

Citrus Peel Flavonoids as Potential Cancer Prevention Agents

Nooshin Koolaji,^{1,2} Balakrishnan Shammugasamy,^{1,2} Aaron Schindeler,^{1,2,3} Qihan Dong,^{4,5,6} Fariba Dehghani,^{1,2} and Peter Valtchev^{1,2}

¹School of Chemical and Biomolecular Engineering, University of Sydney, Sydney, Australia; ²Center for Advanced Food Enginomics, University of Sydney, Sydney, Australia; ³Bioengineering & Molecular Medicine, The Children's Hospital at Westmead, Sydney, Australia; ⁴School of Science and Health, Western Sydney University, Sydney, Australia; ⁵Greg Brown Laboratory, Central Clinical School and Charles Perkins Centre, University of Sydney, Sydney, Australia; and ⁶Department of Endocrinology, Royal Prince Alfred Hospital, Sydney, Australia

ABSTRACT

Citrus fruit and in particular flavonoid compounds from citrus peel have been identified as agents with utility in the treatment of cancer. This review provides a background and overview regarding the compounds found within citrus peel with putative anticancer potential as well as the associated in vitro and in vivo studies. Historical studies have identified a number of cellular processes that can be modulated by citrus peel flavonoids including cell proliferation, cell cycle regulation, apoptosis, metastasis, and angiogenesis. More recently, molecular studies have started to elucidate the underlying cell signaling pathways that are responsible for the flavonoids' mechanism of action. These growing data support further research into the chemopreventative potential of citrus peel extracts, and purified flavonoids in particular. This critical review highlights new research in the field and synthesizes the pathways modulated by flavonoids and other polyphenolic compounds into a generalized schema. *Curr Dev Nutr* 2020;4:nzaa025.

Keywords: citrus peel extract, flavonoids, anticancer, inflammation, mechanism of action, apoptosis

Copyright [©] The Author(s) on behalf of the American Society for Nutrition 2020. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Manuscript received July 25, 2019. Initial review completed December 11, 2019. Revision accepted February 24, 2020. Published online March 13, 2020. doi: https://doi.org/10.1093/cdn/nzaa025 Supported by the Australian Research Council (IC14012006) and Faculty of Engineering and Information Technologies at the University of Sydney for the award of postgraduate scholarship to BS. Author disclosures: The authors report no conflicts of interest.

NK and BS contributed equally to this work. FD and PV are equal senior authors.

Address correspondence to PV (e-mail: peter.valtchev@sydney.edu.au).

Abbreviations used: ACF, aberrant crypt foci; Akt, serine specific protein kinase; Bax, proapototic member of Bcl-family; Bcl, apoptosis regulator; CDC, cell division cycle; CDK, cyclin-dependent kinase; COX, cyclooxygenase; CPE, citrus peel extract; CYP, cytochrome P450; DMBA, 7,12-dimethylbenz(a)anthracene; EMT, epithelial mesenchymal transition; ER, estrogen receptor; ERK, extracellular signal regulated kinase; Fas, a receptor protein of the tumor necrosis family (TFN) receptor family that induces apoptosis; FasL, Fas ligand; HUVEC, human umbilical vein endothelial cell; IAP, inhibitor of apoptosis; iNOS, inducible nitric oxide synthase; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; mTOR, mechanistic target of rapamycin; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PMF, polymethoxylated flavone; Ras, reticular activating system; STAT3, signal transducer and activator of transcription 3; VEGF, vascular endothelial growth factor.

Medicinal Properties of Citrus Fruits

Citrus fruits such as mandarin, pomelo, orange, lime, lemon, and grapefruit have been recognized as having high contents of bioactive compounds (1). Between the pulp and the peel, such fruits contain folate, vitamin C, dietary fiber, and bioactive compounds such as flavonoids. Flavonoids are widely distributed in aromatic plants such as mint and tea but are present in high concentrations in citrus fruits and their peels (2).

Citrus peel has untapped potential as a source of medicinal compounds because it contains carotenes, essential oils, pectin, and a range of polyphenolic compounds (3). Epidemiological studies have suggested that high consumption of fruits and vegetables (>400 g/d) can reduce cancer risk by \geq 20% (4). The Mediterranean diet is rich in fruit pulp and juice, and the associated high intake of fiber, antioxidants, and polyphenol compounds is linked with a lower cancer risk (5, 6).

The medicinal use of citrus peels can be traced back to the 10th century, but the biological activities of specific chemicals within the peel have only recently been characterized (7, 8). Citrus peels are rich in polyphenolic compounds, which are secondary plant metabolites with diverse and essential biological functions (9, 10).

Polyphenolic compounds consist of various classes of bioactive compounds including flavonoids, limonoids, coumarins, phenolic acids, terpenoids, tannins, stilbenes, lignans, and carotenoids (11–13). They contain heterocycles including aromatic rings with hydroxyl groups in their basic structure (14) and exist in the free state or as glycosides. Flavonoids are likely to be key bioactive compounds in citrus peel, particularly in terms of their anticancer activity (15–17) as well as in the prevention of infectious and degenerative diseases (18–20). Although it is appealing to identify specific molecules with high anticancer activity, there is growing evidence to suggest synergy between bioactive molecules in citrus peel extract (CPE). Whole CPEs have been shown to have higher anticancer activity than the fractionated extracts and isolated single compounds. Indeed, the methanolic extracts and freeze-dried CPEs are correlated with higher concentrations of total phenolic and flavonoid contents (21–23).



FIGURE 1 Main skeleton of flavonoids and their classes.

Several salient reviews should be noted. Cirmi et al. (4) detail the range of individual flavonoid and polyphenolic compounds found within citrus fruits and summarize the preclinical and epidemiological evidence for their utility in cancer treatment. Kandaswami et al. (24) describe the general utility of flavonoid compounds (not specifically from citrus) in modulating cell signaling pathways. This critical review focuses on the bioactive compounds that are enriched in citrus peel and examines their underlying mechanism of action. This is timely based on growing efforts to utilize CPEs as chemopreventive agents (25), as well as to leverage their antiatherogenic, anticarcinogenic, anti-inflammatory (26), anticancer (27), antidiarrheal, and antimicrobial properties (3, 28). In this extensive field, such studies are challenging to compare due to a lack of standardized in vitro and in vivo methodologies, as well as the use of whole CPE compared with individual polyphenolics, flavonoids, flavonols, flavones, and polymethoxylated flavones. However, this review explores a range of common mechanisms that feature in preclinical studies including motivation of carcinogen detoxification, scavenging of free radical species, control of cell cycle progression, preventing the initiation of cancer, inhibiting cell proliferation, increasing apoptosis, reducing oncogene activity, prohibiting metastasis and angiogenesis, as well as modulating hormone or growth factor activity (4, 29-32). This involves highlighting both recent and historical reports and synthesizing a model for the different biological functions of CPE bioactives. In most cases there has been no proper follow-up, either in vivo or in clinical research.

Flavonoid Subtypes within CPE

Flavonoids are low molecular weight compounds that are responsible for the vivid color of fruit peels, pulp, and leaves (11). They are found abundantly in citrus fruits, seeds, olive oil, red wine, and tea. More than 9000 flavonoids have been identified to date. Flavonoids feature a basic $C_6-C_3-C_6$, 15-carbon skeleton. They are comprised of 2 benzene rings (A and B), which are linked via a heterocyclic pyran ring (C in **Figure 1**). Flavonoids are subdivided according to the presence of an oxy moiety at C4, a double bond between positions 2 and 3, or a hydroxyl group in position 3 of a heterocyclic ring (C in Figure 1).

The biological activities of flavonoids increase with the degree of hydroxylation of the B ring (Figure 1) (24, 33). The basic structure of flavonoids permits a significant number of substitution patterns in the benzene rings A and B within each class of flavonoids: O-sugars, methoxy groups, phenolic hydroxyls, sulfates, and glucuronides (2, 34). The abundance of distinct flavonoids arises from a large number of different combinations of hydroxyl and methoxyl group substitutions. Besides, flavonoids can be classified by variations of the heterocyclic ring C to flavones, flavanones, flavonols, isoflavones, flavans, and anthocyanidins (9, 35). The antioxidant activity of flavonoids is related to *ortho*-dihydroxy substitution in ring B, the presence of a 2,3 double bond and of a 4-oxo moiety in ring C, as well as a 3-hydroxy-4-keto and/or 5-hydroxy-4-keto conformation in rings C and A (36, 37).

Flavonoids with a hydroxyl group in position C3 of the C ring are termed flavonols, and those lacking such an –OH moiety are called flavanones and flavones. Figure 2 illustrates the main structural formulas of some flavonoids isolated from CPE and their structural variations. The main abundant flavonoids in CPE are flavanones such as neohesperidin, naringin, and hesperidin (38–42) as well as nobiletin, sinensetin, and tangeretin (43). The biological activities of flavonoids are related to their antioxidant properties (44). The different degenerative diseases such as brain diseases and Alzheimer disease are affected by flavonoids via their antioxidant properties (42, 45, 46). There is ev-



FIGURE 2 The structural formulas of the main citrus peel flavonoids and their subclasses.

idence linking the pharmacological activity of CPE flavonoids to their ability to reduce the activity of intracellular signaling molecules including topoisomerases, phosphodiesterases, and kinases, as well as other regulatory enzymes (45, 47).

Flavanones (2,3-dihydro-2-phenylchromen-4-one) are a major class of flavonoids and occur mostly in glycoside forms such as hesperidin, neohesperidin, narirutin, naringin, eriocitrin, and neoeriocitrin. The glycosidic forms are divided into 2 types—rutinosides and neohesperidosides. Both rutinose and neohesperidose are glycosylated at position 7 and disaccharides are formed by glucose (Figure 2). The bitter taste of neoeriocitrin, naringin, and neohesperidin is caused by the presence of neohesperidose (rhamnosyl- α -1,2 glucose) in flavanones. Hesperidin, narirutin, and eriocitrin consist of a flavanone bound to rutinose (rhamnosyl- α -1,6 glucose), and they have no taste. The most critical flavanones in aglycone forms are naringenin and hesperetin.

Flavonols (3-hydroxy-2-phenylchromen-4-one), such as kaempferol, quercetin, catechin, and isorhamnetin, are aglycone forms of flavonoids. Flavonols are recognized by the presence of a 2,3-double bond and the 4-oxo group in the C ring. They differ in the presence of 1 additional –OH moiety at position C3 in the C ring. Additionally, the 3-OH group can be glycosylated by different sugars, which significantly increases the number of flavonol isomers (48). The glycoside flavonols such as rutin are found in trace amounts in citrus peel. The predominant types are 3-O-monoglycosides, and glycosylation occurs at the 3-OH group of the C ring (4).

Flavones (2-phenylchromen-4-one) are found in low concentrations in citrus peel. Nevertheless, they can produce important biological activities in vitro and in vivo. For instance, apigenin has shown high anti-inflammatory activity, and diosmin is an important venotonic agent (49, 50). Methylated flavones are the key flavones noted in citrus fruits (51).

Anthocyanidins (2-phenylchromenylium cation) are structurally derived from pyran, flavan, and flavones found only in grapefruit and blood oranges (4). Anthocyanidins are the aglycone counterpart of anthocyanins that are natural pigments of fruits responsible for the fruits' and flowers' violet, red, and blue coloring. The color of the anthocyanin occurs in response to changes in pH, oxygen, temperature, light, and enzymes and also by methylation or acylation at the hydroxyl groups on the A and B rings (52).

Polymethoxylated flavones (PMFs) are a subdivision of flavones with ≥ 2 methoxyl groups on their basic benzo- γ -pyrone skeleton and a carbonyl moiety at the C4 position. Notable PMFs include tangeretin, nobiletin, and sinensetin. PMFs exist exclusively in citrus peels and have been used as herbal (alternative) medicines for decades (49, 53). In research studies, PMFs have shown a broad spectrum of biological activities including anticarcinogenic (54, 55), antioxidant, cardiovascular protection, antiproliferation, antiatherogenic (56, 57), and anti-inflammatory activities (7, 55, 58–60). The permeability of PMFs through biological membranes is higher than other flavonoids because of their planar structure and low polarity (58, 61).

The antioxidant, enzyme-inhibitory, and antiproliferative activities of flavonoids are related to their specific structural features including the presence of glycosylation, the structure oxidation state, and the substituents in both the A and B rings of the flavonoid structure (62, 63). Studies of melanoma cell lines employing several flavonoids of citrus peels have shown the presence of the C2=C3 double bond on the B ring, conjugated with the 4-oxo function, to be critical for this biological activity (64). The presence of \geq 3 hydroxyl/methoxyl groups in each ring (A or B) of the flavonoid skeleton significantly increased the antiproliferative activity in human melanoma B16F10 and SK-MEL-1 cell lines (64, 65).

Up to 62 glucoside and aglycone limonoids have been reported in citrus fruits (66). Obacunone glucoside and nomilin acid glucoside are the major limonoid glucosides in CPEs (67). Coumarins are another class of bioactive compounds mainly present in citrus peel. Coumarins such as 7-methoxy-8-(2-oxo-3-methylbutyl) coumarin, 5-geranyloxy-7-methoxycoumarin, auraptene, limettin, and epoxyaurapten, as well as furanocoumarins such as psoralen, xanthotoxin, bergamottin, and epoxybergamottin have been found in citrus peels (68-71). Cinnamic acids (caffeic, p-coumaric, chlorgenic, ferulic, and sinapic) and benzoic acids (protocatechuic, p-hydroxybenzoic, and vanillic) are phenolic acids found in low concentrations in citrus peel (72, 73). Meanwhile, carotenes (β -carotene) and xanthophylls [β -cryptoxanthin, lutein, β citraurin, violaxanthin, (9Z)-violaxanthin, and zeaxanthin] are the main carotenoids found mostly in citrus peel (72, 74). Apart from the above bioactive compounds, D-limonene is the primary essential oil in citrus peel (75) with anticancer activity in humans (76).

Extraction of Flavonoids from Citrus Waste

In order to maximize the yield of bioactive flavonoid compounds from citrus peel, several different methods for extraction have been reported in the literature (77). Recommended methods include: 1) chemical methods such as hot water extraction (78, 79), solvent extraction (80), and alkaline extraction (81, 82); and 2) advanced methods such as ultrasound-assisted extraction, supercritical fluid extraction (83), microwave-assisted extraction (84), and enzyme-assisted extraction. The goal is to develop processes that are rapid and economical.

Most of the pharmaceutical and food industries use solvents for the extraction of bioactive compounds from citrus. Organic solvents, such as hexane, methanol, ethanol, petroleum ether, benzene, toluene, ethyl acetate, isopropanol, and acetone have been used to extract flavonoids from citrus waste. Phenolic compounds transfer from the solids to the surrounding solvents during the extraction. The temperature and time of extraction are specific for different kinds of flavonoids. The limitations of chemical methods are the several hours needed for extraction, large volumes of solvent, and the extra cost and time to evaporate the residual solvent. In contrast, "green chemistry" has emerged as a principle for the environmentally friendly extraction of high-value compounds. Such methods can be selective, low-energy, time-saving, and produce higher yields at a reduced solvent consumption (78).

The different extraction methods used for citrus flavonoids have their own advantages and limitations. However, combined approaches could ultimately prove superior to any individual method. In general, using food-grade solvents and ultrasound-assisted extraction of flavonoids from citrus waste has a strong potential for future industrial development as an efficient and environmentally friendly process (85).

Mechanism of Action of CPE Flavonoids

CPEs have been reported to show anticancer activity in various cancer lines at different efficacious levels; their activity is directly related to the CPE composition and the cell line sensitivity. The following sections provide an overview of the in vitro and in vivo studies showing that CPEs have potential in reducing the risk of cancer development and progression (Tables 1 and 2).

The following section examines the anticancer effects of CPEs reported in in vitro experiments and animal studies that elucidate the specific mechanisms involved. The anticancer effect of CPEs can be exhibited through suppression of proliferation, cell cycle inhibition, and induction of apoptosis.

Suppression of proliferation

Cancer cells differ from normal cells by their ability to proliferate without control, resistance to apoptosis, ability to form new blood vessels, and metastasis to distant parts of the body. Flavonoids found in CPEs have been shown to suppress these events through modulation of multiple cellular proteins that inhibit cell proliferation by downregulation of oncoproteins.

In human lung carcinoma A549 cells, the methanol extract of Korean *Citrus aurantium* fruit peel inhibited cell proliferation dose dependently and also induced apoptosis (86). Similar inhibitory effects were also observed with flavonoids isolated from Korean *C. aurantium* peel in A549 cancer cells (39).

Quercetin—the aglycone form of polyhydroxylated flavonoids (flavonols) found in onions, berries, grapes, green vegetables, and apple—is one of the most highly studied flavonoids in terms of its effects on cell proliferation. It exhibits growth inhibitory effects against a range of cancer cell lines including immortal human HeLa cells (36), human epidermoid carcinoma (A431), NK/LY ascites tumor cells, gastric cancer cells including NUGC-2, HGC-27, MKN-28, and MKN-7 (39), colon (COLO 320 DM) (39, 87), human breast (87, 88), human squamous, gliosarcoma (89, 90), ovarian (91), human pancreatic, and human liver (HepG2) cancer cells (88, 92). Indeed, quercetin's strong antiproliferative effect might be attributable to inhibition of the protein kinase C (PKC) pathway (93, 94).

Polymethoxylated flavones such as nobiletin, tangeretin, quercetin, and sinensetin showed antiproliferative activity against human lung carcinoma cells (A549), squamous cell carcinoma (HBT43) (90), gastric cancer, leukemia (HL-60), T-cell leukemia (CCRF-HSB-2), and B16 melanoma cells (95). The antiproliferative effect of naringin is correlated with the inhibition of cell survival by binding ATP on a phosphoinositide 3-kinase (PI3K) binding site; prohibition of cell growth and modulation of cell cycle–associated proteins by inhibition of the extracellular signal regulated kinase (ERK)-signaling pathway (96); and/or binding to p21 to increase the cells' nuclear antigens and block DNA synthesis (97). Naringenin and hesperetin exhibited strong antiproliferative activity against a broad spectrum of human [estrogen receptor positive (ER⁻)] MDA-MB-435 and (ER⁺) MCF-7 breast cancer cells, prostate (DU-145), melanoma (SK-MEL5), lung (DMS-114), and colon (HT-29) cancer cell lines (60, 90, 98–100).

Nobiletin, a major polymethoxyflavone, also enhances the cytostatic effect in (ER⁺) MCF-7 breast cancer cells, via upregulation of inhibitors selective for the cytochrome P450 family members CYP1B1 and CYP1A1 (the main oxidizing enzymes which are major determinants of resistance) (101). Moreover, nobiletin has effectively inhibited the proliferation of human endothelial cells of human breast, prostate, skin, and colon carcinoma cells (95, 102); decreased azoxymethane (AOM)-induced cell proliferation in colonic adenocarcinoma cells (103, 104), and exhibited direct cytotoxicity in MKN-45, TMK-1, MKN-74, and KATO-III gastric cancer cells through cell cycle deregulation (105).

TABLE 1 In vitro anticancer effects of citrus peel extract¹

Citrus reticulata D WEHI 3B (<100)	1(06, 107 108 109 68 110 53 38
C. reticulata SNU-668 (~100) I C. sinensis D MCF-7 (10.2–17.9) I C. grandis D U937 (60), HepG2 I* (31), HeLa (287), HCT-15 (87), I*		108 109 68 110 53 38
C. sinensis D MCF-7 (10.2–17.9) — — I — C. grandis D U937 (60), HepG2 — — I* — (31), HeLa (287), HCT-15 (87), HCT-15 (87),		109 68 110 53 38
C. grandis D U937 (60), HepG2 — — I* — (31), HeLa (287), HCT-15 (87),		68 110 53 38
MCF-7 (110), NCI-H460 (73), SNU-16 (90)		110 53 38
17 citrus varieties D HT-29 (31–45) — — — — — —		53 38
C. sunki D HL-60 (25) G2/M — I —	_	38
C. aurantium D AGS (40–60) G2/M I I —	_	50
C. aurantium — U937 (40–60) — — I I		111
C. grandis D HeLa (100–200), — — I — AGS (200–400)	—	70
C. aurantium D A549 (230) G2/M I I —	_	39
C. unshiu — MDA-MB-231(>200) — — — I	_	112
C. junos — HT-29 (>1200) — — — — —	I	113
C. aurantifolia — MCF-7 (59) G2/M — I —	_	114
C. aurantium D A549 — — I I	_	40
C. hassaku D MDA-MB-231 — — — I	_	113
C. reticulata D HepG2 (20–40), — — — — — — — — — — — — — — — — — — —	_	42
C. paradisi, C. D Caco-2, LoVo, — — — — — — — — — — — — — — — — maxima	_	115
C. hassaku D SNU-1 (<25) G1 — I —	_	116
C. paradisi Kasumi-1 (2000) — — — — — — —	_	117
C. reticulata D SKOV3 (~100) — — —		118
C. platymamma D A549 (364) G2/M I I —	I	86
C. sphaerocarpa D MDA-MB-231 — — — I (>200)	I	113
C. iyo D U266 (>400), K562 — I^ I^ I^ I^ (200-400), DU145 (>400), MDA-MB-231 (>400), HepG2 (200-400), RWPE-1 (>400)	Ιv	75
C. platymamma D Hep3B (100–200), G2/M I [#] I [#] I [#] I [#] HepG2 (300–400)	_	119
C. sinensis D HepG2 (>500) G1 I I — C. reticulata — HCT116 — — — — —	_	120 121

¹D, determined; I, induced; *only for U937; ^only for DU145; [#]only for Hep3B.

Cell cycle dysfunction is correlated with cancer development. Cell cycle progression is a complex and highly regulated process and consists of 4 phases: G1, S, G2, and M (122). The progression of cells from one phase to another is controlled by the coordinated interaction of cyclin-dependent kinases (CDKs) and their cyclin subunits to form active complexes. The formation of an active complex is regulated by CDK inhibitors. In normal cells, cell cycle progression is arrested when faulty DNA needs to be repaired, or further cell replication is not required. In the context of cancer, by arresting the cell cycle progression of ma-

lignant cells the tumor or metastatic cancer burden can be reduced or eliminated (123, 124).

CPEs can modulate proteins involved with cell growth such as epidermal growth factor receptor and reticular activating system (Ras), which have a range of downstream pathways including mitogenactivated protein kinases (MAPKs), serine specific protein kinase (Akt), 3-kinase PI3K/Akt, and mechanistic target of rapamycin (mTOR). Methanol extract from freeze-dried Korean *C. platymamma* flavonoids reduced the proliferation of Hep3B cells by inhibiting PI3K and Akt phosphorylation and increased the ERK1/2, c-Jun N-terminal kinase, and p38 MAPK phosphorylation; these reduced PI3K/AKT signaling and increased MAPK activity (119). Methanol extract of the peel of *C. aurantium* also suppressed the phosphorylation of Akt in U937 cells (111), and mTOR in SNU-1 cancer cell lines (116). In A549 cells, the ethanolic extract from *C. aurantifolia* peels inhibited cell proliferation dose dependently while inducing apoptosis (39, 86, 114). The suppression of growth signals was ascribed to Akt, Ras, ERK1/2, and E-cadherin in colon tumor-bearing mice (125). The treated mice showed low concentrations of inactive glycogen synthase kinase-3 β and low accumulation in cell nuclei of β -catenin, which limits the activity of signaling pathways. The oral administration of CPEs from Gold Lotion has been reported to considerably reduce the enzyme ornithine decarboxylase, which controls cell growth and proliferation through the biosynthesis and metabolism of polyamines in treated mice with colorectal cancer (125–127).

Cell cycle inhibition

CPEs suppress cancer cell proliferation by arresting cell cycle progression and modulating cell proliferation signaling pathways that can be reduced or eliminated in malignant cells. Analysis of cell cycle distribution in CPE-treated cells demonstrated that auraptene, the main compound of the supercritical fluid extraction of *C. hassaku* Hort ex. Tanaka peel, caused cell cycle arrest mainly at G1 phase (116, 120). The ethanoic extract of *C. aurantifolia* lime peels at a concentration of 6 μ g/mL induced apoptosis and cell accumulation at G1 phase, whereas the 15- μ g/mL extract induced apoptosis and cell accumulation at G2/M phase (38, 39, 86, 119, 114). CPEs have been shown to upregulate the ex-

 TABLE 2
 In vivo anticancer effects of citrus peel extract¹

Sample	Animal models	Dose (route)	Duration	Effects	Reference
Citrus junos	HT-29 cells implanted mice	100 mg/kg/d (i.p.)	4 wk	Reduced tumor size, disease activity index and colon shortening	113
C. aurantium	A549 cells injected in mice tail vein	Twice weekly (i.p.)	5 wk	Reduced cancer metastasis	40
C. reticulata	Treated leukemic cells injected into mice	_	2/10 wk	Reduced number of tumor cells and increased mice survival time	106
C. sinensis	AOM-induced carcinogenesis in mice	0.2% in diet	26 wk	Reduced number and size of ACF, tumor burden, and incidence	128
C. sinensis	Western diet inducing cancer	0.25%/0.5% in diet	9 wk	Reduced tumor number, multiplicity, and induced apoptosis	129
Multiple citrus	DMBA-induced carcinogenesis in mice	100/200 μL twice weekly (cream application)	20 wk	Reduced epidermal thickness, number of papillomas, tumor incidence, and tumor weight	127
C. unshiu	Double-TPA application to ICR mouse skin	8.1 nmol/30 min	24 h	Inhibit NO and O ₂ - generation	56
Multiple citrus	PC-3 cells implanted in mice	1/2 mg/kg 5 d/wk (i.p.) and 2 or 4 mg/kg 5 d/wk (o.p.)	3 wk	Suppressed tumor size	126
Multiple citrus	AOM-induced carcinogenesis in mice	100/200 μL 5 d/wk (o.p.)	6 wk	Reduced number of ACF	126
С. іуо	DU145 cells implanted in mice	50/200 mg/kg thrice weekly (i.p.)	4 wk	Suppressed tumor growth	75
C. depressa	TEWL and epidermal thickness in UVB-irradiated mouse skin	100 µL of 10%/d	1 wk	Reduce photoaging in mice	130
C. sinensis	HepG2 cells implanted in mice	1/10 mg/kg thrice weekly in diet	3 wk	Reduced tumor growth	120
C. sinensis	AOM-induced carcinogenesis in mice	0.01/0.05% in diet	4/18 wk	Reduced number of ACF	125

¹ACF, aberrant crypt foci; AOM, azoxymethane; DMBA, 7,12-dimethylbenz(α)anthracene; ICR, Institute of Cancer Research; i.p., intraperitoneal injection; o.p., oral injection; TEWL, transepidermal water loss; TPA, tissue plasminogen activator.

pression of p21 (cyclin-dependent kinase inhibitor 1) and/or p53 (tumor suppressor protein) leading to G1 arrest as observed in breast cancer cell line MCF-7 (114), human gastric cells SNU-1 (116), DU145 prostate cancer cells (75), and COLO 205 human colon carcinoma cells (114, 131). The CPEs can also arrest cell cycle at G2/M by increasing the expression of p21 and decreasing the expression of cyclin B1, cell division cycle 25C (CDC25C), and CDC2 in A549, Hep3B, and human gastric cancer AGS cells (38, 39, 86, 119). A water-based extract from *C. sinensis* L. peel (which chiefly contains hesperidin and narirutin) modulated the cell cycle of quiescent (PC-3 and LNCaP) prostate cancer cells, impairing their ability to enter the S phase (2–3% reduction of G0/G1 cells compared with 12–18% reduction of control cells) (132).

Tangeretin induced G1 phase by increasing the expression of p37 and p21 in COLO 205 human colon carcinoma cells (131) and prohibited the growth of estradiol-stimulated T47D cells (133). Nobiletin modulated the cell cycle in MKN-45, TMK-1, and KATO-III human gastric carcinoma cells (105), and MKN-74, and induced G1 phase arrest in MCF-7 and MDA-MB-435 breast cancer cells, and HT-29 colon cancer cell lines (134, 135). Hesperetin decreased the activity of MCF-7 breast cancer cells by accumulating cells in G1 phase through the inhibition of CDK4, CDK2, and cyclin D, upregulation of p21 and p27, and increased binding of p21 and CDK4 (136). Both tangeretin and nobiletin led to the accumulation of cells in the G1/S cell cycle in human colon and breast cancer cells. Naringin induced G1 arrest by upregulation of p21 (96). Apigenin also arrested cell cycle in G2/M phase in both androgen-insensitive PC-3 and androgen-sensitive LNCaP human prostate cancer cell lines by activation of a cyclin kinase suppressor WAF1/p21 (137) (Table 3).

Induction of apoptosis

Apoptosis and necrosis are 2 distinct mechanisms of cell death in eukaryote cells. Apoptosis, or programmed cell death, is involved in embryonic development, hormone-dependent atrophy, and metamorphosis. These processes eliminate damaged or unwanted cells (138). The apoptosis is characterized by plasma blebbing, cell shrinkage, and fragmented nuclei/DNA (139), which are reported in a variety of cancer cells treated with CPE in vitro (38, 40, 42, 53, 68, 70, 75, 111, 116, 119, 117) and in an in vivo mouse model (140).

Citrus peel polymethoxyflavones and CPE from *C. unshiu* induce apoptosis mainly through the intrinsic pathway by reducing antiapoptotic B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma extra large (Bcl-XL) proteins and increasing proapoptotic proteins [Bcl-2 associated X protein (Bax); proapoptotic Bcl-2 protein (Bid); Bcl-2 homologous antagonist killer (Bak); Bcl-2-associated death promoter (Bad)] in different cancer cell lines (105, 141–143). The increase in the ratios of Bax/Bcl-XL and Bax/Bcl-2 allows the release of cytochrome *c* through the permeabilized mitochondrial membrane. Following the binding of cytochrome *c* to the apoptosis protease-activation of caspase-9 and the apoptosis effector protein caspase-3 is achieved (144).

Increase of caspase-9 and caspase-3 was reported following treatment with CPEs (super critical extract of *C. hassaku* peels) for many cancer lines including gastric carcinoma SNU-668 (108) and SNU-1 (116), adenocarcinoma human alveolar basal epithelial cells A549 (40, 86), histiocytic lymphoma U937 (68, 111), metastatic prostate cancer DU145 (75), human gastric cancer AGS (38), hepatocellular carcinoma Hep3B (119) and HepG2 (120), as well as acute myeloblastic leukemia Kasumi-1 (*Citrus* \times *paradisi* Macfad.) (125, 117). CPEs increased the concentrations of cleaved poly ADP-ribose polymerase inhibitors in U937, SNU-1, AGS, Kasumi-1, A549, Hep3B, DU145, and colon cancer cells (38, 68, 75, 86, 111, 116, 119, 125, 117). CPE can also reduce endogenous inhibitor of apoptosis (IAP) proteins such as XIAP, cIAP1, and cIAP2 in U937 (111) and DU145 cancer cells (75).

It was reported that nobiletin could induce apoptosis by increasing Bax and p53 protein expression, inhibiting Bcl-2 protein expression, and elevating the ratio of Bax/Bcl-2 proteins in human lung A549 adenocarcinoma cells (145). Tangeretin induced apoptosis in leukemia HL-60 cells by affecting the mitogen-stimulated blastogenic response of human peripheral blood mononuclear cells (99), and quercetin promoted apoptosis as a consequence of cell cycle arrest in triple-negative breast cancer cells (88, 92, 146).

Accumulated evidence supports that CPE has negligible apoptosisinducing effects through the extrinsic apoptotic pathway. It was shown that CPEs induced apoptosis in U937 cells by increasing caspase-8; however, expression of the death receptors [DR4, DR5, and Fas (a receptor protein of the TFN family)], and proapoptotic ligands such as TFN-related apoptosis-inducing ligand (TRAIL), Fas ligand (FasL), and Fas-associated protein with death domain (FADD) was unchanged (111). Similarly, no reduction in the Fas and FasL proteins was observed in Hep3B cells treated with CPE (119). Further research is required to clarify the precise modulation of extrinsic apoptotic pathways involving cell death receptors by CPEs.

Inhibition of angiogenesis

It is well established that tumor growth is dependent on angiogenesis the growth of new blood vessels around cancer tissue needed to supply nutrients and oxygen to tumor cells (147). Because angiogenesis is essential for the growth of different cancers, vascular targeting has been considered as a potential strategy to reduce tumor growth and metastasis.

Flavonoids are antiangiogenic through a variety of mechanisms: they inhibit vascular endothelial growth factor (VEGF) expression, suppress endothelial cell migration, and decrease matrix metalloproteinases MMP-2 and MMP-9 (148).

The antiangiogenic properties of quercetin include inhibition of MMP-2 and MMP-9 secretion from tumor cells as well as inhibition of endothelial cell proliferation and migration (149). Quercetin reduced tube formation of VEGF-stimulated human umbilical vein endothelial cells (HUVECs) by 40% in vitro (150). Luteolin and apigenin are the most potent angiogenesis inhibitors, acting by inhibiting the release of inflammatory cytokine IL-6 and the signal transducer and activator of transcription 3 (STAT3) pathway (149). Hydroxylated PMFs suppress

the expression of MMPs and VEGF in colonic tumors. For example, sinensetin inhibited angiogenesis by inducing cell cycle arrest in the G0/G1 phase in HUVEC culture and downregulated the mRNA expressions of angiogenesis genes kinase insert domain receptor (kdrl), transforming protein p21 (hras), and Friend leukemia integration 1 transcription factor (FLI1) in zebrafish (150). Nobiletin inhibited angiogenesis by regulating cell cycle progression through G0/G1 arrest in vivo (150). It also suppressed CD36 expression and decreased the expression of thrombospondin 1—an endogenous inhibitor of angiogenesis—and TGF- β 1 (151). Eventually, the expression of VEGF was dramatically modified in DMBA-induced animals by tangeretin treatment (152).

Inhibition of metastasis

In metastasis, the cancer cells break away from a primary tumor to distal sites in the body. Metastasis involves several distinct steps including secretion of metastasis-inducing proteins, cell detachment at a primary site, migration, adhesion, and invasion at the new site. MMPs such as MMP-2 and MMP-9 are the main proteins that are necessary for metastasis because they break down the extracellular matrix and allow the cancer cells to migrate (153).

The antimetastatic effects of CPE extracted by different methodologies have been tested in a range of cancer cell lines (Table 3). CPEs have been shown to reduce MMP protein expression and activity in A549 (40), DU145 (75), Hep3B (119), and MDA-MB-231 breast cancer cells (154), and in Caco-2, LoVo, and LoVo/ADR colon cancer cell lines (115). In a notable study, quercetin decreased the invasion of murine melanoma cells by suppressing MMP-9 via the PKC activator pathway (155). Genistein prohibited the invasion of triple-negative MDA-MB 231 breast cancer cells in vitro, via downregulation of MMP-9 activity (153, 155). Apigenin, quercine, and luteolin can also inhibit MMP-2 and -9 activities (156). Flavonoids with an increasing number of substitutions or hydroxyl groups showed a stronger inhibitory effect on the activity of MMP-9 and -2 (156, 157). Suppression of the MMP proteins by CPE also was observed in in vivo models for colon (125, 126) and prostate tumors (140).

Like the reduction in MMPs, CPEs reduced concentrations of chemokine receptor CXCR4 together with the human epidermal growth factor receptor 2 (HER2)/neu protein that stimulates CXCR4 expression in MDA-MB-231 cells (154). CPE also suppressed the phospholipase-C gamma-1 (PLCG1) protein required for cell migration in U937 cells (111). Furthermore, vascular cell adhesion molecule-1, which promotes the adherence of cells at new sites, was reduced by *C. unshiu* Marc. peel in MDA-MB-231 cells through inhibition of PKC phosphorylation (112). Many proteins related to metastasis such as reduced epithelial mesenchymal transition (EMT) markers (N-cadherin, vimentin, and fibronectin), EMT-associated transcription factors (Slug and Snail), and activated type I receptors (SMADs) were shown to be downregulated by the Ougan (*C. reticulata* cv. Suavissima) flavedo extract in SKOV3 cells (118).

E-cadherin plays an essential role in cell adhesion, and loss of E-cadherin is associated with a tendency for tumor metastasis (158). An increase in the expression of E-cadherin was observed in colon tumor-bearing mice fed hydroxylated polymethoxyflavones in CPE (125). In another study, the Korean *C. aurantium* L. peel showed antimetastatic

properties by preventing the migration and infiltration of A549 cells in an in vitro experiment (40).

Anti-inflammatory activity

Cancer initiation and proliferation are closely associated with inflammation and, in some cases, infection. Inflammation can facilitate the initiation and progression of normal cells to malignancy through the production of inflammatory oxidants such as inducible nitric oxide synthase (iNOS), myeloperoxidase, eosinophil peroxidase, and NAD(P)H oxidase. Chronic inflammation is associated with carcinogenesis and acts as a driving force for cancer progression (159).

The expression of proinflammatory proteins is reduced by CPE in both in vitro and in vivo models (Table 3). iNOS and inducible-type cyclooxygenase (COX) are enzymes that are induced in response to an oxidative environment. Consequently, overexpression of these enzymes contributes to carcinogenesis through promotion of inflammation (7, 56, 136). CPEs downregulated the expression of iNOS and COX-2 enzymes in human histiocytic lymphoma U937 cell lines, DU145, and murine macrophage RAW264.7 cells (75, 113, 160–162). Reduction in these enzymes by CPEs was also observed in colon, skin, and prostate cancer cell lines in in vivo models (125–127). It is reported that CPEs in RAW264.7 cells reduced nitric oxide that is produced by iNOS (163).

NF-*κ*B activation is an essential factor in inflammation. NF-*κ*B is a heterodimeric protein composed of 5 subunits, and presents in an inactive state in the cytoplasm due to the binding of the inhibitory protein, $I\kappa B\alpha$ (164, 165). Upon chemical signaling for the activation of NF-*κ*B, the $I\kappa B\alpha$ degrades and releases the NF-*κ*B from its inactive state in the cytoplasm. The release of NF-*κ*B allows the translocation of NF-*κ*B subunits p50 and p65 to the nucleus, where they activate the transcription of proinflammatory cytokines, chemokines, adhesion molecules, and enzymes. It is documented that CPE treatment reduced NF-*κ*B activation and the nuclear translocation of its p50 and p65 subunits in RAW264.7, A549, MDA-MB-231, and U937 cancer cells (125, 113, 160, 161, 163, 121, 166–168).

Likewise, inhibition of NF- κ B suppresses a range of downstream genes that include proinflammatory cytokines. Sweet orange peel extract with a high amount of PMFs suppressed the expression of TNF- α , intercellular adhesion molecule 1, IL-1 β , IL-6, and IL-8 in inflammation-induced U937 cells (160). The abundances of TNF- α , monocyte chemoattractant protein 1 (MCP-1), IL-6, and phosphorylated p38 proteins were found to be lower in CPE-treated RAW264.7 cells than the control (113).

CPEs also have a suppressive effect on the STAT3 signaling pathway, which is involved in inflammation (75, 169). CPEs reduced the phosphorylation of STAT3 in DU145, PC-3, and prostate cancer cell line M2182 (75). In the same study, Janus-like kinase and a c-Src kinase that mediated the phosphorylation of STAT3 were also found to be suppressed by CPE (125).

The mechanism of action of flavonoids on cancer cells is presented schematically in **Figure 3**. It is highly complex and involves not only certain distinct biological processes but also different modulation of overlapping cell signaling pathways.

	Chemopreventive and anti-inflammatory			
Flavonoids	effects	Mechanisms	Cancer cells	References
Nobiletin (5,6,7,8,3',4'-hexamethoxyflavone)	Cell cycle regulation	Arrested cell cycle progression at G1	MDA-MB-435, MCF-7, HT-29, KATO-III, TMK-1, A549, MKN-45, MKN-74	39, 68, 142, 130, 170–172
	Antiangiogenesis, anti-inflammatory, antimetastasis	Inhibited the activity of extracellular signal regulated kinases 1/2 (ERK1/2) phosphorylation and c-JNK and activation of the caspase pathway	MDA-MB-435, MCF-7, HT-29	
	Co-chemotherapeutic	Increased cytotoxicity of doxorubicin	MCF-7, T47D	
	Suppression of carcinogenesis	Inhibited the activity of CYP1A2	MCF-7, T47D	
	Antioxidant	Scavenge DPPH radicals, hydrogen peroxide scavenging, hydroxyl radical scavenging	—	
	Antimetastasis	Prevented the migration of A549 cancer cells	A549 cells in vitro/in vivo	
	Apoptosis	Downregulated (Bcl-2)/upregulation (Bax)	HeLa, THP-1	
	Anti-inflammation	Decreased activation of AP-1, NF- κ B, and CREB	RAW 264.7 monocyte/macrophage-like cells	
	Anti-inflammation	Prohibited the LPS-induced mRNA and protein expression of iNOS	Skin inflammation	
	Anti-inflammation	Induced the expression of COX-2 by suppressing UVB	Human keratinocytes in vitro	
	Antimetastatic	Inhibited MEK1/2 activity is associated with the suppression of	Human fibrosarcoma HT-1080 cells	
	Antimetastatic	pro-MINIPS Enhanced the expression of TIMP-1 by the activation of PKCβII/epsilon-JNK pathway	Human fibrosarcoma HT-1080 cells	
	Antiproliferation	Decreased differentiation into granulocytes and macrophages by TNF- <i>a</i>	Murine myeloid leukemia WEHI 3B cells	
Tangeretin (4',5,6,7,8-pentamethoxyflavone)	Antioxidant	Scavenge DPPH radicals, hydrogen peroxide scavenging, hydroxyl radical scavenging	_	111, 133, 136, 170, 173–177
Meo OMe	Antioxidant	Inhibited the activity of CYP1A1 and the expression of mRNA	Human intestine Caco-2 cells	—
JMe U	Apoptosis	Triggered apoptosis via p53 pathway	COLO 205, HL-60 cells	

TABLE 3	Mechanisms and ch	emopreventive	effects of citru	us peel extract	flavonoids on	cancer cell lines ¹
---------	-------------------	---------------	------------------	-----------------	---------------	--------------------------------

(Continued)

Downloaded from https://academic.oup.com/cdn/article/4/5/nzaa025/5804723 by guest on 05 November 2020

TABLE 3 (Continued)

	Chemopreventive and anti-inflammatory			
Flavonoids	effects	Mechanisms	Cancer cells	References
	Antiproliferation	Decreased the expression of PROM1 and SNAI1	Cancer stem cell of HT29	
	Antiproliferation, apoptosis	Activated caspase-3	Cocon LOvo/DX cells	
	Co-chemotherapeutic	Increased cytotoxicity of doxorubicin	MCF-7, T47D	
	Cell cycle regulation	Arrested cell cycle at G1 by targeting p53, p21, and p37 pathway	MCF-7, MDA-MB-435, colon cancer line HT-29, upregulate COLO 205 cells	
	Anti-inflammation	Blocked AKT activation	Lung carcinoma cells	
	Anticarcinogenic	Inhibited P450 1A/1A2/3A4	Human liver microsome cells	
	Antimetastatic	Decreased the number of metastatic nodules in Lentini model	Melanoma B16F10 cells	
	Anticarcinogenic	Reduced PhIP-DNA adduct formation in colon	Colon cancer cells	
	Anti-inflammation	Induced LPS-induced	RAW 264.7 cells	
	Anti-inflammation	Inhibited IL-1β-induced production of COX-2 by the activation of JNK, AKT, ERK, and	A549, H1299	
Sinensetin	Cell cycle arrest	Induced cells in G0/G1	HUVEC	149, 178–186
(5,6,7,3',4'-pentamethoxyflavone)	Antiangiogenesis	Downregulated the mRNA expression of angiogenesis flt1, hras,	Zebrafish	
	Antiproliferation, apoptosis	Inhibited iNOS expression, NO production, and PGE ₂ production	_	
	Cell cycle regulation	Inhibited in S phase by DNA elongation	T47D breast cancer cells	
	Antiproliferation, cell cycle block	Captured cells G2/M phase and increased apoptosis, increased the expression of p53 and p21	AGS gastric cancer cells	
	Anti-inflammatory	Inhibited inflammatory gene expression and STAT1 activation, inhibited iNOS, NO, and PGE ₂ production	Carrageenan-induced paw inflammation in the rat	
	Apoptosis	Reactivated oxygen species production, DNA damage, gene 153 expression,	Leukemia cells	_
	Antiproliferation	Activated Ca ²⁺ -dependent	MCF-7 breast cancer cells	
	Apoptosis	Upregulated caspase-3, -8, -9, and poly(ADP-ribose), polymerase (PARP) cleavage	T-cell lymphoma Jurkat cells	

TABLE 3 (Continued)

Flavonoids	Chemopreventive and anti-inflammatory effects	Mechanisms	Cancer cells	References
	Induced autophagy and cell death	Activated reactive oxygen species/c-Jun N-terminal kinase (JNK), blocked Akt/mTOR	T-cell lymphoma Jurkat cells	
	Cell cycle arrest	Arrest cells at G0/G1	HepG2 cells	
	Apoptosis	Downregulated Bcl-xL, upregulated TRAIL and PTEN	HepG2 cells	
Hesperetin (3′,5,7-trihydroxy-4′- methoxyflavanone)	Apoptosis	Induced apoptosis by activation of caspase-3	HL-60 cells	39, 86, 187–193
MeOOOH	Antiproliferation	Inhibited oxidative stress and DNA damage	HT-29 colon adenocarcinoma	
ОН О	Anticarcinogenic	Downregulated the HIF-1a/VEGF/VEGFR2	Xenograft C6 glioma cells in rats	
	Cell cycle arrest	Decreased cyclin D1, CDK4 and Bcl-xL by upregulating the level of p57Kip2	MCF-7 cancer cells	
	Antimetastatic	Induced COX-2, MMP-2, and MMP-9	DMH-induced colon cancer in rat; B16-F10 murine melanoma cells	
	Apoptosis	Activated the mitochondrial pathway by rising concentrations of ROS, Ca ²⁺ , and ATP in mice	Xenograft tumors in mouse model of gastric cancer	
	Apoptosis, antiproliferation	Suppressed the expression of NF-kB, p38, and caspase-3	PC-3 prostate cancer cells	
	Cell cycle arrest	G2/M arrest by controlling the concentration of cyclin B1, CDC2, CDC25C, and p21	A549 lung cancer, MCF-7	
	Apoptosis	Increased the expression of caspase-3, -8, -9, p53, Bax, and Fas death receptor	Cervical cancer SiHa, A549 lung cancer, HL-60 cells	
	Apoptosis	Induced via Bax-dependent mitochondrial pathway	HT-29 cells	
Naringin (4′,5,7-trihydroxyflavanone-7- rhamnoglucoside)	Cell cycle regulation	Upregulated p21, G1-phase arrest, activated Ras/Raf/ERK- mediated, decreased cyclin D1 and cyclin E	5637 bladder cancer cells, MDA-MB-231 xenograft mice	96, 194–200
	Metastasis, anticarcinogenic	Inhibited the activity of PI3K/Akt/mTOR and upregulated	AGS cells	
H0~n	Cell cycle arrest	Cell cycle arrest in S phase	HT-29	

(Continued)

Downloaded from https://academic.oup.com/cdn/article/4/5/nzaa025/5804723 by guest on 05 November 2020

TABLE 3 (Continued)

Flavonoids	Chemopreventive and anti-inflammatory effects	Mechanisms	Cancer cells	References
	Antiproliferation, antioxidant	Modulated gene expression, decreased	SKOV3 ovarian cancer cells	
		DNA methyltransferase activity, downregulated the expression of Bcl2 and Bcl-xL		
	Cell cycle arrest	Increasing p21 and arrest in G1 of cell cycle; inhibited the activity of CDK2	MCF-7	
	Antiproliferative	Inhibited CYP3A4, CYP1A2, CYP2C9, CYP2C19, and CYP2D6		
	Antiproliferation, apoptosis	Decreased the mRNA expression of BID, BAX, caspase-3, cytochrome c, p53, p21, and p27	DU145 prostate cancer cells	
	Apoptosis	Enhanced the expression of caspases, p53, Bax, and Fas death receptor	HT-29	
	Antimetastasis	Downregulation of MMP-9 and repressed the PI3K/AKT/mTOR/p70S6 signaling pathway	MCF-7 K	
	Antiproliferation	Upregulated EGFR and ERK phosphorylation	HeLa and A549 cells	
	Antiproliferation, apoptosis	Suppressed the NF-κB/COX-2/caspase 1	HeLa	
Hesperidin (hesperetin-7-rutinoside)	Antiproliferative	Inhibited MMP-9 by NF-κB and AP-1 signaling	NALM-6 leukemia cells	187, 188, 201–206
	Apoptosis	Inhibited the PI3K/Akt pathway through PTEN-phosphatase	SUN-C4 colon cancer cells	
MOO HO HOH	Antimetastatic, angiogenesis	Suppressing ANGPT1 gene	Laryngeal cancer cells	
		Upregulated the level of p21 and p53	MCF-7 cells	
	Antiproliferation	Inhibition of JAK/STAT signaling pathway	Cutaneous skin cancer cells	
	Apoptosis	Inhibited Aurora-A and Akt-mediated GSK-3β/β catenin cascade	A431 skin cancer cells	
	Antioxidant	Upregulated Nrf2 (nuclear factor-2)	Cutaneous skin cancer cells	
	Anti-inflammation	Downregulated mRNA expression of various cytokines (TNF, IL-1, IL-6)	Cutaneous skin cancer cells	

Downloaded from https://academic.oup.com/cdn/article/4/5/nzaa025/5804723 by guest on 05 November 2020

(Continued)

TABLE 3 (Continued)

Flavonoids	Chemopreventive and anti-inflammatory effects	Mechanisms	Cancer cells	References
	Anti-inflammation	Inhibited IL-6, TNF, COX-2, iNOS inflammatory components	A431 skin cancer cells	
	Antiproliferation	Upregulated BAX and downregulated Bcl-2, decreased the release of cytochrome <i>c</i>	HeLa cervical cancer cells, A2780 ovarian cancer cells	
	Co-chemotherapeutic	Inhibited PgP activity	Human leukemia cells (CEM/ADR5000)	

¹Akt, serine specific protein kinase; ANGPT1, angiopoietin 1; AP-1, activator protein 1; Bax, Bcl2-associated X protein; Bcl, B-cell lyphoma; Bcl-xL, Bcl2-associated extra large protein; BID, a proapoptotic protein; CDK, cyclin-dependent kinase; COX, cyclooxygenase; CREB, c-AMP response element binding protein; CYP, cytochrome P450; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EGFR, epidermal growth factor receptor; ERK, extracellular signal regulated kinase; Fas, a receptor protein of the TNF receptor family; flt, vascular endothelial growth factor receptor 1; GSK, glycogen synthase kinase; HIF, hypoxia inducible factor; hras, transforming protein p21; HUVEC, human umbilical vein endothelial cell; iNOS, inducible nitric oxide synthase; JAK, Janus-like kinase; JNK, c-Jun N-terminal kinase; kdrl, vascular endothelial growth factor receptor kdr-like; Kip2, cyclin-dependent kinase inhibitor; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; mTOR, mechanistic target of rapamycin; PgP permeability glyoprotein; PhIP, 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PROM, prominin-1; PTEN, phosphatase and tensin homolog; p21CIP1/WAFI, cyclin-dependent kinase inhibitor 1; P450, cytochrome P450; Raf, a serine/threonine-specific protein kinase; Ras, reticular activating system; ROS, reactive oxygen species; SNAI, sodium-coupled neutral amino acid transporter 1; STAT, signal transducer and activator of transcription; TIMP, tissue inhibitor of metalloproteinases; TRAIL, TNF-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor receptor.

Functional evidence for citrus anticancer activity in in vivo models

CPE flavonoids have been suggested to play a critical role in cancer prevention and maintaining a healthy lifestyle (207). Individual flavonoids such as apigenin, nobiletin, hesperidin, and tangeretin, all highly enriched in CPE, have demonstrated anticancer activity in preclinical animal models. In addition to single and combined flavonoids, whole CPE has been tested for anticancer activity in rodent models.

A series of studies used preclinical mouse models of colon carcinogenesis to examine the protective effects of crude cold-pressed CPE oil. This oil contained \sim 30% PMFs such as nobiletin, sinensetin, tangeretin, and monohydroxylated analogs. When mice were fed a diet containing 0.2% CPE before, during, and after carcinogen treatment (128), the number of aberrant crypt foci (ACF)-histological biomarkers for colon carcinogenesis-was reduced by 34-66% compared with the control. The low incidence of tumor development could be due to the highly potent flavonoids in CPE (102, 128). Feeding mice a diet containing 0.01% or 0.05% hydroxylated PMFs for 4 wk also reduced the total number of large ACF and tumors in colonic tissue by 40-44% compared with controls (125). When mice were fed hydroxylated PMFs for 20 wk, the number of microadenomas was reduced by $\leq 81\%$ in comparison with controls. Similarly, oral administration of CPE with naringin and hesperidin reduced numbers of ACF by \leq 40% compared with the control group in colon tumor-bearing mice (125). Moreover, the addition of CPE (containing methoxylated flavones, including: tetramethoxyflavone, 13.6%; nobiletin, 12.49%; sinensetin, 9.16%; hexamethoxyflavone, 11.06%; heptamethoxyflavone, 15.24%; and tangeretin, 19.0%) at 0.25% or 0.5% to the new Western-style diet reduced the overall colon tumor number by 26-48% and overall tumor volumes

by 36–63%, and increased the number of apoptotic cells compared with patients who had the Western-style diet alone (129).

In another study, oral administration of ethanol extract of CPE (*C. junos* Tanaka) at 100 mg/kg/d significantly reduced the size of colorectal adenocarcinoma HT-29 tumor cells through reducing COX-2 expression in xenograft mice (113). Administration of methanol/water extract of dried citrus peel (*C. reticulata* Blanco) at a dose of 1000 ppm in the diet reduced total ACF by 75% compared with the control (121). In a similar study, an in vivo model showed that a 70% aqueous methanol extract of CPE (Korean *C. aurantium* L.) could prevent human lung (carcinoma) A549 cells migrating to lungs of mice injected with A549 cells via the tail vein (40). These data suggest that CPE had effects on the regulation of apoptosis and cell migration.

In a 2-stage skin carcinogenesis model, mice were treated with 7,12dimethylbenz(α)anthracene (DMBA) to initiate tumors followed by repeated application of 12-O-tetradecanoylphorbol-13-acetate to promote tumor growth. Topical application of CPE, Gold Lotion (the peels of navel oranges, *C. hassaku*, *C. limon*, *C. natsudaidai*, *C. miyauchi*, and satsuma), at 100 µL and 200 µL on the skin reduced the number of papillomas by 25%, tumor incidence by 18%, tumor weight by 65%, and the number of tumors with a diameter >5 mm by 33% compared with controls (127). The epidermal thickening due to the associated inflammation and edema was decreased by 23–33% compared with the control (127).

Apigenin reduced DMBA-induced skin cancers by inhibiting epidermal ornithine decarboxylase, a key enzyme in cancer prevention (208). Nobiletin was effective in preventing skin carcinogenesis by suppression of DMBA and 12-O-tetradecanoylphorbol-13-acetate and decreasing the inflammatory parameters (56). The daily administration of hesperidin for 45 days inhibited DMBA-induced experimental breast



FIGURE 3 Schematic of the main anticancer molecular mechanism of flavonoids. 1. Antiangiogenesis activity via VEGF by inhibiting HIF-1α/Akt/NF-κB signaling pathways. 2. Anti-inflammation activity by decreasing p38 via MAPK and inhibiting the expression of COX-2. 3. Antimetastasis activity via inhibition of MMP-2/9 by diminishing the Akt/FAK/Ras/PI3K signaling pathways. 4. Antiproliferation activity by inhibiting PI3K/Akt; via cell-cycle arrest in the G0/G1 or G1/S phase by activating p53 and p21, and also inhibiting BAX and Bcl-2; and by increasing cytochrome *c* and activating caspase pathways. Akt, serine specific protein kinase; BAX, Bcl2-associated X protein; Bcl, B-cell lymphoma; BH3, Bcl-2 homology domain 3; Casp, cysteine-aspartic proteases; cdc, cell division cycle; CDK, cyclin-dependent kinase; COX, cyclooxygenase; Cyto-C, cytochrome complex; Erk, extracellular signal-regulated kinase; FADD, Fas-associated protein with death domain; FAK, focal adhesion kinase; FAS, a receptor protein of the TNF receptor family; HIF, hypoxia-inducible factor; IκBα, nuclear factor of kappa light polypeptide gene enhancer; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; mTOR, mechanistic target of rapamycin; PARP, poly ADP-ribose polymerase; PGD2, prostaglandin D2; PGE2, prostaglandin E2; PGH2, prostaglandin H2; PI3K, phosphoinositide 3-kinase; Ras, reticular activating system; STAT3, signal transducer and activator of transcription 3; VEGF, vascular endothelial growth factor.

cancer formation through modification of phase I and phase II metabolizing enzymes, as well as modulating the xenobiotic-metabolizing enzymes during 1,2-dimethylhydrazine-induced colon carcinogenesis in rats (209). Tangeretin, a PMF, significantly arrested DMBA-induced breast cancer in rats (210). The anticancer activity of CPE (Gold Lotion) was also tested in prostate cancer models. In PC-3 prostate tumor-bearing mice, treatment with CPE by intraperitoneal injection of 1 mg/kg/d reduced the tumor weight by 57% and tumor volume by 79% compared with the control (140, 211). For mice treated with 2 mg/kg/d by oral ingestion, tumor weight was reduced by 86% and tumor size by 94%. The strong anticancer activity was attributed to the high concentration of PMFs and other compounds such as hesperidin. Chu et al. (120) showed that the ethyl acetate extracts from sweet orange peel (50-500 µg/mL) reduced human liver cancer HepG2 growth when tested in an in vivo model and exhibited significant cytotoxicity on HepG2 cells.

Despite the growing number of preclinical animal studies, clinical trials involving CPEs are currently limited to a single study. Naringenin isolated from *C. aurantium* peel (Chinese bitter orange) was tested as a therapeutic on 95 postoperative patients with osteosarcoma (212). The treatment group (n = 47) that received 20 mg/d of narin-

genin showed significantly reduced osteosarcoma volume compared with placebo controls.

Conclusions

Citrus fruits are rich in flavonoid compounds; however, much of the literature to date has focused on the effects of fruit pulp (and juice) consumption rather than examining the rich flavonoid profile of CPE. CPE is an underutilized commercial resource. For instance, the US orange juice industry produces 700,000 tons of peel waste annually (213), which represents nearly 40% of the total weight of the fruit (49). Due to the low cost and current nonuse of the peel by industry, citrus peel represents an untapped nutritional source that is rich in bioactive compounds. There is thus a great deal of potential for the application of citrus fruit peels to create products that counter the effects of oxidative stress and have important health benefits (9).

This review has summarized a selection of the key preclinical and clinical studies that show an anticancer utility for citrus-derived flavonoids. This property is linked to the chemical structures of flavonoids, which can dramatically affect a range of molecular and cellular mechanisms for inhibiting cancer initiation and progression. Overall, citrus flavonoids act not only as free radical scavengers but also as modulators of several key molecular events implicated in cell survival and apoptosis. Flavonoids exhibit a remarkable spectrum of biological activities including anti-inflammatory, anticancer, antiproliferation, antiangiogenesis, antioxidant, cell cycle regulation, and antimetastasis effects.

Future Studies

Further studies are needed to address in greater detail the basic science underlying CPE mechanisms, as well as examining pharmacokinetics, pharmacodynamics, and efficacy in a clinical setting. At a fundamental level, there is scope to explore the means by which flavonoids enter cancer cells and potentially accumulate in specific cellular organelles and tissues. This plays into the concept of flavonoid bioavailability, and there has been some discussion regarding innovative methods for enhancing this property (214). Further study could also focus on elucidating signaling pathways by which CPEs can affect critical enzymes such as tyrosine kinases and focal adhesion kinases, PKC, and MMPs.

For clinical translation, trials in both the general population (as health supplements) and in the setting of cancer treatment are needed to build upon cell culture studies and preclinical animal models. Multiple tests indicate that CPEs have a low toxicity profile in vitro and in vivo, making them suitable for further dietary and food product development. Future studies will be required to test the utility of CPEs in a multitargeted pharmacological strategy, either for cancer prevention or as a coadministration in oncological therapies.

Acknowledgments

The authors' responsibilities were as follows—QD, FD, PV: were responsible for the design; NK, BS: were responsible for the writing; AS: was responsible for the final content; and all authors: read and approved the final manuscript.

References

- Darband SG, Kaviani M, Yousefi B, Sadighparvar S, Pakdel FG, Attari JA, Mohebbi I, Naderi S, Majidinia M. Quercetin: a functional dietary flavonoid with potential chemo-preventive properties in colorectal cancer. J Cell Physiol 2018;233(9):6544–60.
- Wang T-Y, Li Q, Bi K-S. Bioactive flavonoids in medicinal plants: structure, activity and biological fate. Asian J Pharm Sci 2018;13(1):12–23.
- 3. Tsitsagi M, Ebralidze K, Chkhaidze M, Rubashvili I, Tsitsishvili V. Sequential extraction of bioactive compounds from tangerine (*Citrus unshiu*) peel. Ann Agrarian Sci 2018;16(2):236–41.
- 4. Cirmi S, Ferlazzo N, Lombardo G, Maugeri A, Calapai G, Gangemi S, Navarra M. Chemopreventive agents and inhibitors of cancer hallmarks: may citrus offer new perspectives? Nutrients 2016;8(11):698.
- Giacosa A, Barale R, Bavaresco L, Gatenby P, Gerbi V, Janssens J, Johnston B, Kas K, La Vecchia C, Mainguet P, et al. Cancer prevention in Europe: the Mediterranean diet as a protective choice. Eur J Cancer Prev 2013;22(1):90– 5.
- Smeriglio A, Cornara L, Denaro M, Barreca D, Burlando B, Xiao J, Trombetta D. Antioxidant and cytoprotective activities of an ancient Mediterranean citrus (*Citrus lumia* Risso) albedo extract: microscopic

observations and polyphenol characterization. Food Chem 2019;279:347–55.

- 7. Li S, Pan M-H, Lo C-Y, Tan D, Wang Y, Shahidi F, Ho C-T. Chemistry and health effects of polymethoxyflavones and hydroxylated polymethoxyflavones. J Funct Foods 2009;1(1):2–12.
- Gómez-Mejía E, Rosales-Conrado N, León-González ME, Madrid Y. Citrus peels waste as a source of value-added compounds: extraction and quantification of bioactive polyphenols. Food Chem 2019;295:289–99.
- Rafiq S, Kaul R, Sofi S, Bashir N, Nazir F, Ahmad Nayik G. Citrus peel as a source of functional ingredient: a review. Journal of the Saudi Society of Agricultural Sciences 2018;17(4):351–58.
- Tomás-Barberan FI, Ferreres F, Gil MI. Antioxidant phenolic metabolites from fruit and vegetables and changes during postharvest storage and processing. In: Atta ur R editor. Studies in Natural Products Chemistry, vol. 23, Elsevier; 2000. p. 739–95.
- 11. Hollman P, Katan M. Dietary flavonoids: intake, health effects and bioavailability. Food Chem Toxicol 1999;37(9):937–42.
- Abudayeh Z, Al Khalifa I, Mohammed S, Ahmad A. Phytochemical content and antioxidant activities of pomelo peel extract. Pharmacogn Res 2019;11(3):244–47.
- Sharma K, Mahato N, Lee Yong R. Extraction, characterization and biological activity of citrus flavonoids. Rev Chem Eng 2018;35(2): 265–84.
- Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem 2006;99(1):191–203.
- Hakim I, Harris R, Ritenbaugh C. Citrus peel use is associated with reduced risk of squamous cell carcinoma of the skin. Nutr Cancer 2000;37(2):161– 68.
- Li W, Kuriyama S, Li Q, Nagai M, Hozawa A, Nishino Y, Tsuji I. Citrus consumption and cancer incidence: the Ohsaki cohort study. Int J Cancer 2010;127(8):1913–22.
- Garcia-Castello EM, Rodriguez-Lopez AD, Mayor L, Ballesteros R, Conidi C, Cassano A. Optimization of conventional and ultrasound assisted extraction of flavonoids from grapefruit (*Citrus paradisi* L.) solid wastes. LWT–Food Sci Technol 2015;64(2):1114–22.
- Kim SS, Park KJ, An HJ, Choi YH. Phytochemical, antioxidant, and antibacterial activities of fermented *Citrus unshiu* byproduct. Food Sci Biotechnol 2017;26(2):461–6.
- Dandekar Deepak V, Jayaprakasha Guddadarangavvanahally K, Patil Bhimanagouda S. Simultaneous extraction of bioactive limonoid aglycones and glucoside from *Citrus aurantium* L. using hydrotropy. Z Naturforsch C 2008;63(3-4):176.
- 20. Chen X-M, Tait AR, Kitts DD. Flavonoid composition of orange peel and its association with antioxidant and anti-inflammatory activities. Food Chem 2017;218:15–21.
- 21. Kroyer G. The antioxidant activity of citrus fruit peels. Z Ernahrungswiss 1986;25(1):63–9.
- 22. Zia ur R. Citrus peel extract a natural source of antioxidant. Food Chem 2006;99(3):450–4.
- 23. Azman NFIN, Khoo HE, Razman MR. Antioxidant properties of fresh and frozen peels of citrus species. Curr Res Nutr Food Sci 2019;7(2):331–9.
- 24. Kandaswami C, Lee L, Lee P, Hwang J, Ke F, Huang Y, Lee M. The antitumor activities of flavonoids. In Vivo 2005;19(5):895–909.
- 25. Yusof S, Ghazali HM, King GS. Naringin content in local citrus fruits. Food Chem 1990;37(2):113–21.
- Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NFkappaB pathway in the treatment of inflammation and cancer. J Clin Invest 2001;107(2):135–42.
- 27. Cazarolli LH, Zanatta L, Alberton EH, Figueiredo MS, Folador P, Damazio RG, Pizzolatti MG, Silva FR. Flavonoids: prospective drug candidates. Mini Rev Med Chem 2008;8(13):1429–40.
- Amutha R, Kavusik T, Sudha A. Analysis of bioactive compounds in citrus fruit peels. Int J Sci Res Rev 2018;6(12):19–26.
- Manson M. Cancer prevention the potential for diet to modulate molecular signalling. Trends Mol Med 2003;9(1):11–18.

- Milner J, McDonald S, Anderson D, Greenwald P. Molecular targets for nutrients involved with cancer prevention. Nutr Cancer 2001;41(1-2):1–16.
- Micali S, Isgro G, Bianchi G, Miceli N, Calapai G, Navarra M. Cranberry and recurrent cystitis: more than marketing? Crit Rev Food Sci Nutr 2014;54(8):1063–75.
- Han X, Shen T, Lou H. Dietary polyphenols and their biological significance. Int J Mol Sci 2007;8(9):950.
- 33. Long X, Zeng X, Yan H, Xu M, Zeng Q, Xu C, Xu Q, Liang Y, Zhang J. Flavonoids composition and antioxidant potential assessment of extracts from Gannanzao Navel Orange (*Citrus sinensis* Osbeck Cv. Gannanzao) peel. Nat Prod Res [Internet] 2019:1–5. doi:10.1080/14786419.2019.1593162.
- Middleton E. Effect of plant flavonoids on immune and inflammatory cell function. Adv Exp Med Biol 1998;439:175–82.
- 35. Peterson J, Dwyer J. Flavonoids: dietary occurrence and biochemical activity. Nutr Res 1998;18(12):1995–2018.
- Mori A, Nishino C, Enoki N, Tawata S. Cytotoxicity of plant flavonoids against HeLa cells. Phytochemistry 1988;27(4):1017–20.
- 37. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem 2002;13(10):572–84.
- 38. Lee D, Park K, Park H, Kang S, Nagappan A, Kim J, Kim E, Lee W, Hah Y, Chung H, et al. Flavonoids isolated from Korea *Citrus aurantium* L. induce G2/M phase arrest and apoptosis in human gastric cancer AGS cells. Evid Based Complement Alternat Med [Internet] 2012. https://doi.org/10.1155/2012/515901.
- 39. Park KI, Park HS, Nagappan A, Hong GE, Lee do H, Kang SR, Kim JA, Zhang J, Kim EH, Lee WS, et al. Induction of the cell cycle arrest and apoptosis by flavonoids isolated from Korean *C itrus aurantium* L. in nonsmall-cell lung cancer cells. Food Chem 2012;135(4):2728–35.
- 40. Park K-I, Park H-S, Kim M-K, Hong G-E, Nagappan A, Lee H-J, Yumnam S, Lee W-S, Won C-K, Shin S-C, et al. Flavonoids identified from Korean *Citrus aurantium* L. inhibit non-small cell lung cancer growth in vivo and in vitro. J Funct Foods 2014;7:287–97.
- 41. Du Q, Chen H. The methoxyflavones in *Citrus reticulata* Blanco cv. Ponkan and their antiproliferative activity against cancer cells. Food Chem 2010;119(2):567–72.
- 42. Zhang J, Wu Y, Zhao X, Luo F, Li X, Zhu H, Sun C, Chen K. Chemopreventive effect of flavonoids from Ougan (*Citrus reticulata* cv. Suavissima) fruit against cancer cell proliferation and migration. J Funct Foods 2014;10:511–19.
- 43. Goh JXH, Tan LT-H, Goh JK, Chan KG, Pusparajah P, Lee L-H, Goh B-H. Nobiletin and derivatives: functional compounds from citrus fruit peel for colon cancer chemoprevention. Cancers 2019;11(6):867.
- 44. Al-Saman MA, Abdella A, Mazrou KE, Tayel AA, Irmak S. Antimicrobial and antioxidant activities of different extracts of the peel of kumquat (*Citrus japonica* Thunb). Food Measure 2019;13:3221.
- 45. Benavente-Garcia O, Castillo J. Update on uses and properties of citrus flavonoids: new findings in anticancer, cardiovascular, and antiinflammatory activity. J Agric Food Chem 2008;56(15):6185–205.
- 46. Nakajima A, Ohizumi Y. Potential benefits of nobiletin, a citrus flavonoid, against alzheimer's disease and parkinson's disease. Int J Mol Sci 2019;20(14):3380.
- 47. Ledesma-Escobar CA, Priego-Capote F, Luque de-Castro MD. Relevance and analysis of citrus flavonoids. In: Watson RR editor. Polyphenols in Plants. 2nd ed. Academic Press; 2019. p. 133–50. Chapter 9.
- Marijan Seruga IT. Influence of chemical structure of some flavonols on their electrochemical behaviour. Int J Electrochem Sci 2017;12(9):7616–37.
- Li S, Lo C-Y, Ho C-T. Hydroxylated polymethoxyflavones and methylated flavonoids in sweet orange (*Citrus sinensis*) peel. J Agric Food Chem 2006;54(12):4176–85.
- Jiang N, Doseff AI, Grotewold E. Flavones: from biosynthesis to health benefits. Plants (Basel, Switzerland) [Internet] 2016;5(2):27.
- Caristi C, Bellocco E, Gargiulli C, Toscano G, Leuzzi U. Flavone-di-Cglycosides in citrus juices from Southern Italy. Food Chem 2006;95(3):431– 7.

- 52. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res [Internet] 2017;61(1):1361779. doi:10.1080/16546628.2017.1361779.
- 53. Ko H-C, Jang M-G, Kang C-H, Lee N-H, Kang S-I, Lee S-R, Park D-B, Kim S-J. Preparation of a polymethoxyflavone-rich fraction (PRF) of *Citrus sunki* Hort. ex Tanaka and its antiproliferative effects. Food Chem 2010;123(2):484–8.
- 54. Tang M, Ogawa K, Asamoto M, Hokaiwado N, Seeni A, Suzuki S, Takahashi S, Tanaka T, Ichikawa K, Shirai T. Protective effects of citrus nobiletin and auraptene in transgenic rats developing adenocarcinoma of the prostate (TRAP) and human prostate carcinoma cells. Cancer Sci 2007;98(4): 471–7.
- 55. Lai C, Li S, Chai C, Lo C, Ho C, Wang Y, Pan M. Inhibitory effect of citrus 5hydroxy-3,6,7,8,3',4'-hexamethoxyflavone on 12-O-tetradecanoylphorbol 13-acetate-induced skin inflammation and tumor promotion in mice. Carcinogenesis 2007;28(12):2581–8.
- 56. Murakami A, Nakamura Y, Torikai K, Tanaka T, Koshiba T, Koshimizu K, Kuwahara S, Takahashi Y, Ogawa K, Yano M, et al. Inhibitory effect of citrus nobiletin on phorbol ester-induced skin inflammation, oxidative stress, and tumor promotion in mice. Cancer Res 2000;60(18):5059–66.
- 57. Murakami A, Shigemori T, Ohigashi H. Zingiberaceous and citrus constituents, 1'-acetoxychavicol acetate, zerumbone, auraptene, and nobiletin, suppress lipopolysaccharide-induced cyclooxygenase-2 expression in RAW264.7 murine macrophages through different modes of action. J Nutr 2005;135(12 Suppl):2987S.
- Kurowska E, Manthey J. Hypolipidemic effects and absorption of citrus polymethoxylated flavones in hamsters with diet-induced hypercholesterolemia. J Agric Food Chem 2004;52(10):2879–86.
- Nielsen S, Breinholt V, Cornett C, Dragsted L. Biotransformation of the citrus flavone tangeretin in rats. Identification of metabolites with intact flavane nucleus. Food Chem Toxicol 2000;38(9):739–46.
- 60. Manthey J, Guthrie N. Antiproliferative activities of citrus flavonoids against six human cancer cell lines. J Agric Food Chem 2002;50(21):5837–43.
- 61. Manthey J, Bendele P. Anti-inflammatory activity of an orange peel polymethoxylated flavone, 3',4',3,5,6,7,8-heptamethoxyflavone, in the rat carrageenan/paw edema and mouse lipopolysaccharide-challenge assays. J Agric Food Chem 2008;56(20):9399–403.
- 62. Pouget C, Lauthier F, Simon A, Fagnere C, Basly J-P, Delage C, Chulia A-J. Flavonoids: structural requirements for antiproliferative activity on breast cancer cells. Bioorg Med Chem Lett 2001;11(24):3095–7.
- 63. Yanez J, Vicente V, Alcaraz M, Castillo J, Benavente-Garcia O, Canteras M, Teruel JA. Cytotoxicity and antiproliferative activities of several phenolic compounds against three melanocyte cell lines: relationship between structure and activity. Nutr Cancer 2004;49(2):191–9.
- 64. Rodriguez J, Yanez J, Vicente V, Alcaraz M, Benavente-Garcia O, Castillo J, Lorente J, Lozano JA. Effects of several flavonoids on the growth of B16F10 and SK-MEL-1 melanoma cell lines: relationship between structure and activity. Melanoma Res 2002;12(2):99–107.
- 65. Martinez C, Yanez J, Vicente V, Alcaraz M, Benavente-Garcia O, Castillo J, Lorente J, Lozano J. Effects of several polyhydroxylated flavonoids on the growth of B16F10 melanoma and Melan-a melanocyte cell lines: influence of the sequential oxidation state of the flavonoid skeleton. Melanoma Res 2003;13(1):3–9.
- 66. Kim J, Jayaprakasha G, Vikram A, Patil BS. Cancer chemopreventive properties of citrus limonoids. In: Patil BS, Jayaprakasha GK, Chidambara Murthy KN, Seeram NP, editors. Emerging Trends in Dietary Components for Preventing and Combating Disease. American Chemical Society Symposium Series 1093. Washington, DC: American Chemical Society; 2012. p. 37–50.
- Tian Q, Ding X. Screening for limonoid glucosides in *Citrus grandis* L. Osbeck by high performance liquid chromatography-electrospray ionization/mass spectrometry. J Chromatogr A 2000;874:13–19.
- Lim H-K, Moon JY, Kim H, Cho M, Cho SK. Induction of apoptosis in U937 human leukaemia cells by the hexane fraction of an extract of immature *Citrus grandis* Osbeck fruits. Food Chem 2009;114(4):1245–50.

- 69. Miyake Y, Murakami A, Sugiyama Y, Isobe M, Koshimizu K, Ohigashi H. Identification of coumarins from lemon fruit (*Citrus limon*) as inhibitors of in vitro tumor promotion and superoxide and nitric oxide generation. J Agric Food Chem 1999;47(8):3151–7.
- 70. Gyawali R, Jeon DH, Moon J, Kim H, Song YW, Hyun HB, Jeong D, Cho SK. Chemical composition and antiproliferative activity of supercritical extract of *Citrus grandis* (L.) Osbeck fruits from Korea. J Essent Oil Bear Pl 2012;15(6):915–25.
- 71. Dugrand A, Olry A, Duval T, Hehn A, Froelicher Y, Bourgaud F. Coumarin and furanocoumarin quantitation in citrus peel via ultraperformance liquid chromatography coupled with mass spectrometry (UPLC-MS). J Agric Food Chem 2013;61(45):10677–84.
- Wang Y-C, Chuang Y-C, Hsu H-W. The flavonoid, carotenoid and pectin content in peels of citrus cultivated in Taiwan. Food Chem 2008;106(1): 277–84.
- 73. Xu G, Chen J, Liu D, Zhang Y, Jiang P, Ye X. Minerals, phenolic compounds, and antioxidant capacity of citrus peel extract by hot water. J Food Sci 2007;73(1):C11.
- 74. Agócs A, Nagy V, Szabó Z, Márk L, Ohmacht R, Deli J. Comparative study on the carotenoid composition of the peel and the pulp of different citrus species. Innov Food Sci Emerg 2007;8(3):390–4.
- 75. Kim C, Lee IH, Hyun HB, Kim J-C, Gyawali R, Lee S-G, Lee J, Kim S-H, Shim BS, Cho SK, et al. Supercritical fluid extraction of *Citrus iyo* Hort. ex Tanaka pericarp inhibits growth and induces apoptosis through abrogation of STAT3 regulated gene products in human prostate cancer xenograft mouse model. Integr Cancer Ther 2017;16(2):227–43.
- Crowell P. Prevention and therapy of cancer by dietary monoterpenes. J Nutr 1999;129(3):775S.
- 77. Putnik P, Bursac Kovacevic D, Rezek Jambrak A, Barba FJ, Cravotto G, Binello A, Lorenzo JM, Shpigelman A. Innovative "green" and novel strategies for the extraction of bioactive added value compounds from citrus wastes—a review. Molecules 2017;22(5):E680.
- M'hiri N, Ioannou I, Ghoul M, Boudhrioua NM. Extraction methods of citrus peel phenolic compounds. Food Rev Int 2014;30(4):265–90.
- Amanuel L. Extraction of pectic acid from citrus fruit peels and its application as textile printing thickener. Latest Trends in Textile and Fashion Designing 2018;1(3):45–50.
- Li BB, Smith B, Hossain MM. Extraction of phenolics from citrus peels: I. Solvent extraction method. Sep Purif Technol 2006;48(2):182–8.
- Bocco A, Cuvelier M-E, Richard H, Berset C. Antioxidant activity and phenolic composition of citrus peel and seed extracts. J Agric Food Chem 1998;46(6):2123–9.
- Zhou X-M, Wen G-Y, Zhao Y, Liu Y-M, Li J-X. Inhibitory effects of alkaline extract of *Citrus reticulata* on pulmonary fibrosis. J Ethnopharmacol 2013;146(1):372–8.
- Diaz-Reinoso B, Moure A, Dominguez H, Parajo JC. Supercritical CO₂ extraction and purification of compounds with antioxidant activity. J Agric Food Chem 2006;54(7):2441–69.
- 84. Ahmad J, Langrish TAG. Optimisation of total phenolic acids extraction from mandarin peels using microwave energy: the importance of the Maillard reaction. J Food Eng 2012;109(1):162–74.
- Puri M, Verma ML, Mahale K. Processing of citrus peel for the extraction of flavonoids for biotechnological applications. Hauppauge, New York: Nova Science Publishers, Inc; 2017, p. 443–59.
- 86. Nagappan A, Lee HJ, Saralamma VVG, Park HS, Hong GE, Yumnam S, Raha S, Charles SN, Shin SC, Kim EH, et al. Flavonoids isolated from *Citrus platymamma* induced G2/M cell cycle arrest and apoptosis in A549 human lung cancer cells. Oncol Lett 2016;12(2):1394–402.
- 87. Hosokawa N, Hirayoshi K, Nakai A, Hosokawa Y, Marui N, Yoshida M, Sakai T, Nishino H, Aoike A, Kawai K, et al. Flavonoids inhibit the expression of heat shock proteins. Cell Struct Funct 1990;15(6):393–401.
- 88. Huang Y, Hwang J, Lee P, Ke F, Huang J, Huang C, Kandaswami C, Middleton E, Lee M. Effects of luteolin and quercetin, inhibitors of tyrosine kinase, on cell growth and metastasis-associated properties in A431 cells overexpressing epidermal growth factor receptor. Br J Pharmacol 1999;128(5):999–1010.

- Castillo M, Perkins E, Campbell J, Doerr R, Hassett J, Kandaswami C, Middleton E. The effects of the bioflavonoid quercetin on squamous cell carcinoma of head and neck origin. Am J Surg 1989;158(4):351–5.
- Kandaswami C, Perkins E, Soloniuk D, Drzewiecki G, Middleton E. Antitproliferative effects of citrus flavonoids on a human squamous cell carcinoma in vitro. Cancer Lett 1991;56(2):147–52.
- 91. Scambia G, Ranelletti F, Panici P, Piantelli M, Bonanno G, De Vincenzo R, Ferrandina G, Rumi C, Larocca L, Mancuso S. Inhibitory effect of quercetin on OVCA 433 cells and presence of type II oestrogen binding sites in primary ovarian tumours and cultured cells. Br J Cancer 1990;62(6):942– 6.
- 92. Lee L, Huang Y, Hwang J, Lee A, Ke F, Huang C, Kandaswami C, Lee P, Lee M. Transinactivation of the epidermal growth factor receptor tyrosine kinase and focal adhesion kinase phosphorylation by dietary flavonoids: effect on invasive potential of human carcinoma cells. Biochem Pharmacol 2004;67(11):2103–14.
- 93. Hofmann J, Ueberall F, Posch L, Maly K, Herrmann DB, Grunicke H. Synergistic enhancement of the antiproliferative activity of cisdiamminedichloroplatinum(II) by the ether lipid analogue BM41440, an inhibitor of protein kinase C. Lipids 1989;24(4):312–17.
- 94. Liao C-Y, Lee C-C, Tsai C-C, Hsueh C-W, Wang C-C, Chen IH, Tsai M-K, Liu M-Y, Hsieh A-T, Su K-J, et al. Novel investigations of flavonoids as chemopreventive agents for hepatocellular carcinoma. Biomed Res Int 2015;2015:840542. doi:10.1155/2015/840542.
- Kawaii S, Tomono Y, Katase E, Ogawa K, Yano M. Antiproliferative activity of flavonoids on several cancer cell lines. Biosci Biotechnol Biochem 1999;63(5):896–9.
- 96. Kim D-I, Lee S-J, Lee S-B, Park K, Kim W-J, Moon S-K. Requirement for Ras/Raf/ERK pathway in naringin-induced G1-cell-cycle arrest via p21WAF1 expression. Carcinogenesis 2008;29(9):1701–9.
- Cayrol C, Knibiehler M, Ducommun B. p21 binding to PCNA causes G1 and G2 cell cycle arrest in p53-deficient cells. Oncogene 1998;16:311.
- Sugiyama S, Umehara K, Kuroyanagi M, Ueno A, Taki T. Studies on the differentiation inducers of myeloid leukemic cells from citrus species. Chem Pharm Bull 1993;41(4):714–19.
- Hirano T, Abe K, Gotoh M, Oka K. Citrus flavone tangeretin inhibits leukaemic HL-60 cell growth partially through induction of apoptosis with less cytotoxicity on normal lymphocytes. Br J Cancer 1995;72:1380.
- 100. Iwase Y, Takemura Y, Ju-ichi M, Yano M, Ito C, Furukawa H, Mukainaka T, Kuchide M, Tokuda H, Nishino H. Cancer chemopreventive activity of 3,5,6,7,8,3',4'-heptamethoxyflavone from the peel of citrus plants. Cancer Lett 2001;163(1):7–9.
- 101. Surichan S, Androutsopoulos VP, Sifakis S, Koutala E, Tsatsakis A, Arroo RR, Boarder MR. Bioactivation of the citrus flavonoid nobiletin by CYP1 enzymes in MCF7 breast adenocarcinoma cells. Food Chem Toxicol 2012;50(9):3320–8.
- Rawson N, Ho C-T, Li S. Efficacious anti-cancer property of flavonoids from citrus peels. Food Science and Human Wellness 2014;3(3-4):104–9.
- 103. Suzuki R, Kohno H, Murakami A, Koshimizu K, Ohigashi H, Yano M, Tokuda H, Nishino H, Tanaka T. Citrus nobiletin inhibits azoxymethaneinduced large bowel carcinogenesis in rats. Biofactors 2004;21(1-4):111–14.
- 104. Kunimasa K, Ikekita M, Sato M, Ohta T, Yamori Y, Ikeda M, Kuranuki S, Oikawa T. Nobiletin, a citrus polymethoxyflavonoid, suppresses multiple angiogenesis-related endothelial cell functions and angiogenesis in vivo. Cancer Sci 2010;101(11):2462–9.
- 105. Yoshimizu N, Otani Y, Saikawa Y, Kubota T, Yoshida M, Furukawa T, Kumai K, Kameyama K, Fujii M, Yano M, et al. Anti-tumour effects of nobiletin, a citrus flavonoid, on gastric cancer include: antiproliferative effects, induction of apoptosis and cell cycle deregulation. Aliment Pharmacol Ther 2004;20(Suppl 1):95–101.
- 106. Mak NK, Wong-Leung YL, Chan SC, Wen J, Leung KN, Fung MC. Isolation of anti-leukemia compounds from *Citrus reticulata*. Life Sci 1996;58(15):1269–76.
- 107. Wang Y, Qian J, Cao J, Wang D, Liu C, Yang R, Li X, Sun C. Antioxidant capacity, anticancer ability and flavonoids composition of 35 citrus (*Citrus reticulata* Blanco) varieties. Molecules 2017;22(7):E1114.

- 108. Kim M, Park H, Hong M, Park H, Kim M, Leem K, Kim J, Kim Y, Kim H. *Citrus reticulata* Blanco induces apoptosis in human gastric cancer cells SNU-668. Nutr Cancer 2005;51(1):78–82.
- 109. Sergeev IN, Ho CT, Li S, Colby J, Dushenkov S. Apoptosis-inducing activity of hydroxylated polymethoxyflavones and polymethoxyflavones from orange peel in human breast cancer cells. Mol Nutr Food Res 2007;51(12):1478–84.
- 110. Hirata T, Fujii M, Akita K, Yanaka N, Ogawa K, Kuroyanagi M, Hongo D. Identification and physiological evaluation of the components from citrus fruits as potential drugs for anti-corpulence and anticancer. Bioorg Med Chem 2009;17(1):25–8.
- 111. Han M, Lee W, Lu JN, Kim G, Jung J, Ryu C, Kim G, Hwang H, Kwon T, Choi Y. *Citrus aurantium* L. exhibits apoptotic effects on U937 human leukemia cells partly through inhibition of Akt. Int J Oncol 2012;40(6):2090–6.
- 112. Jin H, Lee W, Yun J, Jung J, Yi SM, Kim H, Choi Y, Kim G, Jung J, Ryu C, et al. Flavonoids from *Citrus unshiu* Marc. inhibit cancer cell adhesion to endothelial cells by selective inhibition of VCAM-1. Oncol Rep 2013;30(5):2336–42.
- 113. Kim SH, Shin EJ, Hur HJ, Park JH, Sung MJ, Kwon DY, Hwang J-T. *Citrus junos* Tanaka peel extract attenuates experimental colitis and inhibits tumour growth in a mouse xenograft model. J Funct Foods 2014;8:301–8.
- 114. Adina AB, Goenadi FA, Handoko FF, Nawangsari DA, Hermawan A, Jenie RI, Meiyanto E. Combination of ethanolic extract of *Citrus aurantifolia* peels with doxorubicin modulate cell cycle and increase apoptosis induction on MCF-7 cells. Iran J Pharm Res 2014;13(3):919.
- 115. Ademosun Ayokunle O, Oboh G, Passamonti S, Tramer F, Ziberna L, Boligon Aline A, Athayde Margareth L. Inhibition of metalloproteinase and proteasome activities in colon cancer cells by citrus peel extracts. J Basic Clin Physiol Pharmacol 2015;26(5):471.
- 116. Moon JY, Kim H, Cho SK. Auraptene, a major compound of supercritical fluid extract of phalsak (*Citrus hassaku* Hort ex Tanaka), induces apoptosis through the suppression of mTOR pathways in human gastric cancer SNU-1 Cells. Evid-Based Compl Alt 2015;2015:10. doi:10.1155/2015/402385.
- 117. Wang B, Lin S-Y, Shen Y-Y, Li-qiang W, Chen Z-Z, Jing Li CZ, Wen-Bin Q, Jian-Ping J. Pure total flavonoids from *Citrus paradisi* Macfadyen act synergistically with arsenic trioxide in inducing apoptosis of Kasumi-1 leukemia cells in vitro. J Zhejiang Univ Sci B 2015;16(7):580–5.
- 118. Chang L, Jia S, Fu Y, Zhou T, Cao J, He Q, Yang B, Li X, Sun C, Su D, et al. Ougan (*Citrus reticulata* cv. Suavissima) flavedo extract suppresses cancer motility by interfering with epithelial-to-mesenchymal transition in SKOV3 cells. Chin Med 2015;10:14.
- 119. Hong G, Lee H, Kim J, Yumnam S, Raha S, Venkatarame Gowda Saralamma V, Heo J, Lee S, Kim E, Won C, et al. Korean Byungkyul *Citrus platymamma* Hort. et Tanaka flavonoids induces cell cycle arrest and apoptosis, regulating MMP protein expression in Hep3B hepatocellular carcinoma cells. Int J Oncol 2017;50(2):575–86.
- 120. Chu C-C, Chen S-Y, Chyau C-C, Duh P-D. Antiproliferative effect of sweet orange peel and its bioactive compounds against human hepatoma cells, in vitro and in vivo. J Funct Foods 2017;33(Suppl C):363–75.
- 121. Onuma W, Asai D, Tomono S, Miyamoto S, Fujii G, Hamoya T, Nagano A, Takahashi S, Masumori S, Miyoshi N, et al. Anticarcinogenic effects of dried citrus peel in colon carcinogenesis due to inhibition of oxidative stress. Nutr Cancer 2017;69(6):855–61.
- 122. Molinari M. Cell cycle checkpoints and their inactivation in human cancer. Cell Prolif 2000;33(5):261–74.
- 123. Grana X, Reddy E. Cell cycle control in mammalian cells: role of cyclins, cyclin dependent kinases (CDKs), growth suppressor genes and cyclin-dependent kinase inhibitors (CKIs). Oncogene 1995;11(2):211–19.
- 124. Foster I. Cancer: a cell cycle defect. Radiography 2008;14(2):144-9.
- 125. Lai C, Tsai M, Cheng A, Li S, Lo C, Wang Y, Xiao H, Ho C, Wang Y, Pan M. Chemoprevention of colonic tumorigenesis by dietary hydroxylated polymethoxyflavones in azoxymethane-treated mice. Mol Nutr Food Res 2011;55(2):278–90.
- 126. Lai C, Li S, Liu C, Miyauchi Y, Suzawa M, Ho C, Pan M. Effective suppression of azoxymethane-induced aberrant crypt foci formation in mice with citrus peel flavonoids. Mol Nutr Food Res 2013;57(3): 551–5.

- 127. Pan M-H, Li S, Lai C-S, Miyauchi Y, Suzawa M, Ho C-T. Inhibition of citrus flavonoids on 12-O-tetradecanoylphorbol 13-acetate-induced skin inflammation and tumorigenesis in mice. Food Science and Human Wellness 2012;1(1):65–73.
- 128. Wei D, Yue L, Robert TR, Geetha G, Mou-Tuan H. Inhibitory effects of oral administration of an extract of orange peel in the diet on azoxymethaneinduced formation of aberrant crypt foci and colon tumor in CF-1 mice. In: Food Factors in Health Promotion and Disease Prevention. American Chemical Society Symposium Series 851. Washington, DC: American Chemical Society; 2003. p. 213–23.
- 129. Fan K, Kurihara N, Abe S, Ho C-T, Ghai G, Yang K. Chemopreventive effects of orange peel extract (OPE) I. OPE inhibits intestinal tumor growth in ApcMin/+ mice. J Med Food 2007;10(1):11–17.
- 130. Tanaka S, Sato T, Akimoto N, Yano M, Ito A. Prevention of UVBinduced photoinflammation and photoaging by a polymethoxy flavonoid, nobiletin, in human keratinocytes in vivo and in vitro. Biochem Pharmacol 2004;68(3):433–9.
- 131. Pan M, Chen W, Lin-Shiau S, Ho C, Lin J. Tangeretin induces cell-cycle G1 arrest through inhibiting cyclin-dependent kinases 2 and 4 activities as well as elevating Cdk inhibitors p21 and p27 in human colorectal carcinoma cells. Carcinogenesis 2002;23(10):1677–84.
- 132. Shammugasamy B, Valtchev P, Dong Q, Dehghani F. Effect of citrus peel extracts on the cellular quiescence of prostate cancer cells. Food Funct 2019;10(6):3727–37.
- 133. Van Slambrouck S, Parmar V, Sharma S, De Bondt B, Fore F, Coopman P, Vanhoecke B, Boterberg T, Depypere H, Leclercq G, et al. Tangeretin inhibits extracellular-signal-regulated kinase (ERK) phosphorylation. FEBS Lett 2005;579(7):1665–9.
- 134. Morley K, Ferguson P, Koropatnick J. Tangeretin and nobiletin induce G1 cell cycle arrest but not apoptosis in human breast and colon cancer cells. Cancer Lett 2007;251(1):168–78.
- 135. Wu X, Song M, Gao Z, Sun Y, Wang M, Li F, Zheng J, Xiao H. Nobiletin and its colonic metabolites suppress colitis-associated colon carcinogenesis by down-regulating iNOS, inducing antioxidative enzymes and arresting cell cycle progression. J Nutr Biochem 2017;42:17–25.
- 136. Choi S, Ko H, Ko S, Hwang J, Park J, Kang S, Han S, Yun S, Kim S. Correlation between flavonoid content and the NO production inhibitory activity of peel extracts from various citrus fruits. Biol Pharm Bull 2007;30(4):772–8.
- 137. Vue B, Zhang S, Chen Q. Flavonoids with therapeutic potential in prostate cancer. Anticancer Agents Med Chem 2016;16(10):1205–29.
- 138. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol 2007;35(4):495–516.
- Jin Z, El-Deiry W. Overview of cell death signaling pathways. Cancer Biol Ther 2005;4(2):147.
- 140. Lai C, Li S, Miyauchi Y, Suzawa M, Ho C, Pan M. Potent anti-cancer effects of citrus peel flavonoids in human prostate xenograft tumors. Food Funct 2013;4(6):944–9.
- 141. Kim M, Bo H, Choi E, Kwon D, Kim H, Ahn K, Ji S, Jeong J, Park S, Hong S, et al. Induction of apoptosis by *Citrus unshiu* peel in human breast cancer MCF-7 Cells: involvement of ROS-dependent activation of AMPK. Biol Pharm Bull 2018;41(5):713–21.
- 142. Wang L, Wang J, Fang L, Zheng Z, Zhi D, Wang S, Li S, Ho C-T, Zhao H. Anticancer activities of citrus peel polymethoxyflavones related to angiogenesis and others. Biomed Res Int 2014;2014:1.
- 143. Hata A, Engelman J, Faber A. The BCL2 family: key mediators of the apoptotic response to targeted anticancer therapeutics. Cancer Discov 2015;5(5):475–87.
- 144. Yu S, Andrabi S, Wang H, Kim N, Poirier G, Dawson T, Dawson V. Apoptosis-inducing factor mediates poly(ADP-ribose) (PAR) polymerinduced cell death. Proc Natl Acad Sci U S A 2006;103(48):18314–19.
- 145. Luo G, Guan X, Zhou L. Apoptotic effect of citrus fruit extract nobiletin on lung cancer cell line A549 in vitro and in vivo. Cancer Biol Ther 2008;7(6):966–73.
- 146. Middleton E, Kandaswami C, Theoharides T. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000;52(4):673–751.

- 147. Hanahan D, Weinberg R. Hallmarks of cancer: the next generation. Cell 2011;144(5):646-74.
- 148. Schindler R, Mentlein R. Flavonoids and vitamin E reduce the release of the angiogenic peptide vascular endothelial growth factor from human tumor cells. J Nutr 2006;136(6):1477–82.
- 149. Lamy S, Akla N, Ouanouki A, Lord-Dufour S, Beliveau R. Diet-derived polyphenols inhibit angiogenesis by modulating the interleukin-6/STAT3 pathway. Exp Cell Res 2012;318(13):1586–96.
- 150. Lam I, Alex D, Wang Y, Liu P, Liu A, Du G, Lee S. In vitro and in vivo structure and activity relationship analysis of polymethoxylated flavonoids: identifying sinensetin as a novel antiangiogenesis agent. Mol Nutr Food Res 2012;56(6):945–56.
- 151. Sp N, Kang DY, Kim DH, Park JH, Lee HG, Kim HJ, Darvin P, Park Y-M, Yang YM. Nobiletin inhibits CD36-dependent tumor angiogenesis, migration, invasion, and sphere formation through the Cd36/Stat3/Nf-Kb signaling axis. Nutrients 2018;10(6):E772.
- 152. Arivazhagan L, Sorimuthu Pillai S. Tangeretin, a citrus pentamethoxyflavone, exerts cytostatic effect via p53/p21 up-regulation and suppresses metastasis in 7,12-dimethylbenz(α)anthracene-induced rat mammary carcinoma. J Nutr Biochem 2014;25(11):1140–53.
- 153. Hamdy F, Fadlon E, Cottam D, Lawry J, Thurrell W, Silcocks P, Anderson J, Williams J, Rees R. Matrix metalloproteinase 9 expression in primary human prostatic adenocarcinoma and benign prostatic hyperplasia. Br J Cancer 1994;69(1):177–82.
- 154. Kim C, Kim D, Nam D, Chung W, Ahn K, Kim S, Choi S, Shim B, Cho S, Ahn K. Anti-metastatic effect of supercritical extracts from the *Citrus hassaku* pericarp via inhibition of C-X-C chemokine receptor type 4 (CXCR4) and matrix metalloproteinase-9 (MMP-9). Phytother Res 2014;28(9):1374–82.
- 155. Zhang X, Huang S, Xu Q. Quercetin inhibits the invasion of murine melanoma B16-BL6 cells by decreasing pro-MMP-9 via the PKC pathway. Cancer Chemother Pharmacol 2004;53(1):82–8.
- 156. Bachmeier B, Iancu C, Jochum M, Nerlich A. Matrix metalloproteinases in cancer: comparison of known and novel aspects of their inhibition as a therapeutic approach. Expert Rev Anticancer Ther 2005;5(1):149–63.
- 157. Park J, Shin M, Kim S, Kim H, Kim K, Shin K, Kang K. Polysaccharides from Korean *Citrus hallabong* peels inhibit angiogenesis and breast cancer cell migration. Int J Biol Macromol 2016;85:522–9.
- 158. Onder T, Gupta P, Mani S, Yang J, Lander E, Weinberg R. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. Cancer Res 2008;68(10):3645–54.
- 159. Ohshima H, Tatemichi M, Sawa T. Chemical basis of inflammation-induced carcinogenesis. Arch Biochem Biophys 2003;417(1):3–11.
- 160. Gosslau A, Chen KY, Ho C-T, Li S. Anti-inflammatory effects of characterized orange peel extracts enriched with bioactive polymethoxyflavones. Food Science and Human Wellness 2014;3(1):26–35.
- 161. Oh Y-C, Cho W-K, Jeong YH, Im GY, Yang MC, Hwang Y-H, Ma JY. Antiinflammatory effect of *Citrus unshiu* peel in LPS-stimulated RAW 264.7 macrophage cells. Am J Chin Med 2012;40(03):611–29.
- 162. Shin H-S, Kang S-I, Ko H-C, Kim H-M, Hong Y-S, Yoon S-A, Kim S-J. Antiinflammatory effect of the immature peel extract of Jinkyool (*Citrus sunki* Hort. ex Tanaka). Food Sci Biotechnol 2011;20(5):1235–41.
- 163. Jung KH, Ha E, Kim MJ, Won H-J, Zheng LT, Kim HK, Hong SJ, Chung JH, Yim S-V. Suppressive effects of nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression by *Citrus reticulata* extract in RAW 264.7 macrophage cells. Food Chem Toxicol 2007;45(8):1545–50.
- 164. Karin M, Lin A. NF-kappaB at the crossroads of life and death. Nat Immunol 2002;3(3):221–7.
- 165. Tsai S-H, Lin-Shiau S-Y, Lin J-K. Suppression of nitric oxide synthase and the down-regulation of the activation of NF κ B in macrophages by resveratrol. Br J Pharmacol 1999;126(3):673–80.
- 166. Etoh T, Kim YP, Hayashi M, Suzawa M, Li S, Ho C-T, Komiyama K. Inhibitory effect of a formulated extract from multiple citrus peels on LPSinduced inflammation in RAW 246.7 macrophages. Functional Foods in Health and Disease 2013;3(6):242–53.
- 167. Kang S, Han D, Park K, Park H, Cho Y, Lee H, Lee W, Ryu C, Ha Y, Lee do H, et al. Suppressive effect on lipopolysaccharide-induced

proinflammatory mediators by *Citrus aurantium* L. in macrophage RAW 264.7 cells via NF-kappaB signal pathway. Evid Based Complement Alternat Med 2011;2011:248592. doi:10.1155/2011/248592.

- 168. Wang A-Y, Zhou M-Y, Lin W-C. Antioxidative and anti-inflammatory properties of *Citrus sulcata* extracts. Food Chem 2011;124(3): 958–63.
- 169. Pan M, Ho C. Chemopreventive effects of natural dietary compounds on cancer development. Chem Soc Rev 2008;37(11):2558–74.
- 170. Alshatwi A, Ramesh E, Periasamy V, Subash-Babu P. The apoptotic effect of hesperetin on human cervical cancer cells is mediated through cell cycle arrest, death receptor, and mitochondrial pathways. Fundam Clin Pharmacol 2013;27(6):581–92.
- 171. Kim HJ, Yonezawa T, Teruya T, Woo J-T, Cha B-Y. Nobiletin, a polymethoxy flavonoid, reduced endothelin-1 plus SCF-induced pigmentation in human melanocytes. Photochem Photobiol 2015;91:379– 86.
- 172. Yoshizaki N, Fujii T, Hashizume R, Masaki H. A polymethoxyflavone mixture, extracted from orange peels, suppresses the UVB-induced expression of MMP-1. Exp Dermatol 2016;25(Suppl 3):52–6.
- 173. Charoensinphon N, Qiu P, Dong P, Zheng J, Ngauv P, Cao Y, Li S, Ho C-T, Xiao H. 5-Demethyltangeretin inhibits human nonsmall cell lung cancer cell growth by inducing G2/M cell cycle arrest and apoptosis. Mol Nutr Food Res 2013;57(12):2103–11.
- 174. Lu C, Zhu F, Cho Y, Tang F, Zykova T, Ma W, Bode A, Dong Z. Cell apoptosis: requirement of H2AX in DNA ladder formation, but not for the activation of caspase-3. Mol Cell 2006;23(1):121–32.
- 175. Breinholt V, Lauridsen S, Dragsted L. Differential effects of dietary flavonoids on drug metabolizing and antioxidant enzymes in female rat. Xenobiotica 1999;29(12):1227–40.
- 176. Chen K, Weng M, Lin J. Tangeretin suppresses IL-1beta-induced cyclooxygenase (COX)-2 expression through inhibition of p38 MAPK, JNK, and AKT activation in human lung carcinoma cells. Biochem Pharmacol 2007;73(2):215–27.
- 177. Pereira CV, Duarte M, Silva P, Bento da Silva A, Duarte CMM, Cifuentes A, García-Cañas V, Bronze MR, Albuquerque C, Serra AT. Polymethoxylated flavones target cancer stemness and improve the antiproliferative effect of 5-fluorouracil in a 3D cell model of colorectal cancer. Nutrients 2019;11(2):326.
- 178. Laavola M, Nieminen R, Yam M, Sadikun A, Abdullah M, Basir R, Welling J, Vapaatalo H, Korhonen R, Moilanen E. Flavonoids eupatorin and sinensetin present in *Orthosiphon stamineus* leaves inhibit inflammatory gene expression and STAT1 activation. Planta Med 2012;78: 779–86.
- 179. Arifianti L, Sukardiman S, Hadi Santosa M. Sinensetin-rich fraction solid dispersion inhibits cancer cell cycle. KnE Life Sci 2017;3:436.
- 180. Dong Y, Ji G, Cao A, Shi J, Shi H, Xie J, Wu D. Effects of sinensetin on proliferation and apoptosis of human gastric cancer AGS cells. Zhongguo Zhong Yao Za Zhi 2011;36:790–94.
- 181. Choi C, Sun K, An C, Yoo J, Hahm K, Lee I, Sohng J, Kim Y. Reversal of P-glycoprotein-mediated multidrug resistance by 5,6,7,3',4'pentamethoxyflavone (sinensetin). Biochem Biophys Res Commun 2002;295(4):832–40.
- 182. Androutsopoulos V, Ruparelia K, Arroo R, Tsatsakis A, Spandidos D. CYP1-mediated antiproliferative activity of dietary flavonoids in MDA-MB-468 breast cancer cells. Toxicology 2009;264(3):162–70.
- 183. Sergeev I, Li S, Colby J, Ho C, Dushenkov S. Polymethoxylated flavones induce Ca(2+)-mediated apoptosis in breast cancer cells. Life Sci 2006;80(3):245–53.
- 184. Kim H, Moon J, Mosaddik A, Cho S. Induction of apoptosis in human cervical carcinoma HeLa cells by polymethoxylated flavone-rich *Citrus* grandis Osbeck (Dangyuja) leaf extract. Food Chem Toxicol 2010;48(8-9):2435–42.
- 185. Tan K-T, Lin M-X, Lin S-C, Tung Y-T, Lin S-H, Lin C-C. Sinensetin induces apoptosis and autophagy in the treatment of human T-cell lymphoma. Anticancer Drugs 2019;30(5):485–94.
- 186. Samidurai D, Pandurangan AK, Krishnamoorthi SK, Perumal MK, Nanjian R. Sinensetin isolated from Orthosiphon aristatus inhibits cell proliferation

and induces apoptosis in hepatocellular carcinoma cells. Process Biochem 2019;81:156–64.

- 187. Roohbakhsh A, Parhiz H, Soltani F, Rezaee R, Iranshahi M. Molecular mechanisms behind the biological effects of hesperidin and hesperetin for the prevention of cancer and cardiovascular diseases. Life Sci 2015;124:64– 74.
- 188. Stanisic D, Costa AF, Fávaro WJ, Tasic L, Seabra A, Duran N. Anticancer activities of hesperidin and hesperetin in vivo and their potentiality against bladder cancer. J Nanomed Nanotechnol 2018;9:6. doi:10.4172/2157-7439.1000515.
- 189. Zhang J, Wu D, Vikash SJ, Wang J, Yi J, Dong W. Hesperetin induces the apoptosis of gastric cancer cells via activating mitochondrial pathway by increasing reactive oxygen species. Dig Dis Sci 2015;60(10):2985–95.
- 190. Sak K, Lust H, Kase M, Saar M, Jaal J. Suppression of taxanes cytotoxicity by citrus flavonoid hesperetin in PPC-1 human prostate cancer cells. Anticancer Res 2018;38(11):6209–15.
- 191. Ye L, Chan FL, Chen S, Leung LK. The citrus flavonone hesperetin inhibits growth of aromatase-expressing MCF-7 tumor in ovariectomized athymic mice. J Nutr Biochem 2012;23(10):1230–7.
- 192. Babukumar S, Vinothkumar V, Velu P, Ramachandhiran D, Nirmal MR. Molecular effects of hesperetin, a citrus flavanone on 7,12dimethylbenz(a)anthracene induced buccal pouch squamous cell carcinoma in golden Syrian hamsters. Arch Physiol Biochem 2017;123(4):265–78.
- Chen Y, Shen S, Lin H. Rutinoside at C7 attenuates the apoptosis-inducing activity of flavonoids. Biochem Pharmacol 2003;66(7):1139–50.
- 194. Leslie E, Mao Q, Oleschuk C, Deeley R, Cole S. Modulation of multidrug resistance protein 1 (MRP1/ABCC1) transport and ATPase activities by interaction with dietary flavonoids. Mol Pharmacol 2001;59(5):1171–80.
- 195. Wong K-C, Pang W-Y, Wang X-L, Mok S-K, Lai W-P, Chow H-K, Leung P-C, Yao X-S, Wong M-S. *Drynaria fortunei*-derived total flavonoid fraction and isolated compounds exert oestrogen-like protective effects in bone. Br J Nutr 2013;110(3):475–85.
- 196. Chen R, Qi Q-L, Wang M-T, Li Q-Y. Therapeutic potential of naringin: an overview. Pharm Biol 2016;54(12):3203–10.
- 197. Habauzit V, Sacco SM, Gil-Izquierdo A, Trzeciakiewicz A, Morand C, Barron D, Pinaud S, Offord E, Horcajada M-N. Differential effects of two citrus flavanones on bone quality in senescent male rats in relation to their bioavailability and metabolism. Bone 2011;49(5):1108–16.
- 198. Anwar A, Uddin N, Siddiqui BS, Siddiqui RA, Begum S, Choudhary MI. A natural flavonoid lawsonaringenin induces cell cycle arrest and apoptosis in HT-29 colorectal cancer cells by targeting multiple signalling pathways. Mol Biol Rep 2018;45(5):1339–48.
- 199. Yoshinaga A, Kajiya N, Oishi K, Kamada Y, Ikeda A, Chigwechokha PK, Kibe T, Kishida M, Kishida S, Komatsu M, et al. NEU3 inhibitory effect of naringin suppresses cancer cell growth by attenuation of EGFR signaling through GM3 ganglioside accumulation. Eur J Pharmacol 2016;782:21–9.
- 200. Cai L, Wu H, Tu C, Wen X, Zhou B. Naringin inhibits ovarian tumor growth by promoting apoptosis: an in vivo study. Oncol Lett 2018;16(1): 59–64.

- 201. Nandakumar N, Haribabu L, Perumal S, Balasubramanian MP. Therapeutic effect of hesperidin with reference to biotransformation, lysosomal and mitochondrial TCA cycle enzymes against 7,12dimethylbenz(a)anthracene-induced experimental mammary cellular carcinoma. Biomedicine & Aging Pathology 2011;1(3):158–68.
- 202. Lee K-H, Yeh M-H, Kao S-T, Hung C-M, Liu C-J, Huang Y-Y, Yeh C-C. The inhibitory effect of hesperidin on tumor cell invasiveness occurs via suppression of activator protein 1 and nuclear factor-kappaB in human hepatocellular carcinoma cells. Toxicol Lett 2010;194(1):42–9.
- 203. Febriansah R, Putri DDP, Sarmoko Nurulita NA, Meiyanto E, Nugroho AE. Hesperidin as a preventive resistance agent in MCF-7 breast cancer cells line resistance to doxorubicin. Asian Pac J Trop Biomed 2014;4(3):228–33.
- 204. Kusharyanti I, Larasati L, Susidarti R, Meiyanto E. Hesperidin increase cytotoxic activity of doxorubicin on HeLa cell line through cell cycle modulation and apoptotis induction. Indones J Cancer Chemoprevent 2011;2:267–73.
- 205. Zhao J, Li Y, Gao J, De Y. Hesperidin inhibits ovarian cancer cell viability through endoplasmic reticulum stress signaling pathways. Oncol Lett 2017;14(5):5569–74.
- 206. Hana R, Bawi B. Hesperidin inhibits angiogenesis, induces apoptosis, and suppresses laryngeal cancer cell metastasis. Ibnosina J Med Biomed Sci 2018;10(5):169–73.
- 207. Hursting S, Cantwell M, Sansbury L, Forman M. Nutrition and cancer prevention: targets, strategies, and the importance of early life interventions. Nestle Nutr Workshop Ser Pediatr Program 2006;57:153–202.
- 208. Wei H, Tye L, Bresnick E, Birt D. Inhibitory effect of apigenin, a plant flavonoid, on epidermal ornithine decarboxylase and skin tumor promotion in mice. Cancer Res 1990;50(3):499–502.
- 209. Aranganathan S, Selvam J, Sangeetha N, Nalini N. Modulatory efficacy of hesperetin (citrus flavanone) on xenobiotic-metabolizing enzymes during 1,2-dimethylhydrazine-induced colon carcinogenesis. Chem Biol Interact 2009;180(2):254–61.
- 210. Lakshmi A, Subramanian S. Chemotherapeutic effect of tangeretin, a polymethoxylated flavone studied in 7,12-dimethylbenz(a)anthracene induced mammary carcinoma in experimental rats. Biochimie 2014;99:96– 109.
- 211. Michiko Suzawa LG, Min-Hsiung P, Chi-Tang Ho, Shiming Li. In vivo anticarcinogenic property of a formulated citrus peel extract. Functional Foods in Health and Disease 2014;4(2):120–9.
- 212. Zhang L, Xu X, Jiang T, Wu K, Ding C, Liu Z, Zhang X, Yu T, Song C. *Citrus aurantium* naringenin prevents osteosarcoma progression and recurrence in the patients who underwent osteosarcoma surgery by improving antioxidant capability. Oxid Med Cell Longev 2018;2018:8713263. doi:10.1155/2018/8713263.
- 213. Manthey J, Grohmann K. Phenols in citrus peel byproducts. Concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. J Agric Food Chem 2001;49(7):3268–73.
- 214. Thilakarathna SH, Rupasinghe HPV. Flavonoid bioavailability and attempts for bioavailability enhancement. Nutrients 2013;5(9): 3367–87.