



## Article

# An In Vitro Study of Saffron Carotenoids: The Effect of Crocin Extracts and Dimethylcrocetin on Cancer Cell Lines

Kyriaki Hatziagapiou <sup>1,2,\*</sup>, Olti Nikola <sup>1,†</sup>, Sofia Marka <sup>3</sup>, Eleni Koniari <sup>4</sup>, Eleni Kakouri <sup>5</sup>, Maria-Eleftheria Zografaki <sup>6</sup>, Sophie S. Mavrikou <sup>3</sup>, Charalabos Kanakis <sup>5</sup>, Emmanouil Flemetakis <sup>6</sup>, George P. Chrousos <sup>4</sup>, Spyridon Kintzios <sup>3</sup>, George I. Lambrou <sup>1,\*</sup>, Christina Kanaka-Gantenbein <sup>1</sup> and Petros A. Tarantilis <sup>5</sup>

<sup>1</sup> Choremeio Research Laboratory, First Department of Pediatrics, National and Kapodistrian University of Athens, Thivon & Levadeias 8, 11527 Athens, Greece; khatziag@med.uoa.gr; onikola@med.uoa.gr (O.N.); ckanaka@med.uoa.gr (C.K.-G.)

<sup>2</sup> Physiotherapy Department, Faculty of Health and Care Sciences, State University of West Attica, Agiou Spiridonos 28, Egaleo, 12243 Athens, Greece; khatziag@med.uoa.gr

<sup>3</sup> Laboratory of Cell Technology, Department of Biotechnology, Agricultural University of Athens, EU-CONEXUS European University, 11855 Athens, Greece; smarka@aua.gr (S.M.); sophie\_mav@aua.gr (S.S.M.); skin@aua.gr (S.K.)

<sup>4</sup> University Research Institute of Maternal and Child Health & Precision Medicine, UNESCO Chair on Adolescent Health Care, National and Kapodistrian University of Athens, Thivon & Levadeias 8, 11527 Athens, Greece; hkoniari@med.uoa.gr (E.K.); chrousge@med.uoa.gr (G.P.C.)

<sup>5</sup> Laboratory of Chemistry, Department of Food Science & Human Nutrition, School of Food Biotechnology and Development, Agricultural University of Athens, EU-CONEXUS European University, 11855 Athens, Greece; elenikakouri@aua.gr (E.K.); chkanakis@aua.gr (C.K.); ptara@aua.gr (P.A.T.)

<sup>6</sup> Laboratory of Molecular Biology, Department of Biotechnology, Agricultural University of Athens, EU-CONEXUS European University, 11855 Athens, Greece; mzografaki@aua.gr (M.-E.Z.); mflem@aua.gr (E.F.)

\* Correspondence: khatziag@med.uoa.gr (K.H.); glamprou@med.uoa.gr (G.I.L.)

† These authors contributed equally to this work and should be considered first authors.

SOURCE	TERM_NAME	ADJ. P_VALUE	INTERSECTIONS
GO:MF	BH3 domain binding	0.000200168	BAX, BCL2
GO:MF	BH domain binding	0.000733425	BAX, BCL2
GO:MF	death domain binding	0.000733425	BAX, BCL2
GO: BP	positive regulation of intrinsic apoptotic signaling pathway	1.89054E-06	BAX, BID, BCL2, SOD1
GO: BP	regulation of mitochondrial membrane potential	4.11443E-06	BAX, BID, BCL2, SOD1
GO: BP	positive regulation of apoptotic signaling pathway	3.8264E-05	BAX, BID, BCL2, SOD1
GO: BP	protein insertion into mitochondrial membrane involved in apoptotic signaling pathway	8.32542E-05	BAX, BID, BCL2
GO: BP	regulation of intrinsic apoptotic signaling pathway	0.000104947	BAX, BID, BCL2, SOD1
GO: BP	positive regulation of mitochondrial outer membrane permeabilization involved in apoptotic signaling pathway	0.000146303	BAX, BID, BCL2
GO: BP	regulation of nitrogen utilization	0.000279587	BAX, BCL2
GO: BP	regulation of mitochondrial outer membrane permeabilization involved in apoptotic signaling pathway	0.000310662	BAX, BID, BCL2
GO: BP	protein insertion into mitochondrial membrane	0.00037691	BAX, BID, BCL2
GO: BP	positive regulation of cell death	0.000471175	BAX, BID, BCL2, MYCN, SOD1
GO: BP	establishment of protein localization to mitochondrial membrane	0.000507114	BAX, BID, BCL2
GO: BP	ovarian follicle development	0.000536304	BAX, BCL2, SOD1
GO: BP	neuron apoptotic process	0.00053806	BAX, BID, BCL2, SOD1
GO: BP	nitrogen utilization	0.000559091	BAX, BCL2
GO: BP	response to toxic substance	0.000564259	BAX, BCL2, SOD1, GSTM1
GO: BP	mitochondrial outer membrane permeabilization	0.000597993	BAX, BID, BCL2
GO: BP	release of cytochrome c from mitochondria	0.000664228	BAX, BID, BCL2
GO: BP	positive regulation of mitochondrial membrane permeability involved in apoptotic process	0.000810977	BAX, BID, BCL2
GO: BP	mitochondrial outer membrane permeabilization involved in programmed cell death	0.000891809	BAX, BID, BCL2
GO: BP	positive regulation of mitochondrial membrane permeability	0.000977827	BAX, BID, BCL2
GO: BP	intrinsic apoptotic signaling pathway	0.001054384	BAX, BID, BCL2, SOD1
GO: BP	regulation of mitochondrial membrane permeability involved in apoptotic process	0.00106919	BAX, BID, BCL2
GO: BP	positive regulation of membrane permeability	0.001216602	BAX, BID, BCL2
GO: BP	retinal cell programmed cell death	0.001397316	BAX, BCL2
GO: BP	response to axon injury	0.001738502	BAX, BCL2, SOD1
GO: BP	regulation of mitochondrial membrane permeability	0.001871725	BAX, BID, BCL2
GO: BP	extrinsic apoptotic signaling pathway via death domain receptors	0.002011561	BAX, BID, BCL2
GO: BP	positive regulation of mitochondrion organization	0.00223406	BAX, BID, BCL2
GO: BP	regulation of apoptotic signaling pathway	0.002399642	BAX, BID, BCL2, SOD1
GO: BP	neuron death	0.002399642	BAX, BID, BCL2, SOD1
GO: BP	positive regulation of developmental pigmentation	0.002607555	BAX, BCL2
GO: BP	protein insertion into membrane	0.002998294	BAX, BID, BCL2
GO: BP	female gonad development	0.002998294	BAX, BCL2, SOD1
GO: BP	regulation of membrane permeability	0.003092591	BAX, BID, BCL2
GO: BP	development of primary female sexual characteristics	0.003489463	BAX, BCL2, SOD1
GO: BP	intrinsic apoptotic signaling pathway in response to DNA damage	0.003699981	BAX, BID, BCL2
GO:CC	mitochondrial outer membrane	0.003834007	BAX, BID, BCL2
GO:CC	pore complex	0.004261987	BAX, BCL2
GO:CC	organelle outer membrane	0.005407208	BAX, BID, BCL2
GO:CC	outer membrane	0.005548796	BAX, BID, BCL2
GO:CC	mitochondrial envelope	0.00694808	BAX, BID, BCL2, SOD1
KEGG	Platinum drug resistance	4.13249E-06	BAX, BID, BCL2, GSTM1
KEGG	Apoptosis - multiple species	5.34016E-05	BAX, BID, BCL2
KEGG	p53 signaling pathway	0.000661918	BAX, BID, BCL2
KEGG	Amyotrophic lateral sclerosis	0.002693269	BAX, BID, BCL2, SOD1
KEGG	Sphingolipid signaling pathway	0.002876347	BAX, BID, BCL2

KEGG	<b>Apoptosis</b>	0.004286524	BAX, BID, BCL2
KEGG	Measles	0.004574794	BAX, BID, BCL2
KEGG	Necroptosis	0.006827122	BAX, BID, BCL2
KEGG	Hepatitis B	0.007217276	BAX, BID, BCL2
KEGG	Pathways of neurodegeneration - multiple diseases	0.007744505	BAX, BID, BCL2, SOD1
KEGG	Tuberculosis	0.009076859	BAX, BID, BCL2
KEGG	Pathways in cancer	0.011794425	BAX, BID, BCL2, GSTM1
KEGG	Epstein-Barr virus infection	0.013086894	BAX, BID, BCL2
KEGG	Human immunodeficiency virus 1 infection	0.015573539	BAX, BID, BCL2
KEGG	Lipid and atherosclerosis	0.016466087	BAX, BID, BCL2
REAC	Activation, translocation and oligomerization of BAX	3.15444E-05	BAX, BID
REAC	Intrinsic Pathway for Apoptosis	0.000195569	BAX, BID, BCL2
REAC	BH3-only proteins associate with and inactivate anti-apoptotic BCL-2 members	0.001134101	BID, BCL2
REAC	Activation of BAD and translocation to mitochondria	0.003304058	BID, BCL2
REAC	<b>TP53 Regulates Transcription of Genes Involved in Cytochrome C Release</b>	0.00597314	BAX, BID
REAC	<b>Apoptosis</b>	0.007504833	BAX, BID, BCL2
REAC	<b>Programmed Cell Death</b>	0.009161674	BAX, BID, BCL2
REAC	Activation of BH3-only proteins	0.013649586	BID, BCL2
REAC	TP53 Regulates Transcription of Cell Death Genes	0.029605634	BAX, BID
WP	Amyotrophic lateral sclerosis (ALS)	6.65E-07	BAX, BID, BCL2, SOD1
WP	TP53 Network	2.59376E-05	BAX, BID, BCL2
WP	Nanomaterial induced apoptosis	2.59376E-05	BAX, BID, BCL2
WP	Regulation of Apoptosis by Parathyroid Hormone-related Protein	3.50177E-05	BAX, BID, BCL2
WP	Host-pathogen interaction of human corona viruses - apoptosis	3.50177E-05	BAX, BID, BCL2
WP	Photodynamic therapy-induced AP-1 survival signaling.	0.000469452	BAX, BID, BCL2
WP	<b>Apoptosis</b>	0.002282975	BAX, BID, BCL2
WP	Viral Acute Myocarditis	0.002363801	BAX, BID, BCL2
WP	<b>Apoptosis Modulation and Signaling</b>	0.002888716	BAX, BID, BCL2
WP	Small cell lung cancer	0.003380728	BAX, BID, BCL2
WP	Overview of nanoparticle effects	0.00734644	BAX, BCL2
WP	Unfolded protein response	0.012861208	BID, BCL2
WP	Integrated Breast Cancer Pathway	0.01304607	BAX, BID, BCL2
WP	Hepatitis B infection	0.01304607	BAX, BID, BCL2
WP	miRNA regulation of p53 pathway in prostate cancer	0.022571838	BAX, BID
WP	Photodynamic therapy-induced HIF-1 survival signaling	0.028431074	BAX, BID
WP	Integrated Cancer Pathway	0.042143043	BAX, BCL2
HPA	spleen; cells in white pulp[β%¥Medium]	0.047615873	BAX, BID, BCL2, SOD1, GSTM1
CORUM	BAX homo-oligomer complex	0.024838735	BAX
CORUM	BAX homo-oligomer complex	0.024838735	BAX
CORUM	BCL2-CISD2 complex	0.049670619	BCL2
CORUM	<b>p53-BCL2 complex</b>	0.049670619	BCL2
CORUM	CALM1-FKBP38-BCL2 complex	0.049670619	BCL2

**Table S1.** The detailed description of GO enrichment as derived from the gprofiler web-tool.