Citrus peel flavonoids as potential cancer prevention agents

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List of Abbreviations:

ACF – Aberrant crypt foci

APAF-1 – Apoptosis protease activating factor-1

COX – Cyclooxygenase

CDK – Cyclin-dependent kinases

CPE – Citrus peel extract

DMBA – 7,12-dimethylbenz(α)anthracene

EMT – Epithelial mesenchymal transition

GSK-3β – Glycogen synthase kinase-3β

GST – Glutathione transferase

IAP – Inhibitor of apoptosis

iNOS – Inducible nitric oxide synthase

JAK – Janus-like kinase

MAPK – Mitogen-activated protein kinase

MMP – Matrix metalloproteinase

mTOR – Mechanistic target of rapamycin

NF-κB – Nuclear factor kappa-light-chain-enhancer of activated B cells

ODC – Ornithine decarboxylase enzyme

PARP – Poly ADP-ribose polymerase
PCNA – Proliferating cells’ nuclear antigen

PGP – Permeability glycoprotein

PKC – Protein kinase C

PMF – Polymethoxylated flavone

STAT-3 – Signal transducer and activator of transcription 3

tPA – Tissue plasminogen activator

VCAM – Vascular cell adhesion molecule

**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 anti-cancer 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 started to elucidate the underlying cell signaling pathways that are responsible for the flavonoids’ mechanism of action. This growing data supports further research into the chemo-preventative potential of citrus peel extracts, and purified flavonoids in particular. This critical review highlights new research in the field as well as synthesizes the pathways modulated by flavonoids and other polyphenolic compounds into a generalized schema.
Keywords: citrus peel extract, flavonoids, anti-cancer, inflammation, mechanism of action, apoptosis

1. 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 widely distribute 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 as they contain carotenes, essential oils, pectin and a range of polyphenolic compounds (3). Epidemiological studies have suggested that high consumption of fruits and vegetables (>400g/day) can reduce cancer risk by at least 20% (4). The Mediterranean diet is rich in fruit pulp and juice, and the associated high intake of fiber, antioxidants, and polyphenol compounds are linked with lower cancer risk (5, 6).

The medicinal usage of citrus peels can be traced back to the 10th century, but the biological activities of specific chemicals within the peel have only been recently 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 can be classified into 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 anti-cancer activity (15-17) as well as in the prevention of infectious and degenerative diseases (18-20). While it is appealing to identify specific molecules with high anti-cancer
activity, there is growing evidence to suggest synergy between bioactive molecules in citrus peel extract (CPE). Whole CPEs have been shown to have higher anti-cancer activity than the fractionated extracts and isolated single compounds. Indeed the methanolic extracts and freeze-dried CPEs are correlated to higher concentrations of total phenolic and flavonoid contents (21-23).

Several salient reviews should be noted. Cirmi et al. 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 (4). Kandaswami et al. describe the general utility of flavonoid compounds (not specifically from citrus) in modulating cell signaling pathways (24). This critical review focuses on the bioactive compounds that are enriched in the citrus peel and examines their underlying mechanism of action. This is timely based on growing efforts to utilize CPE as chemopreventive agents (25), as well as leverage their anti-atherogenic, anti-carcinogenic, anti-inflammatory (26), anti-cancer (27), anti-diarrheal and anti-microbial 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 versus 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 the most of cases there has been no proper follow up in vivo or clinical research.

2. Flavonoid Subtypes within Citrus Peel Extract
Flavonoids are low molecular weight compounds that are responsible for the vivid color of fruit peels, pulp, and leaves (11). They are abundantly found in citrus fruits, seeds, olive oil, red wine, and tea. More than 9000 flavonoids have been identified to date. Flavonoids feature a basic C6-C3-C6, 15-carbon skeleton. They are comprised of two benzene rings (A and B), which are linked via a heterocyclic pyran ring (C). Flavonoids are subdivided according to the presence of an oxy moiety at carbon atom 4, a double bond between positions 2 and 3, or a hydroxyl group in position 3 of a heterocyclic ring (C) (Figure 1).

The biological activities of flavonoids increase with the degree of hydroxylation of the B ring (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 the ring B, the presence of a 2,3 double bond and of a 4-oxo moiety in the ring C as well as a 3-hydroxy-4-keto and/or 5-hydroxy-4-keto conformation in the ring C and A (36, 37).

Flavonoids with a hydroxyl group in position C-3 of the C ring are named as flavonols and those lacking such OH moiety flavanones and flavones. Figure 2 illustrates the main structural formula 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's are effected by flavonoids via their antioxidant properties (42, 45, 46). There is evidence 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 the major classes of flavonoids and present mostly in glycoside forms such as hesperidin, neo hesperidin, narirutin, naringin, eriocitrin, and neo eriocitrin. The glycosidic forms are divided into two types – rutinosides and neo hesperidosides. Both rutinose and neo hesperidose are glycosylated at position 7 and disaccharides are formed by glucose (Figure 2). The bitter taste of neo eriocitrin, naringin and neo hesperidin occur by the presence of neo hesperidose (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, 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 one additional -OH moiety at position C-3 in the C ring. Additionally, the 3-OH group can be glycosylated by different sugars, which significantly increases the number of flavonols 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-hydroxyl 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-phenylchromenylum cation) are structurally derived from pyran, flavan, and flavones which are found only in grapefruit and blood oranges (4). Anthocyanidins are the aglycones counterpart of anthocyanins that are natural pigments of fruits that are 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, 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 two or more 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 anti-carcinogenic (54, 55), antioxidant, cardiovascular protection, anti-proliferation, anti-atherogenic (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 anti-proliferative activities of flavonoids are related to their specific structural factors 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 on 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 three or more hydroxyl/methoxyl groups in each rings (A or B) of the flavonoid skeleton significantly increased the anti-proliferative activity in human melanoma B16F10 and SK-MEL-1 cell lines as well (64, 65). Up to 62 glucoside and aglycone limonoids have been reported as present in citrus fruits (66). Obacunone glucoside and nomilin acid glucoside are the major limonoid glucosides in CPEs...
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 were found in citrus peels (68-71). Cinnamic acids (caffeic, p-coumaric, chlorogenic, ferulic, and sinapic) and benzoic acids (protocatechuic, p-hydroxybenzoic, and vanillic) are phenolic acids found in low concentration 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) which has anti-cancer activity in humans (76).

3. Extraction of Flavonoids from Citrus Waste

In order to maximise the yield of bioactive flavonoid compounds from citrus peel, several different methods for the extraction of have been reported in the literature (77). Recommended methods include: 1) chemical methods including: hot water extraction (78, 79), solvent extraction (80), and alkaline extraction (81, 82), 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 transfers from the solids to the surrounding solvents during the extraction. The temperature and time of extraction are
specific for different kind of flavonoids. The limitations of chemical methods are the requirements for several hours 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 environment-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 are used for citrus flavonoids have their own advantages and limitations. However, combined approaches may 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 environment-friendly process (85).

4. Mechanism of Action of Citrus Peel Extract Flavonoids

CPEs have been reported to show anticancer activity in various cancer lines at different efficacious levels, which 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 that CPEs have potential in reducing the risk of cancer development and progression (Table 1 and Table 2).

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

4.1 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 in distant parts of the body.
Flavonoids found in CPEs were shown to suppress these events through modulation of multiple cellular proteins that inhibit cell proliferation by down-regulation of oncoproteins. In human lung carcinoma A549 cells, the methanol extract of Korean *citrus aurantium* fruit peel inhibited cell proliferation dose dependently and inducing apoptosis as well (86). Similar inhibitory effects were also observed with flavonoids isolated from Korean *citrus aurantium* peel in A549 cancer cells (39).

Quercetin - the aglycone form of polyhydroxylated flavonoid (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 a 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 cancer cells (HepG2) (88, 92). Indeed, quercetin’s strong anti-proliferative 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 anti-proliferative 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 anti-proliferation effect of naringin is correlated with the inhibition of cell survival by binding ATP on PI3K binding site; prohibition of cell growth and modulation of cell-cycle-associated proteins by inhibition of ERK-signaling pathway (96); and/or binding to p21 to proliferating cells’ nuclear antigen (PCNA) and blocking DNA synthesis (97). Naringenin and hesperetin exhibited strong anti-proliferative activity against a broad spectrum of human (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 CYP1B1 and CYP1A1-selective (the main oxidizing enzymes which are major determinants of resistance) inhibitors upregulation (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 illustrated direct cytotoxicity in gastric cancer cells MKN-45, TMK-1, MKN-74, and KATO-III cells through cell cycle deregulation (105).

CPEs can modulate proteins involved with cell growth such as epidermal growth factor receptor and Ras that have a range of downstream pathways including mitogen-activated protein kinases (MAPK), phosphatidylinositol Akt, 3-kinase PI3K/Akt, and mechanistic target of rapamycin (mTOR). Methanol extract from freeze dried Korean *citrus platymamma* flavonoids reduced the proliferation of Hep3B cells by inhibiting PI3K and Akt phosphorylation and increased the ERK1/2, JNK, and p38 MAPK phosphorylation; these reduced PI3K/AKT signaling and increased MAPK activity (106). Methanol extract of the peel of *citrus aurantium* L. also suppressed the phosphorylation of Akt in U937 cells (107), and mTOR in SNU-1 cancer cell lines (108). In A549 cells, the ethanolic extract from *citrus aurantifolia* peels inhibited cell proliferation dose-dependently while inducing apoptosis (39, 86, 109). The suppression of growth signals was ascribed to Akt, Ras, ERK1/2, and E-cadherin in colon tumor-bearing mice (110). The treated mice showed low levels of inactive glycogen synthase kinase-3β (GSK-3β) and low accumulation of β-catenin in cell nuclei, which limits the growth of signaling pathways. The oral administration of CPEs from Gold Lotion has been reported to considerably reduce the ornithine decarboxylase enzyme (ODC),
which controls cell growth and proliferation through the biosynthesis and metabolism of polyamines in treated mice with colorectal cancer (110-112).

4.2 Cell Cycle Inhibition

Cell cycle dysfunction is correlated with cancer development. Cell cycle progression is a complex and highly regulated process and consists of four phases: G1, S, G2, and M (113). 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 malignant cells the tumor or metastatic cancer burden can be reduced or eliminated (114, 115).

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 *citrus hassaku* Hort ex. Tanaka peel, caused cell cycle arrest mainly at G1 phase (108, 116). The ethanolic extract of *citrus aurantifolia* lime peels at concentration of 6 μg/mL induced apoptosis and cell accumulation at G1 phase, while the 15 μg/mL induced apoptosis and cell accumulation at G2/M phase (38, 39, 86, 106, 109). CPEs have been shown to up-regulate the expression of p21 (cyclin-dependent kinase inhibitor 1) and/or p53 (tumor suppressor protein) leading to G1 arrest as observed in breast cancer cell lines MCF-7 (109), human gastric cells SNU-1 (108), DU145 prostate cancer cells (75), and COLO 205 human colon carcinoma cells (109, 117). The CPEs can also arrest cell cycle at G2/M by increasing the expression of p21 and decreasing the expression of cyclin B1, CDC25C, and CDC2 in A549, Hep3B, and AGS cells (38, 39, 86, 106). A water-based
extract from citrus sinensis L. peel (that chiefly contains hesperidin and narirutin) modulates the cell cycle of quiescent (PC-3 and LNCaP) prostate cancer cells that was impaired in their ability to enter the S phase (2–3% reduction of G0/G1 cells compared to 12–18% reduction of control cells) (118).

Tangeretin induced G1 phase by increasing the expression of p37 and p21 in COLO 205 human colon carcinoma cells (117) and prohibited the growth of estradiol-stimulated T47D cells (119). Nobiletin modulates cell cycle on MKN-45, TMK-1, KATO-III human gastric carcinoma cells (105), and MKN-74 as well as induced G1 phase arrest in MCF-7, MDA-MB-435 breast cancer cells, and HT-29 colon cancer cell lines (120, 121). Hesperetin decreased MCF-7 breast cancer cells activity by accumulating cells in G1 phase through the inhibition of CDK4, CDK2, and cyclin D; upregulation of p21 and p27; and increasing the binding of p21 and CDK4 (122). 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 up-regulation of p21 (96). Apigenin also arrested both androgen-insensitive PC-3 and androgen-sensitive LNCaP human prostate cancer cell cycle in the G2/M phase by activation of a cyclin kinase suppressor WAF1/p21 (123) (Table 3).

4.3 Induction of Apoptosis

Apoptosis and necrosis are two distinct mechanisms of cell death in eukaryotes cells.

Apoptosis or programmed cell death is involved in embryonic development, hormone-dependent atrophy, and metamorphosis. These processes eliminate damaged or unwanted cells (124). The apoptosis is characterised by plasma blebbing, cell shrinkage, and fragmented nuclei/DNA (125), which is reported in variety of cancer cells treated with CPE extract in vitro (38, 40, 42, 53, 68, 70, 75, 106-108, 126) and in vivo mouse model (127). Citrus peel polymethoxyflavones and citrus peel extract from citrus unshiu induce apoptosis mainly through the intrinsic pathway by reducing anti-apoptotic Bcl-2 proteins (Bcl-2 and
Bcl-XL) and increasing pro-apoptotic proteins (Bax, Bid, Bak, and Bad) in different cancer cell lines (105, 128-130). The increase in the ratios of Bax/Bcl-XL and Bax/Bcl-2 allows the release of cytochrome C through the permeabilised mitochondrial membrane. Following the binding of cytochrome C to the apoptosis protease activating factor-1 (Apaf-1) and formation of an apoptosome complex, activation of caspase-9 and the apoptosis effector protein caspase-3 is achieved (131).

Increasing of caspase-9 and caspase-3 was reported following treatment with CPEs (super critical extract of *citrus hassaku* peels) for many cancer lines including gastric carcinoma SNU-668 (132) and SNU-1 (108), adenocarcinoma human alveolar basal epithelial cells A549 (40, 86), histiocytic lymphoma U937 (68, 107), metastatic prostate cancer DU145 (75), AGS (38), hepatocellular carcinoma Hep3B (106) and HepG2 (116), as well as acute myeloblastic leukemia Kasumi-1 by the extract of citrus peel (*paradise macfad*) (110, 126). CPEs increased the levels of cleaved the poly ADP-ribose polymerase (PARP) inhibitors in U937, SNU-1, AGS, Kasumi-1, A549, Hep3B, DU145 and colon cancer cells (38, 68, 75, 86, 106-108, 110, 126). CPE can also reduce endogenous inhibitor of apoptosis (IAP) proteins such as XIAP, cIAP1, and cIAP2 in U937 (107) 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 protein ratio of Bax/Bcl-2 in human lung adenocarcinoma cell line A549 cells (133). Tangeretin induced apoptosis in leukemia HL-60 cells through 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, 134).

Accumulated evidence supports that CPE has negligible apoptosis inducing 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), pro-
apoptotic ligands such as TRAIL, FasL, and FADD were unchanged (107). Similarly, no reduction in the Fas and FasL proteins was observed in Hep3B cells treated with CPE (106). Further research is required to clarify the precise modulation of extrinsic apoptotic pathways involving cell death receptors by CPEs.

4.4 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 (135). Whereas angiogenesis is essential for the growth of different cancers, vascular targeting was considered as a potential strategy to reduce tumor growth and metastasis. Flavonoids are anti-angiogenic 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 (136). The anti-angiogenic 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 (137). Quercetin reduced tube formation of VEGF-stimulated human umbilical vein endothelial cells (HUVECs) by 40% in vitro (138). Luteolin and apigenin are the most potent angiogenesis inhibitors through inhibiting the release of inflammatory cytokine IL-6 and the STAT3 pathway (137). Hydroxylated PMFs suppress the expression of MMP 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, kdr1, hras, and fl1 in zebrafish (138). Nobiletin inhibited angiogenesis by regulating cell cycle progression through G0/G1 arrest in vivo (138). Nobiletin suppressed CD36 expression and decreased the expression of TSP-1 - an endogenous inhibitor of angiogenesis - and TGF-β1 (139). Eventually, the expression of VEGF was dramatically modified in DMB–induced animals by tangeretin treatment (140).
4.5 Inhibition of Metastasis

In metastasis, the cancer cells break away from a primary tumor to the 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. Matrix metalloproteinases (MMPs) such as MMP-2 and MMP-9 are the main proteins that are necessary for metastasis as they break down the extracellular matrix and allow the cancer cells to migrate (141).

The anti-metastatic 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 (106), MDA-MB-231 breast cancer cells (142), Caco-2, LoVo, and LoVo/ADR colon cancer cell lines (143). In one notable study, quercetin decreased the invasion of murine melanoma cells by suppressing MMP-9 via the PKC (protein kinase C) activator pathway (144). Genistein prohibited the invasion of triple-negative MDA-MB 231 breast cancer cells in vitro, via down-regulation of MMP-9 activity (141, 144). Apigenin, quercine, and luteolin can also inhibit MMP-2 and -9 activities (145). Flavonoids with an increasing number of substitutions or hydroxyl groups illustrated a stronger inhibitory effect on the activity of MMP-9 and -2 (145, 146).

Suppression in the MMP proteins by CPE also was observed in in vivo models for colon (110, 111) and prostate tumors (127).

Likewise the reduction in MMPs, CPEs reduced levels of chemokine receptor CXCR4 together with the HER2/neu protein that stimulates the CXCR4 expression in MDA-MB-231 cells (142). CPE also showed suppression in the phospholipase-C gamma-1 (PLK-γ1) protein that required for cell migration in U937 cells (107). Furthermore, vascular cell adhesion molecule-1 (VCAM-1), which promotes the adherence of cells at new sites, was reduced by *citrus unshiu* Marc. peel in MDA-MB-231 cells through inhibition of protein kinase C (PKC)
phosphorylation (147). Many proteins related to the metastasis such as reduced epithelial mesenchymal transition (EMT) markers (N-cadherin, vimentin, and fibronectin), EMT-associated transcription factors (Slug and Snail), and SMADs were shown to be downregulated by the Ougan (citrus reticulate cv. suavissima) flavedo extract in SKOV3 cells (148).

E-cadherin plays an essential role in cell adhesion, and loss of E-cadherin is associated with a tendency for tumor metastasis (149). An increase in the expression of E-cadherin was observed in colon tumor-bearing mice fed with hydroxylated polymethoxyflavones in CPE (110). In another study, the Korean citrus aurantium L peel showed anti-metastatic properties by preventing the migration of A549 cells to the wounded area in vitro experiment (40).

4.6 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, myeloperoxidase, eosinophil peroxidase, and NADPH oxidase. Chronic inflammation is associated with carcinogenesis and acts as a driving force for cancer progression (150).

The expression of pro-inflammatory proteins is reduced by CPE in both in vitro and in vivo models (Table 3). Inducible nitric oxide synthase (iNOS) and inducible-type cyclooxygenase (COX) are enzymes that were induced in response to oxidative environment. Consequently, overexpression of these enzymes contributes to carcinogenesis through promotion of inflammation (7, 56, 122). CPEs down-regulated the expression of iNOS and COX-2 expression enzymes in human histiocytic lymphoma U937 cell lines, DU145 and murine macrophage RAW264.7 cells (75, 151-154). Reduction in these enzymes by CPEs was also
observed in colon, skin, and prostate cancer cell lines in vivo models (110-112). It is reported that CPEs in RAW264.7 cells reduced nitric oxide that is produced by iNOS (155).

Nuclear factor-kappa B (NF-κB) activation is an essential factor involved in inflammation. NF-κB is a heterodimeric protein composed of five subunits, and NF-κB presents in an inactive state in the cytoplasm due to the binding of inhibitory protein, IκBα (156, 157).

Upon the chemical signaling for the activation of NF-κB, the IκBα degrades and releases the NF-κB from its inactive state in the cytoplasm. The release of NF-κB allows the translocation of NF-κB subunits, p50 and p65, to the nucleus, where it activates the transcription of pro-inflammatory cytokines, chemokines, adhesion molecules, and enzymes. It is documented that CPEs treatment reduced the NF-κB activation and the nuclear translocation of its p50 and p65 subunits in RAW264.7, A549, MDA-MB-231, and U937 cancer cells (110, 151-153, 155, 158-161).

Likewise, inhibition of NF-κB suppresses a range of downstream genes that include pro-inflammatory cytokines. Sweet orange peel extract with high amount of PMFs suppressed the expression of TNF-α, ICAM-1, IL-1β, IL-6, and IL-8 in inflammation-induced U937 cells (151). The TNF-α, MCP-1, IL-6, and phosphorylated-p38 proteins were found to be lower in CPEs treated RAW264.7 cells than the control (153).

CPEs also have a suppressive effect on the signal transducer and activator of transcription 3 (STAT3) signaling pathway, which is involved in inflammation (75, 162). CPEs reduced the phosphorylation of STAT3 in DU145, PC-3, and prostate cancer cell lines M2182 (75). In the same study, janus-like kinase (JAK) and a c-Src kinase that mediated the phosphorylation of STAT3 were also found to be suppressed by CPE (110).

The mechanism of action of flavonoids on cancer cells is presented schematically in Figure 3. It is highly complex and involves not only some separate biological processes but also different modulation of overlapping cell signaling pathways.
5. Functional Evidence for Citrus Anti-Cancer Activity in *In Vivo* Models

CPE flavonoids have been suggested to play a critical role in a cancer prevention and maintaining a healthy lifestyle (163). Individual flavonoids such as apigenin, nobiletin, hesperidin and tangeretin, all highly enriched in CPE, have demonstrated anti-cancer activity in preclinical animal models. In addition to single and combined flavonoids, whole CPE has been tested for anti-cancer 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 approximately 30% polymethoxylated flavones such as nobiletin, sinensetin, tangeretin and monohydroxylated analogs. When mice were fed with a diet containing 0.2% of CPE before, during, and after carcinogen treatment (164), they showed a reduction in the number of aberrant crypt foci (ACF) - a histological biomarker for colon carcinogenesis - by 34-66% compared with control. The low incidence of tumor development could be due to the highly potent flavonoids in CPE (102, 164). Feeding mice with a diet containing 0.01% or 0.05% of hydroxylated PMFs for four weeks also reduced the total number of large ACF and tumors in colonic tissue by 40-44% compared to controls (110). When mice were fed with hydroxylated PMFs for 20 weeks, the number of microadenomas was reduced by up to 81% in comparison with controls. Similarly, oral administration of CPE with naringin and hesperidin reduced numbers of ACF up to 40% compared to the control group in colon tumor-bearing mice (110). Moreover, the addition of CPE (contained 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%, increased the number of apoptotic cells compared to the Western-style diet alone as well (165).
In another study, oral administration of ethanol extract of CPE (*citrus Junos* Tanaka) at 100 mg/kg/day significantly reduced the size of colorectal adenocarcinoma HT-29 tumor cells through reducing COX-2 expression in xenografts mice (153). Administration of methanol/water extract of dried citrus peel (*citrus reticulate* Blanco) at a dose of 1,000 ppm in the diet reduced total ACF by 75% compared to control (161). In a similar study, an in vivo model showed that a 70% aqueous methanol extract of CPE (*Korean citrus aurantium* L) could prevent human long (carcinoma) A549 cells migrating to lungs of mice injected with A549 cells via tail vein (40). These data suggested that CPE had effects on the regulation of apoptosis and cell migration.

In a two-stage skin carcinogenesis model, mice were treated with 7,12-dimethylbenz[a]anthracene 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*, *citrus hassaku*, *citrus limon*, *citrus natsudaidei*, *citrus 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 of above 5 mm by 33% compared to controls (112). The epidermal thickening was decreased 23-33% compared to control as a result of the associated inflammation and edema (112).

Apigenin reduced dimethyl benzanthracene-induced skin cancers by inhibiting epidermal ornithine decarboxylase, a key enzyme in cancer prevention (166). Nobiletin was effective in preventing skin carcinogenesis by suppression of 7,12-dimethylbenz(a)anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) and decreasing the inflammatory parameters (56). The 45-daily administration of hesperidin inhibited DMBA-induced experimental breast 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 (167). Tangeretin, a PMF, significantly arrested DMBA-induced breast cancer in rats (168). The anti-cancer activity of CPE (Gold Lotion) was also tested in prostate cancer models. In prostate cancer PC-3 tumor-bearing mice, treatment with CPE by intraperitoneal injection of 1 mg/kg/day reduced the tumor weight by 57% and tumor volume by 79% compared to control (127, 169). For mice treated with 2 mg/kg/day by oral ingestion, tumor weight was reduced by 86% and tumor size by 94%. The strong anti-cancer activity was attributed to the high concentration of PMFs and other compounds such as hesperidin. Chu, Chen, Chyau and Duh (116) showed the ethyl acetate extracts from sweet orange peel (50-500 µg/ml) reduced the human liver cancer HepG2 growth when tested in in vivo model and exhibits significant cytotoxicity on HepG2 cells.

Despite the growing number of preclinical animal studies, clinical trials involving citrus peel extracts are currently limited to a single study. Naringenin isolated from citrus aurantium peel (Chinese bitter orange) was tested as a therapeutic on 95 postoperative patients with osteosarcoma (170). The treatment group (n=47) that received 20 mg/day of naringenin showed significantly reduced osteosarcoma volume compared to placebo controls.

6. 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 (171) and contributes nearly 40% of the total weight of the fruit (49). Due to the low cost and current non-use of the peel by industry, citrus peel represents an untapped nutritional rich in bioactive compounds. There is a 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 anti-cancer utility for citrus-derived flavonoids. This property is linked to 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, anti-cancer, anti-proliferation, anti-angiogenesis, anti-oxidant, cell cycle regulation, and anti-metastasis effects.

7. Future studies

Further studies are needed to address both the basic science underlying CPE mechanisms in greater detail, as well as examining pharmacokinetics and pharmacodynamics as well as 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 (172). Further study may also focus on elucidating signaling pathways by which PCE can affect critical enzymes such as tyrosine and focal adhesion kinases, protein kinase C, and MMPs.

For clinical translation, trials in both the general population (as health supplements) and in the setting of cancer treatment are both 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 multi-targeted pharmacological strategy, either for cancer prevention or as a co-administration in oncological therapies.

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Figure 1. Main skeleton of flavonoids and their classes
Figure 2. The structural formula of main citrus peel flavonoids and their subclasses

Figure 3. Schematic of the main molecular mechanism of flavonoids on anticancer. 1) The anti-angiogenesis activity via VEGF by inhibiting HIF-1α/Akt/NFkB signaling pathways. 2) The anti-inflammation activity by decreasing p38 via MAPK and inhibiting the expression of COX-2. 3) The anti-metastasis activity via inhibition of MMP-2/9 by diminishing the Akt/FAK/Ras/PI3K signaling pathways. 4) The anti-proliferation activity via inhibiting PI3K/Akt; via cell-cycle arrest the G0/G1 or G1/S phase by activating p53 and p21, and also inhibiting BAX and bcl-2; via increasing cytochrome-c and activating caspase pathways.
Table 1: *In-vitro* anticancer effect of citrus peel extract

| Sample              | Compound Identification | Cell lines (IC50, µg/mL) | Cell cycle arrest | Anti-proliferation | Anti-apoptosis | Anti-metastasis | Anti-inflammatory & anti-angiogenesis | Reference |
|---------------------|-------------------------|--------------------------|-------------------|-------------------|----------------|----------------|--------------------------------------|-----------|
| Citrus reticulata   | D                       | WEHI 3B (<100)           |                   |                   |                |                |                                      | (173, 174) |
| Citrus reticulata   |                         | SNU-668 (=100)           | I                 |                   |                |                |                                      | (132)     |
| Citrus sinensis     | D                       | MCF-7 (10.2-17.9)        |                   | I                 |                |                |                                      | (175)     |
| Citrus grandis      | D                       | U937 (60), HepG2 (31), HeLa (287), HCT-15 (87), MCF-7 (144), NCI-H460 (73), SNU-16 (90) | I*                |                   |                |                |                                      | (68)      |
| 17 citrus varieties | D                       | HT-29 (31-45)            |                   |                   |                |                |                                      | (176)     |
| Citrus sunki        | D                       | HL-60 (25)               | G2/M              | I                 |                |                |                                      | (53)      |
| Citrus aurantium    | D                       | AGS (99)                 | G2/M              | I                 | I              |                |                                      | (38)      |
| Citrus aurantium    |                         | U937 (40-60)             |                   | I                 | I              |                |                                      | (107)     |
| Citrus grandis      | D                       | HeLa (100-200), AGS (200-400) | I             |                   |                |                |                                      | (70)      |
| Citrus aurantium    | D                       | A549 (230)               | G2/M              | I                 | I              |                |                                      | (39)      |
| Citrus unshiu       |                         | MDA-MB-231 (>200)       |                   | I                 |                |                |                                      | (147)     |
| Citrus junos        |                         | HT-29 (>1200)            |                   |                   |                | I              |                                      | (153)     |
| Citrus aurantifolia |                         | MCF-7 (59)               | G2/M              | I                 |                |                |                                      | (109)     |
| Citrus aurantium    | D                       | A549                     |                   | I                 | I              |                |                                      | (40)      |
| Citrus hassaku      | D                       | MDA-MB-231               |                   | I                 |                |                |                                      | (153)     |
| Citrus reticulata   | D                       | HepG2 (20-40), HL-60 (25-50), MDA-MB-231 (25-50) |                   |                   |                |                |                                      | (42)      |
| Citrus paradisi, Citrus sinensis, Citrus maxima | D                   | Caco-2, LoVo, LoVo/ADR |                   |                   |                |                |                                      | (143)     |
| Citrus hassaku      | D                       | SNU-1 (=25)              | G1                | I                 |                |                |                                      | (108)     |
| Citrus paradesi     |                         | Kasumi-1 (2000)          |                   | I                 |                |                |                                      | (126)     |
| Citrus reticulata   | D                       | SKOV3 (≥100)             |                   | I                 | I              |                |                                      | (148)     |
| Citrus platymamma       | D  | A549 (364)  | G2/M | I  | I  | I  | (86) |
| Citrus sphaerocarpa     | D  | MDA-MB-231 (>200) |       |       | I  | I  | (153) |
| Citrus iyo             | D  | U266 (>400), K562 (200–400), DU145 (>400), MDA-MB-231 (>400), HepG2 (200–400), RWPE-1 (>400) | I^  | I^  | I^  | I^  | (75) |
| Citrus platymamma       | D  | Hep3B (100–200), HepG2 (300–400) | G2/M | I^  | I^  | I^  | (106) |
| Citrus sinensis         | D  | HepG2 (>500) | G1   | I  | I  | I  | (116) |
| Citrus reticulata       |     | HCT116      |       |     |     |     | (161) |

D: Determined; I: Induced, * only for U937, ^ only for DU145, # only for Hep3B

### Table 2: In-vivo anticancer effect of citrus peel extract

| Sample                     | Animal models                        | Dose (route) | Duration | Effects                                      | Reference |
|----------------------------|--------------------------------------|--------------|----------|----------------------------------------------|-----------|
| Citrus junos               | HT-29 cells implanted mice           | 100 mg/kg/daily (i.p) | 4 weeks | Reduced tumor size, disease activity index and colon shortening | (153)     |
| Citrus aurantium           | A549 cells injected in mice tail vein | Twice weekly (i.p) | 5 weeks | Reduced cancer metastasis                     | (40)      |
| Citrus reticulata          | Treated leukemic cells injected into mice | 2/10 weeks | Reduced number of tumor cells and increased mice survival time | (173)     |
| Citrus sinensis            | AOM-induced carcinogenesis in mice   | 0.2% in diet | 26 weeks | Reduced number and size of ACF, tumor burden and incidence | (164)     |
| Citrus sinensis            | Western diet inducing cancer         | 0.25%/0.5% in diet | 9 weeks | Reduced tumor number, multiplicity and induced apoptotic | (165)     |
| Multiple citrus            | DMBA-induced carcinogenesis in mice  | 100/200 µL/twice weekly (cream application) | 20 weeks | Reduced epidermal thickness, number of papillomas, tumor incidence and tumor weight | (112)     |
| Citrus unshiu              | double-TPA-application to ICR mouse skin | 8.1 nmol/30 min | 24 hours | Inhibit NO and (O2-) generation | (56)      |
| Multiple citrus            | PC-3 cells implanted mice            | 1/2 mg/kg/five days per week (i.p) and 2 or 4 mg/kg/ five days per week (o.p) | 3 weeks | Suppressed tumor size. | (111)     |
| Multiple citrus            | AOM-induced carcinogenesis in mice   | 100/200 µL/five days per week (o.p) | 6 weeks | Reduced number of ACF | (111)     |
| Citrus iyo                 | DU145 cells implanted mice           | 50/200 mg/kg/thrice weekly (i.p) | 4 weeks | Suppressed tumor growth | (75)      |
| Citrus depressa            | TEWL and epidermal thickness in UVB-irradiated mouse skin | 100µl of 10%/day | 1 week | Reduce photoaging in mice | (177)     |
| Citrus sinensis            | HepG2 cells implanted mice           | 1/10 mg/kg/thrice weekly in diet | 3 weeks | Reduced tumor growth | (116)     |
| Citrus sinensis            | AOM-induced carcinogenesis in mice   | 0.01%/0.05% in diet | 4/18 weeks | Reduced number of ACF | (110)     |

i.p: intraperitoneal injection; o.p: oral injection; ACF: aberrant crypt foci; AOM: azoxymethane; 2, 4-dimethoxybenzaldehyde
| Flavonoids                      | Chemopreventive & anti-inflammatory 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 cancer cells | (39, 68, 129, 177-180)           |
|                               | Anti-angiogenesis, anti-inflammatory, anti-metastasis | 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 cancer cells                                      |                                   |
| Co-chemotherapeutic           | Increased cytotoxicity of doxorubicin        |                                                                           | MCF-7, T47D cancer cells                                                   |                                   |
| Suppressing carcinogenesis    | Inhibited the activity of CYP1A2             |                                                                           | MCF-7, T47D                                                                  |                                   |
| Anti-oxidant                  | Scavenge DPPH radicals, hydrogen peroxide scavenging, hydroxyl radical scavenging |                                                                           |                                                                           |                                   |
| Anti-metastasis               | Prevented the migration                      |                                                                           | A549 cells in vitro/in vivo                                                |                                   |
| Apoptosis                     | Downregulated (Bcl-2)/upregulation (Bax)    |                                                                           | HeLa; THP-1                                                                 |                                   |
| Anti-inflammatory             | Decreased activation of AP-1, NF-kB and CREB |                                                                           | RAW 264.7 monocyte/macrophage-like cells                                    |                                   |
| Anti-inflammatory             | Induced LPS-induced both O2 and N2 generation |                                                                           | Skin inflammation                                                           |                                   |
| Anti-inflammatory             | Induced the expression of COX-2 by suppressing UVB |                                                                           | Human keratinocytes in vitro                                               |                                   |
| Anti-metastatic               | Inhibited MEK1/2 activity is associated with the suppression of pro MMPs |                                                                           | Human fibrosarcoma HT-1080 cells                                           |                                   |
| Anti-metastatic               | Enhanced the expression of TIMP-1 by the activation of PKCbetaII/epsilon-JNK pathway |                                                                           | Human fibrosarcoma HT-1080 cells                                           |                                   |
| Anti-proliferation            | Decrease their differentiation into granulocytes and macrophages by TNF-α |                                                                           | Murine myeloid leukemia WEHI 3B cells                                       |                                   |
| Tangeretin (4′,5,6,7,8-pentamethoxyflavone) | Anti-oxidant | Scavenge DPPH radicals, hydrogen peroxide scavenging, hydroxyl radical scavenging |                                                                           | (107, 119, 122, 178, 181-185)    |
|                               | Anti-oxidant | Inhibited the activity of CYP1A1 and the expression of mRNA |                                                                           |                                   |
|                               | Apoptosis | Triggered apoptosis through p53 pathway |                                                                           | COLO 205, HL-60 cells                                                      |                                   |
|                               | Anti-proliferation | Decreased the expression of PROM1 and SNAI1 |                                                                           | Cancer stem cell of HT29                                                   |                                   |
|                               | Anti-proliferation, Apoptosis | Activated caspase-3 |                                                                           | Cocon LOvo/DX CELLS                                                      |                                   |
|                               | Co-chemotherapeutic | Increased cytotoxicity of doxorubicin |                                                                           | MCF-7, T47D                                                               |                                   |
| Property                          | Effect                                                                 | Cell Type                                    | Tissue/PD                                                            |
|----------------------------------|------------------------------------------------------------------------|----------------------------------------------|----------------------------------------------------------------------|
| 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, upregulated COLO 205 cells |                                             |
| Anti-inflammation                | Blocked AKT activation                                                 | Lung carcinoma cells                         |                                             |
| Anti-carcinogenic                | Inhibited P450 1A1/2/3A4                                               | Human liver microsome                        |                                             |
| Anti-metastatic                  | Decreased the number of metastatic nodules in Lentini’s model         | Melanoma B16F10                              |                                             |
| Anti-carcinogenic                | Reduced PhIP-DNA adduct formation in colon                             | Colon cancer cells                           |                                             |
| Anti-inflammation                | Induced LPS-induced NO production                                      | RAW 264.7 cells                              |                                             |
| Anti-inflammation                | Inhibited IL-1beta-induced production of COX-2 by the activation of JNK, AKT, ERK and p38 MAPK | A549, H1299                                  |                                             |
| **Sinensetin (5,6,7,3',4'-pentamethoxyflavone)** | Cell cycle arrest                                                      | HUVEC (137, 186-194)                        |                                             |
| Anti-angiogenesis, apoptosis     | Downregulated the mRNA expression of angiogenesis, flt1, hras and kdr | Zebrafish                                   |                                             |
| Anti-proliferation, apoptosis    | Inhibited iNOS expression, NO production and PGE2 production          | -                                           |                                             |
| Cell cycle regulation            | Inhibited on S phase by DNA elongation                                 | T47/D breast cancer cells                    |                                             |
| Anti-proliferation, cell cycle block | Captured cells G2/M phase and increased apoptosis, increased expression of p53 and p21 | AGS gastric cancer cells                     |                                             |
| Anti-inflammatory                | Inhibited inflammatory gene expression and STAT1 activation, inhibited iNOS, NO production and PGE2 production | Carrageenan-induced paw inflammation in the rat |                                             |
| Apoptosis                        | Reactivated oxygen species production, DNA damage, caspase activation | Leukemia cells                               |                                             |
| Anti-proliferation               | Activated Ca(2+)-dependent apoptotic proteases                         | MCF-7 breast cancer cells                    |                                             |
| Apoptosis                        | Upregulated caspase-3/8/9 and poly(ADP-ribose) polymerase (PARP) cleavage. | T-cell lymphoma Jurkat cells                 |                                             |
| Induced autophagy and cell death | Activated reactive oxygen species/ c-Jun N-terminal kinase (JNK), blocked the Akt/mTOR | T-cell lymphoma Jurkat cells                 |                                             |
| Cell cycle arrest                | Arrest cells at G0/G1 population                                       | HepG2 cells                                  |                                             |
| Apoptosis                        | Down-regulated Bcl-xL, up-regulated TRAIL and PTEN                    | HepG2 cells                                  |                                             |
| **Hesperetin (3',5,7,3'-trihydroxy-4'-methoxyflavone)** | Apoptosis                                                              | HL-60 cells (39, 86, 195-201)                |                                             |
| Anti-proliferation               | Inhibited oxidative stress and DNA damage                              | HT-29 colon adenocarcinoma                   |                                             |
| Anti-carcinogenic                | Downregulated the HIF-1a/VEGF/VEGFR2 pathway                          | Xenograft C6 gliomas cells in rats           |                                             |
| Cell cycle arrest                | Decreased cyclin D1, CDK4                                              | MCF-7 cancer cells                           |                                             |
| Phenomenon                        | Activity                                                                 | Cell line                                                                 |
|----------------------------------|--------------------------------------------------------------------------|---------------------------------------------------------------------------|
| **Anti-metastatic**              | Induced COX-2, MMP-2 and MMP-9                                            | DMH-induced colon cancer in rat B16-F10 murine melanoma cells             |
| **Apoptosis**                    | Activated of the mitochondrial pathway by rising levels of ROS, Ca2+ and ATP on mice | Xenograft tumors in mice model of gastric cancer                           |
| **Apoptosis, anti-proliferation**| Suppressed the expression of NFκB, p38 and caspase-3                     | PC-3 prostate cancer cells                                                |
| **Cell cycle arrest**            | G2/M arrest by controlling the level of cyclin B1, CDC2, CDC25C and p21   | A549 lung cancer, MCF-7                                                   |
| **Apoptosis**                    | Increased the expression of caspase-3, caspase-8, caspase-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-chamnoglucoside)** | Cell cycle regulation: Upregulated p21, G1-phase arrest, activated Ras/Rat/ERK-mediated, decreased cyclin D1 and cyclin E | 5637 bladder cancer cells, MDA-MB-231 xenograft mice (96, 202-208) |
| **Metastasis, anti-carcinogenic**| Inhibited the activity of PI3K/Akt/mTOR and upregulated p21CIP1/WAFI      | AGS cells                                                                 |
| **Cell cycle arrest**            | Cell cycle arrest in S phase                                              | HT-29                                                                     |
| **Anti-proliferation, anti-oxidant** | Modulated gene expression: Decrease DNA methyltransferase activity, down-regulated the expression of Be12 and Bcl-xL | SKOV3 ovarian cancer cells                                                |
| **Cell cycle arrest**            | Increasing p21 and arrest G1 cell cycle, inhibited the activity of CDK2   | MCF-7                                                                    |
| **Anti-proliferative**           | Inhibited CYP3A4, CYP1A2, CYP2C9, CYP2C19 and CYP2D6                      |                                                                           |
| **Anti-proliferation, apoptosis**| Decreased the mRNA expressions 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                                                                    |
| **Anti-metastasis**              | Downregulation of MMP-9 and repressed the PI3K/akt/mTOR/p70S6K signaling pathway | MCF-7                                                                    |
| **Anti-proliferation**           | Upregulated EGFR and ERK phosphorylation                                  | HeLa and A549 cells                                                      |
| **Anti-proliferation, apoptosis**| Suppressing the NF-κB/COX-2-casppase-1                                    | HeLa                                                                    |
| **Hesperidin (Hesperetin-7-rutinoside)** | Anti-proliferative: Inhibited MMP-9 by NF-κB and AP-1 signaling           | NALM-6 leukemia cells                                                    (195, 196, 209-214) |
| **Apoptosis**                    | Inhibited the PI3K/Akt pathway through PTEN-phosphatase                   | SUN-C4 colon cancer cells                                                |
| **Anti-metastatic, angiogenesis**| Suppressing ANGPT1 gene                                                   | Laryngeal cancer cells                                                   |
| Category                | Effect Description                                                                 | Cells Tested                      |
|-------------------------|-------------------------------------------------------------------------------------|-----------------------------------|
| Anti-proliferation      | Upregulated the level of p21 and p53                                                | MCF-7 cells                       |
| Apoptosis               | Inhibited Aurora-A and Akt mediated GSK-3β/β catenin cascade                        | A431 skin cancer cells            |
| Anti-oxidant            | 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       |
| Anti-inflammation       | Inhibited IL-6, TNF, COX-2, iNOS inflammatory components                              | A431 skin cancer cells            |
| Anti-proliferation      | Upregulated BAX and downregulate Bcl-2, decreased the release of cytochrome c        | HeLa cervical cancer cells, A2780 ovarian cancer cells |
| Co-chemotherapeutic     | Inhibited Pgp activity                                                               | Human leukemia cells (CEM/ADR5000) |