SHORT REPORT

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Flavonoid Constituents from Algerian *Launaea resedifolia* (O.K.) and Their Antimicrobial Activity

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Abstract: A chemical investigation of the aerial parts of *Launea resedifolia* (O.K.) afforded four flavonoids, apigenin **1**, luteolin **2**, apigenin 7-*O*-β-glucoside **3** and apigenin 7-*O*-β-glucuronide **4**. The structures of the isolated compounds were established by chromatographic behaviour and by means of UV, NMR and MS spectral analysis. Moreover, the antimicrobial activity of two isolated flavonoids **3**, **4** and the *n*-BuOH extract against eleven bacteria and one fungus was studied. It was found that the most powerful effect was against *Morganella morgani*; *Streptococcus* Sp; *Enterobacter* Sp. and *Proteus mirabilis*.

Keywords: *Launaea resedifolia* O.K;, Asteraceae; flavonoids; antimicrobial activity.

1. Plant Source

Launaea resedifolia is a perennial herb widely distributed in the arid regions of Mediterranean area, where it is abundant in south east of Algeria. The genus *Launaea* (tribe lactuceae, family Asteraceae) comprises about 40 species in the Algerian flora and is represented by nine species most of which occur in the Sahara [1]. Aerial parts *of Launaea resedifolia* (O.K.) (Asteraceae) were collected during the flowering period in March (2005), 25. km north of Ouargla, Algeria. A voucher specimen was kept at the Herbarium of our Laboratory under the code number SR 101.

2. Previous Studies

Several species of this genus are used in folk medicine in bitter stomachic, skin diseases and reported to have antitumor, insecticide and cytotoxic activities [2]. The antimicrobial activities of coumarin constituents [3] and the neuropharmacological properties [4] have been investigated as well**.** Previous work on members of this genus revealed that the main constituents are flavonoids, coumarins [5-7] and terpenoids [8,9]. *Launaea resedifolia* from Egypt was reported to involve the four flavonoids: apigenin, luteoline-7-*O*-glucoside, apigenin-5-*O*-diglucoside, and 5,7-di-ohcoumarine [5].

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3. Present Study

In continuation of our investigation of *Launaea resedifolia* [10], the *n*-BuOH extract of the aerial parts gave four flavonoids identified as apigenin **1**, luteolin **2**, apigenin 7-*O*-β-glucoside **3** and apigenin 7-*O*-β-glucuronide **4.** The sructures of compounds were determined by UV, MS, and NMR spectra. Moreover the antibacterial and antifungal activities of the crude extract and the compounds **3** and **4** against several gram positive and gram negative bacteria and one fungus were carried out.

All of the bacteria (clinical stains) ; *E.coli, Staphylococcus blanc, Staphylococcus aureous, Proteus vulgaris, proteus mirabilis, Klebsiella pneumonia, Klebsiella oxytoca, Morganella morgani, Streptococcus Sp, Enterobacter Sp., Seratia Sp.*and the fungi *Candida albicans* were obtained from Bacteriology Laboratory Constantine Hospital University (C.H.U), while the fungus strain was isolated from microbiology laboratory, department of biology, Constantine University.

Air dried aerial parts of *Launaea resedifolia* O.K. (1700 g) were macerated four times with 70% MeOH solution by replacing the solution every day with fresh solvent. The hydro-alcoholic solutions were concentrated under reduced pressure to dryness and the residue was dissolved in hot water (500 mL) and kept in cold overnight. After filtration, the aqueous solution was successively extracted with EtOAc and *n*-BuOH for three times for each solvent, then the EtOAc and *n*-BuOH extracts were concentrated to dryness.

The *n*-BuOH extract was subjected to a MN-SC6 polyamide column chromatography being eluted with a gradient of toluene/MeOH with increasing polarity. Five main fractions (A, B, C, D and E) were collected and analyzed by DC6 polyamide TLC using "SI (Toluene: MeCOEt: MeOH 4:3:3)" and "SII (H_2O: MeOH: MeCOEt: Acetylacetone 13:3:3:1)" as solvents systems. Thus, from fractions A and B compounds **1** and **2** were separated by using preparative polyamide TLC using (SI) system as eluent. Compound **3** was obtained as a yellow-brown precipitate from fraction **C**, while compound **4** was obtained from fraction **D** and **E** which were combined and subjected to preparative TLC on silica gel using EtOAc: MeOH: Formic acid (95:5:5) as eluent. Compound **1** was identified previously in the species [5], but compounds **2**, **3** and **4** are identified for the first time in this species.

The anti-microbial activity tests were carried out on flavonoids extract and two isolated flavonoids: apigenin 7-*O*-β-glucoside 3 and apigenin 7-*O*-β-glucuronide 4 using disk diffusion method [11] against eleven human pathogenic bacteria, including Gram positive and Gram-negative bacteria.

The bacterial strains were first grown on Muller Hinton medium (MHI) at 37 ◦C for 24 hours prior to seeding on to the nutrient agar, while the *Candida albicans* at 30^0 C for 48 h. A sterile 6mm diameter filter disk (Whatman paper n° 3) was placed on the infusion agar seeded with bacteria, and each extract suspended in water was dropped on to each paper disk (40 µL per disk) for all of prepared concentrations (8mg/mL, 4mg/mL, 2mg/mL, 1mg/mL, 0.5mg/mL, 0.25mg/mL). The treated Petri disks were kept at 4 ◦C for 1 h, and incubated at 37 ◦C for 24 h. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the disks. Each experiment was carried out in triplicate.

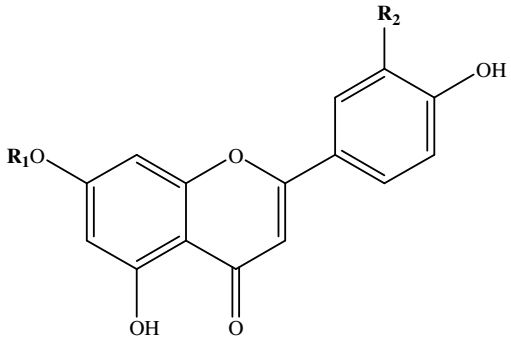
Some flavonoids are formed as antimicrobial barriers in plants response to microbial infection. Therefore, it should not be surprising that they have been found *in vitro* to be effective antimicrobial compounds against a wide array of microorganisms. There has been an enormous increase in the number of studies on flavonoids as potential antimicrobial agents [3]. Antimicrobial activities of apigenin and other derivatives have also been previously reported [12].

The diffusion test was applied to 12 microorganisms including one fungus from *Candida* genus and Gram-positive and negative bacteria. The results summarized in Table 1 showed that the crude extract from *Launaea resedifolia* (O.K) as well as compounds **3** and **4** prevented the growth of all the tested microorganisms and it has been revealed that the medium diameter of inhibition zone increases proportionally with the increase of flavonoids concentration. The obtained inhibition zone varied from 6.0 to 13.25 mm. The highest inhibition zone for the tested compounds (13.25mm) was recorded with *n*-BuOH extract (8 mg/mL) against *Morganella morgani*. However no activity against *E.coli, Staphylococcus blanc and Candida albicans* at low concentration (0.25-2mg/mL and 0.25-1mg/mlL have been recorded. To the best of our knowledge, there are no reports in the literature concerning antimicrobial activity of the crude extract of *Launaea resedifolia* O.K.

Table 1. Antimicrobial activity of *n*-BuOH extract at different concentrations on eleven bacteria and one fungus.

Strain bacteria	0.25mg/mL	0.5mg/mL	1mg/mL	2mg/mL	4mg/mL	8mg/mL
E.coli	..	..	..	..	6.5±0.57	7.0± 0.81
S. blanc	..	..	..	7.5± 1.29	7.75±1.25	10±1.82
S. aureous	6.5 ±0.75	6.75 ±0.95	8.0 ±0.81	8.5 ±0.57	8.5 ±0.57	9.75 ±1.25
P. vulgaris	6.5 ±0.57	7.5 ±1.29	7.75 ±0.95	8.0 ±0.81	8.5 ±0.57	8.5 ±0.57
P. merabilis	8.0 ±1.15	8.0 ±0.81	8.25 ±0.95	9.75 ±0.95	10.0 ±1.15	11.75±1.25
K. pneumonia	6.0 ±0.0	6.25 ±0.5	6.75 ±0.95	6.75 ±0.95	7.25 ±0.95	8.5 ±0.57
K. oxytoca	6.75 ±0.95	7.0 ±1.15	7.5 ±0.57	7.5 ±0.95	7.75 ±1.29	8.25 ±0.95
M. morgani	7.25 ±0.50	7.75 ±0.95	8.0 ±1.15	12.0 ±1.82	12.75±1.70	13.25 ±1.5
Streptococcus Sp.	6.5 ±0.57	7.75 ±0.5	8.75 ±0.95	9.25 ±1.25	11.25 ±1.5	12.25±0.95
Enterobacter Sp.	9.5 ±0.57	9.75 ±1.70	10.5 ±1.73	11.0 ±0.81	11.0 ±1.15	12.0 ±1.41
Seratia Sp.	7.5 ±0.57	8.5 ±0.57	9.25 ±0.5	9.25 ±1.21	9.5 ±1.29	10.0 ±0.81
Candida albicans	..	..	..	..	7.5±0.57	8±0.0

Screening experiments dealing with antimicrobial activity of compounds **3** and **4** were carried out through biological testing. To substantiate the antibacterial results, we screened compounds against an assortement of: *Staphylococcus blanc* (inhibition zone 6 mm and 7.50 mm) , *Klebsella pneumonia* (inhibition zone 6.75 mm and 8.0 mm) and *Morganella morgani* (inhibition zone 8 mm and 8.50 mm) with a concentration of 500µg/mL. The obtained results were closed to those found with *n*-butanolic extract.



	R_1	R_2
1	H	H
2	H	OH
3	Glucose	H
4	Glucuronide acid	H

*Apigenin (**1**):* Rf = 0.64, 0.05 (SI, SII) ; UV (λmax in MeOH) : gives bands at 337 and 268 nm for band I and II, addition of NaOH ; 391, 323 and 275 , NaOAc; 381, 301 and 276, H_3BO_3; 355, 302 and 270, $AlCl_3$; 347, 382, 301 and 274 while HCl; 342, 382, 301 and 276.^{1}H-NMR (400 MHz, DMSO- d_6) δ (ppm): δ 7.7 (2H, d, *J* = 9Hz, H-2′ and H-6′), 6.8 (2H, d, *J* = 9Hz, H-3′ and H-5′), 6.5 (1H, s , H-3) , 6.4 (1H, d, *J* = 1.3 Hz, H-8) , 6.2 (1H, d, *J* = 1.3 Hz, H-6) [12].^{13}C-NMR (100 MHz, DMSO- d_6) δ (ppm) : 181.8 (C-4), 164.1 (C-2), 163.7 (C-7) , 161.5 (C-9), 161.1 (C-4′), 157.3 (C-5), 128.4 (C-6′, C-2′), 121.3 (C-1′), 116.0 (C-3′, C-5′), 103.7 (C-10) , 99.2 (C-6), 94.2 (C-8). EI-MS *m/z* (% rel. int) showed $[M-H]^-$ at 269 (100).

*Luteolin (**2**)*: Rf = 0.48, 0.08 (SI, SII) , UV (λmax in MeOH) : gives bands at 344 and 268 nm for band I and II, addition of NaOH ; 396 and 272, NaOAc; 389 and 273, H_3BO_3; 360 and 266, $AlCl_3$; 413, 353, 273 and 297 while HCl; 348, 382, 274 and 296. ^{1}H-NMR (400 MHz, DMSO- d_6) δ (ppm): δ 7.5 (1H, dd, *J* = 9Hz, *J* = 2Hz, H-6′), 7.4 (1H, d, *J* = 2Hz, H-2′), 6.9 (1H, d, *J* = 9 Hz, H-5′), 6.7 (1H, s ,H-3), 6.5 (1H, d, *J* = 2 Hz, H-8) , 6.2 (1H, d, *J* = 2 Hz, H-6). ^{13}C-NMR (100 MHz, DMSO- d_6) δ (ppm) 182.2 (C-4), 164.7 (C-7), 164.5 (C-2), 162.1 (C-9), 157.9 (C-5), 150.1 (C-4′), 146.2 (C-3′), 121.7 (C-6′), 119.3 (C-1′), 116.4 (C-5′), 113.8 (C-2′), 104.2 (C-10), 99.2 (C-6), 94.2 (C-8) [12]. EI-MS *m/z* (% rel. int) showed $[M-H]^+$ at 285 (100), 286 $[M^+]$ (30).

*Apigenin 7-O-ß-D-glucoside (**3**):* Rf = 0.71, 0.32 system (SI, SII); UV (λmax in MeOH) : gives bands at 334 and 269 nm for band I and II, addition of NaOH ; 385 and 275, NaOAc; 390 and 268, H_3BO_3; 350 and 265, $AlCl_3$; 359, 344 and 280 while HCl; 341 and 281.^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): δ 7.9 (2H, d, *J* = 8.8 Hz, H-2′ and H-6′), 6.9 (2H, d, *J* = 8.8 Hz, H-3′ and H-5′), 6.8 (1H, s, H-3), 6.6 (1H, d, *J* = 2 Hz, H-8), 6.50 (1H, d, *J* = 2 Hz, H-6). Sugar moiety, 5.2 (1H, d, *J* = 7 Hz, H-1″, glucose), 3.4- 4 (ramaining sugar protons) [12]. ^{13}C-NMR (100 MHz, DMSO- d_6): δ (ppm): 182.4 (C-4), 164.7 (C-2), 163.1 (C-7), 161.9 (C-5), 161.4 (C-4′), 157.3 (C-9), 128.9 (C-2′, C-6′), 121.2 (C-1′), 116.4 (C-3′, C-5′), 105.7 (C-10), 104.7 (C-8), 103.4 (C-3) , 99.9 (C-1″, glucose), 95.0 (C-6), 76.1 (C-5″, glucose), 75.2 (C-3″, glucose), 73.9 (C-2″, glucose), 71.0 (C-4″, glucose) , 66.7 (C-6″, glucose) [12]. IR(ν cm^{-1}in KBr) (sugar band = 2858, 3317), CO=1602, 1732.

*Apigenin-7-O- ß-D-glucuronide (**4**)*: Rf = 0.71, 0.60 system (SI, SII); UV (λmax in MeOH) : gives bands at 336 and 280 nm for band I and II, addition of NaOH ; 390 and 270, NaOAc; 390, 330 and 270, H_3BO_3; 330 and 270, $AlCl_3$; 350 and 280 while HCl; 350, 320 and 270. ^{1}H-NMR (400 MHz, DMSO- d_6) δ (ppm): δ 7.9 (2H, d, *J*=7.8 Hz, H-2′ and H-6′), 6.9 (2H,d, *J*=7.8 Hz, H-3′ and H-5′), 6.8

(1H, s, H-3), 6.8 (1H, d, *J*=1.5 Hz, H-8), 6.5 (1H, d, *J*=1.5 Hz, H-6). Sugar moiety, 5.2 (1H, d, *J* = 8 Hz, H-1″), 3.25 - 4.25 (ramaining sugar protons) [12]. ^{13}C-NMR (100 MHz, DMSO- d_6) δ (ppm): 182.39 (C-4), 171.8 (C-6″, glucuronide), 164.7 (C-2), 163.1 (C-7), 162 (C-5), 161.4 (C-4′), 157.3 (C-9), 128.9 (C-2′, C-6′), 121.1 (C-1′), 116.4 (C-3′, C-5′), 105.7 (C-10), 103.2 (C-3), 100 (C-1″, glucuronide), 99.7 (C-6), 95 (C-8), 76.3 (C-5″, glucuronide), 75 (C-4″, glucuronide), 73.2 (C-3″, glucuronide), 72 (C-2″, glucuronide) [12].IR (ν cm^{-1}in KBr) (sugar band =2900, 3315), CO = 1662, 1732.

As a conclusion; antimicrobial activity of the crude *n*-butanolic extract as well as compounds **3** and **4** were reported. Also, four flavonoids isolated and identified from *Launaea resedifolia* and three of them are identified for the first time in this species.

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References

[1] P.Quezel, S.Santa, (1963). Nouvelle flore d'Algérie et des régions désertique méridionales. Paris. *CNRS,* 2 ,162.

[2] S.Rashid, M.Ashraf, S.Bibi and R.Anjum (2000). Insecticidal and Cytotoxic Activities of *Launaea Nudicaulis* (Roxb.) and *Launaea Resedifolia* (Linn.). *Pakistan J. of Biol. Sc*. **3**(5), 08-809.

[3] A.E.Ashraf,A.A.Nabil (2006). Antibacterial coumarins isolated from *launaea resedifolia*. *Chem. o f plant raw material*. **1**, 65-68.

[4] A.Abdu Raazag, Auzi, T.Najat, M.Hawisa, F.Sherif,D. atyajit, Sarker (2007). Neuropharmacological properties of *launaea resedifolia*, *Brazilian J. of Phamacognosy* , **17**(2), 160-165.

[5] M.R.I. Salehh, A.A.M. Habib, M.G. El-Ghazouly, O.M.K. Gabr And F.K. El-Fiky (1981). Chemical constituent from *Launea resedifolia, Egypt J. Pharm.Sc. **29**,* 1-4, 507-513.

[6] R.M.A. Mansour, A.A. Ahmed and N.A.M.Saleh (1983). Flavone glucosides of some *Launea species, Phytochemistry*, **22**, 2630-2631.

[7] R.M.Giner, S.Manez, M.C.Recio, C.Soriano, J.L.Rios (1992). Phenolic of Spanish Species. *Biochem Sys Ecol* . **20**, 187-188.

[8] H. Abd El-Fattah, A. F. Zaghloul, A. F. Halim and ES Waight (1990). Steroid and triterpenoid constituents of *launaea resedifolia* L. Egypt. *J. Pharm. Sci*. **31**, 81-91.

[9] F.Bitam, M.Letizia Ciavata, E.Manzo, A.Dibi and M.Gavagnin (2008).Chemical Characterisation of the Terpenoid Constituents of the Algerian plant *Launea arborescens* . *Phytochemistry*. **69**, 2984- 2992

[10] N.Gheraf, A Zellagui, S.Rhouati (2006). Isolation Of Coumarins and Coumarin glucosides from *Launaea resedifolia*. *Asian j. of Chem* , **18** ,2348-2352.

[11] B.F.Carbonnelle, A.Denis, G.Marmonier and P.Rivargues (1987). Bacteriologie medicale-techniques usuelles.p. 224-243.

[12] K.R.Markham, T.J.Marbry and H.Marbry (1975) . *In The flavonoids*. (J.B.Harborne, T.J.Marbry and H.Marbry). Chapman and Hall. London .

[13] E.Middleton, C.Kandaswami (1986). The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In The flavonoids- Advances in Research since. Chapman & Hall. London.