RESEARCH AND INNOVATION FOCUSING ON BY-PRODUCTS OF GRAPES AND WINES

DEMETRIOS KOURETAS

PROFESSOR

DEPARTMENT OF BIOCHEMISTRY & BIOTECHNOLOGY

UNIVERSITY OF THESSALY, GREECE

Entrepreneurial Discovery Focus Group on wine for Eastern Macedonia and Thrace

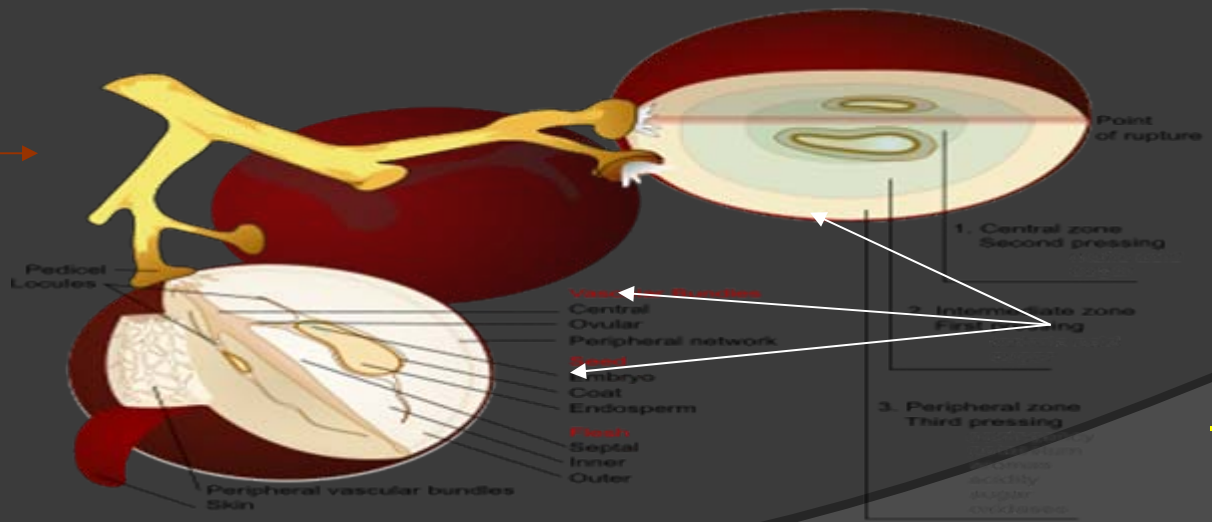
Drama, Greece

- *Vitis vinifera is a plant that presents important biological properties.*

The main by-products after vinification are:

- *Stems, seeds and skin (altogether named as pomace).*

Grape stems



Berries

- **The research on by-products of grapes and wines involves the following stages:**
- **Isolation of extracts containing bioactive compounds.**
- **Chemical characterization of the extracts.**
- **Assessment of the biological activity of the extracts and individual bioactive compounds.**
- **Development of food supplements or biofunctional foods using grape bioactive extracts.**

RESEARCH PROJECT 'WINE & HEALTH

- **Funding: E.U., KEOSOE (GSRT PENED 2001)**

- **This was the first and biggest study on the chemical characteristics and biological properties of the Greek grape varieties and wines.**

- **Participant research organisations:**

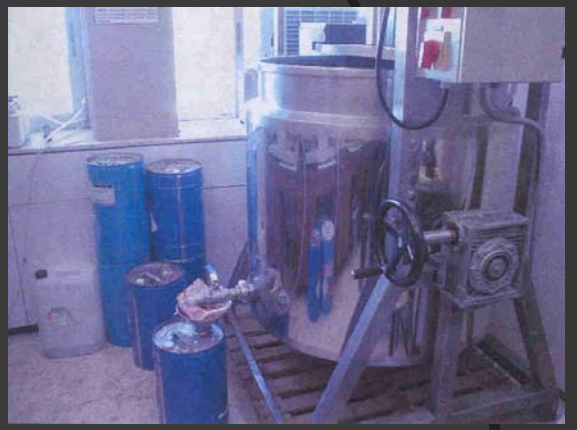
University of Thessaly (Dept. of Biochemistry & Biotechnology), Agricultural University of Athens, National & Kapodistrian University of Athens (Dept. of Pharmacy), National Hellenic Research Institute

- **Research Aims:**

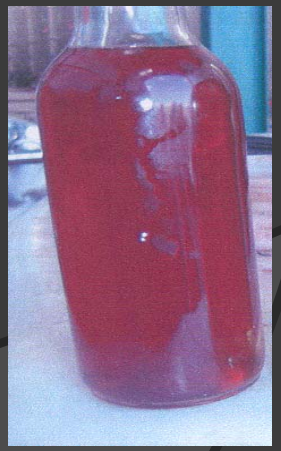
i. **Qualitative and quantitative recording of bioactive compounds found in Greek grape varieties and wines.**
ii. **Assessment of the biological activity of the bioactive compounds.**
iii. **Development of a methodology for the fast identification of bioactive compounds in grapes.**
iv. **Development of the effects of viticulture and vinification methods on the biological properties of**

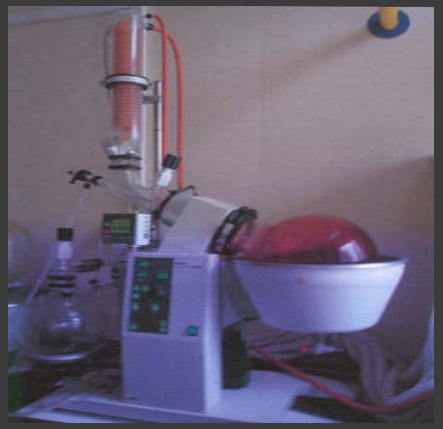
ISOLATION OF BIOACTIVE EXTRACTS FROM GRAPE POMACE

Grape pomace



Extraction
(with methanol or H_20)





Vaporization of the dissolver

Methanolic extracts

Water extracts

- **Grape extracts rich in bioactive polyphenols**

System of columns containing absorptive resins

Extract

Elution with H_2O

Elution with ethyl acetate or methanol

Available online at www.sciencedirect.com
ScienceDirect
Mutation Research 609 (2006) 165–175
Genetic Toxicology and Environmental Mutagenesis
www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Activity of grape extracts from Greek varieties of *Vitis vinifera* against mutagenicity induced by bleomycin and hydrogen peroxide in *Salmonella typhimurium* strain TA102

Demetrios Stagos [a], Georgios Kazantzoglou [b], Demetra Theofanidou [a], Georgia Kakalopoulou [a], Prokopios Magiatis [b], Sofia Mitaku [b], Demetrios Kouretas [a,*]

[a] *Department of Biochemistry and Biotechnology, University of Thessaly, Ploutonos 26 & Aiolou, GR-41221 Larissa, Greece*
[b] *Division of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis Zografou, GR-15771 Athens, Greece*

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- **Methanolic grape pomace extracts rich in polyphenols inhibit ROS-induced mutagenicity.**

- **However, individual polyphenols such as trans-resveratrol and quercetin enhance ROS-induced mutagenicity.**

- **The antimutagenic effects of grape extracts rich in polyphenols may be due not to specific polyphenols but to a synergism between polyphenols and/or between polyphenols and other phytochemical compounds.**

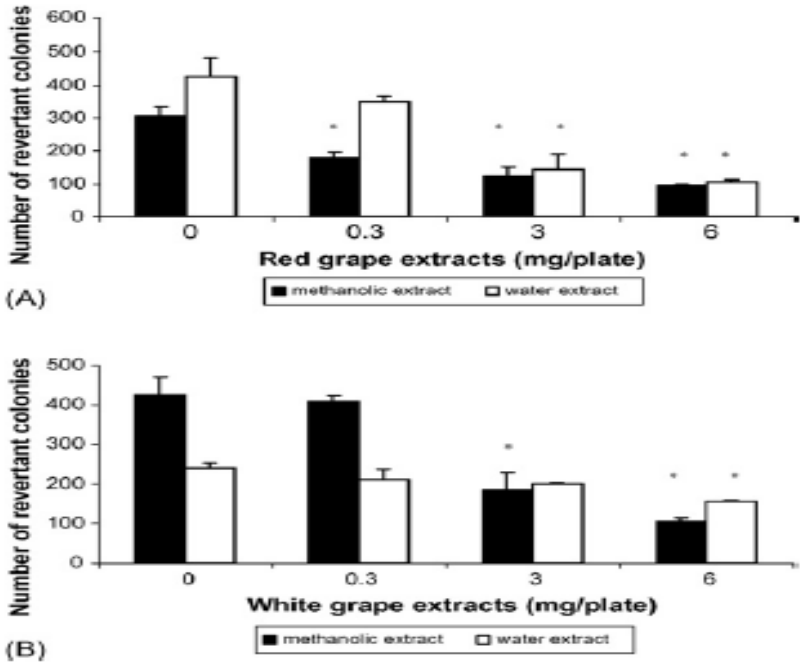


Fig. 2. Antimutagenic effects of grape extracts on BLM-induced mutagenicity in *S. typhimurium* TA102 cells. (A) Mandilaria variety (red grapes) and (B) Assyrtiko variety (white grapes). Values are the mean ± S.D. number of histidine revertants minus the number of spontaneous revertants (356 ± 29) of three independent experiments carried out in triplicate. The concentration of BLM was 0.5 μg/plate. $^{*}p < 0.05$ when compared with positive control.

Table 3
Effects of polyphenols on BLM-induced mutagenicity in *S. typhimurium* TA102 strain

Polyphenol	Dose (μM)	His^{+} revertants/plate[a] (% inhibition or induction)[b] BLM (0.5 μg/plate)
	0	494 ± 31
	1	497 ± 13 (+1)
Trans-resveratrol	10	545 ± 31 (+10)
	100	582 ± 37 (+18)
	500	727 ± 40 (+47)*
	0	332 ± 44
	1	350 ± 14 (+5)
(+)-Catechin	10	365 ± 46 (+10)
	100	371 ± 30 (+12)
	500	415 ± 42 (+25)
	0	416 ± 22
	1	439 ± 38 (+6)
(−)-Epicatechin	10	426 ± 14 (+2)
	100	376 ± 37 (10)
	500	429 ± 23 (+3)
	0	314 ± 45
	1	311 ± 17 (1)
Gallic acid	10	323 ± 8 (+3)
	100	342 ± 26 (+9)
	500	330 ± 14 (+5)
	0	314 ± 45
	1	320 ± 7 (+2)
Protocatechuic acid	10	309 ± 14 (2)
	100	316 ± 10 (+1)
	500	354 ± 14 (+13)
	0	386 ± 8
	1	371 ± 21 (4)
Quercetin	10	419 ± 5 (+9)*
	100	460 ± 6 (+19)*
	500	toxicity

5246 J. Agric. Food Chem. 2007, 55, 5246–5252

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Cytogenetic Effects of Grape Extracts (*Vitis vinifera*) and Polyphenols on Mitomycin C-Induced Sister Chromatid Exchanges (SCEs) in Human Blood Lymphocytes

Dimitrios Stagos,[†,#] Chrysa Spanou,[†] Maria Margariti,[†] Constantinos Stathopoulos,[†] Zissis Mamuris,[†] Georgios Kazantzoglou,[§] Prokopios Magiatis,[§] and Demetrios Kouretas*[,†,#]

- Grape pomace extracts rich in polyphenols exhibited pro-oxidant activity not in normal cells but only in those being under the genotoxic pressure of an oxidant agent (mitomycin-C).

- This selective pro-oxidant activity is thought to be an important chemopreventive mechanism, because it may lead to apoptosis, a programmed cell death, which eliminates cells showing a genomic instability.

Table 2. Effects of Grape Extracts and Polyphenol-Rich Fractions on MMC-Induced SCEs in Cultures of Human Lymphocytes

treatment[a]	dose (μg/mL)	SCE[b] (x + SD, n = 3)	% inhibition/ induction[c]
negative control		8.50 ± 0.74	
ME (white grapes)	300	8.58 ± 0.49	1[d]
MMC[e]		21.19 ± 0.27	
MMC + ME (white grapes)	75	19.84 ± 0.50	+10
	150	23.50 ± 0.20[f]	+18
	300	24.24 ± 0.10[f]	+24
negative control		8.85 ± 0.61	
WE (white grapes)	300	8.52 ± 0.28	+4[d]
MMC		19.91 ± 0.16	
MMC + WE (white grapes)	75	20.44 ± 0.20	+5
	150	22.91 ± 0.10[f]	+27
	300	23.42 ± 0.56[f]	+32
negative control		7.51 ± 0.88	
ME (red grapes)	300	7.74 ± 0.42	+3[d]
MMC		17.18 ± 0.16	
MMC + ME (red grapes)	75	16.75 ± 0.20	3
	150	19.87 ± 0.10[f]	+28
	300	22.63 ± 1.24[f]	+56
negative control		7.51 ± 0.88	
WE (red grapes)	300	8.17 ± 0.32	+9[d]
MMC		17.18 ± 0.16	
MMC + WE (red grapes)	75	17.32 ± 0.77	+1
	150	18.01 ± 0.45	+9
	300	21.50 ± 0.12[f]	+45
negative control		7.65 ± 0.09	
EAF from WE (red grapes)	300	7.73 ± 0.38	+1[d]
MMC		25.10 ± 0.52	
MMC + EAF from WE (red grapes)	75	28.76 ± 0.43[f]	+21
	150	32.60 ± 0.24[f]	+43
	300	37.66 ± 0.83[f]	+72
negative control		7.65 ± 0.09	
MF from WE (red grapes)	300	8.15 ± 0.89	+7[d]
MMC		25.10 ± 0.52	
MMC + MF from WE (red grapes)	75	28.04 ± 0.90	+17
	150	29.22 ± 0.10[f]	+24
	300	33.57 ± 1.76[f]	+49
negative control		8.12 ± 0.96	
MF from ME (red grapes)	300	7.31 ± 0.32	10[d]
MMC		21.15 ± 0.11	
MMC + MF from ME (red grapes)	75	22.26 ± 0.83	+9
	150	20.60 ± 0.30	4
	300	22.53 ± 0.40	+11

[a] ME, methanolic extract; WE, water extract; MF, methanolic fraction; EAF, ethyl acetate fraction. [b] Values are the mean ± SD number of SCEs in three independent experiments (the SCE frequency was scored in 30 s metaphases in each experiment). [c] The (+) denotes the percentage induction in the number of SCEs; otherwise, numbers denote inhibition. [d] Compared with negative controls (untreated cultures). [e] The concentration of MMC was 20 ng/mL in all cultures. Percent inhibition/induction of SCEs caused by test compounds was calculated as described under Materials and Methods. [f] $p < 0.05$ when compared with positive control (cultures with MMC alone) using Dunnett's multiple-comparison test.

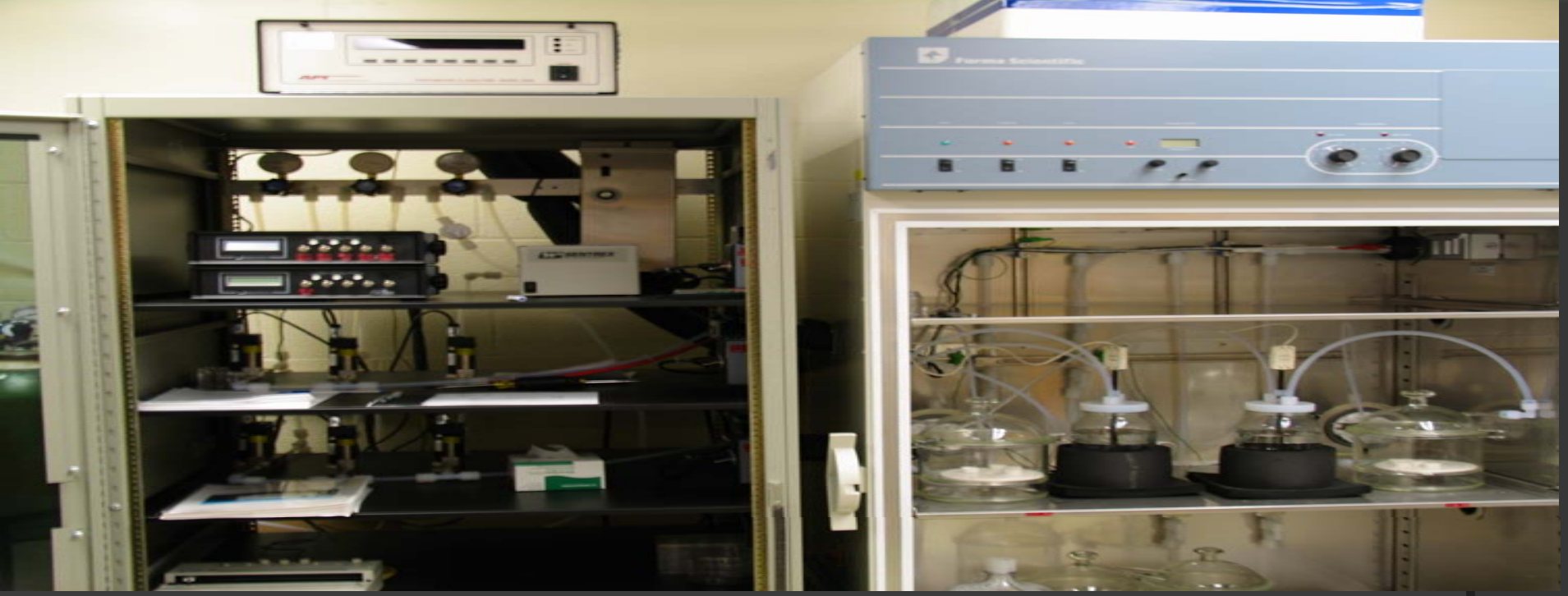
POLYPHENOLS FROM GRAPE POMACE PROTECT LUNG PROTEIN SP-A FROM OZONE-INDUCED OXIDATION

Free Radical Research, March 2007; 41(3): 357–366

informa
healthcare

Inhibition of ozone-induced SP-A oxidation by plant polyphenols

DIMITRIOS STAGOS[1,2], TODD M. UMSTEAD[3], DAVID S. PHELPS[3], LEANDROS SKALTSOUNIS[4], SERKO HAROUTOUNIAN[5], JOANNA FLOROS[2,3,6,†], & DEMETRIOS KOURETAS[1]



•Collaboration with the Medical School of the Pennsylvania State University (USA).

•The experimental system for in vitro ozone exposure of proteins, cells and animals.

•This system delivers precisely controlled flow rates of gases (filtered air with 5% carbon dioxide saturated with water vapor at 37^0C) to the exposure vessels with precise ozone concentrations.

•SP-A protein samples were exposed to ozone (1 ppm) for 4 h.

• Grape pomace polyphenols protect SP-A protein, a molecule that plays an important role in normal lung function and innate host defense, from ozone-induced oxidation.

•Grape pomace polyphenols may be used to prevent the detrimental effects of air pollutants on the lung.

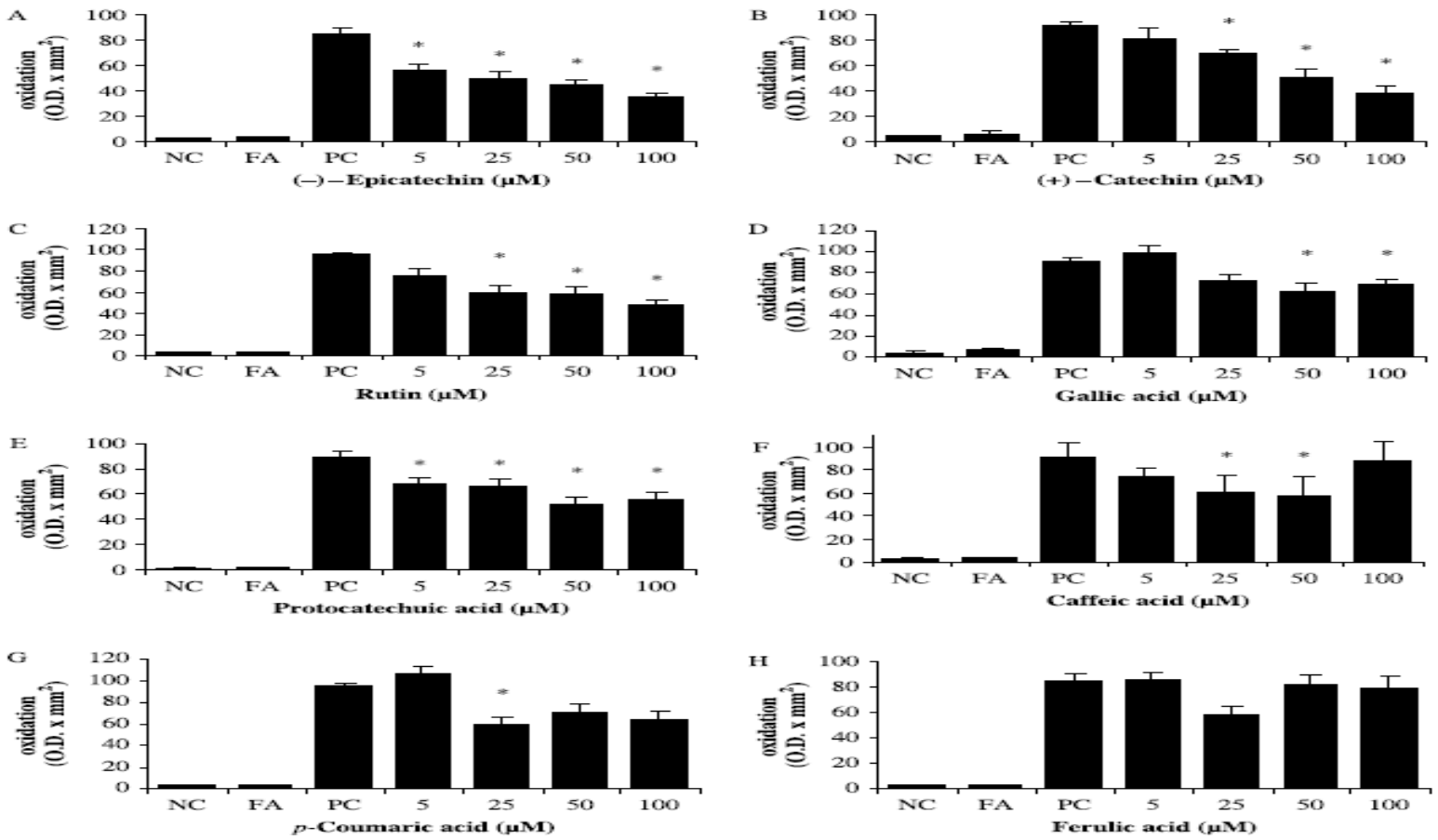


Figure 5. Effects of tested polyphenols on ozone-induced SP-A oxidation. SP-A was exposed to ozone (1 ppm) for 4 h. Oxidation was expressed as the product of optical density (OD) by the area (mm^2) of dots. Values are the mean ± SE from three independent experiments carried out in triplicate. NC: Negative control (SP-A alone exposed to filtered air); PC: Positive control (SP-A alone exposed to ozone); FA: SP-A plus polyphenol at a concentration of 100 μM exposed to filtered air. ANOVA was used for the statistical analysis. $*p < 0.05$ when compared with positive control.

in vivo 25: xxx-xxx (2011)

section for this paper

- ☐ Experimental
- ☐ Clinical
- ☐ Epidemiological

Effects of Grape Extracts on the *In Vitro* Activity of Enzymes Involved in Oxidative Stress Regulation

CHRYSOULA SPANOU[1], ARISTIDIS S. VESKOUKIS[1], DIMITRIOS STAGOS[1], KALLIOPI LIADAKI[1], MARIA ANASTASIADI[2], SERKOS A. HAROUTOUNIAN[2], MARIA TSOUKA[1], ELENI TZANAKOULI[1] and DIMITRIOS KOURETAS[1]

• Grape extracts from different parts (stems, skin, berries) of the plant modify the activity of important antioxidant enzymes.

Table III. *Effects of Vitis vinifera grape extracts on XO, CAT and SOD activities.*

Plant part extract	*Vitis vinifera* grape varieties	IC_{50} (μg/ml) Inhibition XO	TPC at IC_{50}	IC_{50} (μg/ml) Inhibition CAT	TPC at IC_{50}	PC_{50} (μg/ml) Induction SOD	TPC at PC_{50}	Pyrogallol autoxidation % Inhibition
Stems	Athiri Santorini 2007	2.5±0.6	1.2	5±0.5	2.3	300±13	139	NA
	Asyrtiko Santorini 2006	8±1	3.6	1.5±0.1	0.7	NA		NA
	Mavrotragano Santorini 2008	9±0.2	4.2	4.5±0.1	2.1	475±1†	220	15*
	Mandilaria Santorini sun dried 2007	15±0.6	8.1	27±0.6	14.5	480±2	258	NA
	Vilana Sitia 2009	16±1.2	7.3	15±0.3	6.8	260±1	118	NA
	Mavrotragano Santorini 2007	29±2.7	16.9	9±0.3	5.3	NA		NA
	Voidomato Santorini 2006	33±6.4	16.3	22±0.6	10.9	510±2†	252	19*
	Asyrtiko Santorini 2007	33±1.7	18.9	2.5±0.3	1.4	420±5	241	NA
	Asyrtiko Santorini 2008	35±0.2	13.0	18±0.6	6.7	580±12†	216	18*
	Mandilaria Santorini 2006	42±2.3	24.5	11±0.3	6.4	180±1	105	NA
	Athiri Santorini 2006	60±0.6	33.5	8±0.6	4.5	460±2	257	NA
Skin (pomace)	Asyrtiko Santorini 2007	15±1	2.5	4±0.3	0.7	340±6	57	NA
	Mandilaria Santorini 2006	35±4	7.3	34±1.3	7.1	NA		NA
	Asyrtiko Rodos 2006	75±1	34.9	7±0.2	3.3	650±10†	302	13*
Grape (berries)	Batiki Tyrnavos (aqueous) 2006	36±3	23.3	>30‡	>19.4	200±1	130	NA
	Mandilaria Santorini (methanolic) 2003	50±7	11.5	200±0.5	44.6	NA		NA
	Mandilaria Rodos 2006	50±2	23.3	>50‡	>23.3	390±1	182	NA
	Mandilaria Santorini (aqueous) 2003	70±2	14.7	98±1	20.6	480±9	101	NA
	Asyrtiko Santorini (methanolic) 2003	85±14	7.5	80±3.5	7	NA		NA
	Asyrtiko Santorini (aqueous) 2006	140±5	69	40±4.6	19.7	320±2†	158	48*
	Asyrtiko Santorini (aqueous) 2003	250±32	15.7	214±1.2	13.5	NA		NA
Positive controls		IC_{50} (μM)		IC_{50} (μM)				
	Allopurinol	2.1±0.3						
	Sodium azide			0.35±0.01				

TPC: Total Polyphenol Content as mg gallic acid/g extract; NA, no activity. The date accompanying extracts refers to the year of vinification. †Extracts affecting pyrogallol autoxidation. *Statistically significant inhibition of pyrogallol autoxidation at PC_{50} concentrations compared to control, $p<0.05$. ‡Extracts contributing to the optical density. Values are expressed as the mean±SEM (n=3).

Article

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Effects of polyphenolic grape extract on the oxidative status of muscle and endothelial cells

N Goutzourelas[1], D Stagos[1], N Demertzis[1], P Mavridou[1], H Karterolioti[1], S Georgadakis[1], E Kerasioti[1], N Aligiannis[2], L Skaltsounis[2], A Statiri[1], A Tsioutsiouliti[1], AM Tsatsakis[3], AW Hayes[4,5] and D Kouretas[1]

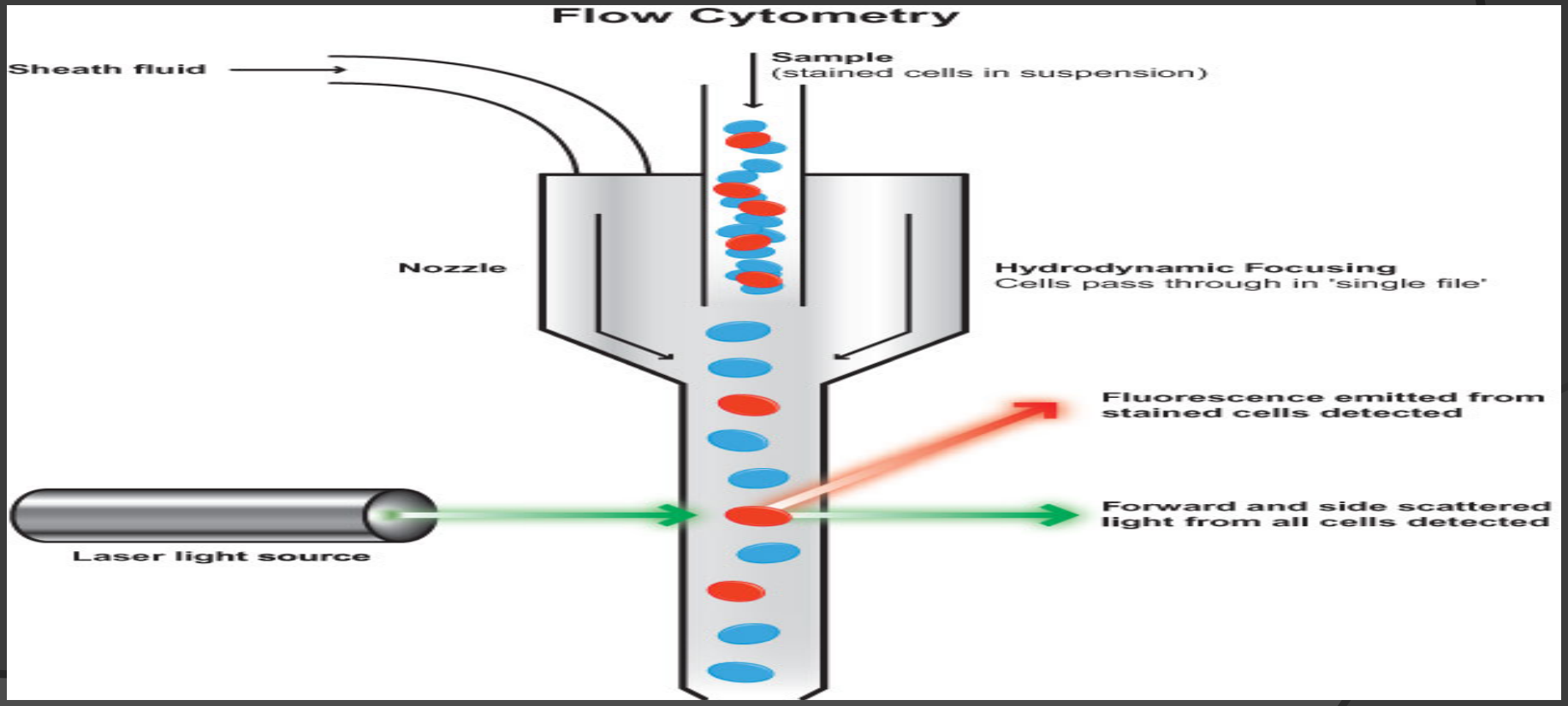
Abstract

A grape pomace extract enhanced antioxidant mechanisms in muscle and endothelial cells both in the absence and in the presence of oxidative stress-induced agent tert-butyl hydroperoxide (tBHP). In particular, muscle (C2C12) and endothelial (EA.hy926) cells were treated with the extract at noncytotoxic concentrations for 24 h, and the oxidative stress markers, total reactive oxygen species (ROS), glutathione (GSH), thiobarbituric reactive substances (TBARS), and protein carbonyl levels were assessed. The results showed that the grape extract treatment reduced significantly ROS, TBARS, and protein carbonyl levels and increased GSH in C2C12 cells, while it increased GSH and decreased protein carbonyl levels in EA.hy926 cells. In the presence of tBHP, the grape extract treatment in C2C12 cells reduced significantly ROS, TBARS, and protein carbonyls and increased GSH compared with tBHP alone treatment, while, in EA.hy926 cells, the extract decreased significantly TBARS and protein carbonyls but increased GSH. The antioxidant potency of the extract was different between muscle and endothelial cells suggesting that the antioxidant activity depends on cell type. Moreover, the antioxidant activity of the grape extract, in both cell lines, exerted, at least in part, through increase in GSH levels. The present work is the first to report the effects of grape extract shown for skeletal muscle cells.

•In this study, the effects of a grape pomace extract on the redox status of cells was assessed with flow cytometry.

• In cell biology, flow cytometry is a laser-based, biophysical technology employed in cell counting, cell sorting and biomarker detection by suspending cells in a stream of fluid and passing them by an electronic detection apparatus.

•It allows simultaneous multiparametric analysis of the physical and chemical characteristics of up to thousands of cells per second.



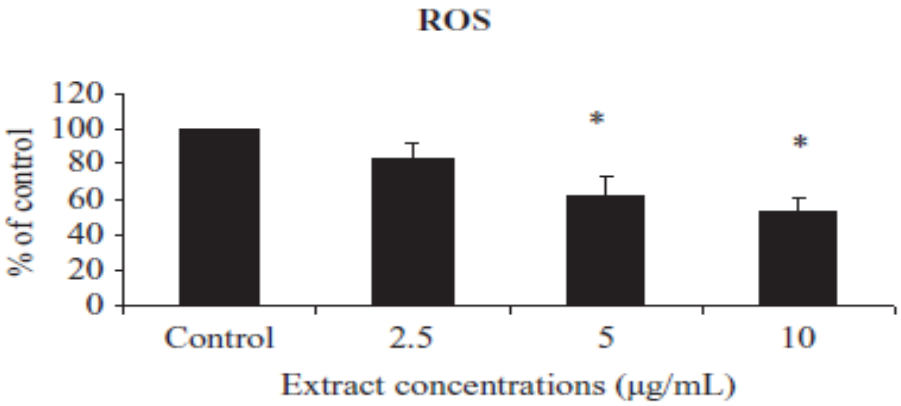
•**Grape pomace extract improved redox status in muscle cells (C2C12):**

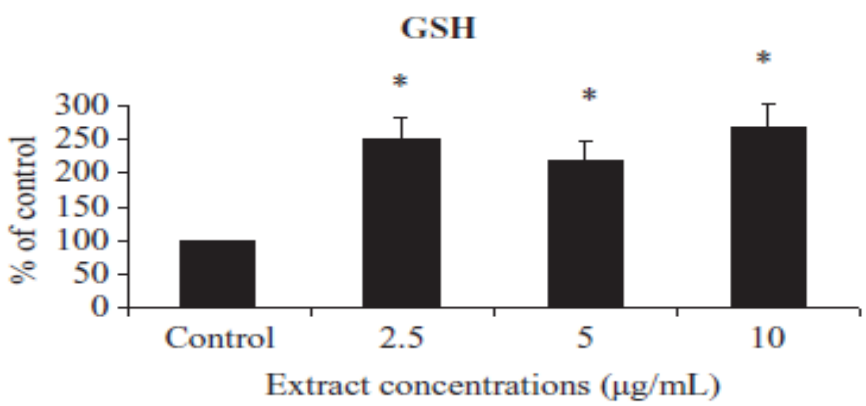
•**Reduced ROS levels**

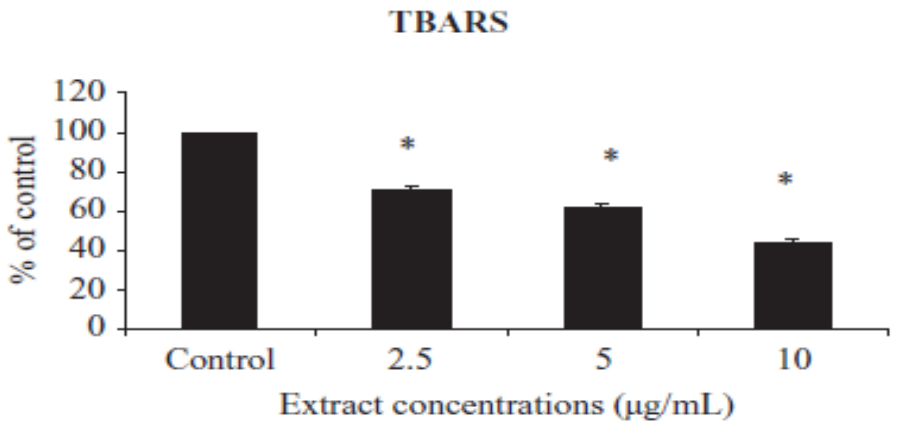
• **Increased GSH, levels, the most important antioxidant molecule in cells**

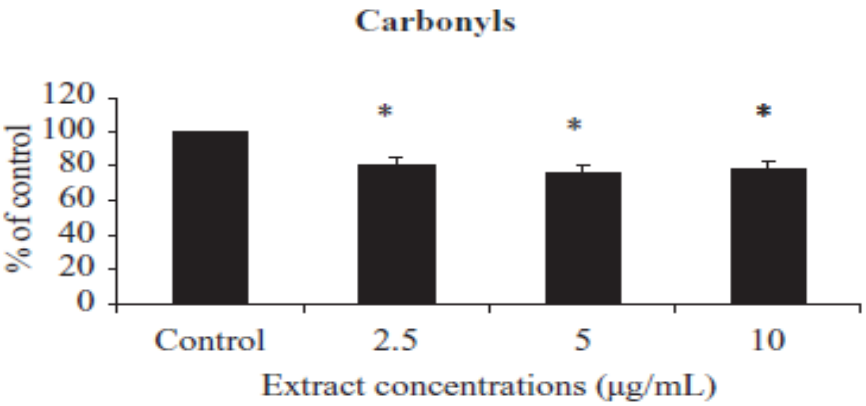
• **Reduced lipid peroxidation**

• **Reduced protein oxidation**









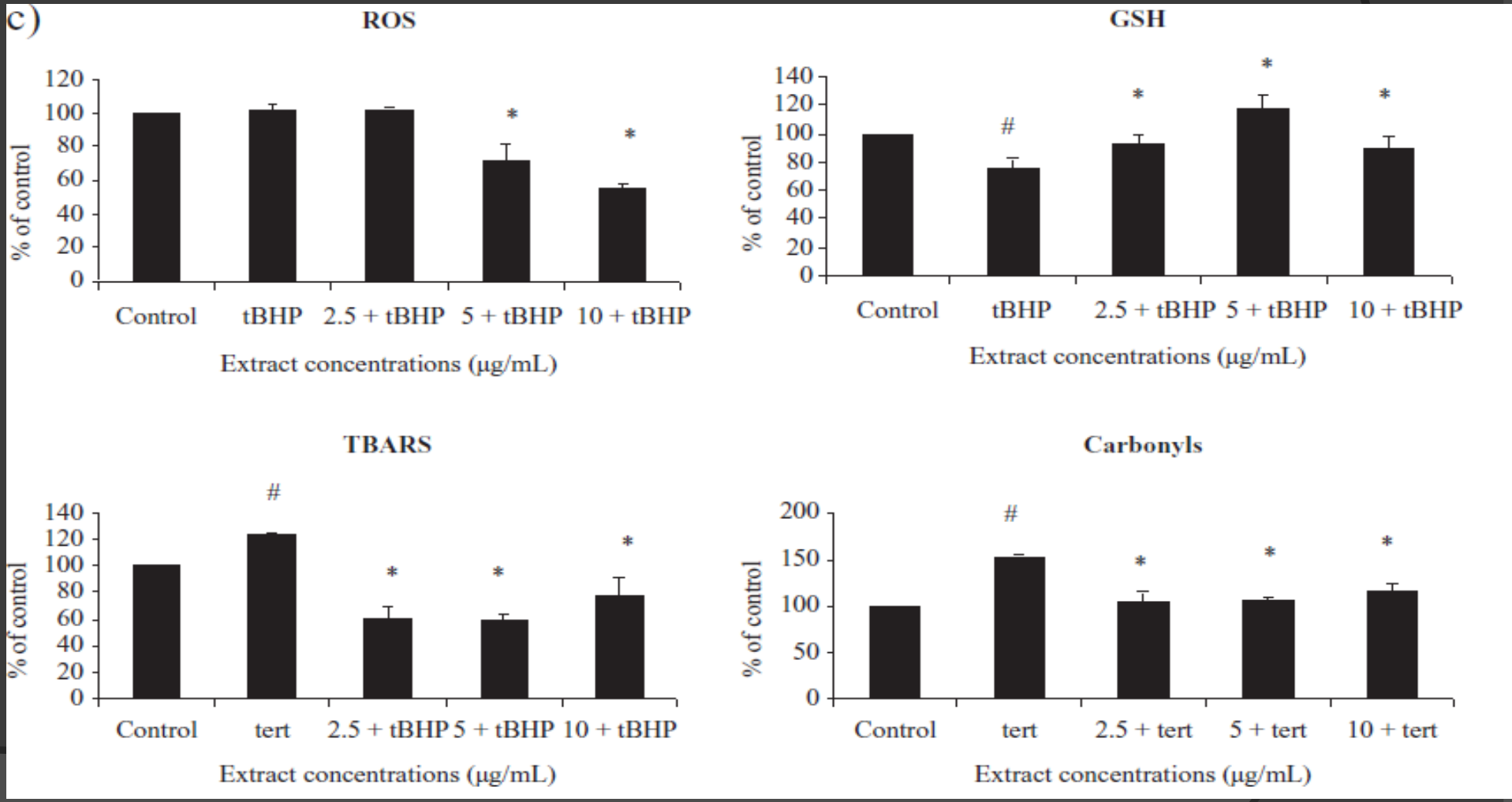
•**Grape pomace extract protected muscle cells (C2C12) from ROS-induced oxidative stress:**

•**Reduced ROS levels**

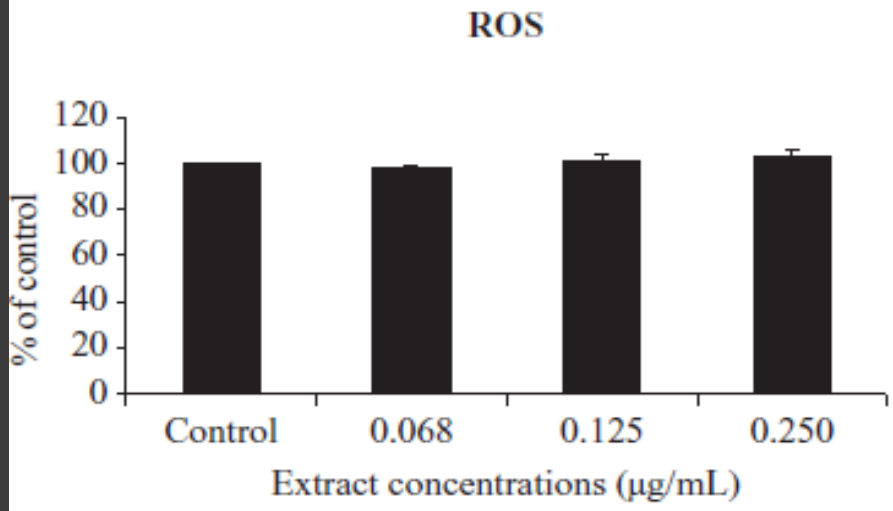
• **Increased GSH, levels**

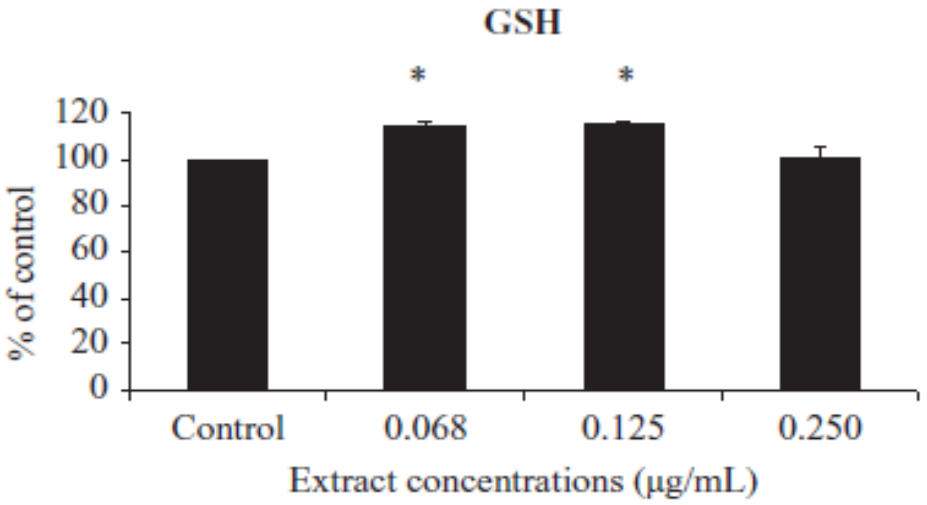
• **Reduced lipid peroxidation**

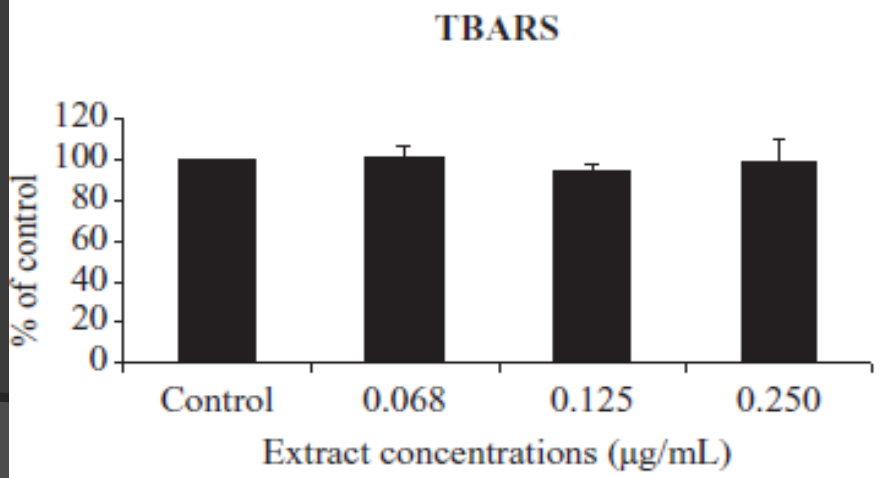
• **Reduced protein oxidation**

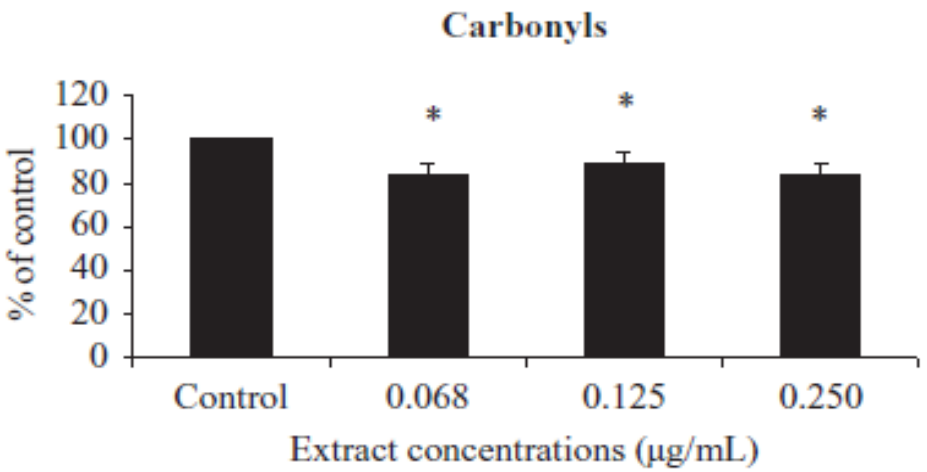


•**Grape pomace extract improved redox status in endothelial cells:**
•**Increased GSH, levels, the most important antioxidant molecule in cells**
• **Reduced protein oxidation**







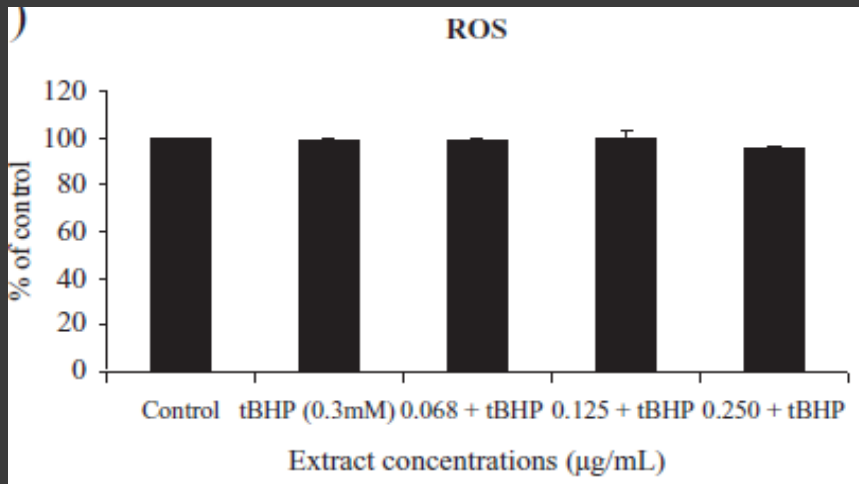


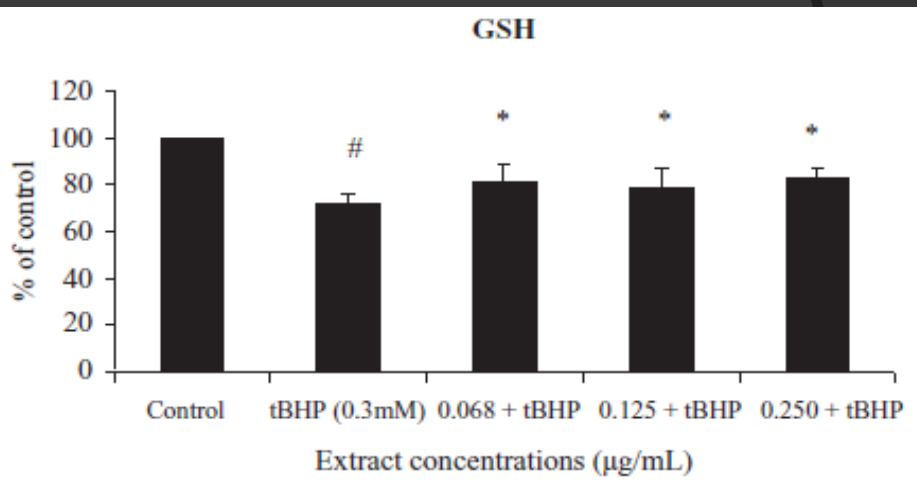
•**Grape pomace extract protected endothelial cells from ROS-induced oxidative stress:**

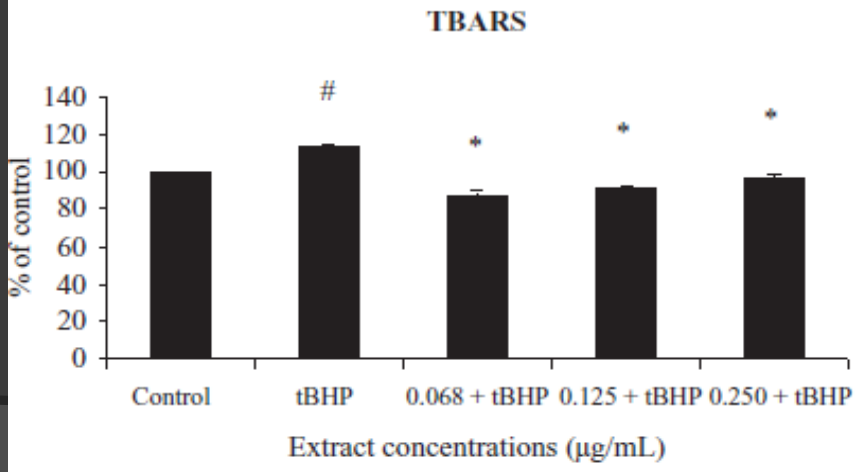
•**Increased GSH, levels, the most important antioxidant molecule in cells**

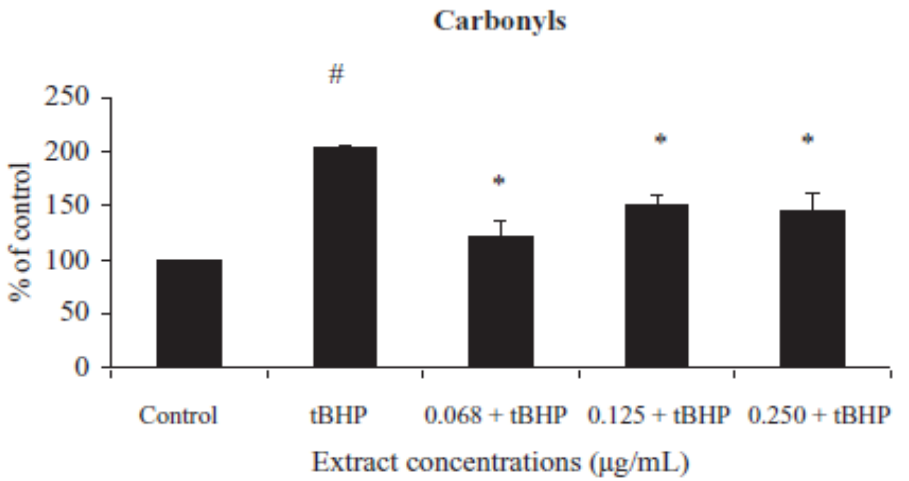
• **Reduced lipid peroxidation**

• **Reduced protein oxidation**









- **This is one of the first studies examining the antioxidant and anticarcinogenic activity of extracts from grape stems, a scarcely investigated by-product.**

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Food and Chemical Toxicology

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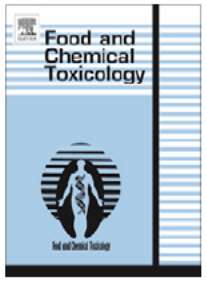


Assessment of polyphenolic content, antioxidant activity, protection against ROS-induced DNA damage and anticancer activity of *Vitis vinifera* stem extracts

Anna Apostolou [b], Dimitrios Stagos [a,*], Elissavet Galitsiou [a], Argiris Spyrou [a], Serko Haroutounian [b], Nikolaos Portesis [a], Ioanna Trizoglou [a], A. Wallace Hayes [c,d], Aristides M. Tsatsakis [e], Dimitrios Kouretas [a,*]

[a] *Department of Biochemistry and Biotechnology, University of Thessaly, Ploutonos 26 & Aiolou, 41221 Larissa, Greece*
[b] *Agricultural University of Athens, Athens, Iera Odos 75, 11855 Athens, Greece*
[c] *Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115, USA*
[d] *Spherix Consulting, Inc., 6430 Rockledge Drive #503, Bethesda, MD 20817, USA*
[e] *Laboratory of Toxicology, School of Medicine, University of Crete, Voutes 71003, Heraklion, Greece*

• **Stem extracts from Greek grape varieties exhibit strong free radical scavenging activities which are comparable to those of extracts from seeds and pomace.**

Table 3
Antioxidant capacity, protective activity against hydroxyl ($OH^{\cdot}$) and peroxyl ($ROO^{\cdot}$) radical-induced DNA damage.

Extract	Plant variety/harvest year	IC_{50} (μg/mL)	
		DPPH· [a]	$ABTS^{\cdot+}$ [a]
Stems	Assyrtiko/2009	4.2 ± 0.5	5.5 ± 0.4
	Assyrtiko/2010	5.5 ± 0.7	3.5 ± 0.2
	Mavrotragano/2009	5.0 ± 0.4	3.5 ± 0.3
	Mandilaria/2009	6.0 ± 0.8	4.0 ± 0.6
	Mandilaria/2006	9.0 ± 1.0	9.0 ± 0.9
	Voidomato/2006	7.0 ± 0.9	3.5 ± 0.5
	Voidomato/2009	8.5 ± 1.0	6.8 ± 0.7
	Vilana/2009	7.0 ± 0.8	11.8 ± 1.3
	Moshato/2009	8.0 ± 0.7	3.5 ± 0.5
	Ksinomavro/2010	9.0 ± 1.1	5.0 ± 0.3
	Vinsanto/2010	10.0 ± 1.1	4.0 ± 0.2
	Athiri/2010	15.0 ± 1.6	5.0 ± 0.4
Seeds	Assyrtiko/2010	3.0 ± 0.2	3.0 ± 0.4
	Ksinomavro/2010	4.7 ± 0.6	3.5 ± 0.3
	Robola/2010	6.0 ± 0.7	2.8 ± 0.2
Pomace	Assyrtiko/2009	5.0 ± 0.5	6.0 ± 0.8

[a] Values are the mean ± SD of at least two separate triplicate experiments.
[b] Values are the mean ± SD from three independent experiments.

• **Stem extracts from Greek grape varieties protect from ROS-induced DNA damage.**

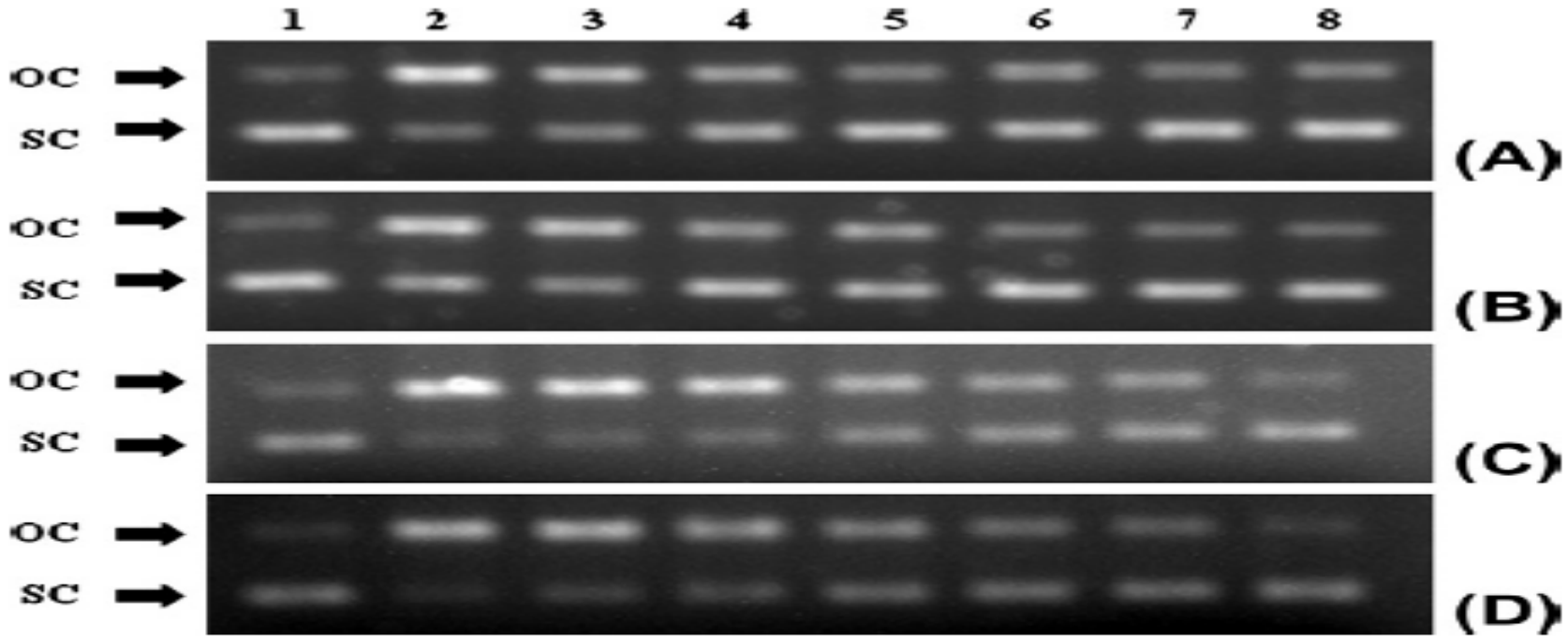


Fig. 1. Protective activity of stem extracts from (A) Mandilaria (2009) and (B) Voidomato (2009) varieties against $ROO^{\cdot}$ radicals: Lane 1, pBluescript-SK+ plasmid DNA without any treatment; lane 2, plasmid DNA exposed to $ROO^{\cdot}$ radicals alone; lanes 3–7 plasmid DNA exposed to $ROO^{\cdot}$ radicals in the presence of 0.25, 0.5, 1, 1.5 and 2 μg/mL of extract respectively; lane 8, plasmid DNA exposed to 2 μg/mL extract alone. Protective activity of stem extracts from (C) Voidomato (2006) and (D) Vinsanto (2010) varieties against $OH^{\cdot}$ radicals: Lane 1, plasmid DNA without any treatment; lane 2, plasmid DNA exposed to UV plus H_2O_2; lanes 3–7, plasmid DNA exposed to UV plus H_2O_2 in the presence of 50, 100, 200, 400 and 800 μg/mL extract respectively; lane 8, plasmid DNA exposed to 800 μg/mL extract alone. OC: open circular; SC: supercoiled.

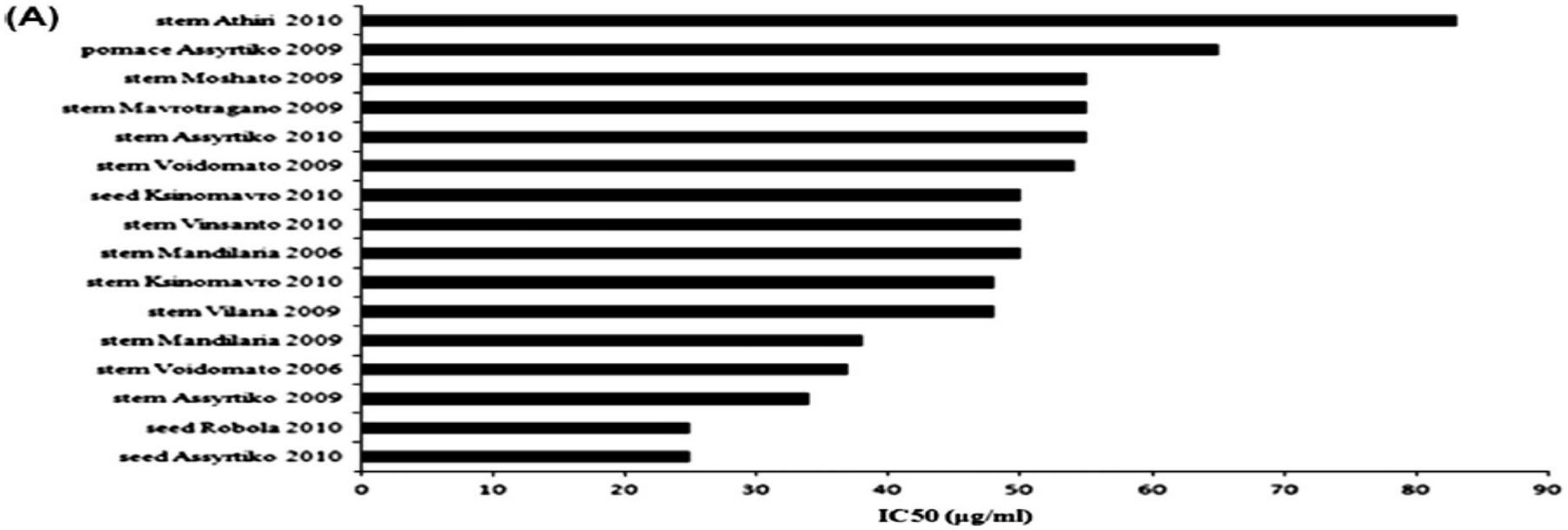
• **The IC50 values of grape stem extracts against ROS-induced DNA damage was comparable to those of grape seeds and pomace.**

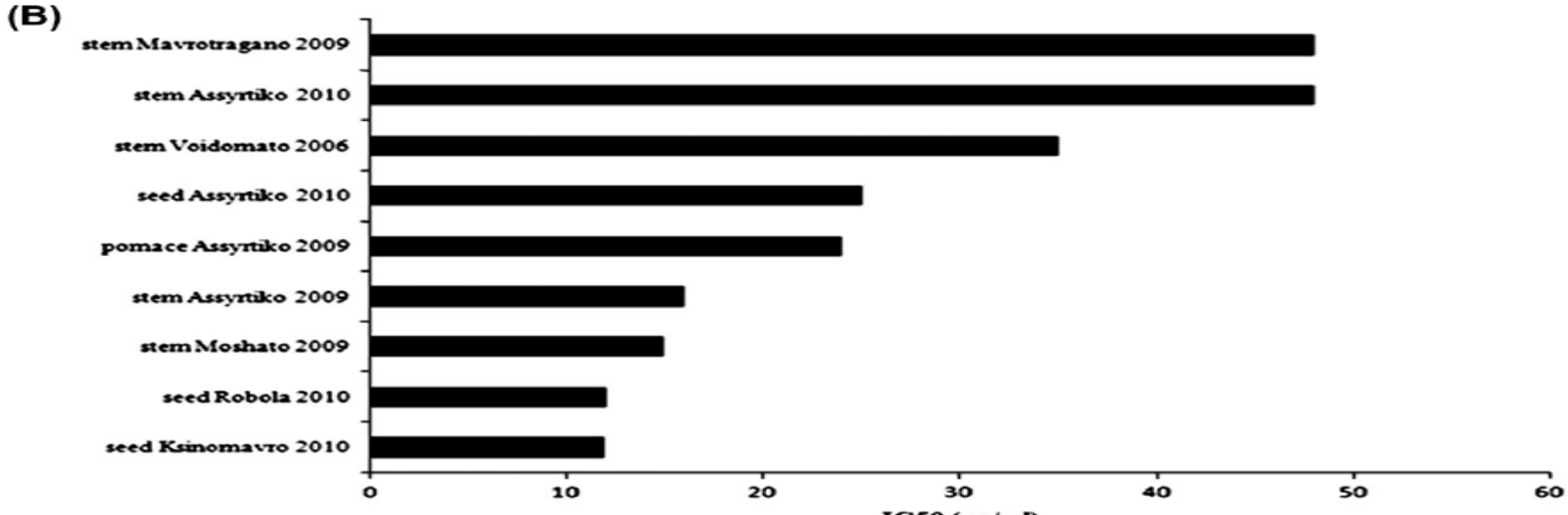
Table 3
Antioxidant capacity, protective activity against hydroxyl (OH·) and peroxyl (ROO·) radical-induced DNA damage.

Extract	Plant variety/harvest year	IC_{50} (μg/mL)			
		DPPH· [a]	ABTS·+ [a]	OH· [b]	ROO· [b]
Stems	Assyrtiko/2009	4.2 ± 0.5	5.5 ± 0.4	460 ± 57	1.15 ± 0.14
	Assyrtiko/2010	5.5 ± 0.7	3.5 ± 0.2	400 ± 41	0.95 ± 0.11
	Mavrotragano/2009	5.0 ± 0.4	3.5 ± 0.3	400 ± 55	0.65 ± 0.07
	Mandilaria/2009	6.0 ± 0.8	4.0 ± 0.6	800 ± 113	0.35 ± 0.02
	Mandilaria/2006	9.0 ± 1.0	9.0 ± 0.9	390 ± 35	1.05 ± 0.12
	Voidomato/2006	7.0 ± 0.9	3.5 ± 0.5	200 ± 24	1.00 ± 0.08
	Voidomato/2009	8.5 ± 1.0	6.8 ± 0.7	800 ± 103	0.55 ± 0.06
	Vilana/2009	7.0 ± 0.8	11.8 ± 1.3	>800	3.70 ± 0.50
	Moshato/2009	8.0 ± 0.7	3.5 ± 0.5	400 ± 39	1.00 ± 0.10
	Ksinomavro/2010	9.0 ± 1.1	5.0 ± 0.3	800 ± 97	1.50 ± 0.14
	Vinsanto/2010	10.0 ± 1.1	4.0 ± 0.2	300 ± 38	0.95 ± 0.08
	Athiri/2010	15.0 ± 1.6	5.0 ± 0.4	310 ± 25	1.05 ± 0.07
Seeds	Assyrtiko/2010	3.0 ± 0.2	3.0 ± 0.4	360 ± 44	0.75 ± 0.09
	Ksinomavro/2010	4.7 ± 0.6	3.5 ± 0.3	170 ± 19	0.85 ± 0.06
	Robola/2010	6.0 ± 0.7	2.8 ± 0.2	300 ± 28	0.70 ± 0.06
Pomace	Assyrtiko/2009	5.0 ± 0.5	6.0 ± 0.8	340 ± 47	0.85 ± 0.11

[a] Values are the mean ± SD of at least two separate triplicate experiments.
[b] Values are the mean ± SD from three independent experiments.

• Grape stem extracts inhibit growth of liver (A) and cervical cancer (B) cell at very low concentrations suggesting that they could be used as chemopreventive agents.





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D. STAGOS[1], A. APOSTOLOU[2], E. POULIOS[1], E. KERMELIOTOU[1], A. MPATZILIOTI[1], K. KREATSOULI[1], S.D. KOULOCHERI[2], S.A. HAROUTOUNIAN[2], D. KOURETAS[1,*]

ANTIANGIOGENIC POTENTIAL OF GRAPE STEM EXTRACT THROUGH INHIBITION OF VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION

[1]Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece;
[2]Agricultural University of Athens, Athens, Greece

The aim of the present study was to investigate the antiangiogenic potential of a grape stem extract against tube formation by human endothelial cells (EA.hy926). The results showed that at low and non-cytotoxic concentrations (50 and 100 μg/ml) the grape stem extract inhibited tube formation, indicating a possible antiangiogenic activity. Moreover, the results showed that this extract inhibited the expression levels of vascular endothelial growth factor (VEGF), one of the most potent proangiogenic factors, suggesting that the tube formation inhibition by the extract may be exerted through inhibition of VEGF levels. Since it is well established that VEGF prevents apoptosis, the previous finding was further supported by the fact that the grape stem extract induced apoptosis in EA.hy926 cells. Furthermore, it was shown that the extract treatment did not change the levels of the proangiogenic molecules hypoxia inducible factor 1 alpha (HIF-1α) and cyclooxygenase-1 (COX-1). Therefore, these findings indicate that the grape stem extract reduces VEGF levels through mechanisms that may be HIF-1μ- and COX-1-independent. The present study is the first showing that grape stem extracts possess antiangiogenic potential. Thus, our findings suggest that since grape stem extracts possess important bioactivities such as antiangiogenic potential, they could be exploited for developing chemopreventive and anticancer agents, while simultaneously protecting the environment through the use of a harmful waste.

• **Grape stem extracts inhibit in vitro angiogenesis suggesting that they could be used as anticancer agents.**

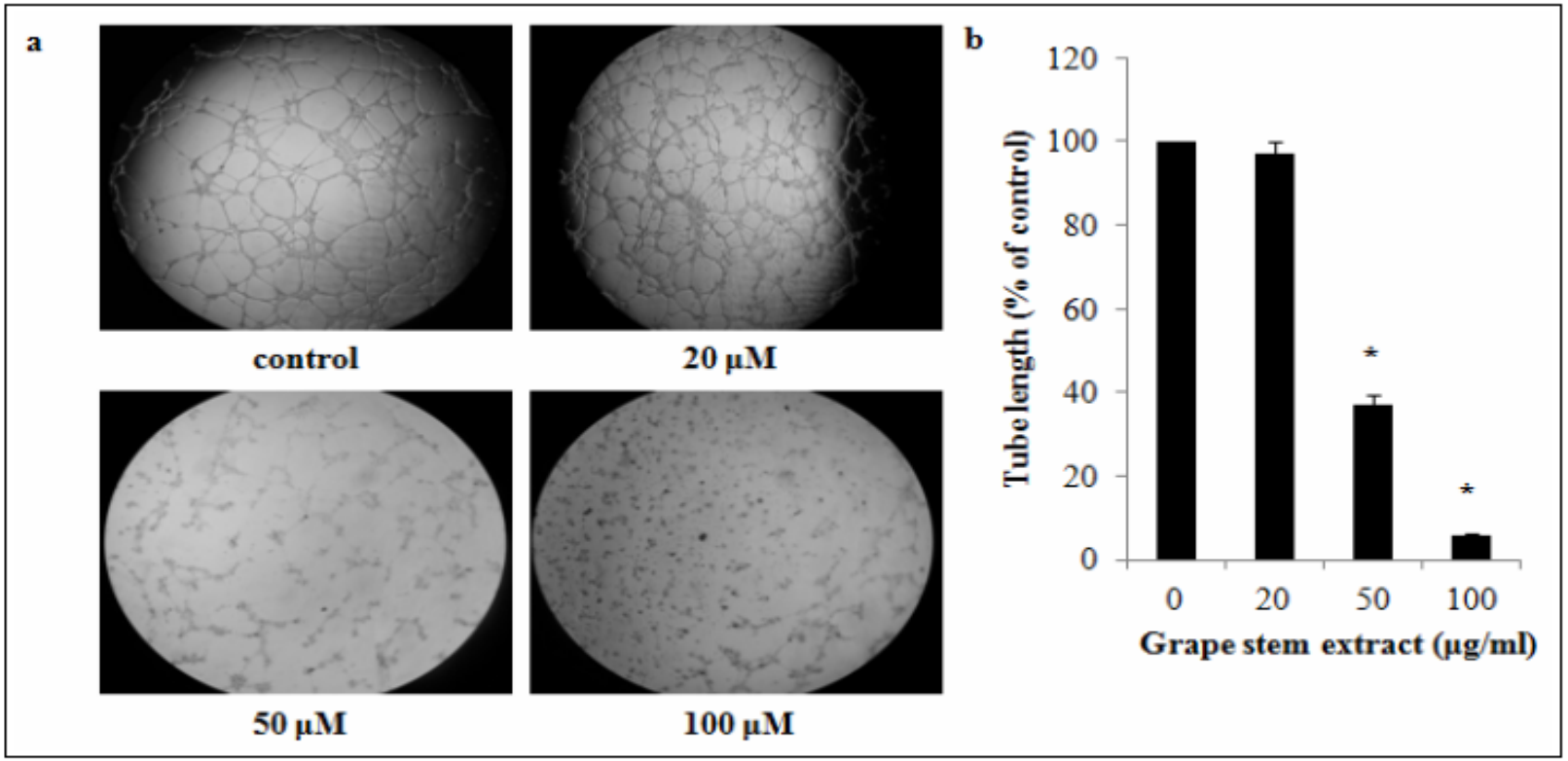
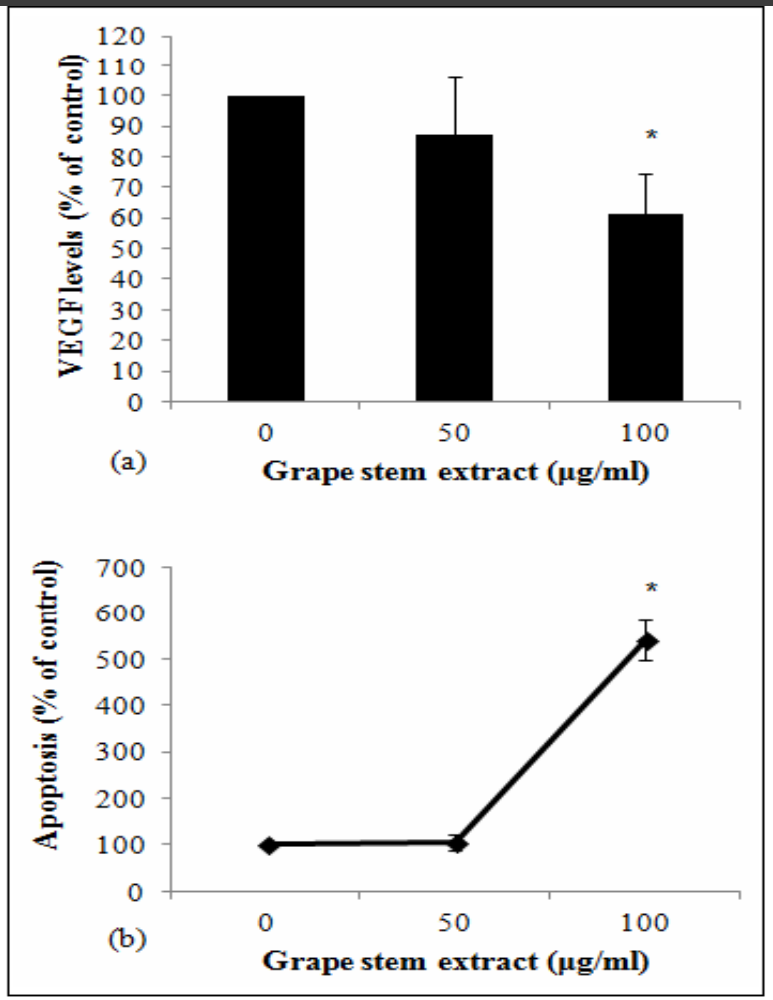
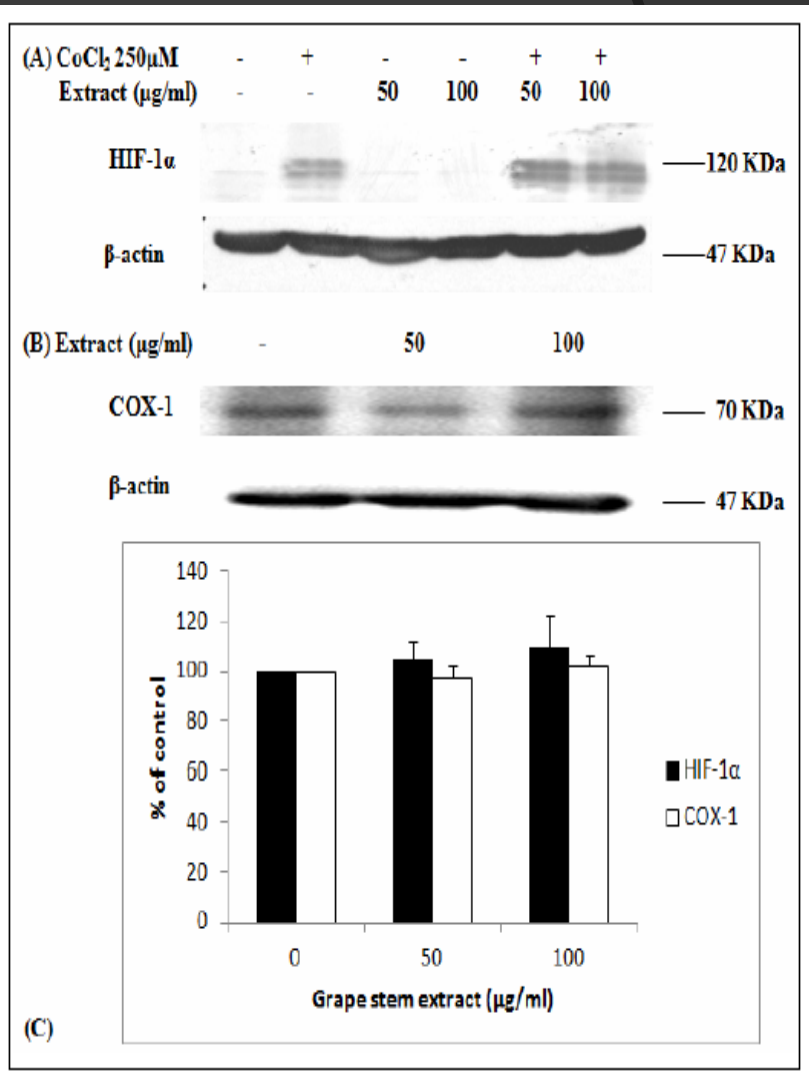


Fig. 2. (*A*) Representative pictures of formation of tube-like structures by EA.hy926 cells. Cells were plated onto Matrigel and treated with different concentrations (20, 50, and 100 µg/ml) of grape stem extract for 16 hours. After treatment, cells were visualized microscopically and photographed at magnification 40×. (*B*) Total tube length was evaluated in 6 optical fields per sample using ImageJ (NIH) software. Each experiment was repeated in duplicate at least three times. Values are means ± S.D. from three independent experiments carried out in duplicate. * $P<0.05$ when compared with the control (untreated cells). Statistical analysis was made by ANOVA followed by Dunnett's *post hoc* test.

• The anti-angiogenic effects of grape stem extracts are exerted through induction of apoptosis and inhibition of VEGF protein, the most important pro-angiogenic factor.

• Grape stem extract-induced VEGF inhibition is regulated by a HIF-1 independent mechanism.





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Biochemical and biological assessment of the inhibitory potency of extracts from vinification byproducts of *Vitis vinifera* extracts against glycogen phosphorylase

Anastassia L. Kantsadi [a], Anna Apostolou [b], Stavroula Theofanous [a], George A. Stravodimos [a], Efthimios Kyriakis [a], Vyron A. Gorgogietas [a], Demetra S.M. Chatzileontiadou [a], Kalliope Pegiou [a], Vassiliki T. Skamnaki [a], Dimitrios Stagos [a], Dimitrios Kouretas [a], Anna-Maria G. Psarra [a], Serkos A. Haroutounian [b], Demetres D. Leonidas [a,*]

[a] *Department of Biochemistry and Biotechnology, University of Thessaly, 26 Ploutonos Str., 41221 Larissa, Greece*
[b] *Department of Animal Sciences and Aquaculture, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece*

• **Stem extracts from Greek grape varieties inhibit in vitro glycogen phosphorylase activity suggesting anti-diabetic properties.**

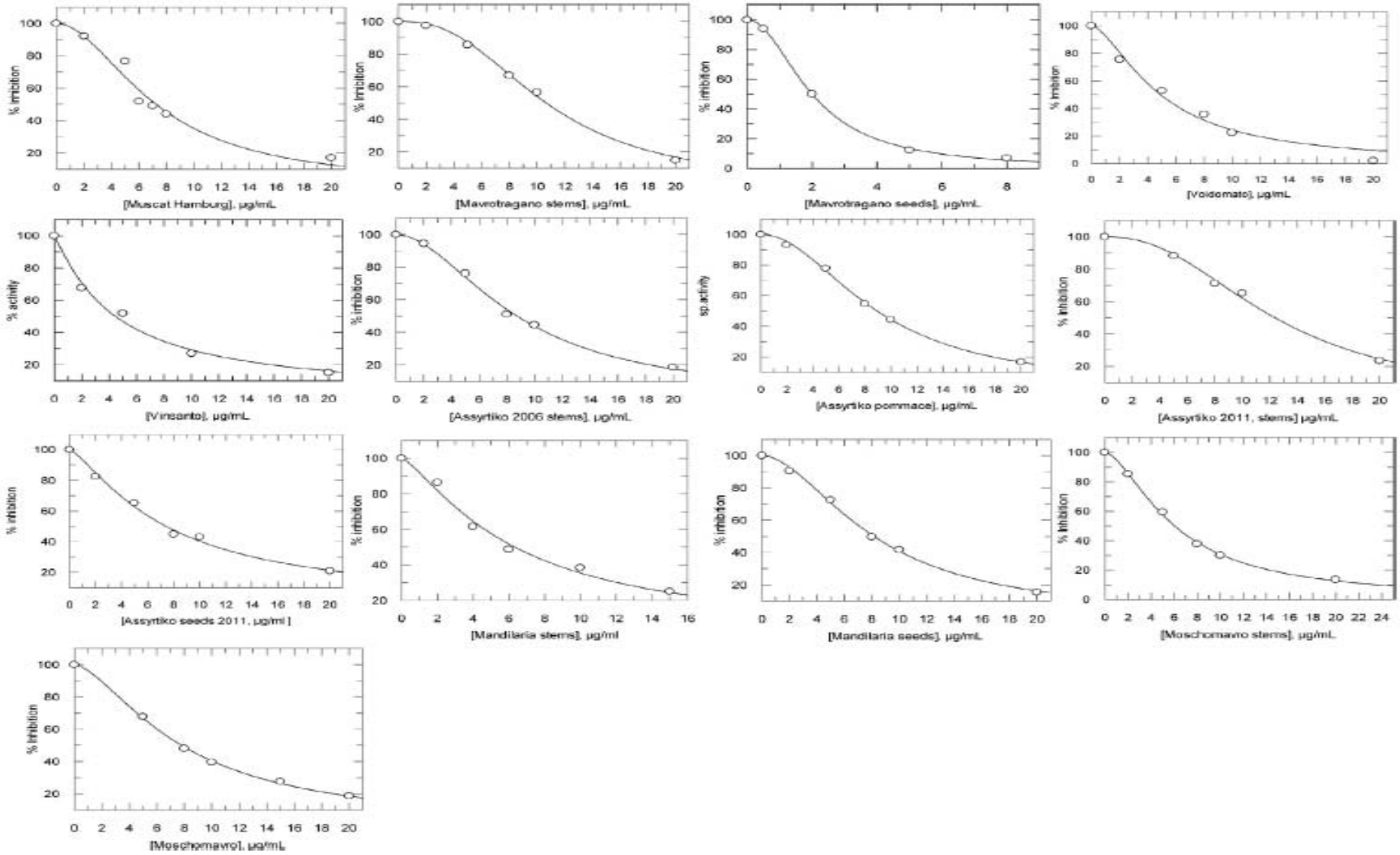


Fig. 2. Inhibition of GPb by the extracts from the Greek *Vitis vinifera* varieties. All assays were performed as described in Section 2.

• **IC50 values of grape stem extracts against glycogen phosphorylase were at low concentrations.**

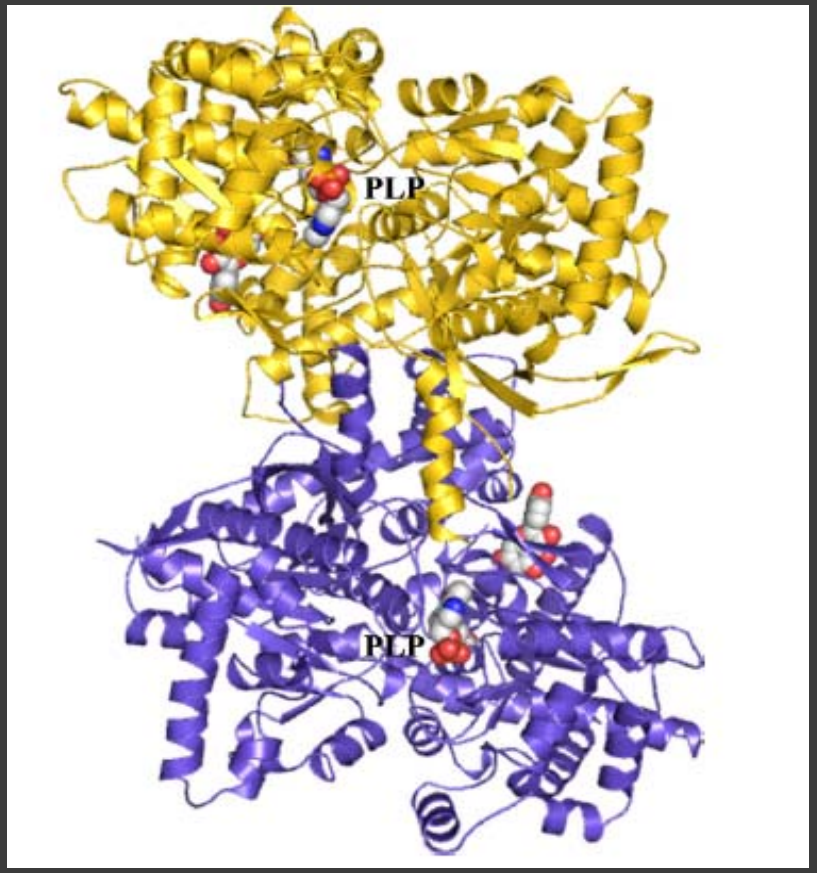
Table 3

The IC_{50} values of the polyphenolic extracts.

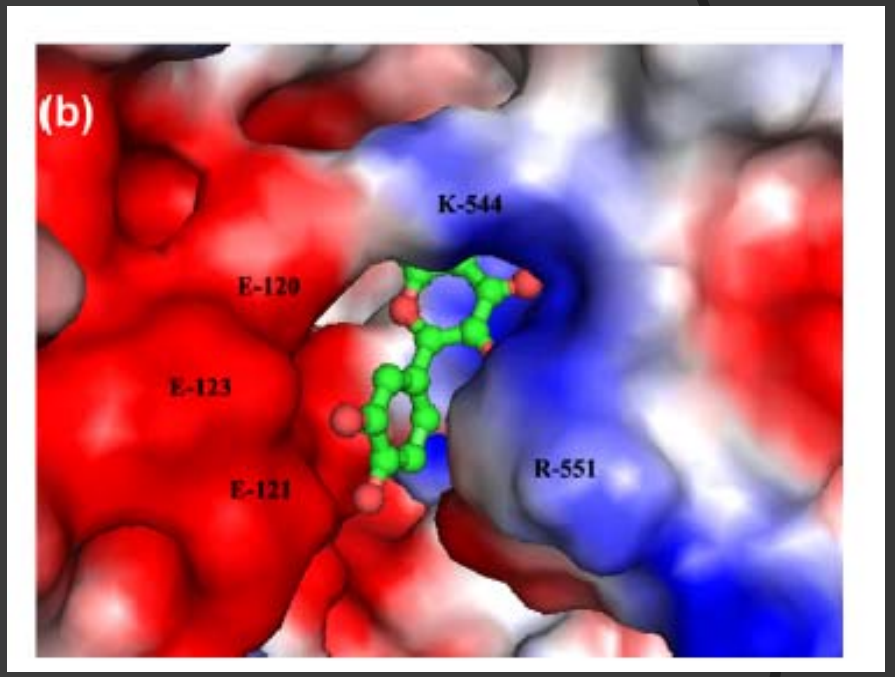
	IC_{50} (μg/ml)		
α-D-glucose	828.7 ± 18.0[a]		
Variety	Stems	Pommace	Seeds
Muscat Hamburg, Thessaly 2008	7.2 ± 0.5		
Mavrotragano Santorini 2009	10.7 ± 0.2		2.0 ± 0.02
Voidomato Santorini 2006	4.5 ± 0.4		
Vinsanto Santorini 2008	7.3 ± 0.4		
Assyrtiko Santorini 2006	8.7 ± 0.2	8.9 ± 0.1	
Assyrtiko Santorini 2011	12.4 ± 0.4		7.4 ± 0.3
Mandilaria Santorini 2011	6.3 ± 0.3		8.1 ± 0.2
Moschomavro, Central Macedonia 2011	6.0 ± 0.1		7.8 ± 0.1

[a] Measured at 4 mM Glc-1-P.

• Crystallography analysis showed that the most potent polyphenol accounting for the inhibition of glycogen phosphorylase was quercetin.



The dimer of glycogen phosphorylase with quercetin bound (the cofactor PLP is also shown to mark the active site of the enzyme).



The accommodation of quercetin at the glycogen phosphorylase surface. Charged residues are indicated by a coloring scheme from blue (positive) to red (negative).

DEVELOPMENT OF BIOFUNCTIONAL FOODS USING BY-PRODUCTS OF VINIFICATION

- The spin-off company (K-Meditura) of the University of Thessaly developed a biofunctional flour containing grape pomace extract with antioxidant activity.

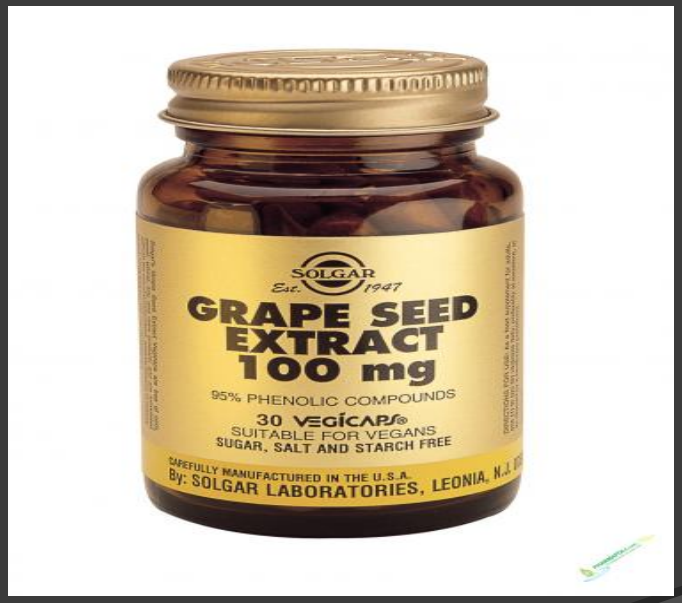
- A biofunctional food is a natural or processed food that apart from its conventional nutritious function, has an additional function (often one related to health-promotion or disease prevention) by adding bioactive compounds.

- These extra functions of a biofunctional food give it a high added value.

Grape pomace extract from Mandilaria Santorini variety incorporated in flour

Flour containing grape pomace extract

- By-products from vinification are used for making food supplements.
- For example, food supplements named as ‘grape seed extracts’ are consumed worldwide for their beneficial properties, especially the antioxidants, on human health.



- However, most of the studies regarding by-products from grapes refer to extracts from seeds.
- Our studies provide a strong evidence that apart from seeds, grape pomace and stems can be used for making food supplements or biofunctional foods.
- Especially, grape stems, a scarcely investigated byproduct produced in large amounts, approximately 5% of the original grape material.
- The amount of grape stems produced during the vinification process in Greece is estimated about 5000 tons per year.

- Our studies have shown that grape stem and pomace extracts are rich in polyphenols and possess strong antioxidant and chemopreventive properties.

- Grape stems and pomace derived from the vinification process are usually used for animal feed and making natural organic fertilizers (compost) which constitute processes of limited economical interest.

- Moreover, wine waste including stems and pomace cause environmental problems.

- Thus, the potential exploitation of grape stem and pomace extracts for developing food supplements or biofunctional foods of high added value is particularly interesting because this combines the profitable venture with environmental protection close to wine-producing zones.

THANK YOU