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# Chemical Composition and Biological Activities of *Allium roseum* L. var. *grandiflorum* Briq. Essential Oil

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**Abstract:** *Allium roseum* L. (Alliaceae) endemic mediterranean specie was represented in the North Africa by 12 different taxa. In the present study, chemical composition, antiproliferative, antioxidant and antimicrobial activities of the essential oil extracted from *A. roseum* var. *grandiflorum* Briq. bulbs collected in the North of Tunisia were investigated. Chemical characterization has shown methyl methanethiosulfinate as major sulphurous compounds. *A. roseum* bulbs essential oil provides interesting antiproliferative activity against two human colonic adenocarcinoma HT29 and CACO2 cell lines in dose-dependent manner with a half-maximal inhibition (IC<sub>50</sub>) of 4.64 µg/mL and 8.22 µg/mL respectively. The antioxidant activity, as determined by FRAP assay, was 285 µmol equivalent Trolox/g of essential oil. The scavenging effect on DPPH radicals of essential oil was estimated as IC<sub>50</sub> values at 156 µg/mL. The inhibition of superoxide anion production in a model of cancer cell lines was significant for both lines HT29 and CACO2 with IC<sub>50</sub> of 20.25 µg/mL and 29.12 µg/mL respectively. *Allium roseum* essential oil exhibited antibacterial and antifungal activities with a high effectiveness against *Candida albicans* given by an MIC value of 0.019 mg/mL. This biological effect appears to be related mainly to the presence of organosulfur compounds.

**Key words:** *Allium roseum* L., essential oil, chemical composition, antiproliferative activity, antioxydant activity, antimicrobial activity

## 1 INTRODUCTION

Alliaceae family, belonging to the order Liliales and comprising 30 genera and 600 species is widely distributed throughout the world. The largest and most important representative genus is *Allium*<sup>1</sup>. Scientific research on these plants started in the second half of the 19th century with Luis Pasteur in 1858, who showed the antibacterial properties of garlic<sup>2</sup>. The antibacterial bioactive principle of garlic was identified in 1944 by Cavallito as diallylthiosulfinate and was given the name allicin<sup>3,4</sup>. *Allium* is still being employed in folk medicine all over the world for the treatment of a variety of diseases. Several biological activities of *Allium* species including anti-cancer, anti-hypertensive,

anti-diabetic, antithrombotic and antimicrobial activity have also been reported<sup>5</sup>. Evidence from several investigations suggests that the biological and medicinal functions of *Allium* species are mainly due to their high organosulfur volatile compounds content through the hydrolysis of flavor precursor, S-alk(en)yl-L-cysteine sulfoxides (ACSOs), by alliinase action when the tissues are damaged<sup>6,7</sup>.

As a very polymorphous species, *Allium roseum* L. is represented in Tunisia by the presence of only three varieties: var. *grandiflorum* Briq., var. *perrotii* Maire. and var. *odoratisimum* (Desf.) Coss.<sup>1,8</sup>. The literature review could ascertain studies about *Allium roseum* species, except for

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the southern varieties odoratisimum which had been considered for the assessment of their possible biological activities<sup>9–11</sup>, but few information about the northern varieties var. grandiflorum, used in culinary preparations as condiment for couscous preparation in the north of Tunisia was reported. The chemical composition of essential oils of leaves, stems and flowers of *A. roseum* var. grandiflorum has been studied recently by Ben Jannet *et al.*<sup>12</sup> and Sakka Rouis-Soussi *et al.*<sup>13</sup> while that from the bulbs has never been investigated. Therefore, the present work investigates for the first time the chemical composition, antiproliferative, antioxidant and antimicrobial activities of the essential oil extracted from *A. roseum* var. grandiflorum Briq. bulbs collected in the North of Tunisia.

## 2 EXPERIMENTAL

### 2.1 Solvents and reagents

Dulbecco's modified Eagle's medium (DMEM) was purchased from GIBCO (Cergy-Pontoise, France) and foetal calf serum (FCS) from BioWhittaker (Fontenay-sous-Bois, France). Methanol was obtained from Merck (Darmstadt, Germany). Penicillin, streptomycin 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), butylated hydroxytoluene (BHT), n-alcane C8-C30, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and 2,2-diphenyl-1-picryl hydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2,4,6-Tripyridyl-s-triazine (TPTZ), ferric chloride hexahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ), sodium acetate and anhydrous sodium sulphate were procured from Fluka Chemical Co. (Buchs, Switzerland).

### 2.2 Plant material

*Allium roseum* bulbs were collected in Avril 2011 from a region of Sidi Thabet, area of Ariana (Northern Tunisia, latitude 36° 54' 45.25" N, longitude 10° 06' 02.10" E, altitude 30 m). Identification was made by Professor S. Ben Saad (Department of Botany, Faculty of Sciences of Tunis) according to the "Flora of Tunisia" handbook<sup>1</sup> and voucher specimens were deposited at the laboratory of Natural Substances mentioned above to serve as a future reference (Voucher no. 15).

### 2.3 Essential oil extraction

Two-hundred fifty grams of fresh bulbs were crushed in blender (IKA, Werke GmbH & Co., Germany) and then subjected to 4 h hydrodistillation using Clevenger apparatus. The obtained essential oil was dried over anhydrous sodium sulphate<sup>14</sup>.

### 2.4 Analysis of essential oil

#### 2.4.1 Gas chromatography

Gas chromatography analysis was performed using a Hewlett-Packard 6890 GC-FID apparatus (Agilent Technologies, Santa Clara, United States). Separation was performed using a non-polar HP5-MS (Phenyl Methyl Siloxane, 30 m × 0.25 mm i.d, film thickness 0.25 μm) capillary column. The oven temperature program is as follows: initially kept at 40°C, then incremented by 2°C/min, and finally held at 350°C for 15 min; injected volume: 1 μL; split ratio, 1/40; Injector temperature, 220°C; carrier gas: helium at a flow rate of 1 mL/min.

#### 2.4.2 Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) analysis was performed on an Autospec 610-M mass spectrometer coupled to a gas chromatograph (Model 6890) from Agilent Technologies. Analysis was performed using the same analytical columns and operating parameters as used in GC analysis. The results obtained were compared to the NIST and Wiley databases.

#### 2.4.3 Qualitative and quantitative analysis

The identity of components was assigned by comparison of their Kovats retention indices (RI) on HP5-MS columns and from their mass spectra. The RI was calculated using a C8–C30 hydrocarbons standard. Acquired mass spectra were compared to the NIST and Wiley mass spectra databases and to literature data. Relative amounts of individual components were calculated by electronic integration of FID peak areas without response factor correction.

### 2.5 Proliferation assay

#### 2.5.1 Cell culture

The cell lines used in this study was two human colonic adenocarcinoma cancer cell lines; (HT29-D4) and (CACO2) was purchased from American type culture Collection Company. Cells were cultivated in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine as a complete growth medium. Cells were maintained in culture and were incubated at 37°C in a humid atmosphere of 5% CO<sub>2</sub> in air. When confluence was about 80%, cells were sub-cultured by splitting with fresh medium at needed density for cytotoxicity, proliferation and antioxidant assay.

#### 2.5.2 Cytotoxicity assay

Cytotoxicity was assessed first by measuring the release of lactate dehydrogenase (LDH) activity into the culture medium upon damage of plasma membrane. Total release of LDH (100% toxicity) was obtained by adding 0.1% Triton-X100 in incubation medium. The supernatants were collected, clarified by centrifugation for 5 min at 600 g and 80 μL were submitted to LDH-based cytotoxicity kit (Sigma) in accordance with the manufacturer's instructions.

In a second time cell viability was assessed by MTT (3-

(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cells were cultured in standard conditions until confluence in microtiter plates. MTT solution (500 µg/mL final concentration) was added to the culture medium 4 h before the end of the treatment. Subsequently, the MTT solution was removed and replaced by 100 µL of DMSO in order to dissolve the precipitated formazan crystals. Finally, the absorbance was measured at 550 nm.

### 2.5.3 Proliferation assay

Cells were obtained in single cell suspension by treatment of subconfluent cell monolayers with Trypsine-EDTA. After centrifugation, cells were washed twice and resuspended with culture medium (5.000 cells/100 µL). After 2 h seed oil was added and cells allowed to proliferate for 72 hours at 37°C in a cell culture incubator. Cells were washed and then fixed by 1% glutaraldehyde. After staining by 0.1% crystal violet, cells were lysed with 1% SDS and the optical density was measured at 600 nm by a microplate reader<sup>15</sup>.

## 2.6 Antioxidant assays

### 2.6.1 DPPH<sup>•</sup> Scavenging assay

The diphenylpicryl hydrazyl radical (DPPH<sup>•</sup>) scavenging activity was evaluated. Bulbs essential oil was diluted in pure methanol at different concentrations ranging from 10 to 200 µg/mL, and then 2 mL were added to 0.5 mL of a DPPH<sup>•</sup> methanol solution (0.2 mmol/L). The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark, and then the absorbance was measured at 517 nm. For each concentration of the essential oil, the DPPH<sup>•</sup> scavenging activity was calculated as  $100 \times (A_0 - A_1)/A_0$ , where  $A_0$  is the absorbance of the control at 30 min, and  $A_1$  is the absorbance of the sample at 30 min. The antiradical activity was finally expressed as 50% inhibiting concentration (IC<sub>50</sub>). A lower IC<sub>50</sub> value corresponds to a higher antioxidant activity of essential oil<sup>16, 17</sup>. All samples were analyzed in triplicate.

### 2.6.2 Ferric reducing/antioxidant power (FRAP) assay

FRAP (Ferric reducing antioxidant power) was assayed. The FRAP, generally measures the antioxidant effect of any substance in the reaction medium as its reducing ability. The reducing ability in FRAP assay was calculated with reference to the reaction given by the FeSO<sub>4</sub>·7H<sub>2</sub>O. The activity was expressed as µmol of Trolox equivalents (TE) per 1g essential oil. A working solution was prepared freshly by mixing 25 mL of acetate buffer 300 mM (pH = 3.6) and 2.5 mL of 2,4,6-tripyridyl-s-triazine (TPTZ) solution 10 mM in 40 mM HCl and 20 mM of FeCl<sub>3</sub>·6H<sub>2</sub>O into a final volume of 2.5 mL. The mixed solution was incubated at 37°C for 30 min and was referred as FRAP solution. A sample (150 µL) was mixed with 2850 µL of FRAP solution and kept for 30 min in dark. The ferrous tripyridyltriazine complex (colored product) was measured by reading the absorbance at 593 nm (JASCO-V530). The standard curve

was prepared using Trolox ranging from 50 to 500 µM<sup>18</sup>.

### 2.6.3 Measurement of ROS: Lucigenin-enhanced chemiluminescence

ROS generation was measured by lucigenin chemiluminescence detecting superoxide ions. HT29 and CACO2 cells were seeded on 96-well microplates, after incubation with the essential oil for 15 min at 37°C, NADPH (1mM) and lucigenin (20 µM) in DMEM without phenol red was added. Luminescence was detected by a Fluoroscan Ascent FL fluorimeter (Labsystems, France). Signal was assessed at each minute over the course of 45 min. All measurements were performed at 37°C. Results are expressed as total ROS measurements and represent the percentage variation relative to untreated control<sup>19</sup>.

## 2.7 Antimicrobial assay

### 2.7.1 Bacterial and fungal strains

The microorganisms, maintained on nutrient Agar, were supplied by the microbiology laboratory of Pasteur institute (Tunis, Tunisia). Five bacteria strains were selected for antibacterial tests, including the Gram positive bacteria, *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 29212) and the Gram negative bacteria *Escherichia coli* (ATCC 8739), *Salmonella thyphymurium* (ATCC 14028), and *Klebsiella pneumoniae* (ATCC 13883) while the selected fungi is *Candida albicans* (UMIP 48.72/ATCC 10231) for antifungal test.

### 2.7.2 Agar diffusion methods

Antibacterial and antifungal activities of essential oil of the plant were assessed using the paper disk agar diffusion following the method described by National Committee for Clinical Laboratory Standard with some modification<sup>20</sup>. Absorbent disks (Whatman disk of 6mm diameter) were impregnated with 15 µL of essential oil or ampicillin and Gentamicin as a positive control and incubated at 37°C for 24 h. Antimicrobial activity was assessed by measuring the inhibition zone. This is the diameter of the zone visibly showing the absence of growth, including the 6mm disk.

### 2.7.3 Minimum Inhibitory Concentration (MIC)

The minimal inhibition concentration (MIC) was determined for all bacterial strains<sup>21</sup>. Essential oil sample that was dissolved in 10% dimethylsulfoxide (DMSO) was first diluted to the highest concentration (5 mg/mL) to be tested, and then serial two-fold dilutions were made in a concentration range from 0.004–5 mg/mL for oils in 5 mL sterile test tubes.

The 96-well plates were prepared by dispensing into each well 95 µL of nutrient broth and 5 µL of the inoculum. A 100 µL aliquot from the stock solutions of each essential oil was added into the first wells. The last well containing 195 µL of nutrient broth without essential oil and 5 µL of the inoculum on each strip was used as the negative control. The final volume in each well was 200 µL. The plates were incubated at 37°C for 24 h. After incubation,

twenty microliters of a p-iodonitro-tetrazolium (MTT) yellow solution (0.04%, w/v) (Sigma, USA) were added to each well. The plates were incubated for a further 30 min, and estimated visually for any change in colour from yellow to pink indicating reduction of the dye due to bacterial growth. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of the microorganisms.

## 2.8 Statistical analysis

All data were expressed as means  $\pm$  standard deviations of three measurements performed in triplicate. Evaluation of the statistical significance for observed differences was performed by one-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test (RStudio, Version 0.97, Boston, USA). Differences between means are considered statistically significant for  $p < 0.05$ .

## 3 RESULTS

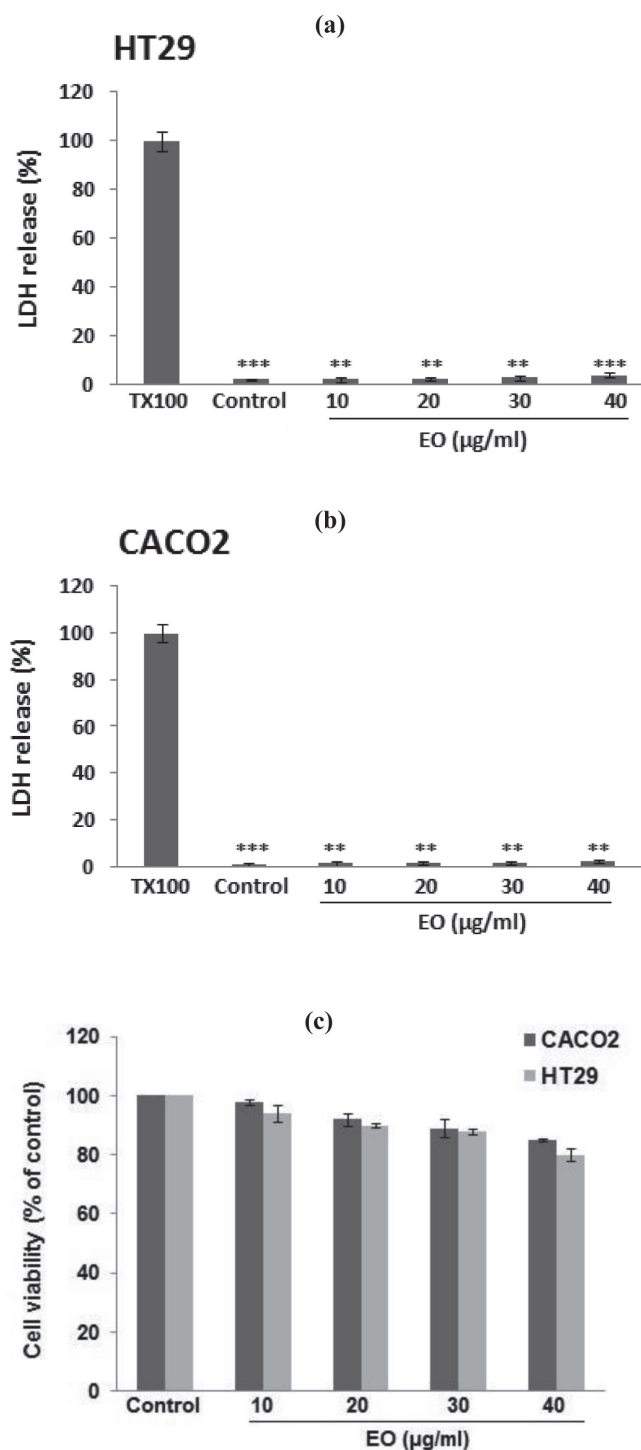
### 3.1 Chemical composition of essential oil

The *A. roseum* fresh bulbs subjected to hydrodistillation lead to a light yellow-colored essential oil with pungent odour at room temperature. The yield of the essential oil from bulbs of *A. roseum* var. *grandiflorum* was 0.024% (w/w).

Volatile compounds identified in the essential oil of *A. roseum* var. *grandiflorum* growing in the North of Tunisia are listed in Table 1, according to the order of their elution on the HP5-MS column, together with their percentage composition. The global chromatographic analysis of this essential oil showed twenty three identified compounds, representing 93.3% of the whole oil constituents. The oil was characterized by large amounts of organic sulphurous compounds making up 43.69% of the total eluted compounds; which may be classified into the following sulfides groups: disulfides (29.57%), trisulfides (11.24%), tetrasulfides (2.78%). The major constituents of sulfur-containing compounds in the volatile sample were methyl methanethiosulfinate (8.91%), followed by 3-vinyl-1,2-dithiacyclohex-

**Table 1** Chemical composition of the essential oil from *Allium roseum* L. var. *grandiflorum* bulbs.

No	Compounds	RI <sup>a</sup>	Percentage
1	2-3 butandiol	782	2.7
2	dimethyl disulfide	789	0.95
3	furfural	818	2.19
4	2-Furanmethanol	846	1.93
5	2-cyclopentene1, 4dione	849	5.19
6	methyl 2-propenyl disulphide	913	3.6
7	2-Furancarboxaldehyde, 5-methyl	965	5.57
8	methyl methanethiosulfinate	994	8.91
9	phenol	998	0.98
10	benzeneacetaldehyde	1045	5.13
11	diallyldisulfide	1077	3.16
12	hexanal	1097	6.23
13	benzeneethanol	1117	4.91
14	didulfide, methyl (methylthio) methyl	1140	4.57
15	3,4-dimethylthiophene	1161	2.59
16	3-vinyl-1,2-dithiacyclohex-4-ene	1180	3.56
17	3-vinyl-1,2-dithiacyclohex-5-ene	1199	6.81
18	dimethyl tetrasulfide	1207	2.78
19	2-furancarboxaldehyde, 5 (hydroxymethyl)-	1224	5.86
20	diallyl Trisulfide	1290	6.76
21	tetradecanoic acid	1760	2.69
22	tricosane	2300	2.55
23	pentacosane	2500	3.69



**Fig. 1** Essential oil (EO) cytotoxicity was assessed by measuring the release of lactate dehydrogenase (LDH) activity into the culture medium. Suspended HT29 (a) and CACO2 (b) cells were preincubated at room temperature with various concentrations of seed oil and then added in 96-well plates (100 µL/well). Upon 5 h at 37°C, 80 µL of clarified supernatant were submitted to LDH activity released by damaged cells using a colorimetric assay. Total release of LDH (100% toxicity) was obtained by adding 0.1% Triton-X100 in the assay medium (TX100). (c) MTT assay: CACO2 and HT29 cells after treatment with different concentrations of EO. Data shown are means (±SD) from 3 experiments performed in triplicate.  $p < 0.05$  was considered statistically significant compared with control.



5-ene (6.81%), diallyl trisulfide (6.76%), disulfide, methyl (methylthio)methyl (4.57%), methyl 2-propenyl disulfide (3.6%), 3-vinyl-1,2-dithiacyclohex-4-ene (3.56%) and diallyldisulfide (3.16%).

Carbonyl compounds (aldehydes and ketones) were the second major components of the volatile sample of *Allium roseum* (16.55%), followed by heterocyclic compounds (16.53%), alcohols (7.61%), alkanes (6.24%), and fatty acid found in relatively low levels (2.69%).

### 3.2 Antiproliferative activity

The cytotoxicity of *A. roseum* bulbs essential oil was investigated towards the human adenocarcinoma HT29 and CACO2 cell lines. Using the LDH assay, we found in the present study that volatile oil did not either significantly affect the viability of cells when incubated with concentrations up to 40  $\mu\text{g/mL}$  for both HT29 (Fig. 1a) and CACO2 (Fig. 1b) cell lines. In addition, cell viability was evaluated by MTT assay. As displayed in Fig. 1c, the viability of CACO2 and HT29 cells incubated for 24 h was not affected by 20  $\mu\text{g/mL}$  oil and only slightly affected by concentrations up to 40  $\mu\text{g/mL}$ .

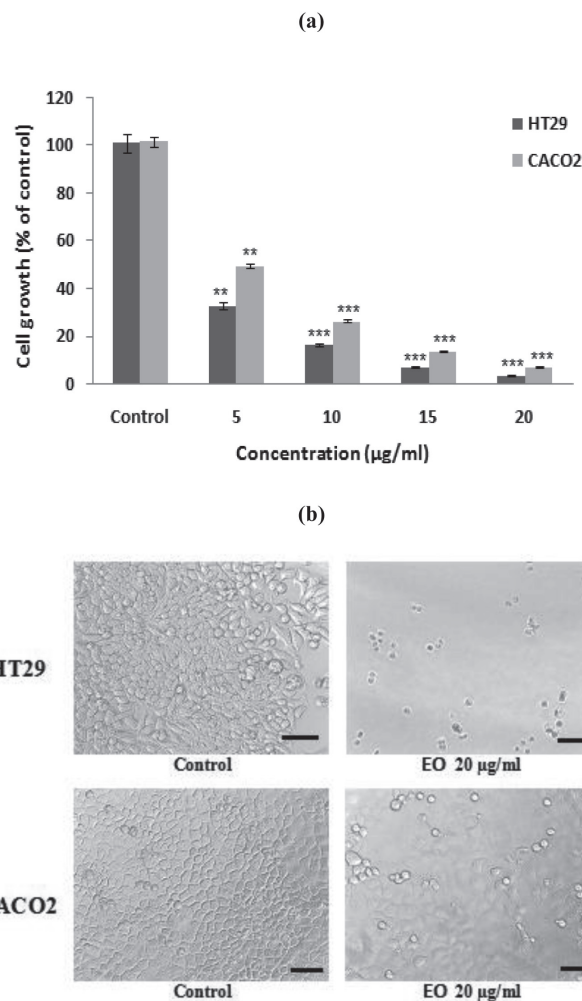
Antiproliferative assay showed that the number of cells was reduced by the presence of the essential oil in culture medium for cell lines without being cytotoxic. The growth-inhibitory effect of tumor cell lines was in dose-dependent manner with a half-maximal inhibition ( $\text{IC}_{50}$ ) respectively of 4.64  $\mu\text{g/mL}$  and 8.22  $\mu\text{g/mL}$  (Fig. 2a, b). The proliferation of HT29 cell lines was completely abolished in the presence of 20  $\mu\text{g/mL}$  of essential oil. The present study was the first report to provide evidence of *A. roseum* activity on tumor HT29 and CACO2 cell lines, but the HT29 was more sensitive.

### 3.3 Antioxidant activities

DPPH was a free radical that accepts an electron or hydrogen radical to become a stable molecule. DPPH radical scavenging was one of the commonly used methods to evaluate antioxidant activity of essential oils and phenolic extracts. Figure 3 illustrates scavenging of the DPPH radical by *A. roseum* essential oil. The scavenging effect of essential oil and standard (BHT) on the DPPH radical expressed as  $\text{IC}_{50}$  values was 156  $\mu\text{g/mL}$  for *A. roseum* oil vs. 31.5  $\mu\text{g/mL}$  for BHT. Therefore, this antioxidative activity of the bulbs essential oil was in a dose dependent manner.

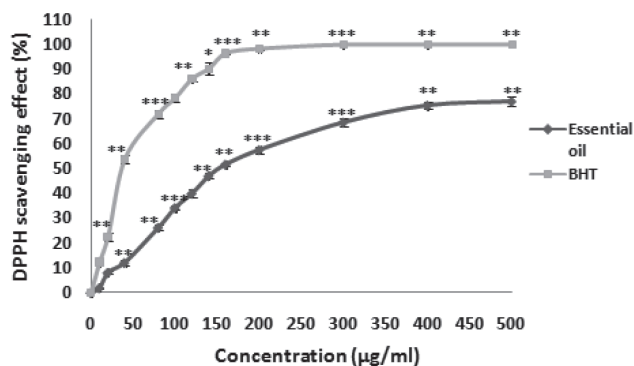
The FRAP assay showed high antioxidant activity and was 285  $\mu\text{mol}$  equivalent Trolox/g essential oil (Table 2). This study revealed that *A. roseum* essential oil was able to reduce the complex of ferric ion ( $\text{Fe}^{3+}$ ) TPTZ to another complex of ferrous ion ( $\text{Fe}^{2+}$ ) TPTZ by releasing an electron.

The present study also investigated the ability of bulbs essential oil of *A. roseum* to inhibit superoxide anion production on human colon adenocarcinoma HT29 and CACO2



**Fig. 2** (a) Essential oil (EO) of *A. roseum* inhibits colon cancer proliferation of adenocarcinoma HT29 and CACO2 human cells lines. Cells were resuspended with culture medium. After 2h different concentrations of oil were added and allowed to proliferate 72 h. Cells were quantified by staining with 0.1% crystal violet, solubilization with 1% SDS and measure of absorbance at 600 nm. Data shown are means ( $\pm$ SD) from 3 experiments performed in triplicate.  $p < 0.05$  was considered statistically significant compared with control. (b) Representative visualization of proliferation assay after treatment of cells without (control) or with 5, 10, 15 or 20  $\mu\text{g/mL}$  oil for 72 h. Metamorph imaging software was used to capture images. Scale bar: 70  $\mu\text{m}$ .

cell lines (Fig. 4). The bulbs essential oil of rosy garlic decreased significantly the luciferin enhanced chemiluminescence responses of the both HT29 and CACO2 cell lines. The inhibition of superoxide anion production was in a



**Fig. 3** Scavenging effect of *Allium roseum* essential oil bulbs on the stable DPPH. The antioxidant activity of essential oil extracted from *Allium roseum* bulbs at different concentration as determined by DPPH radical scavenging effect. DPPH: 1,1-diphenyl-2-picryl-hydroxyl free radicals, BHT: butylated hydroxytoluene. Data shown are means ( $\pm$ SD) from 3 experiments performed in triplicate.  $p < 0.05$  was considered statistically significant compared with control.

**Table 2** Antioxidant activity of *Allium roseum* L. var. grandiflorum bulbs essential oil.

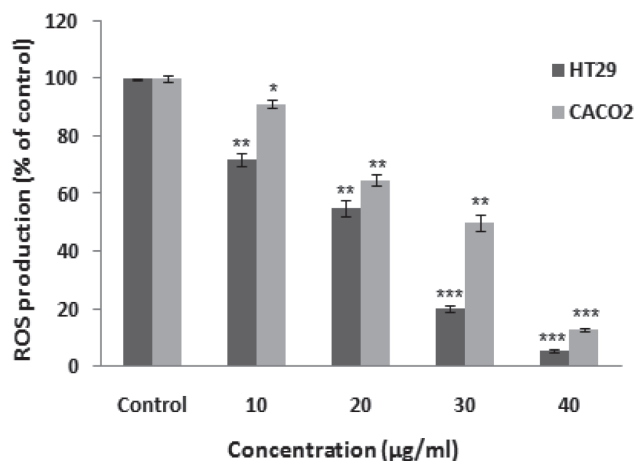
	Essential oil of Tunisian <i>A. roseum</i> bulbs	BHT
DPPH IC <sub>50</sub> (µg/mL)	156	31.5
FRAP (µmol TE/g sample)	285	433

dose related manner with a half-maximal inhibition (IC<sub>50</sub>) of 20.256 µg/mL and 29.123 µg/mL respectively. This inhibition was detectable at concentrations as low as 10 µg/mL. Results showed that bulbs essential oil of *A. roseum* var. grandiflorum was an exogenous ROS (superoxide anion) scavenging activity in a model of cancer cell lines in culture with pronounced effect with HT29 cells.

### 3.4 Antibacterial and antifungal activity

The antimicrobial activities of bulbs essential oil *Allium roseum* against microorganisms examined in the present study and its potency were assessed by the presence or the absence of inhibition zone diameter (Fig. 5) and with the Minimum Inhibitory Concentration (MIC).

Results (Table 3) showed that *A. roseum* essential oil exhibited antibacterial and antifungal activities given by a diameter of inhibition ranging from 11 to 28 mm. The best activities were observed against *C. albicans* with an MIC value of 0.019 mg/mL followed by Gram negative bacteria (*E. coli*, *K. pneumoniae*, and *S. thyphymurium*). A less inhibitory activity was detected against Gram positive bacteria with a minimal inhibitory concentration respectively of 0.625 and 2.5 mg/mL for *S. aureus* and *E. faecalis*.



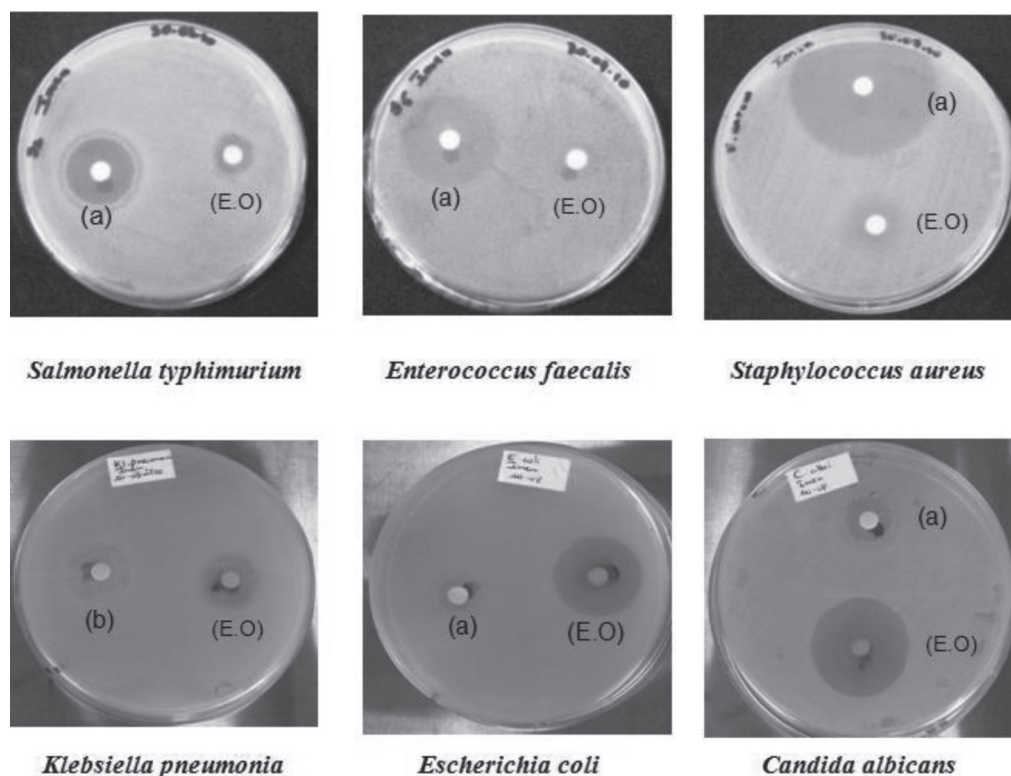
**Fig. 4** Production of anion superoxide (O<sub>2</sub><sup>-</sup>) in Human colon adenocarcinoma (HT29) and CACO2 cancer cell lines exposed to (EO) essential oil of *A. roseum* (10-40 µg/mL). The production of O<sub>2</sub><sup>-</sup> was determined by lucigenin luminescence. Cells were plated at 50×10<sup>3</sup> cell/well. Essential oil of *A. roseum* significantly inhibited O<sub>2</sub><sup>-</sup> production in the HT29 and CACO2 cells compared with untreated cells. Data shown are means ( $\pm$ SD) from 3 experiments performed in triplicate.  $p < 0.05$  was considered statistically significant compared with control.

## 4 DISCUSSION

Sulphurous compounds were the major constituents of volatiles found in *Allium* as shown by previous studies. They comprised about 39.2% of total volatiles recovered from *Allium cepa*, 95% from *Allium ursinum* and 23.6% from *Allium roseum* var. odoratisimum from the South of Tunisia<sup>9, 22, 23</sup>. Comparison of results with previous ones concerning the southern Tunisian varieties odoratisimum showed many differences. The major component of *A. roseum* var. odoratisimum flowers essential oil was identified as methional (17.1%), which is absent in bulbs essential oil of the northern varieties grandiflorum<sup>9</sup>. In fact, some of the var. grandiflorum compounds were not found in the flowers essential oil of the Southern varieties like methyl methanethiosulfinate namely also dimethyl disulphide, diallyldisulfide, diallyl trisulfide and dimethyl tetrasulfide. Ben Jannet *et al.* reported that the Tunisian *Allium roseum* var. grandiflorum flowers gathered in the region of Monastir was rich in sulphur compounds (60.97%) and allyl methyl disulphide (16.06%) and diallyl disulphide (16.57%) was found as major compounds however the stem's oil was rich in fatty acid esters which represent 65.23% of all its components<sup>12</sup>.

The leaf essential oil *A. roseum* var. grandiflorum was rich in carboxylic acids. Hexadecanoic acid was detected as the major component, with 75.9% of the totality of the





**Fig. 5** Antimicrobial activity of essential oil extracted from *Allium roseum* bulbs. Antibacterial and antifungal activity was expressed by inhibition zones after applying 15  $\mu$ L of essential oil from *Allium roseum* bulbs or 15  $\mu$ L of ampicilline and gentamicine. (a): positif contrôle 1 : Ampicilline ; (b): positif contrôle 2 : Gentamicine ; (E.O) : Essential Oil.

**Table 3** Antibacterial and antifungal activity of essential oil extracted from *Allium roseum* L. var. grandiflorum bulbs.

Microorganisms	Inhibition zone diameter (mm)			CMI (mg/mL)
	Essential oil	Ampicilline	Gentamicine	
Gram-positive bacteria				
<i>Staphylococcus aureus</i> ATCC 6538	16	38	n.d	0.625
<i>Enterococcus faecalis</i> ATCC 29212	11	36	n.d	2.5
Gram-negative bacteria				
<i>Escherichia coli</i> ATCC 8739	24	13	n.d	0.078
<i>Salmonella thyphimurium</i> ATCC 14028	16	23	n.d	0.312
<i>Klebsiella pneumonia</i> ATCC 13883	19	0	22	0.156
Yeast strains				
<i>Candida albicans</i> ATCC 10231	28	16	n.d	0.019

n.d : not determined

oil. Four sulfur compounds were identified representing 3.8% of the totality of the oil with di-2-propenyl trisulfide (1.7) as the major sulfurous compounds. In contrast, in our study only smaller percentages of tetradecanoic acid (0.25%) were present in the essential oil of *A. roseum* grandiflorum<sup>13</sup>.

In the present studies we demonstrated that the sulfurous compounds methyl methanethiosulfinate was only present in the bulbs essential oil of *A. roseum* var. grandiflorum.

Observed differences in chemical composition of essential oils depend mainly on climatic, seasonal and geographic

conditions. It should be pointed out that the presence of these sulphurous compounds at high concentrations may be used as a chemo-taxonomic marker of the *A. roseum* essential oil from Tunisia.

Several pieces of evidences suggested that *Allium* genus possess anticancer properties as shown by their ability to suppress tumor proliferation *in vivo* and *in vitro*<sup>2)</sup>. *Allium roseum* belongs to the same biological genus as *Allium sativum* (garlic) and other onions. This species was a medicinal plant in Tunisia that its antiproliferative effects have not received any attention. In this context the present study was carried out to evaluate the *in vitro* antiproliferative activity of the essential oil on the human colonic adenocarcinoma. Potential antiproliferative effect appears to be related mainly to the presence of organosulfur compounds. Previous study showed also that *A. hirtifolium* chloroformic extract rich in organosulfur compounds especially allicin might inhibit proliferation of HeLa (cervical cancer) and MCF-7 (human breast carcinoma) tumor cell lines. Fifty percent inhibition of cells occurred with 20 µg/mL and 24 µg/mL respectively<sup>24)</sup>. The major compounds found in *A. roseum* bulbs essential oil was *S*-methyl methanethiosulfinate analogue of diallyl thiosulphinate (Allicin). Previous results indicate that thiosulfates, and *S*-methyl methanethiosulfinate isolated from *Allium tuberosum* inhibit *in vitro* proliferation of human breast cancer cells (MCF-7) through apoptosis in concentration and time-dependent manners. This compounds have also *in vivo* antitumor activity in sarcoma-180 tumor-bearing mice<sup>25)</sup>. Allyl sulfides isolated from garlic essential oil show various effects on inhibition of proliferation of human liver tumor cell (J5). Diallyl disulfide (DADS) markedly suppressed the growth of cultured human colon tumor (HCT-15) cell lines and induces a G2/M phase arrest<sup>24)</sup>. Studies from various laboratories revealed that several mechanisms have been proposed to explain the benefits of *Allium* organosulfur compounds. The medicinal effect was not limited to a specific species, to a specific tissue or to a specific cancer type. Therefore, the essential oil possibly modify common pathway(s), controlling cell proliferation by the inhibition of DNA adducts formation, apoptosis and free-radical scavenging, but further investigations were needed to elucidate cellular mechanisms involved in the suppression of growth in human colon cancer cell lines.

The antioxidant activity (FRAP and DPPH) of the northern varieties (var. *grandiflorum*) of bulbs essential oil of rosy garlic was higher than that of the leaves methanolic extract of the southern varieties (var. *odoratissimum*) of the same species. DPPH assay show that *A. roseum* var. *grandiflorum* bulbs essential oil present an IC<sub>50</sub> value of 156 µg/mL however previous study show that the leaves methanolic extract of the var. *odoratissimum* present IC<sub>50</sub> value of 240.4 µg/mL<sup>10, 11)</sup>. *A. roseum* var. *grandiflorum* bulbs essential oil exhibited the strongest antiradical activities

when compared with ethyl acetate extract of the stems and leaves of the same varieties with a 50% inhibition concentration (IC<sub>50</sub>) of 0.156 mg/ml and 0.35 mg/mL of DPPH· respectively<sup>13)</sup>.

Luciginin enhanced chemiluminescence assay showed a potential inhibition of the superoxide anion production in a model of human colon adenocarcinoma HT29 and CACO2 cell lines in culture with pronounced effect with HT29 cells.

Many of *Allium* organo-sulfurous compounds have been already identified to have anti-oxidant defense mechanisms, which are mainly mediated by thiols together with their corresponding disulfides, making up an intracellular redox-buffer<sup>26)</sup>. Alliin isolated from *A. sativum* have been reported to have potent reducing abilities when examined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable free radical scavenging assay. Diallyl sulfide, diallyl disulfide and diallyl trisulfide derivatives found in garlic can inhibit superoxide production generated by xanthine-xanthine oxidase which was able to chelate copper ions.

This study demonstrated that decrease of ROS production by using rosy garlic bulbs essential oil might exert a beneficial effect in the prevention of colon cancer diseases related to the generation of ROS. The high content of organo-sulphur compounds in this essential oil can explain the antioxidant activity of this essential oil but more studies were needed to elucidate mechanisms. A promising strategy for targeting redox status of the cells was to use readily available natural substances from *Allium* vegetables food<sup>2, 26)</sup>.

Good correlation between FRAP, DPPH radical scavenging activity and inhibition of superoxide anion production in a model of cancer cell lines in culture assays was observed and suggesting that antioxidants in the essential oil possessed the ability of scavenging the free radicals DPPH and superoxide anion, together with reducing power of ferric ion.

Highlighted antimicrobial bulbs essential oil of rosy garlic can be assumed to be useful in warding off infectious diseases and there is a compelling reason to suppose that anti-infective agents could be active against human pathogens as suggested by folkloric and historical accounts<sup>2)</sup>. Indeed, infections caused by *C. albicans* are among the most difficult to treat with conventional antifungal agents<sup>27)</sup>. Therefore, this herbal product could thus be used as a drug to improve the treatment of candidose or could be used as a food-conserving agent.

*A. roseum* essential oil showed high content of organo-sulphur compounds. Methyl methanethiosulfinate namely also dimethylthiosulfinate was present with high percent. This compound is a thiosulphinate with a similar structure to its analogue found in garlic; diallyl thiosulphinate or Allicin. Diallyl thiosulphinate (allicin) was discovered as the responsible of fresh garlic flavour and its active principle, is only present after rupture of the cell membranes, that

allows the enzyme alliinase to degrade S(+)-allyl-l-cysteine sulfoxide (alliin), producing allicin (thiosulfinate)<sup>3)</sup>. Thiosulfinate diallyl has an important antibacterial activity against both Gram positive and Gram negative bacteria such as *E. coli*, *S. aureus*, *Klebsiella*, *Salmonella*, *Micrococcus*, *B. subtilis*, *Mycobacterium*, and *Clostridium*<sup>2)</sup>. The 3-vinyl-1,2-dithi-4-ene and the 3-vinyl-1,2-dithi-5-ene, products of instantaneous decomposition of thiosulfinate, are also known as an important antimicrobial compounds<sup>5)</sup>.

## Conclusions

This work represents the first attempt to study the chemical composition and the biological activities of the bulbs essential oil from *A. roseum*, var. *grandiflorum*, an endemic Mediterranean specie growing wild in the North of Tunisia. The qualitative and the quantitative analysis of essential oil are characterized by large amounts of organic sulphurous compounds making up 43.69% of the total eluted compounds. We have attempted to find new results on the antiproliferative effects of essential oil rich in organo-sulfur compounds extracted from *A. roseum* on human colon cancer cell lines. These findings demonstrate the potential of this spice as an inhibitor of production of superoxide anion in a model of cancer cell lines in culture and as anticandidose drug. We are also continuously studying to better understand the various mechanisms regulating antiproliferative effects as well as inhibition of ROS production on cancer cell lines.

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