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Two new pentacyclic triterpenoids from the roots of *Achellia Fragrantissima F*. and their biological activity

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Summary. Two new pentacyclic triterpenoids, 2α-hydroxy-3-oxours-12-en-28-oic acid **(1)** and 2α-hydroxy-3-oxours-12,18-dien-28-oic acid **(2)** were isolated from the roots of *Achellia Fragrantissima F*., along with two known terpenes namely, α-amyrine **(3)** and ursolic acid **(4)**. The structures of the new compounds **(1)** & **(2)** were elucidated by spectroscopic data interpretation. The antimicrobial activity of new two terpenoids **(1)** & **(2)** was studied on *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa.*

Keywords: triterpenoids, plant extraction, spectroscopic analyses.

Introduction. *Achellia Fragrantissima F*. (santolina) belongs to the family Compositae and grows in Saudia-Arabia, Iraq and Egypt. It is known as medicinal plant that is used for treatment of rheumatoid arthritis, gout and another form of inflammation [1]. The roots of *Achellia Fragrantissima F*. were selected for analysis of their content by novel chromatographic and spectroscopic methods. Survey of literature on the chemical composition of the roots of *Achellia Fragrantissima F*. found that no chemical work was done on it. The antimicrobial activity of the essential oil from *Achellia Fragrantissima F*. was shown against several Gram-positive and Gram-negative bacterial strains [2]. This paper describes the isolation and characterization of (**1-4**) as constituents of the roots of the *Achellia Fragrantissima F*. including two new compounds, **1** and **2**.

Experimental and methods.

General experimental procedures. Optical rotations were determined on a JASCO P-1020 polarimeter using a 100 mm glass microcell. IR spectra (KBr) were recorded on a Perkin Elmer FTIR-spectrometer 1650. ^{1}H-NMR spectra were obtained on a Varian Equinox 300 MHz Spectrometer with TMS as the internal standard. MS were measured on a GSMS-QP-1000EX gas chromatograph-mass spectrometer Shimadzu (Japan). For column chromatography, silica gel (Merck, 63-200 μm particle size) was used. TLC was carried out with Merck silica gel $^{60}F_{254}$ plates.

1. Plant material. The dried roots of *Achellia Fragrantissima F*. were obtained from North Sini Government, Egypt, in Feb. 2009. The plant material has been deposited at the Laboratory of Botany, Faculty of Science, and Zagazig University, Egypt.

2. Extraction and isolation. The air-dried roots were ground to pass a 60 mesh screen. The dried roots (300 g) were extracted with MeOH (2 L x 2 times) at room temperature for one week. The MeOH extract was exhaustively concentrated to dryness (120 g) and then suspended in H_2O (2 L) and partitioned with EtOAc (2 L x 3 times) to give an EtOAc fraction (15 g). This fraction was chromatographed over a silica gel column and eluted with hexan-EtOAc mixture as the solvent system to obtain four fractions. F-3 (4.2 mg, R_f=0.85, hexan-EtOAc (3:1) and F-4 (2.7 mg, R_f=0.59, hexan-EtOAc (3:1) were identical with the authentic samples of α-amyrine **3** and ursolic acid **4** respectively. Elution with hexan-EtOAc (2:1) also resulted in the isolation of the new compounds **1** (4.3 mg, R_f=0.53) and **2** (1.8 mg, R_f=0.41).

*2α-hydroxy-3-oxours-12-en-28-oic acid **(1)**:*

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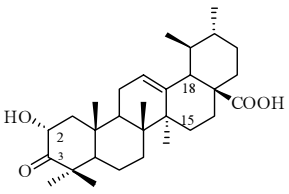
white, amorphous powder, mp 268-69 ˚C; $[\alpha]_D$ +44.6 ($CHCl_3$); IR (KBr) γ_{max} 3420, 1710 and 1720 cm^{-1}; ^1H and ^{13}C NMR see table 1; *m/z* 470 (calcd for $C_{30}H_{46}O_4$ 470.32).

2α-hydroxy-3-oxours-12,18-dien-28-oic acid ***(2)****:* white, amorphous powder; mp 272-74 ˚C; $[\alpha]_D$+42.6 ($CHCl_3$); IR (KBr) γ_{max} 3430, 1710 and 1720 cm^{-1}; ^1H and ^{13}C NMR see table 1; *m/z* 468 (calcd for $C_{30}H_{44}O_4$ 468.67).

3. Antimicrobial activities. *In vitro* antimicrobial activity of two new terpenoids **(1)** & **(2)** against Gram-positive and Gram-negative bacterial species was tested using the conventional paper disc assay [3] (Table 2). A loop of bacteria from stock agar slant cultures was cultured in nutrient broth overnight (at 37 ˚C) and spread with a dry sterile cotton swap onto triplicate set of Petri plates containing 25 ml of sterile solid Mueller Hinton agar medium. After twenty four hours' incubation (at 37 ˚C), diameters of inhibition zones (DIZs) induced by extract-loaded discs (12.5-1000 lg/disc) were measured and approximated.

Results and discussion

1. Structure elucidation. Repeated column chromatography of the methanol extract from the roots of the *Achellia Fragrantissima F.* resulted in the purification of 4 compounds (**1-4**). The structures of two compounds were determined as α-amyrine [4, 5] and ursolic acid [6] by comparing their physicochemical (mp) and spectroscopic data (IR, MS, ^1H and ^{13}C NMR) with those previously reported values.



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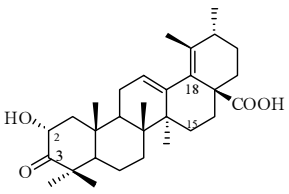

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Compound **1** was isolated as a white, amorphous powder, $[\alpha]_D$+44.6, and gave a positive pink colour in the Liberman-Burchard reaction [7], and a positive Zimmermann test for 3-ketontriterpenes [8]. IR spectrum showed absorption bands for hydroxyl, ketonic and carboxylic groups at γ_{max} 3420, 1710 and 1720 cm^{-1} respectively [9]. In addition, the molecular formula was determined as $C_{30}H_{46}O_4$ from a molecular ion peak at *m/z* 470, also, showed a fragment at *m/z* 203,

Table 1

*^1H and ^{13}C NMR spectroscopic data for compounds **1** & **2***

Position	1		2	
	δ_H(J in H_Z)	δ_C	δ_H(J in H_Z)	δ_C
1	2.00 m, 1,73 m	49.6	2.00 m, 1,73 m	49.5
2	3.95 s	69.05	3.95 s	69.8
3	–	213.0	–	217.05
4	–	47.7	–	47.3
5	1.62 m	55.3	1.62 m	52.4
6	1.52 m, 1.27 m	20.7	1.52 m, 1.37 m	18.6
7	1.56 m, 1.31 m	32.8	1.66 m, 1.35 m	32.7
8	–	38.9	–	38.2
9	–	47.5	–	46.7
10	–	37.5	–	37.3
11	2.04 m, 1.79 m	23.8	2.14 m, 1.89 m	22.9
12	5.11 s	124.2	5.51 s	126.3
13	–	138.1	–	138.9
14	–	42.2	–	44.5
15	1.38 m, 1.13 m	52.8	1.34 m, 1.23 m	134.5
16	1.65 m, 1.4 m	48.1	1.65 m, 1.4 m	39.5
17	–	24.0	–	28.6
18	2.2	29.2	–	135.1
19	1.63 m	39.2	–	29.5
20	1.60 m	38.7	2.1 m	37.4
21	1.74 m, 1.49 m	30.1	1.78 m, 1.29 m	31.2
22	1.97 m, 1.72 m	37.2	1.97 m, 1.74 m	34.3
23	1.25 s	22.1	1.28 s	22.1
24	1.25 s	22.1	1.28 s	22.1
25	1.04 s	16.2	1.14 s	16.3
26	1.04 s	17.3	1.14 s	17.3
27	1.33 s	26.9	1.23 s	19.5
28	–	175.2	–	178.3
29	1.11 d	16.7	1.20 s	19.8
30	1.11 d	28.2	0.96 m	28.3

suggesting that fragmentation is occurring in the manner associated with Δ^{12}-triterpenoid [10]. The ^{13}C NMR spectrum showed resonance for all thirty carbon atoms in the molecule. The spectra revealed the presence of seven methyl, eight methylene, seven methane carbons and eight quaternary carbon atoms. The two downfield quaternary carbon signals at δ, 213.0 (C-3) and 69.05 (C-2) showed the presence of ketonic and one hydroxyl functionality in the molecule [11]. The analytical results obtained from ^{13}C NMR spectrum for this compound were tabulated in table 1.

Compound **2** was also isolated as a white, amorphous powder, $[\alpha]_D$+42.6. The ^1H and ^{13}C NMR spectra of **2** were closely similar to those of **1** but there was no signal of the double bond at C-18. UV spectra of the compound **2** showed the presence of hetero conjugated cissoid diene. It is the first report of the presence of a s-cis diene in triterpenoids of the *Achellia Fragrantissima F.* Its IR spectrum showed absorption bands for

Table 2

*Vitro antimicrobial values of compounds **1** & **2***

Test organism	compound (1)	compound (2)
Escherichia coli	32.0	9.0
Staphylococcus aureus	25.0	8.0
Enterococcus faecalis	10.0	8.0
Pseudomonas aeruinose	7.0	8.0

hydroxyl, ketonic and carboxylic groups at γ_{max} 3420, 1710 and 1720 cm^{-1} respectively. In addition, the molecular formula was determined as $C_{30}H_{44}O_4$ from a molecular ion peak at m/z 468, also, showed a fragment at m/z 203, suggesting that fragmentation is occurring in the manner associated with Δ^{12}-triterpenoid [10]. The ^{13}C NMR spectrum showed resonance for all thirty carbon atoms in the molecule. The spectra revealed the presence of seven methyl, eight methylene, five methane carbons and ten quaternary carbon atoms. The two downfield quaternary carbon signals at δ, 213.0 (C-3) and 79.05 (C-2) showed the presence of ketonic and one hydroxyl functionality in the molecule [11]. The analytical results obtained from ^{13}C NMR spectrum for this compound were tabulated in table 1.

2. Antimicrobial activity. The antimicrobial activity of new two terpenoids **(1)** & **(2)** was studied using the agar diffusion methods. These compounds exhibit antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa.*

Conclusion. *Achellia Fragrantissima F.* is local medicinal plant which grows in west Egypt in great abundance is shown to be in anti-Gram-positive and Gram-negative phytochemicals including pentacyclic triterpenoids. These results give the opportunity to start intensive studies for isolation of these biologically active compounds for local drug design programs.

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Supporting information. Supporting information accompanies this paper on http://pubs.asc.org.

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Два нові пентациклічні тритерпеноїди з коріння *Achellia Fragrantissima F.* та їх біологічна активність

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Резюме. Два нові пентациклічні тритерпеноїди — 2α-гідрокси-3-оксоурс-12-ен-28-ова кислота **(1)** і 2α-гідрокси-3-оксоурс-12,18-дієн-28-ова кислота **(2)** були виділені з коріння *Achellia Fragrantissima F.* разом з двома відомими терпенами — α-амірином **(3)** та урсоловою кислотою **(4)**. Структури нових сполук **1** і **2** встановлено за допомогою спектроскопічної інтерпретації даних. Антимікробну активність нових терпеноїдів **(1)** і **(2)** досліджено на *Escherichia coli, Staphylococcus aureus*, *Enterococcus faecalis* та *Pseudomonas aeruginosa.*

Ключові слова: тритерпеноїди рослинного походження, спектроскопічний аналіз.

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