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Further constituents of *Vernonia cineria* leaves

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ABSTRACT

The genus *Vernonia* consists of over 1000 species and members of the genus are widely used as food and medicine. As part of our continuing research into ethnomedicinal plant of Nigeria for bioactive plant metabolites, the leaves of *Vernonia cineria* was investigated for phytochemical constituents. The pulverized leaves of *Vernonia cineria* were extracted with 70% ethanol and the crude ethanolic extract was suspended in water and partitioned with n-hexane, ethyl acetate and n-butanol. The n-hexane and ethyl acetate soluble parts which showed similar spots on thin layer chromatography were pooled together and coded V1. Extensive column chromatography of V1 afforded the triterpenoids: Lupenol and Oleanan-12.15 dein-28-oic acid. The structures were elucidated using NMR an MS and compared with literature. The oily fraction was subjected to GC-MS equipped with library data to give fatty acids and long chain hydrocarbons. Antibacterial investigation of the ethanol extract at concentration in the range 5-50 mg/ml against the clinical isolates of *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* did not show any activity.

INTRODUCTION

Vernonia cineria is a deciduous plant widely distributed in tropical Africa including Nigeria,Ghana, Senegal and some parts of Europe (Irvine, 1961). The whole plant is used to treat various skin diseases, dressing of wounds and as remedy for diarrhea (Iwalewa *et al.,* 2003). The leaves of the plant also find use as antidote to snake bite and scorpion stings (Saligrama et al,2003). The anti-oxidant activity of the methanolic extract has been reported (Kumar *et al.,* 2003), while the antibacterial activity of the benzene extract of the leaves of this plant was also reported (Gupta *et al.,* 2003). Previously, some flavonoids and triterpenoids have been isolated from this plant (Kuo *et al.,* 2003; Chen *et al*., 2006; Zhu *et al.,* 2009). In this present study, as part of our continuing study on bioactive plant metabolites from the genus *Vernonia*, we report here in the isolation and structure elucidation of a lupane and an oleanane type triterpenoid: Lup-20(29)-en-3-ol (Lupenol) and Oleanan-12(13); 15(16)-dien-28-oic acid and identification of fatty acids alongside long chain hydrocarbon from the oil rich fraction of the n-hexane soluble part of ethanolic extract of the leaves of *Vernonia cineria*. The structures of the isolated compounds were elucidated using NMR, MS and GC-MS respectively.

MATERIALS AND METHOD

The IR spectra were recorded on Nicolet 1s 10 FT-IR (Thermo Scientific Company, USA) in Nujol and KBR disc. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance DRX 250 MHz and 62.5MHz with $CDCl_3$ as solvent and TMS as internal standard. Mass spectroscopy was performed on Thermo finnigan HT mass spectrophotometer at 70 EV.

GC-MS was performed on a Shimadzu 8400 S GC-MS spectrophotometer. Thin later chromatography was performed on pre-coated silica gel (0.2 mm) Silicycle, while column chromatography was carried out on silica gel G (230-400 mesh) Silicycle. TLC spots were detected by spraying with 10% H_2SO_4 and heated in the oven at 110^0c for 10 minutes.

Plant Collection

The leaves of the plant were collected in Uyo, Akwa Ibom state in the month of June, and were identified at the Department of Botany, Faculty of Science University of Uyo where a voucher specimen was deposited.

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Extraction

The powdered dried leaves (400 g) were extracted exhaustively at room temperature with 70 % ethanol for seven days. The concentrated extract after removal of solvent in rotary evaporator gave a dark green mass (23.4 g) (5. 85%w/w). 15 g of this extract was suspended in 100 ml of water and partitioned with (3x200ml) each of n-hexane, ethyl acetate and n-butanol to give 0.9 g of n-hexane soluble part, 0.54 g of ethyl acetate and 1.2 g of n-butanol. A portion of the ethanolic extract was subjected to anti-bacterial screening using agar diffusion assay method as described by (Mendoza *et al.,* 2008).

Isolation

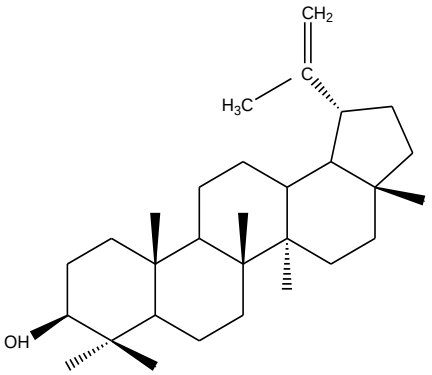
TLC studies of the n-hexane and ethyl acetate soluble parts using the solvent systems n-hexane ethyl acetate (9:1) revealed similar spots, these extracts were pooled together to give 1.4 g and coded V1.

1.3 g of V1 was packed in a column of (2cm x 50cm) with 50 g of silica gel and elution commenced gradiently with n-hexane (100%); n-hexane :ethyl acetate 99:1,98:2; 97:3;96:4; 95:5; 90:10;80:20; 70:30; 60:40; 50:50; 30:70 ;10:90 and ethyl acetate(100%) and finally 5% methanol in ethyl acetate. 50 ml aliquots were collected and the progress of elution were monitored using solvent systems I: n-hexane :ethyl acetate (9:1) and II n-hexane :ethyl acetate (4:1) respectively. Forty nine fractions were collected. Fractions 4 and 5 eluted with 1 and 2% ethyl acetate in n-hexane showed at least six spots on TLC and were pooled together to give a pale yellow oily fraction coded V2. Fractions 12-14 eluted with 5% ethyl acetate in n-hexane showed a single spot on TLC using solvent system I, these fractions were pooled together and removal of the solvent afforded a white solid coded V3 and weighed 18 mg. V3 was found to be soluble in chloroform and dichloromethane and insoluble in methanol.

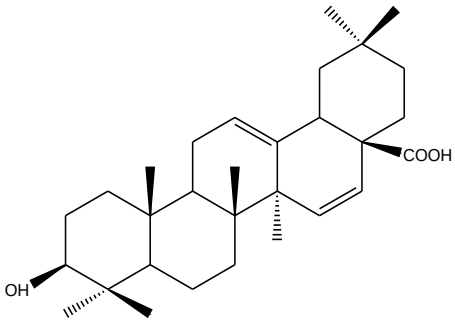
Fractions 27-30 eluted with 20% ethyl acetate in n-hexane gave a single spot on TLC using solvent system II, these fractions were pooled together and removal of the solvent gave a yellowish-white solid coded V4 and weighed 11 mg. Both V3 and V4 gave positive test to Salkowskii test (reddish-brown) suggestive of steroid or triterpenoid. V3 and V4 were subjected to spectral analysis: IR, NMR and MS, while V2 was subjected to GC-MS**.**

Antibacterial activity

Antibacterial activity of the ethanolic extract was evaluated against a panel of clinical isolates *B. subtilis*, *S. aureus*, *E. coli* and *Pseudomonas aeruginosa*. The method described by (Mendoza *et al.,* 2008) was adopted. Different concentrations of the extract were prepared at 5 mg/ml, 10 mg/ml, 20 mg/ml and 50 mg/ml were used in the assay, while 10 µg disc of gentamicin was used as the standard antibiotic. The bacteria isolates were standardized using colony suspension method by matching the bacterial suspension with 0.5 McFarland standard (Duraipandiyan and Ignamicimuthu, 2009). 50 ml of sterilized Mueller Hinton agar was transferred to sterilized plates and were differently inoculated with the standardized suspension of each test organism after solidification: Using a cork borer of 10 mm diameter, holes wells were made in the inoculated agar plate and the bottom sealed with few drops of molten agar. Different concentrations of the extract were introduced aseptically in to the four well labeled wells on each of the inoculated agar plate while the standard antibiotic disc was placed on the surface of each plate. The plates were incubated at 37 ^{o}C for 24 hours after which the zones diameter of inhibition were measured to the nearest millimeters.



Compound 1 V3



Compound II V4

Fig. 1: Structures of isolated compounds.

RESULT

Compound V3, a white solid (18 mg). Gave positive test for triterpenes.

IR (Nujol) cm^{-1}: 3307 (O-H stretching), 2931 (CH_3 stretching), 1037 (C-O stretching for alcohol)

^{1}H-NMR ($CDCl_3$) ð: 0.74,0.78,0.83,0.91,0.94,1.06,1.72 (each 3H (s)) Methyl x7); 3.20 (1H ,d,d H-3); 4.54 (1H (s),H-29a; 4.68 1H s, H-29b).

^{13}C-NMR ($CDCl_3$) ð: 151.6 (C-20),108.6 (C-29),78.4 (C-3),55.1 (C-5),49.7 (C-9),48.2 (C-18),47.8 (C-19),43.2 (C-17),42.6 (C-14),41.8 (C-8),40.2 (C-22),39.2 (C-13),38.6 (C-4),38.0 (C-1),37.3 (C-10),35.6 (C-16),34.1 (C-9),31.0 (C-21),28.2 (C-23),27.6 (C-15),27.5 (C-12),25.2 (C-2),21.1 (C-11),9.5 (C-30),18.1 (C-6),18.0 (C-28),16.8 (C-25),16.4 (C-26),16.0 (C-24),15.1 (C-27).

MS (70 ev): EI-MS M/Z 426(M) ,409 (M-OH), 218(100 %), 189 , 43

Compound V4, a yellowish- white solid (11 mg), gave positive test for triterpenes.

IR (KBr) cm^{-1}: 3300-3000) O-H broad),1712.6 (CO stretching in acid)

^{1}H-NMR($CDCl_3$) ð: 0.82,0.9,1.01,1.20 (CH_3 x3) all for methyl protons, 3.5 (1H, d,d (H-3);5.01 1H,H-15; 5.15 1H ,s, H-16; 5.3 1H s,H-11).

^{13}C-NMR ($CDCl_3$) ð: 179.1 (C-28),141.5 (C-13),138.2 (C-16),129.6 (C-15),121.5 (C-11),73.1 (C-3),56.5 (C-5),53.1 (C-9),42.1 (C-17),43.0 (C-18),40.5 (C-8),50.1 (C-19),35.2 (C-21),33.9 (C-22),30.9 (C-20),44.8 (C-14),28.9 (C-23),37.5 (C-29),23.0 (C-30),18.4 (C-24),15.5 (C-25),25.1 (C-27),17.1 (C-28),15.5 (C-25).

DISCUSSION

Result of the antibacterial activity showed that the extract at different concentrations used (5-50 mg/ml) did not showed any zone of inhibition, while the standard antibiotic Gentamycin at 10µg/ml gave zones of inhibition ranging from 14-20mm.

Compound V3 was isolated as a white powder; its mass spectral data gave a molecular ion peak at M/Z 426 consistent with the molecular formula C_{30} $H_{50}O$. The ^{1}H-NMR spectral data showed seven methyl singlets at ð 0.78, 0.81, 0.85, 0.97, 0.99, 1.05 and 1.70, and one secondary hydroxyl group as doublet of doublets at ð 3.20. The olefin protons at ð 4.54 and 4.68 representing an exocyclic double bond and a characteristic methyl proton signal at ð 1.70 indicating the presence of an isopropenyl group (Jamal *et al.,* 2008). The ^{13}C-NMR spectra of V3 showed 30 signals for the triterpenoid of lupane skeleton (Back *et al.,* 2010), which include a C-OH bond ascribable to C-3 position which appeared at ð 78.4 ppm, while the olefenic carbons of the exocyclic double bond were evident at 108.6 and 151.6 ppm respectively. The above spectral data suggested that V3 is a lupane triterpenoid having a secondary alcohol group. The IR spectra showed a band at 3307 cm^{-1} assigned to O-H stretching of alcohol. These data indicated that V3 is a pentacyclic triterpenoid of lupane series, and comparison of its physical and spectral data with published values confirmed V3 to be lupenol (Privitea and Monaco, 1984; Connolly and Hill, 1999; Chaturvedula and Indra, 2012). Compound V4 was isolated as a yellowish-white solid, the IR spectra showed band at 1712 cm^{-1} assigned to a ketone functional group, and a broad band at 3300-3000cm^{-1} indicating a bonded O-H stretching frequency in carboxylic acid. The ^{1}H-NMR spectra revealed the presence of six methyl signals at ð 0.82, 0.9, 1.01, and 1.20(3x CH_3) which integrated for three methyl protons all suggestive of an oleanane type triterpenoid. The doublet of doublets signal at ð 3.5 was assigned to 3-OH typical of a triterpenoid . Three vinylic protons were observed at 5.01, 5.15 and 5.22 ppm suggesting the presence of at least two double bonds suggesting an oleanane diene skeleton which are not homoannular. The ^{13}C-NMR spectra revealed the presence of 30 signals ascribable to a triterpenoid skeleton (Mahato *et al.,* 1988).The vinylic carbons were evident from the signals at 121.5, 129.6, 138.2 and 141.5. The C-3 signals was discernable at 73.1 ppm, while the six methyl carbons were in the range 15-30 ppm. Complete C-H assignment was based on HSQC, while C-H long range coupling was aided with the HMBC specta. Based on comparison with the literature data, compound V4 was found to be Olean-12(13), 15 (16)dien-28-oic acid (Mahato et al.,1992). Fractions 3-4 coded V2, eluted from 1% ethyl acetate in n-hexane revealed similar spots on TLC; removal of the solvent gave an oily mass which was subjected to GC-MS equipped with library data. The result of the GC-MS table (1), revealed the presence of 18 compounds, 7 of which are major compounds and 11 are minor compounds based on their peak area. The quantitative estimation of each peak area was done with the aid of computer library data of the GC-MS. Identification of each compound was based on the comparison of their retention time, and mass spectral data with that of the library data stored in the GC-MS.

Table 1: GC-MS Analysis of V2.

Fraction	Retention time (min)	Area (%)	Molecular mass	Compound
7	15.409	5.16	270	Pentadecenoic acid ethyl ester
12	18.128	17.7	284	Hexadecenoic acid methyl ester
15	20.79	9.4	310	E-octadecenoic acid methylester
17	21.33	3.94	312	Stearic acid ethyl ester
19	23.33	4.21	340	Eicosanoic acid ethyl ester

Table 2: Antibacterial investigation of Ethanol extract of *V. cineria* leaves.[#]*

Extract	Conc (µg/ml)	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*
Ethanol		-	-	-	-
	5	-	-	-	-
	10	-	-	-	-
	20	-	-	-	-
	50				
Gentamycin	10	14.00	18.00	15.5	16.00

Key (-) no activity.
[#] mean in triplicates.

CONCLUSION

Two triterpenoids were isolated for the first time from the combined n-hexane and ethylacetate soluble part of *Vernonia cineria*, and some fatty acids and long chain hydrocarbons from the oily rich fraction were identified using GC-MS. Anti-bacterial activity on the extract did not show any activity. Further work is ongoing to isolate more compounds and investigate their biological activity.

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REFERENCE

Back MY; Cho JG, Lee DY, Ahm EM, JeogTS and Back, NI. Isolation of Triterpenoids from the stem bark of Albizia julibrissine . Journ of Korean Soc appl.biol.chem, 2010; 53: 310-315

Shashi B. Mahato, Bikas C. Pal, Sudip K. Sarkar. New triterpenoid saponins from *Corchorus acutangulus*. Phytochem, 1992; 27:1433

Charturvedula VSP and Indra P . Isolation and characterization of lupane triterpenoids from Poludium vulgare. Res. Journ of Pharm. Sci, 2012; 1(1):23-27

Chen X, Zhan Z.J and Yue J.M. Sesquiterpenoids from Vernona cineria. Nat.Prod. Res, 2006; 20(20):125-129

Connolly JD and Hill RA. Triterpenoids. Nat. Prod. Rep, 1999; 16: 221-240

Duraipandiyan V and Ignacimithu S. Antibacterial and antifungal activity of medicinal plant. Journal of Ethnopharmacol, 2009; 123: 494-498

Gupta M, Mazumder UK, Manikandan L, Haldar PK, Bhattacharya S and Kandar CC. Antibacterial activity of Vernonia cineria. Fitoterapia, 2003; 74 (1-2): 148-150

Irvine F.R 1961. Woody plants of Ghana : with special reference to their uses. Oxford University Press, London. Pp. 45

Iwalewa E.D, Iwalewa O.J and Adeboya J.O. Analgesic ,Antipyretic and Anti-inflammatory effects of Methanol,chloroform and ether extracts of Vernonia cineria leaves. Journ of ethnopharmacol, 2003; 86(2-3):229-234

Jamal AK, Yaacob WA, Din CB. A chemical study of Phyllanthus reticulatus. Journ of Physic Sci, 2008; 19(2): 45-50

Yao HK, Yu JK, Ang SY, Ming DW, Chi Wo, Li-Myk, Yang KJ, Chieh FC and Shyh YL. Two novel sesquiterpene lactones from Vernoniacinerea. Chem. Pharm. Bull. 2003; 51: 425-426.

Mahato,S,B and Kundu,S.P. ^{13}C-NMR spectra of some pentacyclic triterpenoids.A compilation of some salient features. Phytochem, 1994; 37: 1517-1575

Mendoza,L; Wilkens ,M and Urzua,A . Antimicrobial study of the Diterpenoids and Flavonoids from some Chilean Pseudographalium. Journ of ethnopharmacol,1997; 58(2):246-252

Monaco,P and Privitera,L Isoprenoids from the leaves of Quercus suber. J. Nat. Prod, 1984; 47: 673-676

Saligrama K.R. 2005. Encyclopedia of Indian medicine: material medicia,Bangalore; Herbal drugs.

Zhu H.X, Tang Y.P, Gong and Min Z.D. Studies on bioactive constituents of whole herbs of Vernonia cineria. Zhongguo Zhong Yao Za Zhi. 2009; 33(16): 1986.

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