

Received: 2013.05.05
Accepted: 2014.04.17
Published: 2014.05.14

Synergistic Interactions Between Anticancer Chemotherapeutics and Phenolic Compounds and Anticancer Synergy Between Polyphenols*

Oddziaływania synergistyczne między chemoterapeutykami przeciwnowotworowymi i związkami fenolowymi oraz synergizm przeciwnowotworowy między polifenolami

Urszula Lewandowska¹, Sylwia Gorlach², Katarzyna Owczarek¹, Elżbieta Hrabec², Karolina Szewczyk²

¹ Department of Biochemistry, Medical University of Lodz, Lodz, Poland

² Department of Medical Enzymology, Medical University of Lodz, Lodz, Poland

Summary

Chemoprevention has recently gained a new dimension due to the possibility of studying the mechanisms of action of chemopreventive agents at the molecular level. Many compounds have been proved to inhibit early stages of carcinogenesis in experimental models. These compounds include both recognized drugs (such as tamoxifen and nonsteroidal anti-inflammatory drugs) and natural constituents of edible and therapeutic plants, particularly polyphenols. Phenolics are characterized by high structural diversity and, consequently, a very broad spectrum of biological activities. They are increasingly looked upon as a valuable alternative or a support for synthetic drugs, as evidenced by a growing number of clinical trials regarding the use of phenolic compounds and polyphenol-rich extracts in chemoprevention and therapy. In the present work, we discuss the effectiveness of natural polyphenols as cancer preventive and therapeutic agents resulting from their synergy with synthetic or semisynthetic anticancer drugs as well as with other phenolic compounds of plant origin.

Key words: polyphenols • synthetic drugs • chemoprevention • polyphenol-drug interactions • synergism

Full-text PDF: <http://www.phmd.pl/fulltxt.php?ICID=1102278>

Word count: 5139
Tables: 3
Figures: –
References: 96

* The study was supported by grant no. 4468/B/PO1/2011/40 from the Polish Ministry of Science and Higher Education.

Author's address: Dr. Urszula Lewandowska, Department of Biochemistry, Medical University of Lodz, Mazowiecka 6/8, 92-215 Lodz, Poland; e-mail: urszula.lewandowska@umed.lodz.pl

INTRODUCTION

numerous research groups have focused on anticancer therapies for many years; the progress in techniques of imaging and therapy has also been impressive. Nevertheless, cancer mortality rates are still on the rise. The highest number of cancer-related deaths among women are caused by cancers of the breast, lungs, stomach, colon and/or rectum, and cervix, while among men cancers of the lung, stomach, liver, colon and/or rectum, esophagus, and prostate result in the highest mortality [33,87]. The increase in industrialization and environmental pollution (resulting in food contamination), together with life style (smoking, excessive consumption of highly processed food, long-term stress) are all looked upon as the causes of this phenomenon. Many chemical compounds present in air, water, food, synthetic materials, and other products act as carcinogens [28,47,64]. In spite of the huge progress in understanding the molecular pathogenesis of tumors, surgery combined with chemo- and radiotherapy still remains the most effective therapy. To date, gene therapies have not fulfilled the hopes associated with their application.

An alternative approach is chemoprevention, which consists in using synthetic, semisynthetic or natural agents to inhibit or reverse the process of carcinogenesis, particularly in individuals with a high risk of developing cancer. Epidemiological studies indicate that in Asia the incidence of some cancers (including cancers of the breast, colon, prostate, and lung) is lower than, for instance, in Europe and USA [33,45,82]. This phenomenon may result from the fact that an Asiatic diet is markedly richer in health beneficial plant-derived polyphenols than European or American diets. It may be a consequence of drinking high amounts of green tea (with epigallocatechin gallate/EGCG as the main phenolic constituent) as well as of consuming large amounts of soy products that contain genistein [36,54]. The results of recent studies indicate that natural compounds originating from edible and therapeutic plants could be used in both prevention and therapy of cancer, as they act pleiotropically on cancer cells. Through modulation of some signaling pathways, these compounds can exhibit a variety of biological activities, including antiproliferative [11], proapoptotic [24,25], and anti-angiogenic activities [43,48]. Accordingly, they can inhibit carcinogenesis by various mechanisms, which renders it possible to choose a suitable agent.

There is a growing body of evidence on stronger chemopreventive activities of combinations of phytochemicals than in the case of individual compounds, which is referred to as a synergistic effect [14]. Some combinations of compounds exhibit biological activity that is not detected

when its individual constituents are tested separately. Moreover, polyphenols interact additively, synergistically, or antagonistically not only with other phenolics but also with other food components as well as with drugs of natural or synthetic origin [14,45]. The above-mentioned interactions are looked upon as the reasons for chemopreventive effectiveness of a diet rich in fruits, vegetables, and whole grain cereals against civilization diseases, including cancer [14,15,45].

SYNERGISM OF CHEMOTHERAPEUTICS AND PHENOLIC COMPOUNDS

Chemotherapy, despite many side effects, is still the most popular way of treating cancer [37]. Polyphenolic compounds give hope for an improvement of chemotherapy efficacy as well as reduction of side effects. For instance, cisplatin (cis-diamminedichloroplatinum) is a cytostatic drug widely used in the treatment of different types of cancer; however, its application is limited because of increasing resistance and many undesirable side effects in humans. Olas et al. [59] investigated *in vitro* synergistic action of cisplatin and trans-3,3',5,5'-tetrahydroxy-4'-methoxystilbene on blood cells. The combination of the inorganic platinum derivative with the polyphenolic compound significantly reduced DNA damage in lymphocytes and lipid peroxidation, protein carboxylation, and thiol group oxidation in blood platelets. Other examples of anticancer synergy between chemotherapeutic drugs and phenolic compounds or polyphenol-rich extracts are presented in Table 1 and discussed below.

Genistein

Genistein, a polyphenolic compound occurring in soya seeds (*Glycine soja*), combined with cisplatin significantly reduced proliferation of BxPC-3 pancreatic carcinoma cells and induced their apoptosis. A reduction of tumor growth was also observed *in vivo*, in a murine xenograft model of BxPC-3 cells [54]. Moreover, the regression of cancer was observed in C57B16 mice with Lewis lung carcinoma cells after administration of a single dose of combinations of intraperitoneal cisplatin, 5-fluorouracil, and genistein [51]. It is worth mentioning that in this experiment pharmacotherapy was combined with radiotherapy.

The results of many experiments indicate that overexpression and activation of nuclear factor kB (NF-kB) reduces the efficacy of chemotherapeutics via inhibition of apoptosis [79]. However, polyphenolic compounds can alter this action. Mohammad et al. [54] showed that genistein caused a decrease in expression of antiapoptotic proteins Bcl-2 and BclXL and down-regulated NF-kB in xenografts of pancreatic carcinoma cells in animals fed with the addition of this isoflavone. In *in vitro* studies,

Table 1. Examples of synergy between phenolic compounds and anticancer chemotherapeutics. Animal models are in bold.

Phenolic compounds	Anticancer chemotherapeutics	Experimental models	References	
genistein	cisplatin	human pancreatic carcinoma cell line (BxPC-3), murine xenograft model of BxPC-3 cells	Sharma et al., 2007 [79]	
		human pancreatic carcinoma cell lines (COLO-357 and L3.6pl)	Banerjee et al., 2007 [6]	
	combination of cisplatin and 5-fluorouracil	C57B1/6 mice implanted with Lewis lung carcinoma cells	McDonnell et al., 2004 [51]	
	5-fluorouracil	human colon cancer cell line (HT-29)	Hwang et al., 2005 [29]	
	arsenic trioxide		human monocytic leukemia cell line (U937)	Sanchez et al., 2008 [72]
			human hepatocellular carcinoma cell lines (HepG2, Hep3B, and SK-Hep-1), HepG2 xenografts in BALB/c nude mice	Jiang et al., 2010 [34]
		doxorubicin	hormone-independent human breast cancer cell line (MDA-MB-231)	Lim et al., 2006 [44]
	gemcitabine	murine xenografts of human pancreatic carcinoma cells (COLO 357 and L3.6pl)	Banerjee et al., 2005 [5]	
	camptothecin	human cervical cancer cell line (HeLa), human ovarian carcinoma line (OAW-42)	Papazisis et al., 2006 [62]	
	hydroxycamptothecin	SCID mice xenografts of human bladder carcinoma TCC-SUP cells	Wang et al., 2013 [90]	
combination of genistein and daidzein	cisplatin	human squamous cell carcinoma cell lines (ME-180PT and UMSCC-5)	Ali et al., 2009 [1]	
curcumin	5-fluorouracil, combination of 5-fluorouracil and oxaliplatin	human colon cancer cell line (HT-29)	Du et al., 2006 [17]	
	cisplatin, oxaliplatin	human ovarian carcinoma cell lines (2008 and C13)	Montopoli et al., 2009 [55]	
	cisplatin, etoposide, camptothecin, doxorubicin	human and rat glioblastoma cell lines	Dhandapani et al., 2007 [15]	
(-)-epigallocatechin 3-gallate (EGCG)	doxorubicin	murine xenografts of human carcinoma DOX-resistant cells (KB-A-1)	Zhang et al., 2004 [95]	
	gemcitabine	human cholangiocarcinoma cell line (Mz-ChA-1), murine xenografts of Mz-ChA-1 cells	Lang et al., 2009 [41]	
	cisplatin	human ovarian cancer cell lines (SKOV3, CAOV3, and C200)	Chan et al., 2006 [12]	
quercetin	doxorubicin	neuroblastoma and Ewing's sarcoma cell lines	Zanini et al., 2007 [93]	
	doxorubicin	murine xenografts of 4T1 (murine mammary carcinoma) cells	Du et al., 2010 [19]	
	cisplatin	human laryngeal carcinoma cell line (Hep-2)	Kuhar et al., 2007 [39]	
	arsenic trioxide	human leukemia cell lines (U937 and HL60)	Ramos et al., 2008 [71]	
	temozolomide	human astrocytoma cell line	Jakubowicz-Gil et al., 2010-[31]	
resveratrol	cisplatin	Wistar rats	Do Amaral et al., 2008 [16] Wang et al., 2009 [89]	
	doxorubicin	human acute myeloid leukemia cell lines (ML-2/DX30, AML-2/DX100, and AML-2/DX300)	Kweon et al., 2010 [40]	

pretreatment of pancreatic carcinoma cells COLO 357 and L3.6pl with genistein before incubation with cisplatin inhibited their proliferation and triggered apoptosis by down-regulation of NF- κ B [6].

Similar results were obtained by Ali et al. [1] in experiments carried out on squamous cell carcinoma ME-180PT and UMSCC-5 cell lines using a mixture of isoflavones containing genistein and daidzein combined with cispla-

tin. *In vivo* studies on COLO 357 confirmed that genistein is a repressor of NF- κ B expression [6]. In another study, Hwang et al. [29] showed that genistein and 5-fluorouracil synergism induced p53, p21, and Bax expression in colon cancer HT-29 cells. The induction of these genes was probably caused by genistein. A decrease in cyclooxygenase 2 (COX-2) and prostaglandin secretion caused by 5-fluorouracil was also observed. Furthermore, the combination of arsenic trioxide with genistein increased apoptosis (cytochrome c release and caspase-3 activation) and generated reactive oxygen forms in human leukemia U937 cells [72]. In hepatocellular carcinoma cell lines (HepG2, Hep3B, and SK-Hep-1), a reduction of cell viability and apoptosis stimulation were observed as a result of a synergistic interaction between arsenic trioxide and genistein. Moreover, this synergism was confirmed in HepG2 xenografts in BALB/c nude mice [34].

Studies indicate that genistein might act selectively on hormone-independent breast cancer cells (such as MDA-MB-231 cell line). Genistein sensitized MDA-MB-231 cells to doxorubicin (an anthracycline antibiotic) whereas it had no effect on the hormone-dependent MCF-7 cell line. Probably, genistein induced expression of a gene involved in maintaining cell viability, namely, glucose-regulated protein 78 (GRP78) as well as suppressing glucose uptake in both cell lines [44].

In female nude mice implanted with human pancreatic carcinoma cell lines (COLO 357 and L3.6pl), a combination of genistein and gemcitabine (a nucleoside analog used in chemotherapy) was much more effective as an antitumor agent compared with either agent alone [5]. It was also reported that this phenolic compound and camptothecin synergistically decreased proliferation of HeLa (cervical cancer) and OAW-42 (ovarian cancer) cell lines. Pretreatment of HeLa cells with irinotecan (a semisynthetic analogue of camptothecin) before incubation with genistein inhibited CDK1 phosphorylation, causing abrogation of G2/M checkpoint control and thus enhancing apoptosis (as evidenced by an increase in PARP cleavage) [63]. Recently, Wang et al. [90] reported significant and dose-dependent sensitization of bladder cancer cell lines and bladder epithelial cells to hydroxycamptothecin-triggered apoptosis by genistein both *in vitro* and *in vivo* (in a bladder cancer xenograft model). The sensitization caused double-stranded DNA breaks leading to synergistic activation of ataxia telangiectasia mutated (ATM) kinase, which resulted in a delay in DNA damage repair, reduction in NEMO/NF- κ B/IKK/caspase signal transduction and induction of apoptosis [90]. The isoflavone was previously shown to sensitize various cancer cell lines (including prostate and cervical cancer) to hydroxycamptothecin, which has been used in bladder cancer chemotherapy for nearly 40 years.

Additionally, genistein may exhibit protective properties. Studies revealed that this isoflavone protected murine bone marrow cells against cisplatin-induced clastogenesis and apoptosis [2].

Curcumin

Curcumin, a phenolic compound obtained from the rhizome of *Curcuma longa* L., can also synergize with chemotherapeutics. Importantly, curcumin and other curcuminoids are among the most promising polyphenols for possible future use in co-medication with standard anticancer drugs [88]. Du et al. [17] revealed that curcumin in combination with 5-fluorouracil effectively inhibited the growth of colon cancer HT-29 cells and reduced COX-2 expression in those cells. Moreover, curcumin, 5-fluorouracil, and oxaliplatin synergism resulted in inhibition of HT-29 cell proliferation and induction of their apoptosis. The combination of the three above-mentioned compounds was more effective than curcumin alone or curcumin combined with 5-fluorouracil. On one hand, expression of epidermal growth factor receptor (EGFR), human epidermal growth factor receptor (HER)-2, HER-3, and insulin-like growth factor 1 receptor (IGF-1R) was downregulated. However, on the other hand, an increment in insulin-like growth factor binding protein 3 (IGFBP-3) expression was observed and led to inhibition of the interaction of IGF-1 with the receptor, thus hindering its activation [55].

Furthermore, curcumin combined with cisplatin or with oxaliplatin caused cell cycle arrest in human ovarian carcinoma 2008 and C13 cells and induced their apoptosis. Interestingly, the combination of those compounds was much more effective than when they were used individually [55]. In *in vitro* studies, curcumin sensitized human and rat glioblastoma cells to cisplatin, etoposide, camptothecin, and doxorubicin. The results revealed downregulation of Bcl-2 and of the enzymes taking part in DNA repair [15].

Epigallocatechin gallate

Results of numerous studies indicate that tea polyphenols increase susceptibility of cancer cells to chemotherapeutics; as far as synergy with standard anticancer drugs is concerned, gallic acid derivatives of green tea are among the most promising polyphenols (beside curcuminoids) [88]. For instance, (-)-epigallocatechin 3-gallate (EGCG), a compound isolated from tea (*Camellia sinensis*) leaves, intensified doxorubicin action in human carcinoma KB-A-1 xenografts in mice [95]. EGCG increased doxorubicin concentration in xenografts by 51% and stimulated apoptosis in the tumors. Probably, EGCG inhibited efflux of the drug from cells by altering ATP hydrolysis in P-glycoprotein [69]. The beneficial effect of EGCG was also suggested by the study of Lang et al. [41]. Namely, incubation of human cholangiocarcinoma Mz-ChA-1 cells with EGCG sensitized them to the action of gemcitabine (a nucleoside metabolic inhibitor) by mitochondrial membrane depolarization and cytochrome c release into the cytosol which triggered apoptosis. In *in vivo* studies, EGCG also decreased the growth of Mz-ChA-1 cell xenografts in nude mice and increased their sensitivity to gemcitabine. Combination of EGCG with cisplatin intensified the drug's action,

thus causing oxidative stress in ovarian cancer cell lines (SKOV3, CAOV3, and C200) [12]. Moreover, EGCG sensitized glioma cells to cisplatin and tamoxifen by inhibiting telomerase expression [80].

Molecular mechanisms underlying the synergistic induction of apoptosis of human cancer cells, inhibition of tumor formation in mice, and enhanced suppression of tumor growth in xenograft murine models by combinations of anticancer drugs with EGCG or other green tea catechins were reviewed by Suganuma et al. [84] and Fujiki and Suganuma [22].

Quercetin

Quercetin is a flavonol commonly found in plants, including onion [91]. According to Zanini et al. [93], this phenolic compound might intensify the action of doxorubicin in neuroblastoma cells and, to a smaller extent, in Ewing's sarcoma cells via inhibition of heat shock protein expression. Quercetin combined with cisplatin exhibited a proapoptotic effect toward human laryngeal carcinoma cells [39]. Similar results were obtained by Ramos and Aller [71], who treated human leukemia cells U937 and HL60 with a combination of quercetin and arsenic trioxide.

Furthermore, Du et al. [18,19] investigated synergism between quercetin and doxorubicin. Injection of a combination of the above-mentioned flavonol and chemotherapeutic drug into 4T1 breast tumors implanted subcutaneously in mice revealed inhibition of tumor growth and reduction of doxorubicin side effects, leading to long-term tumor-free survival of the mice. It is known that cytokines secreted by Th1 and Th2 lymphocytes have an influence on the immune response. Quercetin combined with doxorubicin caused an increase in the concentrations of IL-2 and IFN- γ (cytokines inhibiting tumor growth) and a decrease in the concentrations of IL-4 and IL-10 (cytokines promoting tumor growth) in the serum at the same time [19].

Temozolomide is an alkylating chemotherapeutic agent, and quercetin was reported to act synergistically with this autophagy-inducing drug by causing severe necrosis in a human astrocytoma cell line [31]. Interestingly, the observed effect was biphasic: at a low (5 μ M) temozolomide concentration quercetin potentiated its proautophagic effect, while at a higher drug concentration (30 μ M) one form of programmed cell death (i.e. autophagy) switched to another (apoptosis). The migratory phenotype of the glioma cells was also suppressed by the combination of the drug and the flavonol. Moreover, temozolomide attenuated the toxic effect of quercetin. The cytotoxicity of the flavonol (when used alone), the protective effect of the drug, and the concentration dependence of the triggered type of programmed cell death (autophagy vs. apoptosis) were confirmed in a further study by Jakubowicz-Gil et al. [30] on the same cell line. Interestingly, the sequence of administration of temozolomide and quercetin was also important, with the highest number of

dead cells observed after simultaneous administration of both agents or after pre-incubation with temozolomide followed by treatment with quercetin.

Resveratrol

Resveratrol belongs to stilbenes and occurs, among others, in grapes, wine, peanuts, and soy [10]. It is able to attenuate side effects of chemotherapeutics [16,58,89,94,96]. For instance, Do Amaral et al. [16] evaluated the influence of resveratrol on cisplatin-induced renal damage. The study was carried out on male Wistar rats which were treated with resveratrol before cisplatin administration. Then, urine and blood samples were collected and kidneys were removed. Acute tubular cell necrosis was observed only in the group of rats treated with cisplatin without resveratrol pretreatment, whereas in the group treated with resveratrol before cisplatin administration lower levels of creatinine in serum and proteins in urine (markers indicating renal injuries) were reported. Additionally, in comparison with the group of rats treated only with cisplatin, resveratrol was able to decrease the degree of lipid peroxidation and increase the level of reduced glutathione in tissues.

Furthermore, resveratrol was also examined as a preventive agent against cisplatin-induced cardiotoxicity in rats [89]. It is well known that cisplatin treatment might cause cardiac function deterioration and myocardial injury. Moreover, cisplatin increased the levels of lactate dehydrogenase, creatine kinase, and malondialdehyde and decreased the levels of glutathione, glutathione peroxidase, superoxide dismutase, and catalase. Treatment of the rats with resveratrol reduced these side effects of the chemotherapeutic agent in question.

The *in vitro* studies performed by Kweon et al. [40] on acute myeloid leukemia cells revealed that resveratrol might facilitate doxorubicin uptake by the cells, probably by downregulation of the expression of *mrp-1* (*mrp-1* belongs to ATP-binding cassette transporter family, involved in multidrug resistance). It acts as an energy-dependent efflux pump whose overexpression causes a decrease in doxorubicin concentration in the cells [40].

Polyphenol-rich extracts

In addition to individual phenolics, polyphenol-rich extracts were also reported to act synergistically with anticancer chemotherapeutics (Table 2). For instance, an evening primrose seed extract sensitized human metastatic melanoma (HTB-140) and hepatoma (HepG2) cells to vincristine, a mitotic inhibitor used in cancer chemotherapy [32]. The combined use of the extract (25 μ g/mL) and the chemotherapeutic drug (1 μ M) in HTB-140 and HepG2 cells resulted in more than a 4- and a 1.5-fold increase in cytotoxicity, respectively, when compared to vincristine alone. The extract is rich in procyanidins and hydrolyzable tannins (mainly pentagalloyl glucose) which could significantly contribute to the observed effect.

Table 2. Examples of synergy between polyphenol-rich extracts and anticancer chemotherapeutics. Animal models are in bold

Polyphenol-rich extracts and their main phenolic constituents	Anticancer chemotherapeutics	Experimental models	References
evening primrose seed extract (procyanidins and hydrolyzable tannins)	vincristine	human melanoma cell line (HTB-140) and human hepatoma cell line (HepG2)	Jaszewska et al., 2010 [32]
extract of <i>Teucrium polium</i> L. (rutin and apigenin)	vincristine	human cancer cell lines (Skmel-3, Saos-2, SW-480, MCF-7, KB, EJ, and A-431)	Rajabalian et al., 2008 [70]
	vinblastine		
	doxorubicin		
extract of cider apple (procyanidins)	MDL 72527	human colon cancer cell line (SW-620)	Seiler et al., 2006 [75]
	phenylalanine methylester		
	chloroquine		
grape seed extract (procyanidins)	doxorubicin	human breast carcinoma cell lines (MCF-7 and MDA-MB-468)	Sharma et al., 2004 [78]
polyphenolic cocoa extract (flavones and phenolic acids)	tamoxifen	human breast cancer cell lines (MCF-7 and SKBR-3)	Oleaga et al., 2012 [60]
propolis extract (flavonoids and phenolic acids)	irinotecan	Ehrlich ascites tumor-bearing Swiss albino mice	Benkovic et al., 2007 [7] Orsolić et al., 2010 [61]
silymarin (a mixture of seven flavonolignans and one flavonoid)	doxorubicin paclitaxel	human colon carcinoma cell line (LoVo)	Colombo et al., 2011 [13]
extracts of <i>Phyllanthus emblica</i> (ellagitannins, flavonoids, and simple gallic acid derivatives)	doxorubicin	human hepatocellular carcinoma cell line (Hep G2) and human lung cancer cell line (A549)	Pinmai et al., 2008 [67]
	cisplatin		
	doxorubicin		
extracts of <i>Terminalia bellerica</i> (hydrolyzable tannins, including simple gallate esters, ellagic acid derivatives, and glycosides and various ellagitannins such as chebulic ellagitannins)	doxorubicin	human colorectal carcinoma cell lines (DLD-1 and HT-29)	Tai et al., 2013 [85]
	cisplatin		
	docetaxel		
<i>Solanum nigrum</i> leaf extract (gentisic acid, luteolin, apigenin, kaempferol, and <i>m</i> -coumaric acid)	5-fluorouracil	human leukemia cell lines (U937 and HL-60-G, a subclone of human promyelocytic leukemia HL-60 cell line), murine myelomonocytic leukemia cell line (WEHI-3B D-), syngeneic BALB/c mice bearing WEHI-3B D- tumors	Sharabani et al., 2006 [77] Shabtay et al., 2008 [76]
	doxorubicin		
	cisplatin		
rosemary extract (carosic acid)	5-fluorouracil	5-fluorouracil-sensitive and -resistant human colon cancer cell lines	González-Vallinas et al., 2013 [23]
	doxorubicin	human breast cancer cell line (MCF-7)	Sarath et al., 2007 [73]

Different classes of compounds have been isolated so far from various parts of *Teucrium polium* L. (*Lamiaceae*), a wild-growing flowering plant, found abundantly in South-Western Asia, Europe, and North Africa and used traditionally for different pathological conditions (including gastrointestinal disorders, inflammation, diabetes, and rheumatism) [4]. The main groups are flavonoids and terpenoids [4], and the major flavonoid compounds from a methanolic extract of *T. polium* L. are

rutin and apigenin [20]. Rajabalian et al. [70] reported that a methanolic extract of this plant potentiates the cytotoxic and proapoptotic effects of three anticancer drugs (vincristine, vinblastine, and doxorubicin) toward several cancer cell lines (Skmel-3, Saos-2, SW480, MCF-7, KB, EJ, and A431).

Apple procyanidins with a mean degree of polymerization equal to 4, and three lysosomotropic drugs (MDL

72527, phenylalanine methylester, and chloroquine, each used alone), demonstrated synergistic inhibition of the growth of human colon cancer-derived metastatic SW620 cells and induction of their apoptosis [75]. The authors suggested that the major cause of the observed increase in procyanidin cytotoxicity resulting from co-administration with non-toxic concentrations of the above-mentioned drugs was lysosomal membrane permeabilization. Beside apples, grapes are also rich dietary sources of procyanidins; 43 flavan-3-ols (including procyanidin monomers and oligomers with polymerization degrees up to 7 and with various galloylation degrees) were tentatively identified in a grape seed extract [79]. Sharma et al. [78] tested a grape seed extract and doxorubicin (alone and in combination) against estrogen receptor-positive MCF-7 and receptor-negative MDA-MB468 human breast carcinoma cell lines. The obtained results imply a synergistic effect between the procyanidin-rich extract and doxorubicin, independent of estrogen receptor status of the cancer cell. Another important procyanidin source in a daily diet are cocoa-containing foods and beverages; these compounds constitute the major phenolic fraction in cocoa extracts and are followed by flavones and phenolic acids [62]. Oleaga et al. [60] observed synergy between a polyphenolic cocoa extract and tamoxifen; a combination of both was more cytotoxic against MCF-7 and SKBR3 breast cancer cells than either agent alone.

Synergistic interactions between anticancer chemotherapeutics and polyphenol-rich extracts were also observed *in vitro* or *in vivo* for, among others, a combination of irinotecan (a semisynthetic analogue of camptothecin) and propolis extract [7,60]; doxorubicin or paclitaxel combined with silymarin (a standardized extract of *Silybum marianum* (L.) Gaertn. seeds) [13]; doxorubicin or cisplatin and polyphenol-rich extracts of *Phyllanthus emblica* and *Terminalia bellerica* [67]; cisplatin, doxorubicin, docetaxel, or 5-fluorouracil and *Solanum nigrum* leaf extract [85]; the hormonal form of vitamin D (1 α ,25-dihydroxyvitamin D3) and carnosic acid-rich rosemary leaf extract [76,88]; 5-fluorouracil and rosemary leaf extract [23], and doxorubicin and *Artemisia princeps* var. *orientalis* leaf extract [73].

Polyphenols are the major components of the above-mentioned extracts. For instance, among typical phenolic compounds of propolis are apigenin, chrysin, galangin, kaempferol, quercetin, naringenin, pinocembrin, pinostrobin, caffeic acid, cinnamic acid, *p*-coumaric acid, and ferulic acid [8]. Silymarin is a mixture of seven distinct flavonolignans (silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, silydianin) and one flavonoid (taxifolin) isolated from the seeds of *Silybum marianum* (L.) Gaertn. [69]. A methanolic extract of *Phyllanthus emblica* fruit is rich in ellagitannins, flavonoids, and simple gallic acid derivatives [92]. A number of phenolic compounds (mainly hydrolyzable tannins, including simple gallate esters, ellagic acid derivatives, and glycosides and various el-

lagitannins such as chebulic ellagitannins) were identified in a methanolic extract of *Terminalia bellerica* fruit [66]. *Solanum nigrum* leaves were found to be richer in polyphenols than stem and fruit; the highest concentrations of gentisic acid, luteolin, apigenin, kaempferol, and *m*-coumaric acid were detected in the leaves [27]. *Artemisia princeps* Pampanini is also rich in flavonoids [35] and phenolic acids [35,42] and the major rosemary polyphenol is carnosic acid [77].

Despite the differences in phenolic profiles, all the above-mentioned extracts have been demonstrated to act synergistically with one or more anticancer chemotherapeutics (Table 2). Importantly, the synergistic effects were observed not only *in vitro* [15,67,73,76,85] but also in animal models [7,61,76,77]. Importantly, pretreatment of mice bearing Ehrlich ascites tumors with flavonoid-rich propolis extracts before administration of irinotecan (a derivative of camptothecin) decreased the chemotherapeutic-induced cytotoxic and genotoxic effects toward normal cells without affecting the drug's toxicity toward tumor cells [61].

ANTICANCER SYNERGY BETWEEN POLYPHENOLS

Phytochemicals present in plant extracts (including phenolic compounds in polyphenol-rich extracts) can interact synergistically with one another, which results in higher anticancer activity of an extract than the sum of activities of its individual components [14]. Synergistic anticancer effects of combinations of two or more phenolic compounds or polyphenol-rich plant extracts were also observed (Table 3). For instance, OptiBerry IH141 formulation composed of six anthocyanin-rich extracts (from the fruits of wild blueberry, bilberry, cranberry, elderberry, and strawberry, and from raspberry seeds) exhibited superior anti-angiogenic activity both *in vitro* and *in vivo* compared to other combinations tested and, importantly, low toxicity [3]. In the *in vitro* studies, OptiBerry suppressed a key regulator of tumor angiogenesis by significantly inhibiting H₂O₂- and TNF- α -induced vascular endothelial growth factor expression by human keratinocytes. Consistently, the anthocyanin-rich formulation impaired angiogenesis in human microvascular endothelial cells, as assessed by Matrigel assay. Pretreatment of mouse hemangioendothelioma endothelial cells (EOMA cell line) with OptiBerry significantly inhibited the transcription of inducible NF- κ B and of basal monocyte chemoattractant protein-1 (MCP-1, also referred to as chemokine (C-C motif) ligand 2 (CCL2) or small inducible cytokine A2). OptiBerry reduced the ability of the endothelioma cells to form tumors; a pronounced decrease in tumor growth (by more than 50%) was observed in a murine model of hemangioma. Furthermore, infiltration of macrophages into tumors was markedly lower in mice treated with the OptiBerry formulation than in placebo-treated controls. Extensive infiltration of tumors with macrophages was shown to correlate with cancer metastasis and poor prognosis in a variety of human carcinomas [83].

Table 3. Examples of anticancer synergy between phenolic compounds or polyphenol rich extracts. Animal models are in bold.

Phenolic compounds or polyphenol-rich extracts	Experimental models	References
OptiBerry IH141 formulation composed of six anthocyanin-rich extracts (from the fruits of wild blueberry, bilberry, cranberry, elderberry, and strawberry, and from raspberry seeds)	human keratinocytes, human microvascular endothelial cells, murine model of hemangioma	Bagchi et al., 2004 [3]
combination of curcumin and resveratrol	p53-positive (wt) and p53-negative colon cancer HCT-116 cell lines, SCID mice xenografts of HCT-116 (wt) cells	Majumdar et al., 2009 [49]
combination of quercetin and ellagic acid	human leukemia cell line (MOLT-4)	Mertens-Talcott et al., 2003 [53]
combination of resveratrol and ellagic acid or quercetin	human leukemia cell line (MOLT-4)	Mertens-Talcott and Percival, 2005 [52]
combinations of resveratrol and ellagic acid or grape seed extract (additional tested agent: calcium D-glucarate)	SENCAR mice	Kowalczyk et al., 2010 [38]
combination of epicatechin and epigallocatechin gallate (EGCG)	human colon cancer cell line (HT-29)	Shimizu et al., 2005 [81]
combination of EGCG and other green tea catechins; catechin mixtures treated with tannase	human cervical carcinoma cell line (HeLa)	Morré et al., 2003 [56]
combinations of grape extracts (obtained from pulp, skins, juice, seeds, or ground freeze-dried pomace) and decaffeinated green tea extracts	human cervical carcinoma cell line (HeLa), murine mammary carcinoma cell line (4T1), 4T1 tumor-bearing mice	Morré DM and Morré DJ, 2006 [57]
pomegranate fruit juice	human cell lines: oral cancer (KB, CAL27), colon cancer (HT-29, HCT116, SW480, SW620), and prostate cancer (RWPE-1, 22Rv1)	Seeram et al., 2005 [74]
combined extract of clove, oregano, thyme, walnuts, and coffee	human monocytic leukemia cell line transfected with a DNA construct containing three NF- κ B sites from the Ig κ light chain promoter coupled to the gene encoding firefly luciferase (3x- κ B- <i>luc</i>) (U937- κ B), human hepatocellular carcinoma cell line (HepG2), transgenic NF-κB luciferase reporter mice	Paur et al., 2010 [65]
propolis extract	human cell lines: breast cancer (BT-474), undifferentiated lung cancer (ChaCo), hepatocellular carcinoma (HepG2), gastric carcinoma (KATO-III), colon adenocarcinoma (SW-620), and human non-transformed foreskin fibroblast cell line (Hs-27)	Teerasripreecha et al., 2012 [86]
complex virgin olive oil phenolic extracts	human promyelocytic leukemia cell line (HL-60)	Fabiani et al., 2011 [21]
ginger extract and selected ginger phenolics (6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol)	human prostate cancer cell line (PC-3)	Brahmbhatt et al., 2013 [9]
extracts of <i>Rosa roxburghii</i> Tratt. and <i>Fagopyrum cymosum</i>	human cell lines: esophageal squamous carcinoma (CaEs-17), gastric carcinoma (SGC-7901), pulmonary carcinoma (A-549)	Liu et al., 2012 [46]
sweet potato greens extract	prostate tumor xenografts in nude mice	Gundala et al., 2013 [26]

In a study by Mertens-Talcott et al. [53], quercetin and ellagic acid (phenolic compounds abundant in small fruits such as blackberries, strawberries, red raspberries, blueberries and grapes) were shown to synergistically reduce proliferation and viability and trigger apoptosis of human leukemia MOLT-4 cells at the concentrations of 5 and 10 μ M each. Cell cycle kinetics were also considerably altered as a result of the combined treatment. In a further study by Mertens-Talcott and Percival [52], two other combinations of phenolics (el-

lagic acid + resveratrol and quercetin + resveratrol) were tested on the same cell line as potential antiproliferative and apoptosis-inducing agents. Both combinations showed more than additive interactions, with a slightly stronger synergistic effect for the former than for the latter (with a combination index of 0.64 vs. 0.68, respectively).

Another research group demonstrated that resveratrol and curcumin synergistically inhibited the growth

of both p53-positive (wild type) and p53-negative human colon cancer HCT-116 cells [49]. In the *in vivo* studies, this combination of phenolic compounds was also more effective in suppressing the growth of HCT-116 (wt) tumors in SCID mice xenografts than either agent alone. Similarly, resveratrol combinations with ellagic acid, grape seed extract, and other phytochemicals were proved to effectively inhibit skin tumorigenesis in SENCAR mice (7,12-dimethylbenz[a]anthracene-induced skin carcinogenesis model) [38]. Resveratrol was applied topically, grape seed extract was either applied topically or administered in diet, and ellagic acid was administered in diet; another agent tested in the study was calcium D-glucarate (administered in diet). Importantly, all combinations including resveratrol exhibited a synergistic effect against epidermal hyperplasia and Ha-ras mutations.

Synergistic interactions were also reported for green tea catechins; for instance, a combination of EGCG (10 µg/mL) and epicatechin (1 µg/mL) synergistically inhibited the growth of human colon cancer HT-29 cells and triggered their apoptosis [81]. Furthermore, Morr e et al. [56] reported that when EGCG was combined with (-)-epicatechin (EC), (-)-epigallocatechin (EGC) or (-)-epicatechin 3-gallate (ECG), a 10-fold reduction in its concentration required to suppress the growth of human cervical carcinoma HeLa cells and to inhibit the activity of a cancer-associated cell surface-located NADH oxidase (ECTO-NOX, designated tNOX) was observed. Importantly, EC, EGC, and ECG were inactive when tested alone. In further experiments, mixtures based on purified catechins and decaffeinated tea extracts were treated with tannase to reduce the content of ester bond-containing catechins. After the enzymatic treatment, the content of EGCG in the mixtures or extracts varied from 0.065 to 40%. Interestingly, their effectiveness was comparable to that of decaffeinated green tea extracts as long as EGCG was present and the ratio of total catechins to EGCG + EGC was about 1.5.

Grapes and various grape extracts (from pulp, skins, juice, seeds, or ground freeze-dried pomace) were tested and proved to be active in similar experiments, focused on tNOX and on the growth of HeLa cells (*in vitro*) and of murine mammary 4T1 cells (*in vitro* and in 4T1 tumor-bearing mice) [57]. Moreover, synergy between the grape extracts and decaffeinated green tea extracts was observed, both in the inhibition of tNOX activity and in the inhibition of cancer cell growth. In the murine model of breast cancer, the growth of 4T1 tumors was suppressed most effectively by a mixture of a green tea extract and ground freeze-dried grape pomace in the ratio of 25:1, respectively.

Synergy between the constituents of pomegranate juice was demonstrated by Seeram *et al.* [74]. The juice showed the strongest antiproliferative activity against two oral cancer cell lines, four colon cancer cell lines, and two prostate cancer cell lines when compared with its major polyphenol (punicalagin, an ellagitannin) and with a standardized total pomegranate tannin extract.

A combined extract of clove, oregano, thyme, walnuts, and coffee proved to be a potent modulator of NF-kappaB signaling both *in vitro* (in a monocytic cell line) and *in vivo* [65]. The inhibition of lipopolysaccharide (LPS)-induced NF-kappaB activation by the combination extract was synergistic, as evidenced by a comparison with the activities of the individual extracts. A single dose of the combined extract administered to transgenic NF-kappaB luciferase reporter mice prior to LPS treatment resulted in a decrease in whole body LPS-induced NF-kappaB activity after the first 6 hours by 35% when compared with control mice. The expression of genes related to inflammation, cell migration, and proliferation in the liver was also reduced by the combination extract.

Propolis is a honeybee product rich in flavonoids and phenolic acids; its polyphenol-rich dichloromethane and hexane extracts (resulting from a sequential extraction with methanol, dichloromethane, and hexane) acted as antiproliferative/cytotoxic agents toward five cell lines derived from human carcinomas of breast (BT474), lung (Chaco), liver (Hep G2), stomach (KATO-III), and colon (SW620) [86]. The half maximal inhibitory concentration (IC50) values across the above-mentioned cancer cell lines ranged from 41.3 to 52.4 µg/mL for the hexane extract and from 43.8 to 53.5 µg/mL for the dichloromethane extract. Bioactivity-guided fractionation led to isolation of two main active compounds, namely, cardanol and cardol (phenolic lipids). Their IC50 values across the five cancer cell lines and a control (human non-transformed foreskin fibroblast Hs-27 cell line) ranged from 10.8 to 29.3 µg/mL and from < 3.13 to 5.97 µg/mL, respectively. Importantly, after treatment with cardanol or cardol, cytotoxicity and cell death without DNA fragmentation were observed only in cancer cells, whereas in the case of Hs-27 only an antiproliferative effect was observed. However, according to the work in question, the isolated compounds did not account for the net antiproliferative/cytotoxic activity of the crude extracts. Therefore, the authors suggested that either other potent compounds are present in the extracts or synergistic interactions between their constituents are involved [86].

Taking into account that olive is one of the major components of the Mediterranean diet, Fabiani *et al.* [21] compared *in vitro* chemopreventive activities of four complex virgin olive oil phenolic extracts (derived from four Italian cultivars) in a study on the human promyelocytic leukemia HL-60 cell line. The antiproliferative and proapoptotic activities of the extracts were positively correlated with the content of secoiridoids. Representatives of this subgroup of polyphenols are hydroxytyrosol and oleuropein (elenolic acid ester of hydroxytyrosol), the main phenolic compounds of olive oil. Interestingly, the above-mentioned anticancer activities of the tested extracts were negatively correlated with the concentration of two other subgroups of olive oil phenolic compounds, namely, phenyl alcohols and lignans. Importantly, when a complex mixture of the olive oil phenolics was tested, a more pronounced anticancer effect was observed in comparison with individual compounds, which may imply either synergistic interactions or

the presence of other unidentified extract constituent(s) exhibiting antiproliferative and/or proapoptotic activities toward HL-60 leukemia cells.

Brahmbhatt et al. [9] observed growth inhibition of prostate cancer cells and induction of their apoptosis both *in vitro* and *in vivo* as a result of treatment with a ginger extract and decided to investigate the nature of interactions between selected phenolic constituents of the extract (namely, 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol). The obtained results indicate that binary combinations of the above-mentioned ginger phenolics synergistically inhibit proliferation of human prostate cancer PC-3 cells. Furthermore, a combination of ginger extract with its constituents (particularly 6-gingerol) enhanced the extract's antiproliferative activity.

Among traditional Chinese medicines, *Rosa roxburghii* Tratt. and *Fagopyrum cymosum* are recognized to be effective in improving immune responses and enhancing digestive ability as well as to act as antiaging agents [46]. *In vitro* anticancer activities of their extracts were assessed in a study on three human carcinoma cell lines (esophageal squamous carcinoma CaEs-17, gastric carcinoma SGC-7901, and pulmonary carcinoma A549). A combination of extracts of both plants synergistically inhibited cell growth and caused an increase in apoptosis.

As a result of a study demonstrating growth-inhibitory and apoptosis-inducing properties of polyphenol-rich sweet potato greens extract toward human prostate cancer PC-3 cells both *in vitro* and *in vivo* (prostate cancer xenografts in nude mice), Gundala et al. [26] carried out bioactivity-guided fractionation of the extract. A polyphenol-enriched fraction was identified with approximately 100-fold higher activity than the parent extract (as assessed on the basis of IC50 measurements in human prostate cancer cells). The fraction was characterized by a distinct phenolic profile; the authors reported approximately 2.6- and 3.6-fold enrichment of quinic acid and chlorogenic acid, respectively, and a definitive ratiometric relationship between the isochlorogenic acids (4,5-di-caffeoylquinic acid, 3,5-di-caffeoylquinic acid, and 3,4-di-caffeoylquinic acid). Importantly, its subfractionation

resulted in loss of bioactivity, thus suggesting involvement of synergistic interactions among the constituent compounds. In the *in vivo* studies, daily oral administration of the fraction (400 mg/kg body weight) suppressed growth and progression of prostate tumor xenografts in nude mice by approximately 75%.

CONCLUDING REMARKS

The results of *in vitro* and *in vivo* studies reported so far in the literature and briefly discussed above imply that phenolic compounds and polyphenol-rich extracts have a high potential as chemopreventive and therapeutic agents. In the case of extracts, the potential results from the presence of various phenolics in natural proportions, from their complementary biological activities, as well as from synergistic interactions among them. The growing interest in biological activities of polyphenols is a consequence of, among others, an increasingly high incidence of civilization diseases (particularly various cancers) and a need to find safe and effective methods of prophylaxis and therapy. Literature data indicate that among numerous mechanisms underlying the anticancer activity of polyphenols there are, for instance, cell cycle arrest, inhibition of signal transduction pathways, induction of differentiation, downregulation of oncogenes, upregulation of tumor suppressor genes, apoptosis induction, and impairment of angiogenesis. Such a variety of mechanisms of action exhibited by phenolic compounds renders it possible to choose an appropriate anticancer agent among them.

From the point of view of a potential patient, prevention is a more beneficial strategy than therapy, and the use of natural chemopreventive agents bears less risk of adverse side effects, is less expensive, and more environmentally friendly than in the case of synthetic drugs. On the other hand, it is worth emphasizing that in clinical trials relatively few cases of polyphenol overdose and the related toxicity have been reported so far (some of them were caused by intravenous administration of tested preparations) [87]. Therefore, it is essential to develop a set of appropriate biomarkers which would render it possible to precisely monitor the effects of phenolic compounds in cancer chemoprevention and therapy.

REFERENCES

- [1] Ali S., Varghese L., Pereira L., Tulunay-Ugur O.E., Kucuk O., Carey T.E., Wolf G.T., Sarkar F.H.: Sensitization of squamous cell carcinoma to cisplatin induced killing by natural agents. *Cancer Lett.*, 2009; 278: 201-209
- [2] Attia S.M.: The impact of quercetin on cisplatin-induced clastogenesis and apoptosis in murine marrow cells. *Mutagenesis*, 2010; 25: 281-288
- [3] Bagchi D., Sen C.K., Bagchi M., Atalay M.: Anti-angiogenic, antioxidant, and anti-carcinogenic properties of a novel anthocyanin-rich berry extract formula. *Biochemistry*, 2004; 69: 75-80
- [4] Bahramikia S., Yazdanparast R.: Phytochemistry and medicinal properties of *Teucrium polium* L. (*Lamiaceae*). *Phytother. Res.*, 2012; 26: 1581-1593
- [5] Banerjee S., Zhang Y., Ali S., Bhuiyan M., Wang Z., Chiao P.J., Philip P.A., Abbruzzese J., Sarkar F.H.: Molecular evidence for increased antitumor activity of gemcitabine by genistein *in vitro* and *in vivo* using an orthotopic model of pancreatic cancer. *Cancer Res.*, 2005; 65: 9064-9072
- [6] Banerjee S., Zhang Y., Wang Z., Che M., Chiao P.J., Abbruzzese J.L., Sarkar F.H.: *In vitro* and *in vivo* molecular evidence of genistein action in augmenting the efficacy of cisplatin in pancreatic cancer. *Int. J. Cancer*, 2007; 120: 906-917
- [7] Benkovic V., Horvat Knezevic A., Brozovic G., Knezevic F., Dikic D., Bevanda M., Basic I., Orsolic N.: Enhanced antitumor activity of irinotecan combined with propolis and its polyphenolic compounds on Ehrlich ascites tumor in mice. *Biomed. Pharmacother.*, 2007; 61: 292-297

- [8] Bertelli D., Papotti G., Bortolotti L., Marcazzan G.L., Plessi M.: ¹H-NMR simultaneous identification of health-relevant compounds in propolis extracts. *Phytochem. Anal.*, 2012; 23: 260-266
- [9] Brahmabhatt M., Gundala S.R., Asif G., Shamsi S.A., Aneja R.: Ginger phytochemicals exhibit synergy to inhibit prostate cancer cell proliferation. *Nutr. Cancer*, 2013; 65: 263-272
- [10] Burns J., Yokota T., Ashihara H., Lean M.E., Crozier A.: Plant foods and herbal sources of resveratrol. *J. Agric. Food Chem.*, 2002; 50: 3337-3340
- [11] Carvalho M., Silva B.M., Silva R., Valentao P., Andrade P.B., Bastos M.L.: First report on *Cydonia oblonga* Miller anticancer potential: differential antiproliferative effect against human kidney and colon cancer cells. *J. Agric. Food Chem.*, 2010; 58: 3366-3370
- [12] Chan M.M., Soprano K.J., Weinstein K., Fong D.: Epigallocatechin-3-gallate delivers hydrogen peroxide to induce death of ovarian cancer cells and enhances their cisplatin susceptibility. *J. Cell. Physiol.*, 2006; 207: 389-396
- [13] Colombo V., Lupi M., Falcetta F., Forestieri D., D'Incalci M., Ubezio P.: Chemotherapeutic activity of silymarin combined with doxorubicin or paclitaxel in sensitive and multidrug-resistant colon cancer cells. *Cancer Chemother. Pharmacol.*, 2011; 67: 369-379
- [14] de Kok T.M., van Breda S.G., Manson M.M.: Mechanisms of combined action of different chemopreventive dietary compounds: a review. *Eur. J. Nutr.*, 2008; 47 (Suppl. 2): 51-59
- [15] Dhandapani K.M., Mahesh V.B., Brann D.W.: Curcumin suppresses growth and chemoresistance of human glioblastoma cells via AP-1 and NFκB transcription factors. *J. Neurochem.*, 2007; 102: 522-538
- [16] Do Amaral C.L., Francescato H.D., Coimbra T.M., Costa R.S., Darin J.D., Antunes L.M., Bianchi Mde L.: Resveratrol attenuates cisplatin-induced nephrotoxicity in rats. *Arch. Toxicol.*, 2008; 82: 363-370
- [17] Du B., Jiang L., Xia Q., Zhong L.: Synergistic inhibitory effects of curcumin and 5-fluorouracil on the growth of the human colon cancer cell line HT-29. *Chemotherapy*, 2006; 52: 23-28
- [18] Du G., Lin H., Wang M., Zhang S., Wu X., Lu L., Ji L., Yu L.: Quercetin greatly improved therapeutic index of doxorubicin against 4T1 breast cancer by its opposing effects on HIF-1α in tumor and normal cells. *Cancer Chemother. Pharmacol.*, 2010; 65: 277-287
- [19] Du G., Lin H., Yang Y., Zhang S., Wu X., Wang M., Ji L., Lu L., Yu L., Han G.: Dietary quercetin combining intratumoral doxorubicin injection synergistically induces rejection of established breast cancer in mice. *Int. Immunopharmacol.*, 2010; 10: 819-826
- [20] Esmaeili M.A., Zohari F., Sadeghi H.: Antioxidant and protective effects of major flavonoids from *Teucrium polium* on beta-cell destruction in a model of streptozotocin-induced diabetes. *Planta Med.*, 2009; 75: 1418-1420
- [21] Fabiani R., Sepporta M.V., Mazza T., Rosignoli P., Fucelli R., De Bartolomeo A., Crescimanno M., Taticchi A., Esposto S., Servili M., Morozzi G.: Influence of cultivar and concentration of selected phenolic constituents on the *in vitro* chemopreventive potential of olive oil extracts. *J. Agric. Food Chem.*, 2011; 59: 8167-8174
- [22] Fujiki H., Suganuma M.: Green tea: an effective synergist with anticancer drugs for tertiary cancer prevention. *Cancer Lett.*, 2012; 324: 119-125
- [23] González-Vallinas M., Molina S., Vicente G., de la Cueva A., Vargas T., Santoyo S., García-Risco M.R., Fornari T., Reglero G., Ramírez de Molina A.: Antitumor effect of 5-fluorouracil is enhanced by rosemary extract in both drug sensitive and resistant colon cancer cells. *Pharmacol. Res.*, 2013; 72: 61-68
- [24] Goralach S., Wagner W., Podsedek A., Sosnowska D., Dastyh J., Koziolkiewicz M.: Polyphenols from evening primrose (*Oenothera paradoxa*) defatted seeds induce apoptosis in human colon cancer Caco-2 cells. *J. Agric. Food Chem.*, 2011; 59: 6985-6997
- [25] Goralach S., Wagner W., Podsedek A., Szweczyk K., Koziolkiewicz M., Dastyh J.: Procyanidins from Japanese quince (*Chaenomeles japonica*) fruit induce apoptosis in human colon cancer Caco-2 cells in a degree of polymerization-dependent manner. *Nutr. Cancer*, 2011; 63: 1348-1360
- [26] Gundala S.R., Yang C., Lakshminarayana N., Asif G., Gupta M.V., Shamsi S., Aneja R.: Polar biophenolics in sweet potato greens extract synergize to inhibit prostate cancer cell proliferation and *in vivo* tumor growth. *Carcinogenesis*, 2013; 34: 2039-2049
- [27] Huang H.C., Syu K.Y., Lin J.K.: Chemical composition of *Solanum nigrum* Linn. extract and induction of autophagy by leaf water extract and its major flavonoids in AU565 breast cancer cells. *J. Agric. Food Chem.*, 2010; 58: 8699-8708
- [28] Huff J., LaDou J.: Aspartame bioassay findings portend human cancer hazards. *Int. J. Occup. Environ. Health*, 2007; 13: 446-448
- [29] Hwang J.T., Ha J., Park O.J.: Combination of 5-fluorouracil and genistein induces apoptosis synergistically in chemo-resistant cancer cells through the modulation of AMPK and COX-2 signaling pathways. *Biochem. Biophys. Res. Commun.*, 2005; 332: 433-440
- [30] Jakubowicz-Gil J., Langner E., Rzeski W.: Kinetic studies of the effects of Temodal and quercetin on astrocytoma cells. *Pharmacol. Rep.*, 2011; 63: 403-416
- [31] Jakubowicz-Gil J., Langner E., Wertel I., Piersiak T., Rzeski W.: Temozolomide, quercetin and cell death in the MOGGCCM astrocytoma cell line. *Chem. Biol. Interact.*, 2010; 188: 190-203
- [32] Jaszewska E., Kosmider A., Kiss A.K., Naruszewicz M.: *Oenothera paradoxa* defatted seeds extract containing pentagalloylglucose and procyanidins potentiates the cytotoxicity of vincristine. *J. Physiol. Pharmacol.*, 2010; 61: 637-643
- [33] Jemal A., Bray F., Center M.M., Ferlay J., Ward E., Forman D.: Global cancer statistics. *CA Cancer J. Clin.*, 2011; 61: 69-90
- [34] Jiang H., Ma Y., Chen X., Pan S., Sun B., Krissansen G.W., Sun X.: Genistein synergizes with arsenic trioxide to suppress human hepatocellular carcinoma. *Cancer Sci.*, 2010; 101: 975-983
- [35] Ju H.K., Lee H.W., Chung K.S., Choi J.H., Cho J.G., Baek N.I., Chung H.G., Lee K.T.: Standardized flavonoid-rich fraction of *Artemisia princeps* Pampanini cv. Sajabal induces apoptosis via mitochondrial pathway in human cervical cancer HeLa cells. *J. Ethnopharmacol.*, 2012; 141: 460-468
- [36] Khan N., Mukhtar H.: Multitargeted therapy of cancer by green tea polyphenols. *Cancer Lett.*, 2008; 269: 269-280
- [37] Kordek R., Jassem J., Jeziorski A., Kornafel J., Krzakowski M., Pawługa J.: Chemioterapia i hormonoterapia nowotworów: Onkologia. Podręcznik dla studentów i lekarzy (Chemotherapy and hormone therapy for cancers: Oncology. A manual for students and doctors), red.: Ruzicka M., Siemaszko I., Via Medica, Gdańsk, Poland, 2007, 93-100
- [38] Kowalczyk M.C., Kowalczyk P., Tolstykh O., Hanausek M., Walaszek Z., Slaga T.J.: Synergistic effects of combined phytochemicals and skin cancer prevention in SENCAR mice. *Cancer Prev. Res.*, 2010; 3: 170-178
- [39] Kuhar M., Imran S., Singh N.: Curcumin and quercetin combined with cisplatin to induce apoptosis in human laryngeal carcinoma Hep-2 cells through the mitochondrial pathway. *J. Cancer Mol.*, 2007; 3: 121-128
- [40] Kweon S.H., Song J.H., Kim T.S.: Resveratrol-mediated reversal of doxorubicin resistance in acute myeloid leukemia cells via down-regulation of MRP1 expression. *Biochem. Biophys. Res. Commun.*, 2010; 395: 104-110
- [41] Lang M., Henson R., Braconi C., Patel T.: Epigallocatechin-gallate modulates chemotherapy-induced apoptosis in human cholangiocarcinoma cells. *Liver Int.*, 2009; 29: 670-677

- [42] Lee S.G., Lee H., Nam T.G., Eom S.H., Heo H.J., Lee C.Y., Kim D.O.: Neuroprotective effect of caffeoylquinic acids from *Artemisia princeps* Pampanini against oxidative stress-induced toxicity in PC-12 cells. *J. Food Sci.*, 2011; 76: C250-C256
- [43] Lewandowska U., Szewczyk K., Owczarek K., Hrabec Z., Podsedek A., Koziolkiewicz M., Hrabec E.: Flavanols from Japanese quince (*Chaenomeles japonica*) fruit inhibit human prostate and breast cancer cell line invasiveness and cause favorable changes in bax/bcl-2 mRNA ratio. *Nutr. Cancer*, 2013; 65: 273-285
- [44] Lim H.A., Kim J.H., Kim J.H., Sung M.K., Kim M.K., Park J.H., Kim J.S.: Genistein induces glucose-regulated protein 78 in mammary tumor cells. *J. Med. Food*, 2006; 9: 28-32
- [45] Liu R.H.: Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am. J. Clin. Nutr.*, 2003; 78 (Suppl. 3): 517S-520S
- [46] Liu W., Li S.Y., Huang X.E., Cui J.J., Zhao T., Zhang H.: Inhibition of tumor growth *in vitro* by a combination of extracts from *Rosa roxburghii* Tratt and *Fagopyrum cymosum*. *Asian Pac. J. Cancer Prev.*, 2012; 13: 2409-2414
- [47] Loeb L.A., Harris C.C.: Advances in chemical carcinogenesis: a historical review and prospective. *Cancer Res.*, 2008; 68: 6863-6872
- [48] Lu J., Zhang K., Chen S., Wen W.: Grape seed extract inhibits VEGF expression via reducing HIF-1 α protein expression. *Carcinogenesis*, 2009; 30: 636-644
- [49] Majumdar A.P., Banerjee S., Nautiyal J., Patel B.B., Patel V., Du J., Yu Y., Elliott A.A., Levi E., Sarkar F.H.: Curcumin synergizes with resveratrol to inhibit colon cancer. *Nutr. Cancer*, 2009; 61: 544-553
- [50] Mattson M.P.: Dietary factors, hormesis and health. *Ageing Res. Rev.*, 2008; 7: 43-48
- [51] McDonnell C.O., Holden G., Sheridan M.E., Foley D., Moriarty M., Walsh T.N., Bouchier-Hayes D.J.: Improvement in efficacy of chemoradiotherapy by addition of an antiangiogenic agent in a murine tumor model. *J. Surg. Res.*, 2004; 116: 19-23
- [52] Mertens-Talcott S.U., Percival S.S.: Ellagic acid and quercetin interact synergistically with resveratrol in the induction of apoptosis and cause transient cell cycle arrest in human leukemia cells. *Cancer Lett.*, 2005; 218: 141-151
- [53] Mertens-Talcott S.U., Talcott S.T., Percival S.S.: Low concentrations of quercetin and ellagic acid synergistically influence proliferation, cytotoxicity and apoptosis in MOLT-4 human leukemia cells. *J. Nutr.*, 2003; 133: 2669-2674
- [54] Mohammad R.M., Banerjee S., Li Y., Aboukameel A., Kucuk O., Sarkar F.H.: Cisplatin-induced antioxidant activity is potentiated by the soy isoflavone genistein in BxPC-3 pancreatic tumor xenografts. *Cancer*, 2006; 106: 1260-1268
- [55] Montopoli M., Ragazzi E., Froidi G., Caparrotta L.: Cell-cycle inhibition and apoptosis induced by curcumin and cisplatin or oxaliplatin in human ovarian carcinoma cells. *Cell Prolif.*, 2009; 42: 195-206
- [56] Morré D.J., Morré D.M., Sun H., Cooper R., Chang J., Janle E.M.: Tea catechin synergies in inhibition of cancer cell proliferation and of a cancer specific cell surface oxidase (ECTO-NOX). *Pharmacol. Toxicol.*, 2003; 92: 234-241
- [57] Morré D.M., Morré D.J.: Anticancer activity of grape and grape skin extracts alone and combined with green tea infusions. *Cancer Lett.*, 2006; 238: 202-209
- [58] Oktem G., Uysal A., Oral O., Sezer E.D., Olukman M., Erol A., Akgur S.A., Bilir A.: Resveratrol attenuates doxorubicin-induced cellular damage by modulating nitric oxide and apoptosis. *Exp. Toxicol. Pathol.*, 2012; 64: 471-479
- [59] Olas B., Wachowicz B., Majsterek I., Blasiak J., Stochmal A., Oleszek W.: Antioxidant properties of trans-3,3',5,5'-tetrahydroxy-4'-methoxystilbene against modification of variety of biomolecules in human blood cells treated with platinum compounds. *Nutrition*, 2006; 22: 1202-1209
- [60] Oleaga C., García M., Solé A., Ciudad C.J., Izquierdo-Pulido M., Noé V.: CYP1A1 is overexpressed upon incubation of breast cancer cells with a polyphenolic cocoa extract. *Eur. J. Nutr.*, 2012; 51: 465-476
- [61] Oršolić N., Benković V., Lisičić D., Dikić D., Erhardt J., Knežević A.H.: Protective effects of propolis and related polyphenolic/flavonoid compounds against toxicity induced by irinotecan. *Med. Oncol.*, 2010; 27: 1346-1358
- [62] Ortega N., Romero M.P., Macià A., Reguant J., Anglès N., Morelló J.R., Motilva M.J.: Obtention and characterization of phenolic extracts from different cocoa sources. *J. Agric. Food Chem.*, 2008; 56: 9621-9627
- [63] Papazisis K.T., Kalemi T.G., Zambouli D., Geromichalos G.D., Lambropoulos A.F., Kotsis A., Boutis L.L., Kortsaris A.H.: Synergistic effects of protein tyrosine kinase inhibitor genistein with camptothecins against three cell lines *in vitro*. *Cancer Lett.*, 2006; 233: 255-264
- [64] Parzefall W.: Minireview on the toxicity of dietary acrylamide. *Food Chem. Toxicol.*, 2008; 46: 1360-1364
- [65] Paur I., Balstad T.R., Kolberg M., Pedersen M.K., Austenaa L.M., Jacobs D.R. Jr., Blomhoff R.: Extract of oregano, coffee, thyme, clove, and walnuts inhibits NF- κ B in monocytes and in transgenic reporter mice. *Cancer Prev. Res. (Phila)*, 2010; 3: 653-663
- [66] Pfundstein B., El Desouky S.K., Hull W.E., Haubner R., Erben G., Owen R.W.: Polyphenolic compounds in the fruits of Egyptian medicinal plants (*Terminalia bellerica*, *Terminalia chebula* and *Terminalia horrida*): characterization, quantitation and determination of antioxidant capacities. *Phytochemistry*, 2010; 71: 1132-1148
- [67] Pinmai K., Chunlaratthanabhorn S., Ngamkitidechakul C., Soonthornchareon N., Hahnvajanawong C.: Synergistic growth inhibitory effects of *Phyllanthus emblica* and *Terminalia bellerica* extracts with conventional cytotoxic agents: doxorubicin and cisplatin against human hepatocellular carcinoma and lung cancer cells. *World J. Gastroenterol.*, 2008; 14: 1491-1497
- [68] Polyak S.J., Morishima C., Lohmann V., Pal S., Lee D.Y., Liu Y., Graf T.N., Oberlies N.H.: Identification of hepatoprotective flavonolignans from silymarin. *Proc. Natl. Acad. Sci. USA*, 2010; 107: 5995-5999
- [69] Qian F., Wei D., Zhang Q., Yang S.: Modulation of P-glycoprotein function and reversal of multidrug resistance by (-)-epigallocatechin gallate in human cancer cells. *Biomed. Pharmacother.*, 2005; 59: 64-69
- [70] Rajabalian S.: Methanolic extract of *Teucrium polium* L. potentiates the cytotoxic and apoptotic effects of anticancer drugs of vincristine, vinblastine and doxorubicin against a panel of cancerous cell lines. *Exp. Oncol.*, 2008; 30: 133-138
- [71] Ramos A.M., Aller P.: Quercetin decreases intracellular GSH content and potentiates the apoptotic action of the antileukemic drug arsenic trioxide in human leukemia cell lines. *Biochem. Pharmacol.*, 2008; 75: 1912-1923
- [72] Sánchez Y., Amrán D., Fernández C., de Blas E., Aller P.: Genistein selectively potentiates arsenic trioxide-induced apoptosis in human leukemia cells via reactive oxygen species generation and activation of reactive oxygen species-inducible protein kinases (p38-MAPK, AMPK). *Int. J. Cancer*, 2008; 123: 1205-1214
- [73] Sarath V.J., So C.S., Won Y.D., Gollapudi S.: *Artemisia princeps* var *orientalis* induces apoptosis in human breast cancer MCF-7 cells. *Anticancer Res.*, 2007; 27: 3891-3898
- [74] Seeram N.P., Adams L.S., Henning S.M., Niu Y., Zhang Y., Nair M.G., Heber D.: *In vitro* antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *J. Nutr. Biochem.*, 2005; 16: 360-367
- [75] Seiler N., Chaabi M., Roussi S., Gossé F., Lobstein A., Raul F.: Synergism between apple procyanidins and lysosomotropic drugs: potential in chemoprevention. *Anticancer Res.*, 2006; 26: 3381-3385

- [76] Shabtay A., Sharabani H., Barvish Z., Kafka M., Amichay D., Levy J., Sharoni Y., Uskokovic M.R., Studzinski G.P., Danilenko M.: Synergistic antileukemic activity of carnosic acid-rich rosemary extract and the 19-nor Gemini vitamin D analogue in a mouse model of systemic acute myeloid leukemia. *Oncology*, 2008; 75: 203-214
- [77] Sharabani H., Izumchenko E., Wang Q., Kreinin R., Steiner M., Barvish Z., Kafka M., Sharoni Y., Levy J., Uskokovic M., Studzinski G.P., Danilenko M.: Cooperative antitumor effects of vitamin D₃ derivatives and rosemary preparations in a mouse model of myeloid leukemia. *Int. J. Cancer*, 2006; 118: 3012-3021
- [78] Sharma G., Tyagi A.K., Singh R.P., Chan D.C., Agarwal R.: Synergistic anti-cancer effects of grape seed extract and conventional cytotoxic agent doxorubicin against human breast carcinoma cells. *Breast Cancer Res. Treat.*, 2004; 85: 1-12
- [79] Sharma V., Hupp C.D., Tepe J.J.: Enhancement of chemotherapeutic efficacy by small molecule inhibition of NF- κ B and checkpoint kinases. *Curr. Med. Chem.*, 2007; 14: 1061-1074
- [80] Shervington A., Pawar V., Menon S., Thakkar D., Patel R.: The sensitization of glioma cells to cisplatin and tamoxifen by the use of catechin. *Mol. Biol. Rep.*, 2009; 36: 1181-1186
- [81] Shimizu M., Deguchi A., Lim J.T., Moriwaki H., Kopelovich L., Weinstein I.B.: (-)-Epi-gallocatechin gallate and polyphenon E inhibit growth and activation of the epidermal growth factor receptor and human epidermal growth factor receptor-2 signaling pathways in human colon cancer cells. *Clin. Cancer Res.*, 2005; 11: 2735-2746
- [82] Siegel R., Naishadham D., Jemal A.: Cancer statistics, 2012. *CA Cancer J. Clin.*, 2012; 62: 10-29
- [83] Siveen K.S., Kuttan G.: Role of macrophages in tumour progression. *Immunol. Lett.*, 2009; 123: 97-102
- [84] Sukanuma M., Saha A., Fujiki H.: New cancer treatment strategy using combination of green tea catechins and anticancer drugs. *Cancer Sci.*, 2011; 102: 317-323
- [85] Tai C.J., Wang C.K., Tai C.J., Lin Y.F., Lin C.S., Jian J.Y., Chang Y.J., Chang C.C.: Aqueous extract of *Solanum nigrum* leaves induces autophagy and enhances cytotoxicity of cisplatin, doxorubicin, docetaxel, and 5-fluorouracil in human colorectal carcinoma cells. *Evid. Based Complement. Alternat. Med.*, 2013; 2013: 514719
- [86] Teerasripreecha D., Phuwapraisrisan P., Puthong S., Kimura K., Okuyama M., Mori H., Kimura A., Chanchao C.: *In vitro* antiproliferative/cytotoxic activity on cancer cell lines of a cardanol and a cardol enriched from Thai *Apis mellifera* propolis. *BMC Complement. Altern. Med.*, 2012; 12: 27
- [87] Thomasset S.C., Berry D.P., Garcea G., Marczylo T., Steward W.P., Gescher A.J.: Dietary polyphenolic phytochemicals – promising cancer chemopreventive agents in humans? A review of their clinical properties. *Int. J. Cancer*, 2007; 120: 451-458
- [88] Wagner H.: Synergy research: approaching a new generation of phytopharmaceuticals. *Fitoterapia*, 2011; 82: 34-37
- [89] Wang J., He D., Zhang Q., Han Y., Jin S., Qi F.: Resveratrol protects against cisplatin-induced cardiotoxicity by alleviating oxidative damage. *Cancer Biother. Radiopharm.*, 2009; 24: 675-680
- [90] Wang Y., Wang H., Zhang W., Shao C., Xu P., Shi C.H., Shi J.G., Li Y.M., Fu Q., Xue W., Lei Y.H., Gao J.Y., Wang J.Y., Gao X.P., Li J.Q., Yuan J.L., Zhang Y.T.: Genistein sensitizes bladder cancer cells to HCPT treatment in vitro and in vivo via ATM/NF- κ B/IKK pathway-induced apoptosis. *PLoS One*, 2013; 8: e50175
- [91] Watanabe J., Takebayashi J., Takano-Ishikawa Y., Yasui A.: Evaluation of a method to quantify quercetin aglycone in onion (*Allium cepa*) by single- and multi-laboratory validation studies. *Anal. Sci.*, 2012; 28: 1179-1182
- [92] Yang B., Kortensniemi M., Liu P., Karonen M., Salminen J.P.: Analysis of hydrolyzable tannins and other phenolic compounds in emblic leaflet (*Phyllanthus emblica* L.) fruits by high performance liquid chromatography-electrospray ionization mass spectrometry. *J. Agric. Food Chem.*, 2012; 60: 8672-8683
- [93] Zanini C., Giribaldi G., Mandili G., Carta F., Crescenzo N., Bisaro B., Doria A., Foglia L., di Montezemolo L.C., Timeus F., Turrini F.: Inhibition of heat shock proteins (HSP) expression by quercetin and differential doxorubicin sensitization in neuroblastoma and Ewing's sarcoma cell lines. *J. Neurochem.*, 2007; 103: 1344-1354
- [94] Zhang C., Feng Y., Qu S., Wei X., Zhu H., Luo Q., Liu M., Chen G., Xiao X.: Resveratrol attenuates doxorubicin-induced cardiomyocyte apoptosis in mice through SIRT1-mediated deacetylation of p53. *Cardiovasc. Res.*, 2011; 90: 538-545
- [95] Zhang Q., Wei D., Liu J.: *In vivo* reversal of doxorubicin resistance by (-)-epigallocatechin gallate in a solid human carcinoma xenograft. *Cancer Lett.*, 2004; 208: 179-186
- [96] Zhang W., Xue J., Ge M., Yu M., Liu L., Zhang Z.: Resveratrol attenuates hepatotoxicity of rats exposed to arsenic trioxide. *Food Chem. Toxicol.*, 2013; 51: 87-92

The authors have no potential conflicts of interest to declare.