Potterat O, Marston A, Mungai GM, Hostettmann K (1996). Flavonol glycosides from *Vernonia galamensis* ssp. Phytochemistry, 1996, 43(1): 283-286.

Morales-Escobar L, Braca A, Pizza C, Tommasi N (2007). New phenolic derivatives from *Vernonia mapirensis* Gleason. ARKIVOC (Gainesville, FL, United States), (7): 349-358.

Muthaura CN, Rukunga GM, Chhabra SC, Mungai GM, Njagi ENM (2007). Traditional phytotheraphy of some remedies used in treatment of malaria in Meru District of Kenya. S. Afr. J. Bot., 73(3): 402-411.

Oketch-Rabah HA, Lemmich E, Dossaji SF, Theander TG, Olsen CE, Cornett C, Arsalan K, Christensen BS (1997). Two new antiprotozoal 5-methylcoumarins from *Vernonia brachycalyx*. J. Nat. Prod., 60: 458-461.

Rabe T, Mullholland D, van Staden J (2002). Isolation and identification of antibacterial compounds from *Vernonia colorata* leaves. J. Ethnopharmacol., 80: 91-94.

Seebacher W, Simic N, Weis R, Saf R, Kunert O (2003). Complete assignments of ^{1}H and ^{13}C NMR resonances of oleanolic acid, 18α-oleanolic acid, ursolic acid and their 11-oxo derivatives. Magn. Reson. Chem., 41:636-638.

Stepanović S, Vuković D, Dević I, Savić B, Švabić-Vlahović M (2000). A modified Microtiter-plate test for quantification of staphylococcal biofilm formation. J. Microbiol. Meth., 40: 175-179.