Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: http://www.tandfonline.com/loi/iphb20

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To cite this article: Antonella Lisanti, Vincenzo Formica, Federica Ianni, Barbara Albertini, Maura Marinozzi, Roccaldo Sardella & Benedetto Natalini (2015): Antioxidant activity of phenolic extracts from different cultivars of Italian onion (Allium cepa) and relative human immune cell proliferative induction, Pharmaceutical Biology, DOI: 10.3109/13880209.2015.1080733

To link to this article: http://dx.doi.org/10.3109/13880209.2015.1080733

Published online: 09 Oct 2015.

PHARMACEUTICAL BIOLOGY
2015; EARLY ONLINE: 1–8
http://dx.doi.org/10.3109/13880209.2015.1080733

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RESEARCH ARTICLE

Antioxidant activity of phenolic extracts from different cultivars of Italian onion (*Allium cepa*) and relative human immune cell proliferative induction

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ABSTRACT

Context: The total antioxidant activity (TAC) may vary considerably between onion cultivars. Immunological effects of onion phenolic compounds are still underestimated.

Objective: The objective of this study is to determine the total phenol content (TPC) and the relative TAC of three *Allium cepa* L. (Liliaceae) onion cultivars cultivated in Cannara (Italy): *Rossa di Toscana*, *Borettana di Rovato*, and *Dorata di Parma*, and to evaluate the phenol extracts ability to induce human immune cell proliferation.

Materials and methods: TPC was determined by the Folin–Ciocalteu method, TAC with FRAP, TEAC/ABTS, and DPPH methods. Peripheral blood mononuclear cells from healthy human donors were incubated for 24 h at 37 °C with 1 ng/mL of phenolic extract in PBS, immunostained, and then analyzed by 4-color flow cytometry for the phenotypic characterization of T helper cells (CD4+ cells), cytotoxic T lymphocytes (CD8+ cells), T regulatory cells (CD25high CD4+ cells), and natural killer cells/monocytes (CD16+ cells).

Results: *Rossa di Toscana* displayed the highest TPC (6.61 ± 0.87 mg GA equivalents/g onion bulb DW) and the highest TAC with the experienced methods: FRAP, 9.19 ± 2.54 μmol Trolox equivalents/g onion bulb DW; TEAC/ABTS, 21.31 ± 0.41 μmol Trolox equivalents/g onion bulb DW; DPPH, 22.90 ± 0.01 μmol Trolox equivalents/g onion bulb DW. Incubation with *Rossa di Toscana* extract determined an increase in the frequency of the antitumor/anti-infection NK CD16+ immune cells (23.0 ± 0.4%).

Discussion and conclusions: Content of health-promoting phenols and the deriving antioxidant and immunostimulating activity vary considerably among the investigated cultivars. *Rossa di Toscana* can be considered as a potential functional food.

ARTICLE HISTORY
Received 26 February 2015
Revised 22 July 2015
Accepted 4 August 2015
Published online 7 October 2015

KEYWORDS
Immunological effect, onion bulb extracts, total antioxidant capacity, total phenol content

Introduction

The Mediterranean diet is recognized as a very healthy dietary model. Indeed, a plethora of epidemiological studies have clearly shown that people from Mediterranean countries have a longer life expectancy and a lower risk of contracting certain chronic diseases (Dedoussis et al., 2004; Goulet et al., 2003). Vegetables are the most important source of phenolic compounds in the Mediterranean diet, among which flavonoids in particular are thought to be essential bioactive species that provide health benefits (Ninfali et al., 2005; Panico et al., 2005). *Allium cepa* L. (Liliaceae) bulb onion has been recognized as an important reservoir of valuable phytonutrients such as flavonoids, fructo-oligosaccharides, thiosulfinates, and other sulfur containing compounds (Slimestad et al., 2007).

The onion probably originated in central Asia and was then introduced in Europe by the Phoenicians around 2000 years ago; it is cultivated worldwide thanks to its adaptability and ability to occupy a wide range of ecological niches. The onion bulb is not only a food, but it has also well-known medicinal and functional properties, since ancient time; Pliny the Elder, for example, in his *Naturalis Historia*, describes 30 ailments that can be alleviated by onions and pointed out that any onion-containing dishes are, to a certain extent, curative as well as more nourishing and tasty.

Recent literature is rich in both *in vivo* and *in vitro* studies reporting the antithrombotic (Lee et al., 2013), hypolipidemic (Lee et al., 2012b; Srinivasan, 2013), antidiabetic (Jung et al., 2011), antiobesity (Yoshinari et al., 2012), antioxidant (Alpsoy et al., 2013; Lee et al., 2012a), anti-inflammatory (Dorsch et al., 1990), cancer

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chemopreventive (Wang et al., 2012), and antiparasitic (Aboelhadid et al., 2013; Klimpel et al., 2011) properties of onion bulb extracts. Moreover, accumulated epidemiological studies suggest that a diet rich in onions may also have favorable effects on the risk of acute myocardial infarction (Galeone et al., 2009), and benign prostatic hyperplasia (Galeone et al., 2007).

As already anticipated, onion's health benefits have been mainly ascribed to two chemical classes of compounds, namely flavonoids and alk(en)yl cysteine sulfoxides (Griffiths et al., 2002). The first class includes anthocyanins, which impart a red/purple color to some cultivars, and flavonols such as quercetin and its derivatives responsible for the yellow flesh and brown skins of many other cultivars. In Italy, several onion cultivars are farmed although only *Tropea Red Onion*, that was recently awarded with the protected geographical indication (PGI) certification from the European Union, has been the subject of many scientific investigations (Bonaccorsi et al., 2005; Corea et al., 2005; Dini et al., 2005, 2008a, b; Furia et al., 2011; Gennato et al., 2002). Contrariwise, a unique study (Marinozzi et al., 2014) has dealt with the three Italian onion cultivars (*Rossa di Toscana*, *Borettana di Rovato*, and *Dorata di Parma*) widely farmed in Cannara, a small town in the Umbria region, well known since the ancient time for onion growing, that has developed thanks to the water abundance and the favorable geo-pedological characteristics of the soil. *Rossa di Toscana* (Figure 1a), is round and globular with purple tunics, *Borettana di Rovato* (Figure 1b), is characterized by a flat, pale yellow bulb, while *Dorata di Parma* presents a golden bulb similar to a spinning (Figure 1c).

Content of potential health-promoting substances, such as phenol derivatives, and the deriving antioxidant activity, may vary considerably between cultivars and undergo seasonal variation within a cultivar; this fact is often neglected in scientific investigations. In this scenario, the present study determines and compares the total phenol content (TPC) and the relative total antioxidant capacity (TAC) of the bulb of the three onion cultivars farmed in Cannara. TPC was determined by the fully validated Folin–Ciocalteu method while TAC was assayed by using the well-established and here fully validated spectrophotometric methods based on the FRAP, TEAC, and DPPH assays.

Phenolic extracts were also tested *in vitro* for their ability to induce human immune cell proliferation, and in particular growth of CD16+ natural killer cells, which are known to play a protective role against cancer and infection (Taglia et al., 2008).

Materials and methods

Chemicals and reagents

Folin–Ciocalteu reagent, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonate) diammonium salts (ABTS), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrochloric acid (HCl), ferric chloride ($FeCl_3$), sodium acetate (NaOAc), sodium carbonate (Na_2CO_3), acetic acid (AcOH), gallic acid (GA), ethanol (EtOH), phosphate buffered saline (PBS), and potassium persulfate were purchased from Sigma-Aldrich (Milano, Italy).

Immunological tests

Peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors by Ficoll (MP Biomedicals, Segrate, Italy) density gradient separation as per standard procedure and washed twice with PBS. PBMCs were then resuspended in 1 mL of culture medium (Roswell Park Memorial Institute, RPMI, Buffalo, NY) and incubated for 24 h at 37 °C temperature at a concentration of 1×10^7 cells/mL plus either PBS (negative control) or 1 ng/mL of phenolic extract in PBS. After incubation, PBMCs were immunostained and then analyzed by 4-color flow cytometry for the phenotypic characterization of T helper cells (CD4+cells), cytotoxic T lymphocytes (CD8+cells), T regulatory cells (CD25 high CD4+cells) and natural killer cells/monocytes (CD16+cells).

Figure 1. Representative pictures of (a) *Rossa di Toscana*, (b) *Borettana di Rovato*, and (c) *Dorata di Parma* characteristic bulb.

Immunostaining was performed by incubating PBMCs for 30 min at 4 °C with fluorescein isothiocyanate (FITC)-conjugated antiCD25 (from BD Pharmingen, Milan, Italy), phycoerythrin-(PE) conjugated antiCD16 (BD Pharmingen, Milan, Italy), PerCP Cy5.5-conjugated antiCD4 (from eBioscience, Milan, Italy) allophycocyanin (APC)-conjugated antiCD8 (eBioscience, Milan, Italy) antibodies, in PBS. All experiments were performed in triplicate and immunological tests were repeated using PBMCs from three different healthy donors to minimize intra-assay and inter-assay variabilities (results were considered acceptable for variation <±2% from recorded mean values).

Instrumentation

All the UV spectra were recorded at 25 °C with a Varian Cary 100 (Varian Inc., Palo Alto, CA) dual beam, dual chopper spectrophotometer. For the immunological tests, samples were run on a FACSCalibur flow cytometer from BD Biosciences (Erembodegem, Belgium) with 5×10^4 events acquired and data analyzed using the CellQuest software (BD Biosciences, Milan, Italy).

Preparation of onion samples and extraction of phenols

Onion samples were cultivated in a farm belonging to the Cannara Onion Producers Union (Consorzio dei Produttori della Cipolla di Cannara) in the municipality of Cannara and harvested on September 2013. The plant material was carefully verified by the specialized personnel of the Cannara Onion Producers Union before being delivered for the execution of the present scientific study. Soon after harvesting, 10 onion bulbs for each cultivar were skinned, chopped, blended, and finally freeze-dried. The freeze-dried onion bulbs were mixed in a mortar to obtain a fine powder and stored at 4 °C in the dark if not immediately handled.

A water:EtOH (25:75, v/v) solution (3 mL) was added to 0.3 g freeze-dried onion bulb powder. The solution was kept under magnetic stirring at 900 rpm at 25 °C for 30 min and placed in an ultrasonic bath for 20 min. After centrifugation at 3000 rpm at 25 °C for 15 min, the supernatant solution was removed and the pellet twice submitted to the procedure, above described. Finally, the three supernatants were combined and diluted to 10 mL with the extraction solvent.

Statistical analysis

All the statistical analyses were performed by SigmaPlot software within Microsoft Excel (Sigma, St. Louis, MO). Mean values obtained for the variables studied in the different groups were compared by one-way analysis of variation (ANOVA and ANOVA on ranks when the statistical distribution is not normal) (Student–Newman–Keuls method) assuming significant differences among them when the statistical comparison gave $p<0.05$.

Spectrophotometric analysis

Determination of total phenol content by the Folin–Ciocalteu method

Total phenol content (TPC) of each extract was determined by means of the Folin–Ciocalteu method (Sun et al., 2007). The assay is based on the reduction of a complex made by a mixture of phosphomolibdic ($H_3PMo_{12}O_{40}$) and phosphotungstic acid ($H_3PW_{12}O_{40}$) by phenol species to give a blue mixture of oxides (Mo_8O_{23} and W_8O_{23}). A 10-fold dilution of the Folin–Ciocalteu reagent was made with deionised water. Extracts (0.1 mL) were mixed with 0.75 mL of the diluted Folin–Ciocalteu reagent and incubated for 10 min at room temperature. Finally, 0.75 mL of a 2% Na_2CO_3 (w/v) aqueous solution was added and the mixture kept in the dark for 45 min before measuring the absorbance at 765 nm. Analyses were performed in triplicate on each of the three parcels of each of the three extracts from the investigated onion cultivars.

TPC values were determined from a calibration curve prepared with GA standard solutions by applying the same procedure as for the extracted sample. Results are expressed both as mg of GA equivalents/g onion bulb dried weight (DW).

Determination of antioxidant activity by the FRAP method

The assay is based on the ability of the antioxidants to reduce (through an electron transfer mechanism) Fe^{3+} to Fe^{2+} ions in the presence of TPTZ, forming an intense blue Fe^{2+}–TPTZ complex with an absorption maximum at 593 nm. The reaction is pH-dependent (optimum pH 3.6) and the absorbance decrease is proportional to the content of species endowed with antioxidant activity.

The FRAP reagent was prepared by combining 2.5 mL of a TPTZ solution (10 mM) in HCl (40 mM), 2.5 mL of a $FeCl_3$ solution (20 mM), and 25 mL of NaOAc buffer (300 mM, pH 3.6). Then, the FRAP reagent (1.5 mL) was mixed with 100 µL of deionised water and 100 µL of the sample extracts. The reaction mixture was kept for 4 min at room temperature and then the corresponding absorbance was measured in the correspondence of the

absorption maximum (Lu et al., 2011). Analyses were performed in triplicate on each of the three parcels of each of the three extracts from the investigated onion cultivars.

Values were determined from a calibration curve obtained with Trolox solutions at fixed concentrations, following the same procedure as for the extracted sample. Results are expressed both as μmol of Trolox equivalents/g onion bulb DW.

Determination of the radical scavenging capacity by the TEAC method

This method is based on the capacity of antioxidants to neutralize the radical cation blue/green $ABTS^{+\bullet}$ chromophore to a colorless ABTS form. The $ABTS^{+\bullet}$ form is prepared by an oxidation reaction of ABTS with potassium persulfate. A decrease of the concentration is linearly dependent on the antioxidant concentration.

The radical scavenging capacity was measured using a slightly modified version of the TEAC method described by Re et al. (1999). ABTS (7.0 mM) and potassium persulfate (2.45 mM) solutions were separately prepared in water and the resulting solution was diluted in water up to a volume of 10 mL. The flask was then fully covered with aluminum foil and allowed to stand at room temperature for 12 h in the dark (Re et al., 1999). The obtained $ABTS^{\bullet+}$ solution was diluted with EtOH (1:100, v/v) until getting an absorbance of 0.70 (±0.02) at 734 nm. The analysis solution was obtained by mixing 1.96 mL of $ABTS^{\bullet+}$/EtOH solution and 40 μL extract sample or Trolox standard solution.

The absorbance was measured at 734 nm after 6 min standing. The analysis was performed in triplicate for each triplicate extract of each onion cultivar. Values were determined from a calibration curve prepared with Trolox solutions prepared following the same procedure as for the extracted sample. Results are expressed both as μmol of Trolox equivalents/g onion bulb DW

Determination of the radical scavenging capacity by the DPPH method

This method relies on the use of the free radical DPPH. The odd electron in the DPPH free radical produces a strong absorption maximum at 517 nm and produces a color purple. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces as a result of the pairing of the odd electron of DPPH radical with a proton from a free radical scavenging antioxidant to produce DPPH-H.

The radical scavenging capacity was measured using a slightly modified version of the DPPH method described by Floegel et al. (2011). DPPH was progressively solubilized in neat EtOH until a concentration producing an absorbance of 0.65 (±0.02) at 517 nm was reached. The analysis solution was obtained by mixing 2.95 mL DPPH/EtOH solution and 50 μL of extract sample or Trolox standard solution.

The absorbance was measured at 517 nm after 30 min standing in the dark. The analysis was performed in triplicate for each triplicate extract of each onion cultivar. Values were determined from a calibration curve prepared with Trolox solutions prepared following the same procedure as for the extracted sample. Results are expressed as μmol of Trolox equivalents/g onion bulb DW.

Method validation

The analytical methods applied for the assessment of the total phenol content, the antioxidant activity, as well as of the radical scavenging activity were fully validated before their use.

Linearity, LOD, and LOQ

With the Folin–Ciocalteu method, the TPC in the three onion cultivars was established by relying upon a calibration curve built with GA standard solutions with concentration values spanning in the range reported in Table 1 (method 1). The calibration curve in was constructed by plotting the concentration value of each standard solution against the corresponding absorbance recorded at 765 nm. For the FRAP method, the antioxidant activity of the three onion cultivars was established by a calibration curve prepared with Trolox standard solutions with concentration values spanning in the range indicated in Table 1 (method 2). The calibration curve was constructed by plotting the concentration value of each standard solution against the corresponding absorbance recorded at 593 nm. The antioxidant activity of the bulb of the three onion cultivars was also established by the TEAC/ABTS and DPPH assays, using calibration curves prepared with Trolox standard solutions with concentration values spanning in the range reported in Table 1 (methods 3 and 4, respectively). The calibration curve for the TEAC/ABTS assay (Table 1, method 3) was constructed by plotting the concentration value of each standard solution against the corresponding absorbance value recorded at 734 nm, while for the DPPH assay at 517 nm (Table 1, method 4). For all the methods, each solution was previously treated as the real sample, and analyzed in triplicate. As indicated by the R^2 values, the three obtained mathematical model are characterized by a very good linearity.

Table 1. Calibration data for the four spectrophotometric methods: method 1, Folin–Ciocalteu (Abs @ 765 nm); method 2, FRAP(Abs @ 593 nm); method 3, TEAC/ABTS (Abs @ 734 nm); method 4, DPPH (Abs @ 517 nm).

Method #	Regression equation	Eq.	Linearity range (mg/mL)	R^2	LOD (μg/mL)	LOQ (μg/mL)
1	$y = 1.012\ (\pm\ 0.007)x - 0.0045\ (\pm\ 0.001)$	1	0.05–0.25	0.9996	0.5	0.2
2	$y = 2.08\ (\pm\ 0.06)x + 0.014\ (\pm\ 0.004)$	2	0.0037–0.1251	0.9978	0.3	0.9
3	$y = -2.580\ (\pm\ 0.053)x + 0.697\ (\pm\ 0.009)$	3	0.05–0.25	0.9988	0.1	0.3
4	$y = -2.376\ (\pm\ 0.033)x + 0.696\ (\pm\ 0.005)$	4	0.05–0.25	0.9988	0.1	0.4

The LOD and LOQ values (Table 1) were measured from the following equations:

$$C_{LOD} = 3.3\sigma_y/b \quad (1)$$

$$C_{LOQ} = 10\sigma_y/b \quad (2)$$

where C_{LOD} and C_{LOQ} are the sample concentration corresponding to the LOD and LOQ, respectively, σ_y is the standard error of the regression equation and b is the slope of the corresponding regression equation in Table 1.

Intra-day and inter-day precision and accuracy

The methods were validated using external set of two external control solutions with concentration included within the linearity range (Table 2). For each concentration, three separate solutions were prepared and analyzed in triplicate ($n = 9$). This procedure is repeated for three consecutive days. The mathematical models (Table 1) were then used to calculate the concentrations of the external control solutions. The intra-day precision was determined as the relative standard deviation (RSD%) among the concentration values achieved from consecutive analysis carried out within the same day. As evident from the data in Table 2, for all the assays, a good variation of RSD% values was maintained for the two control solutions during the consecutive 3 d of analysis, thus indicating a high reproducibility of the adopted spectrophotometric methods in the short-period.

In all the cases, for each control solution, the variation in a time-frame of three consecutive days ($n = 27$) was employed to determine the inter-day precision. A satisfactory precision was also found when the long-period was considered (Table 3).

The "Recovery test" approach (percentage recovery, recovery%) was selected to estimate the accuracy of the considered spectrophotometric methods. The accuracy values were calculated according to the following equation:

$$\text{Recovery\%} = C_{measured}/C_{theoretical}100 \quad (3)$$

where $C_{measured}$ is the sample concentration (mean observed concentration) as calculated through the regression equation in Table 1, while $C_{theoretical}$ is the concentration of the employed external test solutions (theoretical concentration). By analogy with the estimation of short- and long-term precision, the intra-day and the inter-day accuracy were also calculated (Tables 2 and 3). While the former was determined by considering the nine spectrophotometric analysis of each control solution within a single day ($n = 9$) (Table 2), for the latter, the average value from 27 determinations (during the 3 d of analysis) was considered (Table 3). The recovery% values listed in Tables 2 and 3 are indicative of a good accuracy of each spectrophotometric method both in the short- and the long-period.

Table 2. Statistical analysis for the four selected spectrophotometric methods in the short period (intra-day).

Method #	Solution	Theoretical concentration (mg/mL)	Day	n^a	Precision (RSD%)	Accuracy (recovery%)
1	1	0.12	1	9	0.33	102.30
			2		1.99	100.70
			3		0.36	100.30
	2	0.22	1	9	1.06	100.20
			2		3.15	99.80
			3		0.51	100.10
2	1	0.01	1	9	6.90	99.30
			2		5.03	103.20
			3		2.11	91.30
	2	0.11	1	9	5.53	102.50
			2		3.80	105.20
			3		2.07	95.80
3	1	0.08	1	9	1.81	111.40
			2		0.80	112.30
			3		4.79	99.40
	2	0.23	1	9	2.39	100.70
			2		1.55	101.90
			3		1.69	99.10
4	1	0.08	1	9	5.47	97.00
			2		1.88	97.91
			3		4.61	100.01
	2	0.23	1	9	0.58	97.18
			2		0.54	97.35
			3		1.07	99.05

[a]The number of replicates.

Results and discussion

TPC of each onion extract was determined through the application of the Folin–Ciocalteu method, by using the calibration curve built up with GA standard solutions (Table 4, method 1).

Data in Table 4 show that *Rossa di Toscana* and *Dorata di Parma* cultivars have comparable TPC and higher than that computed for the *Borettana di Rovato* cultivar.

The FRAP assay was used for assessing the TAC of the phenol extracts from the bulb of three investigated onion cultivars. Advantageously, the FRAP method is rather rapid, inexpensive, and characterized by an appreciably overall sensitivity. By using the calibration curve built up with Trolox standard solutions (Table 1, method 2), antioxidant activity of the three onion cultivars was determined. The FRAP assay revealed the phenol extract from the *Rossa di Toscana* cultivar being endowed with a significantly higher antioxidant activity with respect to the other two cultivars. Moreover, the application of the FRAP method indicated a not statistically different TAC between the two onion cultivars *Dorata di Parma* and *Borettana di Rovato*.

Also the TEAC and DPPH methods were applied for the determination of the total antioxidant capacity. The two methods are based on the radical-scavenging capacity of the antioxidant compounds into a complex mixture. By using the calibration curves built up with Trolox standard solutions (Table 1, methods 3 and 4), antioxidant activity of the three onion cultivars was determined. In Table 4, TAC values of the three onion cultivars are shown and the results are expressed as µmol of Trolox equivalents/g onion bulb DW. As a result of the TEAC assay, the phenol extract from *Rossa di Toscana* was found to display the highest values of TAC, followed by *Dorata di Parma* and *Borettana di Rovato*. Also with the DPPH method, the extract from the *Rossa di Toscana* displayed the highest antioxidant activity, while a not statistically difference was revealed between the two onion cultivars *Dorata di Parma* and *Borettana di Rovato*.

Table 3. Statistical analysis for the four selected spectrophotometric methods in the long period (inter-day).

Method #	Solution	Theoretical concentration (mg/mL)	n^a	Precision (RSD%)	Accuracy (recovery%)
1	1	0.120	27	0.44	101.10
	2	0.220		0.94	100.00
2	1	0.013	27	6.67	99.33
	2	0.110		5.31	102.51
3	1	0.075	27	6.31	107.70
	2	0.225		2.05	100.58
4	1	0.075	27	2.86	97.29
	2	0.225		0.43	98.80

[a]The number of replicates.

In line with literature data, for red and white onions, the antioxidant capacity estimated by the TEAC assay is higher that that evaluated through the FRAP method (Gökçe et al., 2010; Lu et al., 2011). Instead, the results of our investigation (Table 4) do not parallel those found by other authors. Indeed, the TAC obtained by Lu et al. (2011) for white, yellow, and red onions with the TEAC assay was sensitively higher than that resulting from both the FRAP and DPPH methods, which were instead comparable. Although their methodological rigor, comparative studies in the literature are carried out with uncertified onion samples, which are readily acquired in local markets. This way to collect samples completely neglects the frequent variability of chemical components, which characterize bulbs belonging to the same cultivar but having different geographical origins. Differently, in our study, a rational sampling and an identical post-harvest treatment of the onion bulbs was made, which enhances the scientific value of the presented results.

Immunological test results

Phenolic extracts from different onion cultivars were found to induce different immunological effects in terms of proliferation of representative immune cell subpopulations. In particular, incubation with extracts from *Rossa di Toscana* onion determined a relevant increase in the frequency of the antitumor/anti-infection NK CD16+ immune cells (23% ± 0.4%), as compared with the baseline value (5%, negative control). Extracts from *Dorata di Parma* onion were associated with a mild increase in NK CD16+ cells (18% ± 0.3%) whereas extracts from *Borettana di Rovato* onion had almost no effect on NK CD16+ cell proliferation (6% ± 0.1%). No significant induction of CD4+, CD8+, or T regulatory cell proliferation was observed in the immunological tests performed. In Figure 2, a representative example of NK CD16+ cell proliferative induction by *Rossa di Toscana* (Figure 2a) and *Borettana di Rovato* (Figure 2b) extracts is depicted.

Table 4. Summary of the measured TPC, FRAP, TEAC, and DPPH reference values of the three different onion cultivars.

Type of assay	*Rossa di Toscana*	*Dorata di Parma*	*Borettana di Rovato*
TPC (mg GA equivalents/g bulb DW)	6.61 ± 0.87[a]	6.53 ± 1.08[a]	4.71 ± 1.05
FRAP (µmol Trolox equivalents/g bulb DW)	9.19 ± 2.54	2.77 ± 1.40[a]	1.98 ± 0.23[a]
TEAC (µmol Trolox equivalents/g bulb DW)	21.31 ± 0.41	18.15 ± 1.15	13.98 ± 1.01
DPPH (µmol Trolox equivalents/g bulb DW)	22.90 ± 0.01	15.08 ± 0.01[a]	15.67 ± 0.01[a]

Data with the 'a' letter in the same line are not statistically different.

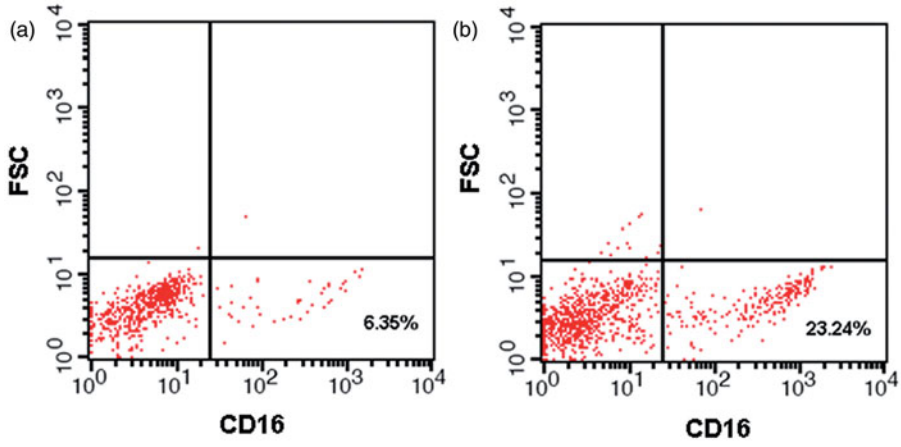

Figure 2. Representative example of NK CD16+ cell proliferative induction by phenol extracts from (a) *Rossa di Toscana* and (b) *Borettana di Rovato*. FSC in the *y*-axis stands for the forward scatter.

Conclusions

In the present study, we have determined the total phenol content (TPC) and the relative total antioxidant capacity (TAC) of the bulbs of three onion cultivars farmed in Cannara: *Rossa di Toscana*, *Dorata di Parma* and *Borettana di Rovato*.

The application of a fully validated Folin–Ciocalteu method showed that *Rossa di Toscana* and *Dorata di Parma* cultivars have comparable TPC and higher than that computed for the *Borettana di Rovato* cultivar. The validated FRAP assay revealed the phenol extract from the *Rossa di Toscana* cultivar being endowed with a significantly higher antioxidant activity with respect to the other two cultivars, which displayed a not statistically different TAC. The evaluation of the TAC by means of the fully validated TEAC/ABTS and DPPH methods indicated that the phenol extract from *Rossa di Toscana* possess the highest values of TAC, followed by *Dorata di Parma* and *Borettana di Rovato*.

Phenolic extracts were also tested *in vitro* for their ability to induce human immune cell proliferation and, in particular, growth of CD16+ natural killer cells, which are known to play a protective role against cancer and infection. The extracts from the different cultivars were found to induce different immunological effects in terms of proliferation of representative immune cell sub-populations. In particular, incubation with extracts from *Rossa di Toscana* onion determined a relevant increase in the frequency of the antitumor/anti-infection NK CD16+ immune cells.

Acknowledgements

The authors acknowledge the Cannara Onion Producers Union (Consorzio dei Produttori della Cipolla di Cannara) for supplying onion samples.

Declaration of interest

The authors declare no conflicts of interest. This research was supported by the grant 2013.0222.021 from "Fondazione Cassa di Risparmio di Perugia".

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