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Biological activity of Coumarins from *Launaea resedifolia*

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ABSTRACT

Four coumarin compounds were isolated from the methylene chloride-methanol (1:1) extract of the aerial parts of *Launaea resedifolia*, namely, Resedin A (I), Resedin B (II), Resedin C (III) and Resedin D (IV). The structures were elucidated by 1D, 2D-NMR and HR-CIMS analysis. Biological activity of isolated compounds were carried out. These compounds showed high antibacterial activity against some Gram-positive bacteria as *Bacillus cereus* and *Staphyllococcus aureus* in minimum inhibitory concentrations of 200 and 400 μg/mL. However, they showed no effect on tested Gram-negative bacteria as *Serratia Sp.*, *Pseudomonas Sp* and *Escherichia coli*).

KEY WORDS: *Launaea resedifolia*, Asteraceae, coumarins, coumarin glucoside, Resedin.

INTRODUCTION

The genus *Launaea* (tribe *Lactucaea*, family Asteracea) comprises about 40 species. Many of its plants are used in folk medicine as bitter stomachic, for skin diseases, as antitumors and as insecticides. The genus *Launaea* presents possesses phytochemical features, such as terpenoids (1-5), phenolics (6) and (7), flavones (8) and coumarins (1), (9), (10-12). Therefore, we investigated the chemical constituents of *Launaea resedifolia* collected from Algeria.

EXPERIMENTAL

General experimental procedures

NMR spectra were recorded with a JEOL ECA500 spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C). NMR chemical shifts were referenced to TMS peaks: EIMS were obtained at 70 ev using a JEOL SX102A mass spectrometer. Column chromotography (CC) was performed using silica gel 60 (Merck, 0.063-0.2 mm). TLC analysis was performed with silica gel (Merck, Kieselgel). Spots were visualized by UV (λ_{max} 259 and 360 nm). HPLC was performed in the reverse phase on knauer pump 64 and different refractometer (column: RP-18, 250×25 mm, flow = 1.7 ml/min, elution with MeOH-H_2O, mixtures, refractive index detector was used for detection.

Plant material

Aerial parts of *L. resedifolia*, were collected in March 2004 from 25 km. North of Ouargla, Algeria, during flowering period. A voucher specimen was deposited at the herbarium of chemistry department, faculty of sciences, Constantine University, under the code number SR 101, Algeria.

Extraction and isolation

The aerial parts of *L. resedifolia* (1 Kg) were dried, powdered and extracted with methylene chloride-methanol (1:1) at room temperature. The solvent was distilled under reduced pressure furnishing a residue (10 g). The residue was subjected to flash column chromatography, being eluted with *n*-hexane, methylene chloride and methanol, increasing the degree of polarity. The extract was prefractionated by CC (6 ×120 cm) a silica gel eluting with *n*-hexane followed by a gradient of *n*-hexane-CH_2Cl_2 up to 100% CH_2Cl_2 and CH_2Cl_2-MeOH up to 15% MeOH. The fraction was further purified by CC (2×40 cm), a Sephadex LH-20 eluted with *n*-hexane-CH_2Cl_2-MeOH (6:4:1) resulted in a complex mixture. The mixture was purified by HPLC (MeOH-H_2O, 65:35, R_t = 5.6 and 6.0 min).

Bioassays

The antibacterial activity of compounds I-IV was determined against Gram-negative strains (*Serratia* sp., *Pseudomonas sp.*, *Escherichia coli*) and Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*), obtained from culture prepared in Department of Microbiology , Faculty of Pharmacy , El-Minia University, Egypt, using Whatman filter paper No. 1, 1 cm. Diameter, disc diffusion assay method. Five replicates were performed for the compounds with two concentrations (200 μg/mL and 400 μg/mL) of each compound were tried. Discs were soaked in the test compound for 30 sec, evaporated, then overloaded on the surface of the nutrient agar media cultured with the tested bacterium. Ampicillin (purchased from ADWIC Comp., Egypt) and amoxillin

(purchased from ADCO Comp., Egypt) were used as a reference compounds.

RESULTS AND DISCUSSION

Investigation of the CH_2Cl_2-MeOH (1:1) extract of the aerial parts of *Launaea resedifolia* afforded four compounds. Compound **I**, colorless oil, EIMS showed a molecular ion peak $(M)^+$ at *m/z* 356 in according with the molecular formula $C_{15}H_{16}O_{10}$. The ^{13}C-NMR spectrum of compound **I** displayed fifteen carbon signals. DEPT experiments indicated these signals as: one carbonyl carbon at δ_C 160.58 (s, C-2), one methylene carbon at δ_C 61.30 (t, C-6`); eight methine carbons at $\delta_C$ 143.60 (d, C-4), 113.01 (d, C-3), 102.97 (d, C-8), 102.98 (d, C-1`), 75.39(d, C-3`), 79.65 (d, C-2`), 73.45 (d, C-4`) and 70.02 (d, C-5`) and five quaternary carbons at δ_C 149.05 (s, C-7), 146.35 (s, C-8_a), 135.18 (s, C-4_a),125.40 (s, C-6) and 149.70 (s, C-5). The ^{1}H-NMR spectrum showed characteristic signals of glucose moiety, whereas, the methylene protons H-6`$_a$ and H-6`$_b$ appeared as two double doublets at δ_H 3.95 (*J* = 12.0, 3.0 Hz) and 3.72 (*J* = 12.0, 3.0 Hz). The anomeric proton H-1` appeared downfield as doublet signal at $\delta_H$ 4.86 (*J* = 7.5 Hz), the other methin protons H-2`, H-3`, H-4` and H-5` appeared at δ_H 3.54 (dd, *J* = 7.5, 8.5 Hz), 3.48 (dd, *J* = 8.5, 9.0 Hz), 3.41(dd, *J* = 9.0, 9.0 Hz) and 3.85 (ddd, *J* = 3.0, 5.0, 9.0 Hz), respectively. The coumarin moiety exhibited characteristic signals as a doublet at δ_H 7.81 (H-4, *J* = 9.5 Hz), which correlated in ^{1}H-^{1}H COSY with doublet at δ_H 6.27 (H-3, *J* = 9.5 Hz). The singlet signal appeared at δ_H 6.82 was assigned for H-8. All proton and carbon signals were assigned by ^{1}H-^{1}H and ^{1}H-^{13}C COSY. In the ^{1}H-^{13}C COSY, the signal at δ_H 4.86 (H-1`) showed correlation with the carbon signal at $\delta_C$ 102.98 (C-1`). The two double doublet signals at δ_H 3.95 and 3.72 correlated with carbon signal at δ_C 61.30 (C-6`). The presence of sugar moiety in position 7 was proved by NOE spectrum (Fig.1), which showed correlation between doublet at $\delta_H$ 7.81 (H-4) and the signal doublet at $\delta_H$ 6.27 (H-3), correlation between singlet at $\delta_H$ 6.82 (H-8) and the doublet at $\delta_H$ 4.86 (H-1`).

The structures of the four compounds were elucidated as follows :

Resedin, yellowish brown oil, HREIMS $(M)^+$, *m/z* (rel. int.) 356 (80), $C_{15}H_{16}O_{10}$, 193 (M-Glu.)$^+$ (75). IR γ_{max}^{KBr} cm^{-1}; 3295.9, 2934.5, 1595.9, 1452.5, 1125.8. ^{1}H-NMR (500 MHz, CD_3OD). ^{13}C NMR (500 MHz, CD_3OD) see Table (2).

Resedin acetate $\mathbf{I_a}$, Compound **I** (14 mg) was refluxed in 1 ml. of AC_2O-C_5H_5N (1:1) for 2h. The mixture was cooled to room temperature and extracted with CH_2Cl_2 to give the acetate $\mathbf{I_a}$ (8 mg). Brownish yellow oil, IR $\boldsymbol{\gamma}_{max}^{KBr}$ cm^{-1} 2960.5, 1588.6, 1445.8; HREIMS *m/z* (rel int.) 608 $(M)^+$ (80), $C_{27}H_{28}O_{16}$; ^{1}H-NMR spectral data (500 MHz, CD_3OD).

Table 1: ^{1}H-NMR spectral data of I-IV (500 MHz, CD_3OD, TMS as int. standard, δvalues)*

	I	I_a^+	II	III	IV
	δ_H	δ_H	δ_H	δ_H	δ_H
3	6.27 (d, 9.5)	6.37	6.15	6.25	6.30
4	7.81 (d, 9.5)	7.65	7.75	7.90	7.65
8	6.82 (s)	6.82	6.52	6.85	6.55
1`	4.86 (d, 7.5)	5.10			
2`	3.54 (dd, 7.5, 8.5)	5.32			
3`	3.48 (dd, 8.5, 9.0)	5.37			
4`	3.41 (dd, 9.0, 9.0)	5.38			
5`	3.85 (ddd, 3.0, 5.0, 9.0)	5.16			
6`	3.95 (dd, 12.0, 3.0)	4.35			
	3.72 (dd, 12.0, 3.0)	4.19			
5-OAc		2.08 (s)			
6-OAc		2.10 (s)			
2`-OAc		2.02 (s)			
3`-OAc		2.01 (s)			
4`-OAc		2.05 (s)			
6`-OAc		2.03 (s)			
OMe				3.80 (s)	3.96 (s)

**Homonuclear ^{1}H-^{1}H COSY spectra were also used for these assignments.*

*Table 2: ^{13}C-NMR spectral data of I-IV (500 MHz, CD_3OD, TMS as int. standard, δvalues)**

	I	I_a^+	II	III	IV
	δ_H	δ_H	δ_H	δ_H	δ_H
2	160.85 (s)	160.85	160.90	160.90	160.90
3	113.01 (d)	113.01	113.41	113.41	113.41
4	143.60 (d)	143.60	143.55	143.55	143.55
4_a	135.18 (s)	140.05	122.30	115.80	111.60
5	149.70 (s)	150.65	150.66	148.85	149.71
6	125.40 (s)	124.95	130.69	139.18	129.77
7	149.05 (s)	152.70	148.99	145.88	147.19
8	102.97 (d)	105.35	103.50	102.92	102.82
8_a	146.35 (s)	151.76	145.89	145.80	145.58
1`	102.98 (d)	98.95			
2`	79.65 (d)	74.85			
3`	75.39 (d)	70.75			
4`	73.45 (d)	71.37			
5`	70.02 (d)	67.91			
6`	61.30 (t)	62.12			
5-OAc		169.12 (s) 20.37 (q)			
6-OAc		169.25 (s) 20.40 (q)			
2`-OAc		170.14 (s) 21.17 (q)			
3`-OAc		170.27 (s) 21.20 (q)			
4`-OAc		170.32 (s) 21.27 (q)			
6`-OAc		170.20 (s) 20.871 (*q)			
OMe				56.59	56.70

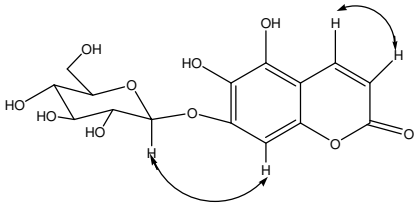
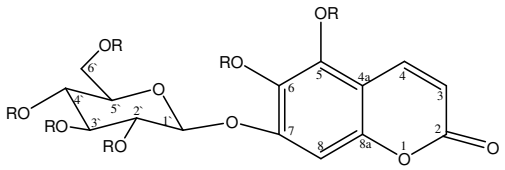
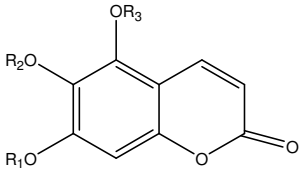


Fig. 1 . Selected NOE Correlations of compound 1



I R= H

I_a R= Ac



II- R_1 = H R_2 = H R_3 = H ; III - R_1 = H R_2 = Me R_3 = H ; IV R_1 = H R_2 = H R_3 = Me

Fig. 2. The structure of the isolated compounds

Resedin **II**, yellowish brown oil, HREIMS $(M)^+$, *m/z* (rel. int.) 194 (95), $C_9H_6O_5$. IR γ_{max}^{KBr} cm^{-1}; 3286.5, 2922.5, 1590.9, 1450.5. ^{1}HNMR (500 MHz, CD_3OD). Resedin **III**, grayish brown oil, HREIMS $(M)^+$, *m/z* (rel. int.) 208 (90), $C_{10}H_8O_5$. IR γ_{max}^{KBr} cm^{-1}; 3230.5, 2900.6, 1700.5, 1452.5. ^{1}HNMR (500 MHz, CD_3OD).

Resedin **IV**, grayish brown oil, HREIMS $(M)^+$, *m/z* (rel. int.) 2083 (93), $C_{10}H_8O_5$. IR γ_{max}^{KBr} cm^{-1}; 3235.5, 2905.6, 1700.6, 1450.5. ^{1}HNMR (500 MHz, CD_3OD). These compounds are explained instrumentally in Table 1 and 2. The structures are illustrated in Fig (1 and 2).

Acetylation of a portion of compound **I** gives the acetylated derivative $\mathbf{I_a}$. HREIMS provides a molecular ion peak $(M)^+$ at *m/z* 608 corresponding to $C_{27}H_{28}O_{16}$. The ^{1}H-NMR spectrum revealed the six acetyl signals at δ_H 2.08, 2.10, 2.02, 2.01, 2.05 and 2.03. The protons of the sugar and coumarin moieties were determined by ^{1}H-^{1}H COSY and given in Table 1. The ^{13}C-NMR data are given in Table 2.

HREIMS of compound **II** showed the molecular ion peak $(M)^+$ at *m/z* 194 in accord with the molecular formula $C_9H_6O_{54}$. ^{1}H-NMR spectrum of **II** showed presence of one singlet signal at δ_H 6.52 (H-8), and the two doublets at δ_H 7.75 (H-4, *J* = 9.5 Hz) and 6.15 (H-3, *J* = 9.5 Hz). The ^{13}C-NMR data are given in Table 2.

The IR spectrum of **III** displayed absorption bonds characteristic of carbonyl group (1700 cm^{-1}, C=O). The HREIMS showed the molecular ion peak $(M)^+$ at *m/z* 208 in accord with the molecular formula $C_{10}H_8O_5$.

The ^{1}H-NMR spectrum of compound **III** revealed the presence of two doublets at δ_H 7.90 (H-4, *J* = 6.0 Hz) and 6.25 (H-3, *J* = 6.0 Hz). The singlet signal appeared at δ_H 6.85 was assigned for the proton H-8. The difference between compound **II** and **III** was the presence of singlet signal at δ_H 3.80, which assigned for a methoxy group. The ^{13}C-NMR data are given in Table 2.

^{1}H-NMR spectrum of compound **IV** was close to compound **III**. The difference in the chemical shifts of the signals suggested that compound **IV** was isomer of compound **III** H-8 of compound **III** appeared as singlet at δ_H 6.85, whereas, H-8 of compound **IV** appeared as singlet at δ_H 6.55. Also, few differences in the chemical shifts for H-3 and H-4 were observed, Table 1 and 2. The HREIMS which revealed a molecular ion peak $(M)^+$ at *m/z* 208 which identical with the molecular formula $C_{10}H_8O_5$.

Results of antibacterial screening

In vitro, screening experiments for antibacterial activities of compounds I-IV was subjected to biological testing. To substantiate the antibacterial results, we screened compounds against an assortment of two Gram-positive bacteria (*Bacillus cereus, Staphylococcus aureus*) and Gram-negative bacteria (*Serratia Sp., Pseudomonos Sp., Escherichia coli*) using ampicillin and amoxillin as a reference standard.

Table 3: Antimicrobial activities of montanone (Dry DMSO as solvent)

Test organism	I[c]	II[c]	III[c]	IV[c]	Ampicillin[d]	Amoxillin[d]
Gram- Positive Strain						
Bacillus cereus	10[a]	10[a]	10[a]	10[a]	10[a]	*N*[a]
	18[b]	18[b]	18[b]	18[b]		
Staphylococcus aureus	N[a]	N[a]	N[a]	N[a]	8[a]	N[a]
	7[b]	7[b]	7[b]	7[b]		
Gram-Negative Strain						*13*[a]
Serratia sp	N[a]	N[a]	N[a]	N[a]	11[a]	
	N[b]	N[b]	N[b]	N[b]		
						13[a]
Pseudomonas sp.	N[a]	N[a]	N[a]	N[a]	11[a]	
	N[b]	N[b]	N[b]	N[b]		
						13[a]
Escherichia coli	N[a]	N[a]	N[a]	N[a]	11[a]	
	N[b]	N[b]	N[b]	N[b]		

[a] *Values show the zone of inhibition in mm.; conc. of the samples was 200 μg/ml ;* [b] *Values show the zone of inhibition in mm.; conc. of the samples was 400μg/ml;* [c] *Data are the mean of five measurements with neglected standard errors.*

[d] *Refference antibiotics were carried out at 200 μg/ml only ; N = No effect*

The minimum inhibitory concentrations (MICs, µg/ml) were determined using standard agar dilution method (13). The MIC value is summarized in Table 3. From the obtained data, it is clear that compounds I-IV posses higher activity against Gram-positive strain, particulerly *Bacillus cereus*. On the contrary, Gram-negative strains not affected at tested concentrations as shown in Table 3. Our results are in agreement with those reported earliear by Joklik et al. (14), they reported that some antibiotics such as ampicillin and amoxillin have been developed as inhibitors of cell wall synthesis of bacterial cell. In conclusion, compounds I – IV exhibited antibacterial activities.

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