

# Microarray-based mRNA Expression Profiling of Leukemia Cells Treated with the Flavonoid, Casticin

CHIARA RIGHESCHI<sup>1\*</sup>, TOLGA EICHHORN<sup>2\*</sup>, ANASTASIA KARIOTI<sup>1</sup>,  
ANNA RITA BILIA<sup>1</sup> and THOMAS EFFERTH<sup>2</sup>

<sup>1</sup>Department of Pharmaceutical Sciences, University of Florence, Florence, Italy;

<sup>2</sup>Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry,  
University of Mainz, Mainz, Germany

**Abstract.** Natural polyphenols play an important role in tumor inhibition. We used a doxorubicin-sensitive acute T-lymphoblastic leukemia cell line (CCRF-CEM) and its multidrug-resistant subline (CEM/ADR5000) to evaluate the activity of 15 plant polyphenols isolated in our laboratory (hypericin and pseudohypericin, verbascoside, ellagic acid, casticin, kaempferol-3-O-(2'',6''-di-E-p-coumaroyl)-glucopyranoside, kaempferol-3-O-(3,4-diacetyl-2,6-di-E-p-coumaroyl)-glucopyranoside, tiliroside, salvianolic acid B, oleuropein, rosmarinic acid, bergenin) or of others from commercial sources (curcumin, epigallocatechin-3-gallate, silymarin). Casticin was the most potent compound ( $IC_{50}$  values of  $0.28 \pm 0.02 \mu M$  in CCRF-CEM and  $0.44 \pm 0.17 \mu M$  in CEM/ADR5000 cells. The  $IC_{50}$  values of the other compounds tested ranged from  $1.52 \mu M$  to  $164.1 \mu M$ . A microarray-based mRNA expression profiling of CCRF-CEM cells treated with casticin was performed in order to identify genes with altered expression following casticin treatment. Networks related to NF- $\kappa$ B, p38MAPK, histones H3 and H4, and follicle stimulating hormone were identified.

Leukemic diseases are a heterogeneous group of malignant diseases, some of which have a poor prognosis. Natural compounds are of considerable therapeutic interest for treatment of these diseases (1). The search for anticancer agents with improved pharmacological features continues

and the huge structural diversity of natural compounds derived from plants, marine flora and microorganisms has resulted in the discovery of lead compounds for semi-synthetic derivatization and improvement of their therapeutic potential (2).

Polyphenols constitute one of the largest and most ubiquitous group of phytochemicals. They are also present in large amounts in many vegetables and are thought to play a major role in health (3). The daily intake of polyphenols by an average person is estimated to range between 25 mg and 1 g (4). A lower incidence of cancer has been associated with the consumption of certain nutrients and especially of polyphenol-rich diets in certain populations (5). There is an increasing interest in polyphenols as chemopreventive agents and in reducing cancer-associated morbidity and mortality by delaying the process of carcinogenesis (6).

Polyphenols scavenge reactive oxygen species, thereby protecting critical cellular targets (*i.e.* DNA, proteins, and lipids) from oxidative insult (7). They also interfere with intracellular signaling pathways regulating proliferation, induction of apoptosis, and response to oxidative stress (8). Several polyphenols have been shown to induce cell cycle arrest and apoptosis in tumor but not normal cells, *e.g.* resveratrol, quercetin, curcumin, ellagic acid, and epigallocatechin gallate (6). Such activity has also been shown *in vivo* (9). While the potential of polyphenols has been shown for some examples, there are still many polyphenolic structures whose activity needs to be explored.

Considering the growing interest in this field, a doxorubicin-sensitive acute T-lymphoblastic leukemia cell line (CCRF-CEM) and its multidrug-resistant subline (CEM/ADR5000) were used to evaluate the activity of 15 polyphenolic constituents derived from medicinal plants. In order to gain insight into possible molecular modes of action, we performed microarray-based analyses to identify genes that are differentially regulated in treated cells compared to untreated controls.

\*These Authors contributed equally to this work.

**Correspondence to:** Professor Dr. Thomas Efferth, Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University Mainz, Staudinger Weg 5, 55128 Mainz, Germany. Tel: +49 61313925751, Fax: +49 61313923752, e-mail: efferth@uni-mainz.de

**Key Words:** Cancer, flavonoids, microarrays, multidrug resistance, natural products, pharmacogenomics, CCRF-CEM, CEM/ADR5000 cell lines, casticin.

Table I. Phytochemical compounds and their plant origins.

	MW	Purity	Medicinal plant of origin
Commercial compounds:			
Curcumin	368	≥98%	<i>Curcuma longa</i>
Epigallocatechin-3-gallate	458	≥98%	<i>Camellia sinensis</i>
Silybin (silymarin)	482	–	<i>Silybum marianum</i>
Compounds isolated in-house:			
Bergenin	328	≥98%	<i>Ardisia crenata</i>
Casticin	374	≥98%	<i>Vitex agnus castus</i>
Ellagic acid	302	≥98%	<i>Quercus ilex</i>
Hypericin	504	≥98%	<i>Hypericum perforatum</i>
Kaempferol-3-O-(2'',6''-di- <i>E-p</i> -coumaroyl)-glucopyranoside	740	≥98%	<i>Quercus ilex</i>
Kaempferol-3-O-(3'',4''-diacetyl-2'',6''-di- <i>E-p</i> -coumaroyl)-glucopyranoside	824	≥98%	<i>Quercus ilex</i>
Oleuropein	540	≥98%	<i>Olea europaea</i>
Pseudo-hypericin	522	≥98%	<i>Hypericum perforatum</i>
Rosmarinic acid	360	≥99%	<i>Salvia miltiorrhiza</i>
Salvianolic acid B	718	≥98%	<i>Salvia miltiorrhiza</i>
Tiliroside	594	≥98%	<i>Quercus ilex</i>
Verbascoside	624	≥98%	<i>Lippia alba</i>

## Materials and Methods

**Polyphenols.** The following compounds were isolated from plants and were fully characterized by nuclear magnetic resonance spectroscopy ( $^1\text{H}$  NMR) and high pressure liquid chromatography-array detector-electrospray ionization-mass spectrometry (HPLC-DAD-ESI-MS) analyses as previously described (10-14): hypericin and pseudohypericin (*Hypericum perforatum*), rosmarinic acid and salvianolic acid B (*Salvia miltiorrhiza*), bergenin (*Ardisia crenata*), kaempferol-3-O-(2'',6''-di-*E-p*-coumaroyl)- $\beta$ -glucopyranoside, kaempferol-3-O-(3'',4''-diacetyl-2'',6''-di-*E-p*-coumaroyl)- $\beta$ -glucopyranoside, tiliroside and ellagic acid (*Quercus ilex*), oleuropein (*Olea europaea*), verbascoside (*Lippia alba*), and casticin (*Vitex agnus castus*) (Table I, Figure 1). Curcumin, epigallocatechin-3-gallate, and silymarin, were purchased from Extrasynthese (Genay, France). Vehicle was used as negative control.

**Cell lines.** Sensitive CCRF-CEM and multidrug resistant CEM/ADR5000 cells were obtained from Dr. Axel Sauerbrey (University of Jena, Department of Pediatrics, Jena, Germany). CEM/ADR5000 cells specifically overexpress P-glycoprotein (*ABCB1*), but none of the other resistance-related ATP-binding cassette (ABC) transporters (15, 16). The cross-resistance profile of CEM/ADR5000 cells to established anticancer drugs and an assortment of natural products has been previously published (17, 18). CCRF-CEM and CEM/ADR5000 (19) were maintained in RPMI-1640 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS, antibiotics (1% of a 10,000 U/ml penicillin G and 10 mg/ml streptomycin), in a humidified atmosphere with 5%  $\text{CO}_2$  at 37°C.

**XTT assay.** The cytotoxicity of compounds was determined by means of Cell Proliferation Kit II (Roche Diagnostics, Mannheim, Germany). This test is based on the cleavage of the yellow 2,3-bis[2-methoxy-4-nitro-5-sulphophenyl]-2H-tetrazolium-5-carboxanilide

inner salt (XTT) by ubiquitous dehydrogenases leading to the formation of an orange formazan dye. The performance of the test has been described elsewhere (19). All *in vitro* experiments were carried out on two microplates with six contiguous wells for each treatment. All values are expressed as the mean $\pm$ SD of two independent experiments, each with six-fold parallel measurements. Vehicles were used as negative controls.

**RNA isolation.** Total RNA was isolated from CCRF-CEM cells using RNeasy Kit (Qiagen, Hamburg, Germany) according to the manufacturer's instructions. The RNA quality was analyzed on an Agilent 2100 Bioanalyzer (Agilent Technologies GmbH, Berlin, Germany). Only samples with RNA index values greater than 8.5 were used further. RNA concentrations were determined using NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

**Probe labeling and Illumina Sentrix BeadChip array hybridization.** Biotin-labeled cRNA samples from CCRF-CEM cells for hybridization on Illumina Human Sentrix-8 BeadChip arrays (Illumina, Inc.) were prepared according to Illumina's recommended sample labeling procedure based on the modified Eberwine protocol (20). Hybridization with containing 19,730 genes per microarray was performed as previously described (19).

**Scanning and data analysis.** Microarray scanning was performed using a Beadstation array scanner. Data collection was performed individually for each bead and measurements were discarded as outliers if the median absolute deviations (MAD) were greater than 2.5. Data analysis was carried out by normalization of signals using the quantile normalization algorithm without background subtraction. Differentially regulated genes were defined by calculating the standard deviation differences of a given sample in one-by-one comparisons of samples or groups (19). Genes were further filtered using Chipster software (<http://chipster.csc.fi/>) by discarding genes with a *p*-value lower than 0.05 and having a differential expression more than one-fold the

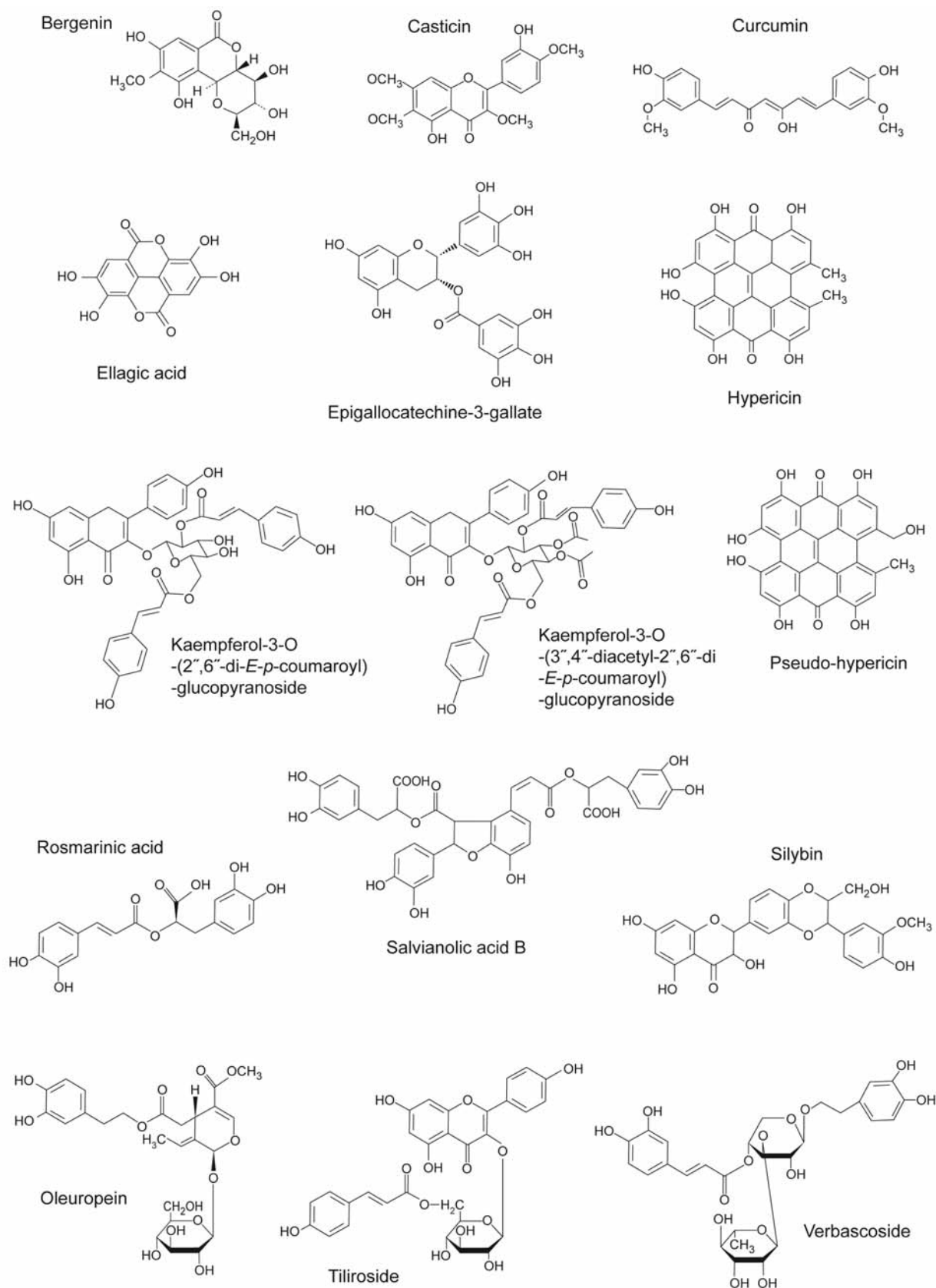


Figure 1. Chemical structures of the compounds investigated.

Table II. 50% inhibition concentration ( $IC_{50}$ ) values (expressed as the mean $\pm$ SD of two independent experiments each with six-fold determination) and relative resistance to natural compounds in CCRF-CEM cells and multidrug-resistant CEM/ADR5000 cells as determined by the XTT test. The  $IC_{50}$  values were calculated from dose-response curves using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA).

Compound	CCRF-CEM ( $IC_{50}$ )	CEM-ADR5000 ( $IC_{50}$ )	Relative resistance*
Bergenin	Inactive	Inactive	–
Casticin	0.28 $\pm$ 0.02 $\mu$ M	0.44 $\pm$ 0.17 $\mu$ M	1.57
Curcumin	3.74 $\pm$ 2.26 $\mu$ M	63.50 $\mu$ M	16.98
Ellagic acid	164.10 $\pm$ 30.69 $\mu$ M	Inactive	–
Epigallocatechin-3-gallate	16.04 $\pm$ 1.56 $\mu$ M	Inactive	–
Hypericin	1.52 $\pm$ 0.23 $\mu$ M	1.34 $\pm$ 0.11 $\mu$ M	0.88
Kaempferol-3-O-(2'',6''-di- <i>E-p</i> -coumaroyl)-glucopyranoside	57.43 $\pm$ 22.75 $\mu$ M	111.92 $\pm$ 19.49 $\mu$ M	1.95
Kaempferol-3-O-(3'',4''-diacetyl-2'',6''-di- <i>E-p</i> -coumaroyl)-glucopyranoside	2.15 $\pm$ 0.37 $\mu$ M	Inactive	–
Oleuropein	66.14 $\pm$ 15.75 $\mu$ M	70.05 $\pm$ 1.30 $\mu$ M	1.06
Pseudo-hypericin	12.14 $\pm$ 5.10 $\mu$ M	11.99 $\pm$ 4.8 $\mu$ M	0.99
Rosmarinic acid	80.95 $\pm$ 15.97 $\mu$ M	Inactive	–
Salvianolic acid B	Inactive	Inactive	–
Silymarin	68.12 $\pm$ 16.33 $\mu$ M	Inactive	–
Tiliroside	Inactive	Inactive	–
Verbascoside	97.27 $\pm$ 3.65 $\mu$ M	81.90 $\pm$ 23.84 $\mu$ M	0.84

\*Relative resistance was calculated by dividing the  $IC_{50}$  of CCRF-CEM cells by the  $IC_{50}$  of CEM/ADR5000 cells.

standard deviation. The final set of filtered genes was analyzed using the Ingenuity Pathway Analysis Software (<http://www.ingenuity.com/>) to identify affected networks of genes and pathways.

## Results

*Cytotoxicity of phytochemicals towards sensitive and multidrug-resistant leukemia cells.* The cytotoxicity of casticin was determined towards wild-type CCRF-CEM leukemia cells. For comparison, 14 other phytochemicals were also tested. The 50% inhibition concentration ( $IC_{50}$ ) values calculated from dose-response curves are shown in Table II. The phytochemicals most active towards CCRF-CEM cells were casticin, hypericin, and kaempferol-3-O-(3'',4''-diacetyl-2'',6''-di-*E-p*-coumaroyl)-glucopyranoside. These compounds were tested in a multidrug-resistant subline of CCRF-CEM, CEM/ADR5000. Of them, casticin had the lowest  $IC_{50}$  value for both cell lines. CEM/ADR5000 exhibited a low degree of cross-resistance towards casticin (1.57-fold). No or only weak cross-resistance was observed towards pseudo-hypericin, hypericin, oleuropein, verbascoside and kaempferol-3-O-(3'',4''-diacetyl-2'',6''-di-*E-p*-coumaroyl)- $\beta$ -glucopyranoside (0.84-to 1.95-fold). CEM/ADR5000 exhibited a higher degree of cross-resistance towards curcumin (16.98-fold) (Table II).

*Differential gene expression in casticin-treated and untreated cells.* The effect of casticin treatment on gene expression was determined by microarray-based mRNA hybridization. CCRF-CEM cells were treated with the  $IC_{50}$  dose of casticin (0.28  $\mu$ M) for 24 or 72 h or left untreated. A total of 186

genes were found to be up-regulated and 302 genes were down-regulated upon treatment for 24 h, compared to untreated cells. After 72 h treatment, 219 genes were found to be up-regulated and 72 down-regulated.

In order to further analyze the molecular modes of action of casticin, we performed a signaling pathway analysis by means of the Ingenuity Pathway Analysis software. The most prominent functional groups of genes differentially regulated upon casticin treatment for 24 h ( $p < 0.05$ ) were those involved in drug metabolism, lipid metabolism, small molecule biochemistry, and the cell cycle (Figure 2A). Treatment of CCRF-CEM cells for 72 h affected genes involved in the cell cycle, in cellular assembly and organization, in DNA replication and repair and cell death (Figure 2B).

As a next step, we asked whether the genes from these diverse functional classes act independently or whether they act together beyond the constraints of canonical pathways in a concerted manner after casticin challenge. For this reason, we carried out dynamic network analyses using the differentially regulated genes. As shown in Figure 3, specific pathways of interacting genes after casticin treatment were revealed. A specific molecular network with a connection to cell death and cellular growth and proliferation was determined in CCRF-CEM cells treated for 24 h with casticin (Figure 3A). A comparable molecular network related to cellular growth and proliferation was identified in CCRF-CEM cells after 72 h treatment with casticin (Figure 3B). Interestingly, both networks were related to the same cellular regulators, *i.e.* NF- $\kappa$ B, p38MAPK, histones H3 and

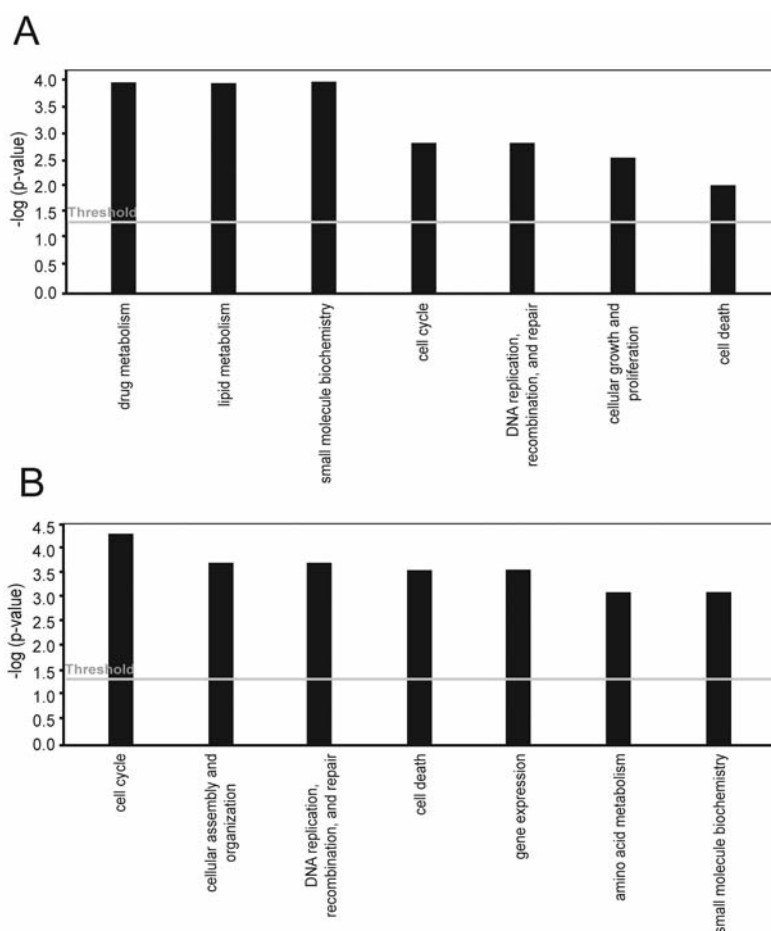


Figure 2. mRNA-based microarray analyses of CCRF-CEM cells treated with casticin. Microarray hybridizations were performed using Illumina Sentrix BeadChip arrays (see Material and Methods). Identification of functional groups of genes regulated upon casticin treatment for 24 h (A) and 72 h (B) in CEM-CCRF cells. Gene expressions of cells treated with 50% inhibition concentration of casticin were compared to those in untreated cells. The evaluation of differentially expressed genes was performed using the Ingenuity Pathway Analysis software, version 5.5.

H4, and FSH, indicating important functions for activity of casticin in CCRF-CEM cells.

## Discussion

In the present study, casticin was the most cytotoxic polyphenol towards CCRF-CEM leukemia cells among a group of 15 polyphenols. Casticin is an unusual polymethoxy-flavonoid bearing a methoxyl moiety at C-3 of the C-ring, which is an essential structure activity requirement of flavones for cytotoxicity (21). The presence of this methoxyl group at C-3 may explain why casticin was more cytotoxic than the other polyphenols investigated by us.

Polymethoxyflavones, bearing two or more methoxy groups on their basic benzo- $\gamma$ -pyrone skeleton with a carbonyl group at the C4 position, are found in a limited number of plant families. Polymethoxyflavones structurally

related to casticin display chemoprotective effects by inhibiting cancer cell growth, induction of apoptosis, and blocking metastasis (22). The rate of methylation of polyphenols in chemoprevention is not fully understood. The enhanced permeability of cellular membranes to polymethoxyflavones due to their lipophilic nature may lead to enhanced cellular absorption and bioactivity. In addition, methylation may increase the oral bioavailability of these compounds. In the past, polymethoxyflavones raised only modest interest for chemoprevention because of their lack of antioxidant properties, which was suggested as a main mechanism of action. However, recent findings point to signal transduction pathways as being the main targets for flavonoids (23).

Polyphenols may modulate various targets either by direct interaction or by modulation of gene expression. To gain insight into the molecular networks and pathways related to



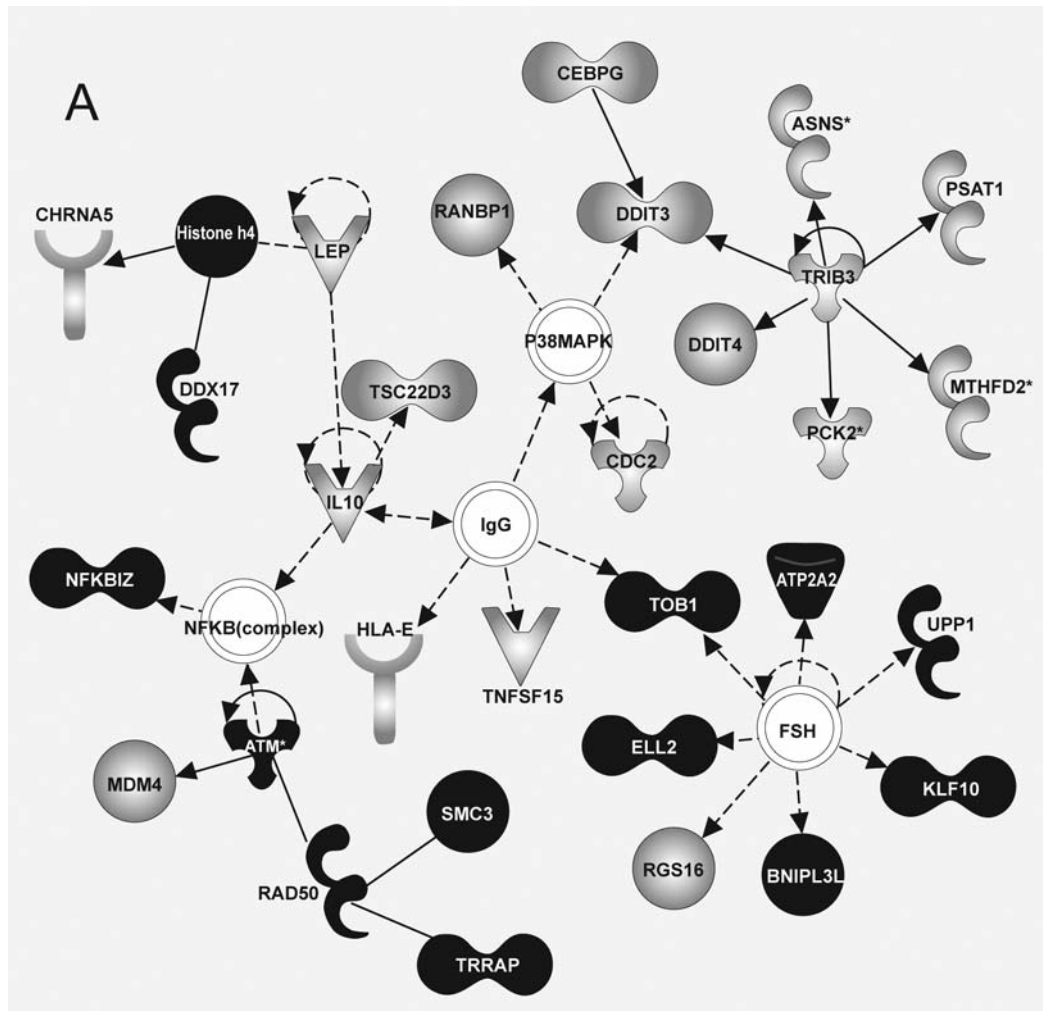


Figure 3. Continued

the cytotoxicity of casticin towards cancer cells, we performed mRNA-based microarray analysis. Upon treatment of CCRF-CEM cells with casticin, several genes that form a network related to the transcription factor NF- $\kappa$ B were differentially expressed. Hence, casticin may act in part by affecting NF- $\kappa$ B signaling. Casticin has been described to inhibit NF- $\kappa$ B and many other flavonoids are also well known NF- $\kappa$ B inhibitors (25, 16). NF- $\kappa$ B plays a major role in regulating immune response, cell proliferation, and apoptosis. Seven related proteins (NF- $\kappa$ B1 and NF- $\kappa$ B2, each with two alternatively spliced forms, and REL-A, REL-B and c-REL) can form dimers, which are able to bind specific DNA motifs in the promoters of target genes (25, 27, 28). These heterodimers can activate the transcription of about 200 target genes (29). Inactive NF- $\kappa$ B1 or NF- $\kappa$ B2 proteins are complexed with I $\kappa$ B $\alpha$  (inhibitory  $\kappa$ B) proteins in the cytosol. Phosphorylation of I $\kappa$ B $\alpha$  by I $\kappa$ B $\alpha$  kinase (IKK)

leads to I $\kappa$ B degradation and translocation of NF- $\kappa$ B1 and NF- $\kappa$ B2 into the nucleus. Casticin demonstrated anti-inflammatory effects *in vivo* (31) confirming the use of plants from the genus *Vitex* for the treatment of inflammation in European, Asian and Chinese traditional medicines. In addition, casticin inhibited leukemia and breast cancer growth *in vitro* (31-33). The results of our microarray analysis testify to NF- $\kappa$ B as being involved in the underlying mechanism of casticin against cancer cells.

p38MAPK is another protein in the network affected by casticin treatment of CCRF-CEM cells. MAPKs form signaling cascades regulating important cellular functions such as cell growth, differentiation and apoptosis (34, 35). They are activated by stress stimuli including cancer therapy (36). No effect on p38MAPK signaling by casticin has yet been described, but p38MAPK inhibition is known for other polyphenols (37-40). p38MAPK is known to act upstream of

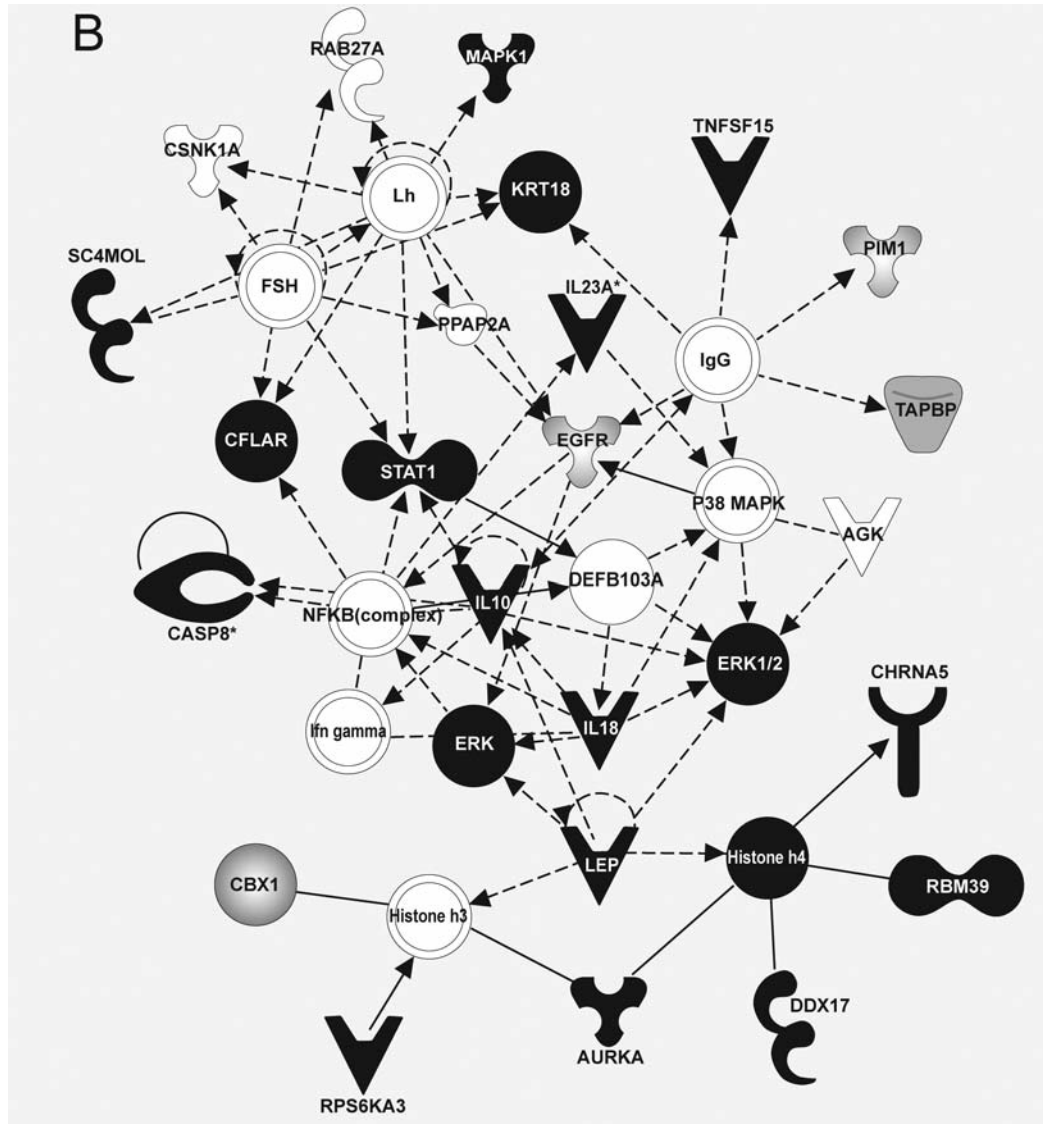


Figure 3. Signaling network of CCRF-CEM leukemia cells treated with casticin (50% inhibition concentration) for 24 h (A) or 72 h (B).

NF- $\kappa$ B and to activate NF- $\kappa$ B (41) indicating that the effect of casticin on both these signaling molecules may contribute to its cytotoxic properties. Interestingly, another polyphenol, furosin, has been reported to suppress RANKL-induced osteoclast differentiation and function through inhibition of MAPK activation (42). Together with our results concerning casticin, this may speak for a more general role of polyphenols affecting RANKL, NF- $\kappa$ B, and MAPK signaling pathways.

Casticin treatment led to differential expression of a network of genes with different functions, including histones. Histones are constituents of nuclear chromatin and organize DNA condensation during meiotic and mitotic chromosome formation. The histones H3 and H4, which appeared in our

casticin-specific genetic network are core histones whose epigenetic modifications (*e.g.* methylation, acetylation, phosphorylation SUMOylation, and ubiquitinylation) play a role in gene regulation (43). While casticin is not yet known to affect histones, the role of flavonoids in chromatin remodeling during inflammatory processes is well described (44-46). Epigenetic modification by flavonoids may represent an attractive novel treatment strategy for cancer, and as such deserves further exploration.

In our microarray-based network, another set of genes centered on FSH. In both females and males, FSH regulates the maturation of germ cells, and the development, growth and pubertal maturation of the organism (47). Interestingly,

endothelia of tumor blood vessels frequently reveal elevated FSH receptor levels, and FSH seems to contribute to neoangiogenesis in tumors by vascular endothelial growth factor-dependent and independent pathways (48). Hence, inhibition of FSH signaling may represent a strategy for anti-angiogenic cancer therapy. Our finding that casticin affected FSH-associated genes in the signaling network may be taken as a hint that casticin might inhibit tumor angiogenesis. This speculation merits further analysis.

In conclusion, the cytotoxicity of casticin towards sensitive and multidrug-resistant leukemia cells was associated with multiple effects on different signal transduction pathways. A general feature of natural products is their ability to act on multiple targets and pathways rather than one single target (49). This plays a major role in preventing the development of resistance, which has been an important selection advantage during the evolution of life. In the case of casticin, this feature might be explained in future therapy of cancers.

## References

- 1 Efferth T, Davey M, Olbrich A, Rücker G, Gebhart E and Davey R: Activity of drugs from Traditional Chinese Medicine toward sensitive and MDR1- or MRP1-overexpressing multidrug-resistant human CCRF-CEM leukemia cells. *Blood Cells Mol Dis* 28: 160-168, 2002.
- 2 Gordaliza M: Natural products as leads to anticancer drugs. *Clin Transl Oncol* 9: 767-776, 2007.
- 3 Hounsborne N, Hounsborne B, Tomos D and Edwards-Jones G: Plant metabolites and nutritional quality of vegetables. *J Food Sci* 73: R48-R65, 2008.
- 4 Kühnau J: The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev. Nutr Diet* 24: 117-191, 1976.
- 5 Sporn MB and Suh N: Chemoprevention: an essential approach to controlling cancer. *Nat Rev Cancer* 2: 537-543, 2002.
- 6 Araújo JR, Gonçalves P and Martel F: Chemopreventive effect of dietary polyphenols in colorectal cancer cell lines. *Nutr Res* 31: 77-87, 2011.
- 7 Rafter JJ: Scientific basis of biomarkers and benefits of functional foods for reduction of disease risk: cancer. *Br J Nutr* 88: S219-S224, 2002.
- 8 Loo G: Redox-sensitive mechanisms of phytochemical-mediated inhibition of cancer cell proliferation. *J Nutr Biochem* 14: 64-73, 2003.
- 9 Duvoix A, Blasius R, Delhalle S, Schnekenburger M, Morceau F and Henry E: Chemopreventive and therapeutic effects of curcumin. *Cancer Lett* 223: 181-190, 2005.
- 10 Timóteo P, Karioti A, Leitão SG, Vincieri FF and Bilia AR: HPLC/DAD/ESI-MS analysis of non-volatile constituents of three Brazilian chemotypes of *Lippia alba* (Mill.) N.E. Brown. *Nat Prod Commun* 3: 2017-2020, 2008.
- 11 Karioti A, Vincieri FF and Bilia AR: Rapid and efficient purification of naphthodianthrone from St. John's Wort extract by using liquid-liquid extraction and SEC. *J Sep Sci* 32: 1374-1382, 2009a.
- 12 Karioti A, Bilia AR, Gabbiani C, Messori L and Skaltsa H: Proanthocyanidin glycosides from the leaves of *Quercus ilex* L. (Fagaceae). *Tetrahedron Lett* 50: 1771-1776, 2009b.
- 13 Karioti A, Bilia AR and Skaltsa H: *Quercus ilex* L: A rich source of polyacylated flavonoid glucosides. *Food Chem* 123: 131-142, 2010.
- 14 Wang M, Karioti A, Bergonzi MC, Gao X, Liu Z, Vincieri FF and Bilia AR: Rapid Isolation of Salvianolic acid B from polar extracts of *Salvia miltiorrhiza* (Danshen). International Symposium, Traditional Chinese Medicine, Modernization of Acupuncture and Herbal Medicine Research, May 14-16, Graz, Austria, 2009.
- 15 Efferth T, Sauerbrey A, Olbrich A, Gebhart E, Rauch P, Weber HO, Hengstler JG, Halatsch ME, Volm M, Tew KD, Ross DD and Funk JO: Molecular modes of action of artesunate in tumor cell lines. *Mol Pharmacol* 64: 382-394, 2003.
- 16 Gillet JP, Efferth T, Steinbach D, Hamels J, de Longueville F, Bertholet V and Remacle J: Microarray-based detection of multidrug resistance in human tumor cells by expression profiling of ATP-binding cassette transporter genes. *Cancer Res* 64: 8987-8993, 2004.
- 17 Efferth T, Konkimalla VB, Wang YF, Sauerbrey A, Meinhardt S, Zintl F, Mattern J and Volm M: Prediction of broad spectrum resistance of tumors towards anticancer drugs. *Clin Cancer Res* 14: 2405-2412, 2008.
- 18 Konkimalla VB and Efferth T: Inhibition of epidermal growth factor receptor over-expressing cancer cells by the aporphine-type isoquinoline alkaloid, dicentrine. *Biochem Pharmacol* 79: 1092-1099, 2010.
- 19 Kimmig A, Gekeler V, Neumann M, Frese G, Handgretinger R, Kardos G, Diddens H and Niethammer D: Susceptibility of multidrug-resistant human leukemia cell lines to human interleukin 2-activated killer cells. *Cancer Res* 50: 6793-6799, 1990.
- 20 Eberwine J, Yeh H, Miyashiro K, Cao Y, Nair S, Finnell R, Zettel M and Coleman P: Analysis of gene expression in single live neurons. *Proc Natl Acad Sci USA* 89: 3010-3014, 1992.
- 21 Beutler JA, Hamel E, Vlietinck AJ, Haemers A, Rajan P, Roitman JN, Cardellina JH 2nd and Boyd MR: Structure-activity requirements for flavone cytotoxicity and binding to tubulin. *J Med Chem* 41: 2333-2338, 1998.
- 22 Li S, Pan M, Lo C, Tan D, Wang Y, Shahidi F and Ho C: Chemistry and health effects of polymethoxyflavones and hydroxylated polymethoxyflavones. *J Functional Food* 1: 2-12, 2009.
- 23 Williams RJ, Spencer JPE and Rice-Evans C: Flavonoids: antioxidants and signaling molecules? *Free Radic Biol Med* 36: 838-849, 2004.
- 24 Koh DJ, Ahn HS, Chung HS, Lee H, Kim Y, Lee JY, Kim DG, Hong M, Shin M and Bae H: Inhibitory effects of casticin on migration of eosinophil and expression of chemokines and adhesion molecules in A549 lung epithelial cells via NF- $\kappa$ B inactivation. *J Ethnopharmacol* 136: 399-405, 2011.
- 25 Nam NH: Naturally occurring NF- $\kappa$ B inhibitors. *Mini Rev Med Chem* 6: 945-951, 2006.
- 26 Guo W, Kong E and Meydani M: Dietary polyphenols, inflammation, and cancer. *Nutr Cancer* 61: 807-810, 2009.
- 27 Gilmore TD: Introduction to NF- $\kappa$ B: players, pathways, perspectives. *Oncogene* 25: 6680-6684, 2006.
- 28 Brasier AR: The NF- $\kappa$ B regulatory network. *Cardiovasc Toxicol* 6: 111-130, 2006.



- 29 Perkins ND: Integrating cell-signalling pathways with NF- $\kappa$ B and IKK function. *Nat Rev Mol Cell Biol* 8: 49-62, 2007.
- 30 Lin S, Zhang H, Han T, Wu JZ, Rahman K and Qin LP: *In vivo* effect of casticin on acute inflammation. *Zhong Xi Yi Jie He Xue Bao* 5: 573-576, 2007. [Article in Chinese]
- 31 Kobayakawa J, Sato-Nishimori F, Moriyasu M and Matsukawa Y: G<sub>2</sub>/M arrest and antimetabolic activity mediated by casticin a flavonoid isolated from *Vitex rotundifolia* Linne fil.). *Cancer Lett* 208: 59-64, 2004.
- 32 Wang HY, Cai B, Cui CB, Zhang DY and Yang BF: Vitexicarpin, a flavonoid from *Vitex trifolia* L., induces apoptosis in K562 cells *via* mitochondria-controlled apoptotic pathway. *Yao Xue Xue Bao* 40: 27-31, 2005. [Article in Chinese]
- 33 Haidara K, Zamir L, Shi QW and Batist G: The flavonoid casticin has multiple mechanisms of tumor cytotoxicity action. *Cancer Lett* 242: 180-190, 2006.
- 34 Vakifahmetoglu H, Olsson M and Zhivotovsky B: Death through a tragedy: mitotic catastrophe. *Cell Death Differ* 15: 1153-1162, 2008.
- 35 Pearson G and Robinson F: Mitogen-activated protein (MAP) kinase pathways: Regulation and physiological functions. *Endocr Rev* 22: 153-183, 2001.
- 36 Vega MI, Huerta-Yepaz S, Garban H, Jazirehi A, Emmanouilides C and Bonavida B: Rituximab inhibits p38 MAPK activity in 2F7 B NHL and decreases IL-10 transcription: pivotal role of p38 MAPK in drug resistance. *Oncogene* 23: 3530-3540, 2004.
- 37 Choi JS, Choi YJ, Shin SY, Li J, Kang SW, Bae JY, Kim DS, Ji GE, Kang JS and Kang YH: Dietary flavonoids differentially reduce oxidized LDL-induced apoptosis in human endothelial cells: role of MAPK and JAK/STAT signaling. *J Nutr* 13: 983-990, 2008.
- 38 Li Y, Ying C, Zuo X, Yi H, Yi W, Meng Y, Ikeda K, Ye X, Yamori Y and Sun X: Green tea polyphenols down-regulate caveolin-1 expression *via* ERK1/2 and p38MAPK in endothelial cells. *J Nutr Biochem* 20: 1021-1027, 2009.
- 39 Adhikary A, Mohanty S, Lahiry L, Hossain DM, Chakraborty S and Das T: Theaflavins retard human breast cancer cell migration by inhibiting NF- $\kappa$ B *via* p53-ROS cross-talk. *FEBS Lett* 584: 7-14, 2010.
- 40 Ho HH, Chang CS, Ho WC, Liao SY, Wu CH and Wang CJ: Anti-metastasis effects of gallic acid on gastric cancer cells involves inhibition of NF- $\kappa$ B activity and down-regulation of PI3K/AKT/small GTPase signals. *Food Chem Toxicol* 48: 2508-2516, 2010.
- 41 Itatsu K, Sasaki M, Harada K, Yamaguchi J, Ikeda H, Sato Y, Ohta T, Sato H, Nagino M, Nimura Y and Nakanuma Y: Phosphorylation of extracellular signal-regulated kinase 1/2, p38 mitogen-activated protein kinase and nuclear translocation of nuclear factor- $\kappa$ B are involved in up-regulation of matrix metalloproteinase-9 by tumour necrosis factor- $\alpha$ . *Liver Int* 29: 291-298, 2009.
- 42 Park EK, Kim MS, Lee SH, Kim KH, Park JY, Kim TH, Lee IS, Woo JT, Jung JC, Shin HI, Choi JY and Kim SY: Furosin, an ellagitannin, suppresses RANKL-induced osteoclast differentiation and function through inhibition of MAP kinase activation and actin ring formation. *Biochem Biophys Res Commun* 325: 1472-1480, 2004.
- 43 Rosenfeld JA, Wang Z, Schones DE, Zhao K, DeSalle R and Zhang MQ: Determination of enriched histone modifications in non-genic portions of the human genome. *BMC Genomics* 10: 143, 2009.
- 44 Rahman I, Biswas SK and Kirkham PA: Regulation of inflammation and redox signaling by dietary polyphenols. *Biochem Pharmacol* 72: 1439-1452, 2006.
- 45 Gilbert ER and Liu D: Flavonoids influence epigenetic-modifying enzyme activity: structure - function relationships and the therapeutic potential for cancer. *Curr Med Chem* 17: 1756-1768, 2010.
- 46 Rahman I and Chung S: Dietary polyphenols, deacetylases and chromatin remodeling in inflammation. *World Rev Nutr Diet* 101: 84-94, 2010.
- 47 Dickerson LM, Shrader SP and Diaz VA: Chapter 8: Contraception. *In: Pharmacotherapy: a pathophysiologic approach*. Wells BG, DiPiro, JT, Talbert RL, Yee GC, and Matzke GR (eds.): McGraw-Hill Medical, New York, NY, USA pp. 1313-1328, 2008.
- 48 Radu A, Pichon C, Camparo P, Antoine M, Allory Y, Couvelard A, Fromont G, Hai MT and Ghinea N: Expression of follicle-stimulating hormone receptor in tumor blood vessels. *N Engl J Med* 363: 1621-1630, 2010.
- 49 Efferth T and Koch E: Complex interactions between phytochemicals. The multitarget therapeutic concept of phytotherapy. *Curr Drug Targets* 12: 122-132, 2011.

Received February 13, 2012

Revised March 9, 2012

Accepted March 12, 2012