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Glucoside flavonoids from the antimicriobial extract of the saharan medicinal plant *Limoniastrum feei*

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ABSTRACT

Phytochemical investiguation of the antimicrobial extract from the endemic saharan medicinal plant *Limoniastrum feei* (Plumbaginaceae) led to the isolation of two major constituents by liquid chromatography methods, the compounds were identified using spectroscopic analysis as flavonoid glycosides: *6-diméthyl 2,5 hexyl-7-O-glucopyranoside (1-6)glucopyranoside-5,7,3',4'hydroxyl flavone* **(1)** and *3-O-glucopyranoside 6-ester 3-méthyle hexanone 1 diene 2,4 -7-O-glucopyranosyle (1-6)(2-6)glucopyranoside trihydroxyl 5,3',4'-flavone* **(2)**.

KEYWORDS

Limoniastrum feei;
Flavonoid;
Antimicrobial;
Endemic;
Sahara.

INTRODUCTION

Natural products have served as an important source of drugs since ancient times and about half of the useful drugs today have been derived from plants. So, the diversity of structures obtained and the different therapeutic activities shown that the isolation, identification, synthesis and biosynthesis of new natural compounds continue to be a field of enormous interest. Only a small part of the 400.000 vegetable species known were investigated from phytochemical and pharmacological aspects, and that each species can contain up to several thousands of different components[1-5]

Limoniastrum feei (Plumbaginaceae) is a herbaceous medicinal plant endemic to Algeria and Morocco sahara. The aerial part of this plant was used in Sahara folk medicine for treating gastrointestinal tracts, fever, icter and various diseases[6,7].

According to our prvious results on biological screening of several extracts from the aerial parts (leave, stem and twig) of *Limoniastrum feei* (Plumbaginaceae) against fungi and bacteria realised by disc diffusion method[6-8], we interested to isolate the natural products responsible of this antimicrobial activities.

MATERIALS AND METHODS

General experimental procedure

UV spectra were obtained in MeOH solvent with UNICAM UV300 spectrophotometer. IR spectra were obtained with AVATAR 320 FT-IR spectrophotometer. The NMR spectra were taken on a Bruker

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GP 250 (^{1}H, 300 MHZ; ^{13}C, 75 MHz) Spectrometer. TLC was carried out on silica gel 60 F_{254} plates (Merck, Germany). Column chromatography was performed over silica gel 60 (Merck, particle size 230-400 mesh).

Plant materials

The whole plants of *Limoniastrum feei* were collected in March 2010 from Saoura region (Southwest of Algeria). Voucher specimen is conserved at the herbarium of Phytochemistry and Organic Synthesis Laboratory (POSL) under to accession number CA99/14 [9, 10]. The aerial parts were dried and grounded into powder using a grinder.

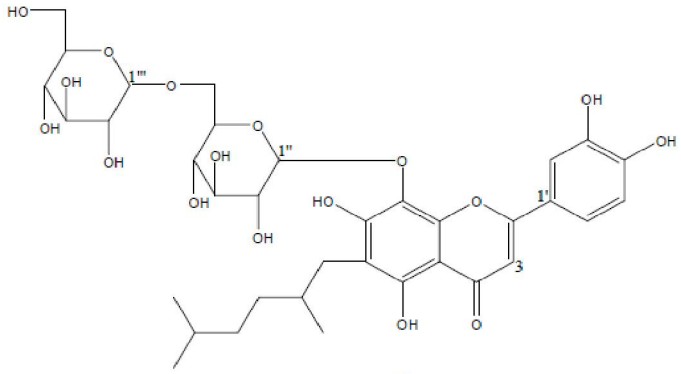
Extraction and isolation

The dried powder of the aerial parts of *Limoniastrum feei* (100 g) were extracted for 4 hours with 80% MeOH (400 mL) using soxhlet apparatus. The extract was evaporated in vacuo apparatus, and the brown obtained residue was diluted with 100 mL of distilled water. This aquous residue was partitioned sequentially with Ethyl ether, Etyle acetate and n-Butanol[11-13].

The residue (1.4 g) of the Ethyl acetate fraction was analyzed by vaccum liquid column chromatography using four solvents of polarity growing respectively (Hexane, AcOEt, Acetone, MeOH), followed by second column chromatography (20mm / 300mm) full with a silica gel stationary phase and eluting with mobile phase Acetone / Toluene/ Formic Acid (60:80:10)[14]. The structures of the two major constituents **(1)** and **(2)** were elucidated by IR, UV, ^{1}H and ^{13}C-NMR analysis[15, 16].

RESULTS AND DISCUSSION

Phytochemical investiguation of the antimicrobial extract from this endemic saharan medecinal specie led to isolation for the first time of two major constituents by liquid chromatography method, the compounds were identified on the basis of their spectral analysis as glucosylated flavonoids: *6-diméthyl 2,5 hexyl-7-O-glucopyranoside (1-6)glucopyranoside-5,7,3',4'hydroxyl flavone* **(1)** and *3-O-glucopyranoside 6-ester 3-méthyle hexanone 1 diene 2,4 -7-O-glucopyranosyle (1-6)(2-6)glucopyranoside trihydroxyl 5,3',4'-flavone* **(2)**.



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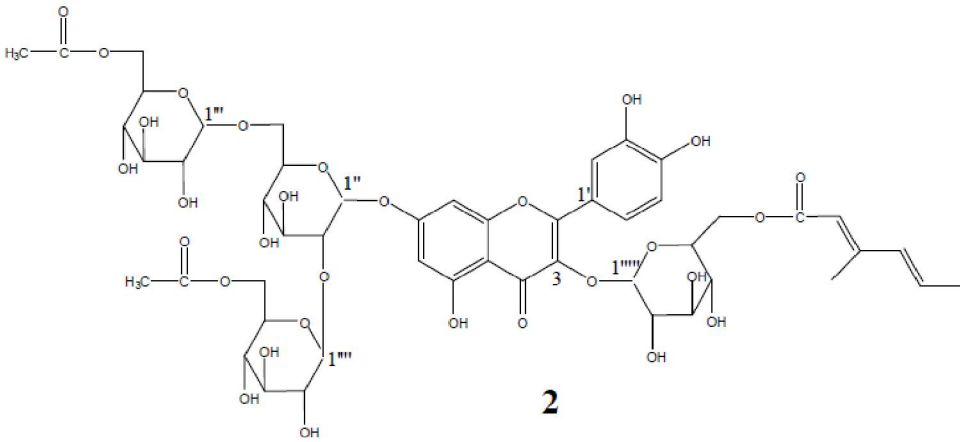

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In UV spectra analysis of compound **(1)**, the addition of sodium acetate reagent lead to a bathochrom displacement of band I (+400 nm) in UV spectrum, what shows the presence of hydroxyl group in position 7[16], the apparition of a second maximum in the band I toward 400 nm observed with the $AlCl_3$ reagent, is revealing of bathochrom displacement induced by hydroxyl groups in position 4 and 5[15]. In relation to this spectrum, the addition of the $AlCl_3$ solution in acid medium shows other bathochrom displacement of the band I (+ 400 nm), indicates the presence of two ortho-hydroxyl groups in the B part of the genine flavone[17]. The addition of MeONa confirms the presence of a group hydroxyl in position 5 by a displacement toward 400 nm (TABLE 1).

TABLE 1 : Results of UV spectra displacement with chemical reagents of compounds (1) and (2)

Chemical reagent	Compound 1		Compound 2	
	Bande I (nm)	Bande II (nm)	Bande I (nm)	Bande II (nm)
MeOH	325	263, 270, 285	304 - 350	262 - 270
MeOH +$AlCl_3$	+ 400	270	+ 400	270
MeOH+ $AlCl_3$/ H^+	+ 400	270	+ 400	262 - 270
MeOH + MeONa	+ 400	270	+ 400	262 - 270
MeOH + AcONa	+ 400	270	350	262 - 270

The NMR analysis of the compound presents a singular signal at 6.72ppm, assigned to the proton H-3, two other more unarmored under shape two doublet, with a $J^3$and J^4 coupling assigned to the aromatic protons H-2' and H-6', the proton H-5' rezoned in a doublet toward 7,12 ppm, the alkyle substituent in position 6 has an asymmetric center in carbon C-10 that returns the proton of methylene H-9 and the protons of methylene H-11 to diastereoisotopic protons, that are not chemically equivalent[18]. The proton of glucose appears of the resonance signals toward 3 to 5ppm. According to the spectroscopic data[19-21] compared with our experiment spectra, the compound **(1)** are identified as: *6-diméthyle 2,5 hexyl-7-O-glucopyranoside (1-6) glucopyranoside-5,7,3',4'hydroxyl flavone*.

The ^{1}H NMR results of compound **(2)** showed two signals, under shape two doublet with J^4 coupling assigned to protons H-6 and H-8, but the aromatic protons in position H-2', H-5' and H-6' appears toward unarmored zone respectively in chemical shifts: 7.86, 7.40,7.59 ppm. Following to the results spectroscopic analysis, the compound **(2)** identifed as: *3-O-glucopyranoside 6-ester 3-méthyle hexanone 1 diene 2,4 -7-O-glucopyranosyle (1-6)(2-6)glucopyranoside trihydroxyl 5,3',4'-flavone* **(2)**.

Compound (1)

6-diméthyle 2,5 hexyl-7-O-glucopyranoside (1-6)glucopyranoside-5,7,3',4'hydroxyl flavone : R_f = 0.58 (acetone/ toluene /formic acid, 6:8:1 with blue fluorescence at 365 nm).

UVmax spectra (MeOH): λ = 263, 270, 285, 325 nm.

IR (KBr): ν_{max} 3541, 3475, 3415, 3229(OH), 2956, 2923, 2852 (C-H), 1735 (C=O ester),1640 (C=O α, β insatured), 1510, 1607(C=C), 1093, 1022,1263, 1159 (C-O).

^{1}H NMR: 6.72 (s, H-3), 2.29 (dd, H-9^a, H-9^b), 1.31 (m, H-10), 1.23 (dt, H-11^a, H-11^b), 1.20 (m, H-12, 2H), 1.17 (m, H-13), 0.92 (d, H-14 et H-16, 6H), 2.16 (d, H-15, 3H), 7.35 (dd, H-2'), 7.12(d, H-5'), 7.92(dd, H-6'), 5.10 (1H, H-1''), 3.52 (1H, H-2''), 3.58(1H, H-3''), 3.47 (1H, H-4''), 3.42(1H, H-5''), 3.64(1H, H-6''), 3.91(1H, H-1'''), 3.50 (1H, H-2'''), 3.55 (1H, H-3'''), 3.45 (1H, H-4'''), 3.25(1H, H-5'''), 3.29(1H, H-6''').

^{13}C NMR: 167.21 (C-2), 104.05 (C-3), 178.26 (C-4), 147.56 (C-5), 117.34 (C-6), 164.72 (C-7), 128.26 (C-8), 150.53 (C-8a), 105.41 (C-4a), 31.63(C-9), 36.92(C-10), 38.75(C-11), 38.50(C-12), 34.20(C-13), 29.00(C-14), 29.27(C-15), 29.18(C-16), 127.75 (C-1'), 116.85 (C-2'), 143.96 (C-3'), 147.43 (C-4'), 122.38 (C-5'), 128.11 (C-6'), 105.76 (C-1''), 76.23 (C-2''), 77.60 (C-3''), 71.21 (C-4''), 78.00 (C-5''), 69.38 (C-6''), 101.60 (C-1'''), 72.30 (C-2'''), 73.26 (C-3'''), 73.12 (C-4'''), 74.36 (C-5'''), 63.42 (C-6''').

Compound (2)

3-O-glucopyranoside 6-ester 3-méthyle hexanone 1 diene 2,4 -7-O-glucopyranosyle (1-6)(2-6)glucopyranoside trihydroxyl 5,3',4'-flavone: R_f = 0.56 (acetone/ toluene /formic acid, 6:8:1 with blue fluorescence at 365 nm).

UVmax spectra (MeOH) : λ = 262, 270, 350 nm.

IR (KBr): ν_{max} 3546, 3415, 3333, 3224(OH),

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2972, 2923, 2847 (C-H), 1722 (C=O ester), 1656, 1634 (C=O α, β insatured), 1607(C=C), 1608(C=C aromatic), 1022 (C-O).

^{1}H NMR: 0.95 (3H, CH_3 Glu 2), 1.29 (3H, CH_3 Glu 3), 1.70 (3H,H-12''''), 1.96 (3H, H-13''''), 3.72 (22H, 4Glu), 3.92 (1H, Hb- 6''), 4.10 (1H, Hb-6''''), 4.50 (1H, H-1'''), 4.68 (1H, H-1''''), 5.19 (1H, H-1"), 5.46 (1H, H-1'''''), 6.00 (1H, H -8''''), 6.25 (1H, H-10''''), 6.50 (1H, H -6), 6.76 (1H, H -8), 6.97 (1H, H-11''''), 7.40 (1H, H-5'), 7.86 (1H, H-2'), 7.59 (1H, H-6').

^{13}C NMR: 166.31 (C-2), 103.20 (C-3), 176.54 (C-4),146.17 (C-5), 98.63 (C-6), 164.81(C-7), 92.74 (C-8), 151.20 (C-8a), 106.82 (C-4a), 121.40 (C-1'), 119.60 (C-2'), 145.22 (C-3'), 149.16 (C-4'), 118.42 (C-6'), 103 (C-1"), 92 (C- 8), 82.33 (C – 2''), 77.86 (C – 3''), 71.53 (C – 4''), 78.04 (C – 5''), 62.86 (C – 6''), 71.97 (C – 2'''), 72.85 (C – 3'''), 70.41 (C – 4'''), 73.18 (C – 5'''), 61.29 (C – 6'''), 71.76 (C – 2''''), 72.22 (C – 3''''), 69.87 (C – 4''''), 72.68 (C – 5''''), 60.73 (C – 6''''), 72.95 (C – 2'''''), 73.22 (C – 3'''''), 71.01 (C – 4'''''), 74.24 (C – 5'''''), 61.94 (C – 6'''''), 167.1 (C-7''''), 138.35 (C-8''''), 127.63 (C-9''''), 137.78 (C-10''''), 128.36 (C-11''''), 26.15 (C-12''''), 23.51 (C-13'''').

CONCLUSION

The antimicrobial extract of the aerial parts from the endemic Saharan medecinal plant *Limonastrium feei* was selected for phytochemical investigations. The chromatographic analysis of the Ethyl acetate fraction of the methanolic extract led to the isolation for the first time of two major constituents identified as flavonoid glycosides.

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