

IN VITRO ANTIPROLIFERATIVE ACTIVITY OF FLAVONOLS: FISETIN, QUERCETIN AND KAEMPFEROL AND THEIR CYCLODEXTRIN COMPLEXES

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Abstract

The aim of this study was to evaluate the *in vitro* antiproliferative capacity of the flavonols: fisetin, quercetin and kaempferol alone and incorporated in β -cyclodextrins, on A2780 (human ovarian carcinoma cells), MDA-MB-231 (human breast cancer cells) and SiHa (human cervix cancer cells). On the selected cell lines, among the tested compounds, fisetin was the most active in terms of antiproliferative activity. The most sensitive cell line to the tested flavonols was found to be the human ovarian carcinoma A2780 cell line. The inclusion complexes between the selected flavonols with ramified β -cyclodextrins determined different effects, depending on the cell line and on the tested compound.

Rezumat

Scopul acestui studiu este evaluarea efectului antiproliferativ *in vitro* pentru flavonolii: fisetină, quercetină și kaempferol pe trei linii canceroase și anume: A2780 (cancer ovarian), MDA-MB-231 (cancer mamar) și SiHa (cancer col uterin). Efectul a fost evaluat pentru substanțele pure, respectiv încorporate în beta-ciclodextrine. Rezultatele arată că, dintre cele trei structuri pure testate, fisetina prezintă activitatea antiproliferativă cea mai ridicată pe liniile celulare analizate. Cea mai sensibilă linie celulară pentru compușii testați a fost linia A2780 (cancer ovarian). Incorporarea în beta ciclodextrine ramificate a determinat efecte diferite în funcție de linia celulară testată, respectiv compusul testat.

Keywords: fisetin, quercetin, kaempferol, β -cyclodextrins, antiproliferative, A2780 cell line, MDA-MB-231 cell line, SiHa cell line

Introduction

The most recent publications in the field demonstrate that the research of the XXIst century exhibits an increased attention pointed towards complementary and alternative medicines (CAM) involved in the most debated and problematic disease of these times, cancer. Total extracts or pure active compounds from different parts of the plant including roots, bulbs, barks, stems, leaves, buds, flowers, seeds and others have shown

promising anti-cancer activity both *in vitro* and *in vivo* on different cancer models [3, 11]. Limitations of this approach are given by technical barriers for screening natural compounds in high-throughput assays against molecular targets. However, the recent research in the field presents strategies that have reduced these barriers [22]. Pointing towards plant-derived anticancer drugs used in clinical practice, some of the most popular are the vinca alkaloids, vinblastine and vincristine isolated from

Madagascar periwinkle, *Catharanthus roseus* (L.) G. Don, *Apocynaceae* family, together with the semisynthetic derivatives of the natural product epipodophyllotoxin, etoposide and teniposide [13, 24, 29]. Flavonols are a heterogen group of phenolic secondary metabolites belonging to the group of flavonoids [27]. In this group, fisetin, quercetin and kaempferol are among the most important representatives because of their biological properties [33]. Fisetin was reported as a potential agent used to prevent Alzheimer's disease in animal models [14]. Additionally, it was found that it promotes extracellular signal-regulated kinases (ERK)-dependent long-term potentiation and enhances memory [32]. It was proposed as a medicinal agent for prostate cancer management due to the dual inhibitory activity on PI3K/Akt/mTOR intracellular signalling pathway [2]. It was described to induce apoptosis in colon cancer cells and hepatocellular carcinoma [9, 44]. Fisetin was also pointed as an anti-inflammatory agent in human mast cells (HMC-1) [39]. Quercetin was described as an antioxidant and protective agent against coronary disease [38]. Due to its antioxidant properties, quercetin was reported to exhibit protective properties on lipid peroxidation in phospholipid bilayers [45]. Chan *et al.* showed that this flavonol induces vasorelaxant activity in isolated rat aorta [8]. Kaempferol was described as an anti-inflammatory agent [21]. The flavonol was also depicted as an antioxidant and hypoglycaemic compound [16]. Recent epidemiological studies have found a direct correlation between the consumption of foods containing kaempferol and a diminished risk of developing several diseases such as cancer and cardiovascular disorders [7]. The research was also focused on the *in vitro* beneficial effects of this flavonol against osteoclastic bone resorption [48].

Although reported with a wide range of biological activities, the bioavailability of these three flavonols is limited by their low water solubility [27]. Cyclodextrins (CD's), a family of cyclic oligosaccharides with amphiphilic character due to the hydrophilic outer surface and lipophilic central cavity, have the ability to form host-guest inclusion complexes with a large number of active molecules. Due to these properties, CD's have been often used in the pharmaceutical industry in order to increase the solubility, bioavailability, stability and safety of biological active molecules [15, 40, 42, 43].

The aim of this study was to evaluate the *in vitro* antiproliferative activity of these three flavonols, on A2780 (human ovarian carcinoma cells), MDA-MB-231 (human breast cancer cells) and SiHa (human cervix cancer cells), as pure phyto-compounds and in complexes with β -CD's.

Materials and Methods

Reagents

Fisetin, kaempferol, and quercetin were acquired from Sigma Aldrich (purity \geq 98%). Beta-cyclodextrin (BCD), hydroxylpropyl-beta-cyclodextrin (HPBCD) and randomly-methylated-beta-cyclodextrin (RAMEB) were obtained from Cyclolab Hungary. All used substances were of analytical grade purity.

Flavonol - CD complexes preparation

Different methods were applied for the preparation of the inclusion complexes:

- simple powder mixing, using a mortar and a pestle;
- kneading with a 50% (w/w) water:ethanol solution until the bulk of solvent evaporated and a paste-type product was formed; the mixture was then dried at room temperature for 24 hours and put in the oven, at 105°C, for several hours until reaching constant weight. The final product was pulverized and sieved.

All the binary products were prepared using the active substance and CD's in a 1:2 molar ratio (Fisetin Mw = 286.23 g/mol, Kaempferol Mw = 286.23 g/mol, Quercetin Mw = 302.236 g/mol, BCD Mw = 1134.98 g/mol, HPBCD Mw = 1396 g/mol, Rameb Mw = 1303 g/mol). The molar ratio of 1:2 was chosen in order to achieve a better water solubility for the active phyto-compounds.

MTT proliferation assay

The growth-inhibitory effects of the tested compounds were determined on A2780, MDA-MB-231 and SiHa cells isolated from ovarian, breast and cervical cancer, respectively. The cell lines were purchased from ECACC (European Collection of Cell Cultures, Salisbury, UK). The cells were cultivated in minimal essential medium (Sigma-Aldrich, Budapest, Hungary) supplemented with 10% foetal bovine serum, 1% non-essential amino acids and an antibiotic-antimycotic mixture. Near-confluent cells were plated into a 96-well plate at the density of 5000 cells/well and were seeded and left overnight, and finally, the medium containing the tested substances was added at a final concentration of 30 μ g/mL. Following a 72 h incubation period in a humidified atmosphere of 5% CO₂ at 37°C, the living cells were assayed by the addition of 20 μ L of 5 mg/mL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution. MTT was converted by intact mitochondrial reductase and precipitated as blue crystals during a 4 h contact period. The medium was then removed, the formazan crystals were solubilized in 100 μ L DMSO during a 60 min period of shaking at 25°C, and the absorbance was read at 545 nm with a microplate reader. The wells containing untreated cells were used as controls. All *in vitro* experiments were carried out with five

parallel wells. Stock solutions of the tested substances (10 mM) were prepared with DMSO. The DMSO concentration (0.3%) of the medium did not have any significant effect on cell proliferation.

Statistics

The Prism software package (Graph Pad Prism 4.03 for Windows) was used for data presentation. The experiment was assayed in triplicate and the results are presented as mean ± SD. One-way ANOVA was applied to evaluate statistical significance.

Results and Discussion

In the case of A2780 human ovarian carcinoma cell line (Figure 1) fisetin was found to be the most active antiproliferative compound, leading to an inhibitory activity of 79.25%. Inclusion in ramified β-CD's did not improve the biological activity, on the contrary, it was decreased. Quercetin inhibited the growth of these cells in a percentage of 35.91%. Inclusion in ramified β-CD's led to a similar activity for BCD and HPBCD complexes. RAMEB complex exhibited an improved effect, namely a

percentage of 48.50% inhibited cells. Kaempferol was the least active compound on this cell line with a percentage of 25.35% inhibited cells. Modulations upon its solubility by inclusion in β-cyclodextrins decreased the biological activity. Also, for human breast cancer MDA-MB-231 cell line, fisetin exhibited the highest percentage of inhibition, namely 40.84% (Figure 2). Inclusion in CD's decreased its activity. For quercetin, the inhibitory activity was only 12.14%. The BCD complex had a slightly increased activity of 17.30%, while the other two complexes decreased the antiproliferative effect. Kaempferol and its complexes had a contrary proliferative effect. Also on the third analysed cell line, human cervix SiHa cell line, fisetin was the most active, leading to an inhibitory activity of 42.63% (Figure 3). Inclusion in ramified β-cyclodextrins, slightly decreased the biological activity. Quercetin produced an inhibition of 19.13%. HPBCD and RAMEB complexes produced a similar activity while the BCD complex led to an inhibitory activity of 29.44%. BCD, HPBCD and RAMEB had no effect on any of the tested cell lines.

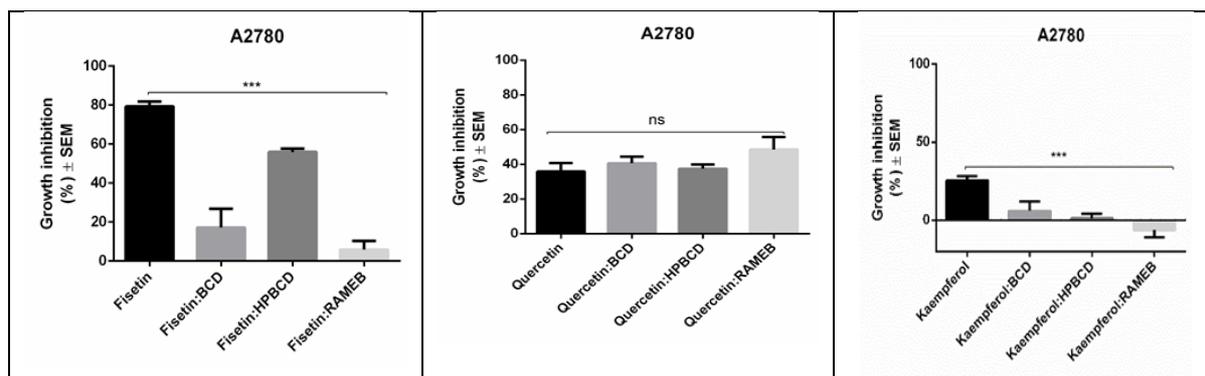


Figure 1.

Antiproliferative activity of selected compounds on human ovarian carcinoma A2780 cell line

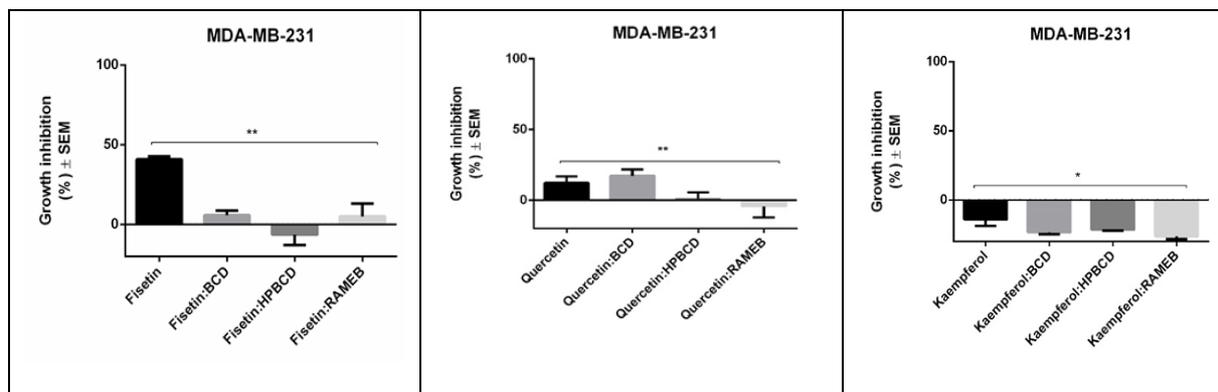


Figure 2.

Antiproliferative activity of selected compounds on human breast cancer MDA-MB-231 cell line

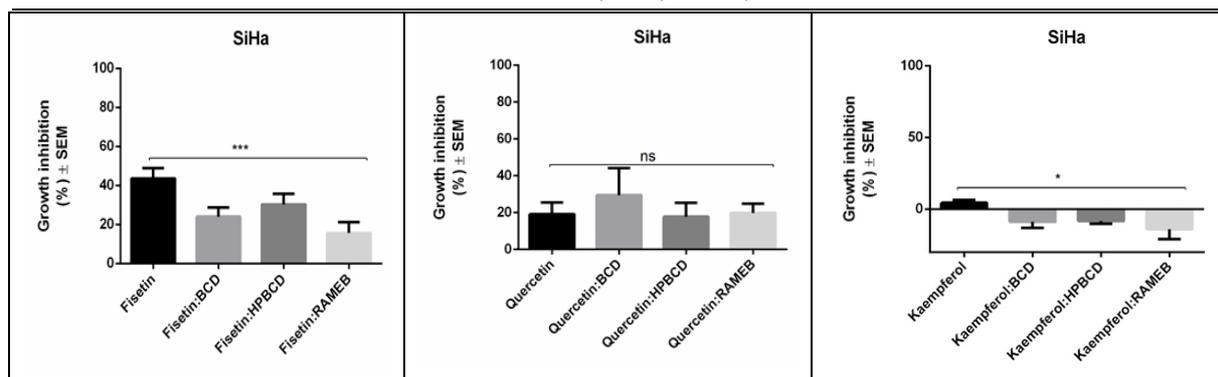


Figure 3.

Antiproliferative activity of selected compounds on human cervix SiHa cell line

Recent studies have shown that fisetin is an *in vitro* active agent against human prostate cancer cells, human colon cancer cells, human squamous carcinoma cells protected by ascorbic acid, human promyeloleukemic cells, hepatic stellate cells [10, 25, 28, 30, 54]. In the case of HeLa, human cervical cancer cell line, fisetin was described to induce apoptosis through ERK1/2-mediated activation of caspase-8/-caspase-3-dependent pathway [52]. Another recent study has shown that fisetin activates p53 protein in human breast cancer, and it is more effective as an antiproliferative agent on MCF-7 cells compared to MDA-MB-231 cells [51]. Fisetin was reported to have a synergist action with cyclophosphamide on Lewis lung carcinoma cells [46]. Regarding the chemopreventive mechanism of fisetin, previous studies have shown that it induces a forced exit from mitosis by targeting the mitotic spindle checkpoint [41]. The flavonol was also studied for direct implications in angioprevention and negative regulation of the pro-inflammatory nuclear factor- κ B [5, 20]. Kaempferol glycosides were depicted as cytotoxic agents against human leukaemic cell lines *in vitro* [17]. The flavonol was found to have an *in vitro* protective effect on human colonocyte DNA against the oxidative attack [19]. It was reported as an active *in vitro* antiangiogenic compound by inhibition of vascular endothelial growth factor (VEGF) expression through a novel ERK-NF κ B-cMyc-p21 pathway [31]. It is an antiproliferative compound for murine hepatoma cells by inducing the anticarcinogenic marker enzyme, quinone reductase [47]. Kaempferol was reported with antiproliferative and pro-apoptotic activity on A549 lung cancer cells by activating the MEK-MAPK (mitogen activated protein kinase) pathways [36]. As a tribute to the importance of the influence of dietary nutrients, Ackland *et al.*, have shown the synergistic antiproliferative action of the flavonols quercetin and kaempferol against human gut (HuTu-80 and Caco-2) and breast cancer cells (PMC42) [1]. The flavonol was also reported as a pro-apoptotic agent in breast cancer cells by

sustained ERK activation [26]. Kaempferol and quercetin were found to stimulate one of the weapons of the immune system, the granulocyte-macrophage colony-stimulating factor, in the case of human prostate cancer cells [4]. The mechanism of quercetin's multitarget prevention involves the modulation of several signal transduction pathways among which the MEK/ERK and Nrf2/keap1 pathways can be mentioned [35]. The *in vitro* anticancer mechanism also involves inhibition of angiogenesis [23]. Quercetin was found to be an active *in vitro* agent by inducing the arrest of proliferation and /or apoptosis on human gastric cancer cells, human colon cancer cells, human lung cancer cells, human breast cancer cells [14, 36, 53]. The anticancer effect in case of LNCaP prostate cancer cells was found to be directly related to the inhibition of the expression and function of the androgen receptor [49].

Inclusion in ramified beta or gamma cyclodextrins was proved to be a good choice for increasing the biological activity for other active agents. For example, Yadav *et al.* have shown that in the case of curcumin, inclusion in hydroxypropyl- γ -cyclodextrin leads to a complex with increased anti-inflammatory and antiproliferative activity compared to the pure compound. The mechanism implies higher cellular uptake [50]. The discussion goes in the same line also for pyrazolo[3,4-d]-pyrimidines, inhibitors of tyrosine kinase phosphorylation, when incorporated in 2-hydroxypropyl- β -cyclodextrin. The complexes were tested on leukemic (K-652, KU-812 and HL-60) and osteosarcoma (SaOS-2) cell lines and showed increased activity compared to the pure compound [18]. On the other hand, another study shows that the activity of hydroxyferrocifen compounds, a new class of drug candidates against breast cancer, have similar activity against MDA-MB23 breast cancer cell whether or not incorporated in beta-cyclodextrins [6]. Increased solubility but similar biological activity was reported also by Meinguet *et al.* when tri-

substituted harmine derivatives were included in 2-hydroxypropyl- β -cyclodextrin [34].

Conclusions

On the selected cell lines, among the tested compounds, fisetin was the most active in terms of antiproliferative activity. The most sensitive cell line to the tested flavonols proved to be the human ovarian carcinoma A2780 cell line. Incorporation in ramified β cyclodextrins caused different effects, depending on the cell line and on the tested compound.

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References

- Ackland M.L., Waarsenburg S.V.D., Jones R., Synergistic Antiproliferative action of the flavonols quercetin and kaempferol in cultured human cancer cell lines. *In Vivo*, 2005; 19(1): 69-76.
- Adhami V.M., Syed D.N., Khan N., Mukhtar H., Dietary flavonoid fisetin: a novel dual inhibitor of PI3K/Akt and mTOR for prostate cancer management. *Biochem. Pharmacol.*, 2012; 84(10): 1277-1281.
- Agarwal N., Majee C., Chakraborty G.S., Natural herbs as anticancer drugs. *Int. J. of PharmTech Research*, 2012; 4(3): 1142-1153.
- Bandyopadhyay S., Romero J.R., Chattopadhyay N., Kaempferol and quercetin stimulate granulocyte-macrophage colony-stimulating factor secretion in human prostate cancer cells. *Molecular and Cellular Endocrinology*, 2008; 287(1-2): 57-64.
- Bhat T.A., Nambiar D., Pal A., Agarwal R., Singh R.P., Fisetin inhibits various attributes of angiogenesis *in vitro* and *in vivo*-implications for angioprevention. *Carcinogenesis*, 2012; 33(2): 385-393.
- Buriez O., Heldt J.M., Labbé E., Vessières A., Jaouen G., Amatore C., Reactivity and antiproliferative activity of ferrocenyl-tamoxifen adducts with cyclodextrins against hormone-independent breast-cancer cell lines. *Chemistry*, 2008; 14(27): 8195-203. doi: 10.1002/chem.200800507.
- Calderon-Montano J.M., Burgos-Moron E., Perez-Guerrero C., Lopez-Lazaro M., A Review on the Dietary Flavonoid Kaempferol. *Mini Reviews in Medicinal Chemistry*, 2011; 11(4): 298-344.
- Chan E.C., Pannangpetch P., Woodman O.L., Relaxation to flavones and flavonols in rat isolated thoracic aorta: mechanism of action and structure-activity relationships. *J. Cardiovasc. Pharmacol.*, 2000; 35(2): 326-333.
- Chen Y.C., Shen S.C., Lee W.R., Lin H.Y., Ko C.H., Shih C.M., Wogonin and fisetin induction of apoptosis through activation of caspase 3 cascade and alternative expression of p21 protein in hepatocellular carcinoma cells SK-HEP-1. *Arch Toxicol.*, 2002; 76(5-6): 351-359.
- Chien C.S., Shen K.H., Huang J.S., Ko S.C., Shih Y.W., Antimetastatic potential of fisetin involves inactivation of the PI3K/Akt and JNK signaling pathways with downregulation of MMP-2/9 expressions in prostate cancer PC-3 cells. *Mol. Cell Biochem.*, 2009; 333(1-2): 169-180.
- Chinembiri T.N., du Plessis L.H., Gerber M., Hamman J.H., du Plessis J., Review of natural compounds for potential skin cancer treatment. *Molecules*, 2014; 19(8): 11679-11721.
- Choi J.A., Kim J.Y., Lee J.Y., Kang C.M., Kwon H.J., Yoo Y.D., Induction of cell cycle arrest and apoptosis in human breast cancer cells by quercetin. *Int. J. Oncol.*, 2001; 19(4): 837-844.
- Cragg G.M., Newman D.J., Natural products: a continuing source of novel drug leads. *Biochim. Biophys. Acta*, 2013; 1830(6): 3670-3695.
- Currais A., Prior M., Dargusch R., Armando A., Ehren J., Schubert D., Modulation of p25 and inflammatory pathways by fisetin maintains cognitive function in Alzheimer's disease transgenic mice. *Aging Cell*, 2014; 13(2): 379-390.
- Danciu C., Soica C., Oltean M., Avram S., Borcan F., Csanyi E., Genistein in 1:1 inclusion complexes with ramified cyclodextrins: theoretical, physico-chemical and biological evaluation. *Int. J. Mol. Sci.*, 2014; 15(2): 1962-1982.
- de Sousa E., Zanatta L., Seifriz I., Creczynski-Pasa T.B., Pizzolatti M.G., Szpoganicz B., Hypoglycemic effect and antioxidant potential of kaempferol-3,7-o-(α)-dirhamnoside from *Bauhinia forficata* leaves. *J. Nat. Prod.*, 2004; 67(5): 829-832.
- Dimas K., Demetzos C., Mitaku S., Marselos M., Tzavaras T., Kokkinopoulos D., Cytotoxic activity of kaempferol glycosides against human leukaemic cell lines *in vitro*. *Pharmacological Research*, 2000; 41(1): 83-86.
- Dreassi E., Zizzari A.T., Mori M., Filippi I., Belfiore A., Naldini A., 2-Hydroxypropyl- β -cyclodextrin strongly improves water solubility and anti-proliferative activity of pyrazolo[3,4-d]pyrimidines Src-Abl dual inhibitors. *European Journal of Medicinal Chemistry*, 2010; 45(12): 5958-5964.
- Duthie S.J., Dobson V.L., Dietary flavonoids protect human colonocyte DNA from oxidative attack *in vitro*. *Eur. J. Nutr.*, 1999; 38(1): 28-34.
- Goh F.Y., Upton N., Guan S., Cheng C., Shanmugam M.K., Sethi G., Fisetin, a bioactive flavonol, attenuates allergic airway inflammation through negative regulation of NF- κ B. *European Journal of Pharmacology*, 2012; 679(1-3): 109-116.
- Hämäläinen M., Nieminen R., Vuorela P., Heinonen M., Moilanen E., Anti-Inflammatory Effects of Flavonoids: Genistein, Kaempferol, Quercetin, and Daidzein Inhibit STAT-1 and NF- κ B Activations, Whereas Flavone, Isorhamnetin, Naringenin, and Pelargonidin Inhibit only NF- κ B Activation along with Their Inhibitory Effect on iNOS Expression and NO Production in Activated Macrophages. *Mediators of Inflammation*, 2007; 2007: e45673.

22. Ranković B., Kosanić M., Stanojković T., *Stereocaulon paschale* lichen as antioxidant, antimicrobial and anticancer agent. *Farmacia*, 2014; 62(2): 306-317.
23. Igura K., Ohta T., Kuroda Y., Kaji K., Resveratrol and quercetin inhibit angiogenesis *in vitro*. *Cancer Letters*, 2001; 171(1): 11-16.
24. Sevastre B., Sárpataki O., Olah N.K., Stan R.L., Taulescu M., Marcus I., Cătoi C., Anti-tumor effect of *Euonymus europaeus* on ehrlich tumor cells *in vivo*. *Farmacia*, 2014; 62(5): 907-917.
25. Kandaswami C., Perkins E., Soloniuk D.S., Drzewiecki G., Middleton E.Jr., Ascorbic acid-enhanced antiproliferative effect of flavonoids on squamous cell carcinoma *in vitro*. *Anticancer Drugs*, 1993; 4(1): 91-96.
26. Kim B.W., Lee E.R., Min H.M., Jeong H.S., Ahn J.Y., Kim J.H., Sustained ERK activation is involved in the kaempferol-induced apoptosis of breast cancer cells and is more evident under 3-D culture condition. *Cancer Biology & Therapy*, 2008; 7(7): 1080-1089.
27. Kumar S., Pandey A.K., Chemistry and biological activities of flavonoids: an overview. *ScientificWorldJournal*, 2013; 2013: 162750. doi: 10.1155/2013/162750.
28. Lee W.R., Shen S.C., Lin H.Y., Hou W.C., Yang L.L., Chen Y.C., Wogonin and fisetin induce apoptosis in human promyeloleukemic cells, accompanied by a decrease of reactive oxygen species, and activation of caspase 3 and Ca²⁺-dependent endonuclease. *Biochemical Pharmacology*, 2002; 63(2): 225-236.
29. Li S., Yuan W., Yang P., Antoun M.D., Balick M.J., Cragg G.M., Pharmaceutical crops: an overview. *Pharmaceutical Crops*, 2010; 1: 1-17.
30. Lu X., in Jung J., Cho H.J., Lim D.Y., Lee H.S., Chun H.S., Fisetin Inhibits the Activities of Cyclin-Dependent Kinases Leading to Cell Cycle Arrest in HT-29 Human Colon Cancer Cells. *J. Nutr.*, 2005; 135(12): 2884-2890.
31. Luo H., Rankin G.O., Juliano N., Jiang B.H., Chen Y.C., Kaempferol inhibits VEGF expression and *in vitro* angiogenesis through a novel ERK-NFκB-cMyc-p21 pathway. *Food Chemistry*, 2012; 130(2): 321-328.
32. Maher P., Akaishi T., Abe K., Flavonoid fisetin promotes ERK-dependent long-term potentiation and enhances memory. *PNAS*, 2006; 103(44): 16568-16573.
33. Manach C., Scalbert A., Morand C., Rémésy C., Jiménez L., Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.*, 2004; 79(5): 727-747.
34. Stănculescu E.C., Pisoschi C.G., Rău G., Andrei A.M., Baniță M., Proteasome inhibition: recent advances in antitumoral therapy. *Farmacia*, 2014; 62(2): 245-253.
35. Murakami A., Ashida H., Terao J., Multitargeted cancer prevention by quercetin. *Cancer Letters*, 2008; 269(2): 315-325.
36. Nguyen T.T., Tran E., Ong C.K., Lee S.K., Do P.T., Huynh T.T., Nguyen T.H., Lee J.J., Tan Y., Ong C.S., Huynh H., Kaempferol-induced growth inhibition and apoptosis in A549 lung cancer cells is mediated by activation of MEK-MAPK. *J Cell Physiol.*, 2003; 197(1): 110-121.
37. Nguyen T.T.T., Tran E., Nguyen T.H., Do P.T., Huynh T.H., Huynh H., The role of activated MEK-ERK pathway in quercetin-induced growth inhibition and apoptosis in A549 lung cancer cells. *Carcinogenesis*, 2004; 25(5): 647-659.
38. Pace-Asciak C.R., Hahn S., Diamandis E.P., Soleas G., Goldberg D.M., The red wine phenolics trans-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: Implications for protection against coronary heart disease. *Clinica Chimica Acta*, 1995; 235(2): 207-219.
39. Park H.H., Lee S., Oh J.M., Lee M.S., Yoon K.H., Park B.H., Anti-inflammatory activity of fisetin in human mast cells (HMC-1). *Pharmacological Research*, 2007; 55(1): 31-37.
40. Rasheed A., Cyclodextrins as Drug Carrier Molecule: A Review. *Scientia Pharmaceutica*, 2008; 76(4): 567-598.
41. Salmela A.L., Pouwels J., Varis A., Kukkonen A.M., Toivonen P., Halonen P.K., Dietary flavonoid fisetin induces a forced exit from mitosis by targeting the mitotic spindle checkpoint. *Carcinogenesis*, 2009; 30(6): 1032-1040.
42. Soica C.M., Peev C., Ciurlea S., Ambrus R., Dehelean C., Physico-chemical and toxicological evaluations of betulin and betulinic acid interactions with hydrophilic cyclodextrins. *Farmacia*, 2010; 58(5): 611-619.
43. Soica C., Danciu C., Savoiu-Balint G., Borcan F., Ambrus R., Zupko I., Betulinic acid in complex with a gamma-cyclodextrin derivative decreases proliferation and *in vivo* tumor development of non-metastatic and metastatic B164A5 cells. *Int. J. Mol. Sci.*, 2014; 15(5): 8235-8255.
44. Suh Y., Afaq F., Johnson J.J., Mukhtar H., A plant flavonoid fisetin induces apoptosis in colon cancer cells by inhibition of COX₂ and Wnt/EGFR/NF-κB-signaling pathways. *Carcinogenesis*, 2009; 30(2): 300-307.
45. Terao J., Piskula M., Yao Q., Protective Effect of Epicatechin, Epicatechin Gallate, and Quercetin on Lipid Peroxidation in Phospholipid Bilayers. *Archives of Biochemistry and Biophysics*, 1994; 308(1): 278-284.
46. Touil Y.S., Seguin J., Scherman D., Chabot G.G., Improved antiangiogenic and antitumour activity of the combination of the natural flavonoid fisetin and cyclophosphamide in Lewis lung carcinoma-bearing mice. *Cancer Chemother Pharmacol.*, 2010; 68(2): 445-455.
47. Uda Y., Price K.R., Williamson G., Rhodes M.J.C., Induction of the anticarcinogenic marker enzyme, quinone reductase, in murine hepatoma cells *in vitro* by flavonoids. *Cancer Letters*, 1997; 120(2): 213-216.
48. Wattel A., Kamel S., Mentaverri R., Lorget F., Prouillet C., Petit J.P., Potent inhibitory effect of naturally occurring flavonoids quercetin and kaempferol on *in vitro* osteoclastic bone resorption. *Biochemical Pharmacology*, 2003; 65(1): 35-42.

49. Xing N., Chen Y., Mitchell S.H., Young C.Y.F., Quercetin inhibits the expression and function of the androgen receptor in LNCaP prostate cancer cells. *Carcinogenesis*, 2001; 22(3): 409-414.
50. Yadav V.R., Prasad S., Kannappan R., Ravindran J., Chaturvedi M.M., Vaahtera L., Cyclodextrin-Complexed Curcumin Exhibits Anti-inflammatory and Antiproliferative Activities Superior to Those of Curcumin Through Higher Cellular Uptake. *Biochem. Pharmacol.*, 2010; 80(7): 1021-1032.
51. Yang P.M., Tseng H.H., Peng C.W., Chen W.S., Chiu S.J., Dietary flavonoid fisetin targets caspase-3-deficient human breast cancer MCF-7 cells by induction of caspase-7-associated apoptosis and inhibition of autophagy. *International Journal of Oncology*, 2012; 40(2): 469-478.
52. Ying T.H., Yang S.F., Tsai S.J., Hsieh S.C., Huang Y.C., Bau D.T., Fisetin induces apoptosis in human cervical cancer HeLa cells through ERK1/2-mediated activation of caspase-8-/caspase-3-dependent pathway. *Arch. Toxicol.*, 2011; 86(2): 263-273.
53. Yoshida M., Sakai T., Hosokawa N., Marui N., Matsumoto K., Fujioka A., The effect of quercetin on cell cycle progression and growth of human gastric cancer cells. *FEBS Letters*, 1990; 260(1): 10-13.
54. Zhang M., Zhang J.P., Ji H.T., Wang J.S., Qian D.H., Effect of six flavonoids on proliferation of hepatic stellate cells *in vitro*. *Acta Pharmacol. Sin.*, 2000; 21(3): 253-256.