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SHORT COMMUNICATION

Antioxidant and cytotoxic activities investigation of tomato seed extracts

Giuseppina Tommonaro[a]*, Angelamaria Caporale[b], Laura De Martino[b], Ada Popolo[b], Rocco De Prisco[a], Barbara Nicolaus[a], Gennaro Roberto Abbamondi[a] and Carmela Saturnino[b]*

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Biological activities of different varieties of tomato seed extracts were evaluated to verify the potential antioxidant and/or antiproliferative activity of the bioactive metabolites present in them. Findings demonstrated that among all the varieties investigated (San Marzano Rosso, San Marzano Giallo, Corbarino, Black Tomato and San Marzano/Black Tomato hybrid) San Marzano Rosso seed extract exhibited the highest free radical-scavenging activity with 68% of 2,2-Diphenyl-1-picrylhydrazyl radical inhibition, and the best cytotoxic activity evaluated by using the brine shrimp test (LD_{50}: 23,198 ppm) and 3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2H-tetrazolium bromide assay on A375 cell line (IC_{50}: 137.7 μg/mL).

Keywords: Tomato; antioxidant activity; cytotoxicity; seed extracts

1. Introduction

Oxidative stress is considered to be very important in the initiation and development of many present-day diseases. A growing amount of evidence indicates a role of reactive oxygen species (ROS) as well as of reactive nitrogen species in the pathophysiology of ageing and different degenerative diseases such as inflammation, cancer, cardiovascular diseases and neurodegenerative diseases (Davies 2000; Fenkel & Holbrook 2000; Ksouri et al. 2011). Living cells possess a protective system of antioxidants which prevents excessive formation and enables the inactivation of ROS. Oxidative stress occurs if the antioxidant defence in the organism is not adequate (Blomhoff et al. 2006). Natural antioxidants, such as phenolic acids, flavonoids and terpenoids, are mainly secondary metabolites of vegetables and fruits, and have been reported to be more effective antioxidants than vitamin C or E and carotenoids (Ksouri et al. 2011). Therefore, the ingestion of fresh fruit and vegetables rich in natural antioxidants has been associated with the prevention of cancer and cardiovascular diseases (Rao & Agarwal 2000; Willcox et al. 2004; Amin et al. 2009).

Tomatoes (*Solanum lycopersicum* L.) are a rich source of antioxidants and contribute to the daily intake of a significant amount of these molecules. In fact, tomato fruit is a reservoir of diverse antioxidant molecules, such as carotenoids, phenolics, flavonoids, vitamins and tocopherols (George et al. 2004; Mitchell et al. 2007; Strazzullo et al. 2007; Tommonaro et al. 2012). Antioxidant components of tomatoes are distributed in different sections of tomato fruit (skin, pulp and seeds). Skin and pulp portions of tomato fruit are richer in antioxidant metabolites (lycopene, anthocyanin, ascorbic acid and phenolic compounds) than seeds. In different cultivars, seeds showed a higher content of phenolic compounds than the pulp of tomato, but less than that of skin (Chandra & Ramalingam 2011; Chandra et al. 2012).

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In this work, we propose to proceed with the extraction of the seeds of six different varieties of *S. lycopersicum* (tomato) to verify the potential antioxidant and/or anti-tumour activity of bioactive metabolites present in them.

2. Results and discussion

2.1. *Sampling*

Seeds of different varieties of tomato, supplied and certified by a local farm, were extracted with chloroform and methanol in order to perform the evaluation of biological activities of extracts linked to the presence of bioactive compounds.

2.2. *Extraction and biological activities*

The weight of the seeds and the yield of the chloroformic and methanolic extractions are reported in Table 1.

We performed the antioxidant assay on the methanolic extracts by using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay (Blois 1958), and we estimated the polyphenol content by using the Folin–Ciocalteu method (Singleton & Rossi 1965). Results are reported in Figure 1. San Marzano Rosso and Black Tomato varieties showed the best radical-scavenging capacity, expressed as percentage of inhibition of DPPH radical. It was 68% and 53% for San Marzano Rosso and Black Tomato, respectively, at the maximum concentration tested (50 μL of a 10^{-2} μM solution). Moreover antioxidant activity was evaluated for all chloroformic extracts as well, by using the ABTS assay (Miller et al. 1996) (Figure 2). Results showed that the Giallo Tondo varieties were the most active with a percentage of radical cation $ABTS^{+}$ inhibition of 35% at the maximum amount tested (5 μL of a 20 mg/mL solution).

The methanolic seed extracts of Giallo Tondo, San Marzano Rosso and San Marzano/Black Tomato hybrid exhibited a greater amount of polyphenolic content than other varieties, as estimated by means of Folin–Ciocalteu method. The estimated amounts were 3.9, 3.6 and 3.5 mmol (in 100 mg of methanolic extract, calculated on the basis of the standard quercetin used in the assay) of polyphenols in Giallo Tondo, San Marzano Rosso and San Marzano/Black Tomato hybrid, respectively.

A general bioassay that appears capable of detecting a board spectrum of bioactivity present in crude extracts is the brine shrimp lethality bioassay (Meyer et al. 1982; Anderson et al. 1991). This crustacean, at larval stage (48 h after hatching), was used to test the cytotoxicity of the samples and to calculate the LD_{50}, which is the minimum lethal dose for which we observe a 50% mortality of larvae. Brine shrimp lethality activity of the seed extracts of different varieties of tomato is shown in Table 2.

Results obtained by using this test showed that among all methanolic extracts of seeds, San Marzano Rosso, San Marzano Giallo and Giallo Tondo exhibited cytotoxicity towards brine

Table 1. Chloroformic and methanolic extraction yield of tomato seeds.

Seed tomato varieties	Seed weight (g)	Chloroformic extract (mg)	Methanolic extract (mg)
Black Tomato	1.7981	40.3	29.5
Corbarino	6.7308	18.6	18.3
San Marzano Rosso	1.8993	24.6	8.7
San Marzano Giallo	2.2816	14.0	15.3
San Marzano/Black Tomato hybrid	3.6861	251.7	67.0
Giallo Tondo	3.3822	34.4	28.3

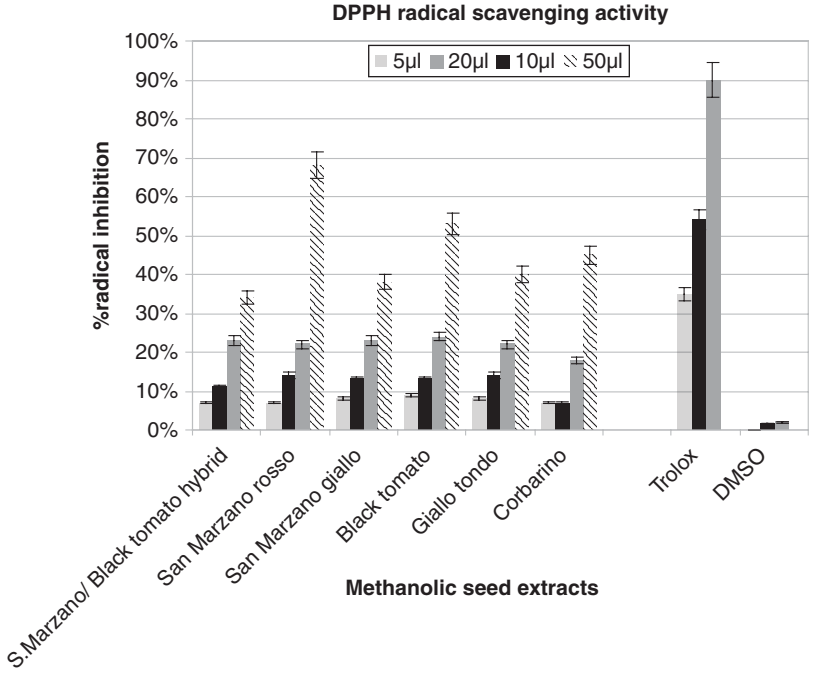

Figure 1. Free radical scavenger capacity of methanolic extracts of tomato seeds. The assay was performed on different amounts (5, 10, 20 and 50 µL) of a 10^{-2} µM solution of methanolic seed extracts.

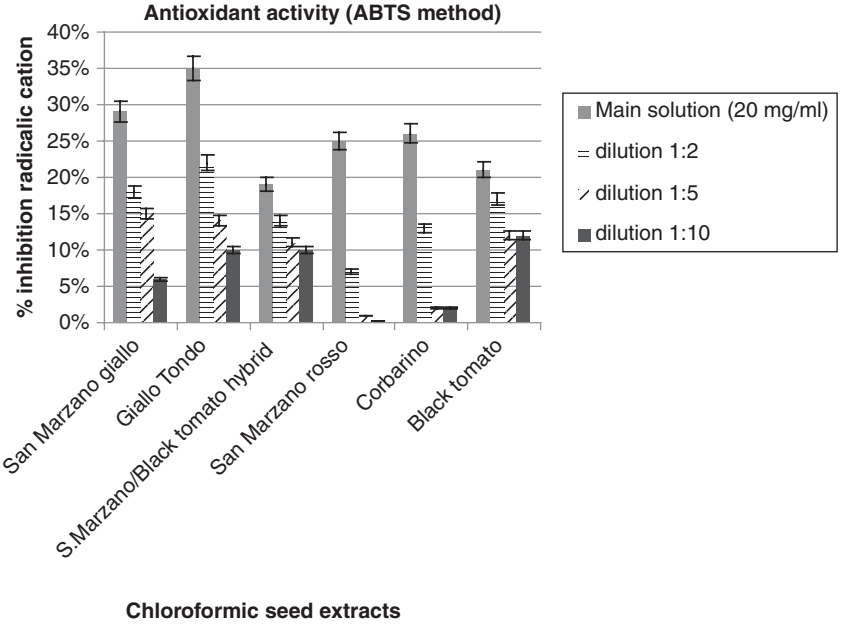

Figure 2. Antioxidant activity of chloroformic extracts of tomato seeds, starting from a stock solution (20 mg/mL) and its dilution (1:2, 1:5, 1:10), evaluated by using the ABTS method.

Table 2. Cytotoxicity by brine shrimp test.

Seeds extracts	LD_{50} (in ppm)
San Marzano/Black Tomato hybrid	169,816 (328,450/92,118)[a]
San Marzano Rosso	23,198 (41,771/10,566)[a]
San Marzano Giallo	31,752 (61,993/12,590)[a]
Black Tomato	161,827 (286,591/92,954)[a]
Giallo Tondo	73,622 (148,586/33,772)[a]
Corbarino	n.a.

[a] LD_{50} values expressed in ppm with 95% confidence intervals.

shrimp larvae (LD_{50} 2.3198, 3.1752 and 7.3622 ppm, respectively). Chloroformic extracts showed LD_{50} values more than 100 ppm, than they were not considered significantly active.

Considering the antiproliferative capacity of the seed extracts, better results were obtained in A375 cell line. In fact, chloroform extracts of San Marzano/Black Tomato hybrid, San Marzano Giallo and San Marzano Rosso e Corbarino showed IC_{50} values of 166, 156, 137.7 and 171 μg/mL, respectively, in these cells after 72 h of incubation. In A549 and HeLa cells, IC_{50} values were higher than 100 μg/mL. Methanolic extracts did not show any antiproliferative activity on cell lines used at tested concentrations.

The results of our study have shown that, among all tested extracts, the San Marzano Rosso seed extracts possess significant free radical-scavenging and cytotoxic activities. These effects could be correlated with the total polyphenolic content of the seeds, as polyphenols are known antioxidant compounds. The obtained results are supported by a previous study, which reports that the seeds have more phenolic content than the pulp of the tomato. The skin fraction of the tomato has more phenolic content than the seeds and pulp (Chandra & Ramalingam 2011). The antioxidants in plants protect them against the oxidative damage produced by various biotic and abiotic stresses. The health benefit of tomato is due to the presence, in different fractions (skin, pulp and seeds), of antioxidant metabolites which inhibit the reactions mediated by ROS. Moreover, the tomato genotype is also a determinant factor of antioxidant content of tomato fruits.

3. Conclusion

In this study, we have investigated the biological activity of seed extracts of different varieties of tomato from southern Italy in order to determine the antioxidant and *in vitro* antiproliferative activities of the extracts. Findings suggest that tomato seeds are a valuable source of natural antioxidant and cytotoxic compounds that could be useful for cosmetic or pharmaceutical applications.

Supplementary material

Supplementary material relating to this article is available online.

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