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Research Article

FLAVONOID FROM METHANOLIC EXTRACT OF *LIMONIASTRUM FEEI* (GIRARD) BATT (*PLUMBAGINACEAE*)

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ABSTRACT

According to the biological screening results of several extracts from three parts (leaves, stems and twigs) of *Limoniastrum feei* (*plumbagenaceae*) against fungi (*Candida albican*, sacharomyce), and bacteria (*Escherechia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebseilla entercoccus*) realized by disc diffusion method, we interested to isolate the natural products responsible of these activities. Our investigation led to the isolation of three phytochemical constituents by the liquid chromatography method, the compounds were identified by spectroscopic analysis as flavonoids. The isolated compounds were identified as Quercetin 1, kaempferol-3-o-β-d-glucopyranoside (astragalin) 2 and quercetin-7-o-β-d-glucopyranoside 3. The presence of different types of bioactive constituents in the *L. feei* extract may explain it wide use by the local population.

Keywords: *Limoniastrum feei*, Bioactive extract, Phytochemical screening, Flavonoid.

INTRODUCTION

The search for drugs derived from plants has accelerated in recent years. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are raking the earth for new phytoconstituents, which could be used for the treatment of infectious diseases. It is interesting to note that local people believe that the medical effects of wild plants are better than those of cultivated species [1].

South Algeria with its rich floral resources and ethnobotanical history is an ideal place to screen plants for biological activity and as a source of new pharmacological compounds [2].

Plumbaginaceae is a cosmopolitan family, which includes 836 species grouped in 27 genera, including *Limoniastrum feei* (Plombagenaceae) which is a plant traditionally used to treat gastric disorders, prepared by decoction in water, and taking a cup of tea per day. The phytochemical screening of the active extracts has noted the presence of flavonoids, saponins and tannins in the methanolic, aqueous and heptane extracts of the three parts of the plant. For that we have realized a correlation essay between the existing substances in plant extracts and both antibacterial and anti-fungal activity, which permit us to conclude that: The bioactive extracts contain flavonoïds, saponins and tannins [1].

The purpose of this study was to isolate the components of the methanol extract of the leaves and stems of *L. feei*, we concluded that the crude extract has good antimicrobial activity [3-5].

METHODS

General experimental procedure

Ultraviolet (UV) spectra were obtained in MeOH solvent with Unicam UV300 spectrophotometer. Infrared (IR) spectra were obtained with an AVATAR 320 Fourier transform-IR spectrophotometer. The nuclear magnetic resonance (NMR) spectra were taken on a Bruker GP 250 (1H, 300 MHZ; 13C, 75 MHz) spectrometer. Thin layer chromatography was carried out on silica gel 60 F254 plates (Merck, Germany). Column chromatography was performed over silica gel 60 (Merck, particle size 230-400 mesh).

Plant materials

The whole plant was collected in March 2012 from Kenadsa (region of Bechar) Algeria, and identified by the National Agency of Nature Protection (ANN), Bechar, Algeria. The leaves, stems and twigs were separated and dried. Stems and leaves were ground into powder form using the grinder [1,6,7].

Extraction and isolation

The dried leaves (100 g) of *L. feei* were extracted with 80% MeOH (400 ml) using a soxhlet apparatus; reflux for 4 hrs was performed. The residue was evaporated in a vacuum apparatus, according to the procedures of chemical screening, to determine the presence of natural products in the bioactive extract [8]. That residue was then dissolved in 100 ml of distilled water and gave a brown aqueous solution. This aqueous residue was then partitioned sequentially with ethyl ether, ethyl acetate and n-butanol [9-11]. Both compounds were identified, and they are getting in the ethyl acetate fraction. To purify and identify the components of the ethyl acetate fraction (1.4 g), separations were achieved by liquid vacuum chromatography using four solvents of decreasing polarities (hexane, ethyl acetate, acetone, MeOH) and second column chromatography, using a column type class: 20 mm/300 mm (29/39) complete with a stationary phase of silica gel (0.20 mm) and the mobile phase chosen for this separation was: Acetone/toluene/formic acid. (60:80:10) [12,13].

RESULTS AND DISCUSSION

After several separations and chromatographic analysis, the results were regrouped (Table 1).

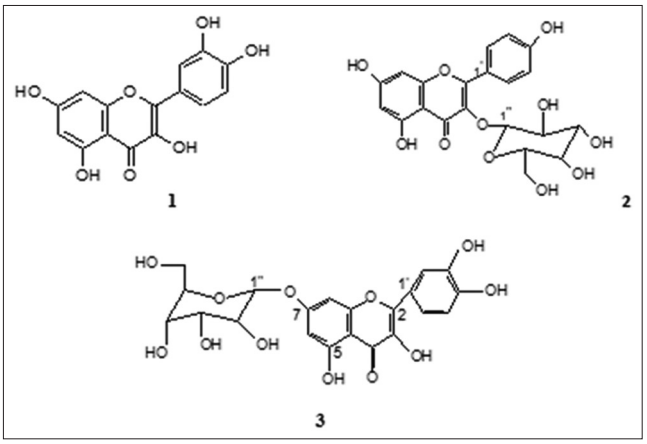

Table 1: Chromatographic features of phenolic fractions of leaves

Physical sample	Aspect weight (g)	Column chromatography			
		Compounds	Weight (g)	Yield (%)	Rf
Orange solid	1.4	Fr-1	0.19	10.88	0.71
		Fr-2	0.59	39.33	0.61
		Fr-3	0.35	25.11	0.49

Quercetin 1: Tf=189°C, Rf=0.71 (acetone/toluene/formic acid, 6:8:1 with blue fluorescence at 365 nm), MW=302.23, $C_{15}H_{10}O_7$, 59.60% (C), 3.31% (H), 37.08% (O), UVmax spectra: λ=262, 270, et 350 nm, IR (KBr) ν_{max}: 3546, 3415, 3333, 3224 (OH), 3102 (CH), 1656, 1634 (C=O α, β unsaturated), 1607, 1508 (C=C), 1153, 1022 (C-O-C). ^{1}H NMR ($CDCl_3$): 6.20 (1H, d, J=2.1 Hz, H-6), 6.40 (1H, d, J=2.1 Hz, H-8), 7.66 (1H, d, J=2Hz, H-2'), 6.85 (1H, d, J=8.4Hz, H-5'), 7.50 (dd, J=8.4, 2Hz, H-6').

^{13}C NRM: 154.5 (C-2), 128.11 (C-3), 187.48 (C-4), 162.16 (C-5), 97.6 (C-6), 167.21 (C-7), 97 (C-8), 154.33 (C-9), 109 (C-10), 131.34 (C-1'), 110.47 (C-2'), 142.18 (C-3'), 134.15 (C-4'), 118 (C-5'), 129.13 (C-6'). ESI-MS/MS m/z: 302 137 149 153 165.

Kaempferol-3-O-β-D-glucopyranoside (astragalin) 2: Tf=214°C, Rf=0.61, MW=448.21, $C_{21}H_{20}O_{11}$, 54.40% (C), 4.31% (H), 41.50% (O), UV max (MeOH): 246, 270, 360. IR (KBr): 3 415, 3 229, 2 956, 2 852, 1 640, 1 454, 1 377, 1 263, 1 093/cm.

^{1}H NMR: 6.31 (H-6), 6.50 (H-8), 7.34 (H-2', H-6', m), 6.98 (H-3', H-5', d), 5.27 (H-1''), 3.39 (H-2''), 3.21 (H-3''), 3.16 (H-4, m), 4.06 (H-5'', m), 3.75 (H-6''a, m), 4.34 (H-6''b).

^{13}CNMR: 159.78 (C-2), 151.36 (C-3), 180.46 (C-4), 163.89 (C-5), 99.65(C 6), 166.50 (C-7), 95.51 (C-8), 158.27 (C-9), 110.15 (C-10),123.11 (C-1'), 135.25 (C-2', C-6'), 122.70 (C-3', C-5'), 147.37 (C-4'), 106.12 (C-1''), 75.14 (C-2''), 78.10 (C-3''), 73.52 (C-4''),79.02 (C-5''), 62.89 (C-6'').

Quercetin-7-O-β-D-glucopyranoside 3: Tf=271°C, Rf=0.49, MW=463.16, $C_{21}H_{20}O_{12}$: 463.20, 56.25% (C), 4.46% (H), 39.28% (O), UV max (MeOH): 250, 276, 360. IR (KBr): 3 315, 2 913, 1 656, 1 508, 1 483, 1 308, 1 153, 1 022/cm

^{1}H NMR: 5.42 (H-2, dd, J=12.2; 2.9Hz), 6.41 (H-3), 6.19 (H-6), 6.58 (H 8), 7.26 (H-2',m), 6.94 (H-5', d, J=8.4 Hz), 7.35 (H-6', d, J=8.4 Hz), 5.37 (H 1''), 3.27 (H-2''), 3.35 (H-3'', m), 3.20 (H-4), 3.73 (H-5'', m), 3.45 (H-6''a), 4.98 (H-6''b, m).

^{13}CNMR: 157.88 (C-2), 106.36 (C-3), 171.46 (C-4), 156.19 (C-5), 99.35 (C-6), 169.10 (C-7), 96.50 (C-8), 153.17 (C-9), 107.55 (C-10), 129.10 (C-1'), 110.45 (C-2'), 132.70 (C-3'), 152.17 (C-4'), 115.32 (C-5'), 121.30 (C-6'), 104.52 (C-1''), 74.44 (C-2''), 75.10 (C-3''), 73.82 (C-4''), 76.32 (C 5''), 63.60 (C-6'').

The ^{1}H NMR data of compound 1 show two signals in the form of two doublets with coupling J4 assigned to protons H-6 and H-8, and the aromatic protons at positions H-2 ', H-5' and H-6' which appear to the area without armor, with the chemical shifts: 7.66, 6.85, 7.50 ppm, respectively. After analyzing the results of the spectroscopic analysis (UV, IR and NMR), the compound 1 was identified as: Quercetin.

The UV spectrum of astragalin 2 showed characteristic flavonol absorptions at 270 and 360 nm. UV analysis using the shift reagent and $AlCl_3$ indicates the 5-hydroxy position is free. The absence of a band, I shift with NaOAc suggests that the hydroxyl group at C-7 is substituted. A bathochromic shift of the band I with sodium methoxide suggested that there was free 4'-hydroxyl group on the ring B [14].

In the infrared spectra, the bands matched to (3229-3415/cm) corresponding to the stretching vibration of the OH, vibration frequencies located at (1640/cm) corresponding to the valence vibrations of C=O, α, β unsaturated.

The ^{1}H NMR spectrum of compound 2 showed two meta-coupled protons J^4 at δ_H 6.31 and 6.50 which were assigned to H-6 and H-8, respectively.

The B ring moiety was oxygenated only at C-4' because of the two doublets at 6.98 and 7.34 assigned to H-3', H-5' and H-2', H-6', respectively.

In UV spectra analysis of compound 3, the addition of $AlCl_3$ reagent leads a bathochrom displacement of band I (+400 nm) in UV spectrum, what shows the presence of a hydroxyl group in position 5 (P. Waridel 2003), in relation to this spectrum, the addition of $AlCl_3$ solution in acid medium shows other bathochrom displacement of the band I (+400 nm), which indicates the presence of two groups ortho-hydroxyls in the B part of the genine flavonol [15]. Its IR spectrum showed a broad band at 3415/cm typical of a hydroxyl group chelated to a conjugated carbonyl group at 1656/cm.

The ^{1}H NMR spectrum of the compound 3 presents two other more unarmored signals, as two doublet, with a J^3 and J^4 coupling assigned to the aromatic protons H-2' at δ_H 7.26 ppm and H-6' at δ_H 7.34 ppm, the proton H-5' rezoned in a doublet toward 6.94 ppm, and two signals appears toward 6.19 and 6.58, corresponding to protons H-6 and H-8, respectively.

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