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Phytochemical and Antioxidant Activity of *Limonium Pruinosum* (L.).

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Abstract

From the ethyl acetate extract of *Limonium pruinosum* (L.) we have identified two methylated flavonols: 3',7dimethoxyquercetin (**1)** and 3'methoxyquercetin **(2)** isolated for the first time from *Limonium pruinosum* **;** Ethyl acetate extract show a high antioxidant activity against DPPH radicals better than ascorbic acid with an IC_{50}value of 7,77 µg/ml. The pure compounds showed an important free radical-scavenging activity towards the DPPH radical.

Keywords: Flavonoids ; *Limonium prunosium*; Antioxidant activity; total phenolics; DPPH free radical assay.

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INTRODUCTION

Plumbaginaceae is a small family of 22 genera and 440 species, distributed arround the world, mostly in dry areas with salin soils, especially in the Mediterranean and Western Asia [1-2]. The genus *Limonium*, formerly known as Statice, is a member of the Plumbaginaceae family and involves 300 wild species. These species are widely distributed in coastal regions, and plains throughout the world in both tropical and temperate zones such as Middle East, latin America and Africa [3]. In algeria, this genus is represented by 23 species [4]. In search of new molecules from genus limonium, we have isolated the Flavonoids, the most extensively investigated chemical property of these compounds is their antioxidant activity. Antioxidants are capable of neutralizing free radicals which are present in food as well as in cells of a human body [5-6]. The antioxidant properties of flavonoids compounds are linked to their ability to transfer hydrogen or an electron, additionally antioxidant activity is often accompanied by antiviral and antibacterial activity of these compounds [7]. In continuation of our previous chemical investigation of our search concerning flavonoid compounds in *Limonium pruinosum* species [8], The present study deals with two known flavonoids isolated for the first time from *Limonium pruinosum* in addition to antioxidant activities.

MATERIALS AND METHODS

Plant material

Limonium pruinosum was collected from Batna, east of Algeria in March 2010. A voucher specimen has been deposited in the Herbarium of the VARENBIOMOL research unit, University of Constantine 1.

Extraction and isolation

Air-dried flowers (2600 g) of *L. pruinosum* were macerated at room temperature with MeOH/H_2O (70:30 v/v) followed by filtration concentration and successive liquid-liquid extractions using chloroform, ethyl acetate and *n*-butanol to give chloroform (0.8 g), EtOAc (5.67 g) and *n*-butanol (30 g) extracts. The EtOAc extract (4g) was chromatographed on a silica gel (230-400 mesh) column using chloroform containing increasing amounts of acetone (0-100 %), to yield 40 fractions (1- 40) obtained by combining the eluates on the basis of TLC analysis.Fraction 06 (30mg) eluted with chloroform-acetone (98:2) gave a yellow precipitate which was washed with chloroform to give compound**1** (15mg).Fraction 23 (35 mg) eluted with chloroform-acetone (95:5) was submitted to preparative TLC ($CHCl_3$/CH_3COCH_3 5:1) gave compound **2** (16mg).

Determination of total phenolics content

The total phenolics content of ethyl acetate extracts of *L. pruinosum* was determined using Folin-Ciocalteu reagent according to the method of Singleton[9]. The reaction mixture was composed by mixing 20µl of sample solution, 1580 µL of distilled water, 100 µL of the Folin–Ciocalteu reagent. The mixture was shaken. After 3 to 8 min 3000 µl of 20 % sodium carbonate was added and the mixture was allowed to stand for 30 min at 37 C° in bath water. The absorption at 765 nm was measured against a blank, which contained 20µl of methanol in place of sample. The total phenolics contents were expressed as micrograms gallic acid equivalents (µg GAE) per gram of methanol extract. All samples were analyzed in three replicates.

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Determination of total flavonoids content

Total flavonoids content was determined using the method of Ordonez [10]. A volume of 0.5 ml of 2% $AlCl_3$ of methanolic solution was added to 0.5ml at sample solution. After one hour at room temperature, the absorbance was measured at 420nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/ml were calculated as quercetin (µg /mg) using the calibration curve. Results were expressed as µg quercetin equivalents (QE)/ mg extract. Measurements were performed at least in triplicate.

DPPH-free radical scavenging activity

The hydrogen atoms or electron-donation ability of the corresponding extract (ethyl acetate fractions from *L. pruinosum* and isolated flavonoids was examined in the presence of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical using the method described by Braca et al.[11]. 3 ml of methanol DPPH solution (0.004%) was added to various concentrations (1, 2.5, 5, 10, 15, 20, 30, 50, 75 µg/ml) of the test samples in methanol. The mixture was shaken vigorously and left standing at room temperature for 30 min. Absorbance of the solutions was measured at 517 nm. The Tests were carried out in triplicate. Inhibition of free radical DPPH in percent (I %) was calculated using the following equation:

$$I\% = (A\ control - A\ sample/A\ control) \times 100.$$

Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration. Ascorbic acid was used as the control.

RESULTS AND DISCUSSION

Fractionation and purification of ethyl acetate extract by combination of chromatographic techniques, led to the isolation of two known flavonols isolated for the first time from *L. pruinosum* : 3',7dimethoxyquercetin (**1)** and3'methoxyquercetin **(2).**

Compound 1

3',7dimethoxyquercetin; yellow powder ; HRESI-MS(-): m/z 329 (calcd. 329.0286 for $C_{17}H_{13}O_6$), which led to $C_{17}H_{14}O_7$ as molecular formula for this compound. UV,(MeOH) λ_{max} nm: 255, 371; +NaOH: 277,425 ; +$AlCl_3$: 264,430 ; +$AlCl_3$ /HCl:264,429 ; +NaOAc: 271, 389 ; +NaOAc/H_3BO_3 : 255, 374, ^{1}H- NMR (500 MHz, DMSO) □ (ppm) 12.46 (1H, s, OH-5), 7.78 (1H, d, *J* = 2.05 Hz, H-2'), 7.74 (1H, dd, *J* = 8.47,2.05 Hz, H-6'), 6.94 (1H, d, *J* = 8.47 Hz,H-5'), 6.79 (1H, d, *J* = 2.2 Hz, H-8), 6.36 (1H, d, *J* = 2.2 Hz, H-6), 3.87 (3H, s, 3'-OCH_3), 3.86 (3H, s, 7-OCH_3); 13C-NMR (500 MHz, DMSO): δ (ppm)

175.97 (C, C-4),164.90 (C, C-7), 160.30 (C, C-5), 156.06 (C, C-9), 148,93(C, C-4'),147.38 (C, C-3'), 147.03 (C, C-2), 136.10 (C, C-3), 121.86 (CH, C-6'), 115.51 (CH, C-5'), 111.56 (CH, C-2'), 103.99 (C, C-10), 97.48 (CH, C-8), 92.07 (CH, C-6), 55.81(CH3, OCH_3, C-3'), 55.03(CH3, OCH3, C-7)[12].

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Compound 2

3'methoxyquercetin; yellow powder ; HRESI-MS (-): m/z 315 (calcd. 315.0510 for $C_{16}H_{11}O_7$), which led to $C_{16}H_{12}O_7$ as molecular formula for this compound.

UV, (MeOH) λ_{max} nm: 255, 371; +NaOH: 277,321,422 ; +$AlCl_3$: 264,429 ; +$AlCl_3$ /HCl: 264,428; +NaOAc: 275, 389 ; +NaOAc/H_3BO_3 : 255, 375.1H-NMR (500 MHz, DMSO) δ (ppm), 7.75 (1H, d, *J* = 2.5 Hz, H-2'), 7.69 (1H, dd, *J* = 10.5,2.5 Hz, H-6'), 6.94 (1H, d, *J* = 10.5 Hz, H-5'), 6.47 (1H, d, *J* = 2.5 Hz, H-8), 6.19 (1H, d, *J* = 2.5 Hz, H-6), 3.84 (3H, s, 3'-OCH_3). 13C -NMR (500 MHz, DMSO): δ (ppm) 176.15 (C, C-4), 164.2 (C, C-7), 161.03 (C, C-5), 156.45 (C, C-9), 148,01(C, C-4'), 147.30 (C, C-3'), 147.11 (C, C-2), 136.05 (C, C-3), 122.26 (C, C-1'), 120.32 (CH, C-6'), 115.90 (CH, C-5'), 115.30 (CH, C-2'), 103.30 (C, C-10), 98.51 (CH, C-6), 93.69 (CH, C-8), 55.0 (CH3,OCH_3, C-3'), 122.26 (C, C-1'), 120.32 (CH, C-6'), 115.90 (CH, C-5'), 115.30 (CH, C-2'), 103.30 (C, C-10), 98.51 (CH, C-6), 93.69 (CH, C-8), 55 (CH_3,OCH_3,C-7)[13].

Compounds **1**and **2** are isolated for the first time from *Limonium pruinosum*. The identity of compounds was confirmed on the basis of UV, HRESI-MS ,^{1} H,^{13}C NMR spectra in comparison with literature data [12-14].

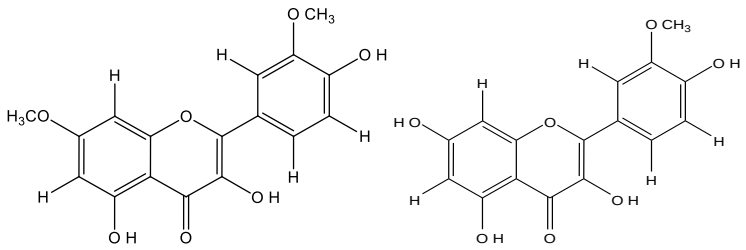

Figure 1: structures of compound1 compound2

Antioxidant activity

Total phenol content is expressed in microgram of gallic acid per milligram of freeze dried sample. The total phenolic content of *L. pruinosum* of ethyl acetate extract was estimated to be 260±0,003 µg gallic acid equivalent/mg however it is not surprising that the flavonoids content was high also 324.70± 0,002 µg Quercertin equivalent/mg extract. This high potential of phenolic and flavonoids explain the strong scavenging activity of ethyl acetate extract of *L. pruinosum* with an IC_{50}value of 7.77µg/ml better than ascorbic acid (vitamin C) IC_{50}=5µg/ml. Compounds1 and 2 were assayed also for DPPH radical scavenging. This study illustrated a significant decrease of DPPH radical due to the scavenging ability of tested samples which correlates with a dose effect dependent .the potency of DPPH free radical-scavenging activity of the tested flavonoids was in the order of vitamin C>3'methoxyquercetin(**2**) > 3',7dimethoxyquercetin (**1**). The difference between compounds **2** and **1** was that the last one had the methoxyl substituent linked at C-7, this suggested that the methylation of 7-hydroxy function reduced scavenging abilities of flavonols [15] (Table1.)

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Table1: IC $_{50}$Values of the two pure compounds

Compounds	IC $_{50}$± SD(µg/ml)
2	8.55±0.075
1	16.59±0.3

CONCLUSION

In conclusion this study allowed the isolation and the structural elucidation of two flavonoids from *L. pruinosum* for the first time, the result of the biological part of this work clearly indicated that *L. pruinosum* has powerful antioxidant capacity against the DPPH radical in vitro.

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