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Isolation of bioactive antioxidant compounds from the aerial parts of *Allium roseum* var. *grandiflorum* subvar. *typicum* Regel.

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ABSTRACT

Objective: To assess the antioxidant activity of aerial parts of *Allium roseum* var. *grandiflorum* subvar. *typicum* Regel. (*A. roseum* var. *grandiflorum* subvar. *typicum* Regel.) for the first time, as well as to isolate the main bioactive compounds.

Methods: The chloroformic extract of *Allium roseum* (*A. roseum*) and their fractions obtained by subjection to a chromatographic study were tested for their antioxidant activities by using 2, 2-diphenyl-2-picrylhydrazyl and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) assays. An activity-guided purification was conducted to isolate five compounds in pure form where their structures were identified by means of nuclear magnetic resonance analyses, including 1D and 2D nuclear magnetic resonance experiments.

Results: The evaluation of the antioxidant activity of chloroformic extract and their fractions from *A. roseum* var. *grandiflorum* subvar. *typicum* Regel. showed interesting results. The active chloroformic extract afforded five isolated compounds where their structures were identified as β-sitosterol (1), chrysoeriol (2), luteolin (3), apigenin (4), and β-sitosterol 3-O-β-D-glucoside (5). All the compounds were isolated for the first time from the *A. roseum* var. *grandiflorum* subvar. *typicum* Regel. The three flavonoids (2–4) exhibited the highest antioxidant activity with IC_{50} values of 62.28, 21.26 and 513.42 μg/mL, respectively (2, 2-diphenyl-2-picrylhydrazyl assay) and 218.00, 73.50 and 877.66 μg/mL, respectively [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) assay]. An important value of trolox equivalent antioxidant capacity (2.10 mmol/L) was reported for luteolin (3).

Conclusions: These results may suggest that the *A. roseum* var. *grandiflorum* subvar. *typicum* Regel. have great potential as a source of a natural preservative ingredient in beneficial for natural health products.

1. Introduction

Nowadays, much attention has been focused on the use of antioxidants, especially natural antioxidants. Fruits and vegetables are rich in natural oxidants and have health promoting effects and these positive effects have been related to their antioxidant activity[1]. Numerous authors have investigated the antioxidant activity of flavonoids, and many attempts have been made to establish the relationship between flavonoid structure and their radical scavenging activity[2,3]. This radical potency of flavonoids is mostly related to the presence of O–H groups at specific positions on the flavonoid core[4].

The genus *Allium* L. comprises more than 800 species[5], including edible and culinary species[6]. They are perennial bulbous plants that produce chemical compounds, and many are used as medicinal plants[7]. Several species of *Allium*, as *Allium roseum* var. *grandiflorum*[8], *Allium fistulosum* auct.[9] and *Allium ascalonicum* L.[10] have been found to have antioxidant properties. *Allium* plants are an important source of dietary flavonoids[11]. Those phenolic compounds have been found in this genus and especially in *Allium myrianthum* Boiss[12], *Allium victorialis* var. *platyphyllum*[13], *Allium cepa* L.[14], *Allium vineale*[15] and *Allium roseum* var. *odoratissimum*[16].

Allium roseum (*A. roseum*) is a highly polymorphous species in North Africa. It presents twelve different taxa: four varieties, four subvarieties and four forms[17]. The uses of this edible spontaneous species as functional food ingredients and/or supplements were also reported[18]. Only two papers related to *A. roseum* var. *grandiflorum*

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subvar. *typicum* Regel. have been published so far[8,19]. Therefore, in the second work, in addition to the study of the chemical composition of the leaf essential oil, nine extracts were obtained from flowers, stems and leaves. Bulbs and bulblets were tested for their antioxidant activities. The study reported that the extracts from flowers and from both stems and leaves parts of *A. roseum* possessed higher 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, so they showed the best antioxidant activity. The chloroform extract showed the highest content in total flavonoids[8]. According to these data previously reported we are motivated in the present work, to carry out a bio-guided chromatographic study of the whole aerial part (flowers, leaves and stems) chloroformic extract (CE) of *A. roseum* var. *grandiflorum* subvar. *typicum* Regel., to isolate pure compounds and to evaluate their possible beneficial antioxidant potency.

2. Materials and methods

2.1. Plant collection

A. roseum var. *grandiflorum* subvar. *typicum* was collected in the region of Sousse (coastal region, in the middle-east of Tunisia, geographical coordinates are 35°49'32" North, 10°38'28" East), during their blooming stage in March 2011. A voucher specimen (Al.104) has been deposited at the Herbarium of the Laboratory of Genetic, Biodiversity and Valorisation of Bioressources, High Institute of Biotechnology of Monastir, Tunisia.

2.2. Preparation of A. roseum var. grandiflorum subvar. typicum organic CE

The aerial part (fresh flowers and both stems and leaves) of *A. roseum* var. *grandiflorum* subvar. *typicum* was air-dried for five weeks then ground into fine powder. The obtained powder (2 096 g) was soaked in a mixture of acetone-H_2O (8:2, v/v). Extraction was performed twice for 5 days at room temperature. The resulting extract was filtered and the solution was evaporated to remove acetone under reduced pressure in a rotary evaporator (Büchi Rotavapor R-200, Büchi Heating Bath B-490). The remaining aqueous solution was extracted sequentially with chloroform. The CE was concentrated with a rotary evaporator under reduced pressure and stored at 4 °C until tested.

2.3. Isolation and purification

The CE (18.4 g) was subjected to a silica gel column chromatography (120 cm × 2.5 cm; silica gel 60 Merck 7734, 400 g) and then eluted with solvents with an increasing gradient polarity. Varying proportions of petroleum ether and ethyl acetate, then 100% of acetone and finally 100% of methanol were used. Collected fractions were combined in 19 homogeneous ones (Fr1-Fr19). All of them were tested for their antioxidant activity. Those showing interesting activity were selected for further separation by column chromatography.

Fraction 4 (2.326 g) in presence of methanol gave a white solid (180 mg) which appeared a pure compound when tested and reviewed with H_2SO_4 reagent (compound 1).

Precipitation of bioactive fraction 9 (1.436 g) in methanol allowed recovery of a white solid. The latter was purified on preparative silica gel thin-layer chromatography plates and eluted with a $CHCl_3$/methanol (9.2:0.8, v/v), leading to obtaining of a pure product weighing 5.9 mg (compound 2).

The most active fraction 10 (1.473 g) was subjected to a silica gel column chromatography (70.0 cm × 1.5 cm; silica gel 60 Merck 7734; 70 g) eluted with $CHCl_3$ and $CHCl_3$/methanol (8:2, v/v) gradients to give sixteen subfractions. The fifth subfraction (256 mg) was purified by a silica gel column chromatography (45.0 cm × 1.5 cm; silica gel 60 Merck 7734; 40 g) using CH_2Cl_2/methanol (9.5:0.5, v/v), two subfractions were obtained. The second subfraction (55 mg) purified by preparative silica gel thin-layer chromatography plates eluted with a $CHCl_3$/methanol (9.75:0.25/v:v) leads to obtain two pure products weighing 5.0 mg and 9.2 mg, respectively (compounds 3 and 4).

Fraction 15 (1.028 g) gave in presence of methanol a white solid (100 mg) which was acetylated (Ac_2O/pyridine, room temperature, 12 h) and then loaded on silica gel column chromatography (45.0 cm × 1.5 cm; silica gel 60 Merck 7734; 30 g) eluted with EP/ethyl acetate (7:3, v/v) to give 80 mg of a pure product (compound 5).

2.4. Spectroscopic analysis

^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR of compounds 1–5 were measured on a Bruker AM 300 NMR spectrometer, at 300 and 75 MHz, respectively, with CD_3OD (compounds 1, 3 and 4) and C_5D_5N (compounds 2 and 5). The residual solvent resonances were used as the internal references. Coupling constants were given in Hertz. The chemical shifts were expressed in δ ppm. Correlation spectroscopy (COSY) and nuclear overhauser effect spectroscopy (NOESY) spectra were run on a Bruker AM 300 NMR spectrometer.

2.5. Determination of free radical scavenging activity

All samples were diluted with absolute ethanol in a concentration range from 0.5 to 5.0 mg/mL for CE and for the different fractions (Fr1–Fr19) and from 0.01 to 1.00 mg/mL for the pure compounds 2, 3 and 4 (only flavonoids compounds were tested).

2.5.1. DPPH radical scavenging assay

Free radical scavenging activities of the CE and its fractions and of the purified compounds were determined in accordance with Ramadan *et al.* method[20], which is based on the principle of scavenging the DPPH (2,2-diphenyl-2-picrylhydrazyl) radical. An aliquot (50 µL) of methanol solution containing different amounts of sample was added to 950 µL of a daily prepared methanol DPPH solution (10^{-4} mol/L). The mixture was shaken gently and left to stand at room temperature in the dark for 30 min. Thereafter, the absorbance was measured at 515 nm. The scavenging activity was measured as a decrease in absorbance of the samples versus DPPH standard solution. Trolox was used as positive control. Results were expressed as IC_{50} value which was defined as the amount of sample (mg extract/mL) necessary to decrease the initial DPPH radical concentration by 50%. All tests were carried out in triplicate.

2.5.2. 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid ($ABTS^+$) radical cation scavenging

The antioxidant activity was measured by $ABTS^+$ radicals according to the method of Re *et al.*[21]. Briefly, the radical cation $ABTS^+$ was produced by reacting 7 mmol/L ABTS aqueous solution with 2.45 mmol/L potassium persulfate ($K_2S_2O_8$) in the dark for 12–16 h at room temperature. The blue-green ABTS solution was diluted in ethanol (1:80, v/v) to give an absorbance of (0.70 ± 0.03) at 734 nm prior to assay. The diluted ABTS solution (1 000 µL) was mixed with 10 µL of sample or trolox standard. The mixture was left to stand at room temperature in the dark for 20 min, and then the absorbance was measured at 734 nm. Trolox was used as positive control. All tests were performed in triplicate. The antioxidant

capacity of test samples was expressed as IC_{50} values, which indicated the concentration of sample (mg extract/mL ethanol) required to scavenge 50% of ABTS radical, expressed also as trolox equivalent antioxidant capacity (TEAC). The TEAC value of a sample represents the concentration of a Trolox solution that has the same antioxidant capacity as this sample.

2.6 Statistical analysis

All data were reported as mean ± SD of three replicates. Statistical analyses were performed using a One-way ANOVA and the significance of the difference between means was determined by Duncan's test at $P < 0.05$.

3. Results

In order to quantify the antioxidant activity of *A. roseum* var. *grandiflorum* subvar. *typicum*, the IC_{50} of the CE and its fractions (Fr1–Fr19) are calculated and reported in Table 1 and the results were comparable to that of the standard Trolox. The IC_{50} value in the DPPH test ranged from (0.290 ± 0.110) to (4.160 ± 0.390) mg/mL. For the lowest IC_{50} value corresponded the greatest free radical-scavenging activity. Fr9 and Fr10 exhibited the lowest IC_{50} [(0.730 ± 0.020) and (0.290 ± 0.110) mg/mL, respectively] and so the highest DPPH radical-scavenging when compared to the rest of the fractions isolated from *A. roseum* var. *grandiflorum* subvar. *typicum* CE. Fractions Fr1–Fr7 were not active. The antioxidant activity of the CE [(1.970 ± 0.330) mg/mL] was less than those for Fr15, Fr17, Fr14, Fr12, Fr9 and Fr10 which may result from the active components through condensation effects during the solvent-solvent partitioning process.

Table 1

Antioxidant activity of *A. roseum* var. *grandiflorum* subvar. *typicum* Regel CE and its fractions (Fr1–Fr19) evaluated with DPPH and ABTS assays.

Samples	DPPH assay	ABTS assay	TEAC (mmol/L)
	IC_{50} (mg/mL)	IC_{50} (mg/mL)	
CE	1.970 ± 0.330^{de}	2.570 ± 0.210^{g}	0.720 ± 0.080^{e}
Fr8	3.300 ± 0.210^{g}	1.560 ± 0.060^{cdef}	0.830 ± 0.050^{d}
Fr9	0.730 ± 0.020^{b}	0.910 ± 0.050^{b}	1.150 ± 0.030^{a}
Fr10	0.290 ± 0.110^{a}	0.840 ± 0.030^{b}	1.200 ± 0.020^{a}
Fr11	4.160 ± 0.390^{h}	1.120 ± 0.100^{bc}	1.020 ± 0.050^{b}
Fr12	1.430 ± 0.050^{c}	1.300 ± 0.140^{bcde}	0.910 ± 0.050^{c}
Fr13	2.060 ± 0.110^{e}	1.280 ± 0.040^{bcde}	1.000 ± 0.020^{bc}
Fr14	1.500 ± 0.160^{c}	1.700 ± 0.020^{ef}	0.770 ± 0.030^{de}
Fr15	1.690 ± 0.020^{cd}	1.200 ± 0.000^{bcd}	1.010 ± 0.010^{b}
Fr16	3.450 ± 0.060^{g}	4.430 ± 0.750^{h}	0.510 ± 0.030^{f}
Fr17	1.530 ± 0.100^{c}	2.930 ± 0.090^{g}	0.700 ± 0.000^{e}
Fr18	2.760 ± 0.090^{f}	1.610 ± 0.280^{def}	0.810 ± 0.070^{d}
Fr19	2.980 ± 0.120^{f}	1.920 ± 0.330^{f}	0.770 ± 0.060^{de}
Trolox	0.078 ± 0.400^{a}	0.273 ± 1.670^{a}	1.000 ± 0.000^{b}

Fractions Fr1–Fr7 were not active; Data are expressed as mean ± SD ($n = 3$). The same superscript letter in a column were not significantly different at $P > 0.05$.

The antioxidant abilities of the CE and its fractions in the ABTS test, determined by IC_{50} and TEAC values, are also shown in Table 1. The IC_{50} values with the ABTS test ranged from (0.840 ± 0.030) to (4.430 ± 0.750) mg/mL compared to Trolox [IC_{50} = (0.273 ± 1.670) mg/mL]. The highest ABTS scavenging rate was found for Fr10 and Fr9 [IC_{50} = (0.840 ± 0.030) mg/mL and (0.910 ± 0.050) mg/mL, respectively], while the lowest was found for Fr16. The TEAC value for CE was (0.720 ± 0.080) mmol/L and for its fractions the values ranged from (0.510 ± 0.030) to (1.200 ± 0.020) mmol/L. The most important values of TEAC were performed by the Fr9 [(1.150 ± 0.030 mmol/L)] and [Fr10 (1.200 ± 0.020 mmol/L)]. These two fractions showed important free radical scavenging activities in both tests (ABTS and DPPH assays) compared to the other fractions. Furthermore, CE was more efficient on DPPH radical scavenging than the ABTS free radical-scavenging (Table 1). The very important antioxidant activity of the two fractions, Fr10 and Fr9, against DPPH and ABTS radicals motivated us to choose them for a simplification on a silica gel column chromatography in order to identify the principal active compounds.

As shown in Figure 1, structures of five pure compounds isolated from the organic extract CE were identified by interpretation of their spectral data (^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY and NOESY). This work was the first phytochemical investigation involving *A. roseum* var. *grandiflorum* subvar. *typicum*.

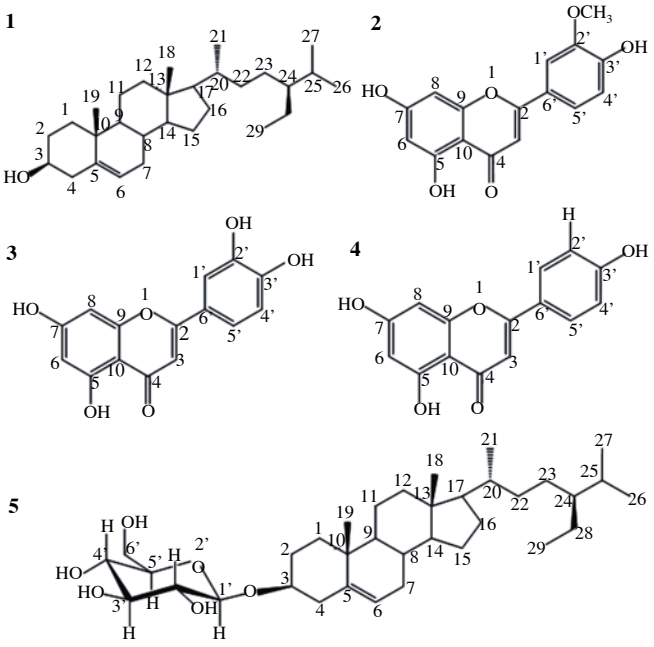

Figure 1. Chemical structures of five compounds isolated from the *A. roseum* var. *grandiflorum* subvar. *typicum* Regel.
Two steroids (1, 5): β-sitosterol (1), and β-sitosterol 3-O-β-D-glucoside (5), and three flavonoids (2–4): chrysoeriol (2), luteolin (3), and apigenin (4).

Compound 1 isolated from Fr4 and compound 5 isolated from Fr15 were identified as β-sitosterol and β-sitosterol 3-O-β-D-glucoside, respectively (Figure 1) based on their spectral data compared to the literature[22].

The NMR (^{1}H and ^{13}C) presented peaks from which it was possible to calculate the chemical shift (δ) in ppm and the coupling constants in Hertz (Table 2). Compound 2: ^{1}H NMR (300 MHz, C_5D_5N), δH (ppm) *J* (Hz), 7.06 (1H, s, H-3), 6.95 (1H, d, *J* = 2.1, H-6), 6.85 (1H, d, *J* = 1.8, H-8), 7.7 (1H, d, *J* = 2.1, H-2'), 7.37 (1H, d, *J* = 8.1, H-5'), 7.73 (1H, dd, *J* = 8.4; 2.1, H-6') and 3.9 (3H, s, OCH_3-1''). ^{13}C NMR spectrum revealed 16 carbon signals. ^{1}H and ^{13}C NMR assignments were carried out with the aid of the detailed 2D analyses (COSY, NOESY) and the resulting NMR evidence revealed 1 to be defined as chrysoeriol which agreed well with the data reported[23].

Compound 3: ^{1}H NMR (300 MHz, CD_3OD), ^{1}H (ppm) *J* (Hz), 6.38 (1H, s, H-3), 6.03 (1H, s, H-6), 6.30 (1H, s, H-8), 6.84 (1H, s, H-2'), 7.31 (1H, s, H-5') and 7.76 (1H, dd, *J* = 8.1; 2.2, H-6'). ^{13}C NMR spectrum revealed 15 carbon signals. Compared with literature data allowed to assign the luteolin structure[24].

Compound 4: ^{1}H NMR (300 MHz, CD_3OD), ^{1}H (ppm) *J* (Hz), 6.32 (1H, s, H-3), 5.95 (1H, d, *J* = 2.1, H-6), 6.15 (1H, d, *J* = 1.8, H-8), 7.70 (1H, d, *J* = 8.7, H-2'), 6.74 (1H, d, *J* = 8.7, H-3', H-5') and 7.70 (1H,

d, J = 8.7, H-6'). Based on the spectral analysis of compounds 2 and 3 already identified and compared to literature[23], compound 4 was identified as apigenin. Structures of all these compounds were reported for the first time in *A. roseum* var. *grandiflorum* subvar. *typicum*.

Table 2

NMR spectral data of compound 2 (C_5D_5N), compounds 3 and 4 (CD_3OD, δ ppm).

P	Compound 2		Compound 3		Compound 4	
	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H
1	-	-	-	-	-	-
2	165.19[a]	-	166.10	-	164.80	-
3	106.22	7.06 (s)[b]	103.01	6.38 (s)	104.61	6.32 (s)
4	184.75	-	183.35	-	182.90	-
5	166.56	-	163.05	-	163.90	-
6	102.00	6.95 (d, 2.1)	103.31	6.03 (s)	101.11	5.95 (d, 2.1)
7	167.90	-	166.10	-	166.40	-
8	96.95	6.85 (d, 1.8)	97.35	6.30 (s)	98.10	6.15 (d, 1.8)
9	165.19	-	160.33	-	160.14	-
10	107.02	-	105.74	-	105.50	-
1'	123.33	-	120.49	-	122.90	-
2'	118.96	7.7 (d, 2.1)	113.74	6.84	119.80	7.70 (d, 8.7)
3'	160.56	-	148.16	-	115.20	6.74 (d, 8.7)
4'	154.42	-	153.39	-	155.70	-
5'	112.37	7.37 (d, 8.1)	117.33	7.31	115.29	6.74 (d,8.7)
6'	124.60	7.73 (dd, 8.4, 2.1)	117.90	7.76 (dd, 8.1, 2.2)	120.15	7.70 (d, 8.7)
C'	58.04	3.9 (s)	-	-	-	-

[a]: Values in ppm (δ); [b]: Values in parentheses were coupling constants (in Hz). P: Position; C': C'$_3$-OCH_3.

In order to continue the bio-guided chromatographic study, the antioxidant activity of purified compounds of *A. roseum* var. *grandiflorum* subvar. *typicum* was tested only for the three flavonoids isolated from the most active fractions Fr9 and Fr10 and these results were reported in Table 3. In the DPPH test, the three flavonoids (luteolin 3, chrysoeriol 2 and apigenin 4) showed significant scavenging activity with IC_{50} values of 21.26; 62.28 to 513.42 µg/mL. Luteolin exhibited the lowest IC_{50} and so the highest DPPH radical-scavenging activity. By comparing the three compounds, the IC_{50}/DPPH free radical-scavenging activities decreased in the order of apigenin followed by chrysoeriol and finally luteolin. The free radical-scavenging activity of Fr9 and Fr10 [(0.730 ± 0.020) and (0.290 ± 0.110) mg/mL, respectively] was less important than the 3 compounds from which they have been isolated. In the ABTS test, the highest scavenging activity was found for luteolin [IC_{50} = (73.50 ± 1.17) µg/mL] and the lowest for apigenin [(887.66 ± 1.65) µg/mL]. The free radical scavenging property was comparable to that of the Trolox (IC_{50} = 273 µg/mL) (Table 3). The antioxidant activity values were decreased in the same rank order compared with that of DPPH assay. TEAC values were (1.19 ± 0.00), (1.87 ± 0.02) and (2.10 ± 0.01) mmol/L for apigenin, chrysoreiol and luteolin, respectively (Table 3). The most important values of TEAC were performed by luteolin. These compounds were more active than the Trolox.

Table 3

Antioxidant activity of the three flavonoid compounds isolated from aerial parts CE of *A. roseum* var. *grandiflorum* subvar. *typicum* Regel. evaluated with DPPH and ABTS assays.

Flavonoid compounds	DPPH assay	ABTS assay	TEAC
	IC_{50} (µg/mL)	IC_{50} (µg/mL)	(mmol/L)
Luteolin	21.26 ± 1.26[a]	73.50 ± 1.17[a]	2.10 ± 0.01[a]
Chrysoeriol	62.28 ± 0.98[b]	218.00 ± 1.73[b]	1.87 ± 0.02[b]
Apigenin	513.42 ± 2.18[d]	887.66 ± 1.65[d]	1.19 ± 0.00[c]
Trolox	78.00 ± 0.40[c]	273.00 ± 1.67[c]	1.00 ± 0.00[d]

Data were expressed as mean ± SD (n = 3). The same superscript letter in a column were not significantly different at $P > 0.05$.

4. Discussion

The current study was carried out to determine the bio-guided antioxidant activity of the organic CE from aerial parts of *A. roseum* var. *grandiflorum* subvar. *typicum* Regel. and it's semi-purified fractions. The chemical composition of the bioactive antioxidant pure compounds was determined by both 1D and 2D NMR experiments.

Previously, the IC_{50}/DPPH values for the same species were determined for the flowers and for both stems and leaves CEs [(2.38 ± 0.09) and (0.96 ± 0.01) mg/mL, respectively][8]. Nencini *et al.*[25] also signaled that the IC_{50}/DPPH values for the Italian *A. roseum* flowers and leaves aqueous extracts ranged from (1.01 ± 0.032) to (3.08 ± 0.108) mg/mL, respectively. Whereas, *Allium subhirsutum* L. flowers has an IC_{50} = (2.05 ± 0.183) mg/mL. Some results reported in the literature showed that the TEAC values for ethanolic extracts of *Allium* species were in the order of (0.98 ± 0.05) mmol/L for *Allium cepa*, (4.97 ± 0.02) mmol/L for *Allium schoenoprasum* and (8.5 ± 0.2) mmol/L for *Allium sativum*[26]. Antioxidative activity of some *Allium* species using ABTS free radical has been previously reported for *Allium sativum*[27] and *Allium oschaninii* L.[28] but no work has been reported for *A. roseum* var. *grandiflorum* subvar. *typicum* Regel using this radical.

The chemical analysis of the organic extract (CE) revealed the presence of three flavonoids: chrysoeriol (2); luteolin (3) and apigenin (4) and two sterols: β-sitosterol (1) and β-sitosterol 3-O-β-D-glucoside (5). All these compounds are reported for the first time in *A. roseum* var. *grandiflorum* subvar. *typicum*. Many flavonoid compounds have been previously identified in multiple *Allium* species. Chrysoeriol was isolated from *Allium vineale* L. leaves[15], both luteolin and apigenin were found in *Allium obliquum* L.[29,30]. In other studies, all these three flavonoids detected in this study were identified in *Allium flavum*[31]. Several studies reported that flavonoids have antioxidant activity[3]. In this study, by comparing the three flavonoids isolated, the IC_{50}/DPPH free radical-scavenging activities were decreased in the order of apigenin followed by chrysoeriol and finally luteolin. In addition, the most important values of TEAC were performed by luteolin. These compounds were more active than the Trolox. According to the literature, apigenin and luteolin have TEAC values of 2.10 and 1.45 mmol/L, respectively[4]. The TEAC values for the three flavonoids correlate well with the number of aromatic hydroxyl groups, nOH. This number and, especially, their position seem to be essential for radical scavenging[32]. The increasing nOH could be related to the increasing ability of H atom abstraction and so enhanced scavenging of free radicals[4]. Luteolin has a higher antioxidant activity (with 4 OH groups), than that of chrysoeriol and apigenin (with 3 OH groups). We can conclude that the isolated compounds including chrysoeriol, luteolin and apigenin are predominate contributors to the overall antioxidant activity of the plant.

This study reported the bio-guided isolation of antioxidant constituents of the CE of *A. roseum* var. *grandiflorum* subvar. *typicum* aerial parts. The antioxidant activity was evaluated by two complementary tests: DPPH and ABTS assays. Three flavonoids were isolated and identified (chrysoeriol, luteolin and apigenin) for the first time in the Tunisian *A. roseum* var. *grandiflorum* subvar. *typicum*. These compounds possessed high ability to scavenge the DPPH and ABTS radicals. The antioxidant activity of this species may be attributed to these flavonoids. In conclusion, this work suggested that plant may be considered as a good source of natural antioxidant compounds suitable for application in supplementary food and in pharmaceutical field.

Conflict of interest statement

We declare that we have no conflict of interest.

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