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Chemical composition of *Limonium thouinii* (viv.) kuntze (Plumbaginaceae) and the DPPH free radical scavenging activity

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The present work considers the phytochemical investigation and DPPH free radical-scavenging activity of the aerial parts of *Limonium thouinii* (Viv.) Kuntze (Plumbaginaceae). The aerial parts of Limonium thouinii (Viv.) Kuntze allow the isolation of four flavonoids: Quercetin, Vitexin, Isoorientin and Cannabiscitrin. Their structures were elucidated on the basis of spectroscopic analysis, including UV, MS and NMR techniques. The DPPH free radical-scavenging activity was evaluated on crude extracts (MeOH, EtOAc and n-BuOH extracts).

Keywords: Plumbaginaceae; *Limonium thouinii*; Flavonoids; Free DPPH radical scavenging activity.

INTRODUCTION

The genus *Limonium* Miller belongs to the Plumbaginaceae family [1]. This genus is represented by 350 species which are growing throughout the world [2]. The flora of Algeria contains 20 species of *Limonium* among which 8 are endemic [3]. Many Limonium species were well known in folk medicine for their cure proprieties, such as *L. wrightii* species which is used for the treatment of fever or arthritis [4], the roots of *L. gmelinii* are used in folk medicine as an astringent and for acute gastrointestinal diseases [5]. Furthermore, *L. brasiliense* are employed as an antioxidant medicinal herb [6], whereas *L. sinense* exhibits antiviral activity [7]. Previous phytochemical studies of *Limonium* revealed the presence of different classes of flavonoids such as flavanes, aurones, flavonols and flavonol glycosides [8-14]. Furthermore, it is observed that *Limonium thouinii* has been tested as an inhibitor of corrosion [15]. The present study was aimed to investigate, for the first time, the constituent aerial parts of *L. thouinii*. The fractionation, isolation and structural elucidation yielded four compounds, which are shown in Fig. 1. The crude extracts were evaluated for their DPPH free radical-scavenging activity. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, due to the presence of the hydroxyl group in their chemical structures [16].

RESULTS AND DISCUSSION

Structures elucidation compounds 1-4 (fig.1) were identified by means of spectral data as quercetin (**1**), Apigenin 8-C-glucoside (2), Luteolin 6-C-glucoside (**3**) and Myricetin-3'-O-glucoside (4). These compounds are identified by spectral data, co-chromatography with authentic sampling when possible and confirmed by comparison with data from the literature [16-20]. Compound 1 was identified by spectroscopic techniques (UV-visible and mass spectroscopy), while 2, 3 and 4 were identified by UV-visible and 1H, 13C NMR spectra and mass spectroscopy.

Quercetin **1**. UV (λmax, nm), MeOH: 255-368. Mass spectrum (IE) m/z: 302 [M] +, 153 [A1 + H], 137 [B2]. [17].

Vitexin (Apigenin 8-C-glucoside) 2. UV (λmax, nm), MeOH: 266-336. Mass spectrum (ES) m/z : 433 [M+H]. δ ^{1}H NMR (300 MHz, DMSO-d6): 6.71 (1H, s, H-3), 6.21(1H, s, H-6), 8 (2H, d, J= 8.6 Hz, H-2', H-6'), 6.9 (2H, d, J= 8.6 Hz, H-5', H-3'),4.75 (1H, d, J= 9.9 Hz,H-1"), sugar protons (3.5- 4), 13.13 (1H, s, OH-5). δ ^{13}C NMR (75 MHz, DMSO-d6): 163.8 (C-2), 102.4 (C-3), 182.0 (C-4), 161.2 (C-5), 98.3 (C-6), 163.2 (C-7), 104.6 (C-8), 156.0 (C-9), 104.6 (C-10), 121.6 (C-1'), 128.9 (C-2'), 115.8 (C-3'), 160.4 (C-4'), 115.8 (C-5'), 128.9 (C-6'), 73.4 (C-1"), 70.9 (C-2"), 78.7 (C-3"), 70.5 (C-4"), 81.8 (C-5"), 61.3 (C-6"). [18].

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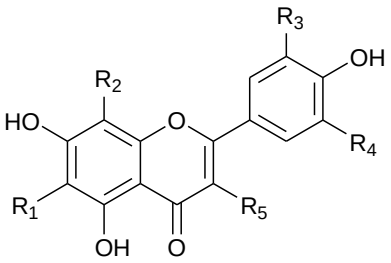

	R1	R2	R3	R4	R5
1	H	H	OH	H	OH
2	H	Glu	H	H	H
3	Glu	H	OH	H	H
4	H	H	OH	O-glu	OH

Fig. 1. Chemical structures of compounds 1-4.

Isoorientin *(Luteolin 6-C-glucoside)* ***3***. UV (λmax, nm), MeOH: 266-336. Mass spectrum (ES) m/z : 447 [M-H].

δ ^{1}H NMR (300 MHz, CD3OD): 6.57 (1H, s, H-3), 6.52(1H, s, H-8), 7.39(1H, d, J= 2.2 Hz, H-2'), 7.41 (1H, dd, J= 2.2; 8.6 Hz, H-6'), 6.93(1H, d, J= 8.6 Hz H-5'), 4.92 (1H, d, J= 9.9 Hz ,H-1"). Sugar protons (3.5- 3.9). δ ^{13}C NMR (75 MHz, CD3OD): 166.3 (C-2), 103.9 (C-3), 184.0 (C-4), 162.0 (C-5), 109.2 (C-6), 165.0 (C-7), 95.2 (C-8), 158.7 (C-9), 105.2 (C-10), 123.5 (C-1'), 114.1 (C-2'), 147.0 (C-3'), 151.0 (C-4'), 116.8 (C-5'), 120.3 (C-6'), 72.5 (C-1"), 75.3 (C-2"), 80.1 (C-3"), 71.7 (C-4"), 82.6 (C-5"), 62.8 (C-6"). [19].

Cannabiscitrin *(Myricetin-3'-O-glucoside)* ***4***, UV (λmax, nm), MeOH: 254-371. Mass spectrum (ES) m/z: 479 [M-H], 317[M-H-162]. δ ^{1}H NMR (300 MHz, CD3OD): 6.18 (1H, d, J= 2.2Hz, H-6), 6.44 (1H, d, J= 2.2Hz, H-8), 7.57 (1H, d, J= 2.2 Hz, H-2'), 7.73 (1H, d, J= 2.2 Hz, H-6'), 4.93 (1H, H1"). Sugar protons (3.5- 3.96). δ ^{13}C NMR (75 MHz, CD3OD): 147.2 (C-2), 138.6 (C-3), 177.3 (C-4), 162.0 (C-5), 99.3 (C-6), 165.7 (C-7), 94.5 (C-8), 158.0 (C-9), 104.4 (C-10), 123.3 (C-1'), 109.9 (C2'), 146.9 (C-3', C-5'), 138.6 (C-4'), 111.6 (C-6'), 104.4 (C1"), 74.8 (C-2"), 77.6 (C-3"), 71.2 (C-4"), 78.4 (C-5"), 62.4 (C-6"). [20, 21].

DPPH free radical-scavenging activity

The anti-radical activity extracts of *L. thouinii* was evaluated through the ability to scavenge DPPH radicals. The results are represented in Table 1.

The results shown in table1 indicate that all extracts present a weak DPPH free radical-scavenging activity compared to Trolox (TEAC $_{MeOH}$ = 0.119, TEAC $_{n\text{-}BuOH}$ = 0.081, TEAC $_{EtOAc}$ = 0.037).

Table 1. DPPH free radical-scavenging activity of L. thouinii extracts

	IC_{50} (µg/ml)	ARP	TEAC
Trolox	0.106	9.43	1
MeOH	0.89	1.13	0.119
EtOAc	2.87	0.354	0.037
n-BuOH	1.30	0.77	0.081

CONCLUSIONS

The phytochemical investigation of *Limonium thouinii* (Viv.) Kuntze revealed the presence of flavonol (Quercetin), two *C*-glucoside flavones (Vitexin, Isoorientin) and one *O*-glucoside flavonol (Cannabiscitrin), these four flavonoids are identified for the first time in this species, but Cannabiscitrin is a new compound in the Plumbaginaceae family. The tested extracts of the plant showed a weak DPPH free radical scavenging activity. We have also found the relationship of total flavonoids contents in these extracts with antioxidant activity, may be the hydroxyl group in these chemical structures.

EXPERIMENTAL SECTION

The aerial parts of *Limonium thouinii* (Viv.) Kuntze (Plumbaginaceae) was collected during the flowering period, from Setif in the east of Algeria and identified by Prof. H. Laouer (biology and plant ecology department, University of Setif, Algeria). A voucher specimen was deposited in the Herbarium of our laboratory. Voucher specimens of the plant material are deposited in the Herbarium at the department of biology and ecology vegetal, University of Setif (UFAS).

Extraction and isolation

Air dried aerial parts of *L. thouinii* (300 g) were soaked in methanol solvent (70%) at room temperature for 72 hours. The residue was filtered and concentrated under reduced pressure to dryness and the residue was dissolved in hot water and kept in the cold overnight. After filtration the aqueous solution was successively extracted with ethyl acetate and n-BuOH. The n-BuOH extract (5g) was subjected to a column polyamide MN SC6 and eluted with a gradient of Toluene-MeOH with increasing polarity to give ten fractions (F1-F10). The fractions F5 and F6 were applied to a preparative PC on Watman n°3 paper using acetic acid 15%, then by preparative TLC on polyamide DC6 to yield compounds: 1 (10mg), 2 (8mg), 3 (30mg) and 4 (11mg).The structures of the isolated compounds were elucidated by spectral analysis

mainly MS, UV, H 1 NMR and C 13 NMR as well as by comparing their spectroscopic data with those reported in the literature.

DPPH free radical-scavenging activity

The free radical scavenging capacity of the *L. thouinii* extracts were determined by using DPPH• (1, 1-diphenyl-2-picryl-hydrazyl), according to the method of Brand-Williams [22]. Five concentrations of each extract were prepared from the stock solution, added in equal volume, to the methanolic solution of DPPH on 96 well micro plates. After an incubation of 30 min at room temperature, the absorbance was determined at 515 nm. Trolox was used as a standard control.

The percentage inhibition of the DPPH free radical was calculated as per the following formula:

% inhibition of DPPH radical = [(DO control – -DO sample)/DO control]x100

The IC50 value was defined as the concentration of antioxidant necessary to decrease the initial DPPH concentration by 50% [23] and determined from the results by linear regression analysis. The lower IC50 values designate the greater antiradical activity. The antiradical power (ARP) was calculated as 1/IC50: the highest ARP values indicate the greater DPPH scavenging effect. The evaluation of free radical-scavenging activity was performed by the Trolox Equivalent Antioxidant Capacity (TEAC). The TEAC value is based on the ability of the antioxidant to scavenge the DPPH radical and was calculated by the following formula:

TEAC=ARP (compound)/ARP (Trolox).

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ХИМИЧЕН СЪСТАВ НА *Limonium thouinii* (viv.) kuntze (Plumbaginaceae) и DPPH-АКТИВНОСТТА ЗА ОТСТРАНЯВАНЕ НА СВОБОДНИ РАДИКАЛИ

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(Резюме)

Настоящата работа разглежда фитохимичните изследвания и DPPH-отстраняващата активност към свободните радикали на надземните части на растението Limonium thouinii (Viv.) kuntze (Plumbaginaceae). Надземните части на това растение позволяват да се изолират четири флавоноида: куерцетин, витексин, изо-ориентин и канабисцитрин. Тяхната структура се определя на базата на спектроскопски анализи, включващи УВ, МС и ЯМР – техники. Способността им да отстраняват DPPH свободни радикали е определяна върху сурови екстракти в метанол, етилацетат и n-бутанол.