

Phytochemical Investigation of Bioactive Extract from Endemic Medicinal Plant *Limoniastrum feei* (Girard) Batt (Plumbaginaceae)

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The phytochemical investigation of bioactive extract of aerial part of *Limoniastrum feei* led to isolation of eleven polyphenol compounds, with two major constituents obtained from the bioactive extract of stems part, identified as glucosylated flavonoids: 3-O-(glucoside-6"-malonyl, 2"-ramnosyl)-7,4',5'-trimethoxy-3-hydroxy flavonol and tetraacetate 7,8-dihydroxy-4'-methoxyisoflavone 8-O-β-glucopyranoside. The compounds were isolated for the first time from the plant extracts by column chromatography and prep-HPLC and structural elucidation was carried out on the basis of their spectral analysis.

Keywords: *Limoniastrum feei*, Plumbaginaceae, Flavones, Isoflavone, LC-UV, TLC.

INTRODUCTION

Limoniastrum feei (plumbaginaceae) is a herbaceous medicinal plant endemic to Algeria and Morocco Sahara. The aerial parts of this plant were used in Sahara folk medicine for treating gastrointestinal tract ailments, fever, icterus and various diseases. The plant is native to the southeast of Algeria (Saoura, region of Bechar), Northern Africa¹⁻³.

According to our previous results, biological activity of several extracts of aerial parts (leaf, stem and twig) of *L. feei* against fungi and bacteria was evaluated by disc diffusion method. Now we are interested in isolating the natural compounds responsible of this biological activities⁴⁻⁶.

EXPERIMENTAL

The IR spectra (ν_{\max}) were determined on a AVATAR 320 FT-IR spectrophotometer. 1D and 2D NMR spectra were obtained on a Bruker Avance DRX 300 FT spectrometer operating at 300 MHz for ¹H NMR and 75 MHz for ¹³C NMR. The LC system consisted of a liquid chromatography (Si-gel-230-400 mesh, MerK- were used for column chromatography) operating at room temperature with a flow rate of 1 mL min⁻¹ and the UNICAM UV 300 spectrophotometer detector operating at 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 & Organic Synthesis

Laboratory. The assigned specimen number was (CA99/14)^{3,6}. The leaves, stems and twigs were separated, dried and ground into powder using a grinder.

Extraction and fractionation: Dried and ground stems, twigs and leaves of *Limoniastrum feei* (350 g) were extracted with acetone-water (400 mL, 75 %). The extract was concentrated in water and partitioned with hexan, diethyle ether, ethyle acetate and *n*-butanol (50 mL in 3 steps)⁷. To purify and identify the constituents of three fractions; ethyle ether, ethyle acetate and butanol, separation by liquid chromatography coupled with UV detector (LC-UV) was developed in our laboratory^{8,9}.

RESULTS AND DISCUSSION

The protocol used for isolation of these natural substances of the plant is made in two stages: In the first one makes a solid-liquid extraction with a solvent appropriate to the ground plant organ. The second step is a liquid-liquid fractionation of the crude extract of plant organs in three solvents of different polarity¹⁰.

The yield mass fractions are related to the polarity of the solvent, it is noted that the butanol extract is the most important (0.81-1.51 %). The TLC analysis, was performed; on TLC plate of silica gel eluting by two systems of different polarity with: acetone/toluene/formic acid (6/8/1)^{4,11}. The results of this study and analytical extraction are summarized Table-1. Table-1 shows the presence of several compounds in the ether fraction and ethyl acetate, by visualizing a spot against in the butanol

fraction at $R_f = 0.85$, with absorbance at 254 nm color purple but this spot we had not seen, when eluting with E1.

TLC analysis of stem extract shows 4 components in all three fractions, 3 components in both ether fraction and ethyl acetate and were sometimes separated a compound in the butanol extract has an $R_f = 0.57$. We reverse the order of fractionation starts with the most polar solvent (Table-1).

TLC analysis also shows the presence of 4 components in all three fractions, two in butanol extract (first extract). It has happened has separated the same compound ($R_f = 0.57$) in the ether fraction and another is in the extract of ethyl acetate $R_f = 0.17$.

The analytical results indicate the presence of 5 total compounds (5 spots), including the compound at $R_f = 0.50$ appears in all three fractions. It is noted that the two fractions ether and ethyl acetate, contain almost the same constituents. Compared against a sample of stem, we note the presence of the compound at $R_f = 0.66$ in both fraction (ether and ethyl acetate) of the two parts of the plant.

The phytochemical screening of three parts of *Limoniastrum feei* on tannins and glycosylated flavonoid, show presence of flavonoid in ethyl acetate and butanol fraction in considerable quantities and total absence in ethyl ether, but the tannin derivative are present in all parts.

It is also observed that the extracts of ethyl acetate and butanol are the richest in anthocyanins by contribution to other extracts *e.g.*, aqueous and ether extracts.

Analytical results obtained previously, we opted for the purification of two fractions butanol and ethyl acetate extract of acetone-water (*L. feei*).

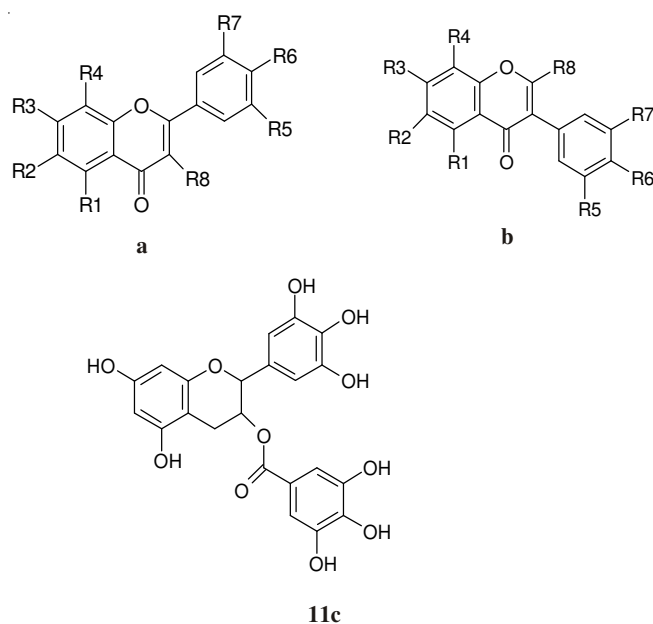
The spectroscopic analysis was performed on 11 compounds isolated from the acetone-water extract distributed in the three organs as follows: 6 from twigs, 4 from stems and 1 from leaves. The spectroscopic analysis (UV, IR and NMR) shows that six compounds isolated from twigs fraction were identified as flavones structures (**1a-6a**)^{12,13}, four products isolated from stems fraction identified as isoflavone structures (**7b-10b**)^{14,15}, and one compound isolated from leaves extract attributed to the tannin class (**11c**).

Pentamethoxy-6,7,8,3',4'-hydroxy-5 flavone (1a): UV(MeOH): maxima at 281 and 342 nm, ESI-MS: m/z [MH^+]: 389. NMR H: 12.70 (s, 1H), 7.63 (dd, 9Hz, 2Hz, 1H), 7.50 (d, 2Hz, 1H), 7.09 (d, 9Hz, 1H), 6.99 (s, 1H), 4.02 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H) NMR ¹³C: 163.50 (C2), 103.45 (C3), 182.1 (C4), 148.49 (C5), 135.70 (C6), 152.40 (C7), 132.51 (C8), 145.00 (C9), 106.10 (C10), 122.60 (C1'), 111.70 (C2'), 149.00 (C3'), 152.30 (C4'), 109.05 (C5'), 119.90 (C6'), 60.50 (6-OMe), 61.70 (7-OMe), 61.50 (8-OMe), 55.71 (3'-OMe), 55.65 (4'-OMe).

5,3',4'-Trimethoxy-7-hydroxy flavone (2a): 163.24 (C-2), 103.77 (C-3), 181.75 (C-4), 151.02 (C-5), 98.78 (C-6), 164.15 (C-7), 132.90 (C-8), 146.63 (C-9), 116.07 (C-10), 122.82 (C-1'), 109.35 (C-2'), 148.93 (C-3'), 152.05 (C-4'), 111.60 (C-5'), 119.96 (C-6'), 55.60 (3'-OMe), 55.54 (4'-OMe).

TABLE-1
EXTRACTION AND TLC ANALYSIS OF LEAVES, STEM AND TWIG FRACTIONS OF WATER-ACETONE EXTRACT (E1: ACETONE /TOLUENE/AC FORMIC 6/8/1, E2: ETHYL ACETATE/MeOH/WATER (10/1.5/1))

Leaves extract						
Fraction	Yield (%)	Colour fraction	R_f (E1)	R_f (E2)	Colour fluorescent	λ (nm) UV
Ethyl ether	0.30	Yellow	0.30	0.70	Purple	254
			0.49	0.80		
			0.57	0.90		
			0.69			
Ethyl acetate	0.62	Yellow enib	0.06	0.55	Purple	254
			0.13	0.60		
			0.20	0.80		
			0.27			
			0.46			
Butanol	0.81	Red brick	-	0.85	Purple	254
Stems extract						
Ethyl ether	0.15	Pale yellow	0.53	-	Violet	365
			0.66			
			0.77			
Ethyl acetate	0.46	Yellow	0.53	-	Violet	365
			0.66			
			0.77			
Butanol	1.51	Lemon yellow	0.57	-	Violet	365
Twigs extract						
Ethyl ether	0.37	Pale yellow	0.30	-	Violet	365
			0.50			
			0.60			
			0.66			
Ethyl acetate	0.48	Yellow	0.36	-	Purple	254
			0.50			
			0.60			
			0.66			
Butanol	0.34	Maron	0.30	-	Purple	365
			0.50			



	R1	R2	R3	R4	R5	R6	R7	R8
1a	OH	OMe	OMe	OMe	OMe	OMe	H	H
2a	OMe	H	OH	H	OMe	OMe	H	H
3a	OH	H	OH	OH	OMe	OMe	H	H
4a	OH	OH	OH	H	OMe	OMe	OMe	H
5a	OH	H	OMe	H	OH	OMe	OMe	OG1
6a	OH	H	OMe	H	OH	OMe	OMe	OH
7b	OH	OMe	OMe	OMe	OMe	OMe	H	H
8b	OH	H	OH	H	OMe	OMe	H	H
9b	H	H	OMe	G2	H	OMe	H	H
10b	H	H	OH	G3	H	OMe	H	H

G1= Glucoside-6''-malonyl, 2''-ramnosyl

5,7,8-Trihydroxy-3',4'-dimethoxy flavone (3a): 161.84 (C-2), 104.89 (C-3), 176.78 (C-4), 161.35 (C-5), 113.84 (C-6), 150.47 (C-7), 132.90 (C-8), 146.63 (C-9), 116.86 (C-10), 123.77 (C-1'), 109.59 (C-2'), 148.92 (C-3'), 151.69 (C-4'), 111.67 (C-5'), 119.90 (C-6'), 55.67 (3'-OMe), 55.61 (4'-OMe).

5,6,7-Trihydroxy-3',4'-5-trimethoxy flavone (4a): UV (MeOH): maxima at 288 and 329 nm; ¹³C NMR: 163.0 (C2), 102.8 (C3), 181.9 (C4), 147.0 (C5), 129.1 (C6), 153.3 (C7), 93.8 (C8), 149.6 (C9), 104.0 (C10), 122.70 (C1'), 111.73 (C2'), 149.00 (C3'), 152.20 (C4'), 109.0 (C5'), 119.90 (C6'), 55.70 (3'-OMe), 55.60 (4'-OMe), 55.71 (5'-OMe).

3-O-(Glucoside-6''-malonyl, 2''-ramnosyl)-7,4',5'-trimethoxy-5,3'-dihydroxy flavonol (5a): UV (MeOH): 281, 296, 334, IR (KBr, ν_{\max} , cm^{-1}): 3393 (OH), 2951, 2923, 2862 (CH_3 , CH_2), 1711 (CH_3COO), 1640 (C=O, C-4), 1601 (C=C) 1514 (C=C arom), 1028, 1121 (C-O) ¹H NMR: 6.70 (H-6), 6.84 (H-8), 7.20 (H-2'), 6.93 (H-6'), 3.117, 3.057, 3.229 (O- CH_3) 3-glycosyl: 5.55 (H-1), 3.697 (H-2), 3.57 (H-3), 3.397 (H-4), 3.292 (H-5), 3.801 (H-Ga), 3.397 (H-G-b). 2''-ramnosyl: 5.21 (H-1), 4.702 (H-2'), 3.729 (H-3) 3.397 (H-4), 4.1 (H-5), 1.03 (H-6, CH_3), 1.912, 1.988, 2.086, 2.177, 2.235, 2.027 (CH_3COO). ¹³C NMR: 162.24 (C-2), 129.9 (C-3), 174.06 (C-4), 151.67 (C-5), 129.9 (C-6), 140.1 (C-7), 99.79 (C-8), 158.44 (C-9), 105.91 (C-10), 122.53 (C-1'), 109.14 (C-2'), 140.1 (C-3'), 145.81 (C-4'), 137.81 (C-5'), 109.14 (C-6'), 47.77, 48.11,

48.44, 48.78 ($\text{CH}_3\text{-O}$), 3-glycosyl : 100.37 (C-1), 79.17 (C-2), 77.23 (C-3), 71.49 (C-4), 73.65 (C-5), 63.37 (C-6) 2''-ramnosyl: 102.62 (C-1), 72.68 (C-2), 72.35 (C-3), 75.07 (C-4), 70.43 (C-5), 18.3 (C-6), 6''-malonyl: 41.4 (CH_2), 28.99, 30.07, 34.9, 38.68, (CH_3COO).

5,3'-Dihydroxy-7,4',5'-trimethoxy flavonol (6a): UV (MeOH): maxima a 286 and 312 nm, IR (KBr, ν_{\max} , cm^{-1}): 3410, 2934, 2847, 1689, 1558, 1430, 1377, 1115, 1033, 771. ¹H NMR: 6.75 (H-6), 6.89 (H-8), 7.19 (H-2'), 6.93 (H-6'), 3.12, 3.06, 3.23 (O- CH_3), ¹³C NMR: 162.16 (C-2), 130.0 (C-3), 174.20 (C-4), 151.67 (C-5), 130.0 (C-6), 140.1 (C-7), 99.78 (C-8), 158.44 (C-9), 105.91 (C-10), 122.53 (C-1'), 109.0 (C-2'), 140.1 (C-3'), 145.81 (C-4'), 137.81 (C-5'), 109.0 (C-6'), 56.16 (7-OMe), 55.68 (5'-OMe), 55.69 (4'-OMe).

6,7,8,3',4'-Pentamethoxy 5-hydroxy isoflavone (7b): ¹H NMR: 8.03 (H-2, s), 7.11 (H-2', d, 1.51), 6.98 (H-5', d, 8.4), 6.97 (H-6', dd, 1.51, 8.4), 3.81 (4'-Me, s), 3.78 (3'-Me, s), ¹³C NMR: 154.55 (C-2), 126.0 (C-3), 181.9 (C-4), 164.51 (C-5), 103.30 (C-6) 161.62 (C-7), 99.28 (C-8), 159.40 (C-9), 108.0 (C-10), 124.91 (C-1'), 117.33 (C-2'), 147.50 (C-3'), 149.40 (C-4'), 60.58 (6-OMe), 61.79 (7-OMe), 61.49 (8-OMe), 56.43 (4'-OMe), 55.67 (3'-OMe).

5,7-Dihydroxy 3',4'-dimethoxy isoflavone (8b): ¹H NMR: 8.12 (s, H-2), 6.57 (H-6, d, 2.0), 6.73 (H-8, d, 2.0), 7.06 (H-2', d, 1.50), 6.98 (H-5', d, 8.5), 6.9è (H-6', dd, 1.5, 8.5), 3.87 (4'-Me, s), 3.81 (3'-Me, s), ¹³C NMR: 155.55 (C-2), 125.01 (C-3), 182.3 (C-4), 164.73 (C-5), 101.30 (C-6) 163.62 (C-7), 99.98 (C-8), 159.30 (C-9), 108.10 (C-10), 124.94 (C-1'), 117.31 (C-2'), 147.54 (C-3'), 149.40 (C-4'), 56.48 (4'-OMe), 55.68 (3'-OMe).

Hexacetate 8-hydroxy-4',7-dimethoxy isoflavone 8-O- $[\alpha$ -rhamnopyranosyl-(1-6)]- β -glucopyranoside (9b): $\text{C}_{41}\text{H}_{47}\text{O}_{20}$, $[\text{M} + \text{H}]^+$ a $m/z = 859$, UV (MeOH): 212, 253, 306 nm. IR (KBr, ν_{\max} , cm^{-1}): 3404, 3235, 2918, 2841, 1629, 1514, 1377, 1115, 1039. ¹H NMR: 8.25 (s, H-2), 8.00 (d, 9.0, H-5), 7.50 (d, 8.5, H-2', H-6'), 7.27 (d, 9.0, H-6), 6.99 (d, 8.5, H-3', H-5'), 5.07 (d, 7.5, H-1''), 4.56 (d, 1.5, H-1'''), 4.03 (s, OMe), 3.82 (s, OMe) 3.84-3.37 (m, glucose and rhamnose protons), 1.06 (d, 6.5, H-6'''). ¹³C NMR: 152.7 (C-2), 124.7 (C-3), 176.0 (C-4), 119.4 (C-10), 123.4 (C-5), 110.3 (C-6), 156.1 (C-7), 132.4 (C-8), 150.8 (C-9), 124.2 (C-1'), 130.5 (C-2', C-6'), 114.2 (C-3', C-5'), 159.9 (C-4'), 101.2 (C-1''), 72.1 (C-2''), 72.9 (C-3'') 69.2 (C-4''), 74.4 (C-5''), 66.5 (C-6''), 97.9 (C-1'''), 69.6 (C-2'''), 69.3 (C-3'''), 70.9 (C-4'''), 66.9 (C-5'''), 17.5 (C-6'''), 56.8 (7-OMe), 55.6 (4'-OMe), 21.0, 20.9, 20.9, 20.8 (CH_3CO). 170.5, 170.3, 170.2, 170.1, 19.9, 169.6 (COCH_3).

Tetraacetate 7,8-dihydroxy-4'-methoxyisoflavone 8-O- β -glucopyranoside (10b): UV (MeOH): 211, 254, 306, IR (KBr, ν_{\max} , cm^{-1}): 3448, 2918, 2852, 1727, 1590, 1519, 1170, 1022, 1465, 716. ¹H NMR: 7.85 (s, H-2), 8.05 (d, 9.0, H-5), 7.05 (d, 9.0, H-6), 7.47 (d, 9.0, H-2', H-6'), 6.98 (d, 9.0, H-3', H-5'), 4.99 (d, 8.1, H-1''), 5.4 (dd, 9.8, 8.1, H-2''), 5.33 (t, 9.8, H-3''), 5.2 (t, 9.8, H-4''), 3.82 (ddd, 9.8, 5.6, 2.8, H-5''), 4.3 (dd, 12.6, 5.6, H-6''), 3.84 (s, 4'-OMe), 2.20, 2.12, 2.07, 2.06 (CH_3CO). ¹³C NMR: 151.3 (C-2), 125.1 (C-3), 175.6 (C-4), 118.7 (C-10), 124.3 (C-5), 115.3 (C-6), 154.4 (C-7), 131.1 (C-8), 150.2 (C-9), 123.5 (C-1'), 130.2 (C-2', C-6'), 114.1 (C-3', C-5'), 159.9 (C-4'), 103.4 (C-1''), 71.0 (C-2''), 72.1 (C-3'') 67.9 (C-4''), 72.7 (C-5''), 61.3 (C-6''), 55.4 (4'-OMe), 20.6, 20.7 (CH_3CO), 17.6, 170.1, 169.3, 169.2 (COCH_3).

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