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Chemical constituents of *Linaria reflexa* Desf. (Scrophulariaceae)

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ABSTRACT

*The phytochemical study on the aerial parts of Linaria reflexa Desf., belonging to the scrophulariaceae family led the isolation and structural elucidation of pectolinarin (**1**), linariin (**2**), methyl linoleate (**3**), apigenin (**4**), mannitol (**5**) and pectolinarigenin (**6**). These compounds were identified by 1D and 2D NMR as well as by mass spectrometry.*

Keywords: Chemical Constituents; *Linaria reflexa* Desf.; Scrophulariaceae.

INTRODUCTION

The genus *Linaria*, one of 220 genus of the Scrophulariaceae family, comprises about 200 species [1], widely distributed in the Mediterranean basin and eastern Asia. Several *Linaria* species are used in folk medicine in many areas for treatment of various diseases, for example fresh or dried flowers of *L. vulgaris* are used internally to help digestive problems and urinary disorders. Externally, the plant is applied in the treatment of hemorrhoids, ulcus cruris, for ablution of festering wounds, and skin rashes. It is also reported to have anti-inflammatory effect [2] and to treat coughs and asthma [3]. *L. japonica* known under the name "unran" is used as diuretic, purgative [4] and laxative [5]. *L. cymbalaria* is used for its diuretic, tonic and antiscorbutic effects [6].

Previous phytochemical studies on this genus showed the presence of iridoids [7-12], flavonoids [13-16], diterpenoids [17-20], monoterpenoids [21], triterpenoids [22, 23], alkaloids [24, 25] and phenylethanoids [4]. Many biological effects have been reported for some isolated compounds from *L. saxatilis* var. *saxatilis* and *L. reflexa* Desf. such as neo-clerodane diterpenoids and flavonoids [26, 27].

As a part of our on-going program of research on Algerian plants [28-35], we report our results on the phytochemical investigation of the aerial parts of *L. reflexa* Desf., a North African folk medicine herb used for the treatment of certain skin diseases [36]. A previous study on this plant, reports the isolation of flavones, iridoids, sterol and the cytotoxic activity of the isolated compounds [26]. In the present work on the chemical constituents the aqueous-MeOH extract of the aerial parts, we have obtained 6 compounds (**1-6**) among which three (**3-5**) are reported for the first time from this spice (Figure 1). The structures were established by spectral analysis, mainly ESIMS, UV and 2D-NMR experiments (COSY, NOESY, HSQC and HMBC).

MATERIALS AND METHODS

Plant Material

The aerial parts of *Linaria reflexa* Desf., were collected in March 2012 from El-Meridj near Constantine city, Algeria. The plant was identified by Dr. D. Sarri on the basis of Quezel and Santa [37]. A voucher specimen has been deposited in the Herbarium of the *VARENBIOMOL* Unit Research, University of Constantine1 under n° 06/2012/SLR.

Extraction and isolation

Air-dried aerial parts of *L. reflexa* (1.5 kg) were macerated with 80% aqueous methanol at room temperature four times. After filtration, a yellow precipitate appeared in the solution. This precipitate which was filtered, led to the isolation of compound **1** (40 mg). The filtrate was concentrated and dissolved in H_2O (600 ml). The resulting solution was successively extracted with petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH. In the EtOAc phase yellow needles appeared giving after filtration the compound **2** (700 mg). The $CHCl_3$ extract (5 g) was further fractioned by silica gel column chromatography, eluted with *n*-hexane containing increasing percentages of EtOAc to obtain ten fractions (F1-F10). F1 (45 mg) and F2 (27 mg) were purified on preparative plates of silica gel *n*-hexane/EtOAc 7:3 and *n*-hexane/EtOAc 8:2 respectively, to obtain compound **3** (5 mg) and compound **4** (4 mg) respectively. During the concentration, the *n*-BuOH extract gave a white precipitate which was filtered to obtain compound **5** (48 mg). Compound **6** is the result of the acid hydrolysis using the microwave method, in which 30 mg of compound **2** were added to 2 ml of ethanol and 100 µl of concentrated HCl, after 30 min in the microwave, the aglycone part was purified on PLC plates.

RESULTS AND DISCUSSION

Isolated and identified compounds

Compound 1: $C_{29}H_{34}O_{15}$; ^{1}HNMR (400 MHz, DMSO-d_6, δ_{ppm}, *J*/Hz): 12.96 (1H, s, OH-5), 8.05 (2H, d, *J*=8.8, H-2', H-6'), 7.18 (2H, d, *J*=8.8, H-3', H-5'), 6.95 (1H, s, H-3), 6.94 (1H, s, H-8), 5.13 (1H, d, *J*=6.9, H-1"Glc), 4.58 (1H, br. s, H-1'"Rhm), 3.87 (3H, s, 4'-OCH_3), 3.70 (3H, s, 6-OCH_3), 1.07 (3H, d, *J*=6.2, CH_3 Rhm); ^{13}C NMR (DMSO-d_6, 100 MHz, δ_{ppm}) 164.1 (C-2), 103.4 (C-3), 182.4 (C-4), 152.5 (C-5), 132.7 (C-6), 156.5 (C-7), 94.3 (C-8), 152.2 (C-9), 105.9 (C-10), 122.7 (C-1'), 128.4 (C-2', C-6'), 114.8 (C-3', C-5'), 162.4 (C-4'), 100.4 (C-1"), 73.1 (C-2"), 75.7 (C-3"), 69.5 (C-4"), 76.4 (C-5"), 65.9 (C-6"), 100.4 (C-1'"), 70.4 (C-2'"), 70.8 (C-3'"), 72.0 (C-4'"), 68.3 (C-5'"), 17.7 (C-6'"), 55.6 (4'-OMe), 60.31 (6-OMe); HR-ESIMS (+): *m/z* 645.1810 $[M+Na]^+$ corresponding to $C_{29}H_{34}O_{15}Na$ (calculated: 645.1795). This compound was characterized as pectolinarin [16].

Compound 2: $C_{31}H_{36}O_{16}$; ^{1}HNMR (400 MHz, DMSO-d_6, δ_{ppm}, *J*/Hz): 12.83 (1H, s, OH-5), 7.92 (2H, d, *J*=9.2, H-2', H-6'), 7.40 (2H, d, *J*=9.2, H-3', H-5'), 6.88 (1H, s, H-3), 6.74 (1H, s, H-8), 5.12 (1H, d, *J*=7.2, H-1"Glc), 4.59 (1H, br. s, H-1'"Rhm), 3.81 (3H, s, 4'-OCH_3), 3.80 (3H, s, 6-OCH_3), 1.91 (3H, s, CH_3CO), 0.77 (3H, d, *J*=6, CH_3Rhm); ^{13}C NMR (DMSO-d_6, 100 MHz, δ_{ppm}) 164.1 (C-2), 103 (C-3), 182.2 (C-4), 152.1 (C-5), 132.7 (C-6), 156.1 (C-7), 94.4 (C-8), 152.3 (C-9), 105.8 (C-10), 122.4 (C-1'), 128.2 (C-2', C-6'), 114.6 (C-3', C-5'), 162.4 (C-4'), 99.9 (C-1"), 73.1 (C-2"), 76.2 (C-3"), 69.1 (C-4"), 75 (C-5"), 65.3 (C-6"), 99.7 (C-1'"), 70.2 (C-2'"), 68.1 (C-3'"), 73.7 (C-4'"), 66.7 (C-5'"), 20.6 (CH_3CO), 16.9 (C-6'"), 170.3 (CO), 55.4 (4'-OCH_3), 60.3 (6-OCH_3). HRESI-MS (+): *m/z* 687.1899, $[M+Na]^+$, corresponding to $C_{31}H_{36}O_{16}Na$ (calculated: 687.1901). This compound was characterized as linariin [16].

Compound 3: $C_{19}H_{34}O_2$; ^{1}HNMR (400 MHz, $CDCl_3$, δ_{ppm}, *J*/Hz): 5.35 (4H, m, H-9, H-10, H-12, H-13), 3.65 (3H, s, OCH_3), 2.80 (2H, m, H_2-11), 2.29 (2H, t, H_2-2), 2.05 (4H, m, H_2-8, H_2-14), 1.61 (2H, m, H_2-3), 1.24 (14H, br. s, H_2-4, H_2-5, H_2-6, H_2-7, H_2-15, H_2-16, H_2-17), 0.9 (3H, t, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz, δ_{ppm}) 174.1 (C-1), 127.5 (C-9, C-10, C-12, C-13), 51.3 (OCH_3), 33.7 (C-2), 29.2 (C-4, C-5, C-6, C-7, C-15, C-16), 26.6 (C-8, C-14), 25.3 (C-11), 24.9 (C-3), 23.1 (C-17), 12.9 (C-18). This compound was identified as methyl linoleate [38].

Compound 4: $C_{15}H_{10}O_5$; ^{1}HNMR (400 MHz, MeOH-d_4, δ_{ppm}, *J*/Hz): 7.89 (2H, d, *J*=8.8, H-2', H-6'), 7.00 (2H, d, *J*=8.8, H-3', H-5'), 6.52 (1H, s, H-3), 6.45 (1H, d, *J*=1.6, H-8), 6.18 (1H, d, *J*=1.6, H-6); ^{13}C NMR (MeOH-d_4, 100 MHz, δ_{ppm}) 163.3 (C-2), 101.2 (C-3), 179.5 (C-4), 162.2 (C-5), 100.3 (C-6), 172.7 (C-7), 95.1 (C-8), 158.23 (C-9), 102.1 (C-10), 118.4 (C-1'), 127.6 (C-2', C-6'), 115.9 (C-3', C-5'), 164.4 (C-4'); ESI-MS (-): *m/z* 269 $[M-H]^-$. This compound was identified as apigenin [12].

Compound 5: $C_6H_{14}O_6$; ^{1}HNMR (400 MHz, D_2O, δ_{ppm}, *J*/Hz): 4.40 (2H, d, *J*=5.6, 2OH), 4.31 (2H, t, *J*=5.6, 2OH), 4.13 (2H, d, *J*=7.1, 2OH), 3.60 (2H, m, H-1a, H-6a), 3.54 (2H, m, H-3, H-4), 3.46 (2H, m, H-2, H-5), 3.38 (2H, m, H-1b, H-6b); ^{13}C NMR (D_2O, 100 MHz, δ_{ppm}) 71.3 (C-2, C-5), 69.7 (C-3, C-4), 63.8 (C-1, C-6); HRESI-MS (+): *m/z*

205.0689 $[M+Na]^+$, corresponding to $C_6H_{14}O_6Na$ (calculated: 205.0688). This compound was characterized as mannitol [15].

Pectolinarigenin (6)
$C_{17}H_{14}O_6$; ^{1}HNMR (400 MHz, MeOH-d_4, δ_{ppm}, *J*/Hz): 7.94 (2H, d, *J*=8.8, H-2', H-6'), 7.08 (2H, d, *J*=8.8, H-3', H-5'), 6.64 (1H, s, H-3), 6.58 (1H, s, H-8), .88 (6H, s, 2OCH_3); ^{13}C NMR (MeOH-d_4, 100 MHz, δ_{ppm}) 164.4 (C-2), 102.2 (C-3), 182.8 (C-4), 152.5 (C-5), 131.3 (C-6), 157.3 (C-7), 94.1 (C-8), 153.2 (C-9), 104.4 (C-10), 122.8 (C-1'), 128.5 (C-2', C-6'), 114.1 (C-3', C-5'), 164 (C-4'), 59 (6-OCH_3), 54.7 (4'-OCH_3) [39].

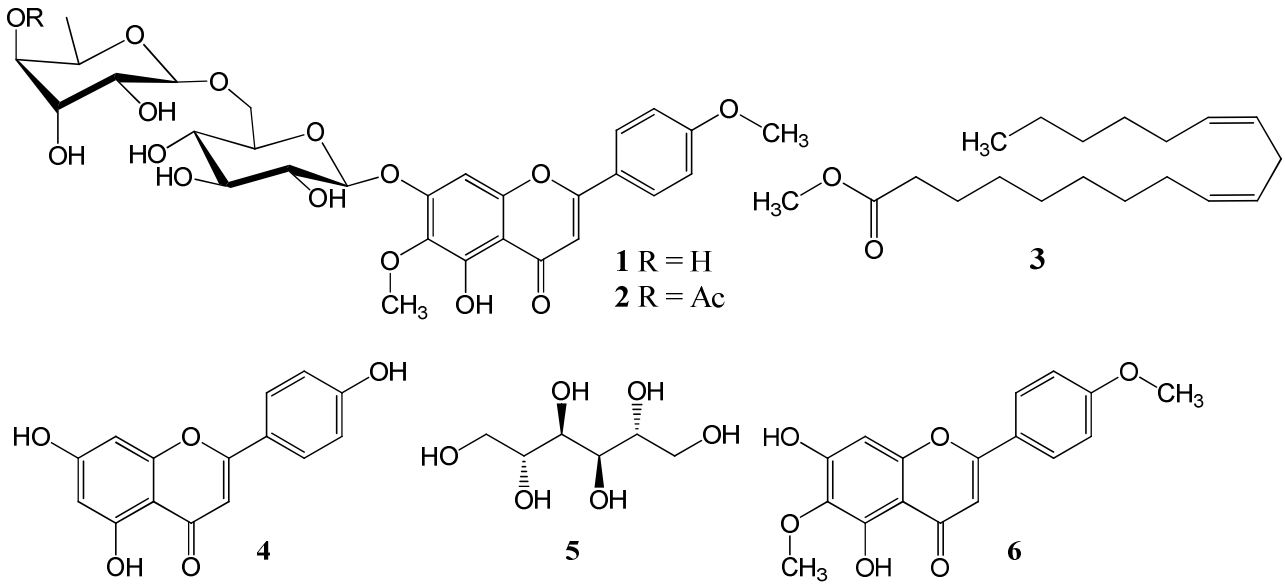

Figure 1: Structure of compounds 1-6

CONCLUSION

The previous study reported the presence of seven constituents such as flavonoids, iridoids and sterol in both the flowers and stems of *L. reflexa* Desf. In this investigation six compounds mainly, pectolinarin (**1**); linariin (**2**); methyl linoleate (**3**); apigenin (**4**); mannitol (**5**) and pectolinarigenin (**6**) have been obtained from the aerial parts of *L. reflexa*. Compounds **3**, **4** and **5** were reported for the first time from *L. reflexa* Desf.

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