ANTICANCER EFFECTS OF COUMARIN COMPOUNDS OSTHOLE AND IMPERATORIN, ALONE AND IN COMBINATION WITH 5-FLUOROURACIL IN COLON CARCINOMA CELLS

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Abstract: Colon cancer is one of the causes of cancer-related mortality. Therefore, more efficient therapy strategies are required. There is an increasing interest in natural products due to the potential cytotoxic activity in various cancer cell lines. Osthole and imperatorin are major active coumarins found in a variety of plants. The present study aimed to assess the cytotoxic effects of two natural coumarins, osthole, and imperatorin, administered separately and combined with 5-fluorouracil (5-FU) in human colon carcinoma cells and to identify the action of a mechanism. Real-time cell analysis with the xCELLigence System was used to screen for appropriate concentrations of osthole, imperatorin, 5-FU, and API-1 with cytotoxic or cytostatic potential and to monitor cell proliferation and viability in CoLo 205 cells continuously. Furthermore, the effects of the compounds on p38MAPK-α levels and the expression of Akt mRNA were evaluated. As a result, osthole showed considerable antiproliferative activity in CoLo 205 cells and increased the efficacy of 5-FU by accelerating the cytotoxic effect. Osthole was found to be as effective as protein kinase inhibitor API-1. Osthole, osthole+5-FU, and imperatorin treatments were significantly able to increase the ratio of phospho-p38MAPK-α/p38MAPK-α. It was demonstrated that osthole, 5-FU, and API-I+5-FU were significantly suppressed the Akt mRNA expression as positive control API-1. The findings indicate that osthole may be a promising anti-cancer agent in the treatment of colon cancer and may increase the efficacy of 5-FU and, therefore, may reduce common side effects of traditional chemotherapy in colon cancer with 5-FU.

Keywords: osthole, imperatorin, 5-fluorouracil, colo 205, cytotoxicity, xCELLigence

Abbreviations: 5-FU: 5-flourourasil, API-1: 4-Amino-5,8-dihydro-5-oxo-b-D-ribofuranosyl-pyrido[2,3-d]pyrimidine-6-carboxamide, BIRB-796: N-[3-(1,1-Dimethylethyl)-1-(4-methyl phenyl)-1H-pyrazol-5-yl]-N’-[4-(2-[4 morpholinyl]ethoxy)-1-naphthalenyl]urea, DMSO: dimethyl sulfoxide, EMT: epithelial-to-mesenchymal transition, FBS: fetal bovine serum, GSK3β: Glycogen synthase kinase 3 beta, HGF: hepatocyte growth factor, IC₅₀: half maximal inhibitory concentration, IGF-1: insulin-like growth factor-1, MAPK: mitogen-activated protein kinase, mTOR: mammalian target of rapamycin, MI:MT: MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, PI3K: phosphatidylinositol 3 kinase, Akt: protein kinase-B, RTCA: The Real Time Cell Analyser, v-Akt: viral oncogene homolog kinase.
likely to have combinatorial efficacy on carcinogenesis and tumor progression. Osthole has been suggested to modulate the pathway of phosphatidylinositol 3 kinase (PI3K)/protein kinase-B (Akt), leading to G2/M arrest and apoptosis in lung cancer cells (11). The anti-cancer mechanisms of action, including the PI3K/Akt/mitogen-activated protein kinase (MAPK) pathway of osthole on several cancer types, have been well-reviewed Shokohinia et al. (12).

Imperatorin (8-isopentenyloxypsoralen), an active furanocoumarin isolated from the root of Angelica dahurica (13), has been reported to have many pharmacological activities such as anti-tumoral, anticonvulsant, vasodilator, antihypertensive, analgesic, anti-inflammatory, and anticoagulant (14, 15). In some studies, imperatorin’s various biological effects, such as antiproliferative and induction of apoptosis, have been demonstrated in cancer cell types (14, 16-18). On the other hand, the underlying anti-carcinogenic mechanisms are not well understood (19).

One of the important pathways shown to be affected by coumarins in different cancer types is the PI3K/Akt/MAPK pathway. Mitogen-activated protein kinases (MAPKs) phosphorylate specific serines and threonines of target protein substrates and regulate cellular functions (20). Some evidence suggests that functional inactivation of p38 MAPK seems to be a defining feature of many various cancers (21). It was shown that p38 MAPK activation is essential for cancer cell death initiated by anticancer agents (22). Therefore, p38 MAPK signaling appears to be an attractive target in cancer therapy, and MAPKs inhibitors are one of the main groups of drugs developed for the therapy of diseases (23). Activation of Akt, a serine-threonine protein kinase, plays a central role in fundamental cellular functions such as cell proliferation differentiation, apoptosis, and tumorigenesis. Akt’s overexpression has been observed in chemoresistance, which affects the efficacy of cancer therapies (24). When Akt is deregulated, it may contribute to cancer development or progression. Therefore, there is a potential utility in targeting the Akt pathway components for cancer treatment and possibly cancer prevention (25). The combination of an inhibitor with varied cytotoxic agents increases the antitumor action. Thus, specific inhibition of the Akt activation may be an effective approach in treating human malignancies and overcoming cancer cells’ chemoresistance (26).

5-Flourouracil (5-FU), the nucleotide analog, is the priority for chemotherapy of colorectal cancers (27). It is the first designed antimetabolite and provides its therapeutic efficacy by inhibiting the enzyme thymidylate synthase required to synthesize and repair DNA (28). Nevertheless, tumor resistance is a great limiting factor of its use as an anti-cancer drug (4). Therefore, chemotherapeutic drugs are essential for therapy and other effective compounds to increase their tumor-killing potential (29).

There is insufficient data to suggest that natural coumarin derivatives have a possible anti-cancer effect in human colon carcinoma cells (30). Therefore, we aimed to evaluate the cytotoxic effects of osthole or imperatorin alone and the combination of each compound with 5-FU on CoLo 205 cells and to identify the mechanism of action.

MATERIALS AND METHODS

Reagents and apparatus

4-Amino-5,8-dihydro-5-oxo-8-b-D-ribofuranosyl-pyrido[2,3-d] pyrimidine-6-carboxamide (API-1, Cat. No. 3897), dimethyl sulfoxide (DMSO, Cat. No. A3672,0100), and RPMI 1640 medium (Cat. No. 11875093) were purchased from Tocris Bioscience (Bristol UK), Applichem (Darmstadt Germany), Gibco™ (Carlsbad USA), respectively. 5-FU (Cat. No. F6627), fetal bovine serum (FBS, Cat. No. F2442), L-glutamine (Cat. No. G7513), imperatorin (Cat. No. I6659), osthole (Cat. No. 78889), penicillin-streptomycin (Cat. No. P4333), RNAzol (Cat. No. R4533), and trypsin-EDTA (Cat. No. T3924) were supplied from Sigma-Aldrich (St. Louis USA). The p38 MAPK-α (Cat. No. Ab126425, Cambridge UK) commercial ELISA kit was obtained from Abcam. FastStart Essential DNA Probes Master (Cat. No. 06 402 682 001), Transcriptor High Fidelity cDNA Synthesis Kit (Cat. No. 05 081 955 001), RealTime ready human β-actin and Akt catalog as- says (Cat. No. 05 532 957 001) were supplied from Roche (Mannheim Germany).

The Real-Time Cell Analyzer (RTCA, xCEL-Ligence) System was performed to evaluate the cytotoxicity as defined by the supplier’s instructions (Roche Applied Science and ACEA Biosciences, San Diego USA).

Cell culture and treatments

Human colorectal cancer cell line CoLo 205 (CCL-222) was obtained from American Type Culture Collection (ATCC). CoLo 205 cells (25 000 cells/well) were maintained in RPMI 1640 medium supplemented with 10% FBS, 1% L-glutamine, 100 U/mL of penicillin, and 100 µg/mL of streptomycin at 37°C in an incubator containing 5% CO₂. After reaching approximately 80% confluence, the cells were detached by trypsin-EDTA.
The cells were centrifuged at 1000 rpm for 5 min at 25°C and then seeded into the E-plate 96 for analysis. When cells came into the log growth phase about 24 h after seeding to E-plate, cells were exposed to either 0.1% DMSO alone (control) or other substances in various concentrations, e.g., 0.1-500 µM imperatorin, 0.1-500 µM osthole, 12.5-50 µM API-1, 1-64 µM 5-FU.

**Cytotoxicity assay**

At first, the optimal seeding concentration of cells was detected, and then the cells were seeded into the 96-wells E-plate. Cell proliferation, attachment, and spreading were viewed every 15 min through E-plate’s impedance by the xCELLigence RTCA system. About 24 h after seeding, the cells were treated with substances in the log growth phase. All analyses were performed approximately 72 h and replicated 4-times, and calculations were done with the RTCA-integrated software of the xCELLigence System (31).

**Quantitative real-time PCR**

CoLo 205 cells were seeded into a plate at a density of 1×10^6 cells/well and grown for 24 h and then treated with API-1 (25 µM), N-[3-(1,1-Dimethylethyl)-1-(4-methyl phenyl)-1H-pyrazol-5-yl]-N'-[4-[(2-[4 morpholinyl] ethoxy]-1-naphthalenyl] urea (BIRB-796) (20 µM), 5-FU (64 µM), osthole (400 µM), imperatorin (200 µM), API-1+5-FU, imperatorin+5-FU, and osthole+5-FU. Total RNA was extracted with RNAzol. The concentration and purity of RNA were identified by monitoring the absorbance with a NanoDrop (DeNovix, Wilmington USA) device at 260 and 280 nm. Total RNA (1000 ng) was used for cDNA synthesis according to a Transcriptor High Fidelity cDNA Synthesis Kit description. The qRT-PCR was carried out with the FastStart Essential DNA Probes Master with the primer.

**p38 MAPK-α Enzyme-linked immunosorbent assay (ELISA)**

ELISA measured the level of p38 MAPKα (Thr180/Tyr182) to assess the inhibitory activity of test compounds on MAPK activation by using a specific commercial kit according to the manufacturer’s protocols. Firstly, cells are seeded into a plate and fixed after various treatments. After blocking, anti-phospho-p38 MAPK (Thr180/Tyr182) or anti-p38 MAPK (primary antibody) were added to the wells and incubated. Following the washing steps, HRP-conjugated anti-mouse IgG (secondary antibody) and tetramethylbenzidine substrate solution were added to the wells, respectively, and color developed. The color from blue to yellow was changed by stop solution, and the color intensity was measured at 450 nm.

**Statistical analysis**

GraphPad Prism (Version 7.01) Software was used to compare differences between groups. The one-way analysis of variance (ANOVA) followed by Dunnett’s test for qPCR and the two-way analysis of variance (ANOVA) followed by Sidak’s multiple comparisons tests for p38 MAPKα (Thr180/Tyr182) were performed. Data were given as mean ± S.D. Significant differences were considered P < 0.1, P < 0.01, and P < 0.001, indicating by asterisks in the figures. At the end of the experiment, all the xCELLigence system calculations were done with the RTCA-integrated software.

**RESULTS**

**Profiling of the compounds’ effect on the cell viability using xCELLigence System**

Firstly, a general dose scan was performed using the xCELLigence System. Imperatorin was observed to be non-toxic until 250 µM (Figure 1a). Osthole was observed to be cytotoxic at 500 µM concentration (Figure 1b). It was found that API-1 was cytotoxic at studied concentrations and reached E_{max} at 12.5 µM (Figure 1c). 5-FU was observed to be proliferative at all the concentrations except for the 64 µM according to the real-time profile results. It was observed that 5-FU had a cytostatic effect at 64 µM concentration (Figure 1d), and this concentration was selected in combination with imperatorin and osthole.

A synergistic effect was observed in combination with both compounds with 5-FU (64 µM) (Figure 2a, 2b, and 2c). Osthole was found to be as effective as protein kinase inhibitor API-1 (25 µM). The cytotoxic effect profiles are like each other in a real-time cell analyzer (Figure 2c).

The IC_{50} values obtained in the studied concentrations for 24 h and 48 h incubations in CoLo 205 cell are demonstrated in Table 1.

**Inhibition of Akt mRNA expression**

RT-qPCR determined Akt mRNA expression, and our results showed that osthole (400 µM), 5-FU (64 µM), and API-1+5-FU treatments significantly suppressed the mRNA expression of Akt (Figure 3) as positive control protein kinase B inhibitor API-1 (25 µM). However, imperatorin (200 µM) and imperatorin+5-FU showed no inhibitory efficacy.
on Akt mRNA expression. Besides, osthole+5-FU treatment significantly increased the expression of Akt mRNA as compared with the control group.

**Inhibition of p38 MAPK phosphorylation**

This ELISA method was used for the qualitative measurement of p38 MAPK phosphorylation in CoLo 205 cell lines. In our study, we determined that osthole (400 µM), osthole+5-FU, and imperatorin (200 µM) treatments were significantly able to increase the ratio of phospho-p38 MAPK-α/p38 MAPK-α (Figure 4). However, BIRB-796 (20 µM) and API-1+5-FU treatments did not have a significant effect. Also, API-1 (25 µM), 5-FU (64 µM), and imperatorin+5-FU treatments were able to decrease the ratio of phospho-p38 MAPK-α/p38 MAPK-α to a significant extent.

**DISCUSSION**

The growing interest in the potential anticancer properties of antioxidants derived from natural compounds in colon cancer therapy has encouraged us to investigate the cytotoxic effect of osthole and imperatorin to modulate the chemo-sensitivity of 5-FU treated CoLo 205 colon cancer cells. Natural products, including antiproliferative and anti-cancer effects, have always been excellent sources as chemotherapeutic agents due to their main and various pharmacological activities (32). In contrast to other chemotherapeutics, coumarin derivatives are relatively low toxic for normal cells (7). Coumarin compounds can inhibit the growth, proliferation, and metastasis of various tumor cells through a variety of mechanisms, including inhibition of PI3K/AKT/mTOR signaling pathway (33).

Osthole has been shown to decrease human cervical cancer cells (HeLa) in a time- and dose-dependent manner while exhibiting low cytotoxicity in primary cultured normal cervical fibroblasts (34). Our study stated that for the first time, the cytotoxicity of osthole and imperatorin was applied separately and in combination with 5-FU in human colon carcinoma cells and clarified the mechanisms of action.

Osthole is known to have potential anticancer activities and has been exhibited to inhibit cancer cell proliferation in several human cancer cell lines in vitro (7, 12). However, the cytotoxicity mechanism is still unclear. Our study detected that osthole demonstrated significant antiproliferative activity in CoLo 205 cells and enhanced the efficacy of 5-FU by accelerating the cytotoxic effect. Similarly, Yang et al. (2003) observed that cytotoxic effect of osthole from *Cnidium monnieri* by the MTT assay on human CoLo 205, leukemia (HL-60), and HeLa cell lines for 24 h, including the IC_{50} values was 29.9 µg/mL, 14.9, and 31.7, respectively (35). A report on the cytotoxicity of
osthole indicated that it has an antiproliferative effect on human hepatocyte carcinoma cells (HepG2) after 24 h (36). In another study, Jiang et al. (2016) determined that osthole treatment considerably reduced the viability of ovarian cancer cells without toxic effect on normal ovarian cells and suppressed cells' proliferation (37). Another study also demonstrated the cytotoxic potential of osthole depending on the dose and time in prostatic cancer cells (PC3) (38) and the inhibition of the hepatocellular carcinoma cell (39) and intrahepatic cholangiocarcinoma cells proliferation (40). It was also shown that osthole dose-dependently reduced the viability and proliferation of cells in cervical cancer HeLa, SiHa, C33A, and CaSkI cell lines (41) and human medulloblastoma (TE671) and laryngeal cancer (RK33) cell lines (7). Xu et al. (2011) identified that application of human lung carcinoma cells (A549) with osthole eased the protein expression of p-Akt by way of dose-dependent, while the levels of total Akt protein remained stable during the treatment of osthole (42). Lin et al. (2014) detected that osthole decreased the phosphorylation of Akt and GSK3β (9). Their results showed that osthole prevented insulin-like growth factor-I (IGF-1)-produced the epithelial-to-mesenchymal transition (EMT) by blocking the PI3K/Akt pathway. Significantly, Hung et al. (2011) determined osthole can inhibit hepatocyte growth factor (HGF)-induced EMT via down-regulation of phosphorylated Akt and mammalian target of rapamycin (mTOR), providing a new natural therapeutic compound for breast cancer (43). Furthermore, Lin et al. (2010) demonstrated that osthole blocked the phosphorylation of Akt and mTOR in HER2-overexpressing cancer cells (44). Osthole was also found to have an anti-cancer effect by blocking the signaling pathways of PI3K / Akt and MAPK in rat glioma (45). Zhu et al. (2017) observed that p-Akt and PI3K expression levels were considerably reduced after exposed to osthole (38). Jo et al. (2020) investigated the synergistic effect of docetaxel and osthole encapsulated in micelles, using mPEG-b-PCL, and investigated the synergistic effects of the two drugs. It was found that the synergistic effect of docetaxel and osthole offers a promising treatment strategy for lung cancer. The findings from the study revealed that the combination of docetaxel and osthole for the treatment of lung cancer effectively inhibits cancer cells and exhibits a synergistic effect (46).

In the present study, we observed that imperatorin was to be non-toxic until 250 μM. Zheng et al. (2016) reported that the antiproliferative activity of imperatorin in the colon cancer cell (H.T. 29) line and its inhibitory effect on cell viability (IC50) values were 239 μM, 101 μM, and 78 μM after incubation for 24 h, 48 h, and 72 h, respectively (2). Badziul et al. (2014) and Mi et al. (2017) exhibited that imperatorin inhibited cell proliferation in HeLa and laryngeal Hep-2 cells (14) and HCT116 cells (15). On the other hand, Luo et al. (2011) detected that imperatorin significantly prevented the proliferation of HepG2 cells.
in a time- and dose-dependent manner (IC\textsubscript{50} values were 101.2, 60.5, and 22.4 μM for 24, 48, and 72 h, respectively) (47). Also, Yang et al. (2003) demonstrated that cytotoxicity of imperatorin showed the highest sensitivity to HL-60 cells and the least cytotoxicity to normal peripheral blood mononuclear cells for 24 h (35).

In our study, osthole and imperatorin show such a similar activity, but in order to explain the molecular mechanism of the difference between them, it is necessary to explain the difference in the chemical structure of coumarins.

Coumarin is characterized by a simple structure, benzopyrone, on which there are multiple substitution sites. According to the different substituents, coumarin compounds are classified into several types, i.e. including simple coumarins, furocoumarins, pyranocoumarins, dicoumarin, and isocoumarin. Imperatorin is a furocoumarin and osthole is a simple coumarin. (33, 48). The structure of furanocoumarins is based on the furan ring attached to the coumarin backbone. Depending on the location of the substituent, the linear and angelic types are distinguished (49, 50). The type, location, and the number of substituents attached determine the physicochemical and biological properties of the compound (51, 52).

Isopentenyloxy derivatives of psoralen, such as imperatorin, have been shown in the literature to induce significant inhibition of cancer cell proliferation. However, it was found less effective than psoralen and methoxy derivatives in eliminating tumor cells. Thus, it was concluded that the isoprenyl substituent reduced antiproliferative properties (50). In our study, we thought that the molecular basis of osthole to be more effective than imperatorin may be due to the fact that the substitution of a methoxy group at the 7-position of the osthole and that imperatorin is an isoprenyloxy derivative.

5-FU is one of the most effective anti-cancer agents utilized in colorectal cancer treatment, but tumor chemoresistance is an important restrictive factor of its use (1). Therefore, along with the natural compounds, we tried to vary the anti-cancer properties of 5-FU. According to the real-time profile results, we observed that 5-FU was proliferative at all concentrations except for the concentration of 64 μM, and it had a cytostatic effect at this concentration. Therefore, we selected 64 μM concentration for the combination with imperatorin and osthole. The combination of osthole at 400 μM and imperatorin at 200 μM with 5-FU synergistically enhanced the inhibitory effect of 5-FU
in the survival of CoLo 205 cells. Similarly, Flis and Splawinski (2009) have shown that the combination of 5-FU with sulindac sulfide strongly inhibits the growth of colorectal carcinoma cells (CRC) in vitro (53). Lopes-Costa et al. (2017) determined that 5-FU lowered the cell viability in a concentration-dependent manner in human colorectal cancer cells (HCT116 and HT29) and observed that co-administration of 5-FU with phloroglucinol at 300 μM (in HT29 cell) and fucosanin at 10 μM (in HCT116 and HT29 cells) enhanced the cytotoxic effect of 5-FU (4). Vinod et al. (2013) suggested that 5-FU (10 μM) and curcumin (10 μM) stimulate a synergistic cytotoxic effect in different breast cancer cells (MCF7, MDA-MB-231, SK-BR-3, T47D) through the enhancement of apoptosis (28).

The signaling pathway of MAPK is associated with various physiological effects, including apoptosis, cell proliferation, and senescence (54). The activation of p38 MAPK is essential for cancer cell death initiated by anti-cancer agents (22). Current clinical trials continue to investigate colon cancer treatment by including more effective combination chemotherapies (23). Signaling pathways of MAPK and PI3K/ murine thymoma viral oncogene homolog kinase (v-Akt) are commonly dysregulated and hyperactivated by genetic and epigenetic aberrations in various cancers, including CRC (55). In the present study, we observed that osthole (400 μM), osthole+5-FU, and imperatorin (200 μM) treatments significantly increased the ratio of phospho-p38 MAPK-α/ p38 MAPK-α. This might be the substantial mechanism of osthole suppressed the growth of the colon cancer cells. However, BIRB-796 (20 μM) and API-I+5-FU treatments did not have a significant effect. Also, API-I (25 μM), 5-FU (64 μM), and imperatorin+5-FU treatments showed the ability to reduce the ratio of phospho-p38 MAPK-α/ p38 MAPK-α to a significant extent. Mi et al. (2017) reported that imperatorin could block the MAPK signaling pathway by inhibiting the expression of phospho-p38 in the tumor tissue (15). Shin et al. (2018) also observed that p38 MAPK activation was significantly increased by hydrangenol (dihydroisocoumarin) with 200 μM treatment compared to the vehicle-treated control in bladder cancer cells (56). Han et al. (2018) determined that shikonin markedly increased the phosphorylation of MAPKs, which indicated that the anti-cancer effect of shikonin in the colon cancer cell (SNU-407) line is mediated by activation of the MAPK pathway (57). Dou et al. (2018) detected that treatment with baikalein and/or baicalin significantly increased phosphorylation of p38 by activating p38 selectively in colon cancer cells (HCT116 and SW480) (54).

The novel Akt inhibitor API-1 acts by binding to Akt and preventing its membrane translocation (58). The effects of API-1 on cell proliferation and/or cell death as a single agent or in combination with other inhibitors were described in different cancer cells (55). We investigated the anti-proliferative efficacy of the selective Akt inhibitor API-1 in CoLo 205 cells in our study, and we identified that osthole (400 μM), 5-FU (64 μM), and API-I+5-FU treatments significantly suppressed the mRNA expression of Akt as positive control protein kinase B inhibitor API-1 (25 μM). However, imperatorin (200 μM) and imperatorin+5-FU did not significantly affect Akt mRNA expression. Yar Saglam et al. (2016) also observed that the combination of FR180204 (selective ERK1/2 inhibitor) with API-1 resulted in substantial cytotoxicity in colorectal cancer cells (LoVo, DLD-1) depending on time compared to any single agent alone (55).

In terms of the clinical use of coumarin, there are many problems that need to be resolved before it can be used clinically. First, most of the work on the anti-tumor mechanisms of coumarins has not been thoroughly studied and has been stalled by a relatively simple mechanism. Second, most studies are limited to in vitro experiments. This requires more research. Third, coumarin compounds are poorly soluble in water. Improvement may be needed to increase their bioavailability. In addition, structure modification or the addition of new functional groups can provide new performance to coumarin compounds.

In conclusion, we have shown that osthole has significant antiproliferative activity in CoLo 205 cells. It is also found that Akt mRNA expression and the activity p38 MAPK-α were significantly reduced after treatment with osthole. Taken together, these findings suggest that osthole may be a potential anti-cancer agent for the treatment of human colon cancer and may increase the efficacy of 5-FU and therefore decrease the common side effects of traditional chemotherapy in colon cancer with 5-FU. Coumarin and coumarin-derived compounds are potential sources of anticancer drugs that need further research and are important in the development of new anticancer drugs.

Disclosure of interest

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REFERENCES

1. Hotnog D., Mihaile M., Iancu I.V., Matei G.G., Hotnog C., et al.: Roum. Arch. Microbiol. Immunol. 72, 255 (2013).
2. Zheng Y.M., Lu A.X., Shen J.Z., Kwok A.H., Ho W.S.: Oncol. Rep. 35, 1995 (2016).
3. Zheng Y.M., Shen J.Z., Wang Y., Lu A.X., Ho W.S.: Phytomedicine 23, 1267 (2016).
4. Lopes-Costa E., Abreu M., Gargiulo D., Rocha E., Ramos A.: J. Toxicol. Environ. Health A 80, 776 (2017).
5. Barot K.P., Jain S.V., Kremer L., Singh S., Ghate M.D.: Med. Chem. Res. 24, 2771 (2015).
6. Venugopala K.N., Rashmi V., Odhav B.: Biomed. Res. Int. 2013, 963248 (2013).
7. Jarzab A., Grabarska A., Kielbus M., Jeleniewicz W., Dmoszynska-Graniczka M., et al.: Anticancer Res. 34, 6473 (2014).
8. Chao X., Zhou J., Chen T., Liu W., Dong W., et al.: Brain Res. 1363, 206 (2010).
9. Lin Y.C., Lin J.C., Hung C.M., Chen Y., Liu L.C., et al.: J. Agric. Food Chem. 62, 5061 (2014).
10. Kao S.J., Su J.L., Chen C.K., Yu M.C., Bai K.J., et al.: Toxicol. Appl. Pharmacol. 261, 105 (2012).
11. Zhang Z.R., Leung W.N., Cheung H.Y., Chan C.W.: Evid. Based Complement. Alternat. Med. 2015, 919616, 10 pages (2015).
12. Shokoohinia Y., Jafari F., Mohammadi Z., Bazvandi L., Hosseinizadeh L., et al.: Nutrients 10, 16 pages (2018).
13. Choochuay K., Chunhacha P., Pongrakhananon V., Ruechapudiporn R., Chanvorachote P.: J. Nat. Med. 67, 599 (2013).
14. Badziul D., Jakubowicz-Gil J., Paduch R., Glowniak K., Gawron A.: Mol. Cell Biochem. 392, 213 (2014).
15. Mi C., Ma J., Wang K.S., Zuo H.X., Wang Z., et al.: J. Ethnopharmacol. 203, 27 (2017).
16. Appendino G., Bianchi F., Bader A., Campagnulo C., Fattorusso E., et al.: J. Nat. Prod. 67, 532 (2004).
17. Sigurdsson S., Ogmundsdottir H.M., Gudbjarnason S.: Z. Naturforsch. C. J. Biosci. 59, 523 (2004).
18. Kim Y.K., Kim Y.S., Ryu SY.: Phytother. Res. 21, 288 (2007).
19. Pae H.O., Oh H., Yun Y.G., Oh G.S., Jang S.I., et al.: Pharmacol. Toxicol. 9, 40 (2002).
20. Koizumi K., Tanno S., Nakano Y., Habiro A., Izawa T., et al.: Anticancer Res. 25, 3347 (2005).
21. Johnson G.L., Lapadat R.: Science 298, 1911 (2002).
22. Olson J.M., Hallahan A.R.: Trends Mol. Med. 10, 125 (2004).
23. Grossi V., Peserico A., Tezil T., Simone C.: World J. Gastroenterol. 20, 9744 (2014).
24. Zhang J., Zhang L.L., Shen L., Xu X.M., Yu H.G.: Oncol. Lett. 5, 756 (2013).
25. Altomare D.A., Testa J.R.: Oncogene 24, 7455 (2005).
26. Osaki M., Oshimura M., Ito H.: Apoptosis 9, 667 (2004).
27. Liu L.Y., Huang W.J., Lin R.J., Lin S.Y., Liang Y.C.: Chem. Res. Toxicol. 26, 1683 (2013).
28. Vinod B.S., Antony J., Nair H.H., Puliyappadamba V.T., Saikia M., et al.: Cell Death Dis. 4, 505 (2013).
29. Pariente R., Bejarano I., Rodriguez A.B., Pariente J.A., Espino J.: Mol. Cell. Biochem. 440, 43 (2017).
30. Huang S.M., Tsai C.F., Chen D.R., Wang M.Y., Yeh W.L.: Biomed. Res. Int. 2014, 175247, 9 pages (2014).
31. Urcan E., Haertel U., Styllou M., Hickel R., Scherthan H., et al.: Dent. Mater. 26, 51 (2010).
32. Wang L., Peng Y., Shi K., Wang H., Lu J., et al.: J. Biomed. Res. 29, 132 (2015).
33. Wu Y., Xu J., Liu Y., Zeng Y., Wu G.: Front. Oncol. 10, 592853, 11 pages (2020).
34. Chou S.Y., Hsu C.S., Wang K.T., Wang M.C., Wang C.C.: Phytother. Res. 21, 226 (2007).
35. Yang L.L., Wang M.C., Chen L.G., Wang C.C.: Planta Med. 69, 1091 (2003).
36. Chao X., Zhou X., Zheng G., Dong C., Zhang W., et al.: Pharm. Biol. 52, 544 (2014).
37. Jiang G., Liu J., Ren B., Tang Y., Owusu L., et al.: J. Ethnopharmacol. 193, 368 (2016).
38. Zhu X., Song X., Xie K., Zhang X., He W., et al.: Int. J. Mol. Med. 40, 1143 (2017).
39. Shokoohinia Y., Hosseinizadeh L., Alipour M., Mostafaie A., Mohammadi-Motlagh H.R.: Adv. Pharmacolac. Sci. 2014, 847574, 8 pages (2014).
40. Zhang L., Jiang G., Yao F., He Y., Liang G., et al.: PLoS One 7, 1 (2012).
41. Che Y., Li J., Li Z., Li J., Wang S., et al.: Oncol. Rep. 40, 737 (2018).
42. Xu X., Zhang Y., Qu D., Jiang T., Li S.: J. Exp. Clin. Cancer Res. 30, 2 (2011).
43. Hung C.M., Kuo D.H., Chou C.H., Su Y.C., Ho C.T., et al.: J. Agric. Food Chem. 59, 9683 (2011).
44. Lin V.C.H., Chou C.H., Lin Y.C., Lin J.N., Yu C.C. et al.: J. Agric. Food Chem. 58, 4786 (2010).
45. Ding D., Wei S., Song Y., Li L., Du G., et al.: Cell. Physiol. Biochem. 32, 1751 (2013).
46. Jo M.J., Lee Y.J., Park C.W., Chung Y.B., Kim J.S., et al.: Int. J. Mol. Sci. 22, 231 (2021).
47. Luo K.W., Sun J.G., Chan J.Y.W., Yang L., Wu S.H., et al.: Chemotherapy 57, 449 (2011).
48. Okamoto T., Kobayashi T., Yoshida S.: Curr. Med. Chem. Anticancer Agents 5, 47 (2005).
49. Ahmed S., Khan H., Aschner M., Mirzae H., Küpeli Akkol E., et al.: Int. J. Mol. Sci. 21, 5622 (2020).
50. Sumorek-Wiadro J., Zając A., Maciejczyk A., Jakubowicz-Gil J.: Fitoterapia 142, 104492 (2020).
51. Küpeli Akkol E., Genç Y., Karpuz B., Sobarzo-Sánchez E., Capasso R.: Cancers 12, 1959 (2020).
52. Selim Y., El-Ahwany M.: Chem. Heterocycl. Compd. 53, 867 (2017).
53. Flis S., Splawinski J.: Anticancer Res. 29, 435 (2009).
54. Dou J., Wang Z., Ma L., Peng B., Mao K., et al.: Oncotarget 9, 20089 (2018).
55. Yar Saglam A.S., Alp E., Elmazoglu Z., Menevse E.S.: Oncol. Lett. 12, 2463 (2016).
56. Shin S.S., Ko M.C., Park Y.J., Hwang B., Park S.L., et al.: EXCLI J. 17, 531 (2018).
57. Han X., Kang K.A., Piao M.J., Zhen A.X., Hyun Y.J., et al.: Biomol. Ther. 27, 41 (2019).
58. Li B., Ren H., Yue P., Chen M., Khuri F.R., et al.: Cancer Prev. Res. 5, 612 (2012).

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