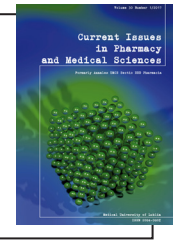
DOI: 10.1515/cipms-2017-0001 Curr. Issues Pharm. Med. Sci., Vol. 30, No. 1, Pages 5-8

Current Issues in Pharmacy and Medical Sciences
Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: http://www.curipms.umlub.pl/



Evaluation of the antioxidant activity of extracts and flavonoids obtained from *Bunium alpinum* Waldst. & Kit. (Apiaceae) and *Tamarix gallica* L. (Tamaricaceae)

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ARTICLE INFO

Received 19 October 2016
Accepted 24 January 2017

Keywords:
B. alpinum,
T. gallica,
flavonoids,
antioxidant activity,
DPPH assay,
EC_{50}.

ABSTRACT

The aim of the present work was to evaluate the antioxidant activity of extracts and four flavonoids that had been isolated from the aerial parts of *Bunium alpinum* Waldst. et Kit. (Apiaceae) and *Tamarix gallica* L. (Tamaricaceae). In this work, the four flavonoids were first extracted via various solvents, then purified through column chromatography (CC) and thin layer chromatography (TLC). The four compounds were subsequently identified by spectroscopic methods, including: UV, mass spectrum ^{1}H NMR and ^{13}C NMR. The EtOAc extract of *Bunium alpinum* Waldst. et Kit yielded quercetin-3-O-β-glucoside (3',4',5,7-Tetrahydroxyflavone-3-β-D-glucopyranoside) (1), while the EtOAc and n-BuOH extracts of *Tamarix gallica* L. afforded 3,5,3'-trihydroxy-7,4'-dimethoxyflavone (2), 3,5,7-trihydroxy-4'-methoxyflavone (3) and 5-hydroxy-3,7,4'-trimethoxyflavone (4).
The antioxidant activity of the extracts and the flavonoids were then evaluated through DPPH free radical-scavenging assay. Of all studied extracts, the n-Butanol extract of Bunium alpinum (EC_{50} = 1.84 µg/ml) showed the best antioxidant activity against (DPPH). In contrast, the isolates demonstrated varying degrees of antioxidant activity: compound (1) was the more active (EC_{50} = 0.28 µg/ml), followed by compound (3) and (2) (EC_{50} = 0.309µg/ml, EC_{50} = 0.406 µg/ml, respectively), compound (4) showed the lowest activity. All the isolated flavonoids exhibited antioxidant activity, but this was lower than the control (Trolox). In conclusion, due to the presence of flavonoids in their ariel parts, the studied plants could be natural sources of several important antioxidant agents.

INTRODUCTION

In modern medicine, herbal remedies still make a significant contribution to health care, being the origin source of most drugs. In this context, several researches continue to study plants which could be the source of novel remedies.

The genus *Bunium* (Apiaceae) involves about 50 to 100 species in the world, which are frequently distributed in: Algeria, Italy, Pakistan, Iran and South Africa. In the Algerian flora, this genus includes seven species, four of which are endemic [24]. Due to their medicinal and nutriment benefits, several species of this genus are often used in folk medicine; for example, the species *Bunium persicum* is generally employed as a antispasmodic, a carminative, an anti-obesity and a lactogage [27]. Furthermore, the fruits of this plant are employed in treating digestive and urinary system complaints, while the seeds have some important effects as hypoglycemics, anticonvulsants and antiemetics [14]. Several researches have shown that the essential oil and the extracts from some *Bunium* species have antihistaminic, antfungal, antibacterial [8] and antioxidant activities [26]. Indeed, phytochemical studies on the genus *Bunium* have revealed the presence of coumarins [5,9] and sesquiterpenes [4], while, especially, the essential oil (monoterppenoids) is seen as a source of metabolites [25]. Of note: it has been

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documented that in Algeria, the roots of *Bunium incrassatum* are usually eaten as a potato substitute [9].

The genus Tamarix L. (Tamaricaceae) is represented in Algeria by ten species [24]. Several species of this genus are employed in folk medicine as astringents, aperitifs, as stimuli of perspiration and as diuretics. Moreover, in Algeria and surrounding areas, some species are used to treat rheumatism and diarrhea [11]. In addition, some Tamarix species (*Tamarix ramosissima* and *Tamarix hispida*) have noted antioxidant effects [28] and antibacterial activities (Tamarix gallica) [20]. Furthermore, previous phytochemical investigations have reported that aerial parts of *Tamarix* species contain lipophilic methylated flavonoids such as: kaempferol-7,4'-dimethylether [13], chrysoeriol, isorhamnetin, rhamnazin [30], tamarixetin [29,30], 7,3',4'-trihydroxy-5-methoxyflavone, 3,7,4'-trihydroxy-5-meth-oxyflavone, 3,5,7-trihydroxy-3',4'-dimeth-oxyflavone[31], 7-O-sinapoylKaem-pferide [7]. The objective of the study was to evaluate the antioxidant activity of extracts and four isolated flavonoids obtained from two plants growing in Algeria: *Bunium alpinum* Waldst. & Kit (Apiaceae) and *Tamarix gallica* L. (Tamaricaceae).

MATERIALS AND METHODS

Plant material

Aerial parts of *Bunium alpinum* Waldst. & Kit (Apiaceae) were collected from Setif (east of Algeria) in May 2012, the plant having been identified by Prof Houcine Laouar (Department of Biology, Setif University, Algeria). While the aerial parts of *Tamarix gallica* L. (Tamaricaceae) were collected from Tebessa in the east of Algeria in March, 2006. A voucher specimen of each species was deposited in the herbarium of our laboratory.

Extraction and isolation

Bunium alpinum Waldst. & Kit (Apiaceae): Air dried aerial parts (300 g) of plant were firstly extracted three times with MeOH/CH_2Cl_2 (1:1). The extract MeOH/CH_2Cl_2 was evaporated till dryness; the obtained residue was dissolved in water, then filtered. The filtrate was extracted with EtOAc (2 g) and n-BuOH (6 g), successively. The EtOAc extract was then chromatographed on silica gel column and eluted with a gradient of EtOAc-MeOH with increasing polarity. Fractions eluted with EtOAc-MeOH 60-40 gave a yellow precipitate, which was washed with MeOH to offer compound **(1)** (30 mg).

Tamarix gallica L. (Tamaricaceae): Air dried aerial parts (500 g) of the plant were extracted three times with boiling 70% MeOH [6]. The extracted MeOH was evaporated till dryness, and the residue were dissolved in boiling water, then filtered. The filtrate was subsequently extracted with ethyl acetate EtOAc (9.32 g) and n-BuOH (25.69 g), successively. The EtOAc extract was chromatographed on polyamide MN-SC6 column, and eluted with a gradient of Toluene-MeOH with increasing polarity. Fractions eluted with toluene-MeOH 75-25 gave a yellow precipitate, which was washed by MeOH to give compound **(2)** (12.4 mg).

The n-BuOH extract was subjected to a MN-SC6 polyamide column chromatography, being eluted with a gradient of toluene/MeOH by increasing polarity. Five main fractions (A, B-G and H) were collected and analyzed via DC6 polyamide TLC, using Toluene: MeCOEt: MeOH) (4:3:3) and H_2O: MeOH: MeCOEt: Acetylacetone (13:3:3:1) as solvent systems. From fraction B, compound **(3)** (10 mg) and compound **(4)** (14 mg) were separated via preparative polyamide TLC, using a Toluene: MeCOEt: MeOH) (4:3:3) system as eluent.

Evaluation of antioxidant activity

Antioxidant activity of the extracts and purified compounds were evaluated with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [17] according to the method described by Brand-William [10]. The molecule DPPH is characterized as being a stable-free radical by virtue of the delocalization of spare electron over the molecule. This delocalization gives a rise to a deep violet color, which is revealed by an absorption band in methanol solution centered at 515 nm spectrophotoscopically. Five main dilutions of each extract and compound were prepared from the stock solution. These were added at an equal volume to a methanolic solution of DPPH, and the product was then transferred to a 96 well microplate and incubated at room temperature for 30 min. After this, the absorbance was measured at 515 nm. Trolox was used as standard control. The percentage of DPPH remaining was calculated as a function:

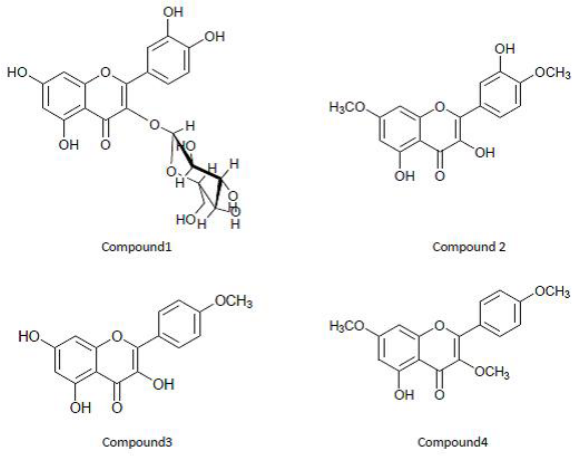
$$DPPH\% = [(DO_{control} - DO_{sample})/DO_{control}] \times 100$$

The EC_{50} value, was defined as the effective concentration of antioxidant that was necessary to decrease the initial DPPH concentration by 50% [3]. This was calculated from the results through linear regression analysis.

RESULTS AND DISCUSSION

Structures elucidation

The isolated compounds were identified by spectral data, as well as: UV-visible, 1H, 13C NMR spectra and mass spectroscopy, their structures (Fig. 1) were confirmed by comparison with literature data.



Compound **(1)**: quercetin-3-*O*-*β*-glucoside $C_{21}H_{12}O_{16}$; isolated from an EtOAc extract of *Bunium alpinum* Waldst. & Kit (Apiaceae) as a yellow powder;

$UV_{\lambda max}$ (MeOH) nm: 271-357, +NaOH; 411, 275; +$AlCl_3$; 426, 273; +$AlCl_3$/HCl; 402, 268; +CH_3COONa; 366, 260. Mass spectrum (ESI^+-MS): 487$[M+Na]^+$, 303[M-162+H], ^{1}H-NMR (300 MHz, DMSO-d6, δ in ppm) data: 6.21 (1H, d, J = 2 Hz, H-6), 6.42 (1H, d, J = 2 Hz H-8), 6.84 (1H, d, J = 8.5 Hz, H-5'), 7.54 (1H, d, J = 2 Hz, H-2'), 7.70 (1H, dd, J = 8.5-2 Hz, H-6'), 12.69 (1H, s, H-5), 5.41 (1H, d, J = 7.6 Hz, H-1''), sugar protons (3.5-4, m).

^{13}C-NMR (75 MHz, DMSO-d6, δ in ppm) data: 156.25 (C-2), 133.4 (C-3), 177.4 (C-4), 161.1 (C-5), 98.6 (C-6), 164.0 (C-7), 93.5 (C-8), 156.2 (C-9), 103.8 (C-10), 101.7 (C-1'), 115.1 (C-2'), 144.7 (C-3'), 148.4 (C-4'), 115.9 (C-5'), 121.8 (C-6'), 101.7 (C-1''), 71.1 (C-2''), 73.0 (C-3''), 67.86 (C-4''), 75.7 (C-5''), 60.0 (C-6'') [12].

Compound **(2)**: 3',3,5-trihydroxy-4',7-diméthoxyflavone $C_{17}H_{14}O_7$: isolated from an EtOAc extract of *Tamarix gallica* L. (Tamaricaceae) as a yellow powder.

$UV_{\lambda max}$ (MeOH) nm: 370, 252, +NaOH; 398, 275; +$AlCl_3$; 419, 254, 246; +$AlCl_3$/HCl; 418, 254, 246; +CH_3COONa; 371, 271. Mass spectrum (ESI+-MS): 353[M+Na] +, ^{1}H-NMR (300 MHz, MeOH-d4, δ in ppm) data: 6.16 (1H, d, J = 2.5 Hz, H-6), 6.4 9 (1H, d, J = 2.5 Hz H-8), 7.15 (1H, d, J = 8.6Hz, H-5'), 7.75 (1H, d, J = 2.5 Hz, H-2'), 7.78 (1H, dd, J = 8.6-2.5 Hz, H-6'), 12.69 (1H, s, H-5), 3.83, 3.84 (6H, s, 2-OCH3), ^{13}C-NMR (75 MHz, DMSO-d6 δ in ppm) data: 176.6 (C-4), 166.7 (C-7), 161.6 (C-9), 157.7 (C-5), 150.2 (C-4'), 147.2 (C-3'), 146.8 (C-2), 137.2 (C-3), 124.7 (C-1'), 121.2 (C-6'), 115.1 (C-2'), 112.1 (C-5'), 104.8 (C-10), 98.4 (C-6), 92.7 (C-8), 56.4 (3'-OCH3), 56.3 (7'-OCH3), [18,32].

Compound **(3):** 3,5,7-Trihydroxy-4-methoxyflavone $C_{16}H_{12}O_6$**;** isolated from a n-Butanol extract of *Tamarix gallica* L. (Tamaricaceae) as a yellow crystal.

UV spectrum (MeOH, λ_{max} in MeOH) 261, 363 nm; NaOH: 275, 390; +AlCl3: 269, 425; +AlCl3/HCl: 270, 426-421; +CH_3COONa: 269, 370. Mass spectrum (EI, 70ev, m/z): 300[M]+, 285[M-15]+, 257[M-15-28]+, ^{1}HNMR spectrum (250 MHz, MeOH-d_4, δ in ppm) data: 6.20(1H, d, J = 2 Hz, H-6), 6.42 (1H, d, J = 2Hz, H-8), 7.1(2H, d, J = 7.9 Hz, H-3, H-5'), 8.16 (2H, d, J = 7.9 Hz, H-2', H-6), 3.91 (3H, s,-OCH3), ^{13}C-NMR (75 MHz, DMSO-d6 δ in ppm) data:146.3 (C-2), 136.1 (C-73), 176.1 (C-4), 160.8 (C-5), 98.3 (C-6), 164.1 (C-7), 93.6 (C-8), 156.3 (C-9), 103.7 (C-10), 123.3 (C-1'), 129.4 (C-2'), 114.1 (C-3'), 160.6 (C-4'), 114.1 (C-5'), 129.4 (C-6'), 55.4 (4'-OCH3) [15].

Compound **(4):** 5-Hydroxy-3,7,4-tri-methoxyflavone $C_{18}H_{16}O_6$; isolated from a n-Butanol extract of *Tamarix gallica* L. (Tamaricaceae) as a white powder.

UV (λ_{max} in MeOH): 344, 268 nm; NaOH ; 360, 275 nm; +$AlCl_3$; 396, 346, 276 nm; +$AlCl_3$/HCl: 396, 350, 276 nm; +CH_3COONa; 346, 269 nm. Mass spectrum (EI, 70ev, m/z): 330 [M+H+1]+, 314[M+H-15]+, 300[M-28]+, 285[M-28-15]+, ^{1}HNMR spectrum (250 MHz, DMSO-d6, δ in ppm) data: 6.25 (1H, d, J = 2Hz, H-6), 6.42 (1H, d, J = 2 Hz, H-8), 7(2H, d, J = 9 Hz, H-3, H-5), 8.25(2H, d, J = 9 Hz, H-2, H-6), 3.84- 3,88(9H, s,-OCH3). ^{13}C-NMR (75 MHz, DMSO-d6 δ in ppm) data: 156.7 (C-2), 138.5 (C-73)176.1(C-4), 161.2 (C-5), 98.0 (C-6), 166.0 (C-7), 92.2 (C-8), 157.0 (C-9), 104.0 (C-10), 122.6 (C-1'), 129.9 (C-2'), 114.0 (C-3'), 161.9 (C-4'), 114.0 (C-5'), 129.9 (C-6'), 59.5 (3'-OCH_3), 55.9 (7-OCH_3), 55.5 (4'-OCH_3) [1].

The antioxidant activity

The antioxidant (DPPH scavenging) activity of extracts and flavonoids of the two plants was evaluated through the ability to scavenge DPPH free radicals. Parameter EC_{50} is defined as the effective concentration of antioxidant that are necessary to decrease the initial DPPH concentration by 50%. In this regard, the lower value of EC_{50} associates with the higher antioxidant potential [22]. The antioxidant activity of the two plant extracts are given in (Tab. 1). All extracts from the two plants showed antioxidant activity, but the n-Butanol extract of *Bunium alpinum* was the most effective (EC_{50} = 1.84 µg/ml). The antioxidant activity of these extracts may be linked to the presence of flavonoids in their compositions. The antioxidant activities of the isolated flavonoids are shown in (Tab. 2). The derived antioxidant activity increased in the following order: compound **(4)** < compound **(2)** < compound **(3)** < compound **(1)**.

The evaluation of antioxidant activity by the DPPH method uncovered a large difference among the investigated flavonoids. This may be associated with differences in their structures. In this regard, compound **(1)** flavone,5,7,3',4'-tetrahydroxy-3-beta-D-glucopyranoside, EC_{50} = 0.28 µg/ml), which was isolated from *Bunium Alpinum*, showed a greater antioxidant activity than did the other isolated flavonoids. Such a difference may be explained by the presence of poly-hydroxylated groups on the ring B. Indeed, Gordana *et al.* suggested that the presence of polyhydroxylated groups in the B ring increase antioxidant activity [16]. Furthermore, the higher antioxidant activity of compound **(1)** can also be attributed to the 3',4'-catechol structure in the ring B [21]. Among the three flavonoids isolated from *Tamarix gallica* L.; compound **(3)** (3,5,7-trihydroxy-4'-methoxyflavone EC_{50} = 0.309 µg/ml) was the most active, while, compound **(4)** (5-hydroxy-3,7,4'-trimetoxyflavone, EC_{50} = 0.725 µg/ml) exhibited the lowest scavenging activity.

Table 1. Antioxidant activity of extracts

	Extract	EC_{50} (µg/ml)
B. alpinum	MeOH:CH_2Cl_2 AcOEt n-BuOH	2.89 2.11 1.84
T. gallica	MeOH AcOEt	3.89 2.66
Standard	Trolox	0.106

Table 2. Antioxidant activity of isolated flavonoids

	flavonoids	EC_{50} (µg/ml)
B. alpinum	compound **(1)**	0.28
T. gallica	compound **(2)** compound **(3)** compound **(4)**	0.406 0.309 0.725
Standard	Trolox	0.106

The weak activity showed by compound **(4)** could be linked to the substitution of this flavonoid by various number of OMe groups. This observation is in agreement with the earlier study of Jeong *et al.*, who found that the substitution of flavonoids by various numbers of OMe groups decreased

antioxidant activity [19]. In addition, it appears that the presence of 3-OH groups in the structures of flavonoids compound **(2)** and **(3)** increase significantly the antioxidant activity when compared to that with 3-OMe groups of compound **(4)**. This observation is in accordance with that of Op de Beck *et al.* [23] and with that of Amic *et al.*, who confirmed that the presence of 3-OH groups significantly enhances antioxidant activity [2]. All the flavonoids isolated from the two plants showed antioxidant activity, but less than the Trolox used as control (EC_{50} = 0.106 µg/ml).

CONCLUSION

The present study reported the antioxidant activity of extracts, as well as that of four flavonoids isolated from the aerial parts of *Bunium alpinum* Waldst. & Kit (Apiaceae) and *Tamarix gallica* L. (Tamaricaceae). The findings of this study reveal that *Bunium alpinum* Waldst. & Kit and *Tamarix gallica* L. have potential to be used as natural antioxidant agents. This study, hence, suggests that further research be taken so as to ascertain the other pharmacological potential of these species.

ACKNOWLEDGMENTS

The Ecologie Microbienne, CESN, ISPB, 8 Avenue Rockefeller, F69373 Lyon cedex, France is acknowledged for the biological activities facilities.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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