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Prunus spinosa Fresh Fruit Juice: Antioxidant Activity in Cell-free and Cellular Systems

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The antioxidant activity was assessed of fresh juice from *Prunus spinosa* L. fruit (Rosaceae) growing wild in Urbino (central Italy) by using different cell-free *in vitro* analytical methods: 5-lipoxygenase test, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, and oxygen radical absorbance capacity (ORAC). Trolox was used as the reference antioxidant compound. In the 5-lipoxygenase and DPPH tests the fresh fruit juice of *P. spinosa* showed good antioxidant activity when compared with Trolox, while the ORAC value was 36.0 μmol eq. Trolox /g of fruit. These values are in accord with data reported in the literature for small fruits such as *Vaccinium*, *Rubus* and *Ribes*. The antioxidant capacity in cell-free systems of *P. spinosa* juice has been compared with its cytoprotective – *bona fide* antioxidant activity in cultured human promonocytes (U937 cells) exposed to hydrogen peroxide. The antioxidant activity of red berries has been correlated with their anthocyanin content. The results of this study indicate that the three most representative anthocyanins in *P.spinosa* fruit juice (cyanidin-3-rutinoside, peonidin-3-rutinoside and cyanidin-3-glucoside) are likely to play an important role in its antioxidant properties.

Keywords: *Prunus spinosa* L., fruit juice, antioxidant activity, oxidative stress, cytotoxicity.

Prunus spinosa L. (Rosaceae), known as "blackthorn" or "sloe", is a wild shrub native to Scotland and commonly found in European deciduous forests and temperate countries of Asia, especially in central, north, west and south Anatolia [1]. It is resistant to cold, drought, and calcareous soils and represents one of the ancestors of *P. domestica* [2]. The fruits are bluish black, bloomy, globular drupes, 5-7 mm with green astringent flesh; they are popularly called "sloes" and despite their succulent appearance are far too bitter for human consumption, except as flavoring in liqueurs and wine. In the Marche region (central Italy), *P. spinosa* fruits are used for the preparation of a wine named "Lacrima di Spino Nero" [3].

The medicinal properties of blackthorn fruit extracts (purgative, diuretic, detoxicant) render them suitable for the preparation of natural medicines [2]. Anthocyanins were detected in sloe fruits by Werner *et al.* [4], Ramos and Macheix [5], Casado-Redin *et al.* [6], and Deineka *et al.* [7]; cyanidin-3-glucoside, cyanidin-3-rutinoside, peonidin-3-glucoside and peonidin-3-rutinoside were identified as the main anthocyanins, whereas caffeoil-3'-quinic acid was the most abundant hydroxycinnamic derivative. Different quercetin glycosides were also detected.

Anthocyanins are widely distributed among fruits and vegetables and have been reported to be absorbed unmodified from the diet [8], and to be incorporated in cell cultures, both in the plasma membrane and in the cytosol [9]. They are one of the main classes of flavonoids, contribute significantly to the antioxidant activities of the compounds [10], and are well known for their ability to give red, blue, and purple colors to plants. Anthocyanins can potentially interact with biological systems, conferring enzyme-inhibiting, antibacterial, cardiovascular protection and antioxidant effects [11,12]. Wang and Lin [13] found

a strong correlation between antioxidant capacity, total phenols and anthocyanins, while, on the other hand, some investigations also indicated that anthocyanins may be less significantly correlated with the antioxidant properties [14-17].

It has been proposed, on the basis of experimental data, that anthocyanins may exert therapeutic activities on human diseases associated with oxidative stress, for example coronary heart disease and cancer [18]. These effects have been related mainly to the antioxidant properties of anthocyanins, as demonstrated by experiments both *in vitro* and *in vivo* [19-22].

Various mechanisms have been proposed to explain the antioxidant activity of anthocyanins, such as their ability to scavenge free radicals [19-22], to chelate metal ions [20], to inhibit lipoprotein oxidation [19,23,24], and to form complexes with DNA [25].

As it is generally accepted that oxidative stress plays a role in a number of chronic and degenerative pathologies [26], including the above mentioned ones, considerable effort is being devoted to the search for naturally-occurring antioxidants from edible plants and fruits to prevent the onset and counteract the progression of these maladies [26-28]. In this light, this study has been aimed to investigate whether the natural microfiltered juice of the fresh fruit of *P. spinosa* (PJ), rich in anthocyanins, was effective either in acting as an antioxidant in established cell-free systems or in protecting cultured human promonocytes against the oxidative insult caused by hydrogen peroxide. It is worth noting that only seed extracts [29] and aqueous extracts (thea) from dried fruits of *P. spinosa* [30] have been tested by other groups for their antioxidant activity.

The results obtained in the first set of experiments, involving the three different acellular *in vitro* assays (namely DPPH, 5-lipoxygenase and ORAC), showed that PJ exerts a significant antioxidant capacity as compared with that of the established antioxidant Trolox (Figure 1A, 1B).

In many fruits and vegetables, the antioxidant activity can be attributed to the level of total polyphenols [31-33]. Therefore, the total polyphenol levels were measured for PJ, and a value of 83.5 ± 2.5 mg/g DW was found. One of the dominant classes of polyphenols in fruits are anthocyanins [34]. Tzulcher *et al.* [35] reported a positive correlation between total polyphenols, anthocyanin content and antioxidant activity of the fruit juice of *Punica granatum*. Similarly Ranilla *et al.* [36] found a positive correlation between antioxidant activity and anthocyanin content in seed coats from Brazilian and Peruvian cultivars of *Phaseolus vulgaris*. The total level of anthocyanins in our juice was 55.1 $\pm$5.6 mg/g DW.

In accordance with data reported in the literature for different fruits and vegetables, the total polyphenol and anthocyanin content appeared to contribute significantly to the antioxidant activity of sloe fruits, which indeed are rich in cyanidin-3-rutinoside (53.5%), peonidin-3-rutinoside (32.4%) and cyanidin-3-glucoside (11.4%) [7].

Elisia *et al.* [37] found that cyanidin-3-glucoside showed marked antioxidant efficacy in both *in vivo* and *in vitro* models, as compared with 13 other anthocyanins tested; moreover, anthocyanins, as compared to other classic natural antioxidants, have been recognised as potent inhibitors of lipid peroxidation [38].

Results shown in Figure 1A indicate that PJ exerted a remarkable antioxidant activity using the lipoxygenase assay, in which Trolox was less active. Again, using the DPPH assay PJ exerted a significant antioxidant activity, although lower than that displayed by Trolox.

Oki *et al.* [39] reported that anthocyanins are the most likely contributors to the DPPH scavenging activity of mulberry mature fruits. Interestingly these fruits contain fairly high amounts of cyanidin-3-glucoside and cyanidin-3-rutinoside [39], which are well represented in our juice.

PJ also displayed good antioxidant capacity (36.0 μmol Trolox equivalents/g of fruits), as measured with ORAC (Figure 1B). These data are in accordance with previously published ORAC values [37] for other small fruits such as *Vaccinium*, *Rubus* and *Ribes*, which displayed Trolox equivalent values ranging from 33.3 to 78.8 μmol/g fresh weight [37]. Monagas *et al.* [40] observed that the ORAC values in the grape skin were very close to the anthocyanins content, once again suggesting that the antioxidant capacity was mainly due to this type of compounds**.**

The second set of experiments was aimed at assessing the antioxidant activity of PJ in oxidatively-

Fig. 1A

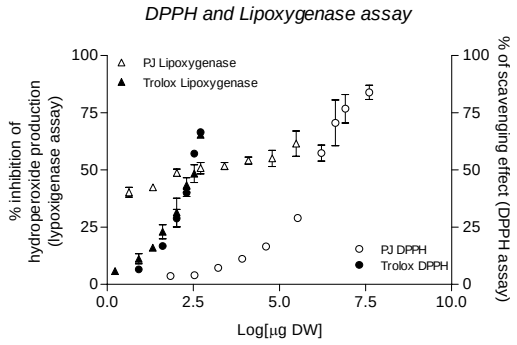

Fig. 1B

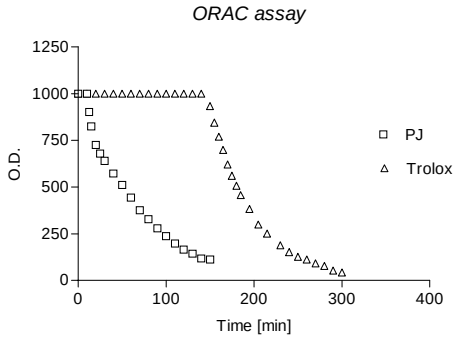

Figure 1A: Antioxidant capacity of PJ in lipoxygenase and DPPH assays. Results represent the percent inhibition of hydroperoxides production and the scavenging effect on DPPH radicals as a function of sample concentration. Trolox (closed symbols) was used as reference. The results represent the means +/- S.E.M. from 3 separate determinations.

Figure 1B: FL fluorescence decay curves induced by AAPH in the presence of 0.05 mg/mL of PJ and of 5 μM Trolox.

injured cultured cells. For this purpose, the effect of the juice on the cytotoxic response of U937 promonocytic cells exposed to H_2O_2 (Figure 2) was investigated. PJ was utilized in the same way in cell-free determinations, and the concentrations selected for this set of experiments(up to 0.01% v/v) were not cytotoxic *per se* (Figure 2, dotted line). Under the selected exposure conditions (see the Experimental section), treatment with 0.3 mM H_2O_2 caused a significant reduction of surviving cells (Figure 2) and, according to previously published observations [26,41-42], the mode of cell death was mainly necrotic. According to the data obtained in cell-free experiments, which indicate a valuable antioxidant activity for PJ, the juice was capable of significantly protecting cells from the oxidative insult in a dose-response fashion (Figure 2).

In conclusion *Prunus spinosa* fruits and juice could be considered as a valuable source of antioxidant

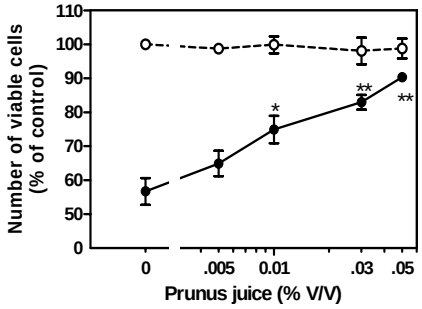

Figure 2: Effect of PJ on the cytotoxic response of U937 cells exposed to H_2O_2.
Cells were treated with 0.3 mM H_2O_2 in the absence or presence of increasing concentrations of PJ, as detailed in the Experimental section. The number of viable cells was determined 48 h post-challenge growth with the Trypan blue exclusion assay. The effect of PJ alone on the viability of U937 cells is also shown (open circles-dotted line). Results represent the means ± S.E.M. from three-five separate experiments, each performed in duplicate. * P <.01 and ** P < .001 (unpaired *t* test) compared to H_2O_2 alone -treated cells.

compounds for nutritional supplementation, as well as of herbal medicine.

Experimental

Plant material: *P. spinosa* fruits were collected in Urbino, Marche, central Italy, at 500 m above sea level, in November 2007 and identified by D. Fraternale. A voucher specimen is deposited in the herbarium of the Botanical Garden of the University of Urbino (P.S.F. 163).

Preparation of juice: PJ was prepared as follows: 10g of sloe pulp was crushed in a mortar, filtered over Whatman No 1 paper under vacuum, and the residue squeezed until exhaustion. The resulting juice, 6 mL, was then centrifuged at 2500 rpm for 10 min. All these steps were performed at ice bath temperature. The supernatant from the centrifugation step was recovered, microfiltered, aliquoted and immediately stored at – 20°C.

Total polyphenol content: Total content of polyphenolic compounds in fresh juice was determined by the Prussian Blue method described by Hagerman and Butler [43], with slight modifications. The optical density of the mixture was determined at 720 nm (Jasco V-530 spectrophotometer). Quercetin (Sigma) was used as standard to construct a calibration curve.

Total anthocyanin content (TA): Total anthocyanin content of the fruit juice of sloe was measured using the pH differential method reported by Elisia *et al.* [37] and Tzulker *et al.* [35]. Absorbancies were read

at 510 and 700 nm against a blank cell containing distilled and deionized water.

DPPH assay: Radical scavenging activity was determined by a spectrophotometric method based on the reduction of an ethanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) [44]. The absorbance decrease at 517 nm was recorded after 10 min and the percent decrease (corrected for the control, without antioxidant agents added) was taken as an index of the antioxidant capacity. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), was used as positive control and purchased from Sigma.

Lipoxygenase test: Inhibition of lipid peroxide formation was evaluated by the 5-lipoxygenase (purchased from Sigma) test in the sample and positive controls. The activity of the enzyme was assayed spectrophotometrically according to Holman. This method was modified by Sud'ina *et al.* [45]. The formation of hydroperoxides from linoleic acid was observed spectrophotometrically at 235 nm at 20°C.

ORAC: The original method of Cao *et al.* [46] was used with slight modifications. Fluorescein (3^{l},6^{l}-dihydroxy-spiro[isobenzofuran-1[3*H*],9^{l}[9*H*]-xanthen]-3-one) (purchased from Sigma) was chosen as fluorescent probe instead of B-phycoerythrin (B-PE) [47]. The area under the curve (AUC) of fluorescence decay was proportional to the antioxidant capacity of the sample, and a comparative evaluation with Trolox was performed. The fluorescence was measured every 10 sec at 37°C using a JASCO FP-6200 spectrofluorometer at 485 nm excitation, and 520 nm emission until zero fluorescence was detected. The ORAC value was calculated with a Spectra Manager for Windows 95/NT (Spectra Analysis) program.

Cell culture and treatments: Cells were grown at 37°C in an atmosphere of 95% air and 5% CO_2. Human promonocytic U937 cells were cultured in suspension in RPMI 1640 medium supplemented with antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin), 1.2 mM glutamine and 10% fetal bovine serum. For experiments, cells were resuspended at a number of 4 x 10^5 cells/treatment condition in 2 mL of Saline A (8.182 g/L NaCl, 0.372 g/L KCl, 0.336 g/L $NaHCO_3$ and 0.9 g/L glucose), prewarmed at 37°C. PJ was added to the cultures and equilibrated for 20 min before treatment with freshly prepared H_2O_2 (0.3 mM for 1 h). Cells were then washed and grown in extract- and H_2O_2–free culture medium.

Cytotoxicity assays: The cytotoxic response was evaluated with the trypan blue exclusion assay after 48 h post-challenge growth in complete culture medium: this time interval allows the quantification of the extent of growth arrest and cell death caused by the oxidant in treated cultures *vs* logarithmically growing control cells.

Briefly, after the post-challenge growth stage, an aliquot of each experimental sample's cell suspension was diluted 1:1 with 0.4% trypan blue and the cells were counted with a hemocytometer. Results are expressed as percent ratio of viable (unstained) cells in treated *vs* untreated samples.

References

[1] Kumarasamy Y, Cox PJ, Jaspars M, Nahar L, Sarker SD. (**2003**) Cyanogenic glycosides from *Prunus spinosa* (Rosaceae). *Biochemical Systematics and Ecology*, ***31***, 1063-1065.

[2] Aparajita M, Juan Pedro M, Itziar A (**2002**) Population genetic analysis of European *Prunus spinosa* (Rosaceae) using chloroplast DNA markers. *American Journal of Botany*, ***89***, 1223-1228.

[3] Bollettino Ufficiale della Regione Marche. (**2002**) Anno XXXIII, n**.** ***36***, 20 maggio.

[4] Werner DJ, Maness EP, Ballinger WE. (**1989**) Fruit anthocyanins in three sections of *Prunus* (Rosaceae). *Horticultural Science*, ***24***, 488-489.

[5] Ramos T, Macheix JJ. (**1990**) Phenols of blackthorn (*Prunus spinosa* L.) fruit. *Plant Medicine and Phytotherapy*, ***24***, 14-20.

[6] Casado-Redin I, Fernandez M, Vega FA. (**1992**) Polyphenols in *Prunus spinosa* L. fruits during ripening period. *Bulletin de Liaison-Groupe Polyphenols*, ***16***, 108-111.

[7] Deineka VI, Deineka LA, Sirotin AA. (**2005**) Anthocyans from fruit of certain plants of the genus *Prunus. Chemistry of Natural Compounds*, ***41***, 230-231.

[8] Cao G, Muccitelli HU, Sanchez-Moreno C, Prior RL. (**2001**) Anthocyanins are adsorbed in glycated forms in elderly women: a pharmacokinetic study. *American Journal of Clinical Nutrition*, ***73***, 920-926.

[9] Youdim KA, Martin A, Joseph JA. (**2000**) Incorporation of the elderberry anthocyanins by endothelian cells increases protection against oxidative stress. *Free Radical Biology and Medicine*, ***29***, 51-60.

[10] Lapidot T, Harel SS, Akiri B, Granit R, Kanner J. (**1999**) pH-dependent forms of red wine anthocyanins as antioxidants. *Journal of Agricultural and Food Chemistry*, ***47***, 67-70.

[11] De Groot H, Rauen U. (**1998**) Tissue injury by reactive oxygen species and the protective effects of flavonoids. *Fundamental and Clinical Pharmacology*, ***12,*** 249-255.

[12] Cowan MM. (**1999**) Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, ***12***, 564-582.

[13] Wang SY, Lin S. (**2000**) Antioxidant activity in fruits and leaves of blackberry, raspberry and strawberry varies with cultivar and development stage. *Journal of Agricultural and Food Chemistry*, ***48***, 140-146.

[14] Frankel EN, Waterhouse AL, Teissedre PL. (**1995**) Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *Journal of Agricultural and Food Chemistry*, ***43***, 890-894.

[15] Burns J, Gardner PT, O'Neil J, Crawford S, Morecroft I, McPhail DB, Lister C, Matthews D, MacLean MR, Lean ME, Duthie GG, Crozier A. (**2000**) Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wines. *Journal of Agricultural and Food Chemistry*, ***48***, 220-230.

[16] Amou A, Makris DP, Kefalas P. (**2002**) Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece. *Journal of Food Composition and Analysis*, ***15***, 655-665.

[17] Kallithraka S, Mohdalya AAA, Makris DP, Kefalas P. (**2005**) Determination of major anthocyanin pigments in Hellenic native grape varieties (*Vitis vinifera* sp.): association with antiradical activity. *Journal of Food Composition and Analysis*, ***18***, 375-386.

[18] Duthie GG, Duthie SJ, Kyle JAM. (**2000**) Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nutrition Research Reviews*, ***13***, 79-106.

[19] Ramirez –Tortosa C, Andersen OM, Gardner PT, Morrice PC, Wood SG, Duthie SJ, Collins AR, Duthie GG. (**2001**) Anthocyanin-rich extract decreases indices of lipid peroxidation and DNA damage in vitamin E-depleted rats. *Free Radical Biology and Medicine*, ***31***, 1033-1037.

[20] Aherne SA, O' Brien M. (**2000**) Mechanism of protection by the flavonoids, quercetin and rutin, against tert-butyl-hydro-peroxide- and menadione-induced DNA single strand breaks in CACO-2 cells. *Free Radical Biology and Medicine*, ***29***, 507-514.

[21] Tsuda T, Shiga K, Ohshima K, Kawakishi S, Osawa T. (**1996**) Inhibition of lipid peroxidation and the active oxygen radical scavenging effect of anthocyanin pigments isolated from *Phaseolus vulgaris* L. *Biochemical Pharmacology*, ***52***, 1033-1039.

[22] Wang CJ, Wang JM, Lin WL, Chu CY, Chou FP, Tseng TH. (**2000**) Protective effects of *Hibiscus* anthocyanins against tert-butyl-hydroperoxide-induced hepatic toxicity in rats. *Food and Chemical Toxicology*, ***38***, 411-416.

[23] Tsuda T, Kato Y, Osawa T. (**2000**) Mechanism for the peroxynitrite scavenging activity by anthocyanins. *FEBS Letters*, ***484***, 207-210.

[24] Kerry NL, Abbey M. (**1997**) Red wine and fractionated phenolic compounds prepared from red wine inhibit low density lipoprotein oxidation *in vitro*. *Atherosclerosis*, ***135***, 93-102.

[25] Sarma AD, Sharma R. (**1999**) Anthocyanin-DNA copigmentation complex: mutual protection against oxidative damage. *Phytochemistry*, ***52***, 1313-1318.

[26] Sestili P, Martinelli C, Ricci D, Fraternale D, Bucchini A, Giamperi L, Curcio R, Piccoli G, Stocchi V. (**2007**) Cytoprotective effect of preparation from various parts of *Punica granatum* L. fruits in oxidatively injured mammalian cells in comparison with their antioxidant capacity in cell free systems. *Pharmacological Research*, ***56***, 18-26.

[27] Kovacic P, Thurn LA. (**2005**) Cardiovascular toxicity from the perspective of oxidative stress, electron transfer, and prevention by antioxidants. *Current Vascular Pharmacology*, ***3,*** 107-117.

[28] Urquiaga I, Leighton F. (**2000**) Plant polyphenol antioxidants and oxidative stress. *Biological Research*, ***33***, 55-64.

[29] Kumarasamy Y, Cox PJ, Jaspars M, Nahar L, Sarker SD. (**2004**) Comparative studies on biological activities of *Prunus padus* and *Prunus spinosa*. *Fitoterapia*, ***75***, 77-80.

[30] Kiselova Y, Ivanova D, Chervenkov T, Gerova D, Galunska B, Yankova T. (**2006**) Correlation between the *in vitro* antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. *Phytotherapy Research*, ***20***, 961-965.

[31] Sellappan S, Akoh CC, Krewer G. (**2002**) Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *Journal of Agricultural and Food Chemistry*, ***50***, 2432-2438.

[32] Rababah TM, Ereifej KI, Howard L. (**2005**) Effect of ascorbic acid and dehydration on concentration of total phenolics, antioxidant capacity, anthocyanins, and color in fruits. *Journal of Agricultural and Food Chemistry*, ***53***, 4444-4447.

[33] Solomon A, Golubowiez S, Yablowiez Z, Grossman S, Bergman M, Gottlieb HE, Altman A, Kerem Z, Flaishman MA. (**2006**) Antioxidant activities and anthocyanin content of fresh fruits of common fig (*Ficus carica* L.). *Journal of Agricultural and Food Chemistry*, ***54***, 7717-7723.

[34] Rapisarda P, Tomaino A, Lo Cascio R, Bonina F, De Pasquale A, Saija A. (**1999**) Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *Journal of Agricultural and Food Chemistry*, ***47***, 4718-4723.

[35] Tzulker R, Glazer I, Bar-Ilan I, Holland D, Aviram M, Amir R. (**2007**) Antioxidant activity, polyphenol content, and related compounds in different fruit juice and homogenates prepared from 29 different pomegranate accessions. *Journal of Agricultural and Food Chemistry*, ***55***, 9559-9570.

[36] Ranilla LG, Genovese MI, Lajolo FM. (**2007**) Polyphenols and antioxidant capacity of seed coat and cotyledon from Brazilian and Peruvian bean cultivars (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry*, ***55***, 90-98.

[37] Elisia I, Hu C, Popovich DG, Kitts DD. (**2007**) Antioxidant assessment of an anthocyanin- enriched blackberry extract. *Food Chemistry*, ***101***, 1052-1058.

[38] Zafra-Stone S, Yasmin T, Bagchi M, Chatterjee A, Vinson JA, Bagchi D. (**2007**) Berry anthocyanins as novel antioxidants in human health and disease prevention. *Molecular Nutrition and Food Research*, ***51***, 675-683.

[39] Oki T, Kobayashi M, Nakamura T, Okuyama A, Masuda M, Shiratsuchi H, Suda I. (**2006**) Changes in radical-scavenging activity and components of mulberry fruit during maturation. *Journal of Food Science*, ***71***, 18-21.

[40] Monagas M, Hernandez-Ledesma B, Gómez-Cordovés C, Bartolomé B. (**2006**) Commercial dietary ingredients from *Vitis vinifera* L. leaves and grape skins: antioxidant and chemical characterization. *Journal of Agricultural and Food Chemistry*, ***54***, 319-327.

[41] Palomba L, Sestili P, Columbaro M, Falcieri E, Cantoni O. (**1999**) Apoptosis and necrosis following exposure of U937 cells to increasing concentrations of hydrogen peroxide: the effect of the poly(ADP-ribose)polymerase inhibitor 3-aminobenzamide. *Biochemical Pharmacology*, ***58***, 1743-1750.

[42] Tommasini I, Sestili P, Cantoni O. (**2002**) Delayed formation of hydrogen peroxide mediates the lethal response evoked by peroxynitrite in U937 cells. *Molecular Pharmacology*, ***61***, 870-878.

[43] Hagerman AE, Butler LG. (**1994**) Assay of condensed tannins or flavonoid oligomers and related flavonoids in plants. *Methods in Enzymology*, ***234***, 429-429.

[44] Mellors A, Tappel AL. (**1966**) The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol. *Journal of Biological Chemistry*, ***241***, 4353-4356.

[45] Sud'ina GF, Mirzoeva OK, Pushkareva MA, Korshunova GA, Sumbatyan NV, Varfolomeev SD. (**1993**) Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties. *FEBS Letters*, ***329***, 21-24.

[46] Cao G, Alessio HM, Culter R. (**1993**) Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology and Medicine*, ***14***, 303-311.

[47] Ou B, Hampsch-Woodill M, Prior L. (**2001**) Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry*, ***49***, 4619-4626.