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Triterpenes from *Salvia argentea* var. *aurasiaca* and their antibacterial and cytotoxic activities

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Abstract

Five undescribed ursane-type triterpenes, identified as $1\beta,3\beta,15\alpha$ -trihydroxy-urs-9(11)-12-diene (**1**), $1\beta,3\beta,15\alpha,28$ -tetrahydroxy-urs-9(11),12-diene (**2**), $1\beta,3\beta$ -dihydroxy-urs-9(11),12-dien-28-al (**3**), $1\beta,3\beta,7\beta,15\alpha,28$ -pentahydroxy-urs-12-ene (**4**), and $1\beta,3\beta,15\alpha$ -trihydroxy-urs-11-en-28-al (**5**), together with five known compounds (**6-10**), were isolated from the acetone extract of the dried aerial parts of *Salvia argentea* L. var. *aurasiaca* (Pomel) Batt. et Trab. (Lamiaceae). Structures were elucidated on the basis of extensive spectroscopic analysis including HR-ESI-MS, 1D- and 2D-NMR techniques and comparison with literature data. The antibacterial evaluation of compounds **1-10** of the acetone extract of the dried aerial parts, in addition to nine compounds (**11-19**) previously isolated from the exudate of the fresh aerial parts, by bioautography on *Staphylococcus aureus* followed by the determination of MIC values of active compounds by serial dilution technique against five bacteria, revealed that two compounds have an antibacterial effect comparable to antibiotics.

The cytotoxic activity evaluation of compounds **1-19** showed that Six compounds exhibited an antiproliferative activity against K562 and HT1080 cells with IC_{50} values ranging from 30.25 to 70.32 μ M.

Keywords: *Salvia argentea* L. var. *aurasiaca*, Lamiaceae, ursane triterpenes.

1. Introduction

The genus *Salvia* (family Lamiaceae), comprising about 900 species, widely distributed in various regions of the world, is represented by about 23 species in the Algerian flora, of which five are endemic [1]. Several investigations showed that *Salvia* species contain triterpenoids, diterpenoids, monoterpenes, and polyphenolics [2-12]. *Salvia* species possess biological properties e.g. antioxidant, antibacterial, anticancer, anti-inflammatory and cytotoxic activities [11-14]. *Salvia argentea* L. is a plant species originating from the Mediterranean region [15]. and specifically in Algeria to treat respiratory diseases. The leaves are the most commonly used part, usually in the form of powder and exclusively administered orally [16]. *Salvia argentea* L. var. *aurasiaca* (Pomel) Batt. et Trab. is a plant species native from Algeria. In a previous paper, we described the isolation of eleven oleanane- and ursane-type triterpenes from the exudate of the fresh aerial parts of this plant (8, 9, and 11-19) (Figs. 1 and 2) [17]. In continuation of our research on the chemical constituents of *S. argentea* var. *aurasiaca*, we describe here the isolation and structural characterization of five new ursane-type triterpenes (1-5) along with five known compounds (6-10) from the acetone extract of its powdered dried aerial parts (Fig. 1). Their structures were elucidated by extensive spectroscopic methods including 1D- (^1H and ^{13}C) and 2D-NMR (^1H - ^1H -COSY, HSQC, HMBC, and NOESY) experiments as well as HR-ESI-MS analysis. The antibacterial activity of compounds 1-19 was tested by bioautography on *Staphylococcus aureus*, followed by the determination of MIC values of active compounds against five bacteria (*Enterococcus faecalis* ATCC 1034, *Staphylococcus aureus* CIP 53.154, *Escherichia coli* CIP 54.127, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* ATCC 9027). The antiproliferative activity of compounds 1-19 was evaluated against K562 and HT1080 cells.

2. Results and discussion

The acetone extract of the aerial parts of *S. argentea* var. *aurasiaca* was fractionated and purified by combination of chromatographic methods to provide five new ursane-type triterpenes (1-5) along

with five known triterpenes (Fig. 1). The known compounds were identified as $1\beta,3\beta$ -dihydroxy-urs-9(11)-12-diene (**6**) [18], 3β -hydroxy-urs-9(11),12-diene (**7**) [19], $1\beta,3\beta,15\alpha,28$ -tetrahydroxy-urs-12-ene (**8**) [17], $1\beta,3\beta,11\alpha,15\alpha$ -tetrahydroxy-urs-12-ene (**9**) [17], and 3β -hydroxy-olean-9(11),12-diene (**10**) [20], by comparison of physical data with literature values and from spectroscopic evidence.

Fig. 1. Chemical structures of compounds **1-10** isolated from the acetone extract of dried aerial parts of *S. argentea* var. *aurasiaca*.

Compound **1** was obtained as white amorphous powder, it possessed the molecular formula C₃₀H₄₈O₃ from its HR-ESI-MS at m/z 457.3688 [M+ H]⁺ (calcd C₃₀H₄₉O₃, 457.3682), indicating 7 degrees of unsaturation. The ¹³C NMR spectrum of **1** showed 30 carbons due to eight methyls (δ_c 11.1, 15.1, 17.4, 19.2, 21.5, 23.0, 27.9, and 29.3), six sp³ methylene carbons, seven sp³ methine

carbons (three oxygenated carbons at δ_C 67.8, 75.6 and, 76.0), five sp^3 quaternary carbons, and four olefinic carbons (two methine carbons at δ_C 118.5 and 124.6, and two quaternary carbons at δ_C 141.7 and 153.2) (Table 1). The 1H NMR spectrum of **1** (Table 1) displayed eight signals, corresponding to six tertiary methyls at δ_H 0.82 (s, CH₃-24), 0.91 (s, CH₃-28), 0.98 (s, CH₃-27), 1.01 (s, CH₃-23), 1.25 (s, CH₃-26), and 1.28 (s, CH₃-25), and two secondary methyls at δ_H 0.85 (d, $J=4.1$ Hz, (s, CH₃-29) and 0.94 (brs, CH₃-30), three oxymethines signals at δ_H 3.34 (dd, $J=12.5$ and 4.1 Hz, H-3), 3.86 (dd, $J=11.5$ and 4.5 Hz, H-1), and 4.35 (dd, $J=11.5$ and 6.0 Hz, H-15), and two doublets ($J=6.1$ Hz) assigned to the olefinic protons H-11 (δ_H 6.69) and H-12 (δ_H 5.55) of $\Delta^{9(11)-12}$ ursane skeleton [18,21]. The location of two trisubstituted double bonds was confirmed by the HMBC experiment. From HMBC spectrum, H-11 showed correlation with δ_C 44.4 (C-8), 153.2 (C-9), 124.6 (C-12), and 141.7 (C-13), whereas H-12 correlated with δ_C 118.5 (C-11), C-13 (141.7), 46.7 (C-14), 57.5 (C-18). By detailed analysis of the NMR spectroscopic data (HSQC, COSY, HMBC and NOESY) and comparison of the 1H and ^{13}C NMR spectra of **1** with $1\beta,3\beta$ -dihydroxy-urs-9(11)-12-diene (**6**), no difference between the two compounds was evident except for the presence of an oxymethine signal instead of those of the methylene signal (H₂-15) in **6**. The oxymethine carbon signal was assigned to the C-15 position (δ_C 67.8) due to the observed HMBC correlations of H-15 with the methyl C-27 and to the carbons C-8, C-13, C-14, C-16 (δ_C 39.5), and C-17 (δ_C 34.9). The position of the oxygenated methine at C-15 was also confirmed by the presence of correlations in COSY spectrum between δ_H 1.30 and 1.94 (H-16) and δ_H 4.35 (H-15). The coupling constants of H-1 (11.5, 4.5 Hz) and H-3 (12.5, 4.1 Hz) indicated their α -axial orientations. This was confirmed by the NOE correlations observed between H-3 α /H-5 α and H-23 α and between H-1 α /H-9 α [17]. The hydroxyl group at C-15 was shown to be α -oriented by the coupling constants of H-15 β -axial ($J_{H-15\beta-ax-H-16\alpha-ax}=11.5$ Hz) and by the NOE correlations between H-15 β /H-26 β and H-15 β /H-18 β . The full assignments of the 1H NMR and ^{13}C NMR data of **1** are listed in Table 1. On

the basis of the above evidence, the structure of compound **1** was established as 1 β ,3 β ,15 α -trihydroxy-urs-9(11)-12-diene.

Compound **2** was obtained as white amorphous powder. Its molecular formula was determined as C₃₀H₄₈O₄ by the HR-ESI-MS at m/z 495.3457 [M + Na]⁺ (calc for C₃₀H₄₈O₄Na, 495.3450). The ¹H and ¹³C spectra of **2** (Table 1) closely resembled to compound **1**, indicating that it was also ursane-9(11)-12-diene triterpene. The difference was the replacement of CH₃-28 by a hydroxymethylene group (Table 1). The attachment of the hydroxymethylene group to C-17 was readily confirmed by the HMBC correlations from H₂-28 to C-22, C-18, and C-16. As expected, a NOE interaction was observed between H-28 and H-18. Thus compound **2** was identified as 1 β ,3 β ,15 α ,28-tetrahydroxy-urs-9(11),12-diene.

Compound **3** was obtained as white amorphous powder with the molecular formula, C₃₀H₄₇O₃, determined by the positive ion HR-ESI-MS at m/z 477.3349 [M+ Na]⁺ (calcd for C₃₀H₄₆O₃Na, 477.3345). Its ¹H and ¹³C NMR data differed from those of compound **1** only in rings D and E (Table 1). CH₃-28 was replaced by a formyl (δ_C 207.4/ δ_H 9.34, s). This was confirmed by HMBC correlations from H-18, H-16 and H-22 to C-28. A NOE interaction between the aldehyde proton and H-18 (δ_C 2.15) indicated the expected β orientation of the aldehyde function. The shielding of C-14 (δ_C 42.9) and C-16 (δ_C 23.9) and the deshielding of C-27 (δ_C 18.3) indicated the absence of hydroxyl at C-15. This was confirmed by the chemical shifts of C-15 (δ_C 26.3) and H₂-15 (δ_H 1.16 and 1.94). CH₃-27 showed a correlation with C-15 in the HMBC spectrum. Therefore, the structure of compound **3** was identified as 1 β ,3 β -dihydroxy-urs-9(11),12-dien-28-al.

Compound **4** was obtained as white amorphous powder with the molecular formula C₃₀H₅₀O₅ (HR-ESI-MS m/z 513.3564 [M+Na]⁺, calcd C₃₀H₅₀O₅Na, 513.3556). By detailed analysis of the 1D- and 2D-NMR spectroscopic data and comparison of the ¹H and ¹³C NMR spectra of **4** (Table 1) with

1 β ,3 β ,15 α ,28-tetrahydroxy-urs-12-ene (**8**) [17], no difference between the two compounds was evident except for the presence of an oxymethine signal instead of those of the methylene signal (H₂-7) in **8**. The oxymethine was assigned to the C-7 position (δ_C 71.5) due to the observed HMBC correlations of H-7 (δ_H 3.85, dd, J =11.3 and 4.8 Hz) to the CH₃-26 (δ_C 9.2), C-6 (δ_C 26.6), C-9 (δ_C 49.2), and C-5 (δ_C 49.7). The COSY correlations observed between H₂-6 (δ_H 1.69,1.77)/H-7 and H₂-6/H-5 (δ_H 0.78) confirmed the position of the extra secondary hydroxyl group at C-7. The multiplicity and coupling constant of H-7 proposed the hydroxyl group to be in the less hindered equatorial orientation (β oriented) as deduced by the axial-axial and axial-equatorial couplings of H-7 with H-6 protons ($J_{H-6ax-H-7ax}$ =11.3 Hz). Thus, compound **4** was deduced to be 1 β ,3 β ,7 β ,15 α ,28-pentahydroxy-urs-12-ene.

The molecular formula of compound **5** was established as C₃₀H₄₈O₄ (HR-ESI-MS m/z 495.3445 [M+Na]⁺, calcd 495.3450). Comparison of the 1D- and 2D-NMR spectroscopic data of compound **5** (Table 1) with those of compounds **1** and **3** showed similarities with the exception of the signals associated with olefinic protons and carbons. Based on these similarities, compound **5** should be a 1 β ,3 β ,15 α -trihydroxy-ursane-28-al derivative. The ¹H-NMR spectrum showed two olefinic proton signals at δ_H 5.57 (H-11) and 6.61 (H-12) (each 1H, dt, J =10.1 and 1.8 Hz), correlated in the HSQC spectrum with two methine carbons at δ_C 131.6 and 127.8, respectively. H-11 correlated in the ¹H-¹H-COSY spectrum with H-12 and with the signal at δ_H 2.09 (brs, H-9), whereas H-12 correlated in the same spectrum, in addition to H-11, with the signal at δ_H 1.88 (dd, J = 10.5 and 1.8 Hz, H-13). The location of the double bond (Δ^{11-12}) was confirmed by the HMBC correlations observed between H-11/C-8 (δ_C 42.6), H-11/C-10 (δ_C 42.1), H-12/C-13 (δ_C 44.2), H-12/C-14 (δ_C 45.3), and H-12/C-18 (δ_C 46.4). Thus, compound **5** was elucidated as 1 β ,3 β ,15 α -trihydroxy-urs-11-en-28-al.

Table 1NMR spectroscopic data of compounds **1-3** in CDCl₃ and **4-5** in CD₃OD.^a

No.	1		2		3		4		5	
	δ_H m (J in Hz)	δ_C	δ_H m (J in Hz)	δ_C	δ_H m (J in Hz)	δ_C	δ_H m (J in Hz)	δ_C	δ_H m (J in Hz)	δ_C
1	3.86 dd (11.5,4.5)	76.0	3.87 dd (11.5,4.5)	76.3	3.78 dd (11.6,4.5)	75.9	3.35 dd (11.5,4.5)	79.0	3.44 dd (11.4,3.3)	79.4
2	1.75 1.95	38.7	1.75 1.97 dt (12.4, 4.4)	38.5	1.74 1.97	38.1	1.76 1.77	37.1	1.79 1.80	37.1
3	3.34 dd (12.5,4.1)	75.6	3.35 dd (12.1,4.3)	75.5	3.35 dd (12.1,4.2)	75.7	3.24 dd (12.0,4.1)	75.1	3.20 dd (12.0,4.6)	75.3
4		38.7	-	39.0		39.1		37.9		38.5
5	0.78	49.1	0.79	49.0	0.78	48.8	0.78 d (11.9)	49.7	0.60 brd (11.8)	51.9
6	1.63 1.70	18.4	1.65 1.70	18.2	1.63, 1.68,	18.2	1.69 1.77	26.6	1.58 1.64	17.6
7	1.53 2.05 dt (12.6,5.6)	34.3	1.51 2.07 td (12.1,5.3)	34.2	1.36 1.68	31.0	3.85 dd (11.3,4.8)	71.5	1.52 2.35	33.7
8		44.4		44.0		40.9		46.9		42.6
9		153.2		153.4		153.5	1.65	49.2	2.09 brs	53.0
10		44.9		45.0		44.9		43.2		42.1
11	6.69 d (6.1)	118.5	6.70 d (6.2)	118.5	6.58 d (6.0)	117.4	2.18 ddd (17.2,11.7,2.2) 2.79 dt (17.2,5.3)	26.2	5.57 dt (10.1,1.8)	131.6
12	5.55 d (6.1)	124.6	5.55 d (6.2)	124.9	5.63 d (6.0)	124.4	5.34 dd (5.3,2.2)	128.2	6.61 dt (10.8,1.8)	127.8
13		141.7		140.6		140.6		137.8	1.88 dd (10.5,1.8)	44.2
14		46.7		47.0		42.9		48.8		45.3
15	4.35 dd (11.5,6.0)	67.8	4.35 dd (11.6,5.9)	66.8	1.16 1.94 m	26.3	4.19 dd (11.8,5.3)	65.6	4.0 dd (10.5,5.8)	66.2
16	1.30 1.94 brt (11.5)	39.5	1.59 m 1.90 brt (12.8)	34.7	1.74 2.05 td (11.5,3.5)	23.9	1.50 dd (13.1,3.9) 1.93 t (13.1)	31.8	1.83 1.88	32.5
17		34.9		39.0	-	49.9		38.3		52.2
18	1.49 d (10.8)	57.5	1.56 m	52.5	2.15 d (11.2)	50.7	1.42 d (11.1)	55.2	1.70 dd (11.8,2.7)	46.4
19	1.31 m	38.9	1.41 m	38.5	1.40 m	38.1	1.45	39.3	1.63	38.9
20	0.94 m	39.1	0.97 m	39.0	1.05 m	38.8	0.90 m	39.2	1.00 m	39.2
21	1.28 m 1.43 m	31.0	1.30 m 1.52	30.3	1.30 m 1.60 m	30.1	1.29 m 1.49 dd (13.1,3.9)	30.3	1.32 m 1.58 m	29.5
22	1.29 m 1.51 m	40.9	1.52 1.67 m	34.8	1.28 m 1.42 m	31.7	1.39 td (13.1,3.8) 1.63 dt (13.1,2.9)	34.6	1.12 m 1.55 m	31.2
23	1.01 s	27.9	1.02 s	27.9	1.00 s	27.8	1.01 s	27.2	0.95 s	26.9
24	0.82 s	15.1	0.83 s	15.2	0.82 s	15.1	0.81 s	14.8	0.76 s	14.1
25	1.28 s	19.2	1.29 s	19.2	1.25 s	18.5	1.05 s	10.6	0.94 s	12.4
26	1.25 s	23.0	1.24 s	22.9	0.94 s	22.0	1.09 s	9.2	0.83 s	16.0
27	0.98 s	11.1	1.01 s	11.1	0.95 s	18.3	1.18 s	15.7	1.04 s	11.2
28	0.91 s	29.3	3.30 d (11.1) 3.57 d (11.1)	70.5	9.34 s	207.4	3.11 d (11.1) 3.50 d (11.1)	69.2	9.25 s	206.1
29	0.85 d (6.4)	17.4	0.88 d (6.5)	17.2	0.92 d (6.4)	16.6	0.88 d (6.1)	16.5	1.12 d (6.2)	17.8
30	0.94 brs	21.5	0.97 s	21.4	01.00 s	21.2	0.96 d (6.3)	20.2	0.97 s	19.4

^a Overlapped protons are reported without designated multiplicity.

In order to screen the antibacterial potential of compounds isolated from the acetone extract of the dried aerial parts (**1-10**), in addition to compounds previously isolated from the exudate of the fresh aerial parts (**11-19**) [17], a bioautography assay was applied on a sensitive strain of *Staphylococcus aureus* CIP 53.154. *S. aureus* is a Gram-positive cocci bacterium frequently found on the skin and in the respiratory tract, and can be responsible for nosocomial infections. Compounds **4**, **8**, and **17-19** were the only active compounds as revealed by the white inhibition zones observed on the TLC plate around the compound spots.

Fig. 2. Chemical structures of compounds **8**, **9**, and **11-19** isolated from the exudate of the fresh aerial parts of *S. argentea* var. *aurasiaca*

A serial liquid dilution technique in 96-well microtiter plates was used to determine the minimum inhibitory concentration (MIC) of these five compounds against the five bacteria, *Enterococcus faecalis* ATCC 1034, *Staphylococcus aureus* CIP 53.154, *Escherichia coli* CIP 54.127, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* ATCC 9027 (Table 2). Compound **19** was the most active one with MIC values similar to gentamicin and vancomycin, used as standards (Table 2). Compound **19** showed potent inhibitory effect against *S. epidermidis* (MIC 3.9 µg/mL) and against *E. coli* and *P. aeruginosa* (MIC 7.8 µg/mL). The results showed also a good inhibitory effect of compounds **4** and **18** against *S. aureus*, *S. epidermidis*, and *E. coli* (MIC 62.5 µg/mL). Compounds **8** and **17** showed a moderate to low inhibitory effect against the five tested bacteria with MIC values ranging from 125 to 500 µg/mL (Table 2).

Table 2

Minimum inhibitory concentration (MIC) values of compounds isolated from *S. argentea* var *aurasiaca* in liquid medium.^a

Compounds	MIC (µg/mL)				
	<i>E. faecalis</i> (ATCC 1034)	<i>S. aureus</i> (CIP53.154)	<i>E. coli</i> (CIP 54.127)	<i>S. epidermidis</i>	<i>P. aeruginosa</i> (ATCC9027)
4	125	62.5	62.5	62.5	62.5
8	125	125	125	125	125
17	500	500	>500	>500	>500
18	125	62.5	62.5	62.5	125
19	125	62.5	7.8	3.9	7.8
Gentamicin*	16	4	4	0.25	8
Vancomycin*	> 64	> 64	16	4	> 64

^aNo bacterial growth inhibition observed in bioautography assay for compounds **1-3**, **5-7**, and **9-16**.

*used as standard.

The cytotoxic activity of compounds **1-19** was evaluated against chronic myeloid leukemia K562 and fibrosarcoma HT1080 cells. Only compounds **2**, **3**, **5**, **8**, **9**, and **11** exhibited a moderate antiproliferative activity with IC₅₀ ranging from 30.25 to 70.32 µM (Table 3).

Table 3

Cytotoxic activity of compounds **1-19** against HT1080 and K562 cells.

Compounds ^a	IC ₅₀ (µM) ^b	
	HT1080	K562
2	30.25±0.41	>100 (26.5 ± 3.98) ^c
3	35.15 ± 0.19	66.81 ± 0.18
5	40.18 ± 0.19	49.15 ± 0.19
8	>100 (36.8 ± 2.76) ^c	70.32 ± 0.19
9	31.12±0.51	50.03 ± 0.18
11	32.14±0.62	54.3 ± 0.18
Doxorubicin*	0.59 ± 0.05	0.59 ± 0.04

^aNo cytotoxic activity was observed for compounds 1,4, 6, 7, 10, and 12-19.

^bData correspond to means ± SD of 3 independent experiments. ^cPercentage of growth inhibition at a concentration of 100 µM.

*used as standard.

In conclusion, the present work reported five new ursane-type triterpenes (**1-5**) along with four known ursane-type triterpenes (**6-9**), and one oleanane-type triterpene (**10**) from the acetone extract of the dried aerial parts of *Salvia argentea* L. var. *aurasiaca* (Pomel) Batt. et Trab. (Lamiaceae) collected from Ain El-Bey in Constantine Province (North-Eastern Algerian). Compounds **8** and **9** were previously obtained from the exudate of the fresh aerial parts of this plant while compounds **7** and **10** were previously isolated from *Salvia* species. Compound **6** was isolated for the first time from *Salvia* species and family Lamiaceae.

Compounds (**1-10**) isolated from the acetone extract of the dried aerial parts and compounds previously isolated from the exudate of the fresh aerial parts (**11-19**) [17], were tested for their

antibacterial and cytotoxic activities. Five compounds showed antibacterial activity against *E. faecalis*, *S. aureus*, *E. coli*, *S. epidermidis*, and *P. aeruginosa*. Compound **19** showed potent inhibitory effect with MIC values similar to gentamicin and vancomycin, used as standards, against *S. epidermidis* (MIC 3.9 µg/mL) and against *E. coli* and *P. aeruginosa* (MIC 7.8 µg/mL).

Six compounds exhibited antiproliferative activity against K562 and HT1080 cells with IC₅₀ ranging from 30.25 to 70.32 µM.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured in MeOH or CHCl₃ using a Perkin-Elmer 341 Polarimeter. ¹H-, ¹³C-NMR and 2D-NMR measurements were recorded in CD₃OD or CDCl₃ on a Bruker Avance III 500 spectrometer (¹H NMR at 500 MHz and ¹³C NMR at 125 MHz) equipped with a 5 mm TCI cryoprobe. 2D-NMR experiments were performed using standard Bruker microprograms (TopSpin 3.2 software). HR-ESI-MS analysis was conducted using a Micromass Q-TOF micro instrument. Flash chromatography was carried out on a Grace Reveleris system equipped with dual UV and ELSD detection using Grace® cartridges (Silica gel or RP-C₁₈). HPLC separations were performed on a Dionex apparatus equipped with an ASI-100 autosampler, an Ultimate 3000 pump, a STH 585 column oven, a diode array detector UVD 340S and a Chromeleon software. A prepacked RP-C₁₈ column (Phenomenex 250 x 10 mm, Luna 5 µ) was used for semi-preparative HPLC. The eluting mobile phase consisted of H₂O with TFA (0.0025%) and CH₃CN with a flow rate of 5 mL/min and the chromatogram was monitored at 205 and 210 nm. Thin-layer chromatography (TLC) was carried out using silica gel 60 F₂₅₄ pre-coated aluminium plates (0.2 mm, Merck). After developing with solvent systems, spots were visualized by spraying with 50% H₂SO₄ followed by heating.

3.2. Plant material

The aerial parts of *Salvia argentea* L. var. *aurasiaca* (Pomel) Batt. & Trab. were collected from Ain El-Bey, Constantine (North-Eastern Algerian) in May 2014, and identified by professor Gérard de

Bélair. A voucher specimen (LOST Saa.05/14) has been deposited at the herbarium of LOST Laboratory, University of Constantine, Algeria.

3.3. Extraction and isolation

The powdered dried aerial parts of *S. argentea* L. var. *aurasiaca* (950 g) were extracted with pure acetone by the use of a soxhlet extraction apparatus. The acetone solution was concentrated under reduced pressure to obtain the acetone extract (30 g). A part of this extract (15 g) was fractionated by column chromatography over silica gel eluted with a gradient of petroleum ether/acetone with increasing polarity (100:0-0:100, v/v), then methanol to yield 24 fractions (Frs A-X). Fraction L (748 mg) was separated by flash chromatography on RP-C₁₈ column (40 g), eluted with gradient MeOH-H₂O (60% to 85% MeOH, in 28 min) with flow rate of 40 mL/min to afford compounds **2** (4 mg, yield: 0.027%), **10** (25 mg, yield: 0.17%) and **7** (10 mg, yield: 0.07%). Fraction Q (600 mg) was subjected to flash chromatography on silica gel (12g), eluted by a gradient system of petroleum ether/EtOAc (100:0 to 40:60, during 45 min; flow rate, 36 mL/min), with 25 mL fractions collected and analyzed by TLC, fractions having similar compositions were combined to yield 29 fractions (Q₁-Q₂₉). Fraction Q₁₅ (34 mg) was purified by semi-prep HPLC using isocratic elution with 40% CH₃CN, in 25 min to give compound **5** (2 mg, yield: 0.013%, *t_R* = 28.4 min). Fraction Q₂₅ was obtained as pure compound **6** (61 mg, yield: 0.41%). Fraction S (669 mg) was purified by flash chromatography on RP-C₁₈ column (40 g), eluted with MeOH-H₂O (50% to 80% MeOH, in 29 min; flow rate, 40 mL/min) to give twenty fractions based on TLC analysis, Frs S₁₋₂₀. Compounds **8** (10 mg, yield: 0.07%) and **1** (25 mg, yield: 0.17%) were obtained as pure compounds in fractions S₇ and S₁₁, respectively, while the fraction S₁₄ (46 mg) was purified by semi-prep HPLC using a gradient of solvents CH₃CN -H₂O (80%-100% CH₃CN, 20 min) to give compound **3** (3 mg, yield: 0.02%, *t_R* = 11.0 min). Fraction U (367 mg) was subjected to flash chromatography RP-C₁₈ column (40 g), eluted with MeOH-H₂O (40%-100% MeOH during 33 min, with flow rate of 40 mL/min) with 25 mL fractions collected, based on TLC analysis were combined to give 20 fractions U₁₋₂₀. The

purification of fraction U₂₀ (58 mg) by semi-prep HPLC using isocratic elution with 40% CH₃CN in 15 min, afford compounds **9** (3 mg, yield: 0.02%, *t_R* = 6.54 min) and **4** (6 mg, yield: 0.04%, *t_R* = 8.4 min).

For the extraction and purification of the exudate, fresh aerial parts (4.5 kg) were immersed in a mixture of toluene and EtOAc (6:4 v/v) for 20 s to yield the exudate crude extract 43 g which was previously fractionated and purified by Lakhal et al. (2014) to yield compounds **8**, **9**, and **11-19**.

3.4. 1 β ,3 β ,15 α -trihydroxy-urs-9(11)-12-diene (**1**)

Amorphous white powder; C₃₀H₄₈O₃; $[\alpha]_D^{25} + 95.1$ (*c* 0.91, CHCl₃); ¹H NMR (500 MHz, CDCl₃) and ¹³C NMR (125 MHz, CDCl₃) data, see Table 1; HR-ESI-MS *m/z* 457.3688 [M+H]⁺ (calcd 457.3682).

3.5. 1 β ,3 β ,15 α ,28-tetrahydrox-urs-9(11),12-diene (**2**)

Amorphous white powder; C₃₀H₄₈O₄; $[\alpha]_D^{25} + 55.0$ (*c* 0.32, CHCl₃); ¹H NMR (500 MHz, CDCl₃) and ¹³C NMR (125 MHz, CDCl₃) of spectroscopic data; see table 1. HR-ESI-MS *m/z* 495.3457 [M+Na]⁺ (calcd 495.3450).

3.6. 1 β ,3 β -dihydroxy-urs-9(11),12-dien-28-al (**3**)

Amorphous white powder; C₃₀H₄₆O₃; $[\alpha]_D^{25} + 49.0$ (*c* 0.21, CHCl₃); ¹H NMR (500 MHz, CDCl₃) and ¹³C NMR (125 MHz, CDCl₃) data, see Table 1; HR-ESI-MS *m/z* 477.3349 [M+Na]⁺ (calcd 477.3345).

3.7. 1 β ,3 β ,7 β ,15 α ,28-pentahydroxy-urs-12-ene (**4**)

Amorphous white powder; C₃₀H₅₀O₅; $[\alpha]_D^{25} + 19.4$ (*c* 0.52, MeOH); ¹H NMR (500 MHz, CD₃OD) and ¹³C NMR (125 MHz, CD₃OD) data, see Table 1; HR-ESI-MS *m/z* 513.3564 [M+Na]⁺ (calcd 513.3556).

3.8. 1 β ,3 β ,15 α -trihydroxy-urs-12-ene-28-al (5)

Amorphous white powder; C₃₀H₄₈O₄; $[\alpha]_D^{25} + 1.1$ (c 0.19, MeOH); ¹H NMR (500 MHz, CD₃OD) and ¹³C NMR (125 MHz, CD₃OD) data, see Table 1; HR-ESI-MS m/z 495.3445 [M+Na]⁺ (calcd 495.3450).

4. Antibacterial Activity.

The antibacterial screening was performed on five bacteria obtained from the Laboratory of Microbiology, faculty of pharmacy from the University of Reims Champagne-Ardenne including the following Gram-positive bacteria: *Enterococcus faecalis* ATCC 1034, *Staphylococcus aureus* CIP 53.154, and *Staphylococcus epidermidis*, and the Gram-negative bacteria *Escherichia coli* CIP 54.127 and *Pseudomonas aeruginosa* ATCC 9027. The five bacteria were incubated overnight at 37 °C in tubes containing Mueller-Hinton (MH) broth medium. The bacteria were then diluted with MH-broth by means of serial dilution to finally reach a concentration of 10⁵ bacteria/mL.

To identify the most promising compounds in terms of antibacterial activity, an immersion bioautography method was adopted [11]. Compounds **1-19** were solubilized in methanol to obtain a solution of 2 mg/mL and 25 μ L of each solution was spotted onto Merck 60 F₂₅₄ pre-coated silica gel plates (10 $\times$ 10 cm). Methanol and Gentamicin (50 μ g) were also spotted on the plates as negative and positive controls, respectively. The TLC plates were directly dried without migration and sterilized. The plates were then covered by Mueller-Hinton (MH) agar medium containing a *Staphylococcus aureus* CIP 53.154 suspension (10⁵ bacteria/mL) in square Petri dishes. After incubation for 24 h at 37°C, bacterial growth was revealed by a 2 mg/mL solution of thiazolyl blue tetrazolium bromide (MTT) and growth inhibition zones were measured. White stains indicated where reduction of MTT to the colored formazan did not take place due to the presence of extracts that inhibited bacterial growth.

A serial dilution technique using 96-well microliter plates was used to determine the MIC values of the most promising compounds as revealed by bioautography. For this purpose, nine concentrations of the most active compounds, from 500 μ g/mL to 2 μ g/mL, were tested. Two wells were

represented as bacteria culture control (positive control) and medium sterility control (negative control). Then the wells were loaded with MH liquid medium and bacterial suspension (10^5 bacteria/mL) giving a final volume of 200 μ L. The plates were incubated overnight at 37°C, sprayed with a 0.2 mg/mL MTT solution and incubated again at 37°C for 30 min. Bacterial growth was indicated by a violet color whatever the color intensity, while bacterial growth inhibition was admitted only for wells which remained clear. MIC values were determined as the lowest concentrations of samples having an inhibitory effect on bacteria growth (clear wells). Gentamicin and vancomycin were used as positive antibacterial controls. Solvents were checked for absence of antibacterial activity.

5. Antiproliferative activity

Chronic myeloid leukemia K562 cells were cultured in suspension in RPMI1640 culture medium, while fibrosarcoma HT1080 cells are adherent and were cultured in MEM culture medium. The culture medium was completed by 10% fetal bovine serum and 1% antibiotics. In order to determine the anticancer activity of the compounds, K562 and HT1080 cells were spread onto 96-well flat-bottom plates at a density of 1000 cells per well, and then incubated for 24 h in RPMI 1640 Medium supplemented with 10% fetal bovine serum and antibiotics. Cells were then treated with compounds **1-19** for 72 h. Cell growth was analyzed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium inner salt (MTS) according to the manufacturer's instructions (Promega Corporation, Charbonnières, France). Doxorubicin was used as a positive control. MTS is bio-reduced by cells into a colored formazan product. Absorbance was analyzed at a wavelength of 540 nm with a Multiskan Ex microplate absorbance reader (Thermo Scientific, Paris, France). Percentage of cell growth was calculated as $100\% \times ((\text{absorbance of the treated cells}) / (\text{absorbance of the negative control cells}))$. Control cells were treated with complete culture medium containing 0.2% DMSO. The values represent averages of three independent experiments.

Supplementary data

HR-ESI-MS spectra and 1D and 2D NMR of **1-5**

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