ISSN 2347-6893
Volume 10 Number2
Journal of Advances in Biology

ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES OF DIFFERENT CULTIVARS OF TOMATOES (LYCOPERSICON ESCULENTUM) ON TUMORAL CELL LINES

Concetta Piscitelli, Margherita Lavorgna, Marina Isidori, Chiara Russo, Rocco De Prisco, Gennaro Roberto Abbamondi, Carmine Iodice, Giuseppina Tommonaro*

Concetta Piscitelli: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università degli Studi della Campania "L. Vanvitelli", Via Vivaldi 43, I-81100 Caserta, Italy – e-mail: concetta.piscitelli@unicampania.it

Margherita Lavorgna: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università degli Studi della Campania "L. Vanvitelli", Via Vivaldi 43, I-81100 Caserta, Italy – e-mail: margherita.lavorgna@unicampania.it

Marina Isidori: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università degli Studi della Campania "L. Vanvitelli", Via Vivaldi 43, I-81100 Caserta, Italy – e-mail: marina.isidori@unicampania.it

Chiara Russo: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università degli Studi della Campania "L. Vanvitelli", Via Vivaldi 43, I-81100 Caserta, Italy – e-mail: chiara.russo@unicampania.it

Rocco De Prisco: Consiglio Nazionale delle Ricerche, Istituto di Chimica Biomolecolare, Via Campi Flegrei, 34, I-80078 Pozzuoli, Italy – e-mail: roccodeprisco1@virgilio.it

Gennaro Roberto Abbamondi: Consiglio Nazionale delle Ricerche, Istituto di Chimica Biomolecolare, Via Campi Flegrei, 34, I-80078 Pozzuoli, Italy – e-mail: gennaroroberto.abbamondi@icb.cnr.it

Carmine Iodice: Consiglio Nazionale delle Ricerche, Istituto di Chimica Biomolecolare, Via Campi Flegrei, 34, I-80078 Pozzuoli, Italy – e-mail: ciodice@icb.cnr.it

Giuseppina Tommonaro: Consiglio Nazionale delle Ricerche, Istituto di Chimica Biomolecolare, Via Campi Flegrei, 34, I-80078 Pozzuoli, Italy – e-mail: gtommonaro@icb.cnr.it

*Corresponding author:

Giuseppina Tommonaro: Consiglio Nazionale delle Ricerche, Istituto di Chimica Biomolecolare, Via Campi Flegrei, 34, I-80078 Pozzuoli, Italy. Phone: +39 0818675029; e-mail: gtommonaro@icb.cnr.it

ABSTRACT

To increase the knowledge about the biological properties of tomatoes, the antioxidant and antiproliferative activities of extracts of nine cultivars of Lycopersicon esculentum, as well as their chemical composition, were studied. The highest antioxidant capacity has been revealed in San Marzano Cirio 3 and Pomodoro Giallo cultivars, both in hydrophilic and lipophilic fractions, while San Marzano, Corbarino di Corbara, and Pomodoro Giallo exhibited the best radical scavenger activity in methanolic fraction. As regards the antiproliferative activity, the median inhibition concentrations of the lipophilic extracts ranged from 272.3 (Pomodoro Giallo) to 364.4 (Corbarino di Corbara) mg dried extract L^{-1} on Hep-G2 and from 324.6 (San Marzano Cirio 3) to 455.4 (Nero di Sicilia) on Caco-2. The methanolic extracts were more active on Caco-2 than Hep-G2, while the hydrophilic extracts were not active. These biological properties could be ascribed to the identified carotenoids and phenolic acids as well as to a pull of minor compounds exerting their synergistic effect.

Key words: Lycopersicon esculentum, antioxidant properties, antiproliferative activity, colon adenocarcinoma cells, hepatocyte carcinoma cells.

Academic Discipline And Sub-Disciplines: Biology, Chemistry

SUBJECT CLASSIFICATION: Agriculture, Agrifood, Biological Activity, Cancer Research

TYPE (METHOD/APPROACH): Experimental research

INTRODUCTION

Tomatoes (Lycopersicon esculentum L.) are the second most widespread vegetable crops all over the world [1]. It is widely cultivated in Mediterranean countries, especially Spain and Italy [2]. Nowadays, among vegetables, tomato has a fundamental role in the so-called "Mediterranean Diet" and its consumption has been increasing not only for the richness in tomato-based food products [1,3] but also for the richness in bioactive compounds known for their beneficial effects on human health [4-6]. Several studies showed that the consumption in daily diet of tomatoes, either as fresh fruits or in processed products, decreases the risk for heart diseases, some kinds of cancer and other chronic diseases [7-10]. These beneficial effects of tomatoes on health are due to their high content in antioxidant metabolites such as carotenoids, flavonoids, vitamins and tocopherols [4,11,12].

In particular, the antioxidant properties of L. esculentum are mainly due to the abundance of polyphenols and carotenoids in peel, pulp and seeds of tomato fruits. Previous studies showed that the main polyphenols found in tomatoes are flavonoids (quercetin, kaempferol and naringenin) and hydroxycinnamic acids (caffeic, chlorogenic, ferulic and p-cumaric acids) [3,11] while the main carotenoid is lycopene (80-90% of total carotenoid content) [13]. Lycopene shows many biological properties such as free radical scavenging activity, an important role in several metabolic pathways (induction of cell-to-cell communications and modulation of immune systems), and a strong antiproliferative activity against prostate, epithelial and lung cancers [12,14,15]. However, the richness in bioactive compounds of tomatoes depends on the kind of cultivar, cultivation conditions as soil fertilization, biotic and abiotic transformations, temperature, light and fruit ripeness [4,12,16,17].

Since the most of the studies concerns the several biological properties of tomato metabolites, the aim of this study was to increase the knowledge about the biological properties of whole tomato fruit. For this purpose, the antioxidant and antiproliferative activities of three different extracts (hydrophilic, methanolic and lipophilic) as well as the partial chemical composition of lipophilic and methanolic fractions were evaluated in nine Italian tomato cultivars. The antioxidant activity was determined using DMPD, DPPH and ABTS decolouration methods, while the antiproliferative activity was tested on two tumoral cell lines, the p-53 and p-450 expressed Hep-G2 (hepatocyte carcinoma) and the enterocytes morphologically characterized Caco-2 (colon adenocarcinoma) by using the MTT assay

MATERIAL AND METHODS

1. Materials

Roswell Park Memorial Institute medium (RPMI 1640), Dulbecco's modified Eagle's medium phenol red-free (DMEM), fetal bovine serum (FBS), HEPES, L-Glutamine, penicillin/streptomycin (10000 U/mL) and non-essential amino acids (NEAA, 100X) were supplied by Lonza Bio Whittaker (Verviers, Belgium). Dimethyl sulfoxide (DMSO) was supplied by Carlo Erba (Cornaredo, Milan). Acetone (HPLC grade) was supplied by Merck (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was from Fluka (Buchs, Switzerland). N,N-Dimethyl-p-phenylenediamine (DMPD), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as the crystallized diammonium salt, analytical-grade solvents and □-carotene, lycopene, hydroxycinnamic acid (caffeic, chlorogenic, ferulic and p-coumaric) standards, 2-propanol, 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyl-tetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (Milano, Italy).

2. Cultivars

The cultivars San Marzano, San Marzano Cirio 3, Corbarino di Corbara, Corbarino di Nocera, Nero di Sicilia were grown in experimental fields of Nocera Inferiore (40,74325° N, 14,63957° E), Campania region, Italy, while Pomodoro Giallo, Superpomodoro, Black Tomato and Corbarino di Accadia were grown in an experimental field named "Vado Cannata" located in Accadia (Foggia, South of Italy). Seeds of tomato hybrids were germinated at the end of March. 45-day old tomato seedlings were transplanted in the Vado Cannata and Nocera Inferiore fields and grown following traditional agronomic techniques. Sampling of fruits was performed in August at the maximum of ripening stage. Samples were taken to the laboratory and kept at – 20 °C until analysis.

3. Sample preparation

Samples were homogenized in a blender and centrifuged at 13848 g for 20 min. The hydrophilic fractions (supernatants) and pellets were collected separately and kept for analysis. Pellets were divided in two aliquots and extracted separately with methanol (methanolic fraction) and diethyl ether (lipophilic fraction) with the aim to extract polyphenols and carotenoids, respectively. Then, the extracts were filtered and concentrated in a rotary evaporator in vacuum and dried under N_2.

4. Antioxidant assays

The antioxidant activity of the three fractions was assessed using the DMPD, DPPH and ABTS methods for the hydrophilic, methanolic and lipophilic fraction respectively. These assays are based on the capacity of the different components to scavenge the DMPD and ABTS radical cations ($DMPD^{\bullet+}$ and $ABTS^{\bullet+}$, respectively) and DPPH radical [18-20]. The antioxidant capacity was expressed as radical percentage inhibition, calculated by the following formula:

$$Abs\ (\%) = (1- Abs_f / Abs_0) \times 100$$

where Abs_0 and Abs_f were the absorbance before and after the addition of sample, respectively.

All the experiments were carried out in triplicate. In particular, DMPD assay was carried out on undiluted hydrophilic fractions (T.Q.) and 1:2, 1:5, 1:10 dilutions. ABTS and DPPH assays were performed on crude extracts (T.Q., 20 mg mL^{-1}) and its dilutions 1:2; 1:5; 1:10.

5. Determination of polyphenol and carotenoids contents

The total polyphenol content was assessed by using the Folin–Ciocalteau colorimetric method [21]. Absorbance was measured at 765 nm (DU spectrophotometer, Beckman Coulter, Brea, CA, USA) and quantification was based on the standard curve generated with quercetin.
The lycopene and □-carotene content was evaluated by means of HPLC analysis (see below). The fractions corresponding to the identified carotenoids were collected, dried and weighted. All determinations were carried out in triplicates.

6. HPLC analysis

Lipophilic and methanolic extracts were analysed by reversed-phase High-Performance-Liquid-Chromatography (HPLC) to evaluate the chromatographic profile of polyphenolic compounds and of the two major carotenoids, lycopene and □-carotene. HPLC experiments were performed using a Shimadzu LC 6A with UV-VIS detector SPD 10A VP, CR 3A recorder, system controller SCL 10A VP, and Chemstation integration software Class–VP 5.0, using with a Kromasil 100A C_{18} column, 5 □m, 250 × 10 mm (Phenomenex). Lipophilic and methanolic extracts were dissolved in 2 mL of HPLC grade dichloromethane and methanol, respectively, and filtered with 0,22 □m PTFE syringe filter. HPLC analysis of lipophilic extracts was performed by using the following chromatographic conditions: gradient elution with acetone (A) and water (B), 25%/75% v/v, A/B for 15 min, 5%/95% A/B for 12 min, 100% B for 5 min and return to starting condition in 5 min before next injection; flow rate, 3 mL/min; wave length of UV detector, 450 nm, sensitivity adjusted to 0.04 AUFS at room temperature. HPLC analysis of methanolic extracts was performed by using the following chromatographic conditions: gradient elution, 90:10 to 70:30, v/v, A/B (A was 0.3% of trifluoroacetic acid in water and B was acetonitrile), linear gradient changed over a period of 10 min and return to starting condition in 10 min before next injection; flow rate, 4 mL/min; the run time was 50 min, UV detector 320 nm, sensitivity adjusted to 0.04 AUFS at room temperature [22].

7. Cell line Culture

Human hepatoma Hep-G2 and human colon adenocarcinoma (Caco-2) cells were grown in culture medium, consisted of RPMI with 10% fetal bovine serum (FBS), 2% HEPES, 2% L-Glutamine, and 1% penicillin/streptomycin (10000 U/mL). For Caco-2 cell line, 1% non-essential amino acids (NEAA, 100X) was added to the culture medium, according to Olejnik and others (2003) [23]. Cells were cultured in T-75-cm^2 tissue culture flasks in humidified atmosphere of 95% air plus 5% CO_2 37°C incubator.

8. MTT assay

The antiproliferative activity of tomato extracts against the human cancer cell lines was tested using the tetrazolium dye colorimetric test (MTT assay) according to Parrella and others (2014) [24]. Briefly, when the cells are at 80%–90% of confluence, they are collected and counted with trypan blue solution. Cells (1 × 104/well) are seeded in quadruplicate in 100 µL of DMEM/well in 96-well microplates. After 24-h incubation at 37 °C, the medium is removed and replaced with 200 µL of different concentrations of tomato extracts solved in DMEM. The plates are then incubated for 72 h at 37 °C. Each plate had negative control wells containing the cell growth medium.

After 72 h, 20µL of yellow MTT solution (5 mg mL^{-1}) is added to each well and the cells further incubated for 4 h at 37°C. Then the purple formazan crystals obtained by mitochondrial reduction of MTT are dissolved with 100 µL of 2-Propanol. The absorbance was recorded at 590 nm using the Ultra Multifunctional Micro plate Reader (TECAN). Cell viability rate was calculated as (compound absorbance – control absorbance) / control absorbance x 100. The concentration inhibiting the 50% cell growth rate (IC_{50}) was calculated as 100 – cell viability rate.

9. Statistical Analysis

The results of the MTT assay, expressed as the mean values with their standard errors, were from three independent experiments. Statistical significances were calculated by One-way ANOVA and Dunnett's multiple comparison test in order to obtain the IC_{50} values using GraphPad Prism 5.0 software. Differences were considered significant at $p < 0.05$.

RESULTS

1. Antioxidant properties, polyphenol contents and HPLC analysis

The yield of extracts obtained from the nine different cultivars is reported in Table 1.

Table 1. Total yield of all tomato extracts.

Tomato cultivar	Hydrophilic fractions (mL)[a]	Methanolic extracts (g)[a]	Lipophilic extracts (g)[a]
San Marzano	402	15.0	0.585
San Marzano Cirio 3	452	10.0	0.439
Corbarino di Corbara	375	15.0	0.739
Corbarino di Nocera	392	13.0	0.480
Nero di Sicilia	420	13.0	0.670
Pomodoro Giallo	403	24.0	0.250
Superpomodoro	407	16.0	0.459
Black Tomato	516	13.0	0.263
Corbarino di Accadia	345	16.0	0.223

[a]The values are reported as mL or g Kg^{-1} of fresh sample weight

Data showing the antioxidant activity of hydrophilic, methanolic and lipophilic fractions of all tomato cultivars are reported in Fig. 1. The antioxidant activity of hydrophilic fractions, calculated as percent inhibition of $DMPD^{\bullet+}$ radical cation, was similar in all tested samples (inhibition percentage ~60% at maximum amount tested, 5 □L, T.Q.). San Marzano Cirio 3 cultivar showed the highest activity (64.7%). Concerning the lipophilic extracts, percent inhibition of $ABTS^{\bullet+}$ radical cation ranged from 79.0 to 100.0%. The highest antioxidant activity was registered for San Marzano Cirio 3 and Pomodoro Giallo cultivars,for which the inhibition percentage was complete (100%) even at the 1:2 dilution.

ISSN 2347-6893
Volume 10 Number2
Journal of Advances in Biology

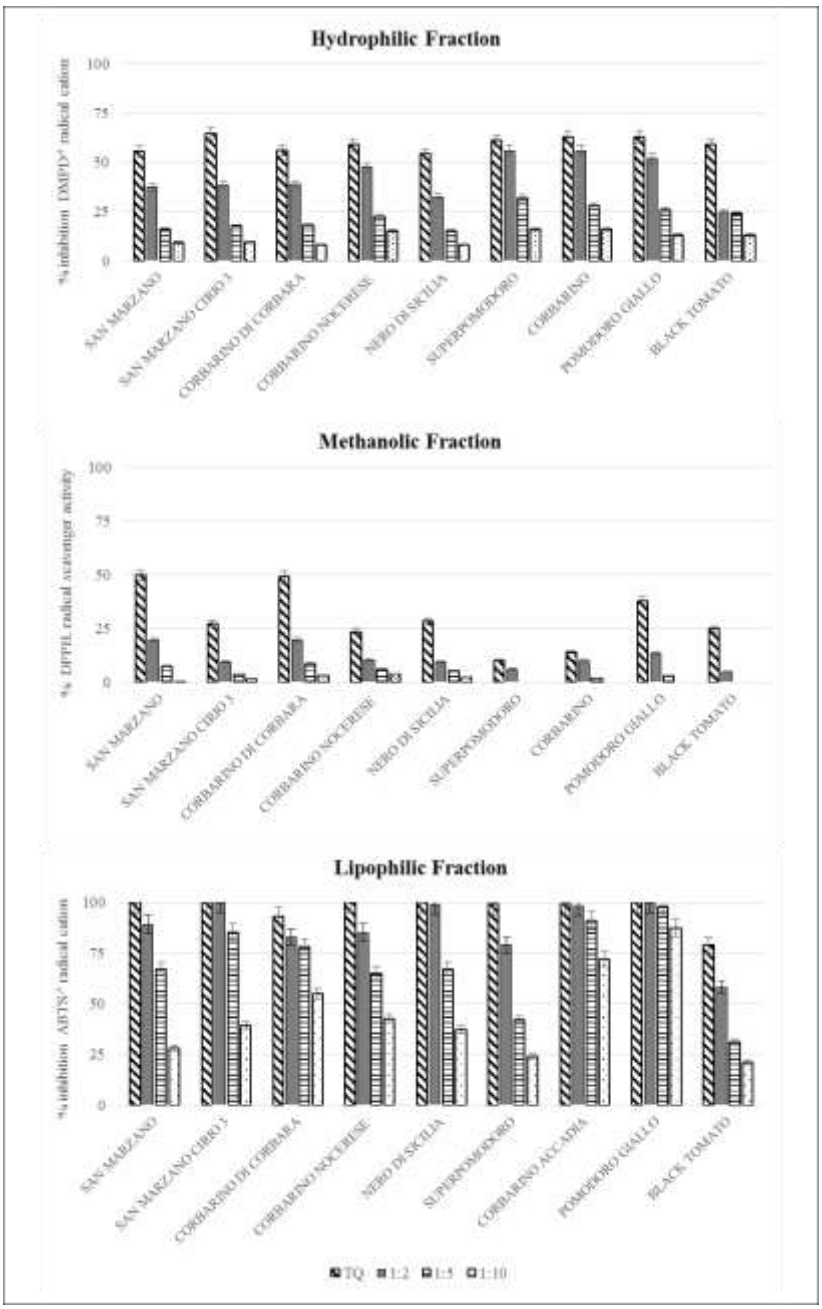

Figure 1. Antioxidant activity of hydrophilic, methanolic and lipophilic of different cultivars, evaluated by using DMPD, DPPH and ABTS methods, respectively. TQ represents undiluted solutions.

As regards the methanolic fraction, San Marzano, Corbarino di Corbara and Pomodoro Giallo revealed the best antioxidant activity, showing a percentage of DPPH radical scavenger activity of 49.8, 49.4 and 38.0%, respectively, at the maximum amount tested (TQ, 20 mg mL^{-1}).

These data were confirmed by the analysis of the polyphenolic contents, measured by Folin–Ciocalteau's method: the cultivar Corbarino di Corbara exhibited the highest total phenols content (830.5 mmol eq quercetin Kg^{-1}) followed by Corbarino di Nocera (649.0), Pomodoro Giallo (474.0) and San Marzano (448.0) (Table 2).

Table 2. Carotenoid and polyphenols contents and hydroxycinnamic acids identified in tomato extracts.

	Lipophilic extract		Methanolic extract	
Tomato cultivars	**Lycopene (g)[a]**	**□-carotene (g)[a]**	**Polyphenol content (mmol eq quercetin)[a]**	**Identified hydroxycinnamic acids**
San Marzano	0.27	0.16	448.0	Cumaric acid
San Marzano Cirio 3	0.87	0.07	51.5	Chlorogenic acid, caffeic acid
Corbarino di Corbara	0.35	0.31	830.5	Chlorogenic acid
Corbarino di Nocera	0.14	0.06	649.0	Chlorogenic acid, caffeic acid, cumaric acid
Nero di Sicilia	0.43	0.08	64.0	Caffeic acid, ferulic acid
Superpomodoro	0.39	0.06	200.0	n.d.
Corbarino di Accadia	0.17	0.13	292.5	Chlorogenic acid, caffeic acid
Pomodoro Giallo	n.d.	0.05	474.0	Caffeic acid
Black Tomato	0.29	0.08	240.0	Chlorogenic acid, caffeic acid

[a]The values are reported as g (carotenoids) and mmol eq quercetin (polyphenols) Kg^{-1} of fresh product; n.d. not detected

The chromatographic profile of lipophilic extracts of the most representative cultivars, san Marzano Cirio 3, Corbarino di Accadia and Pomodoro Giallo, determined by HPLC-UV/Vis method, is shown in Fig. 2. The content of lycopene and □-carotene was calculated in all tomato varieties, except for Pomodoro Giallo in which only □ -carotene was identified (Table 2). Other unidentified compounds were present in the lipophilic extract. San Marzano Ciro 3 exhibited the highest lycopene content (0.87 g Kg^{-1} FW), while Corbarino di Corbara showed the highest □ -carotene content (0.31 g Kg^{-1} FW). The high lycopene content in San Marzano Cirio 3 could be correlated to the highest antioxidant activity exerted by its lipophilic extract (100% $ABTS^{\bullet+}$ inhibition). On the contrary, Pomodoro Giallo showed a high antioxidant activity in lipophilic extract (100% $ABTS^{\bullet+}$ inhibition) but lycopene was not detected and □-carotene content was the lowest (0.05 g Kg^{-1} FW) among all cultivars. These results suggested a synergistic effect of a pull of carotenoids in the evaluation of the antioxidant activity and they were in agree with those reported by other authors [25,26].

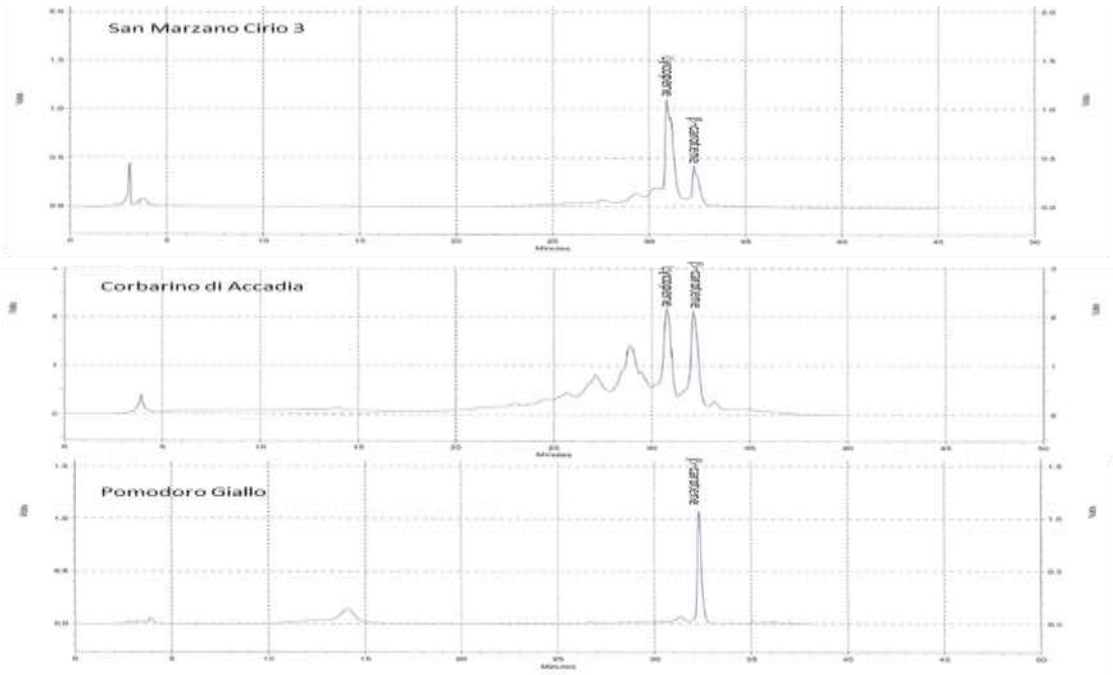

Figure 2. Chromatographic profile of lipophilic extracts of San Marzano Cirio 3, Corbarino di Accadia and Pomodoro Giallo cultivars. Lycopene and □-carotene were identified by comparison with standards.

The chemical differences in polyphenolic profile, in particular in hydroxycinnamic acids, among the different tomato varieties, were finally obtained through the analysis and characterization of the methanolic extracts by HPLC-UV/Vis (Table 2). According to Gomez-Romero and others (2010) [16] who found the hydroxycinnamic acids and their derivates as the most abundant metabolites identified in methanolic fractions of all the tomato cultivars tested, in all our samples one or more hydroxycinnamic acids have been well identified. Corbarino di Nocera contained three phenolic acids such as chlorogenic, caffeic, and p-coumaric acids. On the contrary, in Corbarino di Corbara cultivar only chlorogenic acid was detected, and ferulic acid was identified in Nero di Sicilia but not in the other cultivars. These results might suggest that genotypic factors as well as soil composition could influence the biosynthesis of polyphenols.

2. Antiproliferative activity

The antiproliferative activity of nine cultivars of L. esculentum hydrophilic, methanolic and lipophilic extracts was evaluated in Hep-G2 and Caco-2, using the MTT assay. The results are collected in Figures 3-5. All extracts inhibited Hep-G2 and Caco-2 cell proliferation in a dose-response dependent manner. The results showed that among the tomato fractions tested, the lipophilic extracts had the most evident decrease in cell viability in both the cancer cell lines (Fig. 3) although the lowest concentrations tested (10 and 50 mg L^{-1}) were not statistically different from the control (data not shown). For higher concentrations (125, 250 and 500 mg L^{-1}), as the cell viability decreased, the statistically significant difference increased for all cultivars with the strongest difference (***p<0,0001) at the highest concentration tested (500 mg L^{-1}). The methanolic extracts (Fig. 4) were less active than lipophilic extracts showing the first antiproliferation effects at 500 mg L^{-1} in both cell lines except for Corbarino di Nocera that in Caco-2 showed the first significant effect at 1000 mg L^{-1}. For the hydrophilic extracts (Fig.5), tested up to 1500 mg L^{-1}, a higher cell viability compared to the other extracts was shown especially on Hep-G2 cells. Interestingly, a significant antiproliferative activity (* p<0.05) was observed on Caco-2 at 10 mg L^{-1} for San Marzano Cirio 3, Nero di Sicilia and Black Tomato and for Corbarino di Corbara and Corbarino di Accadia at 100 mg L^{-1}. For all samples tested, the median effective concentration able to inhibit cell proliferation by 50% (IC_{50}) was estimated. The IC_{50} values of the lipophilic extracts ranged from 272.3 (Pomodoro Giallo) to 364.4 (Corbarino di Corbara) mg L^{-1} on Hep-G2 (Table 3) while on Caco-2 (Table 4) ranged from 324.6 (San Marzano Cirio 3) to 455.4 (Nero di Sicilia) showing the lowest IC_{50} values compared to the other extracts. In particular, lipophilic extracts were the only samples able to induce a median inhibition of Hep-G2 cell growth when tested up to 500 mg L^{-1}. On Caco-2 the estimation of IC_{50} was not possible up to 1500 mg L^{-1} only for the hydrophilic extracts while for the methanolic fractions, the IC_{50} values were higher than 1200 mg L^{-1}. Considering the evaluation of the proliferative inhibition percentages, the most active cultivars of methanolic extracts were Pomodoro Giallo (81%), San Marzano Cirio 3 (78%) and San Marzano (67%) at the highest concentration tested on Caco-2 while on Hep-G2, the percentages reached a maximum of 37%. Furthermore, the cultivar Pomodoro Giallo and San Marzano Cirio 3 were the most active also for the lipophilic extracts on both cell lines at 500 mg L^{-1}. Indeed, Pomodoro Giallo reached percentages equal to 87% and 86% in Hep-G2 and Caco-2, respectively, while San Marzano Cirio 3 reached 88% (Hep-G2) and 81% (Caco-2).

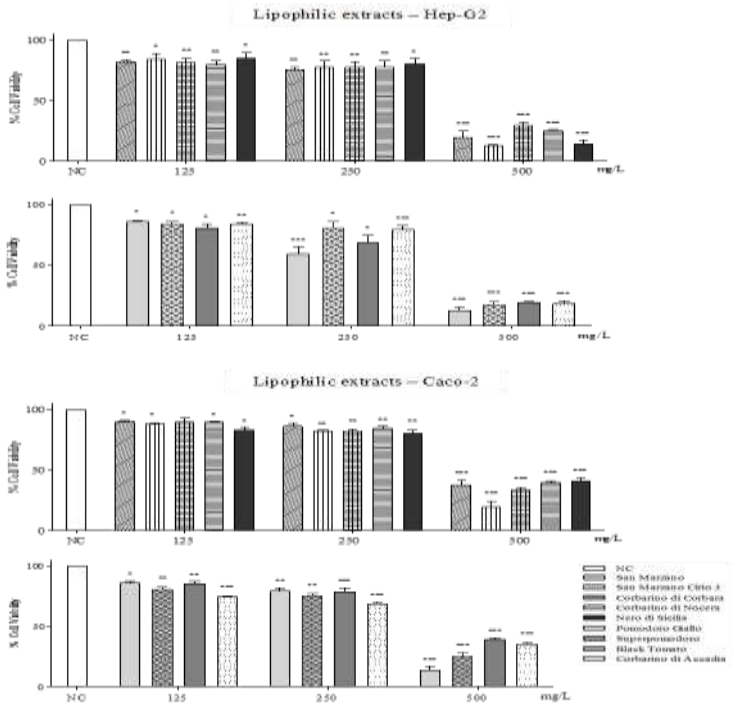

Figure 3. Cell viability, expressed in percentage, of the concentrations of tomato lipophilic extracts of the nine cultivars tested on Hep-G2 and Caco-2 cell lines. Results are expressed as means ± standard error of three independent experiments. Only the statistical different concentrations are reported in the graph. Significant differences from the negative control (NC) are highlighted by asterisks (ANOVA, Dunnett's test - *p< 0.05; **p< 0.001; ***p< 0.0001).

ISSN 2347-6893
Volume 10 Number2
Journal of Advances in Biology

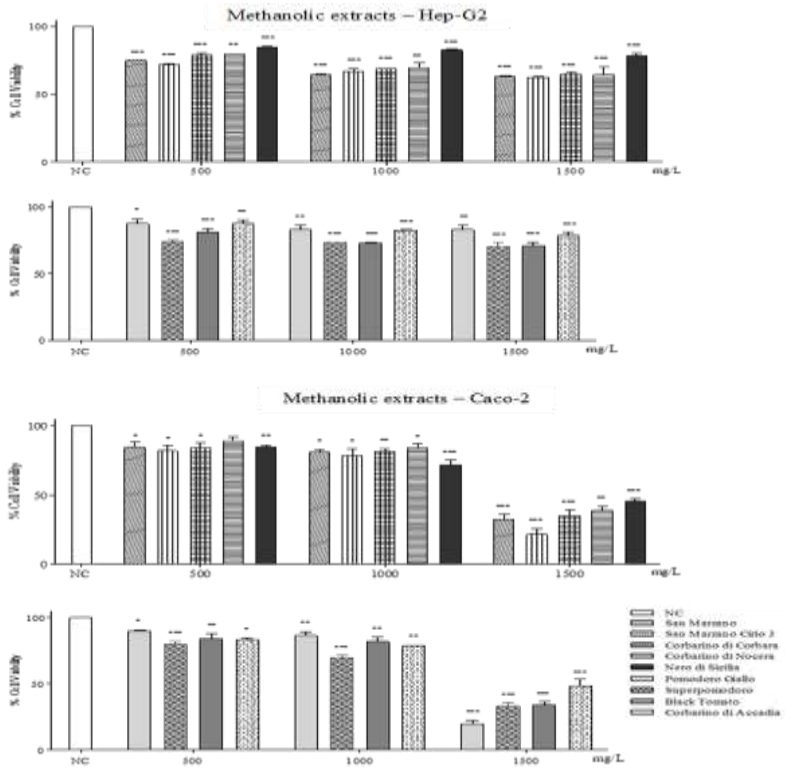

Figure 4. Cell viability, expressed in percentage, of different concentrations of tomato methanolic extracts of the nine cultivars tested on Hep-G2 and Caco-2 cell lines. Results are expressed as means ± standard error of three independent experiments. Only the statistical different concentrations are reported in the graph. Significant differences from the negative control (NC) are highlighted by asterisks (ANOVA, Dunnett's test - *p< 0.05; **p< 0.001; ***p< 0.0001).

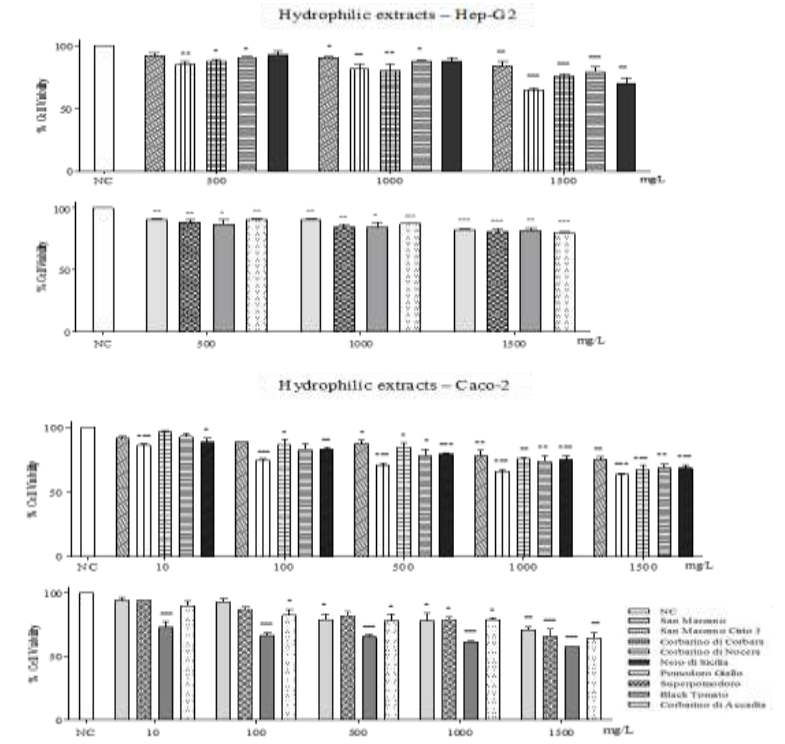

Figure 5. Cell viability, expressed in percentage, of different concentrations of tomato hydrophilic extracts of the nine cultivars tested on Hep-G2 and Caco-2 cell lines. Results are expressed as means ± standard error of three independent experiments. Significant differences from the negative control (NC) are highlighted by asterisks (ANOVA, Dunnett's test - *p< 0.05; **p< 0.001; ***p< 0.0001).

ISSN 2347-6893
Volume 10 Number2
Journal of Advances in Biology

Table 3. IC_{50} (mg L^{-1}) values, with confidence limits (95% probability, in brackets), of hydrophilic, methanolic and lipophilic extracts of nine cultivars of Lycopersicon esculentum in Hep-G2.

	Hydrophilic extracts	Methanolic extracts	Lipophilic extracts
San Marzano	>1500[(a)]	>1500[(a)]	330.5 (276.8 – 394.7)
San Marzano Cirio 3			322.4 (267.7 – 359.5)
Corbarino di Corbara			364.4 (295.9 – 448.6)
Corbarino di Nocera			347.1 (278.3 – 433.0)
Nero di Sicilia			336.2 (286.7 – 394.3)
Pomodoro Giallo			272. 3 (244.2-303.5)
Superpomodoro			343.8 (291.3 – 405.8)
Black Tomato			303.9 (254.7-362.6)
Corbarino di Accadia			343.2 (293.7 – 401.9)

(a) Maximum concentration tested.

Table 4. IC_{50} (mg L^{-1}) values, with confidence limits (95% probability, in brackets), of hydrophilic, methanolic and lipophilic extracts of nine cultivars of *Lycopersicon esculentum* in Caco-2.

	Hydrophilic extracts	Methanolic extracts	Lipophilic extracts
San Marzano	>1500(a)	1304 (1168 – 1455)	425.9 (382.2 – 474.5)
San Marzano Cirio 3		1222 (1077 – 1387)	324.6 (277.7 – 379.5)
Corbarino di Corbara		1329 (1191 – 1482)	399.1 (350.3 – 454.7)
Corbarino di Nocera		1456 (1083 – 1409)	434.0 (382.0 – 493.1)
Nero di Sicilia		1447 (1227 – 1707)	455.4 (327.9 – 605.1)
Pomodoro Giallo		1307 (1169 – 1354)	332.0 (283.0 – 389.3)
Superpomodoro		1221 (1027 – 1451)	338.0 (266.1 – 429.4)
Black Tomato		1320 (1160 – 1503)	420.0 (347.7 – 507.5)
Corbarino di Accadia		1352 (1162 – 1572)	355.8 (283.2 – 447.1)

(a) Maximum concentration tested

DISCUSSION

All cultivars displayed a considerable antioxidant activity linked to the hydrophilic fractions as well as to the lipophilic fractions in which the most representative carotenoids, lycopene and □-carotene, were present. Between these two types of extracts, the lipophilic fractions were more active than the hydrophilic fractions, in particular the cultivars which had the most remarkable antioxidant capacity were San Marzano Cirio 3 and Pomodoro Giallo. These results disagree with those found in the study of Kotíková and others (2011) [27] who demonstrated that the hydrophilic extracts of eight tomato

varieties, cultivated in an experimental field of Czech Republic, showed a more significant impact on total antioxidant activity (83%) than the lipophilic fractions. Moreover, the antioxidant activity of lipophilic extracts could not be ascribed only to the major carotenoids, lycopene and □-carotene, but a synergic activity exerted by a pull of compounds present in lipophilic extract of tomato.

As regards methanolic fractions, San Marzano, Corbarino di Corbara and Pomodoro Giallo revealed the highest antioxidant activity. Our results agree with a previous study where the seed methanolic fraction of San Marzano showed the highest antioxidant activity, inhibiting the DPPH radical of 68% at the maximum concentration tested (20 mg mL^{-1}) [28].

All cultivars, except Pomodoro Giallo, showed the presence of lycopene and □-carotene, in a different ratio, probably depending on the genotype and environmental conditions [29-31]. Considering the antiproliferative activity, the lipophilic extracts, rich in carotenoids, showed the highest antiproliferative activity. Indeed, several studies highlighted the in vitro antiproliferative activity of carotenoids, especially lycopene, against different tumoral cell lines. Salman and others (2007) showed that lycopene induced a significant dose-dependent antiproliferative effect on K562 (human erythroleukemia), HuCC (human colon carcinoma) cell lines and on Raji (a prototype of Burkitt lymphoma cell line) [32]. Furthermore, these carotenoid compounds were able to inhibit cell proliferation arresting the cell cycle in different phases by apoptosis, in breast, colon and prostate cell lines [33]. In a recent study, conducted by Takeshima and others (2014), lycopene exhibited growth inhibitory activities against three different human breast cancer (MCF-7, SK-BR-3 and MDA-MB-468) cell lines, with the highest antiproliferative activity observed in MDA-MB-468 [34]. Moreover, epidemiological studies highlighted the association between the higher dietary intake of lycopene and the lower risk of developing prostate cancer [35,36].

As regards the acid phenols profile, caffeic and chlorogenic acids were the most representative hydroxicinnamic acids in the analyzed cultivars. The potential inhibitory effects of phenolic acids on cancer cell proliferation is discussed in several scientific papers [37-39]. Caffeic acid showed a remarkable anticancer effect in fibrosarcome (HT-1080) cell line. Indeed, caffeic acid significantly decreased the percentage of cell viability in HT-1080 cells, alterating mitochondrial membrane potential and increasing the oxidative DNA damage.37 Moreover, in a study conducted by Puagpraphant and others (2011), caffeic acid inhibited matrix metalloproteinase-9 (MMP-9) activity, involved in cancer metastasis and anti-inflammatory activities [40]. Similarly, chlorogenic acid exhibited a strong cytotoxicity and induced apoptosis in U937, human leukemia cells, through a caspase-dependent pathway [39].

The health benefits of tomato in chronic degenerative diseases are still debated. However, our results underline the biological activities due to the whole tomato, probably obtained by the synergistic effects of single compounds present in the fruit. Indeed, Zanfini and others (2010) demonstrated that the tomato compound mixtures showed a higher antioxidant activity than single molecules. In particular, the best synergistic interactions were observed when lycopene and β-carotene, singularly, were mixed with α-tocopherol or lutein [25]. Moreover, Liu and others (2008) showed the synergistic effects of lycopene-α-tocopherol and β-carotene mixtures [26]. Basically, the activity of single molecules seems to be higher when they are assumed in their natural matrix than as supplement in diet [41,42].

In the present paper, the biological activities of different Italian tomato cultivars showed that the most interesting cultivars, in terms of antioxidant and antiproliferative effects, were San Marzano Cirio 3 and Pomodoro Giallo. These activities could be ascribed to the pull of carotenoids and phenol acids (hydroxycinnamic acids) found in tomato extracts. It is clear that possible synergistic effects of these single bioactive compounds are able to exert human healthy properties so that, the whole tomato rather than single isolated compounds consumption could be advisable in daily diet.

Author Disclosure Statement

No competing financial interests exist

REFERENCES

1. Valleverdù-Queralt, R., Jauregui, O., Di Lecce, G., Andres-Lacueva, C., Lamuela-Raventos, R.M. 2011. Screening of the polyphenol content of tomato-based products through accurate-mass spectrometry (HPLC-ESI-QTOF). Food Chem., 129(3):877–883.
2. Sardaro, M.L.S., Marmiroli, M., Maestri, E., Marmiroli, N. 2013. Genetic characterization of Italian tomato varieties and their traceability in tomato food products. Food Sci. Nutr., 1(1):54–62.
3. Kalogeropoulus, N., Chiou, A., Pyriochou, V., Peristeraki, A., Karathanos, V.T. 2012. Bioactive phytochemicals in industrial tomatoes and their processing byproducts. LWT - Food Sci. Technol., 49(2):213–216.
4. Tommonaro, G., De Prisco, R., Abbamondi, G.R., Marzocco, S., Saturnino, C., Poli, A,, Nicolaus, B. 2012. Evaluation of antioxidant properties, total phenolic content, and biological activities of new tomato hybrids of industrial interest. J. Med. Food, 15(5):483–489.
5. Saturnino, C., Spagnuolo, A., Palladino, C., Popolo, A., Tommonaro, G., De Prisco, R., Pinto, A. 2013. Antiproliferative activity of "*Lycopersicon esculentum*" leaves (var. Paul Robenson): preliminary study. Food Nutr. Sci., 4:632–635.
6. Stajcic, S., Cetkovic, G., Canadanovic-Brunet, J., Djilas, S., Mandic, A., Cetojevic-Simin, D. 2015. Tomato waste: Carotenoids content, antioxidant and cell growth activities. Food Chem., 172:225–232.
7. Chen, L., Stacewicz-Sapuntzakis, M., Duncan, C., Sharifi, R., Ghosh, L., van Breemen, R., Ashton, D., Bowen, P.E. 2001. Oxidative DNA damage in prostate cancer patients consuming tomato sauce-based entrees as a whole-food intervention. J. Natl. Cancer Ins., 93(24):1872–1879.

8. Bowen, P., Chen, L., Stacewicz-Sapuntzakis, M., Duncan, C., Sharifi, R., Ghosh, L., Kim, H.S., Christov-Tzelkov, K., van Breemen, R. 2002. Tomato sauce supplementation and prostate cancer: lycopene accumulation and modulation of biomarkers of carcinogenesis. Exp. Biol. Med., 227(10):886–893.
9. Willcox, J.K., Catignani, G.L., Lazarus, S. 2003. Tomatoes and cardiovascular health. Crit. Rev. Food Sci. Nutr., 43:1–18.
10. Agrawal, R.C., Jain, R., Raja, W., Ovais, M. 2009. Anticarcinogenic effects of *Solanum lycopersicum* fruit extract on Swiss albino and C57 Bl mice. Asian Pacific J. Cancer Prev., 10(3):379–382.
11. Martínez-Valverde, I., Periago, M.J., Provan, G., Chesson, A. 2002. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). J. Sci. Food Agric., 82(3):323–330.
12. Strazzullo, G., De Giulio, A., Tommonaro, G., La Pastina, C., Poli, A., Nicolaus, B. 2007. Antioxidative activity and lycopene and □-carotene contents in different cultivars of tomato (*Lycopersicon esculentum*). Int. J. Food Prop., 10(2):321–329.
13. George, B., Kaur, C., Khurdiya, D.S., Kapoor, H.C. 2004. Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. Food Chem., 84(1):45–51.
14. Etminan, M., Takkouche, B., Caamaño-Isorna, F. 2004. The role of tomato products and lycopene in the prevention of prostate cancer: A meta-analysis of observational studies. Cancer Epidemiol. Biomarkers Prev., 13(3):340–345.
15. Rao, A., Agarwal, S. 2000. Role of antioxidant lycopene in cancer and heart disease. J. Am. Coll. Nutr., 19(5):563–569.
16. Gómez-Romero, M., Segura-Carretero, A., Fernández-Gutiérrez, A. 2010. Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. Phytochemistry, 71(16):1848–1864.
17. Passananti, M., Lavorgna, M., Iesce, M.R., Della Greca, M., Criscuolo, E., Parrella, A., Isidori, M., Temussi, F. 2014. Chlorpropham and phenisopham: phototransformation and ecotoxicity of carbamates in the aquatic environment. Environ. Sci. Process Impacts, 16(4): 823–831.
18. Blois, M. 1958. Antioxidant Determinations by the Use of a Stable Free Radical. Nature, 181:1199–1200.
19. Miller, N.J., Sampson, J., Candeias, L.P., Bramley, P.M., Rice-Evans, C.A. 1996. Antioxidant activities of carotenes and xanthophylls. FEBS Lett., 384(3):240–242.
20. Fogliano, V., Verde, V., Randazzo, G., Ritieni, A. 1999. Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. J. Agric. Food Chem., 47(3):1035–1040.
21. Singleton, V.L., Rossi, J.A. 1965. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am. J. Enol. Vitic., 16(3):144–158.
22. Finore, I., Poli, A., Di Donato, P., Lama, L., Trincone, A., Fagnano, M., Mori, M., Nicolaus, B., Tramice, A. 2016. The hemicellulose extract from *Cynara cardunculus*: a source of value-added biomolecules produced by xylanolytic thermozymes. Green Chem., 18:2460-2472.
23. Olejnik, A., Lewandowska, M., Grajek, W., Czaczyk, K. 2003. New rapid method of Caco-2 cell differentiation. Pol. J. Food Nutr. Sci., 12/53(SI1):60–64.
24. Parrella, A., Lavorgna, M., Criscuolo, E., Russo, C., Isidori, M. 2014. Estrogenic activity and cytotoxicity of six anticancer drugs detected in water systems. Sci. Total Environ., 486(1):216–222.
25. Zanfini, A., Corbini, G., La Rosa, C., Dreassi, E. 2010. Antioxidant activity of tomato lipophilic extracts and interactions between carotenoids and α-tocopherol in synthetic mixtures. LWT - Food Sci. Technol., 43:67–72.
26. Liu, D., Shi, J., Ibarrac, A.C., Kakudac, Y., Xue, S.J. 2008. The scavenging capacity and synergistic effects of lycopene, vitamin E, vitamin C, and β-carotene mixtures on the DPPH free radical. LWT - Food Sci. Technol., 41(7):1344–1349.
27. Kotíková, Z., Lachman, J., Hejtmánková, A., Hejtmánková, K. 2011. Determination of antioxidant activity and antioxidant content in tomato varieties and evaluation of mutual interactions between antioxidants. LWT - Food Sci. Technol., 44(8):1703–1710.
28. Tommonaro, G., Caporale, A., De Martino, L., Popolo, A., De Prisco, R., Nicolaus, B., Abbamondi, G.R., Saturnino, C. 2014. Antioxidant and cytotoxic activities investigation of tomato seed extracts. Nat. Prod. Res., 28(10):764–768.
29. Rosales, M.A., Cervilla, L.M., Ríos, J.J., Blasco, B., Sánchez-Rodríguez, E., Romero, L., Ruiz, J.M. 2009. Environmental conditions affect pectin solubilization in cherry tomato fruits grown in two experimental Mediterranean greenhouses. Environ. Exp. Bot., 67(2):320–327.
30. Roselló, S., Adalid, A.M., Cebolla-Cornejo, J., Nuez, F. 2011. Evaluation of the genotype, environment and their interaction on carotenoid and ascorbic acid accumulation in tomato germplasm. J. Sci. Food Agric., 91(6):1014–1021.
31. Tiwari, U., Cummins, E. 2016. Factors influencing levels of phytochemicals in selected fruit and vegetables during pre- and post-harvest food processing operations. Food Chem., 66(4):72–78.
32. Salman, H., Bergman, M., Djaldetti, M., Bessler, H. 2007. Lycopene affects proliferation and apoptosis of four malignant cell lines. Biomed. Pharmacother., 61(6):366–369.
33. Teodoro, A.J., Oliveira, F., Martins, N., Maia, G., Martucci, R., Borojevic, R. 2012. Effect of lycopene on cell viability and cell cycle progression in human cancer cell lines. Cancer Cell Int., 12(1):36
34. Takeshima, M., Ono, M., Higuchi, T., Chen, C., Hara, T., Nakano, S. 2014. Anti-proliferative and apoptosis-inducing activity of lycopene against three subtypes of human breast cancer cell lines. Cancer Sci., 105(3):252–257.
35. Wei, M.Y., Giovannucci, E.L. 2012. Lycopene, tomato products, and prostate cancer incidence: A review and reassessment in the PSA screening era. J. Oncol., doi:10.1155/2012/271063.

36. Thapa, D., Ghosh, R. 2012. Antioxidants for prostate cancer chemoprevention: Challenges and opportunities. Biochem. Pharmacol., 83(10):1319–1330.
37. Rajendra Prasad, N., Karthikeyan, A., Karthikeyan, S., Venkata Reddy, B. 2011. Inhibitory effect of caffeic acid on cancer cell proliferation by oxidative mechanism in human HT-1080 fibrosarcoma cell line. Mol. Cell. Biochem., 349(1-2):11–19.
38. Weng, C.J., Yen, G.C. 2012. Chemopreventive effects of dietary phytochemicals against cancer invasion and metastasis: Phenolic acids, monophenol, polyphenol, and their derivatives. Cancer Treat. Rev., 38(1):76–87.
39. Yang, J.S., Liu, C.W., Ma, Y.S., Weng, S.W., Tang, N.Y., Wu, S.H., Ji, B.C., Ma, C.Y., Ko, Y.C., Funayama, S., Kuo, C.L. 2012. Chlorogenic acid induces apoptotic cell death in U937 leukemia cells through caspase- and mitochondria-dependent pathways. In Vivo (Brooklyn), 26(91):971–978.
40. Puangpraphant, S., De Mejia, E.G. 2009. Saponins in yerba mate tea (*Ilex paraguariensis* A. St.-Hil) and quercetin synergistically inhibit iNOS and COX-2 in lipopolysaccharide-induced macrophages through NFkappa B pathways. J. Agric. Food Chem. 57(19):8873–8883.
41. Liu, R.H. 2004. Potential synergy of phytochemicals in cancer prevention: mechanism of action. J. Nutr., 134(12):3479S–3485S.
42. Rodriguez-Casado, A. 2014. The Health Potential of Fruits and Vegetables Phytochemicals: Notable Examples. Crit. Rev. Food Sci. Nutr., 56(7):1097–1107.