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ANALYSIS AND ISOLATION OF SAPONINS FROM *Limoniastrum feei* BY LC-UV

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One of the medicinal plants used to treat gastric infections is *Limoniastrum feei* (Plumbagenaceae). The plant is native to the southeast of Algeria (Saoura, region of Bechar) and Northern Africa [1–3]. The other uses of *Limoniastrum feei* are as an antibacterial for the treatment of bronchitis and for stomach infections [4, 5]. LC separation followed by derivatization and detection by fluorometric methods, as described by Waridel et al. [6, 7], allows a high specificity of detection.

The elaboration of a more rapid detection method by LC-UV may be considered for a continuation of this work [8, 9]. The liquid chromatography-UV method has been developed for the analysis and separation of saponin extracts from stems of *Limoniastrum feei*. The separation of saponins using Si-gel phase liquid chromatography and gradient elution with $CHCl_3$: MeOH (65:35) as mobile phase has permitted good separation of all the peaks.

The conditions of detection proposed for the separation are between 15 and 30 s.

TLC analysis ($CHCl_3$:MeOH, 65:35) revealed four constituent in the butanol fraction (yield 0.60%, R_f 0.40, 0.48, 0.53, 0.58), six constituents in the ethyl acetate extract (yield 0.42%, R_f 0.48, 0.58, 0.63, 0.66, 0.78, 0.86), and five constituents in the methylene chloride (DCM) extract (yield 0.31%, R_f 0.6, 0.66, 0.76, 0.80, 0.88).

Liquid chromatography coupled with UV has been used in phytochemical screening and analysis of the isolated constituent from extracts [10, 11].

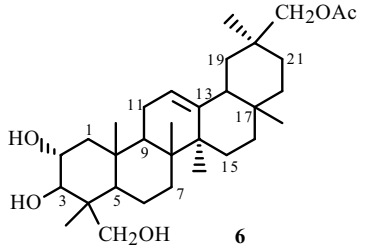
The results obtained from the LC-UV system, retention times, yields, peak areas, and tailing factors, were analyzed The obtained values confirm that this method is suitable for routine analysis [8, 9].

The LC-UV analysis for butanolic extract gave eight constituents. Two fractions, 6 and 7, were isolated and purified by flash chromatography (Si-gel column), eluting with H_2O /MeCN 75:35 and affording 65 mg of **6** and 90 mg of **7**.

In compound **6**, the MS molecular ion was not observed, but two peaks were observed at *m/z* 282 and 234, coming from the retro Diels-Alder cleavage of the $^{12}\Delta$ skeleton in oleanane derivatives. Its ^{13}C NMR spectrum showed 30 carbons and, in particular, two CH_2OH, two CHOH, and an olefinic bond (145.7 and 122.7 ppm).

The trisubstituted nature of the double bond was evidenced by the DEPT spectrum and the presence of one olefinic proton as an undefined triplet at 5.25 ppm in the ^1H NMR spectrum. The DEPT experiment revealed that the 32 carbons consisted of 7 CH_3, 11 CH_2, 6 CH, and 8 quaternary carbons.

The six methyl groups have to be located at quaternary carbons since they resonate as singlets in the ^1H NMR spectrum. The remaining primary alcoholic function has to be placed in position 30 on the basis of a comparison with the ^{13}C NMR data available in the literature [12, 13]. Therefore, compound **6** is 2α,3β,23-trihydroxy-30-acetylolean-12-ene.



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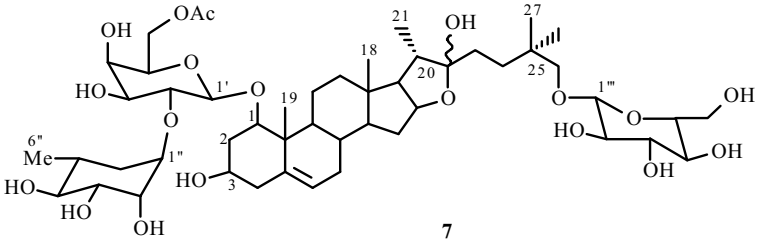

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The LC-UV analysis allowed us to identify **7** as a furostanol saponin, possessing a MW of 960 u. The MS-ESI$^-$ spectrum showed an intense [M–H]$^-$ ion at *m/z* 959, and in the MS^2 spectrum of the [M–H]$^-$ ion, peaks due to the loss of an acetyl moiety at *m/z* 917 (–42 u CH_2CO) and 899 (–60 u CH_3COOH) were detected.

The MS^3 spectrum of ions showed losses of 146 u corresponding to a deoxyhexose group, 162 u corresponding to a hexose group, and 144 u corresponding to a dehydrated hexose group. Peak 8 was identified as a furostanol saponin possessing two hexose, one deoxyhexose, and one acetyl group.

Study of the ^{1}H NMR and ^{13}C NMR spectra showed the presence of α-L-rhamnopyranose,β-D-galactopyranose, and β-D-glucopyranose units and allowed their assignment to the aglycone furost-5-ene-1β,3β,22ξ-26-tetrahydroxy structure.

The presence of the acetyl group was shown by the proton signal at δ 2.00 and the carbon signals at δ 20.8 and 170.5. The $^3J_{CH}$ correlation of the galactose H-6′ with the carbonyl of the acetyl group in the HMBC spectrum indicated that the acetyl moiety is linked at the galactose C-6′ position. Furthermore, the protons H-26 of the aglycone showed a $^3J_{CH}$ correlation with C-1‴ (δ 104.9), allowing us to identify **7** as 1-*O*-[α-L-rhamnopyranosyl-(1)-6-*O*-acetyl-β-D-galactopyranosyl]-1β,3β,22ξ-26-tetrahydroxyfurost-5(6)-ene-26-*O*-β-D-glucopyranoside.

The IR spectra (ν_{max}) were determined on a AVATAR 320 FT-IR spectrophotometer. The 1D and 2D NMR spectra were obtained on a Bruker Avance DRX 300 FT spectrometer operating at 300 MHz for ^{1}H NMR, and 75 MHz for ^{13}C NMR. For the ^{13}C NMR spectra, multiplicities were determined by a polarization transfer (DEPT) experiment. The LC system consisted of a liquid chromatography (Si-gel : 230–400 mesh (Merck) column operating at room temperature with a flow rate of 1 mL/min and a UNICAM UV 300 spectrophotometer detector operating at a wavelength of 210 nm.

Plant Material. The whole plants of *Limoniastrum feei* were collected from Kenadza (region of Bechar), Algeria. The botanical identification and voucher specimen were deposited in the herbarium of the Phytochemical and Organic Synthesis Laboratory. The assigned specimen number was (CA99/14) [4, 14]. The leaf stems and twigs were separated and oven dried (overnight). The plant stems were ground into powder using a grinder.

Extraction and Fractionation. Dried and ground stems of *Limoniastrum feei* (300 g) were extracted with EtOH (70 %). The extract was concentrated, diluted with water, and partitioned with hexane, methylene chloride, ethyl acetate, and *n*-butanol (30 mL in three steps) [7].

LC-UV of Butanol Extract. The residue (1.81 g) of the *n*-butanol extract was analyzed by Si-gel LC-UV eluting with $CHCl_3$/ MeOH (65:35). The structures of the two major constituents **6** and **7** were elucidated by IR, ^{1}H NMR, ^{13}C NMR, mass spectra, and comparative spectra [15, 16].

2α,3β,23-Trihydroxy-30-acetylolean-12-ene (6): $C_{32}H_{52}O_5$; amorphous powder; MS (*m/z*): 282, 234; IR (KBr, cm^{-1}): 3421, 2924, 2847, 1729, 1623, 1459, 1377, 1104, 798. ^{1}H NMR (CD_3OD, δ): 1.61 (H-1), 3.98 (H-2), 4.13 (H-4), 1.65 (H-5), 0.97 (H-6), 1.28 (H-7), 1.92 (H-9), 3.77 (H-11), 5.06 (H-12), 0.95 (H-15), 1.46 (H-16), 2.31 (H-18), 1.36 (H-19), 1.29 (H-21), 1.67 (H-22), 3.77 (H-23), 1.048 (H-24), 0.99 (H-25), 0.96 (H-26), 1.29 (H-27), 1.17 (H-28), 3.6 (H-29), 0.89 (H-30), 2.04 (CH_3CO); ^{13}C NMR (CD_3OD, δ): 171.3 (CH_3CO), 145.7 (C-13), 122.7 (C-12), 78.1 (C-3), 69.2 (C-2), 66.5 (C-23), 64.0 (C-30), 48.6 (C-9), 48.1 (C-5), 47.7 (C-1), 43.8 (C-4), 42.8 (C-14), 42.6 (C-18), 40.2 (C-8), 38.8 (C-10), 34.9 (C-19), 33.8 (C-7), 33.7 (C-20), 33.2 (C-22), 31.5 (C-17), 30.5 (C-16), 28.7 (C-11), 26.4 (C-27), 24.4 (C-15), 24.0 (C-29), 23.7 (C-28), 23.9 (C-21), 20.6 (CH_3CO), 18.9 (C-6), 17.9 (C-25), 17.5 (C-26), 13.8 (C-24).

1-*O*-[α-L-Rhamnopyranosyl-(1)-6-*O*-acetyl-β-D-galactopyranosyl]-1β,3β,22ξ-26-tetrahydroxyfurost-5(6)-ene-26-*O*-β-D-glucopyranoside (7): white amorphous powder; mp 191°C; IR (KBr, cm^{-1}): 3410, 2924, 2852, 1732, 1681, 1454, 1383, 1263, 1033, 979, 805, 771; ESI–MS *m/z*: 959 [M–H] (100). ^{1}H NMR (CD_3OD, δ, J/Hz): 3.78 (dd, J = 11.8, H-1), 2.4 (dd, J = 11.8, H-2a), 2.65 (dd, J = 11.8, H-2b), 3.8 (m, J = 11.8, H-3), 2.56 (dd, J = 11.8, H-4a), 2.68 (dd, J = 11.8, H-4b), 5.61 (br d, J = 5.5, H-6), 1.61 (m, J = 5.5, H-7, H-8, H-9, H-11a), 1.96 (m, J = 5.5, H-7b), 2.96 (m, J = 5.5, H-11b), 1.46 (m, J = 5.5, H-12a), 1.69 (m, J = 5.5, H-12b), 1.29 (m, J = 5.5, H-14), 1.52 (m, J = 5.5, H-15a), 2.07 (m, J = 5.5, H-15b), 4.95 (m, 5.5, H-16), 2.05 (m, J = 5.5, H-17), 0.9 (s, H-18), 1.42 (s, H-19), 2.24 (m, J = 5.5, H-20), 1.27 (d, J = 6.9, H-21), 2.02 (d, 6.9, H-23), 1.68 (m, H-24a), 2.02 (m, H-24b), 1.91 (m, H-25), 3.62 (dd, H-26a), 3.92 (dd, H-26b), 1.00 (d, 6.6, H-27), 4.72 (d, J = 8.1, H-1′), 4.57 (dd, H-2′), 4.15 (m, H-3′), 4.18 (m, H-4′), 3.95 (br dd, H-5′), 4.55 (dd, H-6′a), 4.91 (dd, H-6′b), 2 (s, Ac), 6.36 (br s, H-1″), 4.59 (br d, H-2″), 4.7 (dd, H-3″), 4.27 (dd, H-4″), 4.85 (dq, H-5″), 1.73 (d, J = 6.1, H-6″), 4.8 (d, J = 7.7, H-1‴), 3.94 (dd, H-2‴), 4.19 (m, H-3‴, H-4‴), 3.9 (dd, H-5‴), 4.36 (dd, H-6‴a), 4.48 (dd, H-6‴b); ^{13}C NMR: 84.8 (C-1), 38.0 (C-2), 68.3 (C-3), 43.8 (C-4), 139.6 (C-5), 124.8 (C-6), 32.2 (C-7), 33.2 (C-8), 50.7 (C-9), 42.9 (C-10), 24.2 (C-11), 40.4 (C-12), 40.7 (C-13), 57.2 (C-14), 32.8 (C-15), 81.2 (C-16), 64.0(C-17), 17.0 (C-18), 15.0 (C-19), 40.8(C-20), 16.3 (C-21), 110.7 (C-22),

37.2 (C-23), 28.4 (C-24), 34.3(C-25), 75.3 (C-26), 17.4 (C-27), 100.7 (C-1′), 74.6(C-2′), 76.4 (C-3′), 70.6 (C-4′), 73.2 (C-5′), 64.6 (C-6′), 170.5 (OAc), 20.8 (OAc), 101.7 (C-1″), 72.7 (C-2″), 72.5 (C-3″), 74.3 (C-4″), 69.3 (C-5″), 18.9 (C-6″), 104.9 (C-1‴), 75.2 (C-2‴), 75.6 (C-3‴), 71.9 (C-4‴), 78.4 (C-5‴), 62.9 (C-6‴).

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