Journal of Asian Natural Products Research

ISSN: 1028-6020 (Print) 1477-2213 (Online) Journal homepage: https://www.tandfonline.com/loi/ganp20

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To cite this article: Jenipher Akinyi Odak, Lawrence Onyango Arot Manguro & Keng-Chong Wong (2018) New compounds with antimicrobial activities from *Elaeodendron buchananii* stem bark, Journal of Asian Natural Products Research, 20:6, 510-524, DOI: 10.1080/10286020.2017.1420648

To link to this article: https://doi.org/10.1080/10286020.2017.1420648

Published online: 17 Jan 2018.

JOURNAL OF ASIAN NATURAL PRODUCTS RESEARCH, 2018
VOL. 20, NO. 6, 510–524
https://doi.org/10.1080/10286020.2017.1420648

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New compounds with antimicrobial activities from *Elaeodendron buchananii* stem bark

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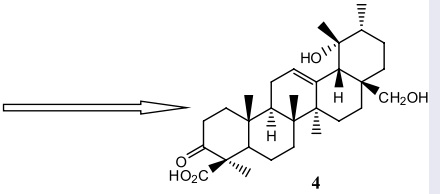
ABSTRACT

The plant species *Elaeodendron buchananii* Loes is widely used in folklore medicine to manage microbial infections in Kenya. Previous studies on the plant fruits and root bark revealed the presence of steroids and terpenoids. The present phytochemical analysis of the plant stem bark has led to the isolation of four new triterpenes characterized as methyl 3β-acetoxy-11α, 19α, 28-trihydroxyurs-12-en-23-oic acid (**1**), 3β, 11α, 19α-trihydroxyurs-12-en-23, 28-dioic acid (**2**), 3β-acetoxy-19α, 23, 28-trihydroxyurs-12-ene (**3**) and 3-oxo-19α, 28-dihydroxyurs-12-en-24-oic acid (**4**), together with ten known ones (**5**–**14**), whose structures were elucidated using spectroscopic techniques. The isolate canophyllol (**8**) showed promising antibacterial activity against *N. meningitides* with MIC value of 31.25 µg/ml.

ARTICLE HISTORY
Received 31 January 2017
Accepted 19 December 2017

KEYWORDS
Elaeodendron buchananii; Celastraceae; triterpenes; antimicrobial activities

Elaeodendron buchananii aerial parts



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1. Introduction

The Celastraceae is a large family of herbs, woody lianas, shrubs, and trees distributed worldwide in tropical and temperate zones. An approximation on the number of species belonging to the family has been inconsistent due to little taxonomic work and also because generic delimitations are controversial [1], a situation which has subjected the family to considerable nomenclatural confusion and name changes [2]. In ethnomedicine, some members of the family have found usage including cleaning of digestive tract and as relief for

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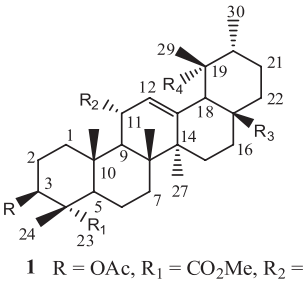
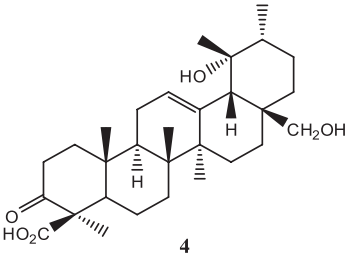
chest congestion [3,4] and also as remedy for HIV infections [5]. The genus *Elaeodendron* is one of the largest genera in the Celastraceae family consisting of approximately 147 species, which are evergreen or rare deciduous trees and shrubs, and is represented by four species, namely *Elaeodendron buchananii* (Loes), E. *schweinfurthianum* (Loes), *E. aquifolium* (Fiori), and *E. schlechteranum* (Loes) in Kenya [6]. These plants have been the focal point of research investigations including their ecology, distribution, and ethnobotanical uses [7,8]. The species *E. buchananii* is a tree that grows up to 20 m high with a strong stout irregular trunk, dense evergreen foliage, and round or irregular crown. In traditional medicine, an infusion of the plant stem bark extract is used to manage malaria [9], HIV [10], fungal [11], and bacterial infections [12]. Previous phytochemical studies on the plant have yielded a number of interesting compounds including steroids and terpenoids [13–15] which have attracted interest on account of their cytotoxicity, antitumour, immunosuppressive, and insect antifeedant activities [16]. In this communication, phytochemical evaluation of the stem bark extracts of this plant afforded four new triterpenes characterized as methyl 3β-acetoxy-11α, 19α, 28-trihydroxyurs-12-en-23-oic acid (**1**), 3β, 11α, 19α-trihydroxyurs-12-en-23, 28-dioic acid (**2**), 3β-acetoxy-19α, 23, 28-trihydroxyurs-12-ene (**3**) and 3-oxo-19α, 28-dihydroxyurs-12-en-24-oic acid (**4**) (Figure 1), together with ten known ones (**5–14**). Their structures were established using physical and spectroscopic techniques as well as comparison with literature data. The extracts and isolated compounds were tested for their antibacterial and antifungal activities.

2. Results and discussion

2.1. Structural elucidation

Compound **1** was obtained as white amorphous powder after crystallization from CH_2Cl_2-MeOH mixture. Its IR spectrum displayed absorption peaks attributable to hydroxyl (3350 cm^{-1}), ester carbonyl (1734 cm^{-1}), methyl carbonyl (1722 cm^{-1}), and double bond (1630 cm^{-1}) functional groups. The HRESI-MS of the compound showed a molecular ion peak at *m/z* 561.7698 $[M + H]^+$ which suggested a $C_{33}H_{52}O_7$ formula containing nine unsaturation equivalents. Besides the molecular ion peak, the EI-MS also exhibited a prominent peak at *m/z* 542 ($C_{33}H_{50}O_6$) representing the loss of H_2O from the molecular ion $[M]^+$. Other characteristic peaks at *m/z* 294 ($C_{17}H_{26}O_4$, rings A/B), 266 ($C_{16}H_{26}O_3$, rings D/E), 248





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1 R = OAc, $R_1 = CO_2Me$, $R_2 = R_4 = OH$, $R_3 = CH_2OH$

2 $R = R_2 = R_4 = OH$, $R_1 = R_3 = CO_2H$

3 R = OAc, $R_2 = H$, $R_1 = R_3 = CH_2OH$, $R_4 = OH$

Figure 1. Compounds **1–4** isolated from *E. buchananii* stem bark.

($C_{16}H_{24}O_2$), and 234 ($C_{15}H_{22}O_2$) (Figure 2) suggested *retro*-Diels-Alder cleavage of ring C typical of ursane-type triterpene with acetoxy and CO_2Me substitutions in A/B rings [17], two additional hydroxyls and a terminal hydroxymethylene in the C/D/E rings [17,18].

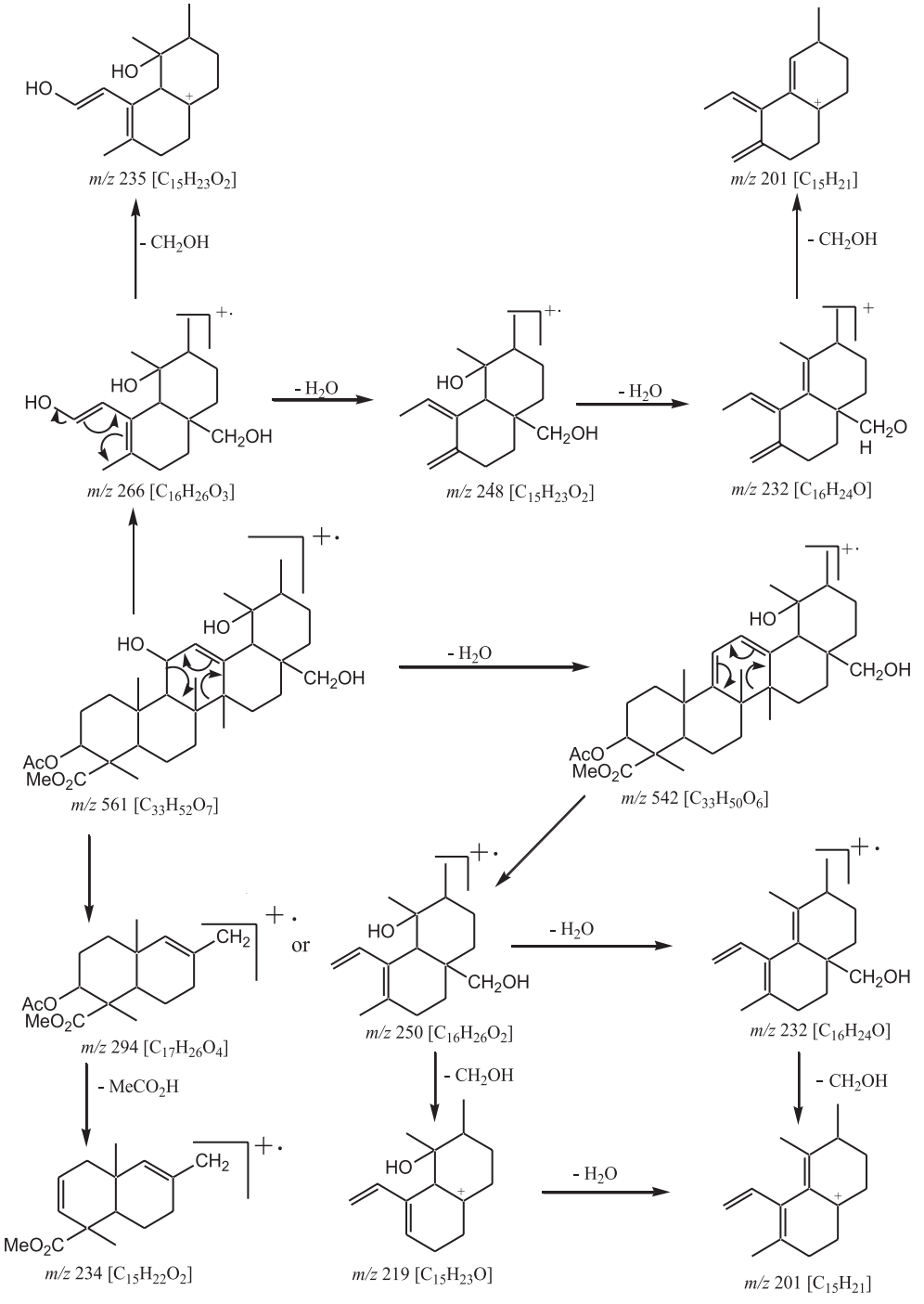

Figure 2. Possible fragmentation pattern of compound **1** in EIMS.

The ^{1}H NMR spectrum of the compound exhibited a vinyl proton (δ 5.30, br t, J = 3.4 Hz, H-12) and two oxygenated methine protons (δ 4.60, dd, J = 12.2, 4.3 Hz, H-3, and 3.50, dd, J = 8.8, 3.3 Hz, H-11) which were observed to correlate with carbon resonances at δ 126.0 (C-12), 78.0 (C-3), and 73.3 (C-11), respectively in the HSQC spectrum (Table 1). The broad band decoupled ^{13}C NMR and DEPT spectra afforded 33 distinct carbon signals accounted for eight methyls, nine methylenes, seven methines including one olefinic and two oxygenated carbons as well as nine quaternary carbons. The acetoxy group at C-3 was deduced to be in equatorial orientation based on axial-axial and axial-equatorial couplings between H-3 and H-2 protons as previously observed in 3β-acetoxy-11α-hydroxyurs-12-ene [19]. This interpretation was further facilitated by HMBC spectrum which exhibited cross-peaks between H-3 and C-23 (δ 180.6), and C-24 (δ 13.7) and in turn with C-5 (δ 54.6), a fact further corroborated by a 1, 3-diaxial correlation between H-3 and H-5 in the NOESY spectrum (Figure 3). The protons attached to each carbon signal observed in the ^{13}C NMR spectrum were deduced by analysis of the DEPT spectrum and in this way, it was established that oxygenated methylene carbon which appeared in the ^{13}C NMR at δ 68.7 signified a terminal CH_2OH [20]. Its position on the ring system was deduced to be at C-17 on the basis of HMBC correlation between H-28 (δ 3.86) and C-18 (δ 53.5)/C-22 (δ 38.3). On

Table 1 ^{1}H NMR spectral data of compounds **1–4** in $CDCl_3$ + drop DMSO-d_6 (600 MHz).

H	**1**	**2**	**3**	**4**
1	1.88–1.86 (m)	1.86–1.83 (m)	1.81–1.79 (m)	1.77- 1.74 (m)
	1.30–1.27 (m)	1.25–1.20 (m)	1.27–1.25 (m)	1.31–1.18 (m)
2	1.96–1.94 (m)	1.97–1.95 (m)	1.91–1.89 (m)	1.94–1.92 (m)
	1.64–1.61 (m)	1.69–1.66 (m)	1.66–1.62 (m)	1.54–1.50 (m)
3	4.60 (dd, J = 12.2, 4.3)	3.56 (dd, J = 12.4, 3.6)	4.70 (d, J = 12.0, 3.4)	
5	0.72–0.67 (m)	0.68–0.65 (m)	0.75–0.70 (m)	0.87–0.85 (m)
6	1.56–1.52 (m)	1.60–1.63 (m)	1.62–1.58 (m)	1.57–1.54 (m)
	1.45–1.40 (m)	1.35–1.31 (m)	1.43–1.40 (m)	1.44–1.39 (m)
7	1.70–1.66 (m)	1.45–1.41 (m)	1.55–1.52 (m)	1.65–1.61 (m)
	1.27–1.23 (m)	1.23–1.21 (m)	1.25–1.20 (m)	1.31–1.27 (m)
9	1.90 (t, J = 9.0)	1.84 (t, J = 9.1)	1.83 (d, J = 8.9, 8.4)	1.88 (dd, J = 9.6, 8.6)
11	3.50 (dd, J = 8.8, 3.3)	3.62 (dd, J = 9.0, 2.8)	2.03–1.97 (m)	
			1.89–1.86 (m)	1.91–1.87 (m)
12	5.30 (br t, J = 3.4)	5.26 (br t, J = 3.1)	5.19 (br t, J = 3.5)	1.78–173 (m)
15	1.58–1.52 (m)	1.41–1.38 (m)	1.48–1.44 (m)	5.31 (br t, J = 2.9)
	1.20–1.17 (m)	1.04–0.96 (m)	1.10–1.05 (m)	1.54–1.50 (m)
16	1.74–1.69 (m)	1.58–1.55 (m)	1.61–1.57 (m)	1.19–1.10 (m)
	1.32–1.27 (m)	1.37–1.30 (m)	1.40–1.37 (m)	1.71–1.66 (m)
18	2.40 (br s)	2.20 (s)	2.24 (s)	1.42–1.39 (m)
20	1.57–1.51 (m)	1.50–1.46 (m)	1.58–1.50 (m)	2.10 (br s)
21	1.49–1.44 (m)	1.42–1.39 (m)	1.47–1.43 (m),	1.60–1.57 (m)
	1.40–1.37 (m)	1.20–1.16 (m)	1.33–1.29 (m)	1.48–1.45 (m)
22	2.14–2.00 (m)	1. 99–1.94 (m)	1.97–1.93 (m)	1.37–1.33 (m)
	1.41–1.38 (m)	1.40–1.37 (m)	1.38–1.34 (m)	2.01–1.97 (m)
23			3.74 (d, J = 10.2)	1.43–1.40 (m)
			3.45 (d, J = 10.2)	0.98 s
24	1.06 s	1.02 s	0.95 s	
25	1.00 s	0.97 s	0.96 s	
26	1.10 s	0.98 s	0.97 s	1.11 s
27	1.25 s	1.13 s	1.23 s	0.89 s
28	3.86 (d, J = 10.5)		3.68 (d, J = 10.5)	1.27 s
	3.73 (d, J = 10.5)		3.50 (d, J = 10.5)	4.10 (d, J = 11.6)
29	1.23 s	1.21 s	1.12 s	3.80 (d, J = 11.6)
30	0.87 (d, J = 6.5)	0.88 (d, J = 6.4)	0.89 (d, J = 6.7)	1.17 s
CO_2Me	3.54 s			0.85 (d, J = 6.1)
OAc	2.01 s		2.02 s	

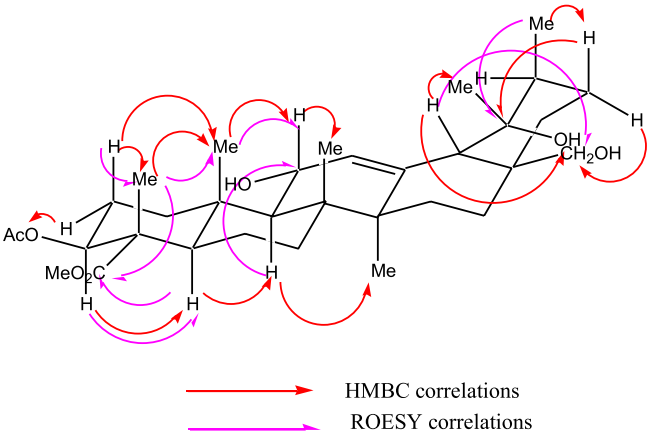

Figure 3. Selected HMBC and ROESY correlations of compound **1.**

the other hand, a comparative analysis of both ^{1}H and ^{13}C NMR spectra of compound **1** with those of 3β-acetoxy-11α-hydroxyurs-12-ene [19] suggested that a hydroxyl group was present at C-11 as indicated by the methine proton peak at δ 3.50 (dd, J = 8.8, 3.3 Hz) and its corresponding ^{13}C NMR peak at δ 73.3. This methine proton was deduced to be β-axial based on the larger coupling constant (J = 8.8 Hz) which represented *trans*-diaxial coupling between H-11 and H-9 (δ 1.90) while the smaller coupling constant (J = 3.3 Hz) was due to interaction of the same proton with the vinyl proton H-12 [21]. In fact, the coupling constants of H-11 with H-9 and H-12 suggested that hydroxyl group had an α-equatorial and the proton H-11 had the β-axial orientations (**22**). The forgoing evidence was further confirmed by the NOESY cross-peak between H-11 and Me-25 (Figure 3), thus corroborating the orientation of H-11 as β. The ^{1}H NMR spectrum also established the presence of a singlet at δ 2.40 which is characteristic of H-18 of ursane-type triterpene with 19-*O* substitution [22], a fact that was corroborated by HMBC correlation between H-18 and C-19 (δ 72.1) and further supported by NOESY correlation between H-18 and Me-29, which in turn showed NOESY cross-peak with H-30. In the HMBC spectrum, the proton signal attributed to H-5 (δ 0.72–0.67, m) showed long range correlation with a carbon signal at δ 180.6 (C-23), corroborating the presence of CO_2Me functionality with a relative orientation α as deduced by the ^{13}C NMR CH_3–24 shift to δ 13.7. This was further supported by the NOESY cross-peak between H-5 and methyl carboxyl. The latter also showed NOESY cross-peak with H-3. The foregoing evidence was further substantiated by the cross-peak observed between H-2 and Me-24 in the NOESY spectrum. Based on spectroscopic evidences as well as comparison with literature data, compound **1** was concluded to be methyl 3β-acetoxy-11α, 19α, 28-trihydroxyurs-12-en-23-oic acid.

Compound **2,** white amorphous powder, exhibited absorption peaks due to hydroxyl (3500 cm^{-1}), acid carbonyl (1710 cm^{-1}), and carbon-carbon double bond (1640 cm^{-1}) groups in the IR spectrum. It was assigned a molecular formula $C_{30}H_{46}O_7$ based on HR-ESIMS molecular ion peak at *m/z* 519.3324 $[M + H]^+$. The EI-MS spectrum on the other hand displayed significant fragments at *m/z* 500 $[M\text{-}H_2O]^+$, 482 $[M\text{-}2H_2O]^+$, 463 $[M\text{-}CO_2H]^+$, 280 ($C_{16}H_{24}O_4$), 262 ($C_{16}H_{22}O_3$), 238 ($C_{14}H_{22}O_3$), 220 ($C_{14}H_{20}O_2$), 219 ($C_{15}H_{23}O$), 201 ($C_{15}H_{21}$), and 187 ($C_{14}H_{19}$). The peaks at *m/z* 238 and 220 are diagnostic *retro*-Diels-Alder fragments

associated with A/B rings containing hydroxyl and CO_2H and double bond between C-12 and C-13 [18]. Similarly, the daughter ion observed at *m/z* 280 was in agreement with *retro*-Diels-Alder cleavage of β-amyrin-type triterpene having two secondary hydroxyls and carboxyl groups in the C/D/E rings [18]. The compound afforded a total of 30 carbon resonances in the ^{13}C NMR spectrum which were sorted out by DEPT into six methyls, eight methylenes, seven methines including one olefinic and two protons on carbons bearing hydroxyl groups as well as nine quaternary carbons. The ^1H NMR spectrum showed the presence of a vinyl proton at δ 5.26 (br t, J = 3.1 Hz, H-12) and two oxygenated methine protons at δ 3.56 (dd, J = 12.4, 3.6 Hz, H-3) and 3.62 (dd, J = 9.0, 2.8 Hz, H-11), which were close to those of compound **1**, and were observed to correlate with carbon peaks at δ 127.3 (C-12), 76.3 (C-3), and 73.1 (C-11), respectively in the HSQC spectrum. The chemical shifts and coupling constants of the hydroxymethines were similar to those of compound **1**, suggesting that the hydroxyls were positioned at C-3 and C-11 and possibly with the same stereochemistry. The hydroxyl group at C-3 was confirmed to be in equatorial orientation from axial-axial and axial-equatorial couplings observed between H-3 and H-2 protons; a fact corroborated by observed NOESY cross-peaks between H-3 and H-5 (δ 0.68–0.65, m), while the orientation of OH-11 in α-position was confirmed by NOESY cross-peak between H-11 and Me-25. In the HMBC spectrum, a correlation was observed between H-18 (δ 2.20, s) and the carboxyl carbon signal at δ 179.7, which in turn correlated with H-22 (δ 1.99–1.94, m), thus confirming the carboxyl group to be at C-28. Similarly, the proton signal attributed to H-5 (δ 0.68–0.65, m) showed the long range correlation with a carbon signal at δ 177.4 (C-23), confirming the presence of second CO_2H moiety with a relative orientation α as substantiated by the relatively up field shift of CH_3–24 to δ 13.1 [18]. This was further evidenced by NOESY correlation between CH_3–24 (δ 1.02, s) and CH_3–25 (δ 0.97, s) which confirmed that CO_2H group at C-4 was α-oriented. On the other hand, a singlet at δ 2.20 which is characteristic for H-18 of an ursane-type triterpene with 19-*O*-substitution, together with five tertiary methyl singlets (δ 0.97, 0.98, 1.02, 1.13, 1.21), a methyl doublet (δ 0.88, J = 6.4 Hz, Me-30), and olefinic proton (δ 5.26, J = 3.1 Hz) further confirmed the 19α-hydroxyurs-12-ene skeleton for compound **2**. Thus, on the basis of spectroscopic data as well as comparison with rotundic acid data [18], compound **2** was concluded to be 3β, 11α, 19α-trihydroxyurs-12-en-23, 28-dioic acid.

Compound **3,** $C_{32}H_{52}O_5$, exhibited a HRESI-MS ion at *m/z* 517.3901 $[M + H]^+$, together with $[\alpha]_D^{25} + 49$ (c 0.4, MeOH). Its IR spectrum showed the presence of hydroxyl (3360 cm^{-1}), ester carbonyl (1737 cm^{-1}), and carbon–carbon double bond (1636 cm^{-1}) functionalities. The ^{13}C NMR spectrum (Table 2) revealed the presence of 32 carbon signals including CH_3 x 7, >CH-O x 1, $>CH_2$-O x 2, >CH- x 4, >C=CH x 1, $>CH_2$- x 9, >C< O x 1, >C-O x1 >C< x 5, -CH=C< x 1 as evidenced by DEPT spectrum. Unequivocal information for the ring system and substitution pattern in compound **3** was substantiated by EI-MS which showed two characteristic peaks at *m/z* 250 ($C_{16}H_{26}O_2$, rel. int. 30) and 266 ($C_{16}H_{24}O_3$, rel. int. 14) suggesting the *retro*-Diels-Alder cleavage of ring C resulting in fragments commonly observed in the spectra of olean-12-ene and/or urs-12-ene derivatives possessing oxymethylene and acetoxy groups in rings A/B and another oxymethylene and a hydroxyl group in rings D/E [18]. The ^1H NMR spectrum (Table 1) of compound **3** showed a singlet peak at δ 2.24 (H-18) which together with six tertiary methyls (δ 0.95, 0.96, 0.97, 1.12, 1.23, and 2.02, each singlet including acetoxy group) and a methyl doublet (δ 0.89, d, J = 6.7 Hz) suggested the presence of ursane-type triterpene with 19-*O*-substitution [21,22], a fact

Table 2. ^{13}C NMR spectral data of compounds **1**–**3** in $CDCl_3$ + drop DMSO-d_6 (600 MHz).

C	1	2	3	4
1	38.4	34.1	37.9	38.9
2	27.5	25.7	28.7	29.3
3	78.0	76.3	80.0	208.1
4	42.3	42.6	42.8	47.2
5	54.6	54.1	52.5	55.6
6	19.3	19.0	18.5	19.8
7	33.3	33.2	33.3	34.0
8	40.9	41.3	39.9	40.2
9	51.1	48.1	48.7	46.6
10	31.0	37.4	37.1	37.5
11	73.3	73.1	24.4	24.5
12	126.0	127.3	128.0	127.9
13	140.4	139.4	140.2	138.8
14	42.5	43.1	41.7	42.0
15	28.4	27.7	30.6	28.7
16	26.0	25.9	27.6	26.6
17	47.8	48.9	47.0	48.4
18	53.5	54.5	54.6	54.3
19	72.1	71.9	72.4	73.2
20	42.5	44.0	39.7	41.8
21	27.4	27.2	26.8	27.0
22	38.3	38.6	38.5	39.1
23	180.6	177.4	68.4	23.5
24	13.7	13.1	12.8	181.1
25	16.7	16.4	17.0	15.0
26	16.9	17.7	16.8	17.0
27	23.9	25.2	25.0	25.3
28	68.7	179.7	64.5	65.4
29	17.4	27.7	27.1	26.9
30	28.6	17.9	16.2	16.8
CO_2CH_3	55.4			
OAc	170.4, 21.7		169.9, 21.2	

confirmed by an EI-MS ion at *m/z* 232 (due to loss of H_2O from the daughter ion at *m/z* 250) and the ^{13}C NMR peak at δ 72.4 (for oxygenated tertiary carbon). Furthermore, from the ^1H NMR spectrum, the methine bearing acetoxy group exhibited doublet of doublet at δ 4.70 (d, J = 12.0, 3.4 Hz) was assignable to H-3α on the basis of large coupling constant characteristic of H-3 and H-2 axial–axial interaction, a fact further evidenced by HMBC correlations between H-5 (δ 0.70–0.75) and C-3 (δ 80.0) and also between H-3 and C-23 (δ 68.4) [18]. From the EI-MS data, the terminal hydroxymethylene attached to ring A was assigned to C-23 on the basis of ^{13}C NMR peak at δ 68.4 which suggested a chemical shift of C-24 methyl to be at δ 12.8 [18,22] and was confirmed by NOESY spectrum which revealed the ^1H–^1H proximity between H-3 and C-23 methylene protons. Similarly, a correlation was also observed between H-3 and H-5. Thus, on the basis of spectroscopic evidences as well as comparison with various literature data, compound **3** was established to be 3β-acetoxy-19α, 23, 28-trihydroxyurs-12-ene.

Compound **4** was obtained as white amorphous powder from CH_2Cl_2-MeOH mixture. Its spectrum revealed the presence of hydroxyl (3390 cm^{-1}), carboxylic acid (1717 cm^{-1}), keto (1705 cm^{-1}), and carbon–carbon double bond (1638 cm^{-1}) functionalities. The ^{13}C NMR spectrum revealed a total 30 carbon signals which were sorted out by 135 DEPT spectrum into ten methylenes, six methyls, five methines, and nine quaternary carbons. Comparison of the ^1H and ^{13}C NMR spectral data of compound **4** with those of 19α, 24-dihydroxy-12-en-3-one-28-oic acid [22] revealed close similarities with notable differences being the

substitution patterns in the A/B and C/D rings in **4** as substantiated by the EI-MS daughter ions observed at *m/z* 236 ($C_{14}H_{20}O_{3,}$ rings A/B) and 250 ($C_{16}H_{26}O_{2}$, rings C/D). The foregoing evidence was further confirmed by the HMBC cross-peak between the keto carbon (δ 208.1) and H-5 (δ 0.87–0.85, m)/CH_3–23 (δ 0.98). H-5 in turn also showed cross-peak with the carboxylic acid carbon at δ 181.1. Comparing the data of compound **4** with those of rotundic acid [18] revealed a shift of an exomethylene peak at δ 65.4 suggesting that the terminal hydroxymethylene group was possibly on C-17, a fact that was further supported by the HMBC cross-peaks between H-28 (δ 4.10) and C-18 (δ 54.3)/ C-22 (δ 39.1). On the other hand, in the HMBC spectrum the proton signal ascribable to H-5 (δ 0.87–0.85, m) exhibited a long range correlation with the carbon signal at δ 23.5 (C-23), suggesting that the carboxyl group was at C-24 with β orientation as exemplified by the NOESY cross-peak between H-5 and C-23 methyl group. Thus, on the basis of spectroscopic data as well as comparison with literature data, compound **4** was established to be 3-oxo-19α, 28-dihydroxyurs-12-en-24-oic acid.

2.2. Biological activities

The n-hexane, ethyl acetate, and methanol extracts as well as isolated compounds from *E. buchananii* stem bark were tested for their potency against bacteria and fungi and the results are shown in Figures 4–6 and Table 3.

The antibacterial activity of n-hexane extract at 1500 µg/ml on both *S. aureus* and *N. meningitidis* was comparable to commercial tetracycline used as positive control with zone diameters of 12.20 ± 0.20 and 14.17 ± 0.15 mm, respectively and 12.13 ± 0.15 and 13.17 ± 0.15 mm, respectively for the tetracycline (Figure 4). However, the extract did not exhibit antifungal activity. Ethyl acetate extract on the other hand, displayed promising antibacterial activities (Figure 5) compared to n-hexane and MeOH extracts. It showed

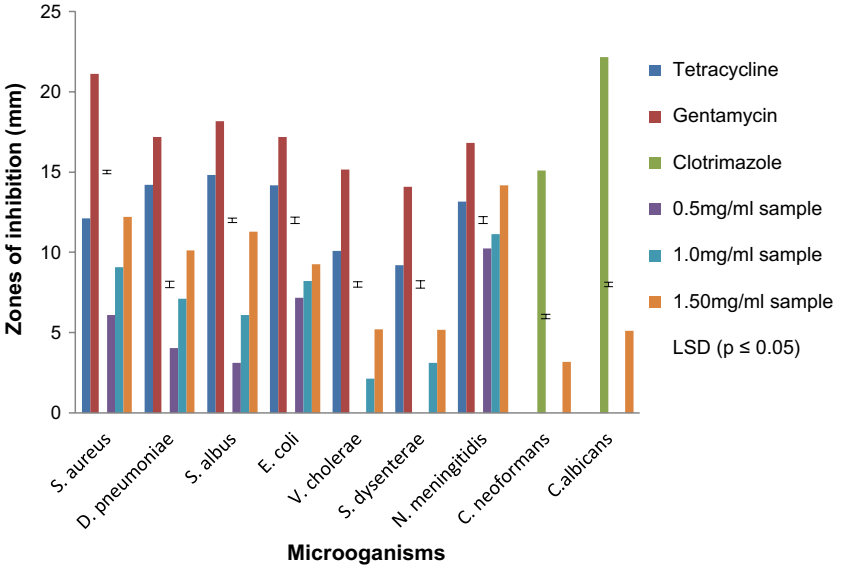

Figure 4. Antibacterial and antifungal activities of n-hexane extract of *E. buchananii* stem bark.

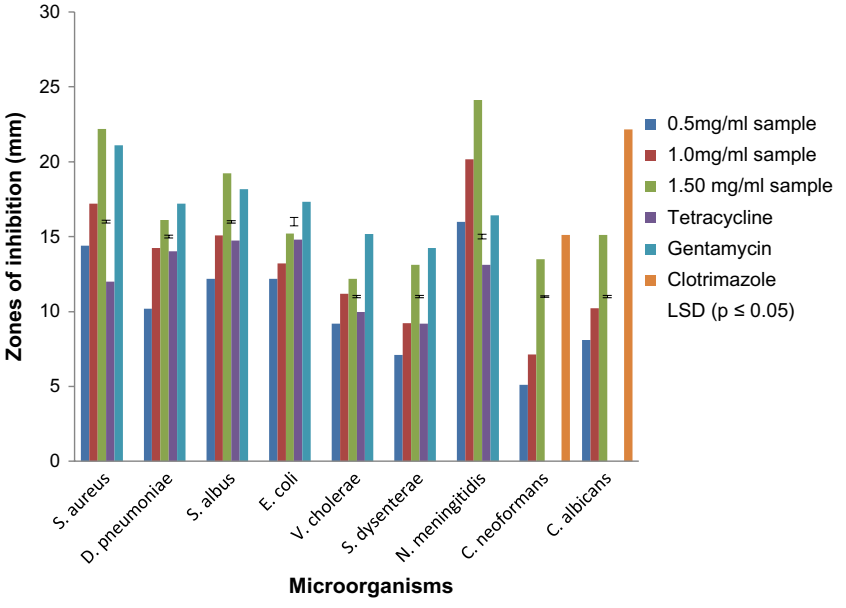

Figure 5. Antibacterial and antifungal activities of ethyl acetate extract of *E. buchananii* stem bark.

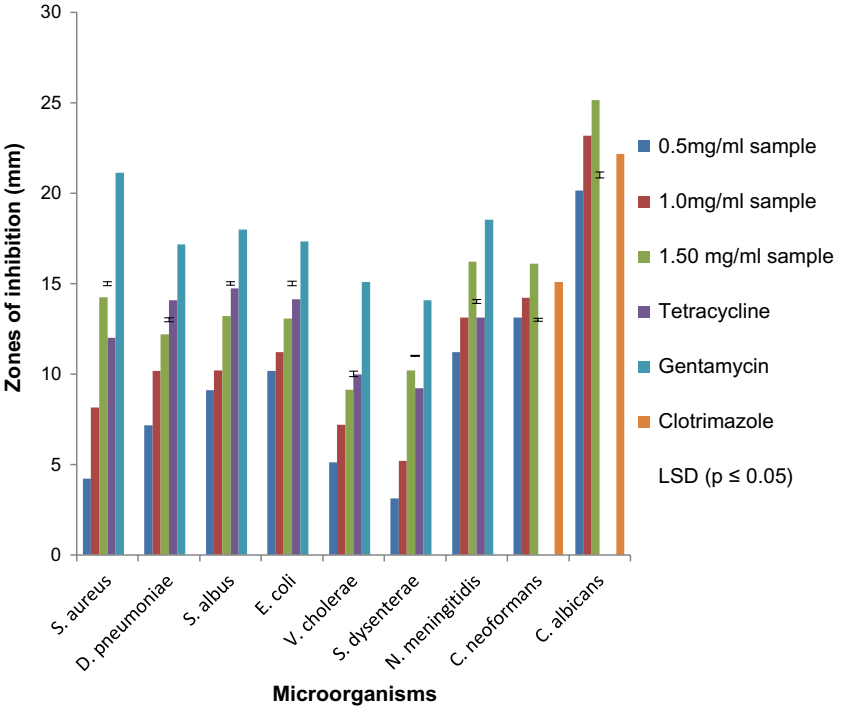

Figure 6. Antibacterial and antifungal activities of methanol extract of *E. buchananii* stem bark.

Table 3. Minimum inhibitory concentration for crude extracts and isolates (μg/ml).

Crude extracts/ compounds	Microorganisms								
	s. aureus	*D. pneumoniae*	*S. albus*	*E. coli*	*V. cholerae*	*S. dysenterae*	*N. meningitidis*	*C. neoformans*	*C. albicans*
Extracts									
n-hexane	250	>500	>500	>500	>500	>500	>500	>500	>500
EtOAc	15.62	62.50	31.25	62.5	125	250	15.62	250	125
MeOH	125	250	125	125	250	250	>500	31.25	15.62
Compounds									
1	250	250	125	250	>250	>250	>250	>250	>250
2	250	250	500	>250	>250	>250	>250	125	250
3	250	125	250	125	125	250	>250	250	250
4	250	>250	>250	>250	250	>250	>250	125	250
5	125	125	250	125	>250	250	250	>250	>250
6	250	125	250	125	250	250	250	250	250
7	62.5	125	62.5	125	250	>250	>250	125	62.5
8	62.5	125	62.5	125	250	250	31.25	>250	>250
9	250	250	250	>250	>250	>250	250	62.5	62.5
10	250	>250	>250	>250	>250	>250	250	125	125
11	250	250	>250	>250	>250	250	250	250	>250
12	>250	>250	>250	>250	>250	>250	250	125.0	>250
13	62.5	125	62.5	125	>250	>250	125	>250	>250
14	>250	>250	>250	>250	>250	250	>250	>250	>250
Gentamycin	7.82	15.62	62.5	62.5	62.5	31.25	15.62		
Clotrimazole								3.91	0.98
DMSO (99.9%)	>500	>500	>500	>500	>500	>500	>500	>500	>500

Notes: Crude extracts: values >62.5 were considered not active. Pure compounds: values >125 were considered not active.

better antibacterial activities on all the tested bacteria than the tetracycline used as the positive control with zone diameters ranging from 12.20 ± 0.10 mm to 24.13 ± 0.06 mm (at 1500 μg/ml) and 9.20 ± 0.10 mm to 14.80 ± 0.06 mm for tetracycline. The extract showed better antibacterial activities against *S. aureus, S. albus,* and *N. meningitides* than gentamycin [(zones of inhibition 22.20 ± 0.01, 19.23 ± 0.06, and 24.13 ± 0.06 mm, respectively) and gentamycin (21.10 ± 0.01, 18.17 ± 0.06, and 16.43 ± 0.06 mm, respectively)]. The extract showed a much lower antifungal activity compared to antibacterial activity with inhibition zones of 15.13 ± 0.10 mm against *C. albicans* and 13.50 ± 0.10 mm against *C. neoformanss*. The methanol extract exhibited mild antibacterial activities but promising antifungal activities on both the fungi tested at the same concentration that was better than clotrimazole used as positive control (Figure 5). The inhibition zones of the extract on *C. neoformans* and *C. albicans* were 16.10 ± 0.01 mm and 25.13 ± 0.15 mm, respectively while those due to clotrimazole on the same fungi were 15.10 ± 0.06 and 22.17 ± 0.15 mm, respectively. The ethyl acetate extract inhibited both Gram-positive and Gram-negative bacteria significantly compared to methanol and n-hexane extracts indicating that most of the active components of the stem bark were extracted by ethyl acetate. The n-hexane showed the least activity against most of the organisms tested, which may be due to the non polar nature of the compounds associated with the extract. The methanol extract was more potent against the fungi which were in agreement with previously reported results [23] that the methanol extract of the stem bark of *E. buchananii* exhibited strong activity against *C. albicans.* The overall results of these extracts are comparable to those of the reference drugs; tetracycline, gentamycin, and clotrimazole, thus indicating promising baseline information

for the potential uses of the methanol and ethyl acetate extracts of *E. buchananii* against pathogens. All the isolated compounds were screened for both antibacterial and antifungal activities (Table 3). The isolate umbelliferone (**9**) showed antifungal activity with MIC value of 62.5 µg/ml against both *Crytococcus neoformans* and *C. albicans.* Both canophyllol (**8**) and friedelin (**13**) exhibited antibacterial activities against *S. aureus* and *S. albus* with MIC value of 62.5 µg/ml. Compound **8** also showed promising antibacterial activity against *N. meningitides* with MIC value of 31.25 µg/ml. Ursolic acid (**7**) displayed both antibacterial and antifungal activities particularly against *S. aureus*, *S. albus,* and *C. albicans* with MIC value of 62.5 µg/ml. These findings compared well with the previous reported data [24] that canophyllol **(8)** demonstrated good activity compared with ambicillin and amoxicillin against *S. aureus* and that friedelin (**13**) exhibited good antifungal activity against *Trichophyton schoenleinii,* a cutaneous fungus occurring in hair, skin, and nails.

3. Experimental

3.1. General experimental procedures

Melting points were determined using Gallen-Kamp electrothermal melting point apparatus (Manchester, U.K) and are uncorrected. Medium pressure chromatography was performed using Eyela flash column chromatography model Ep-100 (Darmstadt, Germany). IR data were recorded on a Bruker Tensor 27 FTIR spectrophotometer (Bruker Corporation, Bremen, Germany) as KBr pellets. The ^{1}H and ^{13}C NMR spectra were recorded on Brucker AM-600 Ultra-Shield (Brucker, Billerica, MA, U.S.A) operating at 600 MHz and 150 MHz, respectively using $CDCl_3$ or $CDCl_3$-DMSO-d_6 and chemical shift are expressed in ppm with tetramethylsilane (TMS) as internal standard. EIMS were recorded on a 70 eV MAT 311A Varian MAT Bremen instrument while ESI- MS data were obtained from Varian MAT 8200 A instrument (Bremen, Germany). Silica gel 60 (0.063–0.20 mm, Merck-Germany) was used for column chromatography while silica gel 60 F254 (Merk-Germany) pre-coated plates were used for thin layer chromatography (Kobian Kenya LTD, Kenya). Paper chromatography was done on standard Whatman No 1 chromatography paper (Kobian Kenya LTD, Kenya). The reagents and solvents used were of analytical grades (Kobian Kenya LTD, Kenya).

3.2. Plant material

The plant material (stem bark of *Elaeodendron buchananii*) was collected from Ngong Forest, Kenya [1°18′13.62″S; 36° 43′ 07.11″ E] in April 2007. The voucher specimens (fruits, leaves, and twigs) were identified by Mr. Mutiso of Botany Department, University of Nairobi after comparison with herbarium authentic samples.

3.3. Extraction and isolation

Two kilograms of the shade-dried ground powdered stem bark were extracted sequentially using n-hexane, ethyl acetate, and methanol (five liters each) with occasional shaking at room temperature. The extracts were concentrated *in vacuo* to give yellow-brown, dark-brown, and red materials in the yields of 25, 80, and 300 g, respectively.

Approximately 20 g of the extract was dissolved in 10 ml of dichloromethane and chromatographed over silica gel packed column (3.0 × 60 cm, SiO_2 300 g) using n-hexane with

increasing amounts of ethyl acetate up to 100% of the latter. A total of 200 fractions, each 20 ml, were collected and their homogeneity monitored by TLC (solvent systems: n-hexane-EtOAc, 9:1 and 4:1). The fractions were grouped into three pools (1-III) depending on TLC profiles. Fractions 26–86 constituted pool I, and a yellow oil which lost color with time was discarded. Pool II (fractions 90–140, 5 g) crystallized out to give a colorless compound which on further re-crystallization gave 3β-acetoxy-β-amyrin (**14,** 90 mg). The mother liquor of this pool upon subjection to further medium pressure chromatography using n-hexane-EtOAc (9:1) afforded more of 3β-acetoxy-β-amyrin (**14,** 40 mg), friedelin (**13,** 65 mg). Pool III (fractions 145–192, 4 g) showed one spot and was further purified by crystallization to give stigmasterol (**12,** 175 mg).

The ethyl acetate extract approximately 75 g was subjected to pass over silica gel column (4.0 × 60 cm, silica gel 500 g) using n-hexane-ethyl acetate (10% increment of ethyl acetate), ethyl acetate neat and finally with CH_2Cl_2-MeOH (with 0.5 and 1% increment of MeOH). Three hundred and fifty fractions (each 20 ml) were sampled and their composition monitored by TLC, eluent: n-hexane-ethyl acetate (4:1, 3:2 and1:1) and CH_2Cl_2-MeOH (99:1, 98:2 and 97:3). Those exhibiting similar TLC profiles were combined resulting into five major pools (A-E). Pool A (fractions 20–80, 6.5 g), upon removal of solvent, afforded a yellow oily paste which lost color with time and was discarded. Pool B (fractions 86–150, 16 g) was similarly subjected to silica gel column and elution with n-hexane-ethyl acetate (4:1) followed by (3:2) afforded 150 fractions of 20 ml each, which were combined into two major fractions (B1 and B2) depending on the TLC profiles. Fraction B1 contained two spots which upon further purification gave friedelin (**13,** 65 mg) and stigmasterol (**12**, 35 mg). Fraction B2 on evaporation of the solvent crystallized out to give white powder, which upon re-crystallization (n-hexane-EtOAc, 3:2) gave coumarin (**11,** 35 mg). Fractions 160–210 constituted pool C (7 g), which showed a single spot on TLC, and upon crystallization (n-hexane-EtOAc, 3:2) afforded 3β-hydroxyfriedelin (**10**, 31 mg). Fractions 200–290 (pool D, 11 g) were resolved into individual components using n-hexane-EtOAc (1:1) followed by same solvent system in the ratio 1:2 to give umbilleferone (**9,** 80 mg) and canophyllol (**8**, 65 mg). Pool E (Fractions 295–335, 6 g), upon repeated medium pressure chromatography over silica gel using CH_2Cl_2-MeOH (99:1) followed by the same solvent system (98:2), afforded ursolic acid (**7,** 80 mg) and 3-oxo-19α, 28-dihydroxyurs-12-en-24-oic acid (**4**, 54 mg).

A portion of MeOH extract (approx. 100 g) was chromatographed over silica gel column using CH_2Cl_2-MeOH (5% increment of MeOH) and MeOH neat to give a total of 200 fractions (each 50 ml). Their homogeneity was monitored by TLC using CH_2Cl_2-MeOH (98:2, 97:3, 95:5, 9:1, and 4:1) and the fractions were grouped into two pools (F and G) depending on TLC profile. Pool F (fractions 15–90, 12 g) on repeated medium pressure chromatographic separation using CH_2Cl_2-MeOH (98:2) followed by the same solvent system in the ratio 97:3 gave **3** (25 mg) and **1** (41 mg). Pool G (9 g) similarly was chromatographed over silica gel column using CH_2Cl_2-MeOH (97:3) to yield elaeodendroside A (**5**, 45 mg), **2** (36 mg) and elaeodendroside B (**6**, 25 mg).

3.3.1. Methyl 3β-acetoxy-11α, 19α, 28-trihydroxyurs-12-en-23-oic acid (1)

White amorphous powder from CH_2C_2-MeOH (9:1); $[\alpha]_D^{25}$ + 1.5 (c 0.1, MeOH); mp 244–246° C; IR ν_{max} (KBr) cm^{-1}: 3350, 2932, 2855, 1734, 1722, 1630, 1455, 1410, 1380, 1250, 1111, 1028, 984; ^1H and ^{13}C NMR spectral data ($CDCl_3$ + drop DMSO-d_6, 600 MHz) see Tables 1 and 2; EI-MS *m/z* (rel. int.): 561 $[M + H]^+$ (2), 560 (4), 542 (2), 524 (4), 500 (3), 294 (13),

266 (11), 248 (22), 234 (100), 232 (5), 219 (50), 201 (21), 175 (31), 133 (60). HR-ESI-MS: *m/z* 561.7698 $[M + H]^+$ (calcd for $C_{33}H_{53}O_5$, 561.7697).

3.3.2. 3β, 11α, 19α-Trihydroxyurs-12-en-23, 28-dioic acid (2)

White amorphous powder from CH_2Cl_2-MeOH (9:1); mp > 250° C; $[\alpha]_D^{25} - 10.6$ (c 0.6, MeOH); IR ν_{max} (KBr) cm^{-1}: 3500, 1710, 1640, 1580, 1450, 1380, 1340, 1250, 1210, 1110, 1028, 960; ^1H and ^{13}C NMR spectral data ($CDCl_3$ + drop DMSO-d_6, 600 MHz) see Tables 1 and 2; EI-MS: *m/z* (rel. int.): 503 $[M\text{-}Me]^+$(9), 500 $[M\text{-}H_2O]^+$ (6), 482 $[M\text{-}2H_2O]^+$(3), 464$[M\text{-}3H_2O]^+$ (11), 473 $[M\text{-}CO_2H]^+$ (15), 280 $[C_{16}H_{24}O_4]^+$ (100), 262 $[C_{16}H_{22}O_3]^+$ (7), 238 $(C_{14}H_{22}O_3]^+$ (11), 220 $[C_{14}H_{20}O_2]^+$ (14), 219 $[C_{15}H_{23}O]^+$(14), 207 $[C_{15}H_{17}O_2]^+$ (31), 202 $[C_{15}H_{22}]^+$ (23), 189 $[C_{14}H_{21}]^+$ (41), 187 $[C_{14}H_{19}]^+$ (21), (175 $[C_{13}H_{19}]^+$ (13), 134 $[C_{10}H_{14}]^+$ (51). HR-ESI-MS: *m/z* 519.3324 $[M + H]^+$ (calcd for $C_{30}H_{47}O_5$, 519.3322).

3.3.3. 3β-Acetoxy-19α, 24, 28-trihydroxyurs-12-ene (3)

White amorphous powder from CH_2Cl_2-MeOH (9:1); mp 192–194° C; $[\alpha]_D^{25} + 49$ (c 0.1, MeOH); IR ν_{max} (KBr) cm^{-1}: 3360, 2930, 2860, 1737, 1636, 1580, 1450, 1020, 888; ^1H and ^{13}C NMR spectral data ($CDCl_3$ + drop DMSO-d_6, 600 MHz) see Tables 1 and 2; EI-MS *m/z* (rel. int.): 516 $[M]^+$, 501 $[M\text{-}Me]^+$ (2), 498 $[M\text{-}Me\text{-}H_2O]^+$ (5), 483 $[M\text{-}2H_2O]^+$ (1), 456 $[M\text{-}MeCO_2H]^+$ (11), 423 $[M\text{-}Me\text{-}H_2O\text{-}MeCO_2H]^+$ (13), 266 $[C_{16}H_{26}O_3]^+$ (14), 250 $[C_{16}H_{26}O_2]^+$ (30), 232 $[C_{16}H_{24}O]^+$ (21), 219 $[C_{15}H_{23}O]^+$ (9), 206 $[C_{14}H_{22}O]^+$ (13), 201 $[C_{15}H_{21}]^+$ (100), 191 $[C_{13}H_{19}O]^+$ (33), 175 $[C_{13}H_{19}]^+$ (21), 133 $[C_{10}H_{14}]^+$ (45). HRESI-MS: *m/z* 517.3901 $[M + H]^+$ (calcd for $C_{32}H_{53}O_5$, 517.3893).

3.3.4. 3-Oxo-19α,28-dihydroxyurs-12-en-24-oic acid (4)

Amorphous white powder; mp > 250° C; $[\alpha]_D^{25} + 32$ (c 0.5, MeOH); IR ν_{max} (KBr) cm^{-1}: 3390, 1717, 1705, 1638, 1447, 1380, 1026., ^1H and ^{13}C NMR spectral data ($CDCl_3$ + drop DMSO-d_6, 600 MHz) see Tables 1 and 2; EI-MS: *m/z* 486 $[M]^+$ (1), 471 $[M\text{-}Me]^+$ (2), 468 $(M\text{-}H_2O]^+$ (6), 250 $[C_{16}H_{26}O_2]^+$ (70), 236 $[C_{14}H_{20}O_3]^+$ (17), 232 $[C_{16}H_{24}O]^+$ (100), 201 $[C_{15}H_{21}]^+$ (13), 191 $[C_{13}H_{19}O]^+$ (25), 133 (20), 73 (50). HRESI-MS: *m/z* 487.3430 $[M + H]^+$ (calcd for $C_{30}H_{47}O_4$, 487.3424).

3.4. Antimicrobiol assays

3.4.1. Test microorganisms

Three Gram-positive (*Staphylococcus aureus* ATCC 25923, *Diplococcus pneumoniae,* and *Staphylococcus albus*) and four Gram-negative (*Eschirichia coli* ATCC 25922, *Vibrio cholerae, Shigella dysenterae, and Neisseria meningitides*) were used for bacteria activity screening. The fungal pathogens used were *Candida albicans* (ATCC 90028) and *Cryptococcus neoformans*. The test organisms *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *C. albicans* ATCC 90028 were obtained from the stock kept at the Microbiology Section of the Jaramogi Oginga Odinga Teaching and Referral Hospital in Kisumu County, Kenya. The rest of the organisms were clinical isolates from the same Hospital.

3.4.2. Disk diffusion assay

The Baur-Kirby procedure which uses known concentrations of antimicrobial substances impregnated on paper disks was used [25]. The anti-biogram patterns were studied using

n-hexane, EtOAc, and MeOH extracts of *E. buchananii* stem bark. Three concentrations 0.5 mg/ml (dissolving 5 mg of each extract in 10 ml of DMSO), 1.0 mg/ml (dissolving 10 mg of each extract in 10 ml of DMSO), and 1.5 mg/ml (dissolving 15 mg of each extract in 10 ml of DMSO) were employed in this study. Sterile paper disks (6 mm of diameter) were impregnated with 20 µl of each solution of the extracts. The impregnated disks were kept in an incubator at 37° C for 30 min for proper diffusion of extracts before putting on to the plates. DMSO loaded to the disks was used as negative controls for the extracts. Overnight cultures of bacteria and fungi species were used for inocula preparation. The inocula were prepared by making microorganisms to grow in a sterile saline and turbidity adjusted to yield 0.5 McFarland standards [approximately $1–2 \times 10^8$ colony-forming units per milliliters (for bacteria) and approximately $1–5 \times 10^6$ colony-forming units per milliliters (for fungi)]. Petri dishes containing Mueller-Hinton agar and Sabouraud dextrose agar were seeded with 100 µl of the prepared bacterial and fungal inocula, respectively. The disks were then incubated at 37° C for bacteria and at 25° C for fungi for 24 h. Similarly, paper disks containing standard concentrations of antibiotics (tetracycline, 30 µg per disk; gentamicin, 10 µg per disk) and antifungal (clotrimazole, 30 µg per disk) were used as positive control. Antimicrobial activities were determined by measuring the zone of growth inhibition (mm) around the disk. The result recorded for each bioassay was the average of three tests and the obtained results were statistically analyzed.

3.4.3. Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of the test sample in which there is no growth of microorganism. The MIC of crude extracts and isolated compounds **1**–**14** was determined by broth microdilution method [25].

Stock solutions of plant extracts (n-hexane, EtOAc, and MeOH) were prepared by dissolving 1 mg of each extract in 1 ml of dimethyl sulfoxide (99.9%) giving the initial concentration of 1 mg/ml (1000 µg/ml). This was diluted using two fold serial dilution by transferring 1 ml of the sterile plant extract (stock solution) into 1 ml of sterile Mueller Hinton broth for bacteria and Sabouraud dextrose broth for fungi to obtain 500 µg/ml concentration. The above process was repeated several times to obtain other dilutions including 250, 125, 62.5, 31.25, 15.6, 7.8, and finally 3.9 µg/ml. For pure isolates, a stock solution of 0.5 mg of each isolate was dissolved in DMSO to afford an initial concentration of 0.5 mg/ml (500 µg/ml) which was similarly two fold serially diluted to obtain final concentrations of 250, 125, 62.5, 31.25, 7.82, and 3.91 µg/ml. The tubes were transferred from the incubator to the laminar flow hood from which an aliquot of 5 µl of standardized suspension of microorganisms was added to 100 µl of each extract or isolate dilution in the tubes. The same procedure was used to determine MIC values for gentamycin and clotrimazole. Each test included two growth controls consisting of the medium with the solvent (DMSO) and medium with bacterial or fungal suspension. The tubes were incubated for 24 h at 37° C and 25° C for bacteria and fungi, respectively. The assays were done in triplicate and analyzed statistically using MSTAT-C statistical package.

Acknowledgments

The authors are grateful to Mr. Mutiso (Taxonomist) of Botany department, University of Nairobi for identification and collection of the plant materials and Mr. James Odhiambo of the Jaramogi Odinga

Teaching and Referral Hospital in Kisumu County, Kenya for provision of microbes and conducting the bioassay experiments. The Institute of Organic Chemistry, University of Saarland, Germany is acknowledged for the spectroscopic data.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Germany Academic Exchange Services (DAAD).

References

[1] M.P. Simmons, C.C. Clevinger, V. Savolainen, R.H. Archer, S. Mathews, and J. Doyle, *Am. J. Bot*. **88**, 313 (2001).
[2] R.H. Archer and A.E. van Wyk, *South Afr. J. Bot*. **64**, 93 (1998).
[3] N. Lall and J.J.M. Meyer, *J. Ethnopharmacol*. **66**, 347 (1999).
[4] T. Yelani, A.A. Hussein, and J.J.M. Meyer, *Nat. Prod. Res*. **24**, 1418 (2010).
[5] G. Prinsloo, J.J.M. Meyer, A.A. Hussein, E. Munoz, and R. Sanchez, *Nat. Prod. Res*. **24**, 1743 (2010).
[6] H.J. Bentjee, *Kenya trees, shrubs and lianas*, 1st ed. (National Museums of Kenya, Nairobi, 1994), pp. 336–338.
[7] C. Keith, *Trees of Southern Africa*, 2nd ed. (Pretoria, PROTA, 1983), p. 510.
[8] T.N. Noad and A.B. Birnie, *Trees of Kenya*, 3rd ed. (PROTA, Nairobi, 1992), p. 71.
[9] J. Kokwaro, *Medicinal Plants of East Africa*, 6th ed. (Nairobi University Press, Nairobi, 2009), p. 254.
[10] P. Gerhard, Ph. D. thesis, University of Pretoria, 2007.
[11] O.J.M. Hamza, V.C.J. Beukel, M.I.N. Moshi, F.H.M. Mikx, O.H. Selemani, H.Z. Mwambo, J.A.M. Vander ven, and E.P. Verweij, *J. Ethnopharmacol*. **108**, 124 (2006).
[12] I.M. Bekalo, E. Keengwe, P. Mathias, and A.L. Mundy, *Ethnoveterinary Medicine in Kenya*, 1st ed. (International Institute of Rural Reconstruction, Nairobi, 1996), p. 226.
[13] I. Kubo and K. Fukuhara, *J. Nat. Prod*. **53**, 968 (1990).
[14] M.K. Tsanuo, A. Hassanali, I.J. Jondiko, and B. Torto, *Phytochemistry* **34**, 665 (1993).
[15] Y. Tsujino, I.J. Ogoche, H. Tazaki, T. Fujimori, and K. Mori, *Phytochemistry* **40**, 753 (1995).
[16] D.J. White, S. Hyunik, K. Tae-Seong, and S. Cutshall, *J. Am. Chem. Soc*. **119**, 2404 (1997).
[17] D. Gupta and J. Singh, *Phytochemistry* **28**, 1197 (1989).
[18] M. Nakatani, Y. Miyazaki, T. Iwashita, H. Naoki, and T. Hase, *Phytochemistry* **28**, 1479 (1989).
[19] Y. Kuo and Y. Chiang, *Chem. Pharm. Bull*. **48**, 593 (2000).
[20] B. Su, Y. Kang, R.E. Pinos, B.D. Santarsiero, A.D. Mesecar, D.D. Soejarto, H.H.S. Fong, J.M. Pezzuto, and A.D. Kinghorn, *Phytochemistry* **64**, 293 (2003).
[21] J. Wandji, F. Tillequin, D.A. Mulholland, J.C. Shirri, N. Tsabang, E. Seguin, P. Verite, F. Libot, and Z.T. Fomum, *Phytochemistry* **64**, 845 (2003).
[22] H. Takahashi, S. Hirata, H. Minami, and Y. Fukuyama, *Phytochemistry* **56**, 875 (2001).
[23] O.J.M. Hamza, C.J.P. van den Bout-van den Beukel, M.N.N. Matee, Mainen J. Moshi, F.H.M. Mikx, H.O. Selemani, Z.H. Mbwambo, A.J.A.M. Van den Ven Van der Ven, and P. E. Verweij, *J. Ethnopharmacol*. **108**, 124 (2006).
[24] S. Sundur, B. Shrivastava, P. Sharma, S.S. Raj, and V.L. Jayasekhar, *Int. J. Adv. Res*. **2**, 599 (2014).
[25] P.R. Murray, E.J. Baron, F.C. Tenover, M.A. Pfaller, and R. H. Myolken, *Antibacterial Susceptibility Tests: Dilution and Disk Diffusion Methods*, 3rd ed. (American Society for Microbiology, International Institute of Rural Reconstruction, 1999), pp. 1526–1543.